

ADOPTED: 14 December 2022

doi: 10.2903/j.efsa.2023.7789

## Welfare of laying hens on farm

EFSA Panel on Animal Health and Animal Welfare (AHAW),  
Søren Saxmose Nielsen, Julio Alvarez, Dominique Joseph Bicot, Paolo Calistri,  
Elisabetta Canali, Julian Ashley Drewe, Bruno Garin-Bastuji, Jose Luis Gonzales Rojas,  
Christian Gortázar Schmidt, Mette Herskin, Miguel Ángel Miranda Chueca, Barbara Padalino,  
Paolo Pasquali, Helen Clare Roberts, Hans Spoolder, Karl Stahl, Antonio Velarde, Arvo Viltrop,  
Christoph Winckler, Inmaculada Estevez, Maryse Guinebretière, Bas Rodenburg,  
Lars Schrader, Inga Tiemann, Thea Van Niekerk, Michele Ardizzone, Sean Ashe,  
Michaela Hempten, Olaf Mosbach-Schulz, Cristina Rojo Gimeno, Yves Van der Stede,  
Marika Vitali and Virginie Michel

### Abstract

This scientific opinion focuses on the welfare of laying hens, pullets and layer breeders on farm. The most relevant husbandry systems used in Europe are described. For each system, highly relevant welfare consequences were identified, as well as related animal-based measures (ABMs), and hazards leading to the welfare consequences. Moreover, measures to prevent or correct the hazards and/or mitigate the welfare consequences are recommended. The highly relevant welfare consequences based on severity, duration and frequency of occurrence are bone lesions, group stress, inability to avoid unwanted sexual behaviour, inability to perform comfort behaviour, inability to perform exploratory or foraging behaviour, isolation stress, predation stress, resting problems, restriction of movement, skin disorders and soft tissue lesions and integument damage. The welfare consequences of non-cage compared to cage systems for laying hens are described and minimum enclosure characteristics are described for laying hens, pullets and layer breeders. Beak trimming, which causes negative welfare consequences and is conducted to reduce the prevalence and severity of pecking, is described as well as the risks associated with rearing of non-beak-trimmed flocks. Alternatives to reduce sharpness of the beak without trimming are suggested. Finally, total mortality, plumage damage, wounds, keel bone fractures and carcass condemnations are the most promising ABMs for collection at slaughterhouses to monitor the level of laying hen welfare on farm. Main recommendations include housing all birds in non-cage systems with easily accessible, elevated platforms and provision of dry and friable litter and access to a covered veranda. It is further recommended to implement protocols to define welfare trait information to encourage progress in genetic selection, implement measures to prevent injurious pecking, rear pullets with dark brooders and reduce male aggression in layer breeders.

© 2023 European Food Safety Authority. *EFSA Journal* published by Wiley-VCH GmbH on behalf of European Food Safety Authority.

**Keywords:** laying hens, on-farm welfare, husbandry systems, welfare consequences, animal-based measures, end the cage age, beak trimming

**Requestor:** European Commission

**Question number:** EFSA-Q-2020-00483

**Correspondence:** ahaw@efsa.europa.eu

**Panel members:** Søren Saxmose Nielsen, Julio Alvarez, Dominique Joseph Bicout, Paolo Calistri, Elisabetta Canali, Julian Ashley Drewe, Bruno Garin-Bastuji, Jose Luis Gonzales Rojas, Christian Gortázar Schmidt, Mette Herskin, Virginie Michel, Miguel Ángel Miranda Chueca, Barbara Padalino, Paolo Pasquali, Helen Clare Roberts, Hans Spoolder, Karl Stahl, Antonio Velarde, Arvo Viltrop and Christoph Winckler.

**Declarations of interest:** If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact [interestmanagement@efsa.europa.eu](mailto:interestmanagement@efsa.europa.eu).

**Acknowledgements:** The Panel wishes to thank the following for the support provided to this scientific output: hearing experts: Stephanie Buijs, Christine Nicol; broiler welfare working group: Ingrid de Jong, Sabine Gebhardt, Linda Keeling, Anja Brinch Riber; EFSA Framework Contract on support services for Expert Knowledge Elicitation: Monique Bestman, Ute Knierim, Karen Laing, Hans-Hermann Thulke; EFSA BIOHAW Unit: Mariana Geffroy, Eléa Bailly-Caumette and Kateryna Chuzhakina. The Panel wishes to acknowledge all European competent institutions, Member State bodies and other organisations that provided data for this scientific output.

**Waiver:** In accordance with Article 21 of the Decision of the Executive Director on Competing Interest Management a waiver was granted to an expert of the Working Group. Pursuant to Article 21(6) of the aforementioned Decision, the concerned expert was allowed to take part in the discussion and in the drafting phase of the EFSA Scientific Opinion concerning the welfare of laying hens, and has not been allowed to be, or act as, a chairman, a vice-chairman or rapporteur of the Working Group. Any competing interests are recorded in the respective minutes of the meetings of the AHAW Panel.

**Suggested citation:** EFSA AHAW Panel (EFSA Panel on Animal Health and Animal Welfare), Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Canali E, Drewe JA, Garin-Bastuji B, Gonzales Rojas JL, Gortázar Schmidt C, Herskin M, Miranda Chueca MA, Padalino B, Pasquali P, Roberts HC, Spoolder H, Stahl K, Velarde A, Viltrop A, Winckler C, Estevez I, Guinebretière M, Rodenburg B, Schrader L, Tiemann I, Van Niekerk T, Ardizzone M, Ashe S, Hempen M, Mosbach-Schulz O, Gimeno Rojo C, Van der Stede Y, Vitali M and Michel V, 2023. Scientific Opinion on the welfare of laying hens on farm. *EFSA Journal* 2023;21(2):7789, 188 pp. <https://doi.org/10.2903/j.efsa.2023.7789>

**ISSN:** 1831-4732

© 2023 European Food Safety Authority. *EFSA Journal* published by Wiley-VCH GmbH on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs](https://creativecommons.org/licenses/by/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.

EFSA may include images or other content for which it does not hold copyright. In such cases, EFSA indicates the copyright holder and users should seek permission to reproduce the content from the original source.



The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.



## Summary

In the framework of its Farm to Fork Strategy, the European Commission is undertaking a comprehensive evaluation of the animal welfare legislation, including Council Directive 1999/74/EC<sup>1</sup>, which lays down minimum standards for the protection of laying hens. Directive 1999/74/EC currently does not apply to hatcheries, pullets or layer breeders. In addition, a European Citizen Initiative (ECI) 'end the cage age', registered in September 2018, calls for banning the use of cages in laying hens, pigs and calves. Against this background, the European Commission requested the European Food Safety Authority (EFSA) to give an independent view on the protection of laying hens during the different phases of the production cycle including laying hen breeders, pullets and hens during production. The European Commission requested EFSA to describe the current husbandry systems and practices of keeping laying hens, pullets and layer breeders, related welfare consequences, animal-based measures (ABMs) and hazards. The European Commission also requested EFSA recommendations to prevent, or correct hazards and mitigate the welfare consequences. For three specific ToRs, the European Commission requests ABMs and preventive and corrective measures with qualitative or quantitative criteria. These scenarios concern (1) a comparison of risks for laying hen welfare associated with non-cage systems compared to cage systems, (2) risks associated with rearing of non-beak trimmed birds, and (3) the assessment of ABMs collected in slaughterhouses to monitor the level of welfare on laying hen farms. To answer these terms of reference, the scientific literature was reviewed, expert elicitations were carried out, a behavioural space model was developed, and a questionnaire was sent to European breeder associations (EFFAB). Sources of uncertainty related to the data collection and the assessment are described and certainty ranges defined. Conclusions for which the certainty is below 90% are presented with their associated certainty level.

Eleven welfare consequences were identified as highly relevant for the welfare of laying hens, pullets and/or layer breeders, based on severity, duration and frequency of occurrence: 'bone lesions', 'group stress', 'inability to avoid unwanted sexual behaviour', 'inability to perform comfort behaviour', 'inability to perform exploratory or foraging behaviour', 'isolation stress', 'predation stress', 'resting problems', 'restriction of movement', 'skin disorders (other than soft tissue lesions and integument damage)' and 'soft tissue lesions and integument damage'.

ABMs were identified for each of the highly relevant welfare consequences. ABMs that are relevant to more than one welfare consequences are referred to as 'iceberg indicators'. The following ABMs were identified as iceberg indicators: 'fear response', 'injurious pecking', 'physiological stress indicators', 'plumage damage', 'pushing and jostling behaviours' and 'wounds'.

Hazards were identified for the different welfare consequences and the currently used husbandry systems. These were related to aspects of the environment within which laying hens, pullets and layer breeders are kept (i.e. group size, surface area, stocking density), the equipment and enrichment material used (i.e. elevated structures, litter, nest, feeders and drinkers) and the management (i.e. light schemes, temperature and humidity, gas and noise). Housing conditions clearly affect the level of aggression and injurious pecking, although in adult laying hens, associations between these behaviours and stocking density are inconsistent. In this Scientific Opinion, it was concluded that for laying hens, pullets aged 6 weeks and older and layer breeders, and with group size larger than 30 birds, the minimum surface areas required to prevent the negative welfare consequences that arise from restriction of space is estimated to be 80 m<sup>2</sup>. If group size is less than 30 birds, it is judged to be 25 m<sup>2</sup> (> 50–100% certainty).

Knowledge elicitation and modelling exercises were used, leading to recommendation for adult laying hens and layer breeders of a maximum stocking density of 4 birds per m<sup>2</sup> to reduce the risk of plumage damage and allow unconstrained performance of motivated behaviours, including those that occupy most space (e.g. wing flapping). In relation to enrichment material, laying hens must be provided with suitable elevated structures such as perches and platforms to fulfil the behavioural need for night-time roosting. In multi-tier systems additional non-slippery perches or ramps with an angle of no more than 45 degrees should be used to facilitate access to the tiers. Pullets should have access to elevated structures from the third week of life. From the age of approximately 6 weeks of age, pullets have the same requirements as laying hens for most resources. In pullets, high stocking densities are associated with decreased plumage score and increased feather pecking. From the literature, a maximum stocking density of 15 pullets per m<sup>2</sup> by the end of the rearing period is recommended.

<sup>1</sup> Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens OJ L 203, 3.8.1999, pp. 53–57.

Litter for laying hens must be kept clean, dry and friable at all times, including in systems with outdoor access. Edible enrichment material (e.g. pecking blocks) should be provided from day one, and at least one nest per 7 hens should be provided, with soft, manipulable material for nest floor allowing nestbuilding behaviour. If group nests are used, there should be at least 1 m<sup>2</sup> of nest space for a maximum of 120 hens. Pullets in their brooding-rearing phase need easy access to the feed and water and need to have access to dark brooders. Sufficient drinkers and feeders should be provided. Light intensity and lighting pattern should be as such that it favours a correct development of laying hens. Only light intensities below 5 lx have been associated with reduced behavioural activity and poor welfare states, but no evidence was provided that light intensity should be higher than the legally required minimum of 20 lx. Birds seem to prefer specific light intensities for specific behaviours and on average spend more time in low than in high light intensities. Laying hens must be provided with an uninterrupted period of darkness of at least 8 h, with dusk and dawn periods. Systems that provide daylight, outdoor access or covered verandas have positive effects on behaviour and help to prevent feather damage. If a range is provided, it should include cover (e.g. vegetation and 50% covered by bushes and trees) and must be well managed to encourage even distribution of birds and to reduce the risk of predation. A covered veranda (covered outdoor area) or outdoor access should have at least 1-m pop-hole width per 500 birds. Hens, pullets and breeding birds should not be exposed to background sound that exceeds 75 dB.

Cage should not be used.

Beak trimming is routinely conducted to reduce the prevalence and severity of pecking (e.g. injurious pecking and vent pecking) in laying hens and laying breeders. Injurious pecking results in serious welfare consequences. The damage incurred by injurious pecking is more severe in non-beak-trimmed flocks than in infrared beak-trimmed flocks. The process of beak trimming itself causes welfare consequences. Alternative approaches to reduce the sharpness of the beak without trimming consist of (i) breeding birds with short or blunt beaks; (ii) achieving and maintaining blunt beaks by using hard, abrasive surface materials that shorten the beak as birds eat (e.g. abrasive feeders); (iii) the use of hard materials as pecking substrates (e.g. pecking stones) to reduce inter-bird pecking by redirecting the pecking behaviour towards the environment. The practice of beak trimming should not be necessary if good management practices are implemented.

Total mortality, plumage damage, wounds, keel bone fractures and carcass condemnations are the most promising ABMs for collection at slaughterhouses to monitor the level of laying hen welfare on farm. However, the readiness for automation is different for the different ABMs. Currently there is a lot of variation in the method used for measuring each ABM and only a few are validated. This impairs standardisation within and between EU member states and subsequently comparison of the resulting data. Unified and standardised scoring systems and protocols across different regions/countries are necessary to monitor and benchmark the welfare of laying.

## Table of contents

Abstract.....	1
Summary.....	3
1. Introduction.....	9
1.1. Background and Terms of Reference as provided by the requestor.....	9
1.2. Interpretation of the Terms of Reference.....	9
2. Data and methodologies.....	11
2.1. Data.....	11
2.2. Methodologies.....	11
2.2.1. General ToRs.....	11
2.2.2. Specific Terms of Reference.....	13
2.2.2.1. The welfare of laying hens and the risks associated with alternative systems (organic, free range and barn) compared to the cage system currently allowed (Specific ToR 1).....	13
2.2.2.2. Welfare of hens in furnished cages, and risks associated with rearing of animals non-beak trimmed (Specific ToR 2).....	17
2.2.2.3. The assessment of animal-based measures collected in slaughterhouses to monitor the level of welfare on laying hen farms (Specific ToR 3).....	17
2.2.3. Uncertainty analysis.....	17
3. Assessment.....	18
3.1. Production cycles of domestic fowl ( <i>Gallus gallus</i> ) related to the production of eggs.....	19
3.1.1. Layer breeders.....	19
3.1.2. Immature birds.....	20
3.1.2.1. Females (pullets).....	20
3.1.2.2. Immature males.....	21
3.1.3. Laying hens during the production of eggs.....	21
3.2. Husbandry systems.....	21
3.2.1. Floor systems with maximum one tier.....	21
3.2.1.1. Laying hens: single-tier.....	21
3.2.1.2. Pullets: floor systems without elevated structures.....	22
3.2.1.3. Pullets: floor systems with single-tier or other elevated structures.....	24
3.2.1.4. Layer breeders: single-tier.....	25
3.2.1.5. Layer breeders: single-tier with slatted floor.....	25
3.2.2. Floor systems with multi-tier.....	25
3.2.2.1. Laying hens: multi-tier.....	25
3.2.2.2. Pullets: multi-tier.....	26
3.2.2.3. Layer breeders: multi-tier.....	28
3.2.3. Systems with access to covered veranda and/or outdoor range.....	28
3.2.3.1. Pullets and laying hens: mobile housing.....	28
3.2.3.2. Covered veranda.....	30
3.2.3.3. Outdoor range.....	30
3.2.4. Collective cages.....	31
3.2.4.1. Laying hens in furnished cages.....	31
3.2.4.2. Pullets.....	32
3.2.4.3. Laying hen breeders.....	33
3.2.5. Individual cages.....	33
3.2.5.1. Laying hen breeders.....	33
3.3. Describing welfare of domestic fowl ( <i>Gallus gallus</i> ) related to the production of eggs.....	34
3.3.1. Bone lesions.....	38
3.3.1.1. Description of the welfare consequence.....	41
3.3.1.2. ABMs.....	42
3.3.1.3. Hazards and preventive or mitigating measures.....	43
3.3.2. Group stress.....	45
3.3.2.1. Description of the welfare consequence.....	45
3.3.2.2. ABMs.....	48
3.3.2.3. Hazards and preventive or mitigating measures.....	49
3.3.3. Inability to avoid unwanted sexual behaviour.....	51
3.3.3.1. Description of the welfare consequence.....	51
3.3.3.2. ABMs.....	52
3.3.3.3. Hazards and preventive or mitigating measures.....	53
3.3.4. Inability to perform comfort behaviour.....	53
3.3.4.1. Description of the welfare consequence.....	53
3.3.4.2. ABMs.....	57

3.3.4.3.	Hazards and preventive or mitigating measures .....	59
3.3.5.	Inability to perform exploratory or foraging behaviour .....	60
3.3.5.1.	Description of the welfare consequence .....	60
3.3.5.2.	ABMs .....	61
3.3.5.3.	Hazards and preventive or mitigating measures .....	62
3.3.6.	Isolation stress .....	63
3.3.6.1.	Description of the welfare consequence .....	63
3.3.6.2.	ABMs .....	63
3.3.6.3.	Hazards and preventive or mitigating measures .....	64
3.3.7.	Predation stress .....	64
3.3.7.1.	Description of the welfare consequence .....	64
3.3.7.2.	ABMs .....	65
3.3.7.3.	Hazards and preventive or mitigating measures .....	66
3.3.8.	Resting problems .....	67
3.3.8.1.	Description of the welfare problem .....	67
3.3.8.2.	ABMs .....	67
3.3.8.3.	Hazards and preventive or mitigating measures .....	68
3.3.9.	Restriction of movement .....	69
3.3.9.1.	Description of the welfare consequence .....	69
3.3.9.2.	ABMs .....	71
3.3.9.3.	Hazards and preventive or mitigating measures .....	72
3.3.10.	Skin disorders (other than soft tissue lesions and integument damage) .....	73
3.3.10.1.	Description of the welfare consequence .....	73
3.3.10.2.	ABMs .....	74
3.3.10.3.	Hazards and preventive or mitigating measures .....	74
3.3.11.	Soft tissue lesions and integument damage .....	74
3.3.11.1.	Description of the welfare consequence .....	74
3.3.11.2.	ABMs .....	75
3.3.11.3.	Hazards and preventive or mitigating measures .....	76
3.3.12.	ABMs related to the assessment of more than one welfare consequence (so-called 'iceberg indicators') .....	76
3.3.13.	Welfare consequences linked to access to covered veranda and outdoor range .....	79
3.3.14.	Welfare of male chicks of the layer breed .....	81
3.3.15.	Welfare consequences linked to feed restriction .....	83
3.3.16.	The impact of laying hen breeding and genetics on welfare .....	83
3.4.	Welfare of laying hens and the risks associated with alternative systems (organic, free range and barn) compared to the cage system currently allowed (Specific ToR 1) .....	84
3.4.1.	Part 1: comparison of cage and non-cage systems for laying hens .....	85
3.4.2.	Part 2: minimum enclosure characteristics .....	86
3.4.2.1.	Environment .....	86
3.4.2.2.	Equipment .....	94
3.4.2.3.	Management .....	100
3.4.2.4.	Access to covered veranda and/or outdoor range .....	103
3.5.	Welfare of hens in furnished cages, and risks associated with rearing of animals non-beak trimmed (Specific ToR 2) .....	104
3.5.1.	Injurious pecking .....	104
3.5.1.1.	Description of different types of injurious pecking .....	104
3.5.1.2.	Prevalence of injurious pecking .....	105
3.5.1.3.	Causes of injurious pecking .....	105
3.5.1.4.	Welfare consequences associated with injurious pecking .....	106
3.5.2.	Beak trimming .....	106
3.5.2.1.	Description of the process of beak trimming .....	106
3.5.2.2.	Welfare consequences associated with beak trimming .....	107
3.5.3.	Risks associated with raising non-beak-trimmed birds .....	110
3.5.3.1.	Laying hens .....	110
3.5.3.2.	Pullets .....	111
3.5.4.	Preventive measures to avoid injurious pecking in non-beak-trimmed hens .....	111
3.5.4.1.	Management strategies .....	111
3.5.4.2.	Genetic strategies .....	113
3.5.4.3.	Alternative strategies to obtain shortened or blunted beaks .....	114
3.5.5.	Mitigating measures to avoid injurious pecking in non-beak-trimmed hens .....	114
3.6.	The assessment of animal-based measures collected in slaughterhouses to monitor the level of welfare on laying hen farms (Specific ToR 3) .....	114

3.6.1.	Mortality (on farm)	115
3.6.1.1.	Description	115
3.6.1.2.	Interpretation	116
3.6.1.3.	Assessment	116
3.6.2.	Plumage damage	117
3.6.2.1.	Description	117
3.6.2.2.	Interpretation	117
3.6.2.3.	Assessment	117
3.6.3.	Wounds	118
3.6.3.1.	Description	118
3.6.3.2.	Interpretation	118
3.6.3.3.	Assessment	118
3.6.4.	Keel bone fracture	119
3.6.4.1.	Description	119
3.6.4.2.	Interpretation	119
3.6.4.3.	Assessment	119
3.6.5.	Carcass condemnations	120
3.6.5.1.	Description	120
3.6.5.2.	Interpretation	120
3.6.5.3.	Assessment	120
4.	Conclusions and recommendations	121
4.1.	Answers to general ToRs	121
4.1.1.	Bone lesions	121
4.1.1.1.	Conclusions	121
4.1.1.2.	Recommendations	122
4.1.2.	Group stress	122
4.1.2.1.	Conclusions	122
4.1.2.2.	Recommendations	123
4.1.3.	Inability to avoid unwanted sexual behaviour	123
4.1.3.1.	Conclusions	123
4.1.3.2.	Recommendations	124
4.1.4.	Inability to perform comfort behaviour	124
4.1.4.1.	Conclusions	124
4.1.4.2.	Recommendations	124
4.1.5.	Inability to perform exploratory or foraging behaviour	125
4.1.5.1.	Conclusions	125
4.1.5.2.	Recommendations	125
4.1.6.	Isolation stress	125
4.1.6.1.	Conclusions	125
4.1.6.2.	Recommendations	125
4.1.7.	Predation stress	125
4.1.7.1.	Conclusions	125
4.1.7.2.	Recommendations	126
4.1.8.	Resting problems	126
4.1.8.1.	Conclusions	126
4.1.8.2.	Recommendations	126
4.1.9.	Restriction of movement	127
4.1.9.1.	Conclusions	127
4.1.9.2.	Recommendations	127
4.1.10.	Skin disorders (other than soft tissue lesions and integument damage)	127
4.1.10.1.	Conclusions	127
4.1.10.2.	Recommendations	127
4.1.11.	Soft tissue lesions and integument damage	128
4.1.11.1.	Conclusions	128
4.1.11.2.	Recommendations	128
4.1.12.	Welfare of male chicks of the layer breed	128
4.1.12.1.	Conclusions	128
4.1.12.2.	Recommendations	128
4.1.13.	Welfare consequences linked to feed restriction	128
4.1.13.1.	Conclusion	128
4.1.13.2.	Recommendation	128
4.1.14.	The impact of laying hen breeding and genetics on welfare	129
4.1.14.1.	Conclusions	129

4.1.14.2.	Recommendations .....	129
4.2.	Answers to Specific ToR 1: The welfare of laying hens and the risks associated with alternative systems (organic, free range and barn) compared to the cage system currently allowed .....	129
4.2.1.	Conclusions.....	129
4.2.2.	Recommendations:.....	132
4.3.	Answers to Specific ToR 2: welfare of hens in furnished cages, and risks associated with rearing of animals non-beak trimmed.....	134
4.3.1.	Conclusions.....	134
4.3.2.	Recommendations .....	135
4.4.	Answers to Specific ToR 3: the assessment of animal-based measures collected in slaughterhouses to monitor the level of welfare on laying hen farms .....	135
4.4.1.	Conclusions.....	135
4.4.2.	Recommendations .....	136
4.5.	Main recommendations concerning more urgent welfare issues in egg production .....	136
	References.....	136
	Abbreviations .....	160
	Appendix A – Request for data to reply to the European Commission on the scientific opinion on the welfare of laying hens .....	162
	Appendix B – Expert knowledge elicitation Report.....	164
	Appendix C – Behavioural space model .....	177
	Appendix D – Assessment of ABMs collected in slaughterhouses to monitor the level of welfare on laying hen farms .....	182
	Appendix E – Uncertainty analysis .....	184
	Appendix F – Measuring welfare consequences.....	187

## 1. Introduction

### 1.1. Background and Terms of Reference as provided by the requestor

The Commission requested EFSA to give an independent view on the protection of domestic fowl (species *Gallus gallus*) related to the production of eggs.

The request includes the different phases of the production cycle:

- 1) The keeping of laying hen breeders
- 2) The keeping of chicks and pullets before they become laying hens
- 3) The keeping of hens during the production of eggs

The killing of domestic fowls in farms and hatcheries is not part of the request.

The EU legislation on the marketing standards for eggs and on organic farming defines minimum standards for production systems for laying hens that are available for the information of consumers.

In the context of the ECI "end of the cage age", the EFSA will explore scientific information on the advantages and disadvantages of the above production systems for the welfare of laying hens, taking into account the possible diversity of the production systems.

The EFSA will also explore how the above-mentioned production systems could be improved regarding animal welfare.

For this request, the EFSA will for each category of animals:

- 1) Describe, based on existing literature and reports, the current husbandry systems and practices of keeping them (General ToR 1);
- 2) Describe the relevant **welfare consequences**. Relevance will not need to be based on a comprehensive risk assessment, but on EFSA's expert opinion regarding the severity, duration and occurrence of each welfare consequence (General ToR 2);
- 3) Define qualitative or quantitative measures to assess the welfare consequences (**animal-based measures**) (General ToR 3);
- 4) Identify the **hazards** leading to these welfare consequences (General ToR 4);
- 5) Provide **recommendations** to prevent, mitigate or correct the welfare consequences (General ToR 5).

For the following scenarios, the Commission has identified practical difficulties or insufficient information in ensuring the welfare of animals. At least for them, the EFSA will propose detailed ABMs and preventive and corrective measures with, where possible, either qualitative (yes/no question) or quantitative (minimum/maximum) criteria (i.e. requirements to prevent and/or mitigate the welfare consequences).

- The welfare of laying hens and the risks associated with alternative systems (organic, free range and barn) compared to the cage system currently allowed (Specific ToR 1);
- Welfare of hens in furnished cages, and risks associated with rearing of animals non-beak trimmed (Specific ToR 2);
- The assessment of ABMs collected in slaughterhouses to monitor the level of welfare on laying hen farms (Specific ToR 3).

### 1.2. Interpretation of the Terms of Reference

The EFSA guidance on risk assessment for animal welfare (EFSA AHAW Panel, 2012) defines welfare consequence as 'the change in welfare that results from the effect of a factor or factors'. Based on that definition, a welfare consequence may be negative or positive; however, for the purpose of this assessment, only those welfare consequences that impair the welfare of animals are assessed (EFSA AHAW Panel, 2022b).

The EFSA guidance on risk assessment for animal welfare (EFSA AHAW Panel, 2012) defines an ABM as 'a response of an animal or an effect on an animal. It can be taken directly from the animal or indirectly and includes the use of animal records. The measure may, for example, be intended to: (i) assess the degree of impaired functioning associated with injury, disease, and malnutrition; (ii) provide information on animals' needs and affective states such as hunger, pain and fear, often by measuring the strength of animals' preferences, motivations and aversions; or (iii) assess the physiological, behavioural and immunological changes or effects that animals show in response to various challenges'.

In this scientific opinion, a list of iceberg indicators is provided based on the ABMs listed for each of the described welfare consequences. Iceberg indicators are indicators that can be used to obtain a quick overview on possible welfare problems, as they may reflect several welfare consequences in an integrative manner. They provide an overall assessment of welfare, just as the protruding tip of an iceberg signals its submerged bulk beneath the water's surface (FAWC, 2009; EFSA AHAW Panel, 2022a,b). For that reason and in the context of this opinion, the listed iceberg indicators have the ability to effectively measure several welfare consequences at the same time.

A *behavioural need* is related to behaviours which are part of the natural repertoire and are primarily motivated by internal causal factors (Weeks and Nicol, 2006). The motivation to perform these behaviours will be present whatever environment the animal is housed in. Animals will often attempt to perform these behaviours even in the absence of resources which facilitate them. In laying hens, the performance of 'sham' dustbathing on a wire floor, in the absence of a preferred substrate, is a good example of the fact that dustbathing is a 'behavioural need' (EFSA AHAW Panel, 2005). Sufficient space and resources should be provided to allow the behaviour to become functional and reach the end goal. A *behavioural preference* indicates the outcome when a bird has a choice, e.g. between different foraging, nesting or dustbathing substrates, or for perches of different characteristics.

The most relevant husbandry systems (i.e. systems existing at commercial level in EU) for laying hens, pullets and layer breeders are identified and described in detail not limiting to those that are commonly called 'cages' (e.g. layer breeders in individual cages and collective cages).

In the background to this mandate, as provided by the European Commission, a cage is described in the following terms:

'In its common meaning "cage" means a box or enclosure having some openwork (e.g., wires, bars) for confining or carrying animals. It can cover either individually confined animals or animals kept in group in a limited space'.

This definition is ambiguous and covers various husbandry systems, e.g. individual and collective cages, with or without enrichment. It is generally understood that cages are enclosures where space is limited (horizontally and vertically) such that monitoring of birds is conducted from outside and not within the same space as the birds.

This scientific opinion addresses the welfare of laying hens, pullets and layer breeders (considered 'animal categories') on farm.

The assessment is divided in two tasks. First, answering the set of ToRs listed in the mandate (so-called 'General ToRs'), and second, addressing three specific scenarios (so-called 'Specific ToRs').

To answer the General ToRs, the risk assessment is conducted by welfare consequences and not by hazards, as many hazards will lead to the same welfare consequences. To avoid repetition, the description of the welfare consequences and associated ABMs is provided at an upper level, i.e. for all husbandry systems and animal categories.

The most relevant husbandry systems (i.e. systems existing at commercial level in EU) for laying hens, pullets and layer breeders are described, including cage and non-cage systems, as well as systems with access to a covered veranda and/or outdoor free range. For each husbandry system, specific elements related to each animal category are described, when applicable.

The General ToRs of the Scientific Opinion contain the description of the five husbandry systems and then for each welfare consequence (i) the ABMs (with specific elements related to different husbandry systems and animal categories, when needed), (ii) a list of hazards that lead to each welfare consequences (with specific elements related to different husbandry systems and animal categories, when needed) and (iii) a list of measures to prevent the hazards and/or mitigate the welfare consequences.

Many of the highly relevant welfare consequences share the same hazards (or exposure variables, e.g. lack of resources). Therefore, in addition to the recommendations for preventing hazards or mitigating the welfare consequences, this opinion proposes recommendations regarding exposure factors, in a holistic way. These recommendations specify 'minimum enclosure characteristics' (Section 3.4.2) fulfilling the needs of animals and intends to cover a wide range of potential housing systems for small or large flocks of birds. The requirements are defined for laying hens, pullets and layer breeders, providing the minimum technical specifications of the enclosure (e.g. size, stocking density, litter) that are expected to prevent the highly relevant negative welfare consequences that have been identified in this scientific opinion. These minimum enclosure characteristics are defined from a bird welfare point of view only.

To reply to the specific ToRs, the description of the ABMs, the hazards, as well as the measures to prevent the hazards and/or to mitigate the welfare consequences as provided in the sections of the general ToRs are used and collated in a narrative text. The following specific scenarios are considered:

- Specific ToR 1: The welfare of laying hens and the risks associated with alternative systems (organic, free range and barn) compared to the cage system currently allowed.

To answer this Specific ToR 1, a summary of the advantages and disadvantages of currently allowed furnished cages and non-cage systems is provided, followed by a specification of the minimum enclosure characteristics that are expected to prevent the highly relevant negative welfare consequences that arise from husbandry conditions. The specifications are related to each hazard (exposure variable) leading to different welfare consequences. These minimum enclosure characteristics are considered based on their potential effect on the highly relevant welfare consequences detailed in this Scientific Opinion. Specifications for other parameters (e.g. feeder and nest design) are also provided.

- Specific ToR 2: Welfare of hens in furnished cages: the risks associated with rearing of non-beak-trimmed layers.

Beak trimming is conducted because of the perceived or actual risk of injurious pecking, a behaviour that can occur in any housing system currently used commercially. The causes, consequences and preventive measures for injurious pecking across all housing systems are therefore summarised. A review of the direct welfare consequences that result from the practice of beak trimming is then provided. Next, the risks associated with keeping birds with intact beaks are considered by comparing data on mortality and soft tissue damage in beak-trimmed and non-beak-trimmed flocks. The direct response to Specific ToR 2 is provided with specific conclusions and recommendations for non-beak-trimmed birds in furnished cages. This is complemented by a set of further conclusions and recommendations that apply to non-beak-trimmed birds housed in alternative systems.

- Specific ToR 3: the assessment of ABMs collected in slaughterhouses to monitor the level of welfare on laying hen farms:

To answer this Specific ToR 3, currently used or potential ABMs are selected that could be applied at the slaughterhouse to monitor the welfare of laying hens on farm.

## 2. Data and methodologies

### 2.1. Data

Information contained in previous EFSA scientific outputs (EFSA AHAW Panel, 2005, 2015), scientific articles and grey literature is used to address the General and Specific ToRs.

Owing to a lack of information on laying hen breeders, a questionnaire is sent to the European Forum of Farm Animal Breeders (EFFAB) to obtain information on current husbandry practices for this animal category (Appendix A).

### 2.2. Methodologies

The methodology applied to answer the ToRs is described in detail in the methodology guidance (EFSA AHAW Panel, 2022a,b).

#### 2.2.1. General ToRs

The General ToRs request EFSA to answer five assessment questions which are divided into more specific sub-questions (EFSA AHAW Panel, 2022a,b; Table 1) based on evidence from the scientific literature and expert opinion.

The identification of welfare consequences follows the approach described in the methodology guidance (EFSA AHAW Panel, 2022a,b). From the list of 33 welfare consequences described in the guidance (EFSA AHAW Panel, 2022a,b; Table 2), the following 11 welfare consequences are identified as highly relevant for laying hens, pullets or layer breeders:

- 1) Bone lesions (incl. fractures and dislocations)
- 2) Group stress
- 3) Inability to avoid unwanted sexual behaviour

- 4) Inability to perform exploratory or foraging behaviour
- 5) Inability to perform comfort behaviour
- 6) Isolation stress
- 7) Predation stress
- 8) Restriction of movement
- 9) Resting problems
- 10) Skin disorders (other than soft tissue lesions and integument damage)
- 11) Soft tissue lesions and integument damage

The approach to identify relevant ABMs for these highly relevant welfare consequences, as well as hazards and measures to prevent the hazards and/or to mitigate the welfare consequences, is described in the methodology guidance (EFSA AHAW Panel, 2022a,b).

For sensitivity of an ABM, the following is considered: the presence of the ABM as its ability to identify animals suffering from the welfare consequence. An ABM that is not systematically present in all animals with the welfare consequence, is less sensitive. For specificity, the following is considered: the absence of the ABM as its ability to identify the animals, which are not experiencing the welfare consequences. An ABM that is present in several welfare consequences tends to be less specific (EFSA AHAW Panel, 2022a,b).

The literature search resulted in few quantitative data on the sensitivity and specificity of the different ABMs towards specific welfare consequences. Therefore, a qualitative judgement on the sensitivity and specificity was provided by the experts, categorising sensitivity and specificity into low, moderate or high as described below:

#### Category 'high':

- High sensitivity: when the welfare consequence is present, the ABM will nearly always show it (i.e. if group stress exists, aggressive interactions are likely to appear): this would correspond, for example, to a sensitivity range from 70% to 100%.
- High specificity: when the welfare consequence is absent, the ABM will nearly always indicate this (e.g. if group stress does not exist, aggressive interactions are rare); this would correspond, for example, to a specificity range from 70% to 100%.

#### Category 'moderate':

- Moderate sensitivity: when the welfare consequence is present, the ABM will often show this, but not in a certain proportion of cases (e.g. if hens cannot perform comfort behaviour, preening bouts duration will be decreased, but not always and only in most drastic cases); this would correspond, for example, to a sensitivity range of 50% to 70%.
- Moderate specificity: when the welfare consequence is absent, the ABM will show this in most animals but not in all of them (e.g. if ability to perform exploratory or foraging behaviour is not limited, the walking/scratching/pecking expression can still be impacted due to other factors such as high animal stocking density or foot disease); this would correspond, for example, to a specificity range of 50% to 70%.

#### Category 'low':

- Low sensitivity: when the welfare consequence is present, the ABM may or may not show it (e.g. if hens have skin disorders, they will not always show restlessness), this would correspond, for example to a sensitivity below 50%.
- Low specificity: when the welfare consequence is absent, the ABM will not indicate the absence in a substantial number of animals (e.g. if ability to perform comfort behaviour is not limited, preening can be impacted by other parameters, like the presence of ectoparasites); this would correspond, for example, to a specificity below 50%.

In this Scientific Opinion, a list of relevant indicators was provided based on the ABMs listed for each of the described welfare consequences. Some of these indicators (so-called 'iceberg indicators') are interpreted as an outcome-based indicators of animal welfare that may provide an overall assessment of welfare.

## 2.2.2. Specific Terms of Reference

The general approach used for answering the specific ToRs is described in the methodology guidance (EFSA AHAW Panel, 2022a,b). The specific methodology applied in this opinion is described below.

### 2.2.2.1. The welfare of laying hens and the risks associated with alternative systems (organic, free range and barn) compared to the cage system currently allowed (Specific ToR 1)

Specific ToR 1 was addressed using data from the literature and expert judgement. In addition, expert knowledge elicitation and modelling is used to estimate maximum stocking density for laying hens.

#### *Expert knowledge elicitation*

A quantitative assessment is carried out on the effect of space allowance on the ABMs 'plumage damage' and 'walking, scratching, pecking' using expert knowledge elicitation (EKE). The model used for the EKE is described in detail in the methodology guidance (EFSA AHAW Panel, 2022a,b). The evidence dossiers with the elicitation results can be found in Appendix B.

#### *Behavioural space model*

In addition to the EKE approach, another quantitative approach is implemented to determine how stocking densities are related to behaviours that are essential for animal welfare. Therefore, specific behavioural needs are selected (Table 1), and data extracted on the proportion of laying hens performing these behaviours within a flock under improved conditions. Improved animal welfare conditions are expected when stocking densities are decreased, cage housing is excluded, and/or enrichment and outdoor area are present. This approach targets to calculate space allowance, i.e. stocking density, but it is acknowledged that increasing animal welfare also requires environmental enrichment, light management, covered verandas or other management improvements that are related to welfare (see Section 3.4). However, for this model and reasons of simplicity, stocking density is considered in isolation.

To calculate the required space allowance per bird to perform the selected behaviours, it is necessary to provide the proportion of birds in the flock showing these behaviours under improved husbandry conditions with the space required to perform them unrestrictedly.

The space allowance (SA) is represented in Equation 1, where  $A_i$  represents the area required by an animal to perform a specific behaviour  $i$  and  $PB_i$  represents the proportion of birds in the flock showing this behaviour (behavioural need):

$$SA = \sum_i^N (A_i \times PB_i) \quad (\text{Equation 1})$$

$i = 1-9$

The space needed to perform the selected behaviours ( $A_i$ ) combines the mean of the space covered by a laying hen performing the behaviour ( $\text{cm}^2$ ) and the interindividual distance, both extracted from the publications.

The elements to calculate space allowance (SA) using Equation 1 are established as follows:

- a) Selection of behaviours
  - b) Calculation of the proportion of birds expressing the selected behaviours (PB)
  - c) Calculation of the area A required by a laying hen to perform a selected behaviour
  - d) Limitations of the model
  - e) Sensitivity analysis
- a) Selection of behaviours

The selection of behaviours for data extraction is based on the welfare consequences and ABMs described in this Scientific Opinion. As space allowance strongly affects restriction of movement, the behavioural ABM locomotory behaviour (walking) is chosen. In addition, the corresponding behaviours standing and sitting are selected. Resting behaviour is combined with passive sitting as this is the typical posture of resting birds and many studies do not differentiate between resting and sitting.

Active sitting, such as pecking while sitting and sleeping, is excluded. Foraging behaviour (ABM walking, scratching and pecking) reflects the welfare consequence 'inability to perform exploratory or foraging behaviour' and the ABMs dustbathing, preening, as well as wing flapping, wing and leg stretching are chosen to reflect the welfare consequence 'inability to perform comfort behaviour'. As drinking and eating are essential to the birds, these behaviours are also integrated into the quantitative approach.

Data in relation to the proportion of animals performing selected behaviours in improved conditions and the space needed to express these behaviours are extracted for two types of behaviours:

- the mainly inactive behavioural categories (e.g. standing, sitting/resting or drinking/eating), in the following referred to as so-called 'stationary' behaviour;
- the behaviours that are associated with locomotory behaviour supporting animal welfare, in the following referred to as so-called 'active' behaviour.

These labels, 'stationary' and 'active', are assigned to classify the behaviours according to the amount of space needed. Behaviours that only require physical body space are labelled as 'stationary' and those that require additional space to perform an activity are labelled as 'active'.

The aim of this labelling is to include those behaviours in the calculation that are part of the general behavioural repertoire of the animals but do not need additional space as compared to current stocking densities (the 'stationary' behaviours). On the other hand, there are behaviours that are associated with an improvement in animal welfare if more space is provided and should therefore be promoted (the 'active' behaviours).

Data are extracted from scientific studies published after 2004 that apply comparable definitions of the selected behaviours and use the scan sampling method providing.

The reason for selecting studies published after 2004 is to provide and extract values that were measured on comparable hybrids as they are currently used commercially. During data extraction, care is taken to ensure that these values originate from the hens' laying period.

Beside improved conditions, standard commercial conditions regarding husbandry system and management are assumed, although some studies are experimental with smaller group sizes.

The selected behaviours, labelled as 'stationary' (3 behaviours) or 'active' (6 behaviours) in the context of space requirements are listed in Table 1 with the number of references from which data are extracted.

**Table 1:** Selected behaviours used in the behavioural model. Given are selected behaviours and the number of references using comparable definition for the behaviours and thus provided data that are extracted for the model

Behaviour	Label	Studies retrieved for extraction (n)
Standing	stationary	5
Sitting/Resting	stationary	5
Walking	active	5
Foraging including scratching	active	7
Dustbathing	active	8
Preening	active	6
Wing/leg stretching	active	2
Wing flapping	active	4
Drinking/Eating	stationary	3

#### b) Calculation of the proportion of birds expressing the selected behaviours (PB)

The proportion of hens expressing a selected behaviour within the flock is based on scan sampling data. Four approaches are explored (mean, median, optimum and stabilised optimum of the extracted data) to calculate the proportion of birds expressing a selected behaviour (PB).

As descriptive statistics, the total number of extracted values/data points (N) as well as their minimum, median, mean and maximum of proportion of birds showing the behaviours are calculated. In addition, so-called stabilised values are calculated to adjust for the variability and small sample size of the available data of some specific behaviours (e.g. wing flapping). The stabilised values are based on the mean of the lowest and highest two values for the stabilised minimum and maximum, respectively.

The values are further processed by including the previously chosen label as 'active' or 'stationary' for four different models:

- median model (median of all selected behaviours)
- mean model (mean of all selected behaviours)
- optimum model (median of 'stationary' and maximum for 'active' behaviours)
- stabilised optimum model (median of 'stationary' and median of the two highest values for 'active' behaviours, i.e. stabilised maximum).

The mean is the most common approach in data analysis. The median is more appropriate when the data range is large, and the data points limited and non-parametric. For the optimum model, the maximum value for selected behaviours is implemented reflecting that, e.g. the highest proportion of laying hens reported in the literature show a behaviour labelled as 'active'. As the optimum model relies on a single maximum value, another approach is added to produce a more robust model, the stabilised optimum, by integrating not only the maximum but also the median of the two highest values as the variability within the selected behaviours was sometimes high and the median buffers this variability. Implementing four mathematical approaches allows more confidence in the outcomes if they are comparable, reflecting the robustness of the model in general.

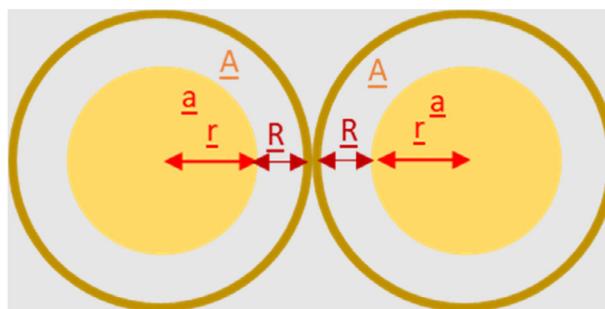
The values of the proportion of birds showing behaviours is standardised to a total of 100%. This is necessary since data points are derived from different studies.

#### c) Calculation of the area A required by a laying hen to perform a selected behaviour

A circular model is chosen to represent a bird performing a specific behaviour as it reflects the oval shape of the birds' body and the packing of the flock. It is preferred to the rectangular model, which is described in Appendix C, for the following reasons:

- the different behaviours expressed simultaneously by birds are in all directions rather than in one (which is assumed by a rectangular model);
- the interindividual distance to all directions is covered more realistically in the circular model;
- the circular model assumes optimal packing in terms of maximal density which in turn results in a higher potential stocking density than the rectangular model. The circular model therefore might underestimate the real space needed and is considered to be a conservative approach;
- the circular model, although assuming +10% additional space between birds (blue area see Figure 2 below), is occupying less space than the rectangular model.

In addition to the space occupied by a bird itself (area  $a$ ), interindividual distance is added to the model, which reflect the behavioural preference of animals to express behaviour in a specific individual distance to the neighbouring hen. The model includes a minimal distance 'D' between hens performing the same behaviour. This interindividual distance (D) is interpreted as additional band of radius ' $R = D/2$ ' around the circle (Figure 1). The area A required to perform a specific behaviour is assumed to be a disc including the area covered by the hen (disc  $a$  of radius  $r$ , in yellow in Figure 1) and an additional band of radius R representing the interindividual distance.



A = total space (area) required to perform a specific behaviour including a = space occupied by the bird itself, with  $r$  = being the radius of  $a$  and  $R$  being the radius added to reflect the interindividual distance  $D/2$  since values for interindividual distances were divided by two birds.

**Figure 1:** The space occupied by two laying hens depicted as two circles with the interindividual space

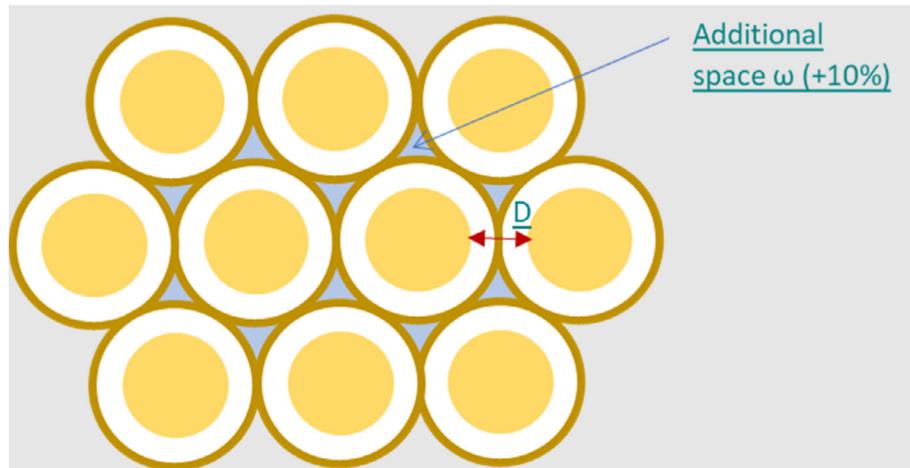
The area ( $A_i$ ) required by an animal to perform a specific behaviour ( $i$ ) is expressed in Equation 2.

$$A_i = \pi \times (r_i + R_i)^2 \quad (\text{Equation 2})$$

$$i = 1-9$$

Assuming, that the hens will optimally and equally distribute in an area, we observe a small part, which is not covered by circles (Figure 2): This area is called  $\omega$  and is calculated with Equation 3 (Steinhaus, 1999).

$$\omega = \frac{A}{0.9069} \quad (\text{Equation 3})$$



**Figure 2:** Depiction of the entire flock with the additional space  $\omega$  between chickens (space covered by chicken plus interindividual distance)

#### d) Limitations

Not all definitions used to extract data in the different behaviour categories are fully congruent. However, as there is no standardised and uniformly used ethogram, the experts allowed a minimal range of deviations, as long as the core of the behaviour was covered.

The extracted data could not always be summed up to 100% in each study and this was due to different reasons, e.g. because only a subset was extracted from the study that fit the predefined categories/definitions. In most studies, only specific behaviours and not complete ethograms were analysed. In addition, the method of scan sampling could differ regarding time of day, frequency and interval, introducing additional variability into the dataset.

The references did not provide data on the space requirement for 'walking'. Therefore, it is assumed that for walking (i) the space requirement is equal to standing and (ii) in addition, the space is doubled to allow free space for walking activity. Similarly, as data for eating/drinking were insufficient, it is assumed that the space required for eating/drinking is equal to that required for standing.

The model might not reflect the circadian rhythm of the birds or social facilitated behaviour, e.g. dust bathing which is preferably shown by several birds simultaneously. The modelling approach does not allow every bird to perform every behaviour at the same time but reflects the proportions of birds showing the range of behaviours selected. The model also demonstrates that an increase of active behaviours relies on the prerequisite that there is more space for each individual.

The distinction between 'stationary' and 'active' is based on the assumption that behaviours indicative of good animal welfare require more space to be shown at higher frequency (labelled 'active') and other behaviours are less dependent on additional space (labelled 'stationary').

A complete ethogram was not compiled, as the primary aim was to present models on those behavioural categories that are directly related to animal welfare. Rather, the definitions that relate the behaviours to the space data and the extracted categories should be kept the same and applied. Therefore, various studies and data points that did not match the definitions were omitted, e.g.

locomotion = walking and hopping, or foraging as pure pecking without scratching. The definitions on which the data are based should be congruent with those on which there are data on space covered.

Average interindividual distances have been applied based on published data. The distribution of the flock might differ from this simplification and, e.g. resting birds might cluster together as well as foraging birds might show greater distances to each other.

#### e) Sensitivity analysis

A sensitivity analysis was conducted to identify the most influential inputs of the model used to estimate the space allowance. To do so @RISK (<https://www.palisade.com/risk/>) was used taking a cumulative distribution of the lowest, median and highest value of the proportion of time and space required for the nine behaviours accounted for in the model. To conduct the sensitivity analysis only one input was calculated as the cumulative function and all the rest inputs were left as discrete values. In total, 1,000 iterations were run for each of the 27 model parameters.

The behaviours with a high space requirement appear to drive the model outcome more than behaviours with low space requirements.

### **2.2.2.2. Welfare of hens in furnished cages, and risks associated with rearing of animals non-beak trimmed (Specific ToR 2)**

Specific ToR 2 is addressed using data from the scientific literature and expert judgement.

### **2.2.2.3. The assessment of animal-based measures collected in slaughterhouses to monitor the level of welfare on laying hen farms (Specific ToR 3)**

Specific ToR 3 considers laying hens that will be sent to the slaughterhouse. It aims at identifying a list of ABMs that can be assessed and collected at slaughter to monitor the welfare condition on farm of a certain population in a flock, farm or region/country. Preferably, these should be 'iceberg indicators', defined as ABMs that help to identify more than one welfare consequence (EFSA AHAW Panel, 2022a,b).

A list of ABMs initially considered potentially relevant for measurement at slaughter in laying hens is identified by EFSA experts on the basis of existing literature (Welfare Quality<sup>®</sup>, 2009; EFSA AHAW Panel, 2005) and further discussed during the EFSA scientific National Contact Points (NCPs) Network annual meeting (2021).

Additional ABMs are added to this list by the EFSA working group based on expert opinion. This list is then subjected to: (i) screening of ABMs and; (ii) selection of ABMs.

The full methodology is described in Appendix D.

### **2.2.3. Uncertainty analysis**

The AHAW Panel agreed to tackle the uncertainty related to the data inputs and the methodology employed to identify welfare consequences, ABMs and related hazards by first describing the potential sources of uncertainty affecting the assessment. Table 3 in Appendix E describes the sources of uncertainty.

The impact of these uncertainties in the assessment of the General ToRs of this Scientific Opinion was assessed collectively following the procedure described in the EFSA guidance on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018) for case-specific assessments, with some modifications. The outcome of the assessment of the General ToRs is the identification and description of the highly relevant welfare consequences, the related ABMs and the hazards causing these welfare consequences for each animal category and in the most relevant husbandry systems. Measures to prevent the hazards and/or to mitigate the welfare consequences are also identified and described. Conclusions and recommendations are formulated on the basis of these elements.

For the General ToRs, EFSA experts agreed to limit the assessment to the quantification of the overall impact of the sources of uncertainty on the summary conclusions developed in Section 4. Experts identified the probability range best reflecting their degree of certainty for each conclusion. Individual answers are then subjected to group discussion during which experts explain the rationale behind their judgement, and a consensus certainty range is formulated. A qualitative translation of the outcome of the uncertainty assessment is also derived (e.g. 'more likely than not' for a certainty range of > 50%–100%) (see Table 2) from the guidance on uncertainty (EFSA, 2019).

For the Specific ToRs, a more quantitative approach is used where possible: in the case of EKEs, the certainty range is assessed as part of the exercise (as described in EFSA AHAW Panel, 2022a,b).

For some of the exposure variables assessed, where EKEs are not possible or not considered relevant, the uncertainty is assessed following the procedure used for the General ToRs.

For the conclusions based on scientific literature, EFSA experts are asked to provide their judgement on the certainty of each conclusion according to three predefined certainty ranges (> 50–100%, 66–100% and 90–100%) derived from the probability classes defined in the EFSA Guidance on Communication of Uncertainty in Scientific Assessments (EFSA, 2019). A group discussion provides the opportunity to explain the rationale behind their judgement, and agree on a consensus certainty range. Three uncertainty classes are defined: (1) 'It is more likely than not', corresponding to certainty greater than 50%; (2) 'from likely to almost certain', corresponding to a certainty greater than 66% and (3) 'from very likely to almost certain', corresponding to a certainty greater than 90%. The uncertainty level corresponding to 'as likely as not' (33–66%) is used to qualify the conclusion about a hazard for keel bone damage, for which the requestor specifically asked EFSA to provide a judgement on the risk associated with this factor.

To improve readability of the conclusions, the certainty range is not indicated in the corresponding text when their certainty range is 90–100%. The certainty range is given for conclusions with higher uncertainty (below 90% certainty).

**Table 2:** Three ranges used to express agreed (consensus) certainty around conclusions (adapted from EFSA, 2019)

Quantitative assessment	Certainty range		
	> 50– 100%	66–100%	90–100%
Qualitative translation	More likely than not	From likely to almost certain	From very likely to almost certain

### 3. Assessment

In 2021, around 376 million laying hens were kept in the EU (27 Member States), producing 6.4 million tonnes of eggs. Overall, around half of the laying hens kept in the EU in 2021 (45%) were kept in furnished cages, 36% in barns, 13% were free-ranging and 7% in organic production systems.<sup>2</sup>

In Section 3.1, the production cycles related to the production of eggs in the EU are described, the most common husbandry systems (Table 3) are described in Section 3.2 (Table 3) and Section 3.3 describes the negative welfare consequences for laying hens, pullets and layer breeders kept in these systems (including ABMs, hazards and preventive or mitigating measures).

**Table 3:** Most common husbandry systems for laying hens, pullets and laying hen breeders included in this opinion

		Laying hens	Pullets	Layer breeders	With exposure to outdoor conditions
<b>Non-cage systems</b>	Floor systems maximum of one tier	Single-tier	<ul style="list-style-type: none"> <li>Floor system without elevation</li> <li>Floor system with elevated structures</li> </ul>	<ul style="list-style-type: none"> <li>Single-tier</li> <li>Single-tier without litter (=slatted floor system)</li> </ul>	<ul style="list-style-type: none"> <li>Mobile housing (single or multi-tier)</li> <li>Covered veranda</li> <li>Outdoor range</li> </ul>
	Floor systems with multi-tier	Multi-tier	Multi-tier	Multi-tier	
<b>Cage systems</b>	Collective cages	Furnished cages	Collective cages	Collective cages	n/a
	Individual cages	n/a	n/a	Individual cages	n/a

n/a: not applicable.

<sup>2</sup> [https://ec.europa.eu/info/sites/default/files/food-farming-fisheries/farming/documents/eggs-dashboard\\_en.pdf](https://ec.europa.eu/info/sites/default/files/food-farming-fisheries/farming/documents/eggs-dashboard_en.pdf)

### 3.1. Production cycles of domestic fowl (*Gallus gallus*) related to the production of eggs

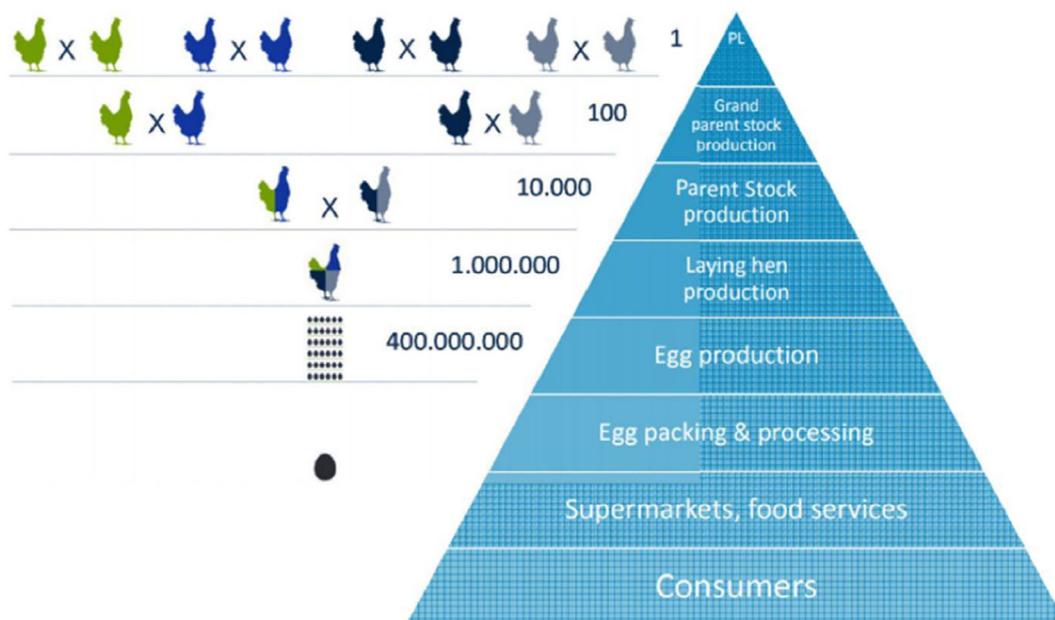
The production cycle for laying hens is described in three parts. First, the structure of the breeding sector is described showing how breeding (parent stock) birds are produced (Section 3.1.1). These layer breeders produce eggs that develop into male and female chicks (Section 3.1.2). The female chicks mature into pullets (Section 3.1.2) and ultimately into the commercial laying hens that produce eggs for human consumption (Section 3.1.3).

#### 3.1.1. Layer breeders

Genetically pure-line birds are highly valuable, kept in isolated and strictly protected biosecure facilities by both of two global breeding companies (Fernyhough et al., 2020). These pure lines are used to produce similarly pure grandparent stock birds and form the top of the breeding pyramid (Figure 3). Grandparents are crossed to produce hybrid males, and separately hybrid female birds, that mature to become the parent stock (also known as layer breeders). The grand-parent and parent stock birds are also valuable and are maintained under strict biosecurity protocols, following shower-in shower-out routines for stockmen, meticulous management practices, and an exhaustive control of working personnel and providers and in closed buildings without outdoor access. There is a lack of available scientific sources of reliable information describing each of the systems used across EU countries for the breeding stock of laying hens.

Layer breeders produce the eggs that develop into commercial pullets and laying hens (Scanes et al., 2020). The number of birds at each stage in this 'pyramid' of production is at least a 100-fold multiplication of the numbers at the previous stage (Fernyhough et al., 2020).

The breeding stock is kept in flocks of less than 10,000 birds with a proportion of 10 females per male. Pure line and grandparent stock can be kept in individual cages for the purpose of individual data collection. Other parent-stock breeders are kept in collective cages (typically unfurnished), or floor systems with single or multi-tier.



The pure line (PL) birds are shown at the top of the pyramid. Successive crosses result in grand-parent and parent (layer breeders) that are genetic hybrids, represented by mixing of colours and diagram.

**Figure 3:** Illustration of the relative number of birds at each stage of the egg production chain (Fernyhough et al., 2020)

Single-tier systems may or may not provide access to perches. When these are available, the perch area may not be sufficient for all birds to perch simultaneously. Lighting programmes are identical to the ones offered to laying hens, with at least 8 h of darkness. Layer breeders are fed ad libitum, and

flock sizes are around 8,000 to 10,000 birds, but these flocks are often subdivided into smaller groups of around 2,500 birds. In many EU countries beak trimming (Section 4.3) is applied regularly to females and males at the hatchery and comb dubbing (cutting the comb) is conducted on males of white layer strains. The large size of the undubbed comb in these birds reduces vision and thus inhibits feed intake.

Female breeding birds are able to store sperm for up to 2 weeks after mating (Pizzari and Birkhead, 2001). This capacity allows females to maintain a high fertility, while the reproductive success of male birds depends on their ability to attract and retain females (McDonald et al., 2017), thus male competition for mating is high. Male–male competition may occur in breeding flocks if the number of females available is not sufficient leading to coercive mating with females, with a high welfare cost for them, described in Section 3.3.1.3.

### 3.1.2. Immature birds

#### 3.1.2.1. Females (pullets)

Approximately 50% of eggs contain female chicks. On hatching these female chicks are vaccinated and beak trimming is applied in some countries and for some housing systems (Section 4.3). Chicks are transported at 'day-old' (24–72 h after hatching) to specific rearing facilities. These growing female birds, called pullets, are maintained in the rearing house. Pullets may be kept in collective cages (generally unfurnished), in floor systems with no elevated structures, in floor systems with elevated structures, in single-tier, or in multi-tier systems. In many systems, female chicks are initially confined to one part of the house and given access to larger areas as they grow. In some free-range rearing systems, birds are moved from a brooding house to a free-range house around 5–7 weeks of age. Some pullets may be given access to a veranda or outdoor range from the age of approximately 8–12 weeks. When pullets approach sexual maturity at 17 weeks ( $\pm 1$  week) they are transferred to the laying housing system.

Food and water must be readily accessible to all birds during this early-life phase. Although it is not usual practice, some companies have developed systems that can provide food and water at the hatchery and during transport to the rearing farm (de Haas, 2020). For the first days on farm, the use of chick paper encourages feeding because food particles can be more easily differentiated from the background litter and because rustling and tapping noises produced by walking and pecking stimulates feeding.

Under natural conditions, chicks would be brooded by a mother. Comparative studies have shown that brooded chicks tend to be less aggressive and have a lower tendency to engage in feather pecking, than non-brooded chicks (Shimmura et al., 2015), although other studies have found only minimal differences (Hewlett and Nordquist, 2019). It remains possible that a lack of brooding could increase the risk of group stress in commercial flocks (Edgar et al., 2016). The use of dark brooders may partially alleviate this deficit.

Pullets continue to learn, and to develop physically and socially throughout the rearing period. Under natural conditions, young chicks imprint on their mother, learning her features and forming a strong attachment. When chicks are raised commercially without a mother, they become somewhat attached to each other (Porter et al., 2005), though any bonds will be disrupted when chicks are transported from the hatchery to the rearing farm.

Pronounced aggressive behaviour does not develop until chicks are 2–3 weeks of age and peaks between 6 and 12 weeks (Estevez et al., 2003). In smaller groups, pullets will establish a network of social dominance relationships and once relative positions have been established, social dominance is signalled by threat and posture, avoiding the need for further overt aggression. However, the appearance of birds is also an important factor influencing aggression. Indeed, in an experiment launched on individual pullets with altered physical appearance (an added black mark to the head), it was shown that these animals received more aggression from conspecifics than the unmarked ones (Campderrich et al., 2017).

Welfare problems in adult birds are strongly influenced by rearing procedures (Janczak and Riber, 2015). Pullets destined for a production life in furnished cages are often raised in collective cages (Section 3.2.4), whereas pullets destined for cage-free systems are usually raised in floor (Section 3.2.1.2), single-tier (Section 3.2.1.3) or multi-tier systems (Section 3.2.2.2). The transfer from the rearing house to the laying system usually takes place just before the onset of lay, when birds are 15–18 weeks of age, but earlier transfer is possible and can improve plumage condition and range use, and reduce floor laying in non-cage system (Janczak and Riber, 2015).

There may be some general benefits of rearing pullets in complex and enriched non-cage environments (e.g. reduced fearfulness (de Haas et al., 2014b; Brantsæter et al., 2016a; Campderrich et al., 2019) and improved general health). For example, hens reared in free-range systems show improved gut microbiome diversity and upregulated gene expression pathways relevant to gut health and immune function in comparison with hens reared in cage systems (Chen et al., 2019). It is important that pullets destined for non-cage systems are reared in systems that (as closely as possible) match their future housing as laying birds and that provide opportunities to develop locomotory and cognitive abilities (Colson et al., 2008; Regmi et al., 2015; Norman et al., 2021). The best rearing system for pullets destined for adult furnished cages is less certain. If pullets are raised in complex environments that provide resources for behavioural needs such as dust bathing, they are likely to become frustrated if they are moved to layer housing that offer less complexity (Janczak and Riber, 2015) and one study has reported significantly higher adult mortality when pullets were moved from non-cage to cage systems, compared to a transfer from cage to cage (Tahamtani et al., 2014). However, non-cage rearing has other general benefits that apply even when adult birds are in furnished cages, including lower fearfulness (Brantsæter et al., 2016b) and reduced keel bone fractures (Casey-Trott et al., 2017).

Stocking densities for pullets are much higher compared to those used for laying hens. In part, this is explained by the fact that pullets are growing.

### 3.1.2.2. Immature males

The other 50% of the eggs contain male chicks. Until recently, these were killed in the hatchery. In some countries (Austria, France, Germany and Luxembourg), after implementing a ban on the killing of day-old chicks, sex of embryos is determined in the hatching eggs (in-ovo-sexing) and male embryos are discarded or the male chicks are raised for meat production. Male layer chicks are not included in this assessment; however, on request from the European Commission, available evidence in relation to their welfare is brought together in Section 3.3.14.

### 3.1.3. Laying hens during the production of eggs

Laying hens produce eggs from 17 weeks of age until 70–100 weeks. In Europe, they are housed in furnished cages (which fall into the category of the EU-Directive 1999/74 'Enriched cage systems') or in single-tier or multi-tier barn systems, with or without a veranda or outdoor range. Since the ban of conventional cage systems in laying hens in 2012, there has been a steady increase in the number of alternative systems such as barn, free-range and organic.

Section 3.1 described animal categories in the order breeders, pullets, then laying hens. However, in the remainder of the Scientific Opinion, information is provided within each section, in the following order: laying hens, pullets then layer breeders. This is because most scientific evidence relates to laying hens.

## 3.2. Husbandry systems

The key features of non-cage systems, and the most relevant welfare consequences pertaining to each system type, are described in Sections 3.2.1 and 3.2.2. This is followed in Section 3.2.3 by a description of the verandas and/or outdoor range areas that can be added to non-cage systems to provide additional space and resources. Because mobile systems are always used in association with an outdoor range they are also described in this section. Sections 3.2.4 and 3.2.5 describe the key features of cage systems.

### 3.2.1. Floor systems with maximum one tier

#### 3.2.1.1. Laying hens: single-tier

Single-tier systems are non-cage systems with a fairly simple design, in which the floor is partly covered with litter and partly with slatted floors. Typically, 1/3 of the floor is covered with litter, but also more litter area can be provided. The slats can be made of wire, plastic or wood. Underneath the slats there is usually a pit, collecting manure over a full laying period. It is an inaccessible area for the birds. As these storages are causing high ammonia concentrations in the air, manure removal systems have been developed (mostly belts, but also scrapers), enabling frequent removal of the manure. Usually, the slatted floor is located in the centre of the house, with litter floors on each side, but can also be on one or both sides of the house, with the litter floor in the middle or on the opposite side.

Nest boxes are usually placed in the middle, on top of the slatted floor. In some situations, the nest boxes are placed separately from the slatted floors in an elevated position at the side walls above the litter floor. Feeders, drinkers and perches are usually provided on the slatted floor. A line of nipple drinkers is often installed close to the entrance of the nest boxes to attract hens to the nest. If nest boxes are installed at the sides above the litter, then a small platform or some perches are installed in front of the nest boxes, to enable the birds to walk along the nest boxes and choose an empty nest. Another drinker line is often installed above that platform as well.

Single-tier systems provide the basic elements needed for the hens to enable their species-specific behaviour. With only one tier, the number of birds in a house is limited and, during colder periods, the house temperature can fall. To prevent this, some farmers reduce ventilation within the house to save energy costs, but this can cause problems with air quality, e.g. high ammonia levels. Currently, A-frames with perches (Figure 4) are positioned on the wire floors, creating resting zones and perching opportunities for the birds. The system can easily be combined with a covered veranda and free range (see Section 3.2.3). If the covered veranda is permanently available to the birds, then the area is counted as usable space allowing a bigger flock size. When the space is not available permanently, the area is not counted as usable space. There is a lower density when covered veranda is open and used.



**Figure 4:** A-frames in a single-tier system (Photo Credit: Wageningen Livestock)

### 3.2.1.2. Pullets: floor systems without elevated structures

Pullets have traditionally been reared in floor systems with solid floors (ground floor or concrete) covered in a substrate such as wood-shavings.

On arrival, day-old chicks are usually confined with a low barrier to a relatively small section of the whole house, termed the brooding area. This confinement area may be provided with a paper floor so that food and water are easy for the young chicks to find (Figure 5).



**Figure 5:** Chicks peck at food on a plain paper background separated from the shavings of litter

It is important to keep chicks warm and this can be achieved through whole-house heating, via brooding lamps or other heat sources including hot air fans. A recent innovation employed in some commercial floor (and single-tier) systems is to use dark brooding, where elevated horizontal heaters provided heated areas that are enclosed by dark curtains, mimicking the combination of darkness and heat provided in natural brooding by a hen (see Figure 6). Dark brooders are associated with a reduction of feather pecking and cannibalism (Sirovnik and Riber, 2022) and reduced fearfulness indicated by shorter tonic immobility durations in tests conducted during rear and early laying periods, more movement in open field tests at 28 weeks, and a closer approach to a novel object at 15 weeks (Riber and Guzman, 2016). In another study pullets reared with dark brooders were, when tested at 16 weeks, more likely to ignore an approaching human than conventionally reared pullets (Gilani et al., 2012).



**Figure 6:** Chicks reared with a dark brooder. The heating element is below the roof of the brooder providing a dark environment which chicks can access through the hanging black curtain (Photo Credit: Anne-Marie Gilani)

Chicks are usually released from the brooding area after the first 7–14 days, and gradually given access to the full house area.

Feed is supplied via feed-tracks running throughout the house and water is generally supplied via nipple drinkers that are adjusted in height as birds grow.

Pullets raised in floor systems are generally kept under artificial light although some commercial producers now allow natural daylight.

A lack of any elevated structures presents a range of welfare consequences directly for pullets and subsequently for the same birds when they become laying hens. Stocking densities for pullets are not regulated by Directive 1999/74/EC. Chicks can be kept at densities of over 100 birds/m<sup>2</sup>, young pullets at densities of 60 birds/m<sup>2</sup> and by the end of the rearing period densities are reportedly variable (12–32 birds/m<sup>2</sup>). The effects of stocking density on pullet welfare are considered in Section 3.4. A high-quality litter substrate throughout the rearing period is important to reduce the risk of feather pecking (Chow and Hogan, 2005; Bestman et al., 2009). Early experience of good pecking substrates is particularly important but continued provision of high-quality foraging and pecking substrates throughout the rearing and laying period is needed to avoid harmful inter-bird pecking (Martins et al., 2000; De Jong et al., 2013a; De Jong et al., 2013b; Hartcher et al., 2015a). Although experimental studies have shown that the provision of additional enrichments such as pecking blocks, stones or forage, can be beneficial in reducing the risk of feather pecking it is not known how often these are provided commercially.

### 3.2.1.3. Pullets: floor systems with single-tier or other elevated structures

Floor systems with single-tier or other elevated structures may have solid floors (covered in a substrate, as described above) with a varying number of elevated structures including raised platforms and perches. They may also use dark brooders for very young chicks (as described above). Separate, small, raised platforms may be placed intermittently within a house, occupying a very small proportion of house space (Figure 7) or may be joined together to form a single tier running continuously along the entire width or length of the house.

The area below the platforms is accessible for pullets. These structures may be fitted with winches so that their height above the ground can be adjusted, or ramps may be provided to facilitate access.



**Figure 7:** Floor system with some raised platforms and perches (Photo Credit: Kate Norman)

In more complex and purpose-built systems, chicks are kept initially on a slatted platform with feed and water provided at the same level. As the chicks age, the part of the platform that contains the water line may be raised so that birds are encouraged to jump. Additional elevated platforms are also made available at age-appropriate heights that can be adjusted using winches.

#### **3.2.1.4. Layer breeders: single-tier**

The single-tier system is probably the most common housing for layer breeders. Following standard location of resources as for egg laying, the house is typically organised around a central row of nest boxes located on a raised slatted platform. Birds can move easily between the litter and the slatted area. The space under the slatted area is inaccessible for the laying hen breeders. The platform is made of a perforated sturdy plastic material that permits the birds to remain in a relatively clean and dry area as faeces fall into the manure pit located underneath. Lines of drinkers and feeders are located on both sides of the nest boxes in the slatted area that connects at each side with litter areas available at floor level. The litter area typically occupies at least 1/3 of the floor area and may or may not be provided with a litter substrate. Laying hen breeders are fed unrestrictedly and both males and females use common feeders that can be round feeders or chain feeders. Nest availability is similar to the laying hen standards, although there are no legal requirements. Perches are often not provided, or not in sufficient numbers to allow all birds to perch simultaneously.

Birds will enter into the production house anywhere between 15 and 18 weeks of age and remain there until the end of their productive period at around 72 weeks of age. Although total flock size can be several thousands of birds (e.g. 6,000–10,000 birds), the groups are often split into 2 or 4 groups of around 200–2,500 birds by a vertical see-through fencing (wire mesh). Rearing density in single-tier houses may vary between 8 and 10 birds per m<sup>2</sup>. Management of breeder stock is particularly sensitive along their reproductive life. Occasionally, due to the lack of stimulation to keep the birds calm, unexpected stimuli may trigger exacerbated fear reactions. In single-tier systems, the litter area is the area where most matings take place, while resting, feeding, drinking and egg laying takes place on the slatted area. Females may retreat to the slatted area to avoid male contact. In single-tier systems, there are reported hybrid differences in male mating activity, which sometimes leads to separate housing of a proportion of the males, to avoid unwanted sexual behaviour and its consequences (female forced copulation and soft tissue lesion and integument damage). Separated males will be released into the main group as the group dynamics settle down. Male mortality can be significantly higher than female mortality, and although the reasons are not well understood, these may include intra-male competition.

#### **3.2.1.5. Layer breeders: single-tier with slatted floor**

Single-tier systems with fully slatted floor mimic the single-tier system as described above with the only difference that, in this case the slatted surface covers the full house area. No litter area is available to the birds, thus limiting foraging or dustbathing opportunities. All resources, feeders, drinkers and nests remain at the same level as described for the single-tier system with a litter area. Rearing densities and management practices are identical in both systems. Perches may be available.

Benefits and welfare risk associated to single-tier systems with slatted floors are similar to the single-tier systems. However, the unavailability of a litter area eliminates the possibilities for layer breeders to perform behaviours such as exploratory and foraging behaviour or comfort behaviours such as dustbathing. The limitations to perform comfort behaviour and foraging behaviour, that occupy a large proportion of time in the birds' behavioural repertoire, leads to an increased risk of feather pecking. Additionally, the permanent contact of the birds' feet with slatted floors may lead to an increased risk of developing foot problems, especially if no perches are available.

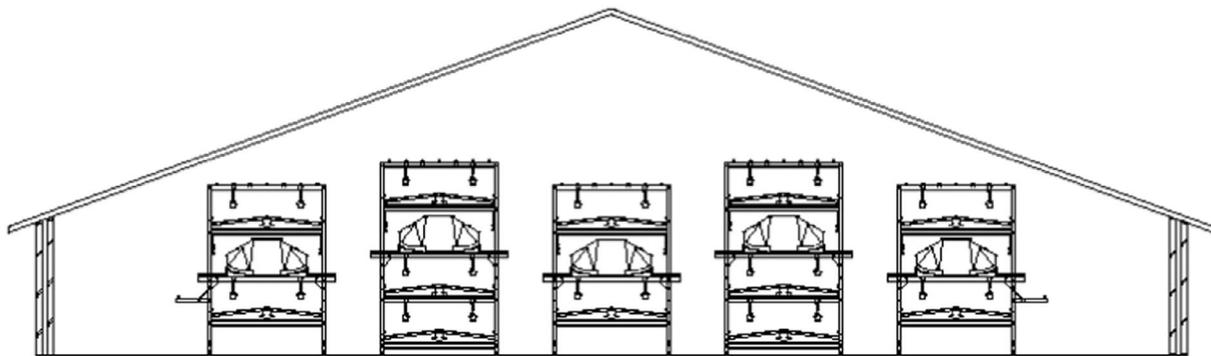
### **3.2.2. Floor systems with multi-tier**

#### **3.2.2.1. Laying hens: multi-tier**

Multi-tier systems, also called aviaries, are housing systems with tiers of floors. These floors consist of manure belts covered with wire mesh or plastic slats. The manure belts are often equipped with manure drying systems. Manure is removed once or twice a week. The tiered floors are equipped with feeders, drinkers and perches. Often at least half of the perches are installed on the top tier to create a resting zone. Some perches or platforms are installed alongside the tiered floors to facilitate the birds to move through the system. In some systems, the tiered floors are placed alongside each other in a way that the birds can use the floors as stairsteps to move up and down. Recently, more systems

are equipped with small sloping wire ramps, that enable birds to walk up and down through the system without the need to fly for accessing levels. Heerkens et al., (2016a) showed that these ramps can help reducing keel bone fractures. Nest boxes are integrated in one level of the tiered floors or, in older designs, in separate tiers. All tiered floor units are placed over a littered floor. This is often a 100% litter floor. All tiers and ground level are available all the time for laying hens, but in some designs there is no access under the first tier for the birds. According to the European legislation (Directive 1999/74/EC) at least 1/3 of the usable area should be covered with litter. The other 2/3 can be slatted floors, provided they are at least 30 cm wide, positioned in a way that prevents manure falling on a lower level and giving the birds at least 45 cm headspace. A maximum of four levels (including ground level) is allowed.

There is a wide variety of designs of multi-tier systems, ranging from fairly simple two-level tiers with aisles in between to very complicated structures, with in some cases even slatted floors running over the top of two tiered floor units (so called portal system). Roughly three variants can be distinguished (EFSA AHAW Panel, 2005): multi-tier with non-integrated nest boxes, multi-tier with integrated nest boxes (Figure 8) and portal systems. Multi-tier with non-integrated nest boxes are mostly older designs and comprise of units with tiered floors without nest boxes, combined with separate units with one or two tiered rows of nest boxes. Birds have to cross the aisles to go from nest boxes to tiered floors with feeders. Water is usually provided both in front of the nest boxes and in the tiered floor units. Multi-tier with integrated nest boxes is comprised of units with tiered slatted floors and one level with nest boxes. Sometimes the tiers with nest boxes also include partially slatted areas. Birds can move to higher or lower floors by crossing aisles or in some systems also by stairways or ramps created within the units. Portal systems are combined units of tiered floors with integrated nest boxes with a top level, that is positioned over two of these tiered floor units. The top level can be a slatted floor with nest boxes in the middle or a two-level system with integrated nest boxes.



**Figure 8:** Multi-tier with integrated nest boxes (from EFSA AHAW Panel, 2005)

### 3.2.2.2. Pullets: multi-tier

Multi-tier systems for rearing of chickens most often consist of rows of at least two tiers placed one above the other. They may have manure belts under the tiers. The height between tiers often is at least 40 cm allowing the pullets to stand fully in an upright position. Pullets can freely move within and between the tiers and have access to the littered ground floor between and often also under the rows of tiers. The floor of the tiers is perforated and made from metal or plastic grid. If manure belts are installed under the tiers they often are ventilated to dry the faeces and, thus, to reduce emissions. Thus, the design of multi-tier systems for pullet rearing resembles those of laying hens (with a reduced scale) and differs from floor systems with single-tier or other elevated structures that have either only one tier or several flexible elevated structures.

During the first 2–4 weeks of life, the lowest and/or the middle tiers can be closed by removable fronts and serve as starter tiers. In many multi-tier rearing systems, these starter tiers are partitioned in compartments by permanent or removable vertical grids such that pullets are kept in small, caged groups during the few first weeks of life.

After the first few weeks, the starter tiers are opened, and pullets have access to all other tiers and the littered ground floor. In many systems, the closings of the starter tiers now serve as terraces, ramps, ladders or perches facilitating the movement of birds between the tiers and to the ground floor (Figures 9 and 10).



**Figure 9:** An example for a multi-tier system for pullets. The slope of the elevated platforms can be adjusted. During the first weeks the pullets are enclosed on the tiers that are open in the picture (Photo Credit: ©Vencomatic Group)



**Figure 10:** In this multi-tier system for pullets the height of platforms, drinkers and feeders can be adapted in height (Photo Credit: Wageningen Livestock Research)

Feed is delivered via a chain or via round troughs in the starter tiers. After opening of all tiers, pullets may have access to additional troughs in the other tiers. Water is delivered within the lower or within all tiers via nipple drinkers. Thus, during the first few weeks of rearing multi-tier systems are quite comparable to cage systems. However, in multi-tier systems perches are integrated within all tiers allowing the chickens to perch from the first day on. Most often, the perches are installed above the troughs and/or the drinking lines. In addition, sometimes litter material already is offered on paper coverings laid on the perforated floor of starter tiers in order to enable the use of litter for foraging and scratching as early as possible. The troughs, the drinkers and the perches can be adjusted in height to follow the growth of pullets. In some systems, additional grid platforms that can be adjusted in height are integrated in the starter tiers. These platforms train the chickens to change between different levels already during their first few weeks of life.

### 3.2.2.3. Layer breeders: multi-tier

The multi-tier system for layer breeders is a niche system. The structure is similar to the multi-tier system used for laying hens, and normally it is organised in three different tiers where resources are located. The multiple tiers allow a better separation of functional areas compared with the single-tier system and also provide the birds with more opportunities to jump and fly and to find a high resting place. Mating takes place in the litter area, while resting, feeding, drinking and egg laying takes place on the tiers. Females can retreat easily from the males in the upper tiers. Male to female ratios, as for other breeding systems is 1 male to 10 females. Groups of several thousands of birds are divided into smaller groups by a vertical wire mesh fence. Density in this system remains around 8 birds/m<sup>2</sup> of useable space, available on the multiple tiers and on the floor.

### 3.2.3. Systems with access to covered veranda and/or outdoor range

Both single-tier and multi-tier systems can be used as full indoor systems or in combination with outdoor access and/or access to a covered veranda. Mobile housing systems are always used in combination with outdoor access, and some also have a covered veranda. Access to an outdoor range, a veranda, or both will mitigate some hazards inherent to non-cage systems. Access to an outdoor range (more so than a veranda) may bring other welfare consequences due to additional hazards.

Pullets destined for adult free-range systems are generally not provided with outdoor access until the laying period. Instead, they are reared in one of the systems described in Sections 3.2.1.2 and 3.2.1.3. As an alternative to access to free-range (open air area with vegetation), pullets may have access to a covered veranda. In contrast, pullets intended for organic systems are usually required to have outdoor access starting at an earlier age (e.g. from 8 weeks of age in the Netherlands and from approximately 10–12 weeks of age in the UK). This can be facilitated by first rearing chicks at a brooding site and then transferring them as pullets at 8–10 weeks of age to a second-stage rearing house, allowing the birds a few weeks to acclimatise before pop-holes are opened. The second-stage accommodation can be a fixed house or a mobile house comprising a floor system with a single-tier or other elevated structures, as described in Sections 3.2.1.3.

#### 3.2.3.1. Pullets and laying hens: mobile housing

Mobile houses are a niche housing system used throughout the EU, mostly in organic production and on farms that sell their eggs directly to consumers. Commercial mobile systems house flocks of 200–4,000 pullets (after approximately 8 weeks of age) or laying hens in either a single-tier or a multi-tier setup. Differently from the systems described previously, mobile housing is always combined with access to a free range (Figure 11). Mobile houses are fitted with wheels or skids which allow them to be pulled to another part of the range by a tractor. This reduces build-up of manure and parasites as well as degradation of range quality in the direct vicinity of the poultry house. However, most houses (especially the larger ones) are designed to only be moved in between two laying cycles. While some mobile houses have fully solid floors, in single-tier mobile houses the litter may be placed directly on top of the bare ground, and the elevated nest area is slatted with bare ground underneath. Although systems with mechanical ventilation exist, most systems depend on natural ventilation through horizontal vents spanning the full length of each side of the house. Roofs and walls are insulated with the intention of maintaining an appropriate temperature in the house, although this can be challenging in cold climates. Houses are equipped with automatic feeding and watering systems and egg collection belts, and multi-tier and some single-tier mobile houses also have manure drying belts. Some mobile houses also include a covered

veranda (Figure 12), but a setup in which the birds move directly from the house onto the range is more common.



**Figure 11:** Indoor (left) and outdoor (right) view of a smaller multi-tier mobile house with gravity ventilation mechanically ventilated © Steiner Automation (top) and a large naturally ventilated single-tier mobile house © 2022 McGregor Structures Ltd (bottom)



**Figure 12:** Indoor (left) and outdoor (right) view of a mobile house including a covered veranda © Steiner Automation

### 3.2.3.2. Covered veranda

A covered veranda (or winter garden) is an additional, roofed, uninsulated, outdoor addition to a building, with an outdoor climate. Covered verandas have a solid roof and at least one side that lacks a solid wall. This side can be closed off with netting (allowing light and air into the veranda), but more commonly this side is opened during daytime to allow hens access to the outdoor range. When the veranda gave access to the free range, 90% of hens used the veranda at least some of the time during a 3-week monitoring period (Gebhardt-Henrich et al., 2014). Furthermore, the majority of the time spent outside the house was spent in the veranda (58% reported by (Larsen et al., 2017); and 71% reported by (Gebhardt-Henrich et al., 2014)). Riedel et al. (2022) reported that 8% of the flock was present in the veranda on average, which was increased to 12% if grain dosing devices were provided. Some newly developed systems have extended covered verandas but allow no or very limited outdoor access. The aim of such systems is to enable a better disease control without compromising birds' welfare. Typically, these systems have a traditional aviary section, called night quarters, a very bright spacious area, called day quarters, an additional small outdoor area, and a low stocking density. Although designed for improved welfare, scientific evidence on the extent of such welfare benefits is currently lacking.

### 3.2.3.3. Outdoor range

The outdoor range as used in egg production is often a mostly grass covered field, although the provision of more extensive cover (e.g. trees, shrubs or artificial structures) is known to increase the number of hens that use the range. Controlled studies report an additional 2–9% of the flock outside in the presence of cover (Hegelund et al., 2005; Zeltner and Hirt, 2008; Buijs et al., 2020), whereas an epidemiological study found that the additional percentage of hens outside increased when the quantity of cover increased (Bestman and Wagenaar, 2003). Many other factors also affect range use (e.g. stocking density, flock size; and design of the indoor area including the pop-holes; design of the outdoor area; weather; and bird characteristics, (Gilani et al., 2014; Pettersson et al., 2016; Gebhardt-Henrich et al., 2014; Campbell et al., 2017a; Buijs et al., 2020)).

The outdoor range characteristics are described in the current legislation (Council Directive 1999/74/EC). Outdoor ranges are typically equipped with shelter from inclement weather and predators and, if necessary, appropriate drinking troughs. It has to be noted that access to outdoor range is rare throughout the EU and in some Member States it is even prohibited for biosecurity reasons (e.g. to avoid attracting wild birds in the context of highly pathogenic avian influenza). At least 2 m of summed pop-hole width per 1,000 hens is required, spread out over the length of the building. In 2020, approximately 18% of the laying hens in the EU had outdoor access (EC, 2021).<sup>2</sup> This type of production is particularly important in Ireland (48% of the hens kept there), Denmark and Austria (both 39%), Germany and France (both 34%), and Luxemburg and the Netherlands (both 24%).

As the hens can choose to go out or to stay inside, the percentage of hens that use the range will affect any benefits that can be derived from outdoor access (whether these are benefits to the hens that choose to go outside themselves, or to those that stay in and experience a lower indoor stocking density due to other birds being outside). The variations in the use of the outdoor area between farms can be explained by climatic conditions, range design or stocking density, while intra-flock differences appear to be related to personality and experience of the hens (Bonnefous et al., 2022). Older studies using direct observation reported relatively low use of the outdoor range (9–30% of the flock outdoors simultaneously (Zeltner and Hirt, 2003; Hegelund et al., 2005)). However, direct observation likely represents an underestimation of true ranging due to undetected birds and omission of peak ranging moments (e.g. dusk). Newer studies using continuous automated monitoring of hens suggest substantially higher range use (52% and 62% of time with access to the range spent outside reported by Campbell et al. (2017a) and Buijs et al. (2020), respectively, and 6 h spent outside daily on average reported by Hartcher et al. (2016). Although range use differs considerably between individuals within a flock (Larsen et al., 2017; Sibanda et al., 2020), a very large percentage of hens go outside at least some of the time (> 95% according to (Hartcher et al., 2016; Campbell et al., 2017b; Larsen et al., 2017; Buijs et al., 2020)), but 71% in (Gebhardt-Henrich et al., 2014). Birds that range more at the start of lay (relative to their flock mates) also range more when older (Rodríguez-Aurrekoetxea and Estevez, 2016) (Chielo et al., 2016). However, hens that visit the free-range more frequently also moved furthest from the house and had larger home ranges (Rodríguez-Aurrekoetxea and Estevez, 2016).

### 3.2.4. Collective cages

#### 3.2.4.1. Laying hens in furnished cages

Furnished cages systems (fall into the category of the EU-Directive 1999/74 'Enriched cage systems') provide 750 cm<sup>2</sup> space per animal, and equipment for feeding, drinking, egg collection, manure removal, insertion and removal of hens, and claw shortening. In addition, they provide equipment intended to enable hens to provide for some of their behavioural needs: perches, nest boxes, a pecking and scratching area. Faeces drop through the mesh floor onto a manure belt for transport and removal. Most of the systems include manure belt ventilation, which dries the manure, enabling reduced ammonia concentrations within the house and reduced environmental emissions.

There are a wide variety of furnished cage designs. Prototype furnished cages initially housed relatively small groups of hens (e.g. 4–20) but for many years, furnished cages have housed around 40–80. Cage dimensions are strongly related to group size and may influence the birds' locomotion possibilities as well as bird inspection and depopulation. The number of birds per cage could have implications for social behaviour, for instance the development of injurious pecking, especially with non-beak-trimmed birds. Positioning and layout of equipment is important to allow proper use and thus contribute to bird welfare, hygiene and performance. Appropriate perches, suitable nest boxes and friable litter may influence their use by the birds.

There are variations of laying and perching behaviour in different laying hen hybrids that need to be taken into account for appropriate cage design (Guinebretière, 2017). The pecking and scratching area may not be adequate to permit full expression of behavioural needs such as foraging and dustbathing since the floor of cages is of wire and therefore it is a challenge to keep friable substrate available at all times for birds.

Nest boxes can be placed at the rear, the side or close to the front of the cage. This can affect bird inspection and the hygiene of eggs and birds. The main types of nest flooring currently available in furnished cages are artificial grass mats (e.g. AstroTurf®) made of malleable polypropylene, perforated or not, and plastic mesh. Plastic floor (open floor to evacuate excrement) are also used (Guinebretière, 2017). Some cages offer nests with litter contained in a tray, which closes automatically outside the laying period (Fiks-van Niekerk et al., 2002). This system is rarely used as it presents a risk for injury to the hens when closing the nest by blocking them. In addition, this option requires automatic closing. Today's cages can accommodate up to 60 or 80 hens in a single cage, with 1 or 2 nests available, open and continuously accessible. The laid eggs roll to the egg belt at the end of the cage.

Furnished cages provide a pecking and scratching area at the same level as the rest of the cage, with a lining instead of a litter tray, over which a friable substrate can be distributed without retaining it in a box. The covering is often artificial grass, perforated or not, or a flat steel or plastic surface covering. This area is continuously accessible and open, but usually of a small surface (Figure 13).



**Figure 13:** Examples of pecking and scratching areas (Photo Credit: M. Guinebretière, ANSES)

The provision of friable substrate imposes technical constraints on the breeding system because the litter must be distributed and renewed frequently. Hard surface materials that can be scratched and pecked are sometimes used replacing both the lining and the friable substrate but not typically used in

cages. They require regular substitution due to the pecking and scratching actions of the animals destroying the substrate. Harder or larger and compact material may resist sufficiently long, but is less friable, or may be too heavy for such cages.

Perches can be arranged in a variety of designs, positions and heights; some are more satisfactory than others. EFSA AHAW Panel (2015) concluded that suitable perches allow hens to grip easily and be stable: non-slippery, wide (3–7 cm), with a suitable shape (round or mushroom shape). In commercial cages, there are different materials and sections of perches (round, square, mushroom; wood, plastic, metal) (Hester, 2014). Perches are generally 4–5 cm in cross-section. The use of perches depends, among other things, on the available perch space, height, material used, shape, ease of perching and experience of the hens (Fiks-van Niekerk et al., 2002; Colson et al., 2008). The height and arrangement of the perches in the cage seem to be important factors for their proper use by the hens. The arrangement of perches in a cage is complex, especially if the cage is small. Several perches can be installed, parallel or perpendicular, at different levels or not. Hens would prefer a position parallel to the feeder (Fiks-van Niekerk et al., 2002). Most of the current cage designs have perches less than 10 cm high.

#### 3.2.4.2. Pullets

Collective (colony) cages for pullets must accommodate growing birds and so the heights of drinkers and feed barriers are always adjustable. Pullet rearing cages are essentially wire mesh boxes. There is no requirement for a sloping floor to collect eggs and so the cage floors are flat. Faeces drop through the mesh floor onto a manure belt for transport and removal. Some systems include manure belt ventilation, which dries the manure, enabling reduced ammonia concentrations within the house and reduced environmental emissions. Most cages for pullets provide no scratching, foraging or perching resources. However, a combi-cage system has been developed with cage-fronts that can either be closed to create furnished cage system or opened to create a multi-tier system.

Water is provided to pullets via nipple drinkers within the cages. The nipple drinkers may have small cups underneath to catch drips. For the youngest birds, these drip cups can be replaced with larger open cups to facilitate early drinking. Feed is distributed via a chain to troughs fitted to the outside of the cages. The height of access increases as the birds grow.

Some cage systems are designed in a way that day-old chicks are distributed to all tiers of the system from the day of their arrival. Other cage systems are designed with a starter (or brooder) tier, so that newly arrived chicks are kept within just one tier for the first few weeks of life. This starter tier may have smaller floor mesh dimensions than other tiers to provide a more supportive floor surface for young chicks. Alternatively, mats may be provided as inserts. As birds grow, they are distributed manually within the other tiers. A pullet rearing house may contain up to 8 tiers of cages.

Chicks will be handled more than adult birds either for vaccinations or for manual distribution. It is therefore important that cage fronts allow easy access to birds. Commercial solutions include push front and sliding doors.

Commercially available cages provide cage heights varying from 39 to 41.5 cm. Cage areas vary from 7,366 to 8,200 cm<sup>2</sup>, with a length of approximately 1.2 m and depth of 0.68 m. Pullets are commonly housed with space allowance varying from 270 cm<sup>2</sup>/bird to 330 cm<sup>2</sup>/bird, with the number of birds per cage varying from approximately 20 to 40. Mortality has been reported at 7% by (Bozkurt et al., 2006) at 16 weeks of age, with no differences attributed to experimental space allowances varying from 212 to 370 cm<sup>2</sup>/bird. In one experiment, where pullets were reared at 315 cm<sup>2</sup>/bird or 945 cm<sup>2</sup>/bird there were no short-term effects on stress, or long-term detectable effects on adult hen welfare (Engel et al., 2019). However, when pullets were reared in experimental floor pens (14 pullets/pen) at spatial allowances that increased progressively with age to either 167 cm<sup>2</sup>/bird, 500 cm<sup>2</sup>/bird or 1,429 cm<sup>2</sup>/bird by the age of 7–10 weeks, more anxious behaviour and higher corticosterone concentrations were found at the lowest space allowance. However, high corticosterone was also found at the highest spatial allowance in comparison with the middle allowance (von Eugen et al., 2019). Another study examining stocking densities within experimental pens housing 46 pullets/pen at either 13 birds/m<sup>2</sup> (769 cm<sup>2</sup>/bird) or 23 birds/m<sup>2</sup> (435 cm<sup>2</sup>/bird), found that by the end of the rearing period, pullets kept at the higher density had lower number of T-lymphocytes and a higher heterophil:lymphocyte ratio, indicating reduced immune function and increased stress levels (Hofmann et al., 2021). The pullets reared under higher density also showed less comfort behaviour, activity and had worse plumage condition (Hofmann et al., 2021). When pullets were reared experimentally in furnished cages compared with conventional cages at equivalent stocking rates providing 1,220 cm<sup>2</sup>/bird, immune function was improved (Matur et al., 2016). When reared in furnished cages at 636 cm<sup>2</sup>/bird

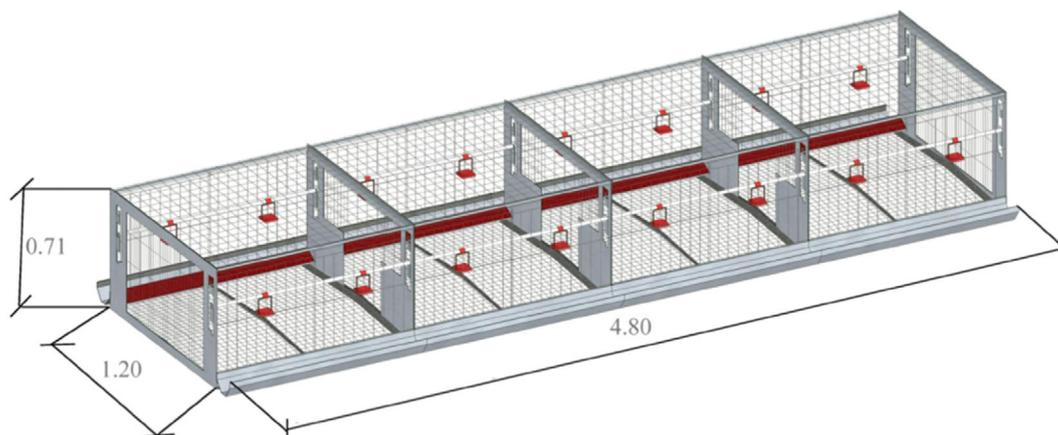
compared with conventional cages at 270cm<sup>2</sup>/bird subsequent eggshell quality in adults was improved, with marginal improvements also to leg bone quality (Khanal et al., 2020). However, stocking density within furnished cages ranging from 247cm<sup>2</sup>/bird to 335cm<sup>2</sup>/bird had little effect on measures of bone and muscle strength and quality (Fawcett et al., 2020), probably because both stocking densities were high, with little difference between them.

There are difficulties in interpreting these studies, given the differing ranges of space allowances and outcomes studied. In some cases, the range of spatial allowance considered may have been too narrow to detect meaningful differences (e.g. if all allowances are inferior or equivalent to 330 cm<sup>2</sup>/bird). Outcome assessments taken at different ages add another layer of complexity as age, size and maturity effects will also play a role. These effects have not been systematically investigated. Despite these caveats, it is clear that some studies have found adverse effects on stress and immune function at space allowances < 500–750 cm<sup>2</sup>/bird but there have been few studies looking at behaviour, few looking at cage enrichment, and (unlike for adult hens) none looking at pullet preferences or motivational demand for space or resources within a cage environment.

### 3.2.4.3. Laying hen breeders

Collective cages used for the breeding stock are large wire mesh cages, with sloped floors provided with nipple drinkers and chain feeders located outside. Collective cages for breeders can be taller (e.g. 4.80 m × 1.20 m × 0.71 m) for 100 birds Shi et al. (2019a) than furnished cages for laying hens to allow mating behaviour. Collective cages may, or may not, be provided with a perch (normally located in the wider axes), and nest boxes or a dustbathing substrate are not available in all countries. The number of birds per collective cage is variable, normally ranging from 20 up to 100 birds, but maintaining a ratio of 1 male to 10 females. Rearing densities in some countries are high, with 600 cm<sup>2</sup> space availability per bird, although, normally, 750 cm<sup>2</sup> per bird is available. Fertilisation in this system is through natural mating. The colony cage system may contain up to 3 tiers with a manure belt collector in between tiers. Birds are maintained under a standard lighting program including 8 h of darkness.

As indicated in Section 3.2.4.1 on furnished cages in laying hens, group size may influence bird inspection, catching procedures at depopulation, and locomotion possibilities for animals. This system offers no possibility for females to get away from males, and also restricts the birds' comfort behaviour, because behaviours like dustbathing will not be shown in an environment where litter material is mostly lacking or limited. Exceptionally, collective cages with females only can be found. These females are artificially inseminated with male ejaculates from individuals that are maintained in the same facility (Figure 14).



**Figure 14:** Standard collective cage for 100-layer breeders (as shown by Shi et al., 2019a)

### 3.2.5. Individual cages

#### 3.2.5.1. Laying hen breeders

Male and female pure line selection candidates at breeding companies are often housed in individual cages to enable detailed record keeping of individual feed intake, egg production (number of eggs, egg quality) and fertility. Individual cages are normally simple metal mesh enclosures, with

sloped floors, and only occasionally, birds are provided with perches. No nest boxes are available in these types of cages. Cages normally have nipple drinkers and individual feeders located externally that allow feed intake control. No legal requirements exist for minimal space per bird or provision of equipment to satisfy the biological needs of breeders. Thus, these individual breeding cages resemble conventional laying hen (battery) cages that are prohibited for laying hens according to Directive 1999/74/EC. Birds are normally placed in these cages at around 16–18 weeks of age, where they will remain for their entire reproductive life (until 60–70 weeks of age). Females are artificially inseminated at regular intervals (normally twice per week) with standardised male ejaculates, thus requiring regular handling. The cage system may contain a manure belt collector in between levels.

The limited size of individual cages seriously impairs the possibility of layer breeders of expressing much of their natural behaviour and seriously limit birds' mobility and exercising possibilities, as well as social interactions.

### 3.3. Describing welfare of domestic fowl (*Gallus gallus*) related to the production of eggs

This section provides a detailed description of the highly relevant welfare consequences for laying hens, pullets and layer breeders kept in the above-described husbandry systems. For each welfare consequence, related ABMs, hazards as well as measures to prevent the hazards and/or to mitigate the welfare consequences are described taking into account the specificity of the different husbandry systems and animal categories, when needed. An overview is provided in Tables 4 and 5. The method adopted to select welfare consequences for further consideration was conducted *within* each housing system and not between housing systems. Within each system and animal category, only those of the highly relevant welfare consequences that the experts considered to be substantially more relevant than the other welfare consequences were considered. Therefore, an empty cell in Table 4 does not mean that the welfare consequence is absent for that particular system and/or animal category, but that it is considered less relevant than those consequences that have been marked with an x for that system/category. Similarly, if a welfare consequence is marked as relevant for two systems and/or animal category, this does not mean that its impact in the two systems/categories is equal. Thus, the summed number of highly relevant welfare consequences of a system/category should not be used to conclude that overall welfare is better in one system than in another.

ABMs to measure the welfare consequences are related to body condition (e.g. plumage score), behaviours (e.g. time spent doing a certain activity), clinical measure (e.g. cloacal temperature) or health status (e.g. sick or dead animals).

Body condition, health status and physiological ABMs are either binary (yes/no: e.g. dead, sick, having a broken bone), categorical (e.g. scoring of plumage (0/1/2)) or continuous (e.g. weight, cloacal temperature, level of hormone). There are different ways to express the results after the measurement of the ABMs: it can be a prevalence (e.g. proportion of birds with a bone fracture; proportion of birds with plumage score 2) or summary scores (e.g. mean/median plumage score of the birds observed) or measurements of continuous variables (e.g. mean weight loss). The measurements can take place during the handling itself (e.g. scoring plumage, weighing), by observing the animals without handling (e.g. transect method) or by automatic measurement (e.g. automatic weighing, infrared camera temperature measurement, video scoring system of feather loss). For physiological data, the sampling is either invasive (e.g. blood sampling) or not (faecal sampling or feather collection) and results can be treated either as continuous values (e.g. corticosterone level per animal or in a pooled sample from multiple animals) or dichotomised (e.g. animal with corticosterone level above a certain threshold, which can then be used to calculate proportions).

More detail about measurement of ABMs is provided in the sections describing the welfare consequences and their related ABMs and in Appendix F for methodological points.

**Table 4:** Highly relevant welfare consequences for the different housing systems and their related ABMs in laying hens, pullets and layer breeders. Because the relative importance of welfare consequences was considered within systems and not between systems, the total number of welfare consequences indicated as highly relevant per column is not necessarily reflective of overall welfare in that system (x = indicates in which husbandry system the welfare consequence is identified as highly relevant)

Highly relevant welfare consequence and related ABMs	Laying hens				Pullets					Breeders				
	Furnished cage	Floor system with single-tier	Floor system with multi-tier	Mobile housing	Collective cage	Floor system without elevated structure	Floor system with maximum one tier	Floor system with multi-tier	Mobile housing	Individual cage	Collective cage	Floor system with single-tier	Floor system with single tier slatted floor	Floor system with multi-tier
<b>Bone lesions (keel bone fracture)</b> – Keel bone fracture	X	X	X	X							X	X		X
<b>Group stress</b> – Aggressive interactions – Fear responses – Injurious pecking – Physiological stress indicators – Piling behaviour – Plumage damage	X	X	X		X	X	X	X			X	X	X	X
<b>Inability to avoid unwanted sexual behaviour</b> – Avoidance of the litter area by the females – Forced copulation											X	X	X	X

Highly relevant welfare consequence and related ABMs	Laying hens				Pullets					Breeder				
	Furnished cage	Floor system with single-tier	Floor system with multi-tier	Mobile housing	Collective cage	Floor system without elevated structure	Floor system with maximum one tier	Floor system with multi-tier	Mobile housing	Individual cage	Collective cage	Floor system with single-tier	Floor system with single tier slatted floor	Floor system with multi-tier
<ul style="list-style-type: none"> <li>– Plumage damage</li> <li>– Wounds to skin</li> </ul>														
<b>Inability to perform comfort behaviour</b> <ul style="list-style-type: none"> <li>– Lack of dustbathing</li> <li>– Preening</li> <li>– Wing flapping</li> <li>– Wing and leg stretching</li> </ul>	X				X					X	X			
<b>Inability to perform exploratory or foraging behaviour</b> <ul style="list-style-type: none"> <li>– Injurious pecking</li> <li>– Plumage damage</li> <li>– Walking, scratching and pecking</li> </ul>	X				X					X	X			
<b>Isolation stress</b> <ul style="list-style-type: none"> <li>– Fear response</li> <li>– Pacing</li> <li>– Physiological stress indicators</li> </ul>										X				

Highly relevant welfare consequence and related ABMs	Laying hens				Pullets					Breeder				
	Furnished cage	Floor system with single-tier	Floor system with multi-tier	Mobile housing	Collective cage	Floor system without elevated structure	Floor system with maximum one tier	Floor system with multi-tier	Mobile housing	Individual cage	Collective cage	Floor system with single-tier	Floor system with single tier slatted floor	Floor system with multi-tier
<b>Predation Stress</b> – Dead birds or surviving birds with signs of predation or remains of birds – Excess missing birds above the recorded mortality – Fear responses – Physiological stress indicators Reluctance to use free-range				X					X					
<b>Resting problems</b> – Balance movements – Pushing and jostling behaviours – Resting birds on elevated structures					X	X					X	X	X	
<b>Restriction of movement</b> – Reduced bone quality	X				X					X	X			

Highly relevant welfare consequence and related ABMs	Laying hens				Pullets					Breeder				
	Furnished cage	Floor system with single-tier	Floor system with multi-tier	Mobile housing	Collective cage	Floor system without elevated structure	Floor system with maximum one tier	Floor system with multi-tier	Mobile housing	Individual cage	Collective cage	Floor system with single-tier	Floor system with single tier slatted floor	Floor system with multi-tier
<ul style="list-style-type: none"> <li>– Locomotor behaviours (e.g. jumping, flying, walking, running)</li> <li>– Pushing and jostling behaviours</li> <li>– Plumage damage</li> </ul>														
<b>Skin disorders (other than soft tissue lesions and integument damage)</b> <ul style="list-style-type: none"> <li>– Restlessness</li> </ul>	X	X	X	X										
<b>Soft tissue lesions and integument damage</b> <ul style="list-style-type: none"> <li>– Beak shape and length</li> <li>– Bruises</li> <li>– Plumage damage</li> <li>– Wounds</li> </ul>	X	X	X	X						X	X	X	X	

**Table 5:** Overview table of hazards identified for each of the highly relevant welfare consequences. Only the direct causal relations are represented here. (x = indicates in which hazards is the main causal factor highly relevant welfare consequence)

Hazards	Highly relevant welfare consequences										
	Bone lesions	Group stress	Inability to avoid unwanted sexual behaviour	Inability to perform comfort behaviour	Inability to perform exploratory or foraging behaviour	Isolation stress	Predation stress	Resting problems	Restriction of movement	Skin disorders	Soft tissue lesions and integument damage
<b>Hazards related to behaviour and/or genetics</b>											
Bone quality	x										
Genetics	x	x									
Aggressive interactions and injurious pecking											x
Sexual development asynchronicity			x								
Inappropriate responses to predators							x				
<b>Hazards linked to the environment</b>											
Absence of conspecifics						x					
Male and female ratio		x	x								
Insufficient space					x	x			x		
High stocking density		x			x				x		
<b>Hazards linked to the equipment</b>											
Insufficient/uneven resource allocation		x							x		
Falls and collisions	x										
Insufficient height of enclosure									x		
Lack of elevated structures		x	x		x			x	x		

Hazards	Highly relevant welfare consequences										
	Bone lesions	Group stress	Inability to avoid unwanted sexual behaviour	Inability to perform comfort behaviour	Inability to perform exploratory or foraging behaviour	Isolation stress	Predation stress	Resting problems	Restriction of movement	Skin disorders	Soft tissue lesions and integument damage
Inappropriate design of elevated structures								X			
Inappropriate litter material				X	X						
Insufficient litter				X	X						
Inadequate pecking and scratching area				X							
Abrasive or sharp housing system elements											X
Insufficient cover on range							X				
Predator access		X					X				
<b>Hazards linked to the management</b>											
Red mite infestation										X	
Inappropriate light				X							
Mutilations											X

### 3.3.1. Bone lesions

#### 3.3.1.1. Description of the welfare consequence

Bone lesions were identified as a highly relevant welfare consequence for laying hens and layer breeders in collective cages as well as single- and multi-tier systems, including mobile housing.

Bone lesions are defined as fractures or dislocations of the bones causing negative affective states such as pain, discomfort and/or distress – excluding those fractures leading to locomotor disorders (EFSA AHAW Panel, 2022a,b).

In this opinion, only keel bone fractures are considered within the welfare consequence 'bone lesions'. Consequences on other bone lesions, e.g. wing or leg fractures, are excluded, as well as bone dislocations or deviations owing to the lack of data on their frequency or impact on welfare.

The severity of keel bone fractures is considered to be high. Compared to birds without fractures, laying hens with keel bone fractures show differences in time spent on highly motivated behaviours (e.g. perching, nest use, locomotion), indicating reduced mobility and potentially negative affective states including pain, discomfort and distress (Riber et al., 2018). Furthermore, keel bone fractures are associated with inflammation and decreased feed intake.

The duration of the welfare consequence varies as bone lesions can occur at any time during the laying period. Richards et al. (2011) reported that 80% of fractures heal within 35 days. The consequences can, however, last beyond the bone fracture healing period (Wei et al., 2020) and some fractures may not heal at all. Recent research on bone lesions in layers focuses almost exclusively on keel bone fractures, although wing and leg fractures also occur during lay and may cause negative affective states and even mortality in laying hens (Fulton, 2017). Older research on fracture risks has included leg and wing bones but focused on bone mineralisation and bone strength (Whitehead, 2004). While these parameters are related to fracture risk (Wei et al., 2021), other factors such as the occurrence of falls and collisions seem to be at least as important for the prevalence of keel bone damage (Gebhardt-Henrich et al., 2017). Collisions are common in hens in non-cage systems. Campbell et al. (2016c) observed that 9–20% of all flights included a collision with the floor, a housing element or the ground. The assumption that the impact resulting from such falls and collisions is a main cause for keel bone fractures is widespread. Indeed, the layout of multi-tier systems and the possibilities for the birds to move around without the need to negotiate distances were found to influence the incidence of keel bone fractures (Stratmann et al., 2015; Heerkens et al., 2016a; Heerkens et al., 2016b). This complies with the finding that hens in furnished cages have the lowest incidence of keel bone fractures, even though bone strength is lower in caged birds compared to non-caged birds (Wilkins et al., 2011). However, the role of falls and collisions has recently been challenged, especially for fractures occurring near the caudal part of the keel (last third). Thøfner et al. (2020) found that > 96% of the fractures are located at the caudal end of the keel bone and suggested that these may be related to the depletion of the hen's body reserves (as a result of breeding for a smaller bird and a higher egg production), biomechanical effects of the egg laying process on the tip of the keel bone, or wing flapping. Further research indicates a breed predisposition and relations with hen size and high (early) production levels (Riber and Hinrichsen, 2016; Candelotto et al., 2020; Wei et al., 2019; Toscano et al., 2020). More specifically Thøfner et al. (2021) suggested that fracture risk was greater for hens that were younger at the onset of lay, that laid heavier eggs at the onset of lay, and those that had a lower body weight at end-of-lay.

Young pullets are more likely to display high-intensity physical activity than older birds (Kozak et al., 2016). It is important for pullets to develop their motor skills and strength before they move to cage-free systems. Chicks will use elevated structures and perches from as young as 2 weeks of age (Kozak et al., 2016) and this early exercise increases the subsequent ability of hens to reach elevated structures (Gunnarsson et al., 2000b; Heikkilä et al., 2006; Colson et al., 2008).

#### Description of bone lesions in non-cage systems

##### Laying hens

The frequency of keel bone fractures in hens over 49 weeks old in single-tier systems shows high variation between different studies and system design, with study averages ranging from 5% to 100% (Rufener and Makagon, 2020).

Direct comparison indicates that keel bone fractures are more frequent in single tier systems than in furnished cages (Wilkins et al., 2011), but less frequent in single-tier systems than in multi-tier systems (Riber and Hinrichsen, 2016). This is likely to be associated with the greater opportunity and

need for flying, jumping and running in single-tier systems as compared to furnished cages, and the smaller need for such behaviour than in the multi-tier systems. This is because such locomotory behaviour increases the risk of high impact falls and collisions with the structures of the housing system (Toscano et al., 2020; Rufener and Makagon, 2020). Recent changes in the design of non-cage systems (e.g. 3D layout and the provision of ramps) may have affected the frequency of keel bone fractures. Some support for this expectation comes from a study using data collected between 2016 and 2019. In contrast to studies on older non-cage systems, this study found no significant difference in the prevalence of birds with at least one keel bone fracture between furnished cages and non-cage systems, although more keel bone fractures per bird were found in non-cage systems (Thøfner et al., 2021).

Although there are no specific figures for the prevalence of keel bone fractures in mobile systems, this is likely to be similar to the prevalence in single-tier and multi-tier systems with outdoor access, which vary a lot between flocks (from 3% to 88%, (Jung et al., 2020)). It is likely that factors such as genetic selection; health status; diet; system height; perch availability, placement and coating; the opportunity and need for flight at difficult angles; and the provision of light are all applicable to mobile systems as well. The compactness of mobile systems varies greatly and this is likely to impact on collision risk and thus keel fractures, but systematic research is currently lacking.

### Layer breeders

To date, there is no information on layer breeders housed in single or multi-tier systems specifically. Although a priori the occurrence can be expected to be similar to that in laying hens. The absence of perches for layer breeders may reduce the risk of this welfare consequence in breeding layers housed in single-tier systems.

### Description of bone lesions in collective cages

In furnished cages, the behaviour of laying hens with keel fractures differs from that of those without, although the exact differences are not consistent over studies. While Casey-Trott et al. (2015) reported a decrease in standing and an increase in perching, Wei et al. (2020) reported reduced perching, as well as reduced feeding, walking and jumping. Bodyweight, feed intake and egg production were also reduced in animals with keel bone fractures (Wei et al., 2020). This suggests an important effect on overall well-being even under circumstances where hens have to move relatively little to fulfil their basic needs like eating, drinking and resting.

In the furnished cage, there is a greater percentage of hens having caudal fractures only (rather than a combination of caudal and medial fractures) and such fractures are more difficult to detect by palpation (Thøfner et al., 2021). This may lead to a greater underestimation of keel bone fractures in hens in furnished cages than in non-cage housing systems.

#### 3.3.1.2. ABMs

The commonly used palpation method is by far the most feasible for the assessment of live hens on farm. The sensitivity of this method is high when applied to the keelbone as a whole or to its medial part (i.e. 75–93% of keelbone fractures are detected by palpation: (Wilkins et al., 2004; Buijs et al., 2019). However, sensitivity is low for fractures of the caudal part of the keel (i.e. only 37% of such keel bone fractures were detected by experienced assessors (Buijs et al., 2019). This is especially relevant because in some cases as much as 96% of the fractures may occur in the caudal part (Thøfner et al., 2021). Palpation can have moderate specificity (i.e. the percentage of keels without a fracture that were correctly classified as fracture-free varied between 54% (Wilkins et al., 2004) and 88% (Thøfner et al., 2021)). The accuracy of the palpation method may also vary between housing systems, as fractures with little callus formation (which are harder to detect by palpation) are common in caged birds, whereas fractures with large calluses are typically found in non-caged birds (Thøfner et al., 2021). The high prevalence of keel bone fractures in most flocks coupled with this limited sensitivity means that, in most cases, inaccuracy in the palpation method will lead to an underestimation of the occurrence of this problem (Buijs et al., 2019; Thøfner et al., 2021). In addition, keel bone fractures are only one type of bone lesion, and their occurrence is not necessarily linked to the occurrence of other bone lesions. This means that the sensitivity of the method to detect bone lesions in general can be compromised even when using a keel bone fracture detection method that is (near to) perfect (as post-dissection or radiographic scores are often considered to be). As different methods to assess keel bone fractures differing in sensitivity have been used to evaluate

different housing systems, meta-analysis of existing research does not give a reliable estimate of the effect of housing system (Rufener and Makagon, 2020). This represents a current lack of knowledge.

ABMs to assess 'bone lesions' are described in Table 6.

**Table 6:** ABMs for the assessment of 'bone lesions (incl. fractures and dislocations)'

ABM	NOTES
<b>Keel bone fracture</b>	
Definition	Complete or partial break in the keel bone, which may, or may not, have healed resulting in a callus (Casey-Trott et al., 2015).
Measurement	The occurrence of healed or acute keel bone fractures can be assessed by palpation, computed tomography, ultrasound, radiography or an automated 3D camera system (Rufener and Makagon, 2020; Jung et al., 2021). Importantly, the assessment method affects the percentage of fractures that are detected as these methods vary in sensitivity (Rufener and Makagon, 2020; Jung et al., 2021). The commonly used palpation method is mostly focused on detecting healed fractures (Casey-Trott et al., 2015) although some authors have also included acute fractures if these can be identified (which is usually only possible for complete fracture of the keel bone).
Interpretation	More keel bone fractures are interpreted as a greater impairment of welfare due to bone lesions.
Sensitivity and specificity	Sensitivity is considered moderate, ranging from moderate to high for the main part of the keel and low for the caudal part (Wilkins et al., 2004; Buijs et al., 2019; Thøfner et al., 2021) Specificity is considered moderate (Wilkins et al., 2004).

### 3.3.1.3. Hazards and preventive or mitigating measures

#### Hazards relevant for bone lesions in single- and multi-tier systems

In both single- and multi-tier systems, hazards are high impact collisions and falls and poor bone quality.

Compared to the single tier, the multi-tier not only provides more options for vertical movement but also requires the hen to make more of such vertical movements to satisfy all her needs (e.g. feeding, resting, laying, foraging). Thus, the risk of a fall or collision during such movement also increases, which in turn increases the risk of keel bone fractures. However, falls and collisions during vertical and horizontal movements are also highly relevant hazards in the single-tier system. Furthermore, multi-level perches and a high number of perchable items have been identified as hazards for keel bone fracture (Wilkins et al., 2011), probably because hens can collide with these or fall down while trying to move between such structures. However, this seems at odds with the finding that low perch space per hen and crowded perches are also a hazard as lack of space makes it more difficult to move between perches without slipping or missing the perch (Moinard et al., 2005; Edwards and Hemsworth, 2021). This suggests that providing sufficient perchable items at locations where they would be unlikely to result in collisions or high falls may be critical. Artificial light affects manoeuvring of birds: very low light intensities (< 5 lx) make hens more reluctant to jump (Taylor et al., 2003; Moinard et al., 2004). This is likely because performing the jump successfully (i.e. without falling or colliding with housing elements) is more challenging. Under low-light conditions reluctance to jump can be reduced by using a perch colour that contrasts well with the environment (Taylor et al., 2003). Reluctance to jump also increases when the distance to be covered is greater (Scott et al., 1999; Taylor et al., 2003). As this reluctance to jump likely reflects the hens' own estimate of their chance of jumping successfully (i.e. not falling or colliding as a result of the jump), low light and poorly contrasting perches likely increase the risk of keel bone fractures. Longer jumps and steeper jumping angles lead to more balancing movements upon landing and greater forces on the keel (Scholz et al., 2014; Rufener and Makagon, 2020). There is also a genetic influence on laying hens' jumping ability which may be due to differences in body weight between different lines (Scholz et al., 2014).

#### Hazards relevant for bone lesions in collective cages

Specifically relevant for keel bone fracture risk in collective cages is poor bone quality due to the lack of vigorous activity like flight and high jumps, as the cage impedes these behaviours. Due to the limited size of the cage, falls from great heights and collisions with the housing system during flight

are unlikely to happen (in contrast to non-cage systems where these are frequent). Collisions with the cage floor and walls as a result of falling off unsuitably designed perches and panic reactions may contribute to the occurrence of keel fractures, but direct evidence on the effects of these specific behaviours obtained within furnished cages is currently very limited (Harlander-Matauschek et al., 2015; Baker et al., 2020). However, it has been shown that the presence of perches increases keel bone fractures in the furnished cage (Hester et al., 2013). Although the exact way in which the perches increase keel bone fractures is unknown, the majority of collisions in furnished cages with a perch is reported occur during ascent of the perch (Baker et al., 2020), and this may be when the fractures occur.

### Prevention of hazards

In cage-free systems:

Three-dimensional complexity during rearing, i.e. providing pullets with the opportunity to move up and down structures, contributes to reducing bone lesions by improving bone strength (Pufall et al., 2021) and spatial awareness (Norman et al., 2021) which reduces the risk of falls and collisions. Even if hens are kept in a cage system during lay, aviary rearing decreases the occurrence of keel fractures during lay (Casey-Trott et al., 2017).

Rearing with perches reduces subsequent collisions for adult hens housed in colony cages (Pullin et al., 2020), and aids movement and reduces collisions in aviary systems (Ali et al., 2019), although as older birds adapt, the influence of the rearing period diminishes (MacLachlan et al., 2020). Conversely, delaying birds' access to perches and elevated nests (from 17 weeks to 25 weeks) compromises movement and increases the risk of injury from falls (Ali et al., 2019).

Providing young chicks and pullets with ramps to enable easier access to elevated structures has additional benefits, with grid ramps preferred over ladders (Pettersson et al., 2017; Zheng et al., 2019) and shallow ramp angles (< 30–40) over steep angles (Zheng et al., 2019). Pullets reared commercially with elevated structures and additional ramps from 3 weeks of age were more mobile and confident in moving between tiers at 12–14 weeks of age than pullets reared with elevated structures but no ramps (Norman et al., 2018). The additional beneficial effects of early ramp access can persist through to the laying period (Norman et al., 2021).

Further methods to reduce keel bone fractures by preventing falls and collisions include: optimising light conditions and providing sufficient space on safely accessible perches. Sufficient light is essential to facilitate orientation and safe manoeuvring. More artificial light increases the chance that jumps are successful (suggesting that this will reduce keel bone fractures due to falls). Furthermore, the presence of daylight in the house has been linked directly to a lower frequency of keel bone fractures in non-cage systems (Jung et al., 2019). Visibility can also be improved by the use of contrast colours for perches. Although perches themselves can be a hazard as hens may fall from or collide with them, improved perch design can improve the ease of perching thereby reducing the risk of such falls and collisions. This includes, for instance, the use of materials less slippery than steel and avoiding thin round perches (Scholz et al., 2014). Furthermore, covering hard perches with soft polyurethane material can reduce keel fractures in non-cage systems by absorbing part of kinetic energy occurring during collisions (Stratmann et al., 2015). Access to perches during rear is also of importance, as hens reared without perches have difficulty using perches later in life. This may be due to low muscle strength, a lack of motor skills and the inability to keep balance, or impairment of their spatial skills (EFSA AHAW Panel, 2015).

An additional preventive measure that can be used in multi-tier systems is the provision of ramps that connect different parts of the system, which reduce the need for flight and long jumps at difficult angles. This in turn reduces collision risk as longer jumps and steeper angles are more difficult for hens to navigate and associated with greater forces at the keel (Scholz et al., 2014; Rufener et al., 2020). Ramps have been shown to reduce keel bone fracture occurrence (Stratmann et al., 2015; Heerkens et al., 2016a).

In addition to the hazards already described, some authors found an association between higher use of the free range and a lower prevalence of keel bone fractures, although others did not find this association (Larsen et al., 2018; Bari et al., 2020). Furthermore, the reduced use of the outdoor area may be the result of the fractures rather than a way of preventing fractures (i.e. fractures may make hens unwilling or unable to go out).

## Furnished cage

Keel bone fractures in furnished cages can be reduced by optimising bone strength. This can be achieved by genetic selection for bone strength rather than egg production (Toscano et al., 2020; Eusemann et al., 2020; Dunn et al., 2021); by feeding diets that promote bone strength and ensuring that gut health is sufficient so all nutrients in these diets can be absorbed appropriately (Toscano et al. 2020); and by avoiding bone disease (Toscano et al., 2020). In addition, providing hens with the option to perform vigorous activity or high jumps would be beneficial for their bone strength (Toscano et al., 2020), but this will require a significant increase of cage height. However, stimulating such behaviour during rearing (for instance by rearing in an aviary system) can reduce fractures in adult layers in cages (Casey-Trott et al., 2017). The latter may be due to a combination of improved bone strength as well as a reduction in collisions due to improved spatial awareness. In addition, improved perch design and more perch space per hen may reduce keel bone fractures in furnished cages due to collisions (Baker et al., 2020; Edwards and Hemsworth, 2021). As such collisions mainly seem to occur when hens ascend the perches in this system (Baker et al., 2020), improvements in perch design could focus on creating more easily ascendable perches.

### Mitigation of welfare consequence

Once hens have been placed in the system, and thus exposed to the hazards, there is little that can be done to mitigate the welfare consequence.

Once keel bone fractures occur, they have to heal. The only possible mitigation strategy that can be considered is moving severely injured birds to a hospital pen and providing analgesics or culling the birds according to best practices.

## 3.3.2. Group stress

### 3.3.2.1. Description of the welfare consequence

Group stress was identified as a highly relevant welfare consequence for laying hens, pullets and layer breeders in all husbandry systems except mobile housing. It does not apply to layer breeders in individual cages.

Group stress is defined as the animal experiencing stress and/or negative affective states such as pain, fear and/or frustration resulting from a high incidence of aggressive and other types of negative social interactions. These negative affective states can be indicated by changes in physiology, vocalisation, patterns of movement or an increased level of fearful responses in standardised tests.

The prevalence of group stress is highly variable and dependent on the cause. Group stress often arises from social conflict leading to aggression, from injurious pecking or from competition for resources or mates. Severity may be high for those birds that experience repeated aggression, resource competition or injurious pecking but the number of birds affected within a flock can range from very low to very high. Severity may also be high if negative social interactions lead to piling and smothering behaviour.

The general definition of group stress mentions social factors, but it is important to appreciate that management and housing will influence these. For example, factors such as high stocking density or uneven/insufficient resource allocation are likely to increase competition and social conflict. These factors should be considered as hazards for group stress. In addition, group stress can also result from non-social welfare consequences such as predation stress or sensory overstimulation, which are subsequently transmitted socially within the flock. For example, an alarm call emitted by one bird may produce fearful responses in others.

Birds that are in pain, fearful or frustrated, from either social or non-social causes, are more likely in turn to resort to negative social interactions. Pain, for example, may result in an increase in defensive aggression. This feedback loop means that indicators of group stress (e.g. aggressive interactions) can also be a cause of further group stress (e.g. because aggression will result in an increase in alarm vocalisations, escape attempts and fearfulness).

### Social dynamics, resource competition and aggression

Group stress is a complex concept, and it is important to understand the social dynamics of domestic fowl to understand what may go wrong.

Laying hens in small groups (6–10 birds) form structured social hierarchies comparable to that of their wild ancestor, the red jungle fowl (Collias and Collias, 1996). These hierarchies are often linear

and established through aggressive displays in early phases of development (Guhl and Ortman, 1953; Rushen, 1982). Once the hierarchy is established, dominant birds can access resources first; thus, aggressive encounters decline, simultaneously reducing the costs and risks of continuous fighting (Enquist and Leimar, 1990; Creel, 2001). In junglefowl, previous social experience, parasite status, morphological characteristics and possibly age can be important factors in establishing a hen's social rank (Kim and Zuk, 2000). In domestic hens, bodyweight, comb size and colour can all predict high social status (e.g. Cloutier and Newberry (2000)). Animals establish and sustain stable relationships, which creates a positive social environment and allows animals to better cope with stressors (Fraser et al., 2013). But, if the hierarchy of a stable social group is changed, this will bring higher levels of aggression and stress (Rose and Croft, 2015). There is no evidence that hens have particular bonds with other individuals in stable small groups (Abeyesinghe et al., 2013).

In slightly larger groups, hens are unable to form a stable social hierarchy, leading to social instability, higher levels of aggressive encounters and reduced egg production (Al-Rawi and Craig, 1975; Banks et al., 1979). Hughes and Wood-Gush (1977) found a positive correlation between group size and rate of aggression with more aggression per bird in larger groups. It has been argued that the number of group members that laying hens can recognise might be around one hundred (Nicol et al., 1999), and thus it might be expected that laying hens would prefer social groups of approximately this size.

In group sizes that are much larger than 120 birds, aggression is generally low (Estevez et al., 2002; D'Eath and Keeling, 2003). Nicol et al. (1999) reported lower aggression in groups of more than 200 compared with groups of 72. Aggressive interactions in chickens are known to be reduced at high vs low densities (Zimmerman et al., 2006; Al-Rawi and Craig, 1975), and normally in commercial multi-tier systems interactions take place in the frugal open areas that emerge from flock movements, rather than in highly populated areas (Rodriguez-Aurrekoetxea and Estevez, 2014). The social system in larger groups is based on determining dominance through direct assessment and 'status signalling' rather than the remembered individual assessment of a small group pecking order.

However, abundant literature on social dynamics of laying hens suggest that the relationships are more complex and that social relationships change as the size of the group increases (Estevez, 2020) as well as according to resource accessibility (Leone and Estévez, 2008b), and the availability of space and structures to escape from conflict situations (Estevez, 2009). Within a group, hens tend to synchronise behaviours like dustbathing, foraging, egg laying and perching. When most birds are engaged in the same activity at the same time this can lead to uneven distributions of birds across the housing system (Channing et al., 2001). Depending on the distribution of resources like feeders, perches and nest boxes this can lead to an increase or a decrease in competition and thus stress due to conflicts (Leone and Estévez, 2008b). In breeding flocks, there can also be competition for mates. Occasionally, birds that differ from their conspecifics in physical appearance or behaviour may attract excessive levels of aggressive behaviour, and have been called victim (Freire et al., 2003) or pariah (Estevez, 2020) birds'.

In general, although aggression is generally low in large groups, other factors contributing to group stress are more difficult to manage and control in a large flock. For example, feather pecking can spread rapidly within large flocks and panic reactions can lead to smothering. Piling can also occur for other (as yet unclear) reasons, also resulting in smothering.

### **Group stress: Laying hens in furnished cages**

In furnished cages, the occurrence of group stress due to aggression can be high due to some instability in social hierarchy and due to competition for resources. Dominant birds can exclude subordinate conspecifics from resources such as the dustbath (Shimmura et al., 2007; Shimmura et al., 2008b) and low-ranking birds may use the nest to escape from conspecifics and therefore not show full nesting (Shimmura et al., 2008a).

Severity is high and duration is long because furnished cages are a closed and small environment from where hens cannot escape from aggressive or negative social interactions, and it lasts the entire production period.

Injurious pecking occurs in furnished cages at similar rates to non-cage systems, but the range of potential preventive measure is much smaller in furnished cages (Section 3.5).

Smothering is less prevalent in cage systems than in non-cage systems (EFSA, 2005). This may be due to the limited number of animals who can pile on top of each other in a cage.

### Group stress: Laying hens in non-cage systems

In non-cage systems, aggression is generally low with birds using direct assessments (e.g. of relative size) to determine access to resources. Resource competition is also likely less than for cages because birds can share access to resources more easily.

Injurious pecking can be a significant cause of group stress in non-cage systems, but many potential management strategies can be applied to reduce this hazard (Section 3.5).

Smothering has been reported to occur, at least occasionally, in as many as 56% of free-range flocks (Barrett et al., 2014) and it can account for 15% of total flock mortality in these systems (Nicol, 2015). Smothering can occur in case of panic reactions, in young flocks in the nests not only at the onset of lay, but also throughout the laying period in the litter area or in the free-range (Rodenburg et al., 2020).

Little is known about the causal factors of piling but in a Swiss study, most piles occurred between 5 and 10 h after light onset (Winter et al., 2021) and in UK flocks housed in single-tier free-range and mobile systems at 4–8 h after light onset (Winter et al., 2022) when birds are most likely to be using the litter area for dustbathing and other activities.

Piling can also occur in multi-tier systems. Campbell et al. (2016c) observed smothering on the litter area of two commercial aviary flocks (> 49,000 birds per flock; aviary divided into sections containing either 852 or 1,704 birds). Duration of piling events ranged from 1 min to 6 h, with 10–230 hens participating. Average pile sizes showed approximately 4–5% of birds participated in piling. Piles were dynamic with birds leaving and joining, and some birds attempting to access the centre of the pile. Fewer than 7% of piling events were due to a disturbance. In this and other studies, most piling occurs due to recurrent, apparently calm, 'creeping' behaviour, whereby pullets and hens move towards and underneath others. Potential underlying causes for piling behaviour are numerous. Smothering due to panic reactions is more likely when group-housed birds are stressed.

### Group stress: Pullets in collective cages

There have been only few studies of group stress in pullets and there is no information on the frequency or severity of aggression, injurious pecking, fearfulness or smothering in collective cages.

However, the group sizes in some experimental studies of pullets housed in small pens are similar to the group sizes for pullets housed commercially in collective cages, so these experiments are of some potential relevance in this context. Pullets housed in group sizes varying across studies from 10 to 120, tended to show lower rates of aggression in larger groups (Estevez et al., 2002; Estevez et al., 2003; Liste et al., 2015; Campderrich et al., 2017). However, aggression was not evenly distributed, and some individuals received more aggression in larger groups than in smaller groups (Estevez et al., 2003).

The prevalence of smothering in collective cages is not known. Van Staaveren et al. (2019) reported that smothering accounted for 15% of all mortality in Canadian pullet flocks housed in cages, single-tier and multi-tier systems. The proportion in each system was not differentiated.

### Group stress: pullets in non-cage systems

Piling and smothering are important causes of group stress in non-cage systems for pullets. Smothering has been reported to account for 25% of mortality in organic pullet flocks (Sparks et al., 2008). Smothering due to panic reactions is perceived by farmers to be more likely when birds in a group are stressed for other reasons (Winter et al., 2022).

Aggression in pullets housed in multi-tier systems is not an area of concern, although research is limited. In two related studies of a commercial multi-tier system, aggression was rarely observed. In many areas of the aviary no aggression was seen, and even the highest rates seen in older birds (0.007 incidents/bird/3 min) and on litter areas (0.008 incidents/bird/3 min) were very infrequent. Enrichment significantly reduced aggression even further (Zepp et al., 2018). When the physical condition of the same flocks was examined, head injuries indicative of aggression occurred at a mean rate of only 0.02 per individual examined across all ages. Head injuries increased with age but were not affected by either stocking density (18 birds/m<sup>2</sup> vs 22–23 birds/m<sup>2</sup>) or enrichment (Liebers et al., 2019).

Fearfulness is reduced in pullets reared with dark brooders (Riber and Guzman, 2016).

## Group stress: layer breeders in collective cages

Group housing of laying hen breeders can cause group stress from the causes previously described for laying hens. However, in the case of breeding birds, group stress may also arise from male conflict over access to females and from forced matings. Limited data exist regarding the intensity of social conflict in layer breeders specifically.

Aggressive interactions in laying hen breeders are normally not too high unless there is a problem with the distribution of resources.

Group size in collective cages may be anywhere between 20 and 120 birds, and there is no evidence on the potential incidence of aggression due to social conflict. The reduced complexity of a collective cage may limit the chance of getting away from other birds in conflictive situations and this includes aggressive interactions due to mating competition, as well as the possibilities to avoid injurious pecking. Shi et al. (2019b) reported feather pecking problems in groups of 100 breeders in collective cages.

## Group stress: layer breeders in non-cage systems

No published data exists regarding the frequency of this ABM for layer breeders in single- or multi-tier systems. de Haas et al. (2014a) recorded feather damage in 10 commercial flocks of layer breeders at 40 weeks of age. They found considerable variation in feather damage between flocks. Furthermore, feather damage in the breeders predicted feather pecking in the offspring during the first weeks of life underlining the importance of managing injurious pecking (de Haas et al., 2014a). It is expected that large group rearing conditions in single and multi-tier housing will cause stress and will have a negative impact on affective states such as frustration resulting from an excessive number of social interactions, both of which may result in soft tissue and integument damage, fear and heightened levels of physiological stress.

### 3.3.2.2. ABMs

The ABMs 'fear response', 'injurious pecking', 'physiological stress indicators' and 'plumage damage' are useful to assess 'group stress' but may also be related to other welfare consequences (Table 7). For this reason, they are described in Section 3.3.12. The ABM 'injurious pecking' is described in Section 3.5. Additional ABMs to assess this welfare consequence are described in Table 5.

**Table 7:** ABMs for the assessment of 'group stress'

ABM	NOTES
<b>Aggressive interactions</b>	
Definition	Interaction between birds including pecks to the head area, threats, chases and fights (Estevez et al., 2002).
Measurement	Directly observable: number of aggressive interactions recorded during a period of time, with direct observation or analysis of video recordings. These behaviours occur sporadically and with brief duration therefore require time for observation (e.g. focal sampling, video recording) to be assessed correctly. Indirectly observable: in commercial settings, comb wounds are often used as a proxy to assess aggressive interactions (Welfare Quality, 2009).
Interpretation	Aggressive interactions are both a hazard for group stress and may also be a consequence of group stress. Group stress increases aggressive interactions and comb wounds compared to situations without group stress.
Sensitivity and specificity	Sensitivity is moderate: if group stress exists, aggressive interactions are likely to appear. If stocking density is too high then aggressive interactions may not be presented but group stress is still present Specificity is high. If group stress does not exist, aggressive interactions are kept at a low level.
<b>Piling behaviour</b>	
Definition	The dense clustering of birds, whereby some hens position themselves on top of others, increasing the risk of smothering (death by suffocation) (Bright and Johnson, 2011; Winter et al., 2021)

ABM	NOTES
Measurement	<p>Directly observable: the number of piles can be measured by observation at defined times of day. Piling occurs more frequently during the afternoon in any area of the house, including the nest-boxes. Piling is often observed in the corners of houses but also against walls or in central areas. The size of piles can be estimated by counting the number of piling birds that are visible or can be assessed more accurately by walking gently towards the pile and dispersing the birds. The duration of piling can be assessed by video observation.</p> <p>Piling occurs sporadically and is best detected by frequent and regular observations of the birds. If piling has resulted in smothering, then the number of birds that died by suffocation should be recorded as a specific type of mortality.</p>
Interpretation	The exact cause of piling is not known, but it is an abnormal and harmful behaviour within groups of layers. Its relation to group stress is not yet clear, although it is likely that (as for aggression), piling can be seen as both a hazard for, and a consequence of, group stress. There is currently insufficient research to be certain, but this ABM could be useful in the future, after more research is developed.
Sensitivity and specificity	The ABM 'piling behaviour' is moderately specific but not a sensitive ABM to assess this welfare consequence.

### 3.3.2.3. Hazards and preventive or mitigating measures

#### Laying hens: Hazards for group stress

Group stress results from a high incidence of aversive social interactions (aggressive behaviours, injurious pecking, piling, smothering) leading to negative affective states such as pain, fear or frustration that may exacerbate both individual and group level of stress. It can also result from predation stress or sensory overstimulation.

Fear response in large groups may differ between brown and white hybrids: brown hens tend to actively avoid perceived threats by moving away, whereas white hens use passive avoidance by freezing (Nelson et al., 2020).

Behaviours such as aggression and injurious pecking are hazards (and also ABMs, see above).

Hazards for aggression include factors that may increase competition or prevent escape. Thus, insufficient space allowance per bird, insufficient total area of the enclosure and barren environments (absence or insufficient availability, poor location or design of resources) may increase the risk of group stress due to increases in resource competition causing frustration. Insufficient space allowance for feeding or drinking will increase resource competition. Barren environments and a lack of elevated structures are hazards because of the reduced possibilities for birds to escape or hide.

Hazards for injurious pecking are described in Section 3.5. and include genetic predisposition, diet, insufficient access to a dry friable foraging material, and environmental conditions (e.g. temperature, humidity, ammonia, noises, light). If environmental factors promote injurious pecking this will cause group stress as birds attempt to escape from pecking conspecifics.

Hazards for piling and smothering are not well understood.

Smothering arises when birds crowd or pile on top of each other causing death by suffocation for hens in the middle or at the bottom of a pile (Bright and Johnson, 2011; Barrett et al., 2014; Gray et al., 2020; Herbert et al., 2021). Crowding and piling can occur relatively frequently and do not always lead to lethal smothering, but smothering is usually preceded by crowding or piling. It can also arise when birds panic and flee to the same area. Panic and smothering events are sporadic and therefore difficult to investigate, but the occurrence of crowding and piling can be monitored. It has been proposed that piling may cause heat stress, injury and breathing difficulties (Gray et al., 2020), all of which would increase overall group stress.

#### Pullets: Hazards for group stress

The hazards described for laying hens apply also to pullets. In addition, low accessibility of elevated structures or tiers (such as the absence of ramps or intermediate perches) will lead to difficulty in moving between tiers and may increase the risk of group stress in growing birds. When aggressive episodes occur birds may be less able to move away from the perpetrator.

Collective cages are a hazard for group stress as the lack of any enrichment or resources may lead to competition and conflict as birds search for absent resources (Nicol and Guilford, 1991).

In addition, the relatively barren environment of a floor system with no elevated structures may increase the risk of group stress as when aggressive episodes occur birds may be less able to move away from the perpetrator. Insufficient space allowance for feeding or drinking may increase the risk of group stress due to competition. Floor systems that provide only a few elevated structures are also hazards for group stress. If only a few elevated structures are provided such that birds cannot rest simultaneously, this may increase resource competition and aggression. Too few elevated structures will also reduce the opportunities for birds to escape.

### **Layer breeders: Hazards for group stress**

The hazards described for laying hens apply also to breeders.

Collective cages are a hazard for group stress as the lack of any enrichment or resources may lead to conflict as birds search for absent resources (Nicol and Guilford, 1991).

In breeding flocks is male–male interactions when competing for female access (Pizzari, 2016). Factors that may increase male–male competition, and thus group stress, may include high male to female ratio, asynchrony in sexual development of males and females.

It is not common to provide access to perches to layer breeders kept in single-tier systems, which may increase the risk of group stress as birds are unable to escape by jumping on a perch.

Insufficient space and unavailability of nests and other equipment are considered main hazards. Competition for space and resources, including access to females can enhance group stress in breeders in collective cages. Differences between bird hybrids may occur: white birds appear to be less affected compared to brown birds.

### **Prevention of hazard**

Choosing hybrids that are less prone to group stress.

Avoiding situations that cause fear (e.g. predators) or pain (e.g. mutilations).

Taking steps to reduce fearfulness will reduce panic events and group stress, e.g. fearfulness is reduced for adult birds that have been reared with dark brooders (Riber and Guzman, 2016).

Preventive strategies to control group stress due to injurious pecking (and the reverse: to control injurious pecking due to group stress) are included in Specific ToR 2 (see Section 3.5).

Preventive strategies to control aggression include sufficient provision of resources to minimise resource competition and of perches and panels to provide laying hens, pullets and layer breeders the opportunity to get away from aggressive conspecifics in the flock (Estévez and Newberry, 2017).

Using non-cage systems will reduce resource competition and increase opportunities for birds to escape or hide.

In non-cage systems, cover panels may limit the risk of smothering by acting as retention barriers of small groups of birds around them in the event of panic episodes (Estévez and Newberry, 2017). In this way, the sudden migration of a large number of birds towards the end house walls can be prevented but this has not been tested.

Separating large flocks into smaller colonies using appropriate fencing can facilitate better bird control and reduce the likelihood of aggressions being perpetrated in other compartment by the same aggressor. However, this is more difficult when birds have access to large free-range areas and may be difficult to apply in practice.

For layer breeders, preventive strategies are regular surveillance of female and male growth to prevent asynchrony in sexual development, as well as assuring the right male to female ratio to minimise the risk of competition for female access may help to reduce group stress due to sexual conflict.

Free-range access potentially reduces the welfare consequence as it gives the choice to birds to several places and more space availability, decreasing as well the density. Mobile housing, as a free-range access housing system, has the potential to reduce the welfare consequence 'group stress', although no data is available on this specific problem in this system.

### **Mitigation of welfare consequence:**

Once cages are installed, it is no longer possible to change the housing dimensions and furniture.

For existing collective cage systems for pullets, a reduction in severity of the hazard of the welfare consequence could be achieved by setting minimum, age adjusted spatial allowances per pullet and by furnishing cages. For pullets and layer breeders housed in unfurnished collective cages, some mitigation may be achieved by providing perches, pecking and scratching areas and, where

appropriate, nesting areas or environmental enrichment, but effective provision within a cage environment is unlikely to be successful.

For existing furnished cage systems for laying hens, the only way to mitigate the welfare consequence is to increase space allowance per bird, and resources availability, and deliver litter frequently in a large quantity in order to have permanent substrate, in sufficient amounts for all hens. However, the effective provision of friable litter in a sufficient amount in furnished cages cannot be effective, so mitigation measures are deemed to fail in furnished cages. Reducing environmental causes of stress (e.g. noise, handling, temperature) may help to mitigate group stress.

In all systems, assurance of good accessibility of feeders, drinkers and other essential resources will reduce or eliminate social conflict due to competition for resources. Ensuring good environmental conditions (temperature, humidity, ammonia, noise and light). A thorough examination of the flock to detect and remove birds that may be targeted due to deviations in phenotype (delayed laying, with wounds, sick) helps to prevent unnecessary suffering of targeted birds and may reduce the spread of behaviours such as aggression or injurious pecking.

In non-cage systems, ensuring that birds do not become trapped in corners by placing barriers may help to reduce mortality during a piling or smothering episode.

Another method of mitigation of the welfare consequence would be to reduce the light intensity, but this should only be used as a last resort and for a limited time period, as low light intensity also negatively affects welfare.

### 3.3.3. Inability to avoid unwanted sexual behaviour

#### 3.3.3.1. Description of the welfare consequence

Inability to avoid unwanted sexual behaviour has been identified as a highly relevant welfare consequence for layer breeders in all husbandry systems except individual cages.

Inability to avoid unwanted sexual behaviour is defined as the animal experiencing stress and/or negative affective states such as pain and/or fear resulting from inability to avoid forced mating (EFSA AHAW Panel, 2022a,b).

The severity is assumed to be high since it induces acute pain and fear to females.

The duration is assumed to be short except for integument damage which often has long-lasting consequences and may lead to mortality.

The frequency is assumed to vary from flock to flock but may reach high levels as all females are exposed to this welfare consequences during all their breeding life.

In systems where fertilised eggs are produced for laying hen breeding, males are kept with the females in a proportion of 1 male per 10 females. Information regarding unwanted sexual behaviour from laying hens is rare. This topic has mainly been studied in broiler breeders (McGary et al., 2003a; McGary et al., 2003b; Bilcik and Estevez, 2005), but extensive literature from experimental studies in red junglefowl is also relevant (e.g. Pizzari, 2016; McDonald et al., 2017; McDonald et al., 2019; Carleial et al., 2020). As explained by Pizzari et al. (2015) 'intra-sexual conflict arises in males whenever the reproductive success of an individual is limited by competition with other members of the same sex over access to reproductive opportunities. Similarly, conflict between individual males and females occurs whenever their fitness cannot be simultaneously maximised by the same outcome of reproductive interactions'. Domestic fowl males are highly competitive for matings as their reproductive success depends on their ability to access females and a high mating frequency reduces the risk of sperm competition (Pizzari, 2016; McDonald et al., 2017). The high male competition to access females has welfare consequences that are specific to breeding stock, including laying hens.

The most relevant of the welfare consequences for layer breeding flocks is the occurrence of unwanted sexual interactions, first described by Mench (1993; Millman and Duncan, 2000a,b; Duncan, 2009) for broiler breeder flocks. Similar to broiler breeding flocks, in layer breeding flocks, the inability to avoid unwanted sexual behaviour exposes females to male forced mating causing soft tissue lesions and integument damage, typically in the form of deep lacerations to the side around the tail. The integument damage can be severe and can lead to considerable mortality derived from wound infections (Estevez, 2009).

Excessive mating due to the inability to avoid sexual behaviour can also cause soft tissue lesions and integument damage (wounds and severe feather damage) around the head and the back area of the hens. This type of damage may be observed in females that are mated at higher frequencies, although other factors may also be relevant (e.g. genetic factors; Moyle et al., 2010).

It is expected that the causal factors triggering unwanted sexual interactions and its welfare consequence for females in layer breeders are similar to those described in red junglefowl and broiler breeder flocks. Studies on light breeding hybrids are scarce and focused on small groups and on other aspects of their reproductive behaviour (Craig and Bhagwat, 1974; Bhagwat and Craig, 1979; Cheng et al., 1985; Jones and Mench, 1991). However, from practical experience, it is known that the inability to avoid sexual behaviour also occurs in layer breeders. Although differences in body size among male and female broiler breeders may be a risk factor for unwanted sexual interactions, sexual conflict leading to unwanted sexual behaviour is also well documented in the red junglefowl, where differences in size between males and females are evident (Pizzari et al., 2015; Pizzari, 2016).

### 3.3.3.2. ABMs

ABMs to assess this welfare consequence are presence of females in the litter area (a low presence mean they are avoiding forced copulations) and forced copulations themselves, which are described in Table 8. In addition, the ABMs 'plumage damage' and 'wounds to skin' are useful but are also related to other welfare consequences and therefore described in Section 3.3.12 in Table 17.

A high frequency of forced copulations and/or copulation attempts is more frequently observed in the late afternoon when mating behaviour peaks (Bilcik and Estevez, 2005), a strategy that maximises the chances of egg fertilisation (Pizzari and Birkhead, 2001). When the problem with forced copulation or copulation attempts becomes severe, an increasing number of females will show signs of soft tissue and integument damage on the back and around the tail produced by the claws of the males. Females may avoid the litter area to escape from males patrolling the litter area in search of mating opportunities. A higher incidence of unsuccessful matings, mating attempts and chasing of females is reported in single-tier system, especially in the evening (Bilcik and Estevez, 2005).

**Table 8:** ABMs for the assessment of 'Inability to avoid unwanted sexual behaviour'

ABM	NOTES
<b>Avoidance of the litter area by the females</b>	
Definition	Separation of males and female layer breeders as result of females' retreat to protected areas of the barn, e.g. jumping up the slatted area while leaving the litter to the males.
Measurement	Directly or indirectly observable: counting the number of females in the litter area and in the slatted area. Counts can be made at a point of time (scan sampling) or by focal sampling. Although data are scarce, it is expected that avoidance of the litter area by females will be highest in the late afternoon when reproductive activity is highest.
Interpretation	An apparent avoidance of the litter area by females is indicative of females avoiding unwanted sexual behaviour.
Sensitivity and specificity	Sensitivity is high. In flocks with high frequency of unwanted sexual behaviour, a clear female avoidance of the litter area will be observed. Specificity is moderate. If inability to avoid sexual behaviour is not an issue, still some over-frequentation of slatted floor by females may appear at feeding or nesting times which might be miss-interpreted as avoidance of the litter area.
<b>Forced copulation</b>	
Definition	Unsolicited copulations or attempts to copulate that are performed forcefully by males to uncrouched females (modified from (McGary et al., 2003b)).
Measurement	Directly observable: frequency of ABM recorded in a population per unit of time and standardised to the number of birds observed through focal or group sampling. Indirectly observable with video: proportion of animals performing the behaviour either at a precise moment in time (scan sampling) or during an observation period (focal, ad libitum sampling). Observations should be done in the late afternoon when occurrence of reproductive behaviour is higher (Bilcik and Estevez, 2005). Video recording allows a more accurate measure as the behaviour occurs quickly and is easy to miss.

ABM	NOTES
Interpretation	A high frequency of forced copulations in the late afternoon indicates inability of females to avoid unwanted sexual behaviour
Sensitivity and specificity	Sensitivity is high. If hens undergo high frequency of inability to avoid unwanted sexual behaviour, a high incidence of ABM forced copulations will be observed. Measurement during morning or at night decreases sensitivity. Specificity is high. If inability to avoid sexual behaviour is not present, the absence of the ABM force copulations will suggest it is absent.

### 3.3.3.3. Hazards and preventive or mitigating measures

In the absence of sexual synchrony between male and female, females retreat to elevated structures away from male access, resulting in an increased frequency of unwanted sexual behaviour towards accessible females in the litter area (Estevez, 2009).

The risk of unwanted sexual behaviour increases if the male to female ratio is high. Duncan (2009) suggested a ratio of 1 male to 10 females.

Strategies that facilitate a homogeneous female distribution will contribute to the prevention of this hazard which might be easier to achieve in multi-tier systems due to the environmental complexity offered by this housing system. Claw shortening devices like in furnished cages for layers may help to reduce the size of males' claws and the damage to females.

Mitigation of unwanted sexual behaviour in single- or multi-tier systems may be achieved by e.g. installing panels in the litter area to increase complexity and attract females to the litter area (Leone and Estevez, 2008a) in order to restore the ratio of 1 male per 10 females.

Mitigation of unwanted sexual behaviour might be possible by temporarily restricting male access to females (for instance, only access to females in the afternoon) (Jong and Van Emous, 2017) or by reducing the number of males per female in the flock.

'Plumage damage' and 'soft tissue lesions and integument damage' caused by male claws can be prevented by providing abrasive strips.

### 3.3.4. Inability to perform comfort behaviour

#### 3.3.4.1. Description of the welfare consequence

Inability to perform comfort behaviour was identified as highly relevant welfare consequence for laying hens, pullets and layer breeders in cage systems.

Inability to perform comfort behaviour is defined as the animal experiencing stress and/or negative affective states such as discomfort and/or frustration resulting from the thwarting of the motivation to maintain the function and integrity of the integument (e.g. when it cannot keep clean, perform scratching and dust bathing) (EFSA AHAW Panel, 2022a,b) or from the total prevention to perform adequate behaviours in cages.

The severity ranges from low to high, depending on how many behaviours and how the performance of each behaviour is restricted. It is most severe when all the behaviour is totally unfeasible.

The duration is long, probably for the entire life span, at least for laying hens and laying breeders.

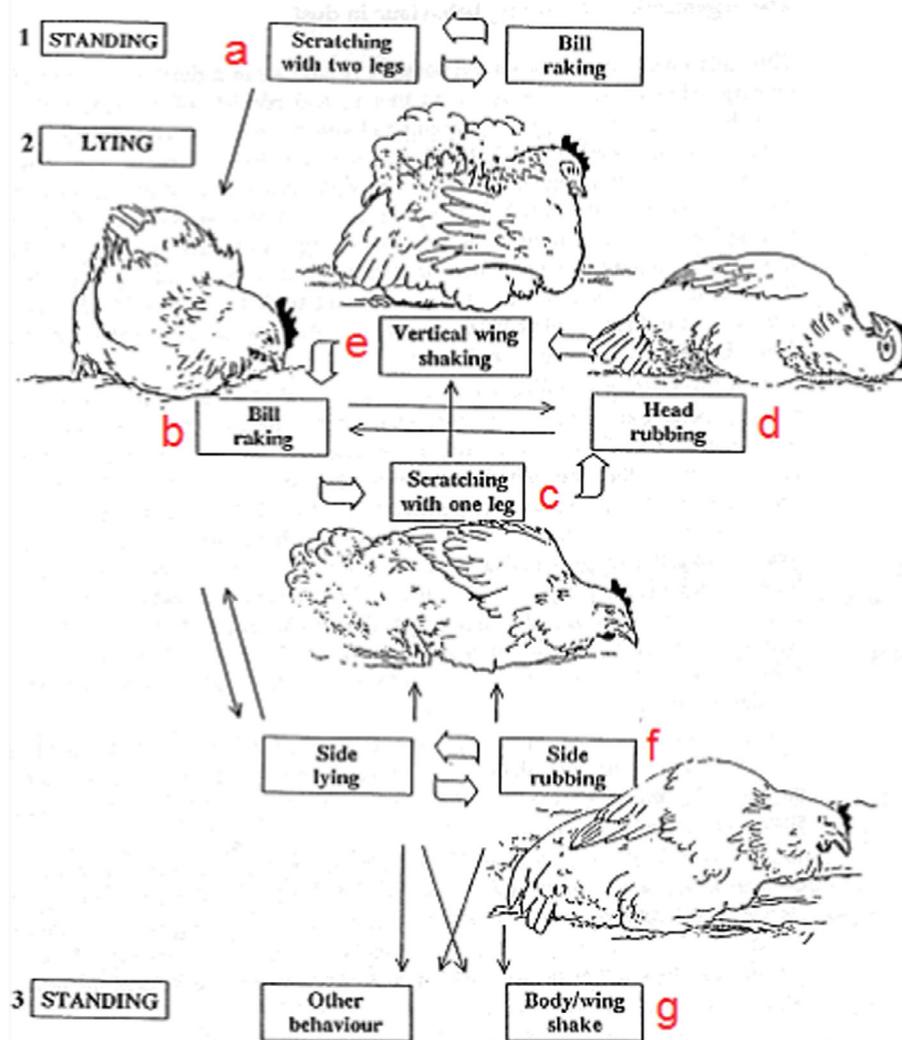
The prevalence is high; all animals in cages are concerned, but depending on space allowance and enrichment, a very limited range of comfort behaviours, such as preening, might still be possible.

Comfort behaviours include behaviours that maintain the function and integrity of the integument as well as behaviours as diverse as wing stretching/flapping, dust bathing, leg stretching, preening, tail wagging, sunbathing, feather raising/body shaking (Nicol et al., 2009), scratching self (Nicol et al., 2009) and head scratching (Black and Hughes, 1974).

Feather raising and body shaking consists in raising feathers with or without vigorous rotation of body around an axial plane, followed by subsidence of feathers back to a smooth position (Nicol et al., 2009). Scratching self consists in leg brought upwards and forwards under the wing to scratch the lowered head, while tail wagging is a rapid side to side movement of the tail. These behaviours occur from time to time and can be considered as comfort behaviours (Nicol et al., 2009), however they are rarely described in literature.

Dustbathing is recognised as a behavioural need for hens due to its strong internally motivated component (Weeks and Nicol, 2006). It is observed in the natural environment in many bird species

(Borchelt and Overmann, 1974; Møller and Erritzøe, 1992; Dawkins, 1989). In the laying hen, as in other species, dustbathing is motivated by maintaining the function and integrity of the plumage (Wichman and Keeling, 2008) by dispersing the excess of lipids on the feathers (van Liere et al., 1990) and by dislodging skin parasites from the feathers (Borchelt and Overmann, 1974). Carried out in a natural environment, dustbathing consists of a sequence of behavioural elements that the hen performs on the floor by means of a fine, dusty substrate (Petherick and Duncan, 1989), thereby collecting dust inside the feathers (Wichman and Keeling, 2008). Thus, dustbathing includes many of the elements of comfort behaviour listed above: wing flapping, head scratching, leg stretching, body shaking (see Figure 15).



**Figure 15:** Complete sequence of Dustbathing behaviour (van Liere, 1992)

When external conditions are optimal from a welfare point of view (in terms of space, time, material), the behavioural sequence of dustbathing is performed in its entirety and lasts between 20 and 30 min (Hoerning, 2004). In general, a hen performs dustbathing every second day (Vestergaard, 1982; Olsson and Keeling, 2005; van Liere, 1992). The sequence of movements, which involves body rotation and leg stretching, may also be considered exercise, as it makes leg muscles stronger, thereby preventing leg deformities and walking incapacity (Shields et al., 2004). The motivation for dustbathing will increase with higher amounts of lipids and parasites in the feathers and with the sight of a suitable substrate (van Liere et al., 1990; Lindberg and Nicol, 1997); Light and warmth favour dustbathing (Hogan and Van Boxel, 1993; Duncan et al., 1998). The social environment also has an impact on its frequency: the sight of fellow hens performing dustbathing influences the motivation of hens to perform dustbathing by imitation. This is called 'social facilitation' (Olsson et al., 2002; Hoppitt et al., 2007).

In addition to the need for an optimal substrate for dustbathing behaviour, free space is needed for dustbathing and other comfort behaviours, like wing or leg stretching. According to Riddle et al. (2018), the space used for dustbathing was between 1,000 and 1,190 cm<sup>2</sup> per hen. Dustbathing can be easily disturbed by external causes (like conspecifics' presence). The dustbathing performed in the absence of a friable substrate is called sham dustbathing. Sham dustbathing is defined as irrespective of the existence of friable substrates, no substrates penetrate the bird's plumage during this behaviour (Olsson and Keeling, 2005). It is not considered a form of satisfying dustbathing (Widowski and Duncan, 2000) and would not fully fulfil the functions of dustbathing (Merrill et al., 2006).

Wing flapping can occur during dustbathing to spread the substrate into the feathers of the wings, or as body shaking observed at the end of a dustbathing sequence. In anticipation of a positive event (sound for a mealworm reward), laying hens showed more wing flapping, which has been associated with a state of relaxation. This comfort behaviour may therefore be associated with a positive emotional state in domestic fowl (Zimmerman et al., 2011). However, in some stressful situations (such as harvesting), wing flapping is considered negatively when associated with escape behaviour (Wolff et al., 2019).

When a bird stretches itself, one wing is straightened outward from the body, while the bird extends its leg on the same side (Rentsch et al., 2019). Schwan-Lardner et al. (2012) reported for broilers that a low frequency of stretching could be a sign of compromised bird health.

Preening is a maintenance behaviour involving the use of the beak to position and align feathers, interlock feather barbules, distribute preen oil from the uropygial gland at the base of the tail to lubricate feathers, and remove ectoparasites. Preening is greatly increased in the presence of mites during both day and night-time periods (Jacobs et al., 2019; Murillo et al., 2020), and in the presence of body lice (Vezzoli et al., 2015). In this context, preening occurs as a direct response to an external threat, and when it is effective in reducing the infestation (particularly in birds with intact beaks; Vezzoli et al., 2015), the level of preening then declines to baseline. Preening in a rapid, shortened form can also occur in situations of frustration or indecision, known as displacement preening (Zimmerman et al., 2003). Thus, ectoparasites (Vezzoli et al., 2015; Jacobs et al., 2019) feather damage or frustration are the main hazards influencing preening expression. However, in most other situations preening is generally considered to be a comfort behaviour, performed when birds are in a relaxed state (Seehuus et al., 2013) and as part of routine feather maintenance. Preening is thus often seen in association with other positive events including anticipation of a positive event (Zimmerman et al., 2011), when perching on stable structures (Skånberg et al., 2021), in preferred environments and locations (Larsen et al., 2017; Rana et al., 2021), and when corticosterone concentrations (an indicator of stress) are low (Jones and Harvey, 1987). Notably, a longer duration of time spent preening is a good indicator of the positive welfare associated with being in a preferred environment (Nicol et al., 2009) while a simple measure of preening frequency is not (Nicol et al., 2011). Preening is positively associated with high feeding efficiency (Clark et al., 2019), better food conversion and reduced aggression (Sorosh et al., 2019) and it is often a synchronised and social activity that birds perform in close proximity (Keeling and Duncan, 1991; Keeling et al., 2017). On the contrary, preening is reduced by inflammation-induced pain in a familiar environment (Gregory et al., 2009), following the administration of a mild air puff stressor to individuals or their conspecifics (Edgar et al., 2015; Edgar and Nicol, 2018) and by spatial restriction (542 cm<sup>2</sup>/bird vs 1,648 cm<sup>2</sup>/bird (Engel et al., 2019); or less than 750 cm<sup>2</sup>/bird, reviewed by Hemsforth and Edwards (2021)) and by small cage size, high stocking density or conventional caging (Li et al., 2016). Due to differences in methods used to measure preening, it is hard to assess conflicting reports about overall levels of preening observed in different housing systems (e.g. more in free-range than in indoor barns (Sokołowicz et al., 2020); or more in furnished cages than in aviary or free-range (Shimmura et al., 2008a)).

Sunbathing is defined as occurring when a bird holds one or both wings out from the body with feathers spread. Duncan et al. (1998) suggested that sunbathing might be triggered by radiant heat and light, changing into dustbathing if environmental factors like dry soil are present. However, sunbathing behaviour and the motivation behind it, is not understood in domesticated poultry species.

Inability to perform comfort behaviour may impair plumage resulting in poor condition and feather breakage and impair normal function of muscles activity. Moreover, the absence of any substrate and, in cages, the rubbing of the feathers against the wire may result in feather abrasion. Feathers have an important role for the body temperature regulation, protection from environmental impact and seasonal changes, and safe flight.

Many studies revealed a clear case of stress in an animal when the environment does not allow the expression of a specific behaviour pattern (i.e. dustbathing) (Vestergaard et al., 1997). For instance, if

hens are moved from conventional cages to a large space after several weeks, rebound of wing flapping, tail wagging and stretching occurs (Nicol, 1987a).

Typically, poultry behaviours are investigated using direct observation, obtaining precise behavioural information at a small scale (Li et al., 2021). However, some comfort behaviours such as stretching and wing flapping, scratching or dust bathing are performed at a low frequency (Nicol, 1987a; Giraldo et al., 2014) meaning that significant effort is needed to monitor and collect sufficient data for statistical analyses.

### Laying hens in furnished cages

The welfare consequence 'Inability to perform comfort behaviour' is highly prevalent in furnished cages, since the pecking and scratching area is often too small with insufficient amount of friable litter. This does not allow dustbathing, which is a strongly motivated behaviour in hens; therefore, the severity of such welfare consequence is high in cages. The duration is long because it lasts the entire production period.

In furnished cages, the provision of litter to meet the dustbathing needs of hens is technically complex. Friable material should be distributed regularly on the floor. However, spilled friable material is quickly scattered (Fiks-van Niekerk et al., 2002; Guinebretière, 2017), especially on flat surfaces. To ensure that the hens can use it properly, the friable material must therefore be regularly distributed in sufficient quantities in each of the cages. Dustbathing will not be shown in its normal form in the absence of friable litter material. Thus, sham dust bathing may be performed (using feed from the trough as litter). However, the rubbing of the feather against the cage wire may result in abrasion of feather breakage leading to another welfare consequence 'soft tissue lesions and integument damage'.

Behaviours that require space, such as wing flapping, and wing or leg stretching might be impaired, or limited if the space available in the cage is too small (Mench and Blatchford, 2014), and the density too high (leading to another welfare consequence 'restriction of movement'). The impossibility to conduct comfort behaviours would lead to negative affective states, such as discomfort and frustration. Preening would be observed at a higher frequency, as it requires less space for its performance as compared to other, more space demanding behaviours, as suggested by Engel et al. (2019), in situations of reduced cage space, but with lower bouts durations.

In Guinebretière et al. (2015), distributing litter (feed or wheat bran) increased the number of dustbathing events observed in the pecking and scratching area (25% more), but the total number of dustbathing in cages and bout duration was not modified by the provision of litter. Thus, providing litter in the pecking and scratching area stimulated hens to dustbathe in it, but available space may not be sufficient for more than 5 hens to dustbathe simultaneously (especially as the authors observed an average of 14.6 hens together in the area limiting the available space even more). Consequently, the increase in the frequency of dustbathing due to the presence of litter in the pecking and scratching area could be limited. van Liere et al. (1990) showed that even if dustbathing bout duration is not improved by litter presence, quality might be different.

### Pullets in collective cages

The only opportunity for pullets to perform dustbathing in collective cages is by using spilled food particles on the wire floor. This will likely result in incomplete dustbathing bouts (sham dustbathing), although the extent to which pullets exhibit sham dustbathing in collective cages is not known. Studies on adult birds show that sham dustbathing is an indicator that the resources needed to permit the behavioural need to dustbathe are inadequate. There is no reason to expect this to differ for younger birds. The severity of the welfare consequence will thus be high.

Cage environments may provide very young (and hence small) chicks with sufficient space to perform comfort movements such as wing and leg stretching, wing flapping and preening but, as the birds grow, spatial restriction will inhibit the performance of these movements. Hofmann et al. (2021) found that the preening behaviour of pullets at 15 weeks of age was reduced from 17.5% of observation time when kept at 769 cm<sup>2</sup>/bird to just 9.75% of time when kept at 435 cm<sup>2</sup>/bird. Collective cages generally provide lower space allowances than 435 cm<sup>2</sup>/bird, so the welfare consequence of reduced preening is likely to be high. Behaviours such as wing flapping and wing/leg stretching require more space than preening. Overall, the occurrence of the welfare consequence of 'Inability to perform comfort behaviour' will be universal as it affects all pullets in collective cages and the severity will be high.

### Layer breeders in collective or individual cages

The welfare consequence inability to perform comfort behaviour in collective cages for laying hen breeders is similar to the one observed for laying hens. Pecking and scratching areas in collective cages for layer breeders are usually not available, thus dust bathing may not be properly performed. Due to the limited space availability and high stocking density in collective cages, behaviours that take space such as wing flapping, and wing or leg stretching might be impaired or drastically reduced. The extent to which this differs from laying hens housed in furnished cages is not known. Perhaps collective cages for laying hen breeders are more restrictive because of the lack of legislation regarding maximum stocking densities for breeding poultry.

Pecking and scratching areas in individual cages are usually absent, thus sham dust bathing may be performed (using feed from the trough as litter). However, due to the limited dimensions of single cages, the absence of any substrate and the rubbing of the feather against the cage wire may result in abrasion of the feathers. Behaviours that take space such as wing flapping, and wing or leg stretching are impaired, or limited if the cage is too small. Inability to perform comfort behaviours will show a high frequency, affecting all birds that are placed in individual cages, the restriction will be severe and will last for the entire housing period. The impossibility to perform comfort behaviours, needed to maintain the function and integrity of the integument, would lead to negative affective states, discomfort and frustration. There is no scientific published data on the frequency of the impairment of comfort behaviour in layer breeders, but we can assume from the knowledge of laying hens in cages that all birds are affected given the restricted housing environment.

#### 3.3.4.2. ABMs

ABMs to assess 'inability to perform comfort behaviour' are described in Table 9.

**Table 9:** ABMs for the assessment of 'Inability to perform comfort behaviour'

ABM	NOTES
<b>Dustbathing</b>	
Definition	A sequence of movements that starts by a hen lying down and tossing loose material onto and between the feathers. Other activities may occur in variable sequence during a dustbathing bout, including side lying, scratching, bill raking, head and body rubbing. A dustbathing bout usually ends with body shaking which removes dust from the plumage (van Liere, 1992).
Measurement	Directly observable: numbers of dustbathing events recorded in an observation period, with direct observation of a cage or part of the cage. Indirectly observable by video of a cage or part of the cage: number of dustbathing events during an observation period. If animals can be identified, duration and/or frequency of components of dustbathing (complete or uncomplete, sham or not). Although this behaviour can be performed through the day, it is more likely to be performed in the afternoon. Thus, attention should be given to conduct the observations to measure this behaviour when the behaviour is more likely to take place.
Interpretation	The inability to perform comfort behaviour decreases complete dustbathing behaviour and can increase incomplete or sham dustbathing behaviour.
Sensitivity and specificity	Performance of complete dustbathing is highly sensitive. Indeed, if hens cannot perform comfort behaviour, it will decrease the complete dustbathing expression, and increase incomplete dustbathing expression. Dustbathing is of high specificity. If ability to perform comfort behaviour is not limited, dustbathing will be present. because there is no other welfare consequence leading to dustbathing reduction.
<b>Preening</b>	
Definition	Lifting feathers and cleaning and realigning them with beak (Nicol, 1987a; Sokołowicz et al., 2020)
Measurement	Directly observable: numbers of preening events recorded in a observation period, with direct observation of a cage or part of the cage, and duration of preening bouts, focussing on one bird, by repeating this observation many times on several birds. Indirectly observable by video of a cage or part of the cage: number of preening events during an observation period, or number of birds preening, and duration of preening

ABM	NOTES
	bouts, focussing on one bird, by repeating this observation many times, on several birds. This behaviour is in any case happening sporadically, therefore requires time for observation (e.g. focal sampling, ad libitum) (Nicol, 1987a).
Interpretation	Longer bouts of preening and overall longer duration of preening are indicators of relaxed comfort behaviour. Short, intense bouts, particularly if seen at night, may indicate ectoparasites, feather damage or frustration.
Sensitivity and specificity	Sensitivity is moderate. If a hen's ability to perform comfort behaviour is reduced, preening behaviour is decreased but only in drastic cases Specificity is low because even in the absence of inability to perform comfort behaviour, preening can be negatively impacted.
<b>Wing and leg stretching</b>	
Definition	Unilateral backward and downward stretch of wing and leg together (Nicol, 1987a; Sokołowicz et al., 2020).
Measurement	Directly observable: numbers of wing and leg stretching events recorded during an observation period, with direct observation of a cage or part of the cage. Indirectly observable by video of a cage or part of the cage: number of wing and leg stretching events during an observation period. This behaviour is in any case happening sporadically and very briefly, therefore requires time for observation (e.g. focal sampling, ad libitum) (Vestergaard et al., 1997; Mollenhorst et al., 2005).
Interpretation	Lack or decrease of occurrence of wing and leg stretching indicates lack of sufficient space to perform the behaviour and therefore the existence of the welfare consequence. Sometimes an attempt to perform the behaviour can be observed without a full stretching position being achieved because of lack of space.
Sensitivity and specificity	Sensitivity is moderate. When inability to perform comfort behaviour is present, it is likely that wing and leg stretching occurrence will be reduced, but probably will be totally absent only in case of high space restriction and high magnitude of the welfare consequence. Specificity is moderate. When ability to perform comfort behaviour is not limited then wing and leg stretching is supposed to be present at normal level of approximately 0.2 to 1.0 stretches per hen per hour (Albentosa and Cooper, 2004; Mishra et al., 2005). However, wing and leg stretching can also be less frequent in case of prostration due to health disorders or injuries.
<b>Wing flapping</b>	
Definition	Bilateral rapid upward and downward movement of the wings performed while standing still (Nicol, 1987a; Sokołowicz et al., 2020).
Measurement	Directly observable: numbers of wing flapping events recorded during an observation period, with direct observation of a cage or part of the cage. Indirectly observable by video of a cage or part of the cage: number of wing flapping events during an observation period. This behaviour is in any case happening sporadically and very briefly, therefore requires time for observation (e.g. focal sampling, ad libitum).
Interpretation	Wing flapping is a comfort behaviour that normally takes place if birds are observed during a meaningful time period. Thus, lack or decrease of occurrence of wing flapping is a clear indication of insufficient space provided that precludes the performance of the behaviour, and therefore the presence of the welfare consequence.
Sensitivity and specificity	Sensitivity is moderate. When inability to perform comfort behaviour is present, it is likely that wing flapping occurrence will be reduced, but probably being totally absent only in case of high space restriction and high magnitude of the welfare consequence. Specificity is high. When the bird is able to perform comfort behaviour, then the wing flapping will be shown at normal level. There is no other welfare consequence leading to reduced wing flapping.

### 3.3.4.3. Hazards and preventive or mitigating measures

#### Laying hens in furnished cages

The main hazard for the inability to perform complete dustbathing behaviour in furnished cages is the absence of a solid floor, the inadequate pecking and scratching area in terms of insufficient suitable space, inappropriate litter material (e.g. unfriable characteristic or feed proposed as substrate), insufficient litter (small and rare quantities of litter delivered) and inappropriate light (Guinebretière et al., 2015).

The absence of a litter substrate will defer birds of performing complete dustbathing behaviour. It is sensitive to the lack of substrate and even to the type of substrate (Monckton et al., 2020) which can affect its frequency of occurrence.

For the inability to perform wing flapping and leg stretching, the main hazard in cages is insufficient space (Albentosa et al., 2007), and the hazards included in the welfare consequence 'restriction of movement'.

The prevention of hazards is more effective before animals are in cages, and even before cages are installed in the house, using large cages with large pecking and scratching areas. Litter substrate must be appropriate in quality and amount and delivered frequently in all cages (Guinebretière et al., 2015). Pecking and scratching areas must be placed in front of lights in each cage, as dustbathing is further increased if the substrate is combined with light and heat (Olsson and Keeling, 2005).

Cage systems do not allow the provision of friable litter in a sufficient amount in a large space, as well as complex environment that encourages exploration. The most effective measure to address the 'inability to perform comfort behaviour' would be to use non-cage housing for laying hens.

A way to mitigate the welfare consequence is to reduce bird density to increase space allowance per bird and deliver litter frequently in sufficient quantities to provide permanent and adequate substrate access. Increasing the number of pecking and scratching areas can be implemented by adding one or several areas in each cage before hens' placement but would then also requires the technical equipment to provide 'litter' on this additional area. Light must be maintained over 5 lx encouraging the expression of comfort behaviours.

#### Pullets in collective cages

Hazards for dustbathing behaviour have not been studied in pullets but are likely to be similar to those described for adult hens. However, adult hens are provided with at least some substrate in furnished cages, whereas none at all is provided for pullets. The hazard for the inability to perform wing flapping, wing and leg stretching and preening components of the welfare consequence is the spatial restriction imposed by high stocking densities in collective cages for pullets (Hofmann et al., 2021).

For existing collective cage systems for pullets, mitigation of the welfare consequence could be achieved by setting minimum spatial allowances per pullet to a level that enable higher levels (if not fully unconstrained) of wing flapping, wing/leg stretching and preening throughout the rearing period. The 2022 EURCAW-Poultry-SFA document suggests that pullets should be housed with at least 600 cm<sup>2</sup> of usable area per bird (or no more than 16 pullets/m<sup>2</sup>) at the end of the rearing period (EURCAW-Poultry-SFA, 2022). This would enable the higher levels of comfort behaviour mentioned above. Addressing the dust-bathing component would at least require the provision of some form of furnished cage for pullets which incorporated a suitable substrate accessible to all birds.

The most effective measure to address the 'inability to perform comfort behaviour' would be to use non-cage housing for pullets.

#### Layer breeders in individual and collective cages

Hazards for individual caged laying hens pure line breeders include insufficient space availability to allow the performance of comfort behaviours that require a large space (preening, wing flapping, wing and leg stretching), and lack of any suitable friable material to perform dustbathing.

Even if it is challenging to get away completely from placing primary breeders in individual cages for individual performance data, the maintenance periods in individual cages should be limited to specific times for data collection that should provide sufficient information for genetic selection. Meanwhile, if individual cages must be used, cages should be large enough to permit breeders to comfortably perform comfort behaviours and make the necessary adjustments in cage design to include a designated dust bath area, a perch and nest box (for females).

Collective cages for larger groups provide more effective space for comfort behaviours as compared to smaller cages, even if density remains constant, due to the increased 'shared space' (Liste et al., 2015). A designated dustbathing area, provided with friable substrate, can be made available even on a temporary basis to satisfy basic dustbathing needs. Cage systems with such an area, that can be automatically opened and closed are already in use in some countries.

The most effective measure to address the 'inability to perform comfort behaviour' would be to use non-cage housing for layer breeders.

### 3.3.5. Inability to perform exploratory or foraging behaviour

#### 3.3.5.1. Description of the welfare consequence

Inability to perform exploratory or foraging behaviour has been identified as highly relevant welfare consequence for laying hens, pullets and layer breeders in cage systems.

Inability to perform exploratory or foraging behaviour is defined as the animal experiencing stress and/or negative affective states such as frustration and/or boredom resulting from the thwarting of the motivation to investigate the environment or to seek for food (i.e. extrinsically and intrinsically motivated exploration) (EFSA AHAW Panel, 2022a,b) or from the total prevention to perform adequate behaviours in cages.

The severity is assumed to range from low to high depending on the extent of restriction on the expression of behaviours. It is high when foraging behaviour is not expressed at all. Depending on space allowance and enrichment, the behaviour might not be completely restricted, some walking and object pecking may still be possible.

The duration is assumed to be long as the animal is affected for its entire life.

The prevalence is assumed to be high as applies to all animals kept in cages.

Domestic fowl have retained most of their ancestor's behavioural repertoire such as foraging behaviours (Collias and Collias, 1967; Appleby et al., 1989). Foraging behaviour comprises an appetitive phase of searching for feeding sources, handling time required to catch, extract and process food into a form that can be swallowed, and the consummatory phase with the ingestion of the prepared food (Estévez and Newberry, 2017). Foraging takes a great proportion of the time budget in wild birds (in semi-natural environments, laying hens may spend more than 60% of their day pecking and 34% scratching (although behaviours were not mutually exclusive)) (Dawkins, 1989). In a natural environment, exploratory behaviour is vital for the survival of the birds. However, in commercial poultry the accessibility of abundant, nutritious, well-balanced feed, has reduced the foraging sequence to the consummatory phase. Despite this, domestic fowl in commercial facilities can be seen foraging in the litter even if no edible items are available and in the nearby presence of feeders and foraging can be considered a behavioural need.

Exploration is another activity closely linked to the search for food and other resources, such as mating possibilities or areas free of predation. In practice, foraging and exploratory behaviours cannot be distinguished. Animals explore their environment to, e.g. find food or water and avoid predators and environmental hazards, which makes this behaviour essential for survival under natural conditions (Wood-Gush and Vestergaard, 1989). Domestic fowl will investigate for a restricted period each day (Newberry, 1999) and engage playfully with small objects found in the litter (Cloutier et al., 2000). By exploring the surroundings animals, including laying hens, learn about the characteristics of their environment, and the behaviour will be performed even if the environment remains constant. Even if there are genetic differences in the intensity in which these behaviours are performed (Meuser et al., 2021), both foraging and exploring are behavioural categories that have major relevance for the welfare of poultry (Wood-Gush and Vestergaard, 1989).

In cages, no or very limited substrate is available. However, the provision of high-quality pecking substrates is suggested to protect against feather pecking and to reduce fearfulness, acting as an enrichment (review in Monckton et al., 2020). These behaviours can be redirected and expressed as feather pecking or cannibalism towards other birds in the absence of foraging material (Blokhuys, 1986; Dixon, 2008). This can lead to the welfare consequence 'soft tissue lesions and integument damage'. Chickens forage in and consume excreta in the absence of substrates (Pokharel et al., 2018; von Waldburg-Zeil et al., 2019).

The absence of foraging substrates could lead to injurious pecking, low feather coverage or skin wounds; however, these are not specific to this welfare consequence (Blokhuys, 1986, Blokhuys and van der Haar, 1989). The severe limitation in opportunities to forage and explore has a relevant negative

impact on the birds' welfare because it results in negative affective states such as distress, boredom and frustration.

### Laying hens in furnished cages

The restrictive environment does not provide opportunities to the birds to be active and explore. Foraging is also impacted by the difficulties of adding adequate amounts of litter in cages, and by the restricted space available (see welfare consequence 'inability to perform comfort behaviour in furnished cage' and 'restriction of movement in furnished cage'). The pecking and scratching area in furnished cages is often, if present, too small. Litter is supplied in insufficient amounts and not friable. The rest of the cage environment is mainly constituted of wire which discourages foraging and exploration, so that the severity of such welfare consequence is high. Wire is known to be less preferred than pads for pecking and scratching behaviours (Merrill et al., 2006; Guinebrière et al., 2015).

The spatial restriction of furnished cages is a secondary factor reducing the possibility of exploratory or foraging behaviour.

### Pullets in collective cages

Whereas adult birds are provided with a small pecking and scratching area, pullet cages do not provide this. The only opportunity for pullets in collective cages to perform foraging is by using spilt food particles on the wire floor, which will rapidly fall through the mesh providing little sustained foraging opportunity.

The spatial restriction of collective cages is a secondary factor reducing the possibility of exploratory or foraging behaviour. Even when a floor substrate is provided, foraging at 15 weeks of age is reduced from 12.2% of observation time for pullets kept at 769 cm<sup>2</sup>/bird to 6.55% of time when kept at 435 cm<sup>2</sup>/bird (Hofmann et al., 2021). Collective cages generally provide lower space allowances than 435 cm<sup>2</sup>/bird.

### Layer breeders in collective or individual cages

In collective cages, the effects on layer breeders will be to a great extent, similar to what is observed for laying hens in furnished cages, but there are also some differences that may affect the ABMs associated with this welfare consequence. For example, some cages for breeders include a pecking and scratching area, but not all do. Such an area is important for birds to show exploratory or foraging behaviour. In some systems, the area is available only during the second part of the day, to avoid mislaid eggs in the pecking and scratching area. These areas are normally offered only in the larger sized cages (> 100 birds). This makes that the area can also be large enough to be able to contain the litter that is offered there. This is important for the area to remain of interest to the birds.

Limited opportunities for exploratory and foraging behaviour will increase the risk of feather pecking, as birds are likely to redirect their exploratory behaviour to the feathers of other birds. All individuals in the cage will be equally affected by the limited possibilities to perform exploratory and foraging behaviours, thus, the welfare consequence has a high frequency and will last throughout the production period. Severity of the restriction will depend on the specific cage design.

The reduced exploring and foraging opportunities (if they exist at all) in individual cages are expected to severely restrict the sequences of walking while scratching the floor and pecking to potential feed particles. However, the severe restriction of exploratory or foraging options may result in increased pecking and development of stereotypic pecking at objects (feeders, drinkers or cage walls), or spot pecking as a form of replacement to fill their time. The increased pecking activity towards objects observed at dusk in caged hens is interpreted as a replacement behaviour for normal foraging behaviour (Moroki and Tanaka, 2016).

#### 3.3.5.2. ABMs

ABMs to assess this welfare consequence are described in Table 10. In addition, the ABMs 'injurious pecking' and 'plumage damage' are useful but are also related to other welfare consequence. For this reason, they are described in Section 3.4.1.12 in Table 17.

**Table 10:** ABMs for the assessment of 'Inability to perform exploratory or foraging behaviour'

ABM	NOTES
<b>Walking, scratching and pecking</b>	as part of foraging or exploratory behaviour
Definition	The birds walk, scratch the ground with one or two legs to expose particles and peck at them. This action is often repeated several times in a row ((Moe et al., 2014) adapted from foraging). The pecking can also be expressed alone, directed towards the available materials.
Measurement	Directly observable or observable by video of a cage or part of the cage: time spent walking, scratching and/or pecking, or numbers of animals walking, scratching and/or pecking available materials, and diversity of materials pecked, recorded in an observation period by direct observation of a cage or part of the cage.
Interpretation	The lower ability to perform exploratory or foraging behaviour, and the less various materials are available, the less animals walk, scratch and peck the physical environment. These behaviours cannot be entirely suppressed, though.
Sensitivity and specificity	Sensitivity is high. If hens cannot perform exploratory or foraging behaviour, walking, scratching and pecking will be decreased, and fewer materials will be explored. Specificity is moderate. If ability to perform exploratory or foraging behaviour is not limited, the walking/scratching/pecking expression can still be impacted. For instance, walking can also be impacted by other factors e.g. animal stocking density, foot disease or bone strength.

### 3.3.5.3. Hazards and preventive or mitigating measures

The main hazard for the inability to perform exploratory and foraging behaviour is the inadequate pecking and scratching area in cages in terms of insufficient suitable space, insufficient litter (small and rare quantities of litter delivered), inappropriate litter (e.g. unfriable characteristic or feed proposed as a substrate) (Guinebretière et al., 2015) and inappropriate light. This is especially relevant for collective cages that do not include a pecking and scratching area.

The lack of environmental complexity and spatial restriction imposed by high stocking densities is also limiting exploratory behaviour.

The preventive methods mentioned in welfare consequence 'inability to perform comfort behaviour in furnished cages', providing enrichment by placing different kind of objects in the cages that birds can interact with may limit the effect of this welfare consequence (Lundén et al., 2022). According to the National Research Council (2011), environmental enrichment should 'enhance animal welfare by providing them sensory and motor stimulation, through structures and resources that facilitate the expression of species-specific behaviour and promote psychological well-being through physical exercise, manipulative activities, and cognitive challenges according to species-specific characteristics'. Enrichment can be provided in various forms such as objects or materials which are suitable for foraging or dustbathing (Campbell et al., 2019). For instance, Guinebretiere et al. (2020) showed that an artificial turf mat placed on a furnished cage floor and different objects hooked onto the cage façade (10 in that study for a cage housing 60 hens: small mobile and coloured objects, plastic transparent pots filled with coloured rings and plastic chain links) limited feather cover damage.

It is not possible to fully prevent the inability to perform exploratory and foraging behaviour without the use of non-cage systems. Cage systems do not allow the provision of friable litter in a sufficient amount in a large space, as well as complex environment that encourages exploration.

Once hens have been placed in the system, and thus exposed to the hazards as described above, it is no longer possible to change the housing dimensions and furniture. A way to mitigate the welfare consequence is to reduce density that will increase space allowance per bird and deliver litter frequently in a large quantity in order to have permanent substrate, in a sufficient amount for all hens. Increase the number of pecking and scratching areas and surfaces can be envisaged by adding areas in each cage. Adding various objects into the cage or on the facade can enrich environment. They should be exchanged frequently. Light must be maintained at a level encouraging exploratory and foraging behaviours.

For existing collective cage systems for pullets, mitigation of the welfare consequence could be achieved by reducing stocking density per pullet and providing a pecking/scratching area or other

substrate within a colony cage. The provision of additional pecking enrichments such as hanging strings may also mitigate this welfare consequence.

It is difficult to increase the frequency of exploratory and foraging behaviour in individual cages, because of the limited cage dimensions. Pecking objects introduced in cages have been shown to result in increased pecking activity at dusk. This could perhaps partly replace foraging (Moroki and Tanaka, 2016) although the effectivity of pecking objects is short lasting. In addition, availability of foraging edible materials other than standard feed, and strategies of environmental enrichment with biological meaning (Estévez and Newberry, 2017) to the single cage housed breeding stock may help to mitigate partially the impact of this welfare consequence.

The most effective measure to prevent the hazard for the welfare consequence 'inability to perform exploratory or foraging behaviour' is to use non-cage housing for laying hens, pullets and layer breeders.

### 3.3.6. Isolation stress

#### 3.3.6.1. Description of the welfare consequence

Isolation stress is considered highly relevant for laying hen breeders in individual cages.

Isolation stress is defined as the animal experiencing stress and/or negative affective states such as frustration and/or fear resulting from the absence of or from limited social contact with conspecifics. (EFSA AHAW Panel, 2022a,b).

The severity is assumed to be high since hens are social animals, thus normally living in groups. Isolation stress is even higher without visual or auditory contact.

The duration is long as the animal is housed alone for most of its life.

The prevalence is assumed to be 100% in animals that are housed alone.

In response to isolation, hens often show repetitive behaviours, e.g. pacing. It is known that poultry will respond negatively to social isolation, especially if birds cannot see or hear other birds (Feltenstein et al., 2002). This is especially true for chicks, for whom it would be very dangerous to be isolated from their mother and their peers under natural conditions (Collias and Collias, 1996). Social isolation is also used as a stress model in behavioural research (Zachar et al., 2019; Peixoto et al., 2020). When hens are kept in individual cages, but can see and hear other birds, signs of isolation stress (pacing, vocalising) tend to be absent. However, this type of housing precludes any form of positive social behaviour, such as allopreening (Zimmerman et al., 2006), and other forms of behavioural synchronisation and facilitation (synchronised resting and dustbathing) (Lundberg and Keeling, 2003; Olsson et al., 2002). Of course, individual housing also precludes negative social interactions, such as aggression, feather pecking and cannibalism. In this case, it is expected that breeders in individual cages experience negative affective states, mainly fear and frustration, by not being able to interact socially with other hens or show synchronised behaviour due to social isolation.

#### 3.3.6.2. ABMs

ABMs to assess isolation stress for birds that are kept in individual cages allowing them to see and hear conspecifics are described in Table 11. In addition, the ABMs 'fear response' and 'physiological stress indicators' are useful ABMs that are also related to other welfare consequences. For this reason, they are described in Section 3.4.1.12 in Table 17.

**Table 11:** ABMs for the assessment of 'isolation stress'

ABM	NOTES
<b>Pacing</b>	
Definition	The birds repeatedly walk rapidly from one side to the other in the cage and back again. This may become increasingly invariant over time.
Measurement	Directly observable: time spent pacing. The amount of pacing may vary throughout the day and can also be influenced by egg laying (Tahamtani et al., 2018) and feeding motivation (Zulkifli et al., 2006) so observations should be spread over the day and the main egg laying period (morning) should be avoided. Indirectly observable with video of a cage. Activity level of individual hens (could also be measured with accelerometers).

ABM	NOTES
Interpretation	The more affected by isolation stress, the more pacing behaviour will be displayed. In a situation where hens can still see and hear hens in adjacent cages, levels of pacing are expected to be low.
Sensitivity and specificity	Sensitivity is high. If hens are severely affected by isolation stress, they will show pacing behaviour. Specificity is moderate. As indicated above, pacing can also be observed in response to an increased egg laying (Tahamtani et al., 2018) or feeding motivation (Zulkifli et al., 2006).

### 3.3.6.3. Hazards and preventive or mitigating measures

Main hazards include insufficient physical space to permit the development of normal behaviour patterns and lack of other group members to interact with. These restrictions can lead to feelings of frustration which can result in chronic stress, which in turn can increase fearfulness and decrease responsivity of the immune system (Jones, 1989). Where single cages are still used, an effort should be made to allow as much social contact between birds in different individual cages as possible. This can be done by using wire mesh partitions instead of closed partitions between neighbouring cages and by allowing face-to-face contact at the feeders. A further method to mitigate the welfare consequences would be to improve human-animal contact, for instance by also creating a positive association with human presence (providing some attractive feed). This would help to prevent excessive fearfulness and stress in individually housed birds.

Avoid the use of single cages for pure line laying hen breeders.

### 3.3.7. Predation stress

#### 3.3.7.1. Description of the welfare consequence

Predation stress has been identified as a highly relevant welfare consequence for laying hens and pullets in non-cage systems with outdoor access, including mobile housing.

Predation stress is defined as the animal experiencing stress and/or negative affective states such as fear and/or pain resulting from being attacked or perceiving a high predation risk (EFSA AHAW Panel, 2022a,b).

This welfare consequence is highly relevant for laying hens and pullets in systems with outdoor access.

The severity is assumed to be high for those animals that are attacked, as they are very likely to be subjected to acute pain, stress and fear. Animals that are not attacked themselves but that perceive an attack or a predator's presence will be affected less severely.

The duration of the attack may be short but the resulting consequences (e.g. wounding, increased fearfulness) may last long or even permanently.

The frequency of predation can be high depending on housing system, anti-predator measures, local wildlife presence and the availability of other prey.

Predation stress includes the fear and pain that birds experience as a result of an actual predator attack, as well as fear due to the risk of predation as perceived by the individual. Predation is one of the main reasons for mortality in flocks with outdoor access (Campbell et al., 2021). Average mortality due to predation varied between different studies, from 4% to 10% of all hens in flocks with outdoor access (with further variation between flocks within each study (Bestman and Bikker-Ouwejan, 2020)). Birds of prey (buzzards and goshawks) and foxes were identified as the most common predators attacking free-range flocks in the Netherlands (Bestman and Bikker-Ouwejan, 2020), although this is likely to differ between EU member states. Actual predation can lead to severe welfare impacts (severe wounding and/or fear during and subsequent to predation). Furthermore, perceived and actual predation risk can lead to pronounced anti-predator behaviour during which nearly all birds that are outdoors move indoors at high speed. However, it has also been observed that in larger flocks, not all hens respond to predator pressure. It has been hypothesised that hens may need to witness an attack on their flock mates before becoming fearful of predators, which may be less common in larger flocks where individual birds are less likely to be close to the attack (Bestman, 2022). Anti-predator behaviour not only suggests fear but is a risk for injury as birds may run into structural elements of the house or smother each other (Sossidou et al., 2015). Similar avoidance reactions and injurious

consequences may occur when a predator enters the house, which is generally easier in systems providing hens with outdoor access. It is often advised (and sometimes required) to provide natural or artificial cover on the range, which increases the number of birds venturing out (Bestman and Wagenaar, 2003; Hegelund et al., 2005; Buijs et al., 2020) presumably because it reduces their fear of being predated. Whether cover does protect hens from predation has been questioned though (Bestman and Bikker-Ouwejan, 2020).

There is marked individual variation in hens' response to predation risk (Nicol et al., 2011) and their response can be inappropriate because it is insufficient or because it is excessive. Hens can sometimes be observed to forage in direct proximity to birds of prey or even eat from the same carcasses that birds of prey are feeding from (Bestman and Bikker-Ouwejan, 2020). Such a blunted response to predator presence may be due to selection for lower fearfulness in modern genetic lines (Agnvall and Jensen, 2016) or not having experienced an attack before (Bestman, 2022). In contrast, other hens may respond strongly, moving away from the predator as fast as they can, which often means that they take cover in the house or under structures on the range.

All mobile housing systems provide access to an outdoor area, and as such there is a risk of predation. There has been no research that specifically focused on predation risk in mobile housing systems, but their occurrence and consequences for hen welfare are likely similar to those described for systems offering outdoor access in general.

### 3.3.7.2. ABMs

ABMs to assess 'predation stress' are described in Table 12. In addition, the ABMs 'fear responses' and 'physiological stress responses' are useful to assess this welfare consequence but as they are also used for other welfare consequences, they are described in Section 3.4.1.12 in Table 17.

**Table 12:** ABMs for the assessment of 'predation stress'

ABM	NOTES
<b>Dead birds, surviving birds with signs of predation or remains of birds</b>	
Definition	Mortality due to predation, animal remains characteristic of predation (carcasses with isolated puncture holes, torn/cut feathers, broken bones with paired-bite marks, or carcasses surrounded by predator hair, feathers or droppings (Stahl et al., 2002) or wounded animals).
Measurement	The outdoor range is inspected for (partial) carcasses of hens. Decapitated carcasses and gnawed feathers are signs of predation by foxes, whereas signs of predation by a bird of prey are partially eaten carcasses with feathers pulled out or only feathers as remains. However, even in the absence of these signs it is assumed that hens found dead on the range are the result of predation (Bestman and Bikker-Ouwejan, 2020).
Interpretation	Greater numbers are associated with increased stress and greater impairment of welfare.
Sensitivity and specificity	Sensitivity is moderate. When animals are predated, and therefore predation stress occurs, dead or mutilated animals can be found, but some might have disappeared. Indeed, predators may remove carcasses from the range and scavengers and other hens in the flock may eat carcasses (Bestman and Bikker-Ouwejan, 2020). Also, it can be difficult to detect all carcasses on ranges with abundant plant growth. Furthermore, birds may be stressed by the presence of predators even in situations where no successful attacks leading to mortality occur. Specificity is moderate. Even in the absence of predation, hens may die while out on the range, for other reasons than predation, but data on the extent to which this happens are lacking.
<b>Excess missing birds above the recorded mortality</b>	
Definition	Any hens whose death goes unaccounted for in the records.
Measurement	Calculated as number of hens supplied at the start of lay minus number of hens removed at depopulation minus recorded mortality not associated with predation

ABM	NOTES
Interpretation	Hens that are unaccounted for are assumed to have been predated, and thus greater numbers are associated with a greater impairment of welfare through predation stress
Sensitivity and specificity	Sensitivity is moderate. Although it is likely that when predation occurs the ABM will reflect this, it cannot be ruled out that hens experience stress as a result of a perceived predatory threat even in the absence of successful predation (and therefore in the absence of missing hens). Thus, the method is considered as moderately sensitive. Specificity is moderate. When there is no predation, birds may still go missing due to other reasons (e.g. escaped from the farm, removed by scavengers after dying from problems unrelated to predation). This leads to an overestimation of predation (the extent of which is unknown), thus reducing the specificity.
<b>Reluctance to use free-range</b>	
Definition	Hens may reduce their use of the outdoor range if they perceive use to be unsafe.
Measurement	Traditionally, range use is determined by estimating the percentage of the flock that is outside, usually by observing the hens from outside the range. Depending on the size and cover percentage of the range, and the intensity and time-pattern of such observations, this may lead to inaccurate or biased estimates. More recently, several kinds of wearable sensors have been used to assess range use continuously and in greater detail (Gebhardt-Henrich et al., 2014; Hartcher et al., 2016; Buijs et al., 2020).
Interpretation	Decreased numbers of hens on the range indicate greater reluctance to use the range, which could be caused by increased fearfulness as a result of a perceived or real threat of predation.
Sensitivity and specificity	The sensitivity of this method is unknown. Although some hens will reliably run into the house when perceiving the presence of a predator or signs of a potential predator, others continue to range in their presence (Bestman and Bikker-Ouwejan, 2020). This may reflect individual differences in the level of stress caused by predators or differences in individual tolerance levels to such stress. However, it could also result from a high motivation to stay outside. Observations of hens staying outside in the presence of predators could also be an indication good range cover (with hens hiding under/behind the cover instead of going into the house, which does not necessarily mean that the predator's presence did not cause fear/stress). The specificity is considered low as even in the absence of predation, hens may choose not to enter the range for reasons other than a (perceived) threat of predation (Pettersson et al., 2016).

### 3.3.7.3. Hazards and preventive or mitigating measures

The main hazards for predation stress are predator access to the free range and/or house, inappropriate responses to predator presence and insufficient cover on the range. Excluding predators from the range and house is easier for terrestrial predators (e.g. foxes) than for aerial predators (birds of prey). Excessive flock movement in response to (perceived) predator presence can lead to smothering (Sossidou et al., 2015).

As many terrestrial predators are crepuscular or nocturnal, it is important to make sure that all birds are inside the house between dusk and dawn, and that there are no gaps in the walls or doors, or pop-holes left open.

Terrestrial predators can be deterred from entering with electrical fencing, whereas providing nets excluding aerial predators from the entire range is considered unfeasible (Bestman and Bikker-Ouwejan, 2020).

Adding extra protection to the range (e.g. cover structures, large animals, roosters) seems to decrease perceived predation risk as more birds will venture out and move further away from the house (Pettersson et al., 2016). However, their effects on actual predation risk are unclear (Bestman and Bikker-Ouwejan, 2020).

Some farms keep other livestock (usually alpacas, donkeys or dogs) in the range to deter predators from entering (Bonnefous et al., 2022). Systematic data on their efficacy are currently lacking. Automated laser devices could be new tools to repel wild birds since they reduced the birds visiting the range by 98% (Elbers and Gonzales, 2021).

Unsuccessful predator attacks may leave hens wounded and depending on the severity of the wounds, culling of hens may be necessary to minimise the impact on welfare.

### 3.3.8. Resting problems

#### 3.3.8.1. Description of the welfare problem

Resting problems have been identified as highly relevant welfare consequence for pullets in collective cages and floor system without elevated structures and for layer breeders in collective cages, single-tier and slatted systems.

Resting problems are defined as the animal experiencing stress and/or negative affective states such as discomfort, and/or frustration due to the inability to lie, rest comfortably or sleep (e.g. due to hard flooring, inability to perch or irritation due to parasites) (EFSA AHAW Panel, 2022a,b).

The severity is assumed to range from medium to high depending on the extent of restriction on the expression of behaviours and on the frequency of disturbance during resting.

The duration is assumed to be long, probably life lasting, at least for layers and layer breeders, and for pullets after about the first 3 weeks of life.

The frequency of resting problems is assumed to be high, as all animals in cages are concerned and even in other systems where perches or other elevated resting sites are not provided. However, depending on space allowance and presence of elevated resting sites, the resting problems might concern a varying number of birds.

Hens demonstrate a strong motivation to seek elevation at night for resting or sleeping (Odén et al., 2002; Olsson and Keeling, 2002; Wichman et al., 2007; Schrader and Müller, 2009; Brendler et al., 2014; Brendler and Schrader, 2016) and they become agitated if roosting is prevented (Olsson and Keeling, 2000). Pullets also seek elevated locations during the day to monitor their environment in safety (Newberry et al., 2001) to preen (Skånberg et al., 2021) to escape from other hens (Cordiner and Savory, 2001). Elevated structures thus tend to reduce fearfulness (Donaldson and O'Connell, 2012) and promote resting behaviour in both young and older birds.

#### 3.3.8.2. ABMs

ABMs to assess 'resting problems' due to a lack of appropriate resting sites are described in Table 13. In case a housing system does not include any elevated resting sites or too few resting sites in relation to the number of birds, 'resting birds on elevated structures' is an ABM for 'resting problems'. In case elevated resting sites are offered but are not appropriate in design (e.g. slippery surface, too small in diameter), 'balance movements' is an ABM. In addition, in case of too few elevated resting sites in relation to the number of birds, 'pushing and jostling behaviours' is an ABM for this welfare consequence, which is also relevant to other welfare consequences, though, and therefore, it is described in Section 3.4.1.12 in Table 17.

**Table 13:** ABMs for the assessment of 'resting problems'

ABM	NOTES
<b>Balance movements</b>	
Definition	'A bird's body tilts in its axis while tail feathers are spread and rapidly moved up- and downwards, once or repeatedly. Bird's neck may be simultaneously reached out. Wings are flapped, once or repeatedly or hen leaves the resting site without focusing a landing point' (Pickel et al., 2010).
Measurement	This ABM occurs sporadically and with brief duration. Therefore, observation by video is recommended (e.g. behavioural sampling).
Interpretation	The restriction of resting due to inadequate resting sites, in particular slippery surfaces, increases balance movements to ensure good stability on the perch, while resting (Pickel et al., 2010).
Specificity and sensitivity	Sensitivity is high. If resting is restricted due to inadequate perch design and materials, balance movements will increase.

ABM	NOTES
	Specificity is high. When resting problems are absent, very few balance movements will be seen when birds are resting on perches or elevated structure. Since there is no other welfare consequence at the origin of balance movement.
<b>Resting birds on elevated structures</b>	
Definition	While resting on an elevated structure (e.g. a perch) birds do not move and resting can be performed in a sitting or a standing position. According to Blokhuis (1984) and Pickel et al., 2010 two types of resting can be distinguished: (a) The head is in a forward position and can be moved. The neck most often is withdrawn. The eyes are either closed or slowly opened and closed. (b) The head is tucked backwards into the feathers above the wing base or behind a wing.
Measurement	Directly observable: the number of birds showing resting can be counted by walking in front (e.g. cages) or through (e.g. single tier systems) the enclosures (Odén et al., 2002; Campbell et al., 2017a). Indirectly observable by video (Brendler and Schrader, 2016) or scouting cameras (Giersberg et al., 2019), that may only cover certain areas of the housing system. In this case, an adjustment must be made to relate the number of birds observed to the total number of birds and the total area. Because during night-time nearly all birds will rest, it is recommended to perform the observations at night. However, observation of daytime resting provides supplementary information and should (if conducted) be done after the peak laying period.
Interpretation	In particular during night-time nearly all birds will rest (Schrader and Malchow, 2020). Birds not resting indicate that resting is impaired by lack of appropriate resting sites (e.g. missing perches), inadequate resting sites (e.g. due to draughts, insufficient resting area or other disturbances such as red mite pest).
Sensitivity and specificity	Sensitivity is moderate. If resting problems are present, the number of resting birds might decrease but it is not systematic. Specificity is high during the night since, when resting problems are absent, all birds are resting and no other welfare consequence is preventing bird from resting on elevated structures

### 3.3.8.3. Hazards and preventive or mitigating measures

The main hazard for resting problems is the absence of perches or other elevated sites.

Unlike laying hens, pullets and layer breeders can be housed in collective cages or simple floor systems that may not offer access to perches or other suitable resting places. Resting problems can occur when perches are unavailable or provide insufficient space for all birds to rest simultaneously or when perches or platforms are of insufficient quality to support comfortable rest and sleep.

Resting problems may also be due to the inappropriate design of the perches. One particular feature of perches is how much grip they allow, when grasped with the feet. Pickel et al. (2010) found that the number of balance movements was related to the perch material as well as the diameter of round perches. With increasing diameter, the number of balance movements during night-time roosting decreased and were lower for perches with a rubber surface compared to perches made of steel or wood.

The hazard associated with resting problems is that birds do not get sufficient rest and sleep and become exhausted. Poor sleep quality during the night may be compensated by increased resting during the day, but this has not been studied extensively. There are indications that poor sleep quality, for instance due to red mite infestation, can result in hens being more likely to develop behavioural problems such as feather pecking (Heerkens et al., 2016b).

Resting problems can be prevented or mitigated by offering perches or other elevated structures (including tiers) in sufficient number and quality. Ideally, perches should be placed at such a height that resting birds are not disturbed by active birds moving across. Provision of cover panels in strategic locations may be effective to reduce disturbances during resting (Cornetto et al., 2002).

In single-tier systems, perches are often supplied in the form of A-frames or directly on the single tier. In the latter case, more disturbances of resting birds are expected than when A-frames are used.

It is not possible to provide fully elevated structures within a conventional-sized cage. Therefore, the prevention of hazards for the welfare consequence 'resting problems' requires non-cage housing with elevated structures (single- or multi-tier systems).

For existing collective cage systems for pullets and layer breeders, mitigation of the welfare consequence could be achieved by installing perches as in furnished cages for laying hens. This would only partly reduce pullet motivation to perch on an elevated structure because perches within cages are necessarily at a very low height. Further mitigation could be achieved by increasing cage height so that perches could be installed at a greater height, but this would still not fully prevent the hazard.

Measures to mitigate the welfare consequence include a reduction in stocking density or a supply of additional perch space in the cage. It would also help to assure there are no environmental conditions that trigger restlessness, as this may lead to increased disturbance of resting birds.

### 3.3.9. Restriction of movement

#### 3.3.9.1. Description of the welfare consequence

Restriction of movement has been identified as highly relevant welfare consequence for laying hens, pullets and layer breeders in cage systems.

Restriction of movement is defined as the animal experiencing stress and/or negative affective states such as pain, fear, discomfort and/or frustration due to the fact that the animal is unable to move freely, or is unable to walk comfortably (e.g. due to overcrowding, unsuitable floors, gates, barriers, perches) (EFSA AHAW Panel, 2022a,b).

The severity ranges from low to high depending on how many behaviours and how the performance of each behaviour is restricted. It is considered severe when the behaviour is highly restricted or totally prevented.

The duration is long, probably for the entire life, at least for laying hens and layer breeders.

The proportion of animals submitted to restriction of movement is high as all animals in cages are concerned, and in other systems where flying is impossible due to lack of elevated structures.

In addition to the limited space determined by cage dimensions, a high density of birds in collective cages can decrease the available space. The body size of birds, depending on hybrid, age and production stage (pullet, laying hen or layer breeder) is also impacting the available space. Furniture in cages also acts as an obstacle to smooth movement of animals and thereby restricts movements to some extent. The restriction of movement strongly reduces behavioural activities leading to frustration, stress and negative affective states. It can also contribute to decreased egg production and increased mortality as detailed below.

Restriction of movement causes physiological stress (reviewed in (Hemsworth and Edwards, 2021)) with floor spaces below 565 cm<sup>2</sup> per bird. For (Mench et al., 1986) reported that reducing space allowance in two-bird cages from 1,394 to 697 cm<sup>2</sup>/bird increased plasma corticosterone concentrations. Kang et al. (2016) found heterophils and the H/L ratio were greater ( $p < 0.01$ ) for 10 birds/m<sup>2</sup> (1,000 cm<sup>2</sup>/bird) than in stock density of 6 or 7 birds/m<sup>2</sup> (1,600–1,400 cm<sup>2</sup>/bird). Serum corticosterone was greater ( $p < 0.01$ ) for 10 birds/m<sup>2</sup> than the other stock densities.

Moreover, the limitation in space and the resulting behavioural restriction may lead to frustration and other negative affective states. Hens show a pronounced rebound in comfort behaviour when they are released from spatial confinement. Behaviours prevented by spatial restriction (e.g. wing stretching and wing flapping) are performed at higher rates than normal until motivation returns to baseline. This indicates that the motivation to perform these behaviours is high during spatial confinement, resulting in frustration, and indicating that hens do not adjust to prolonged spatial restriction (Nicol, 1987a; O'Connor et al., 2011).

Lack of exercise due to restricted space contributes to osteoporosis and weak muscles which can result in fractures (LayWel, 2006). Hens in cages therefore are affected by the poorest bone strength, and the highest number of fractures at depopulation (Widowski et al., 2016) compared to non-caged hens. Various bone parameters (measures of the degree of bone mineralisation) are used as indicators of bone status. These parameters include bone breaking force, bone ash, fat-free dry matter, bone volume and bone density (Zhang and Coon, 1997). According to Zhang and Coon (1997), bone ash concentration may be a better measure to reflect the changes in bone status of the hen as compared to the above-mentioned parameters. The authors suggested that to correctly compare bone ash, fat-free dry matter or bone breaking force in laying hens' studies, data analysis must be adjusted for bone volume as it is correlated with all other parameters. Other methods to estimate bone strength include CT or DEXA scans and also X-ray fluorescent or Raman spectroscopy can be used to determine bone

strength (see (Campbell, 2020)). The amount of medullary bone has been proven to influence bone strength (Fleming et al., 1998).

Elson (2004) found better plumage condition as space allowance increased in furnished cages from 600 to 870 cm<sup>2</sup>/hen. Engel (2016) found better feather condition at the higher space allowance (542 vs 1,648 cm<sup>2</sup>/hen in 8-bird cages), as Widowski et al. (2017), who observed better cleanliness and feather condition when hens were provided with more space (520 vs 750 cm<sup>2</sup>). The link between space allowance and plumage condition is likely to be related to abrasion against cage walls and structures.

Restriction of movement below 565 cm<sup>2</sup> may increase mortality and reduce egg production (reviewed in (Hemsworth and Edwards, 2021)). As floor space decreases, within a range of 650–300 cm<sup>2</sup>/hen, biological function generally decreases, leading to either higher mortality, lower egg production and body weight or poorer feed conversion (Hughes, 1983; Sohail et al., 2004). The increased locomotion associated with a larger space availability increases energy requirements, and therefore requires greater feed consumption by the hens (Fiks-van Niekerk et al., 2002). At 750 cm<sup>2</sup>/bird, Huneau-Salaün et al. (2011) did not find any differences in feed consumption between small (15,000 cm<sup>2</sup> for 20 hens) and large (45,000 cm<sup>2</sup> for 60 hens) furnished cages.

### Laying hens in furnished cages

Furnished cages house different number of hens depending on cage size. Legal dimensions are of minimum 2000 cm<sup>2</sup>, with 750 cm<sup>2</sup> per hen in which 600 cm<sup>2</sup> is usable surface, excluding the nest. The height is 20 cm minimum everywhere, with min 45 cm in the usable area. Nowadays, the majority of furnished cages are 45 cm high everywhere, or 60 cm for certain cages developed in Germany (Guinebrière, 2017). For a cage with 20 hens, this represents 12,000 cm<sup>2</sup> of usable space and a stocking density of 16.6 hens/m<sup>2</sup>. Although the stocking density is arithmetically similar in all cage sizes, available space increases with cage and group size as birds are able to 'share' space to some degree (Appleby, 2004).

The furnished cage dimensions and the placement of perches, nest and pecking areas, in the middle of the cage, does not allow vertical movements (flights and jumps), and limits horizontal movement such as running, distance walking. Some cage designs additionally place feeders within the cage rather than outside. The high density of birds eliminates any free space and only partially allows movements such as wing flapping, wing and leg stretching or dustbathing behaviours (see Section 3.3.4).

Shimmura et al. (2008a) found that dominant hens in small furnished cages of five hens (232 cm<sup>2</sup> of pecking and scratching area per hen and 1,175 cm<sup>2</sup> in total) used more of the pecking and scratching area for dustbathing, pecking and scratching behaviours than other hens; whereas they did not observe these differences in larger cages (18 hens per cage, yet with less surface area per hen: 58 cm<sup>2</sup> and 1,044 cm<sup>2</sup> in total).

In furnished cages, with the same surface per animal, the use of the resources can vary depending on their arrangement inside the cage. The location of the resources like perches, pecking and scratching area and nest depends on the dimensions of the cage (length/depth/height). Therefore, the area required per animal is dependent on the group size and the cage location of resources. In smaller cages (8 vs 50 hens/cage), some places are unusable by birds because of lost space in the corners, close to the perches (Fiks-van Niekerk et al., 2002). Larger cage size makes it easier to arrange resources in the furnished cage, including perches, and provide a wider range of design options, allowing less inter-bird disturbances when moving through the cage than in the small furnished cage, and there is slightly more space for the scratching area (Rodenburg et al., 2005; Guinebrière et al., 2015). In addition, the space freed up when hens are at rest is greater in larger cages. For example, Appleby (2004) calculated that a 20-hen cage frees up a space of 5 hens at rest, whereas a 60-hen cage frees up a space of 15 hens at rest. Same space allowance per bird has very different effects depending on group size (Appleby, 2004), and space available is more efficient the larger the group (Liste et al., 2015).

Good positioning of feeders, drinkers, perches and nest boxes is important, as birds become frustrated if they cannot reach resources easily due to the placement of objects such as perches (DEFRA, 2005). Design features can restrict movement, e.g. usable space per bird is impacted by placement of perches (parallel to the feeders, crossing, in front of the cage, on the back, height etc.) (Struelens et al., 2008a).

## Pullets in collective cages

There is little scientific information on the effects of space allowance on pullets relevant to the welfare consequence 'restriction of movement'. In addition, these studies have explored only a limited range of commercially used stocking densities, all of which may be restricting bird movement to some degree. In considering how collective cages may restrict the movement of pullets, two other considerations are pertinent. First, the effects of a given space allowance per bird will vary with pullet age and size, but this has not been explored scientifically. Second, young chicks are often confined initially to small areas and subsequently allowed greater access to the full cage areas as they grow.

There is a need for longitudinal studies to partition these temporal effects. Assessing the welfare consequences of space allowances of 270 cm<sup>2</sup> to 370 cm<sup>2</sup>/bird requires a degree of generalisation from studies of adult hens, where space allowances of 450 cm<sup>2</sup> to 600 cm<sup>2</sup> are known to impose severe restrictions. In a study of pullets kept in small experimental pens, locomotion at 15 weeks of age was significantly reduced from 1.79% of observation time when kept at 769 cm<sup>2</sup>/bird to just 0.37% of time when kept at 435 cm<sup>2</sup> (Hofmann et al., 2021). One study of pullets during their first 4 weeks in restricted compartments (effectively cages with no litter area) of a larger aviary system found that enrichment had more effect on the development of aggressive and feather pecking behaviour than the space allowance which varied between 94 and 83 cm<sup>2</sup>/bird to 10 days of age, and between 185 and 167 cm<sup>2</sup>/bird to 4 weeks of age (Schwarzer et al., 2022).

The welfare consequence 'restriction of movement' is highly prevalent (affecting all birds), with moderate duration as the restriction is lower during the first few weeks of life but increases as birds grow. There are no scientific studies detailing the responses of chicks to the strategies used commercially where progressive access to greater areas of the cage is provided as pullets grow. However, by 15 weeks of age, collective cages generally provide lower space allowances than 435 cm<sup>2</sup>/bird which demonstrably constrains locomotion. Thus, the severity of the welfare consequence 'restriction of movement' is severe for older birds (e.g. those between approximately 14 and 17 weeks of age).

No studies of the effects of cage height on movement in pullets could be found.

## Layer breeders in individual or collective cages

Housing laying hen breeders in individual or collective cages has major repercussions on the welfare consequence 'restriction of movement'. Although collective cages permit higher activity levels as compared to individual cages, they still impose significant limitations on the movement of laying breeders. Normally large cages, housing 20–100 breeding birds, are used. While birds may be able to perform comfort behaviour activities (depending on the stocking density and the cage size), running, jumping and longer-term movements will be limited. Mobility limitations in collective cages refer especially to distance travel that normally takes place to perform daily routines (Rodriguez-Aurrekoetxea and Estevez, 2016). Perches are only occasionally provided (depending on the country), thus movement in the third dimension, that can be beneficial for the birds (Campbell et al., 2021) is limited. Collective cages for breeders are required to have extra height to permit natural mating. The extra overhead space might provide additional opportunities for the performance of activities requiring more vertical space like wing flapping or wing stretching (described within comfort behaviours).

The dimensions of individual cages severely limit the possibilities for all forms of locomotion (e.g. walking, scratching, running). Perches are normally not provided thus, movement in the third dimension is not possible, adding to the restriction of movement. Such low activity for long periods of time will result in poor bone quality. As individually housed layer breeders must be handled on a regular basis for semen extraction or artificial insemination, the risk of bone fracture is high, especially in birds at high risk of osteoporosis. These rearing conditions will cause stress, negative affective states such as frustration and are likely to affect health and mortality if cage dimensions are limited (Hemsworth and Edwards, 2021).

### 3.3.9.2. ABMs

ABMs to assess 'restriction of movement' are described in Table 14. In addition, 'plumage damage' and 'pushing and jostling behaviours' are ABMs for this welfare consequence, which are described in Section 3.3.12 in Table 17.

**Table 14:** ABMs for the assessment of 'restriction of movement'

ABM	NOTES
<b>Bone quality</b>	
Definition	Aspects of bone composition and structure that contribute to bone strength independently of bone mineral density (Compston, 2006).
Measurement	Most common measurements of bone quality for laying hens include bone breaking force, bone ash, fat-free dry matter, bone volume and bone density, bone elasticity (review by Zhang and Coon (1997)). Only post-mortem measurements, for instance with percentages of fractures counted at the slaughterhouse (but they are impacted by catching also), or before depopulation on a sample of dead animals to measure bone quality.
Interpretation	Lack of opportunity to exercise due to restriction of movement may cause weak bones and muscles which can result in osteoporosis and painful bone fractures (LayWel, 2006). Bone breaking force, bone ash, fat-free dry matter, bone volume and bone density decrease with restriction of movement.
Sensitivity and specificity	Sensitivity is high. If movement is restricted, bone quality will be negatively impacted. For instance, laying hens in cages were shown to have weaker wing bones (Rodenburg et al., 2008; Wilkins et al., 2011). However, sensitivity of bone measurements may vary between different hybrids or feed regimes (Whitehead and Fleming, 2000; Stratmann et al., 2016). Specificity is low. If movement is not restricted, bone quality can still be low for other reasons, e.g. dietary, hybrids, osteoporosis.
<b>Locomotory behaviours (e.g. jumping, flying, walking, running)</b>	
Definition	Self-propelled capacity to move from one place to another using leg and/or wing assisted movements that results in walking, running, jumping and flying activities (Liste et al., 2015).
Measurement	Directly observable: numbers of jumping, flying and running events recorded in an observation period, with direct observation of a cage or part of the cage. Indirectly observable by video of a cage or part of the cage: numbers of jumping, flying and running events during an observation period, and proportion of the time budget dedicated to active behaviours through focal, or scan sampling (Liste et al., 2015). Jumping, flying and running events occur sporadically and with brief duration therefore require time for observation (e.g. focal sampling, ad libitum sampling) to be seen.
Interpretation	The restriction of movement limits active behaviours such as jumping, flying, walking and running events, and the proportion of the time budget dedicated to these.
Sensitivity and specificity	Sensitivity is high. If movement is restricted, locomotory behaviours are highly reduced and will show that the welfare consequence is present. Specificity is low. If movement is not restricted, active behaviours can still be impacted due to other external factors (e.g. low luminosity, lack of suitable materials: e.g. appropriate perches).

### 3.3.9.3. Hazards and preventive or mitigating measures

Main hazards include: insufficient space allowance per bird, insufficient total area of the enclosure, insufficient height of the enclosure, the absence of elevated structures, unsuitable resource distribution (places of nest, perches, pecking and scratching area, feeders in the cage limiting any free space).

To prevent restriction of movement, cage systems should be avoided in favour of single, or multi-tier systems, where bird's behavioural activity is not restricted and there are ample possibilities for locomotion.

If cages are used, then it is preferable to choose larger cages, and a better arrangement of the furniture. Nest and pecking and scratching area should be placed on the opposite part of furnished cage and perches placed parallel to the feeder (Fiks-van Niekerk et al., 2002). Cages must be chosen with the highest possible height, which may allow jumping but probably not flying.

For existing collective cage systems for pullets, mitigation of the welfare consequence could be achieved by setting minimum spatial allowances per pullet. These should be age appropriate. Collective cages could be modified to provide functional areas to separate resting and active birds, increasing the relative amount of space available for locomotion and activity.

Consequences derived from spatial restriction of cages can only be partially mitigated by using collective cages as large as possible and by always providing sufficient perch space, so that a wide range of behavioural activities can be performed. Nevertheless, mobility as measured by distance travelled is severely restricted, which contributes to poor bone strength and a higher risk of bone fractures, especially in female breeders.

Normally, collective cages are not used for elite breeders requiring individual data collection for selection, but rather as a housing option that maximises productivity and is easy to manage. Thus, such populations may be maintained with better welfare conditions in single tier systems or even multi-tier systems (although the latter are only sporadically used). Space availability is quite restrictive, in particular if collective cages are small. Larger cages result in larger 'shared space' for the birds (Liste et al., 2015). In addition, good nutrition to maintain target weight is important to maintain good bone health (Jung et al., 2019), which will be particularly relevant to females with high mobility restrictions.

Individual data acquisition is essential to improve genetic lines with the objective of a continuous improvement of health and performance. However, consideration should be given to mitigate the negative effects of individual housing by reducing the severity and length of the confinement. Even if it is challenging to get away completely from placing primary breeders in individual cages for individual performance data, the maintenance periods in single cages should be limited to specific times for data collection that should provide sufficient information for genetic selection. Meanwhile, if individual cages must be used, cages should be large enough to favour good bone quality and to allow locomotor behaviours.

### 3.3.10. Skin disorders (other than soft tissue lesions and integument damage)

#### 3.3.10.1. Description of the welfare consequence

Skin disorders (other than soft tissue lesions and integument damage) have been identified as highly relevant welfare consequence for laying hens in all systems.

The welfare consequence 'skin disorders (other than soft tissue lesions and integument damage)' is defined as the animal experiencing negative affective states such as pain, discomfort and/or distress due to, e.g. infections (e.g. dermatophytosis/ringworm, pseudomonosis, staphylococcosis, viral diseases), ectoparasites (e.g. mange or red mites), inflammation of the skin or sunburn. In the case of laying hens, skin disorders can be pruritic, somewhat erythematous eruptions composed of papules or more generalised red colouring of the skin, mostly in the belly/cloaca area, sometimes running up to the crup area. Subcutaneous oedema and congestion may also be present (EFSA AHAW Panel, 2022a,b).

The severity is assumed to be moderate since in most cases, inflammation leads to redness but not to swelling or increased temperature of the lesion.

The duration is assumed to be long, depending on the cause and possible cure. Red mite infestations are not easy to control, only a few chemical treatments are available, and they do not prevent re-infestation.

The proportion of animals experiencing skin disorders is assumed to range from low to high depending on housing conditions and management.

Skin irritation caused by parasites like red mites may cause itching leading to discomfort and in certain cases pain and distress, reflected in restless behaviour of the birds. This restlessness is expressed by frequent shifts in posture, ruffling of feathers, frequent short preening bouts and scratching behaviour, the latter mostly directed to the head. This behaviour is expressed during daytime and night-time. The indicated behaviours for restlessness are part of the normal behavioural repertoire of the hens but are performed in higher frequency due to skin irritations. Identification of abnormal frequencies of these behaviours is easier during night-time, when birds are sleeping or resting.

Single-tier systems mostly comprise of a litter area and a slatted floor. Multi-tier systems mostly comprise of a litter area and stacks of slatted floors with manure belts underneath. These structures have many cracks and crevices providing hiding places for mites. Also, the connection of the perches to the A-frames are often hiding places for the mites. Mites can also hide in the nest boxes.

Mobile houses comprise the same structures inside as single or multi-tier housing systems and thus have the same hiding places for the mites. As mites become active at night-time, which is the time the hens are inside, the fact that outside area is available is not reducing the risk for mite-related skin disorders.

### 3.3.10.2. ABMs

The ABMs to assess 'skin disorders' are described in Table 15.

**Table 15:** ABMs for the assessment of 'Skin disorders (other than soft tissue lesions and integument damage)'

ABM	NOTES
<b>Restlessness</b>	
Definition	Restlessness is the inability to sit motionless during resting periods, independently from disturbances by other birds.
Measurement	Restlessness can be observed during night-time, when birds are perching and ideally should be asleep. Restlessness can also be observed during daytime, but it is more difficult to distinguish it from normal activities as birds are more active. Directly observable: Observe resting birds and record if they are calm or frequently moving. Observe the frequency of short preening, ruffling of feathers and scratching bouts. Observations should be done at night. Indirectly observable: Video recordings of hens perching at night can give an indication of birds either sleeping calmly or continuously moving, preening, scratching (Kilpinen et al., 2005).
Interpretation	Red mite affected hens show more preening activity both during daytime and night-time, and head scratching and gentle and severe feather pecking at daytime (Kilpinen et al., 2005; Vezzoli et al., 2015) suggesting increased restlessness. Especially preening with the beak and scratching with the feet are known anti-parasite behaviour of birds (Bush and Clayton, 2018).
Sensitivity and specificity	Sensitivity is low. Hens with skin disorders will not always show restlessness. Specificity is moderate. Low mite infestation may not show systematically restlessness and other welfare consequences can lead to restlessness (e.g. resting problem)

### 3.3.10.3. Hazards and preventive or mitigating measures

Skin disorders may occur due to the presence of parasites, such as mites (Owen et al., 2009; Murillo et al., 2016). The most common haematophagous mite in Europe is the poultry red mite, *Dermanyssus gallinae*, that feed on hens at night, causing skin irritations, seen as erythematous papules. Red mites are known to be a vector for various diseases, among which *E. coli* infection, and thus posing a threat to bird health (Sparagano et al., 2014; Schiavone et al., 2020). The majority of layer flocks in Europe are affected by red mite infestations (Sparagano et al., 2009).

High infestation rates with poultry red mites can cause hyperkeratosis of the skin, abnormal thickening of the epidermis and small abscesses (Sokół and Rotkiewicz, 2010). Kilpinen et al. (2005) indicated that severe health problems are caused by infestations of 150.000 to 200.000 mites/bird. Sparagano et al. (2014) mention even higher numbers, up to 500.000 mites/hen.

Nordenfors et al. (1999) indicated that the optimum reproduction of poultry red mites is between 20 and 25°C and over 70% RH, which is often reached in poultry houses. Lower temperatures and RH will reduce the reproduction rate, although reproduction is seen at temperatures between 5 and 45°C.

Hiding places for mites at daytime are impossible to prevent, but some elements of the equipment will facilitate the mites more than others. The more complicated the housing system is, the more cracks and crevices are present, the more mites will thrive. Heerkens et al. (2015) found more mites in the presence of plastic slats compared to wire mesh.

Control of these parasites is mainly done using synthetic acaricides and diatomaceous earth (Bennett et al., 2011) although new methods are in development, such as vaccinations (Xu et al., 2020). Integrated pest management is used as a systematic approach to mitigate the introduction of mites, monitor their occurrence and decide upon treatments (Sparagano et al., 2014). Between flocks the use of heat treatment is effective in reducing mite populations (Mul et al., 2020).

### 3.3.11. Soft tissue lesions and integument damage

#### 3.3.11.1. Description of the welfare consequence

Soft tissue lesions and integument damage have been identified as highly relevant welfare consequence for laying hens and layer breeders in all systems, except breeders in individual cages.

Soft tissue lesions and integument damage is defined as the animal experiencing negative affective states such as pain, discomfort and/or distress due to physical damage to the integument or underlying tissues, e.g. multiple scratches, open or scabbed wounds, bruises, ulcers, abscesses and feather loss. (EFSA AHAW Panel, 2022a,b). This welfare consequence may result from negative social interactions such as aggression or feather pecking, from handling or from damaging environmental features, or from the mutilation practices of beak trimming or comb dubbing.

The severity is assumed to range from low to high depending on the type of lesion.

The duration is assumed to be long in case of healing problems.

The proportion of birds that become the victim of injurious pecking depends on the occurrence of this problem in a flock and can vary from absent to a high prevalence (see Section 3.5.1).

The proportion of birds experiencing the welfare consequence due to beak trimming or comb dubbing depends on the management decisions. Beak trimming is widely conducted on pullets and layer breeders. Comb dubbing is carried out on male breeder birds of white strains.

The complex issues around injurious pecking (description of different types of pecking, causes, welfare consequences and preventive measures) and around the associated practice of beak trimming (description, welfare consequences, alternative strategies) are described in full in Section 3.5.2.

Other soft tissue damage may be caused by the interaction with housing equipment. For example, bruises may arise from flight collisions, and toes can be trapped in narrow crevices between parts of the frame and slatted floors of aviaries, narrow openings inside partitions of cages, or little holes in structures, which can trap nails. When the birds are trying to free themselves or trying to fly away, the trapped toe can be wounded or even ripped off. Recent types of housing systems have improved as manufacturers are taking possible trapping into account by making the crevices wider.

Abrasion is also important as once plumage cover is eroded by abrasion it can encourage injurious pecking. Abrasion is of high concern for birds housed in collective cages where the constant rubbing against abrasive and sharp edges of the cage may deteriorate plumage condition rendering birds more vulnerable to soft tissue and integument damage.

Another possible risk for toe damage may come from feeding chains. Birds may be trapped with toes between the chain and the feed trough or between the chain and narrow openings to next compartments. Also, corner wheels in chain feeders may constitute a risk for trapped toes. The risk is larger in cases where the bird steps into the feeder. Especially small chicks are known to be at risk of losing toes in the feed chain. Various types may lead to different risks, but no data are available on this. Van Niekerk (personal communication, 2022) found up to 5% birds with healed toe stumps in two flocks originating from the same rearing farm, suggesting an origin in the rearing period. Scratches can also occur from contact with sharp objects (claws, furniture) causing minor superficial skin damage, but also larger and deep wounds.

Group housing of laying hen breeders can cause soft tissue lesions and integument damage not only due to injurious pecking but also to the inability to avoid unwanted sexual behaviour, that may produce damage on the females. Damage from forced matings is mostly to female birds and includes wounds, scratches and lacerations, and also feather damage on the upper neck and back, and occasionally on the back of the head from sexual activity (see Section 3.3.3).

The limited group size in collective cages makes feather pecking outbreaks easier to control compared to non-cage systems (maximum 100 birds per group in collective cages compared to subgroups of up to 6,000 birds in non-cage systems). Similarly, the inability to avoid unwanted sexual behaviours in small groups housed in collective cages appeared to be less of an issue as compared to larger groups in non-cage systems, thus the prevalence of the welfare consequence is expected to be also lower. Nevertheless, some damage may occur related to the ongoing reproductive behaviour.

### 3.3.11.2. ABMs

ABMs to assess 'soft tissue lesions and integument damage' are described in Table 16. ABMs related to soft tissue damage due to beak trimming (ABM 'beak shape and length') are described in Section 3.5.2. Soft tissue damage related to 'inability to avoid sexual behaviour' (ABM 'forced copulations') is described in Section 3.3.3. In addition, the ABM 'plumage damage' and 'wounds' can be used which are described in Section 3.3.12 in Table 17.

**Table 16:** ABMs for the assessment of 'soft tissue lesions and integument damage'

ABM	NOTES
<b>Bruises</b>	
Definition	Bruises comprise injuries appearing as an area of discoloured skin on the body, caused by a blow or impact rupturing underlying blood vessels. Often bruises can be found in the keel area (Heerkens et al., 2016b). Fresh bruises are usually reddish, older bruises can colour yellow, blue and green.
Measurement	Not all bruises are easily visible, especially if feathers cover the skin. Therefore, it is recommended to pick up birds and inspect them one by one. Only birds with extensive feather loss can be visually inspected without handling, as bruises typically are found in areas with low feather coverage
Interpretation	Depending on the size and depth, bruises are more or less painful. As bruises in the keel area are often accompanied by keel bone fractures, they can be seen as visible signs of an underlying painful condition.
Sensitivity and specificity	Sensitivity is moderate. When the welfare consequence is present, bruises are not always present since birds can suffer from other lesions. The specificity is high, as it is highly unlikely that bruises will be detected in birds not affected by this welfare consequence.

### 3.3.11.3. Hazards and preventive or mitigating measures

The hazards for soft tissue and integument damage may originate from the animals themselves, or from the physical elements of the house such as abrasive/sharp housing system elements, or inadequate environmental conditions.

The hazards for injurious pecking and preventive measures are described in Section 3.5.

The inability to avoid unwanted sexual behaviours is more likely to occur in single-tier systems compared to cages, given the larger group size and less opportunity for escape. Asynchrony in sexual development is also a hazard for high incidences of inability to avoid unwanted sexual behaviour.

Regarding the physical environment, abrasive or sharp housing system elements in single- or multi-tier systems can be the edge of the feeder, and the edges of the tier. Usually, most damage by abrasion in single-tier systems is caused by the feeder to the neck area.

The risk of abrasion can be prevented by avoiding sharp edges on the feeders and by providing sufficient feeder space and avoiding feed competition. An increased number of feeding bouts can help to reduce competition, and thus, the risk of soft tissue lesions and integument damage.

The risk of soft tissue damage and wounds is strongly related to group stress, injurious pecking and unwanted sexual behaviour. The preventive measures described for each of these welfare consequences will also prevent or mitigate against soft tissue damage and wounds.

The hazard of plumage damage caused by male claws can be prevented by providing abrasive strips and increased cage complexity may reduce unwanted sexual behaviours.

### 3.3.12. ABMs related to the assessment of more than one welfare consequence (so-called 'iceberg indicators')

ABMs such as fear response, injurious pecking, physiological stress indicators, plumage damage, pushing and jostling behaviours and wounds all have in common that they can be used as iceberg indicators as defined in Section 1.2.

Especially the non-invasive ABMs, such as plumage damage and general fear response, are often used to get an impression on the welfare status of the flock. Observation of injurious pecking behaviour and measurement of physiological indicators can be used to obtain more in-depth information regarding the causes of feather damage or increased fear responses. Flocks with hens with severe plumage damage and an increased level of fear of humans, for instance, would be considered flocks at risk of poor welfare. de Haas et al. (2014b) also showed that increased fear of humans can be a predictor of increased feather damage later in life. These ABMs should be included in any welfare assessment scheme.

Table 17 lists the ABMs that can be used to assess more than one welfare consequence. As the ABMs described in the table are used for different welfare consequences, their sensitivity and specificity towards one specific welfare consequence cannot be assessed. They should be considered as reflecting the general welfare status of the flock.

**Table 17:** ABMs related to the assessment of more than one welfare consequence

ABM	NOTES
<b>Fear response</b>	<b>Reflecting the welfare consequences:</b> <ul style="list-style-type: none"> <li>– ‘group stress’</li> <li>– ‘isolation stress’</li> <li>– ‘predation stress’</li> </ul>
Definition	Fear responses are behavioural or physiological reactions of animals towards sudden, threatening and/or novel stimuli.
Measurement	Fearfulness can be measured by non-invasive tests, such as the tonic immobility test (a specialised restraint test) (Ratner and Thompson, 1960; Jones and Waddington, 1992; Cotter, 2015), the novel arena test (also known as open field test), the novel object test (Welfare Quality, 2009), the human approach test, or the emergence test (Forkman et al., 2007) and avoidance distance test (Whay et al., 2007; Guinebretière et al., 2020). Often, a combination of several tests is used.
Interpretation	The welfare consequences group, predation or isolation stress increase fearfulness. This might result in longer tonic immobility, longer time to first sound/escape attempt, longer approach to a novel object, or attempts to rapidly move away from the test stimulus.
<b>Injurious pecking</b>	<b>Reflecting the welfare consequences:</b> <ul style="list-style-type: none"> <li>– ‘group stress’</li> <li>– ‘inability to perform exploratory or foraging behaviour’</li> <li>– ‘soft tissue lesions and integument damage’</li> </ul>
Definition	Injurious pecking refers to damaging bird-to-bird pecking whereby pecks to the feathers or tissue of another bird cause plumage damage, skin wounds, or tissue damage (De Haas et al., 2021)
Measurement	Directly observable: numbers of injurious pecking events observed per individual and time unit, with direct observation of a cage/pen or part of the cage/pen. Indirectly observable by video of a cage or part of the cage: number of injurious pecking during an observation period. Injurious pecking are short lasting and sporadic events and therefore require time for observation (e.g. focal sampling, video recording) for the behaviour to be observed correctly (Newberry et al., 2007).
Interpretation	The injurious pecking increases with inability to perform exploratory and foraging behaviour, as a redirected behaviour (Blokhuys, 1989) and with group stress (Rodenburg et al., 2013).
<b>Physiological stress indicators</b>	<b>Reflecting the welfare consequences:</b> <ul style="list-style-type: none"> <li>– ‘group stress’</li> <li>– ‘isolation stress’</li> <li>– ‘predation stress’</li> </ul>
Definition	Physiological changes during and after exposure to acute, intermittent or chronic stressful events.
Measurement	Physiological stress measurement can be done in blood but can also be done in faeces or feathers. Indicators used are: Blood Heterophil/Lymphocyte-ratio (Scanes, 2016), plasma corticosterone levels, corticosterone levels in faeces or feathers (Bortolotti et al., 2008), catecholamine levels, especially epinephrine and norepinephrine (Dennis et al., 2008). Previous studies have suggested a positive correlation between the concentration of corticosterone in blood plasma and in egg albumen (Downing and Bryden, 2008). However, more recent research has found that because there is so little corticosterone in the egg albumen it cannot be considered a reliable indicator of bird welfare (Engel et al., 2011; Caulfield and Padula, 2020). Poor body condition is a measure of cumulative chronic stress that has been validated by association with neural biomarkers (Armstrong et al., 2022). In small experimental studies, heart rate can be measured using wearable monitors or blood pressure cuffs (e.g. Paul et al., 2022) but this has limited utility for wider assessment.

ABM	NOTES
Interpretation	Stress most often will result in higher physiological stress response (higher H/L-ratio, corticosterone, catecholamine activity, especially epinephrine and norepinephrine).
<b>Plumage damage</b>	<p><b>Reflecting the welfare consequences:</b></p> <ul style="list-style-type: none"> <li>– <b>'group stress'</b></li> <li>– <b>'inability to avoid unwanted sexual behaviour'</b></li> <li>– <b>'inability to perform exploratory or foraging behaviour'</b></li> <li>– <b>'soft tissue lesions and integument damage'</b></li> </ul>
Definition	Deterioration or loss of plumage due to the action of other birds or by erosion caused by rubbing with the enclosure elements, or birds. Includes damaged feathers or feather loss.
Measurement	<p>Various protocols have been developed to assess plumage damage, many of which require handling of birds. These include the methods of Bilcik and Keeling (1999), Tauson et al. (2005) and the Welfare Quality method (2009). All of them use classifications per body part, but the number of body parts assessed varies from four (head considered separately, neck, back/rump and belly, Welfare Quality), to six (Tauson et al., 2005) or eleven (Bilcik and Keeling, 1999). It has also been recognised that handling of birds in large flock is disadvantageous in terms of stress, time efficiency and feasibility. For this reason, plumage scoring methods that do not require bird handling have also been developed. Bright et al. (2006) proposed a method where five body regions were visually assessed at a 2-m distance and found that this method correlated strongly with assessments of handled birds (<math>r = 0.89</math>). The Assurewel protocol (Main et al., 2012) also relies on visual assessment of unhandled birds providing just one overall score for the head/neck and back/rump regions combined. It should also be noted that scoring systems for each body region vary in the granularity of the scale, which can be 3-points (Assurewel, WQ); 4-points (Tauson et al., 2005), 5-points (Bright et al., 2006), 6-points (Bilcik and Keeling, 1999) and in scale direction, whereby high scores are usually taken as indicators of poorer plumage condition, but sometimes the opposite (e.g. Tauson et al., 2005). Recent evidence suggests that the transect method (Marchewka et al., 2013; Marchewka et al., 2015) is an efficient method to effectively and efficiently quantify plumage damage in commercial flocks housed in loose systems (Vasdal et al., 2022a). Visual inspection of the flock without catching the birds can reveal major problems in plumage coverage. For minor deteriorations or assessment of plumage damage in different body areas, individual bird inspection may be needed.</p>
Interpretation	<p>Although plumage damage is most likely not directly painful, the action that resulted in the damage or the removal of the feather may have been painful (Gentle and Hunter, 1991). Also feather cover damage often leads to naked skin areas, which is a risk for skin damage and thermoregulation issues. Plumage damage increases with 'inability to perform exploratory and foraging behaviour' or 'inability to avoid unwanted sexual behaviour', as a consequence of injurious pecking. Plumage damage is also increasing with 'group stress' and is an ABM for 'soft tissue lesions and integument damage'.</p> <p>Depending on the area where the damage to the feather coverage is found a cause can be identified. Back of the neck and the back and base of the tail are often damaged by feather pecking. The front of the neck can be damaged by the feeder, the wings and tail feathers may be damaged by system components. In breeder flocks the feathers at the back of the head, back and thighs of females may be damaged due to mating behaviour of males.</p>
<b>Pushing and jostling behaviours</b>	<p><b>Reflecting the welfare consequences:</b></p> <ul style="list-style-type: none"> <li>– <b>'resting problems'</b></li> <li>– <b>'restriction of movement'</b></li> </ul>
Definition	A bird makes physical contact with another in such a way that forces the latter to change its original position (adapted from Sirovnik et al. (2018)).
Measurement	Directly observable: numbers of pushing and jostling events recorded in a period of observation, with direct observation of a certain area of the enclosure.

ABM	NOTES
	Indirectly observable by video: number of pushing and jostling events within a certain area during an observation period. For comparison, frequency of occurrences must be standardised by unit of time and number of birds observed. These behaviours occur sporadically and with brief duration therefore requires time for proper observation (e.g. focal sampling, video recording).
Interpretation	Restriction of movement and resting problems increase the occurrence of pushing and jostling behaviours. When the action of pushing and jostling results in disruption of the resting periods of the receiver birds, it is considered a disturbance (Cornetto et al., 2002).
<b>Wounds</b>	<b>Reflecting the welfare consequences:</b> <ul style="list-style-type: none"> <li>– <b>'inability to avoid unwanted sexual behaviour'</b></li> <li>– <b>'soft tissue lesions and integument damage'</b></li> </ul>
Definition	Wounds comprise all lesions to the skin ranging from minor superficial punctiform spots, to scratches and breast blisters/buttons, to large open wounds that go deeper than the skin. Breast blisters can be seen on the skin in the keel area (Heerkens et al., 2016b), and may be related to the impact of collisions with the furniture or contact with the perches.
Measurement	Not all wounds are easily visible, especially if feathers cover the skin. Therefore, the best way to measure is by picking up birds and inspecting them one by one, as suggested in the Welfare Quality protocol (2009), although this may be very time-consuming depending on flock size. For larger wounds often visual inspection without manipulation will do, as these are situated in areas that are less covered by feathers. The transect method is valid for assessment of soft tissue and integument damage in laying hens without picking up birds, involving simple counts of the number of hens noted in a defined flock walk through, including wounds to head, back, tail or feet (Vasdal et al., 2022a).
Interpretation	Wounds are painful and can be a risk for infection. Also, wounds are often attractive to conspecifics to peck at, resulting in increased severity of the affected area, thus further decreasing welfare.

### 3.3.13. Welfare consequences linked to access to covered veranda and outdoor range

The highly relevant welfare consequence 'Predation stress' linked to outdoor access is described in Section 3.3.7. In addition, the welfare consequences 'cold stress', 'heat stress', 'gastro-enteric disorders' and 'respiratory disorders' are relevant for systems with outdoor access and described briefly for completeness. Avian influenza (here categorised under 'respiratory disorders') can have a substantial impact on welfare, both directly by increasing morbidity and mortality and indirectly by restricting outdoor access.

#### Cold stress and heat stress

Laying hens having access to free-range systems are subjected to variable weather conditions but whether they are truly more likely to suffer from thermal stress still needs to be substantiated by scientific evidence (Bonnetous et al., 2022). Access to the range and/or a covered veranda can reduce control over the indoor climate of the house (as air and humidity moves through the popholes). This may expose hens to an increased risk of heat stress as well as cold stress if it results in ambient temperatures outside the hens' thermal comfort zone, and more severe consequences for temperatures outside the thermoneutral zone. In poultry, the range of temperatures encompassed by the thermoneutral zone depends on genetics and physiological status, body weight, the degree of feather cover, acclimatisation, feed and dehydration status (Dawson, 2000; Tao and Xin, 2003) as well as other environmental factors (Lara and Rostagno, 2013). This means that simply monitoring ambient temperature is insufficient to determine if heat or cold stress occurs.

Having access to an outdoor area may also reduce the risk of heat and cold stress under certain circumstances. For instance, well-shaded areas on the range may offer a favourable climate on hot days (Bonnetous et al., 2022) and draw hens out of the house, potentially reducing indoor

temperatures. Similarly, range use may improve plumage condition which makes hens less susceptible to cold stress. Crucially, providing outdoor access allows hens to choose between more areas with different climatic conditions (in temperate climatic areas) and birds can choose to go inside when the weather is aversive.

Systematic data on the likelihood of heat and cold stress occurring in systems with and without outdoor access under the climatic conditions that occur throughout Europe are currently lacking. In Australia, heat stress is a main cause of mortality in free-range laying hen systems (Edwards and Hemsworth, 2021). However, Australian climatic conditions encompass periods of intense heat and sunlight (Campbell et al., 2021), which would be far rarer and less extreme in most of the EU countries where range access is common. Some crossbreeds used in free-range production are more resilient to heat stress than pure breeds (Radwan, 2020). When there is, for example, no shadow available on the range, hens may stay indoors to avoid the sunshine and associated high temperatures. This behaviour may increase the stocking density indoors and hens may suffer from heat stress since housing used for free range production is sometimes not as well ventilated (e.g. no mechanical ventilation) as that used for conventional production systems.

In Southern Mediterranean countries, extreme heat conditions are common during summer and birds have to be protected from such thermal conditions.

### Gastro-enteric disorders

Endoparasites, especially helminths, i.e. nematodes (*Ascaridia galli* and *Heterakis* spp. especially) and cestodes (Raillietina, Choanotaenia, Davainea especially) and protozoa (*Eimeria*, causing coccidiosis can cause gastrointestinal disorders) (Shifaw et al., 2021). Their presence can be detected by post-mortem analysis of the intestinal tract, by counting the number of eggs in the faeces of living hens or by serologic methods (Shifaw et al., 2021). Most poultry nematodes have a direct lifecycle (i.e. no intermediate host is required). The faecal–oral transmission is the main route of infection contributing to the higher susceptibility of poultry in non-cage systems due to access to faeces (Shifaw et al., 2021). Ninety per cent of the eggs of *Ascaridia galli* (the most common laying hen nematode) die within 23–38 weeks after deposition on the range. However, 2% of these eggs may survive and remain infective for at least 2 years (Thapa et al., 2017). Nematod eggs survive indoors as well, but survival can be reduced by cleaning and disinfecting between flocks (although its efficacy in practice has been questioned, (Jansson et al., 2010)).

Cleaning is not feasible for outdoor ranges, which may further contain intermediate or transport hosts (Kaufmann et al., 2011) and water bodies necessary for the hatching of trematode eggs (Shifaw et al., 2021). A meta-analysis including data on layers, broilers and indigenous breeds (Shifaw et al., 2021) found that the pooled prevalence of helminth infections in free-range systems was 85% (CI: 23–100%), and was 71% (CI: 34–95%) in deep litter systems without outdoor access. However, this does not necessarily reflect the impact of helminth infections on welfare in these systems, as infections leading to low worm burdens generally do not cause welfare problems (Sharma et al., 2019). In contrast, infections resulting in a high worm burden in the intestinal tract can obstruct and damage this tract, alter feed intake and nutrient absorption, suppress the immune system, deplete liver lipid and energy reserves, alter hen behaviour and cause mortality (Gauly et al., 2007; Sharma et al., 2019). The quantity of helminth eggs in the hen's faeces (faecal egg counts, FECs) can be used as an indirect indicator of worm burden. FECs have been shown to correlate with worm burdens in experimental and on-farm studies (Jung et al., 2020; Sharma et al., 2019), although these are not always fully informative of worm burdens, e.g. due to differences in the timing of the development of worms and eggs (Sharma et al., 2019). Flocks with an average FEC over 200 have been shown to have increased mortality (Hinrichsen et al., 2016). There is very little research that directly compares worm burdens or FECs between laying hen systems with and without outdoor access. One study (Permin et al., 1999; Grafl et al., 2017) found that access to an outdoor range increased the burden of one species (*Heterakis gallinarum*) but not of other species (*Ascaridia galli* and *Capillaria obsignata*). Instead, *Ascaridia* burden was found to be lower in cage systems than in floor systems with and without outdoor access.

Among flocks with access to an outdoor area, those that use the outdoor area more, and those that have more prolonged access, have a lower risk of helminth infection (Sherwin et al., 2013; Thapa et al., 2015; Jung et al., 2020). This is likely due to greater dispersion of faeces when hens range more (Thapa et al., 2015).

Another level of complexity is added by individual differences in range use. Within flocks with outdoor access those individuals that chose to stay inside mostly during early life had the lowest risk of

helminth infection throughout lay (Sibanda et al., 2020). This does not contradict the flock level association between increased range use and decreased helminth infection risk, as hens that choose to stay inside likely derive benefits when their flock-mates range and thus deposit their faeces outside on the range, where these cannot infect hens that stay inside. This results also highlights the need to further investigate the complex transmission dynamics since a better understanding of the transmission routes in free ranges and their variations with wild fauna behaviour would help to reduce infection in free range hens (Scott et al., 2018).

In organic production systems, prophylactic anthelmintic treatments are prohibited although their therapeutic use is allowed, while both prophylactic and curative treatment is allowed in non-organic systems. Some sources report that anthelmintic use in layers is very limited (e.g. Permin et al., 1999; Kaufmann et al., 2011) due to its cost and concerns about environmental impacts, anthelmintic resistance and residues in animal products (Kaufmann et al., 2011). However, this does not seem to reflect all current systems and EU countries equally. Bestman and Wagenaar (2014) investigated organic flocks in the Netherlands and found that 75% of the flocks in their sample were treated with an anthelmintic in the first 50 weeks of life.

Coccidiosis caused by various species of the genus *Eimeria* (Protozoa), is a ubiquitous problem of floor-reared hens, even when flocks have good management and hygiene (Mathis et al., 2018). It induces intestinal lesions and can reduce weight gain and productivity as well as cause mortality in laying hens (EFSA AHAW Panel, 2005; Fossum et al., 2009; Mathis et al., 2018). Birds acquire resistance towards coccidia in early life (EFSA, 2005), meaning that they will be less or unaffected upon re-exposure to the same strain. Access to an outdoor area that cannot be disinfected may expose hens to coccidia, but studies comparing systems with and without outdoor access are currently lacking.

Grass impaction has been identified in Australian studies as another gastrointestinal problem in free range hens, which can cause high levels of mortality (Groves, 2021). Currently, grass impaction is not reported as a major problem in European systems and was not among the major causes of mortality identified in several European studies (Bestman and Wagenaar, 2014). Hen hybrid, vegetation type and paddock management (mowing and removal of clippings) are reported to affect grass impaction risk (Kjaer and Sørensen, 2002). Therefore, differences in such factors may therefore contribute to the differences between the Australian and European studies. Nonetheless, little information is available on grass impaction in European systems.

### Respiratory disorders

Outdoor access is protective of respiratory disorders caused by poor air quality.

Avian influenza is discussed under respiratory disorders, but there are other effects such as gastro-enteric and neurological symptoms.

Salmonella, mostly *Salmonella* Enteritidis and *Salmonella* Typhimurium (Van Hoorebeke et al., 2010), *Campylobacter* and avian influenza can contaminate flocks after direct or indirect contact with infected wild animals. *Salmonella* and *Campylobacter* having, most of the time, no clinical manifestation, the health and welfare of birds are rarely impacted. But the risk of contamination has been proved to be higher in outdoor systems (Koch and Elbers, 2006) leading to both morbidity and mortality.

Wild birds and their faeces or feathers can introduce pathogenic agents in a flock, like avian influenza. The risk of introducing a low pathogenic strain is estimated to be 6.3 times higher for flocks with outdoor access compared to indoor flocks, probably due to contact with faecal material from wild birds on the range (Bouwstra et al., 2017). Therefore, outdoor access is often withheld in case of an outbreak of highly pathogenic avian influenza in a certain country or region in Europe.

The contamination can occur through direct contact between laying hens and wild birds on the outdoor range, or when the droppings of contaminated wild birds fall on the range and are ingested by poultry. Farms located at short distances (< 500 m) from water bodies or areas with high numbers of migratory wild birds had a higher risk of introduction than farms located at further distances (Bouwstra et al., 2017).

#### 3.3.14. Welfare of male chicks of the layer breed

Male layer chicks were killed in the hatchery and discarded or used as, e.g. food for zoo animals. In the event of a ban on the killing of day-old chicks, as is set in some European countries, the hatching eggs can be sexed in-ovo and male eggs destroyed (often used for feed production) or the male

chicks can be fattened. Current European legislation does not cover this category of birds. As male layer chicks are kept for meat, they are often placed under the regulations for broilers. This, however, is not appropriate as they are more comparable to layer pullets in behaviour, have the same low growth rate and final bodyweight.

Commission Implementing Regulation (EU) 2020/464 (EU, 2020) provides terms for the keeping of organic layer males, in which they are giving the definition: 'brother rooster', referring to a male chicken of laying hen strain intended for meat production.

Male layer chicks are kept up to 8.5–22 weeks of age, depending on the housing and the aimed final body weight (Giersberg and Kemper, 2018). On average, the birds are kept 12–15 weeks to reach a body weight of 1,300–1,500 g (Giersberg and Kemper, 2018; Krautwald-Junghanns, 2021). Feed conversion ratio almost linearly decreases from 2.25/2.65 (brown/white hybrids) at 7 weeks of age to 4/4.5 (brown/white hybrids) at 20 weeks of age (Damme, 2017).

In behaviour, male layer chicks are, like their sisters, more active (Malchow et al., 2018, Malchow et al., 2019a) and their walking ability is much better compared to broiler chickens (Malchow et al., 2019b). In addition, already starting within the second week of life they are very eager to sit on elevated structures, like perches and platforms (grids) (Malchow et al., 2018; Malchow et al., 2019b). When offering perches and grids at 10, 30 and 50 cm height, male layer chickens did not show a preference for a certain height during daytime but in the 9th and 10th week of life (end of the study) they preferred the highest resting site at night (Malchow et al., 2018). The authors did not find a preference of the male layer chickens for perches or platforms during night-time, but during daytime the birds prefer the platforms. The preference for the highest resting site was confirmed in another study in which only platforms of different heights (50, 30, 10 cm) were offered (Malchow et al., 2019a).

To prevent problems with injurious pecking, foraging materials should be provided (Giersberg and Kemper, 2018; Krautwald-Junghanns et al., 2021) and an adequate feed composition is important (Gnauk, 2020). Although Giersberg and Kemper (2018) indicate the use of a broiler diet from 8.5 weeks onwards, commercial companies indicate that as the growing potential of the layer males is limited, feed formulation can be less rich and more comparable to pullet feed, with slightly more energy. Light is important in relation to aggressive behaviour, where both the intensity as well as an even light distribution are mentioned (Gnauk, 2020). The German KAT regulations demand 20 lx light intensity in the house, which is only possible in husbandry situations where ample foraging material and elevated structures are provided (Giersberg and Kemper, 2018).

Lichovnikova et al., 2017 compared brown layer male chicks with male fast growing broiler chicks in the first 7 weeks of their live and found that the layer chicks were more active, whereas broiler chicks were sitting more.

In layer type males, sexual maturation is leading to more aggression around 10 weeks of age. Hillemacher and Tiemann (2018) compared brown layer males with dual purpose males and found more aggression, feather pecking and wounds in the layer males, leading to the necessity to dim the lights, which was not necessary in the dual-purpose breed, where no wounds were found.

Schütz et al. (2018) interviewed commercial people with experience in the keeping of layer males, who all indicated increased pecking behaviour with increasing age, that makes it necessary to provide ample foraging material, pecking stones, range and the possibility to escape each other (perches/platforms).

If no injurious pecking occurs, mortality can be limited to 1 or 2 (Krautwald-Junghanns et al., 2021), but due to poor economic returns the needs of male chicks are often neglected thus resulting in poor welfare and very high mortality such as 30% (van Niekerk and Workamp, 2022).

No clear research is done on space need per bird. Although some regulations take 500 cm<sup>2</sup>/bird as minimum, commercial experiences indicate that 555–526 m<sup>2</sup>/bird is leading to less aggressive behaviour than lower space allowances (Gnauk, 2020).

The behaviour of male layer chicks is comparable to pullets and, therefore, it can be assumed that they have similar requirements such as the provision of elevated structures. Different from pullets, male layer chicks may show aggressive behaviour from 10 weeks on and elevated structures allow the birds to escape from aggressive encounters. More research is recommended about specific needs of male layers.

### 3.3.15. Welfare consequences linked to feed restriction

Feed withdrawal can cause stress and hunger in birds, at levels that increase with duration of removal (EFSA AHAW Panel, 2022a). There is also strong evidence of stress and prolonged hunger in birds that receive low-nutrient diets that do not meet their physiological needs (Nicol et al., 2017; section LH8.3c). Not providing birds with a diet meeting their physiological need is not allowed in EU (Directive 98/58). The practice of 'forced moulting', which partially restores the rate of egg production after a period of feed withdrawal, is often obtained by severe nutritional limitations. Producers may sometimes reduce the quantity or quality of feed, either as hens approach the end of lay, or to obtain the effect of 'forced moulting' extending the period of lay. The practice has been reported in the EU but scientific data about the extent of these practices in the EU are lacking.

### 3.3.16. The impact of laying hen breeding and genetics on welfare

The genetics of laying hens can influence their welfare in several ways: (1) genetic disorders or malformations may appear in offspring, (2) variation may exist in behaviour and welfare traits between different genetic lines and hybrids, and (3) breeding programmes may positively or negatively affect behaviour and welfare traits. These will be discussed in more detail below.

Genetic disorders or malformations are often identified at chick sorting after hatching and will normally result in culling of the chick (Underwood et al., 2021). Disorders include splayed legs, crooked toes and cross beaks (Figure 16).



**Figure 16:** Cross beak (Photo Credit: M. Guinebrière, ANSES)

For instance, for crooked toes genetic variation has been identified linked to the immune system (Ye et al., 2006). However, also incubation conditions (such as a too low incubation temperature) can affect the incidence of disorders such as crooked toes (Oviedo-Rondón et al., 2009). Cross beaks may not always be detected at hatching, as they do not affect the mobility of the chick. When a cross beak is severe, the chick will have problems with eating and will likely not survive the rearing period. Of course, chicks may also be culled at hatching due to a low reactivity or a poor navel score or general chick quality (poor chick health). A poorer chick quality can also be associated with a lower hatchability. Also here, genetic background will play a role, but in interaction with incubation conditions and other factors. For instance, in a large study focusing on data from one of the major breeding companies, it was found that hatchability was affected by the hybrid, breeder age, egg weight uniformity, length of egg storage and season (Bouba et al., 2021).

Second, variation may exist in behaviour and welfare traits between different genetic lines and hybrids. In commercial egg production, both brown hybrids (laying brown eggs) and white hybrids (laying white eggs) are used. Local markets often require a specific egg colour, and sometimes even for a specific type of production system (e.g. white for cage eggs and brown for non-cage eggs). This limits the choices that producers can make regarding hybrid used. Generally, white hybrids tend to be more mobile and flightier than brown hybrids. This has advantages in terms of the white birds' ability to use the three-dimensional space in multi-tier systems (Heerkens et al., 2016b). However, it also means that they are more sensitive to stressful events and human disturbance (de Haas et al., 2014b). The male birds also have larger combs which can reduce their vision and feed intake. Brown hybrids on the other hand, are more docile and less agile than white hybrids (Pufall et al., 2021). This can increase the risk of floor eggs, if hens do not move up towards the nests for egg laying. Brown flocks also tend to be more at risk of piling and smothering, as they seem to have a stronger tendency to

flock together than white flocks. Risk factors for injurious pecking may also differ between white and brown flocks. de Haas et al. (2014b) found that in white flocks, stress and fearfulness played a major role in the risk of outbreaks of injurious pecking. In the brown flocks, injurious pecking was more related to litter restriction and limited opportunities for environmental pecking.

Third, breeding programmes may positively or negatively affect behaviour and welfare traits. Laying hen breeding for a long time was mainly focused on egg production traits. In some cases, this led to negative trade-offs with welfare traits. For instance, the pressure on early maturation and an early onset of egg laying has been shown to be correlated to an increased risk of feather pecking (Jensen et al., 2005). Furthermore, more recently it was shown that early maturation and a small body size in relation to egg size can enhance the risk of caudal keel bone fractures (Thøfner et al., 2021). For keel bone fractures, relationships with bone strength exist. Breeding for increased bone strength may reduce the risk of keel bone fractures, especially in housing systems where the possibilities for exercise are limited (Candelotto et al., 2017; Toscano et al., 2020; Eusemann et al., 2020; Dunn et al., 2021). In the last 10 years, laying hen health and welfare traits have become increasingly important in the breeding programme (Preisinger, 2021); however, further progress in this area is still needed. Fernyhough et al. (2020) argued that more transparency is needed with regard to welfare traits (bone health, cannibalism, morbidity), concomitant with the current level of transparency with regard to productivity traits, meaning that information regarding these welfare traits should be available to the market for each commercial hybrid, similar to information on expected growth and egg production curves. The fact that welfare traits are becoming more important is likely also related to the transition to non-cage systems in Europe. In cage-free systems, behaviour of laying hens plays a much more important role in flock management compared to cage systems. For instance, to reduce the risk of injurious pecking, the major breeders have a system in place where they collect plumage condition and mortality data in family groups in group housing systems. Only those families that maintain a good feather cover and a low mortality level are selected for the next generation. New selection methods, that allow breeders to model the social (or indirect) genetic effects of birds on their group members have been important to support this type of selection (Ellen et al., 2014). At the same time, this type of selection usually still takes place in cage systems, while more and more hens in the EU are housed in non-cage systems. In the future, breeding companies should develop tools to monitor welfare and performance of individual hens in non-cage systems. The information is needed at the individual level, to be able to make the link to the bird's genetic background. Key welfare indicators used by breeders are nesting behaviour, general liveability, good feather cover until the end of the production cycle, and strong bones. The ultimate target will be an extended production period (up to 100 weeks of age) in order to boost lifetime egg production per hen housed (Preisinger, 2021). With the longer laying period, attention should be given to the birds' bone quality, as bones of older hens may become more brittle, increasing the risk of bone fractures due to osteoporosis, having negative impact during laying period (keel bone fracture) and during catching at the population (wing fracture).

### 3.4. Welfare of laying hens and the risks associated with alternative systems (organic, free range and barn) compared to the cage system currently allowed (Specific ToR 1)

Specific ToR 1 is answered in two parts.

In Part 1, a comparison of cage (as described in the mandate) and non-cage systems is presented based on the evidence provided to answer the General ToRs and considering the consequences of these two types of systems on the welfare of laying hens (Section 3.3.1). In laying hens, cage systems are represented by furnished cages, whereas non-cage systems are represented by single-tier and multi-tier systems, as well as mobile housing. Access to covered verandas and/or outdoor ranges can be provided to laying hens kept in any non-cage system, so these are included here too.

In Part 2, parameters called 'minimum enclosure characteristics' are provided that are expected to prevent the negative welfare consequences arising from husbandry conditions. These parameters are based on the evidence provided to answer the General ToRs for non-cage systems (Sections 3.2.1–3.2.3). This second part focuses on laying hens, but when pullets or layer breeders have specific needs compared to the laying hens, they will be specified according to the knowledge available. These 'minimum enclosure characteristics' are related to hazards (exposure variables) which lead to the highly relevant welfare consequences identified. Specifications for the parameters that are necessary to ensure animal needs (e.g. feeder and nest design) are also provided.

### 3.4.1. Part 1: comparison of cage and non-cage systems for laying hens

Table 18 provides a summary of the welfare consequences that laying hens may experience in cage- or non-cage systems. Welfare consequences that were ranked as highly relevant are described in Section 3.3, those ranked as moderately or slightly relevant are mentioned in Table 16 but not described further. For a definition of listed welfare consequences and methodology for ranking of them the reader is referred to EFSA AHAW Panel (2022a,b).

Additional space can be provided through a covered veranda, access to an outdoor range, or both. Verandas can be particularly useful when used in combination with outdoor range, where they provide a buffer zone, reducing the thermal and sensory contrast between indoor and outdoor conditions. The general effects on the welfare consequences of adding access to covered veranda or outdoor range to non-cage systems are presented in Table 16.

**Table 18:** Comparison of welfare consequences that laying hens may experience in cage systems and non-cage systems. (Red: highly relevant; Orange: moderately relevant; Green: slightly relevant) and impact of access to covered veranda or outdoor range on welfare consequences. ↘: decreasing welfare consequence; ↗: increasing welfare consequence, –: no effect)

Welfare consequences	Cage system <sup>(a)</sup>	Non-cage system <sup>(b)</sup>		
		Indoor	With access to covered veranda	With access to outdoor range
Bone lesions	Red	Red	–	–
Group stress	Red	Red	↘	↘
Inability to perform comfort behaviour	Red	Green	↘	↘
Inability to perform exploratory or foraging behaviour	Red	Green	↘	↘
Predation stress	–	–	–	↗
Restriction of movement	Red	Green	↘	↘
Skin disorders	Red	Red	–	–
Soft tissue lesions and integument damage	Red	Red	↘	↘
Resting problems	Orange	Orange	↘	↘
Metabolic disorders <sup>(c)</sup>	Orange	Orange	–	–
Reproductive disorders <sup>(c)</sup>	Orange	Orange	–	–
Stress from visual and/or auditory stimuli (Sensorial stress) <sup>(c)</sup>	Orange	Orange	↘	↘
Inability to express maternal behaviour <sup>(c)</sup>	Orange	Green	–	–
Respiratory disorders <sup>(c)</sup>	Green	Orange	–	–
Eye disorders <sup>(c)</sup>	Green	Green	–	–
Gastro-enteric disorders <sup>(c)</sup>	Green	Green	–	↗
Heat stress <sup>(c)</sup>	Green	Green	↗	↗
Cold stress <sup>(c)</sup>	Green	Green	↗	↗
Muscle disorders <sup>(c)</sup>	Green	Green	–	–
Locomotorily disorders (including lameness) <sup>(c)</sup>	Green	Green	–	–

(a): Furnished cages.

(b): Single- or multi-tier systems; mobile houses are included in non-cage systems with access to covered veranda or outdoor range.

(c): Welfare consequence ranked as moderately relevant or slightly relevant.

The major difference between cage- and non-cage systems is that laying hens in cage systems experience restriction of movement and inability to perform comfort and exploratory or foraging behaviour, which have been identified as highly relevant welfare consequences. In non-cage systems, these are considered only slightly relevant, because comfort and exploratory or foraging behaviour is

observed more frequently owing to larger available spaces and the provision of foraging material and/or litter which is typically difficult to provide adequately in cage systems. These welfare consequences affect all birds in the flock and last for the entire life. Covered verandas and outdoor range areas facilitate the performance of comfort behaviours (such as dustbathing), locomotion, exploratory and foraging behaviours to a greater extent than non-cage systems without these facilities.

Bone lesions (mainly keel bone fractures) are highly relevant for laying hens housed in any housing system, because bone lesions are related to the high biological demands of a high and efficient egg production. In non-cage systems, in which the birds navigate through a more complex environment compared to cage systems, low light intensity or incorrect placement of furniture may increase the risk of collisions, and hence of keel bone fractures. There is no clear benefit from access to verandas or outdoor systems on bone lesions. Birds with bone lesions may be less likely to use outdoor areas provided. On the other hand, outdoor area access allows birds to have more exercise and may thus have a protective effect against bone lesions.

Group stress is highly relevant in all indoor systems due to different hazards such as high stocking density, large group size or uneven/insufficient resource allocation, increasing the risk of injurious pecking, but also the risk of competition and social conflict. Access to a covered veranda and/or an outdoor range may reduce the risk for group stress and its negative welfare consequences (e.g. soft tissue and integument damage due to severe pecking).

Skin disorders (here mainly due to red mite infestation) are ranked as highly relevant for laying hens in any housing system with or without access to outdoor space.

Soft tissue lesions and integument damages (due to injurious pecking or from beak trimming) are highly relevant welfare consequences for laying hens in any housing system. Access to a veranda and/or outdoor access may help to reduce the risk of injurious pecking due to greater space availability, diversity of resources and choices for the birds, as well as presence of structures to enable birds to escape from conspecifics. Nevertheless, it should be considered that in regions with very high light intensity (i.e. Mediterranean countries), access to covered verandas may increase the risk of feather pecking if the birds cannot move to darker areas.

Access to outdoor areas may result in an increase in other welfare consequences including predation stress, gastro-enteric disorders and externally transmitted diseases (such as avian influenza). Particularly in hot or cold conditions, measures may be needed to prevent birds from becoming too hot or too cold in a veranda and outdoor area. In general, though, access to a veranda provides birds with more choice of lighting and temperature conditions and can help to maintain litter quality. Verandas can also be used if full outdoor access has to be prevented in the event of an external disease threat, such as Avian Influenza.

### 3.4.2. Part 2: minimum enclosure characteristics

As illustrated above, and in the answers to the General TORs, the use of cage systems, such as those described in this opinion, is associated with highly relevant welfare consequences for laying hens, pullets and layer breeders.

Based on scientific literature and expert judgement, minimum enclosure characteristics preventing the negative welfare consequences that arise from husbandry conditions, are described below and summarised in Table 3.

The minimum enclosure characteristics are described in the following categories: environment (i.e. group size, surface area and stocking density), equipment (e.g. elevated structures, litter, enrichment, nests, feeder, drinkers, noise) and management of the husbandry conditions including the use of covered veranda and outdoor range (light, temperature, air quality).

#### 3.4.2.1. Environment

##### *Minimum group size*

Keeping birds in individual cages leads to isolation stress (described in Section 3.3.6). Since individual cages are only used for pedigree layer breeders and data on this animal category are very limited, expert judgement assumed that an individual laying hen, pullet or layer breeder would experience isolation stress if kept without a conspecific, independently of enclosure size. Therefore, the recommended minimum group size for laying hens, pullets and layer breeders is two.

However, taking into account the complex social behaviour a bigger group size will be advantageous (e.g. 4–5 birds kept together). However, the minimum number of birds required to allow the expression of complex social behaviours cannot be derived from the literature.

### Maximum group size

Scientific information on the effects of group size on laying hen welfare in indoor systems in commercial settings, is scarce.

Nicol et al. (2006) found no effect of group size from 2,450 to 4,200 birds on any of the welfare indicators measured. Bone fractures or H:L ratio were not affected by either group size or stocking density.

The reduction of aggressive encounters in larger groups of six birds as compared to groups of three was already reported by Hughes and Wood-Gush (1977). Additional evidence of reduced aggression in groups of laying hens (10 vs 120 birds) was provided by D'Eath and Keeling (2003) who showed that hens in groups of 10 discriminated between familiar and unfamiliar subjects by showing more aggression towards unfamiliar ones. In groups of 120 birds, the overall level of aggression was reduced and that attempted fights and aggressive outcomes were no different if directed to unfamiliar or familiar hens. These results that are consistent with the 'tolerance hypothesis' of reduce aggression with increasing group size (Estevez et al., 1997). Hughes et al. (1997) also suggests that agonistic encounters in groups of 700 (free range system) and 300 birds (aviary system) are rare. In deep litter and having access to free range (group of 700 birds) over the entire scanning period of 23.8 h, a total of 10 agonistic interactions was observed.

Behavioural synchrony and spatial clustering are indirect measures of social stress and resource availability. For these measures (Keeling et al., 2017) found no difference between groups of 15, 30, 60 and 120 birds at constant stocking density. The authors concluded that in birds in groups of up to 120, no negative effects on welfare were seen, if sufficient feeders, drinkers and perches were provided at different locations in the pens to allow birds to synchronise and cluster with their immediate neighbours. No evidence of increased aggression with increasing groups size was detected for the same group sizes while maintaining density constant (Estevez et al., 2003).

Channing et al. (2001) compared the spatial distribution and behaviour of laying hens at a constant space allowance (540 cm<sup>2</sup>/birds) in eight pens with colonies of five different sizes (323 birds (N = 1), 374 birds (N = 2), 431 birds (N = 2), 572 birds (N = 1) and 912 birds (N = 2)). Group size did not appear to affect the spatial distribution of birds and similar percentages of birds were observed in the different identified areas. However, birds did not distribute evenly throughout their pens: within specific pen areas, space allowances varied between 1,111 and 243 cm<sup>2</sup>/bird. This variation, which reflects the flux of birds from one part of the pen to another, was the numerically highest, although not statistically significant, for the larger colony sizes and may have adverse implications for welfare.

Gilani et al. (2014) studied systems with outdoor access and found that range use reduces as flock size increases. However, other studies found that increased flock size had no effect on the proportion of birds using the range (Bestman and Wagenaar, 2003; Gebhardt-Henrich et al., 2014). Steinfeldt and Nielsen (2015) found an increment in the use of the outdoor range in smaller groups (going from 396 to 792 individuals), but density was a confounded factor as it increased from 6 to 12 layers/m<sup>2</sup> with no proportional increment in resource availability. Hens at the lowest stocking density and the smallest group size appeared to use the outdoor area more extensively, based on an assessment of vegetation cover, although largest use was of 16% of the birds at the most, and these birds were located primarily within 50 m from the house. A substantially higher frequency of use has been reported in other studies with commercial flocks. From 33% mean use frequency over the production period in groups of 6,000 birds (Rodriguez-Aurrekoetxea and Estevez, 2016), to 68–82% in two flocks with 18,000 birds (Larsen et al., 2017). In both studies welfare benefits were reported for those birds more frequently ranging in the outdoor area (Larsen et al., 2017). From the results of these studies, it is clear that groups size does not, in itself, determine the frequency of use of the outdoor range. Other factors such as the structure of the outdoor range (Buijs et al., 2020), early experience during the production phase (Rodriguez-Aurrekoetxea and Estevez, 2016), genetic strain (Wurtz et al., 2022) and most likely, experience during early rearing are important factors determining frequency of range use. In addition, scientific results have consistently identified large interindividual variations in the level of use of the indoor (Hughes and Wood-Gush, 1977; Rodriguez-Aurrekoetxea and Estevez, 2016) and outdoor (Gebhardt-Henrich et al., 2014; Larsen et al., 2017; Buijs et al., 2020) space.

There is no scientific evidence nor research available defining or showing the maximum group size for layer breeders, under which negative welfare consequences that arise from husbandry conditions can be prevented. However, even if not about group size specifically, in layer breeders, the male to female ratio should be carefully considered not to be lower than 10 females per male at production scale (Ross 308, see Section 3.3.3).

### Minimum size of the area

#### Horizontal space needed for behavioural activities

Parameters used to estimate spatial distribution patterns in chickens (e.g. minimum, mean and maximum distance travelled per time unit, total distance travelled per time unit or net distance) have been shown to be positively affected by the total space available to the birds, in such a way that their values increase or decrease in parallel to the total space available to the birds (Estevez and Christman, 2006; Estevez et al., 2010).

Active behaviours include all actions that birds perform in their normal daily routines for body maintenance, thermoregulation, social contact, exploration of feeding sources or in the search for mates. The spatial area where these daily activities occur is defined as the home range and includes short- and long-distance movements and sporadic movements (e.g. exploring the edges of the home range). The average home range size in single-tier housed commercial laying hen flocks has been reported to be 80 m<sup>2</sup> (Rodriguez-Aurrekoetxea and Estevez, 2016). The home range size is considered appropriate to define the minimum size of the area to house groups of hens larger than 30 individuals. For groups of less than 30 birds, which is typically the size of a family group of layer breeders, experts judged it appropriate to base the minimum size of the area on the activity centre size instead of the home range.

The activity centre is defined as the area of highest activity, with a 50% probability of finding the bird in the calculated area (Leone et al., 2007). Rodriguez-Aurrekoetxea and Estevez (2016) reported an activity centre for laying hens in single-tier systems of  $26.76 \pm 3.7$  m<sup>2</sup> for the age group of 37–53 weeks and  $31.8 \pm 3.7$  m<sup>2</sup> for the age group of 54–69 weeks. Hence the experts judged that the minimum area for the enclosure of groups of less than 30 birds should be at least 25 m<sup>2</sup>. In recent paper (Paul et al., 2022), a relatively large space allowance (0.72 m<sup>2</sup> /bird) combined with other resources resulted in good welfare. If we translate this into a small group of up to 30 birds, this will give an area of 30 birds  $\times$  0.72 m<sup>2</sup> = 21.6 m<sup>2</sup>, which is similar to the recommended minimum area of 25m<sup>2</sup>.

#### Vertical space needed for behavioural activities

Hens require a three-dimensional space to be able to perform basic body movements. Caged hens have a strong preference for cages which have a large vertical space allowance. When filmed in cages of unrestricted head room, nearly 25% of hens' head movements occurred above 40 cm height from the floor (Dawkins, 1985) although Albentosa et al. (2007) only found 15% of observed scans with heads above 35–41 cm. In this study, a cage ceiling explains this difference. They found no differences in behaviour (feather raising, wing raise/flap, wing/leg stretch, body shake) between cage heights of 38 and 45 cm apart from yawning (involving an upward stretch of the head and head scratching (Albentosa et al., 2007)). Vertical movements (fly, jumps) were not counted, and wing flaps and stretching behaviours were infrequent or even absent, explaining the absence of differences. In Nicol (1987b) hens behaved differently when cage heights varied between 30 and 55 cm. At 55 cm, head stretching, head scratching and body shaking were performed at a higher rate and feeding and cage pecking at a lower rate. There was also an increase in the time spent sitting at 55 cm.

Measurements on laying hens have shown that the average height for a laying hen that is standing is 34.8 + 1.3 cm and this increases to 38.6 + 2.3 cm for turning (Mench and Blatchford, 2014). The behaviour that requires most height to perform is wing flapping. Research on laying hens reported that a bird wing flapping is 49.5 + cm high (Mench and Blatchford, 2014), i.e. approximately 15 cm greater than the height of the standing bird.

Considering that commercial hens height is from 35 and 40 cm depending on the breed. Based on a standing height of 40 cm and assuming the 15 cm increase for wing flapping, the requested height would be 55 cm. Breeding birds, especially breeding males, are taller, estimated to be 60 cm when standing (Personnel communication L. Schrader, 2022). These bigger birds will presumably also have longer wings, so the additional height needed to wing flap was increased from 15 cm to 17 cm. As a result, the estimated height of a breeder when wing flapping is proposed to be 77 cm. Accurate measurements of the height of layer breeders are required for more precise estimates of their height when performing different behaviours.

The usable space (above the floor and tiers) should be high enough for the bird to perform all natural behaviours including wing flapping and therefore should be more than 55 cm for layers or 77 cm for breeding birds.

Vertical space is also important for behaviours such as jumping, flying and perching. Tiers in aviaries with perches are designed to fulfil the need of hens to roost on elevated structures (Brendler

and Schrader, 2016). White hybrids have previously been found to prefer height over graspable perches for roosting at night (Schrader and Müller, 2009). Therefore, it is considered that a vertical distance between perches and between the highest perches and the ceiling is the same as the one requested above the usable area. This will allow birds to perform all natural behaviours, as well as landing and taking off without an increase risk of colliding with a perch or the ceiling.

A minimal total height (from floor to ceiling) of 2 m in barn will allow human to enter and check for the birds.

### Used space in relation to density and group size

A few studies have investigated the preferences of hens for space. When given a free choice among different sized cages, individual hens reared in floor pens spent more time in larger enclosures than in smaller ones, although the hens regularly visited and spent short periods of time in the small cages (Nicol, 1987b). Using an operant method, Lagadic and Faure (1987) showed that groups of 4 hens would work to increase space above 400 cm<sup>2</sup>/hen, but only for 25% of the time, suggesting that there may be an intermittent preference for a larger cage that is context dependent (Cooper and Albentosa, 2003; Cooper and Appleby, 2003). Besides the space required for movement, interindividual distance i.e. to maintain separation between individuals, should be accounted for. It is important for group housing even though its relative importance in different group sizes is unknown. Hens may prefer to distance themselves from other individuals, having different preferred interindividual distances that vary with activities as shown extensively in broiler research (Leone and Estevez, 2008a,b). Still, more research is needed to determine the strength of motivation (Widowski et al., 2016) or the environmental and social conditions that lead to changes in interindividual distances.

The effects of space and group size on behaviour have not been investigated as extensively as in broilers, particularly in large groups where birds group together during different times of the day and around resources, leaving other areas largely unoccupied (Channing et al., 2001; Collins et al., 2011). Savory et al. (2006) found that the average nearest interindividual distances in groups of six hens were around 20, 37, 44, 49, 51 and 53 cm at space allowances of 600, 2,400, 4,800, 7,200, 9,600 and 12,000 cm<sup>2</sup>/hen, respectively. This suggests that similar to the findings in broilers, interindividual distances in laying hens will be adjusted according to the space available and socio-spatial conditions, and may distance themselves from other birds, to perform behaviours that require space like for stretching and exercising. It also suggests that there is a 'tailing off' of this effect, with additional space slowing down the increase of interindividual distance.

On farms, laying hens cluster together, rather than spreading out as much as possible (Nicol, 2007; Collins et al., 2011). Clustering can have negative consequences for welfare such as increased competition for resources (Arnould et al., 2001), aggressive behaviour (Bestman et al., 2009), and injuries (Frankenhuis et al., 1991) leading to other types of welfare consequences. Clustering is believed to result from social or resource attraction (Asher et al., 2013). High stocking density may restrict movement, and group size may affect the number of animals performing highly competitive behaviours such as feeding, nesting, or socially facilitated behaviours such as dustbathing (EFSA, 2005) and thus, affect the available free space. Despite the effects of density, in general, movement is facilitated in larger groups even if density is maintained constant (Liste et al., 2015). Thus, space provided to birds in non-cage systems allow greater opportunities for locomotion than in cage systems, allowing the birds to access a mean home range area of approximately 80 m<sup>2</sup> in indoor commercial single-tier systems (Rodriguez-Aurrekoetxea and Estevez, 2016).

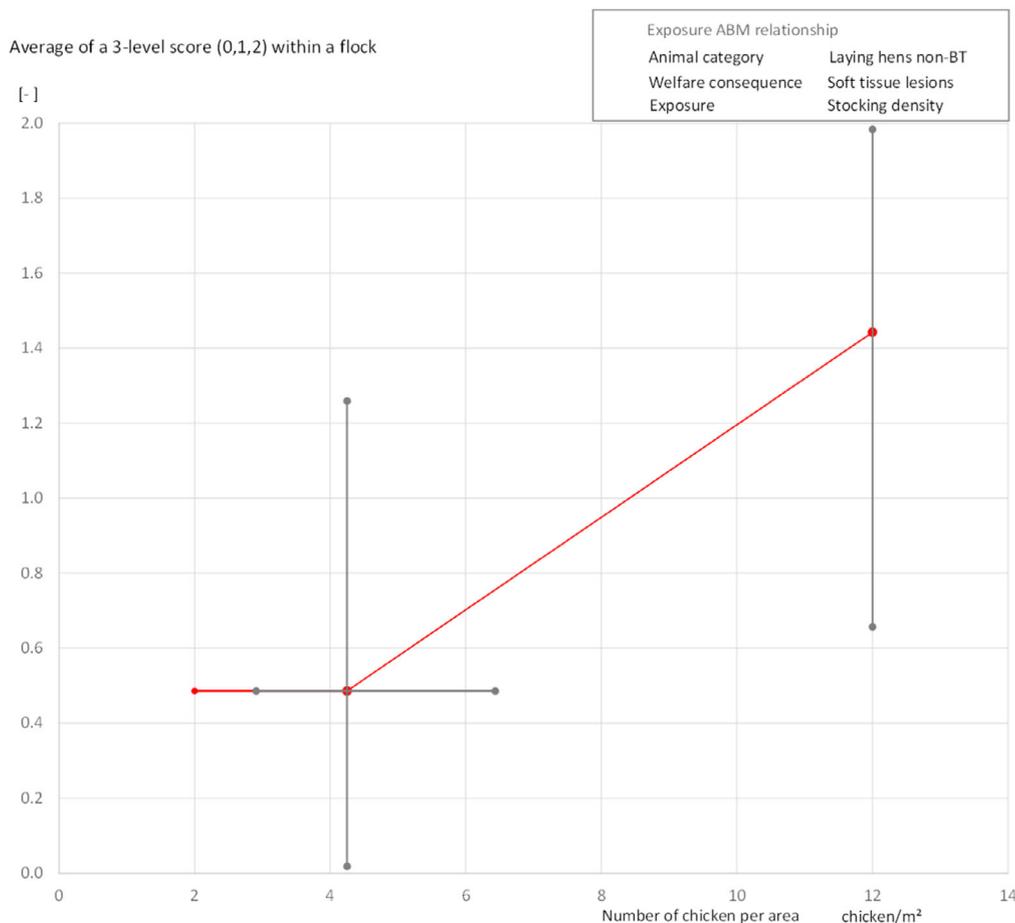
#### *Maximum stocking density*

To define a maximum stocking density, a literature search was carried out, that informed both an expert knowledge elicitation (EKE) and a mathematical model which was developed to estimate space requirements for selected behavioural needs.

### Results from expert knowledge elicitation

An EKE was executed to judge the effect of stocking density on plumage damage score. Experts expressed high uncertainty providing their judgements. At a very low density (2 hens/m<sup>2</sup>), the experts estimated the average plumage score to be 0.45 (interquartile range (IQR): 0.2–0.9) on a scale of 0–2 (0: hen with perfect plumage, 1: hen with some plumage damage, and 2: hen with significant plumage damage). Subsequently, the experts judged that at a density of 12 hens per m<sup>2</sup> the average plumage score would be 1.45 (IQR: 1.0–1.5). Again, there was considerable variation between experts and experts place wide confidence bands around their own estimate. Using EKE, experts agreed on a

maximum stocking density of 4.4 bird/m<sup>2</sup> (2,272 cm<sup>2</sup>/bird) for non-beak-trimmed birds (90% credibility range of EKE from 3 to 6.6 birds/m<sup>2</sup>), above which there is a significant increase in plumage damage (Figure 17). Details are provided in Appendix B.

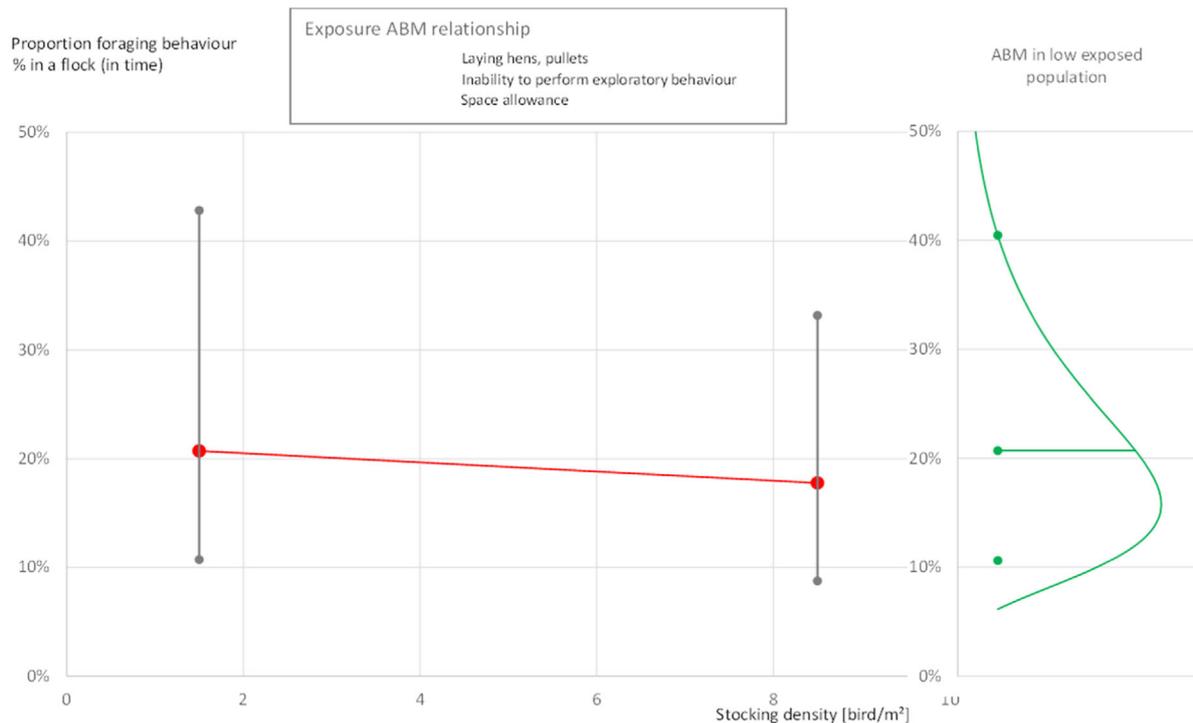


**Figure 17:** Using experts knowledge elicitation, experts agreed on a maximum stocking density of 4.4 birds/m<sup>2</sup> for non-beak-trimmed birds (90% credibility range of EKE from 3 to 6.6 birds/m<sup>2</sup>) above which there is a significant increase in plumage damage

In a second EKE, the effect of stocking density on percentage time performing foraging behaviour (walking, scratching, pecking) was elicited. The experts judged that at a very low stocking density (2 hens/m<sup>2</sup>; 5,000 cm<sup>2</sup>/hen), hens would spend 20% of their time foraging (IQR:15–30) whereas at a high stocking density (9 hens/m<sup>2</sup>; 1,111 cm<sup>2</sup>/hen) hens would spend 17% (IQR: 13–25) of their time foraging. Because the difference in foraging between the lowest and highest density was so small, it was concluded that density did not have an important effect on the foraging behaviour of laying hens (Figure 18) when considering space allowances between 1,111 and 5,000 cm<sup>2</sup> per bird.

Experts agreed that stocking density had only a minimal effect on foraging, whereas other factors, such as the accessibility, size and quality of the litter area or access to outdoor, were much more important.

Details are provided in Appendix B.



**Figure 18:** Consensus graph EKE on effect of stocking density on average percentage of birds showing foraging behaviour (walking, scratching, pecking)

### Results from the behavioural model on space requirement for selected behaviours

In addition to the EKE, a mathematical model was developed to determine a maximum stocking density that would still allow laying hens to show behavioural needs. Improved animal welfare conditions mean that stocking densities were equal or below legislative requirements, cage housing was excluded, and/or enrichment and outdoor area were present. Behaviours considered behavioural needs for laying hens were selected and data extracted on the proportion of hens performing these behaviours within a flock of hens under optimal conditions using mean, median, optimum and stabilised optimum of the extracted data (Table 19). Details of the methodology is described in Section 2.2.2.1, and additional results are presented in Appendix C.

Data were extracted from available references, for each of the behaviours selected. If different hybrids were used in the study, only the data for the high-performing laying hens were used. If different housing environments or stocking densities were tested in the study, the values were extracted from those treatments/environments that were more likely to allow for more animal welfare compared to the current standard. If different values were given over different trials, times of day, etc., these were averaged. If the data were not given as percentages in the results, but were presented graphically, the data were extracted using WebPlotDigitizer (<https://automeris.io/WebPlotDigitizer/>). The data should reflect how high-performing laying hens behave under improved conditions.

In total, 10 studies published after 2004 were chosen for data extraction which resulted in 44 data points (N, specific values for a given behaviour), ranging from 2 data points for wing/leg stretching to 7 data points for foraging and dustbathing. On average, 4.9 data points are extracted per behaviour.

**Table 19:** Selected behaviours and the proportion of hens performing these behaviours within a flock of hens under optimal conditions are provided. The behaviours have been standardised to 100% in each approach of the model (median, mean, optimum, stabilised optimum)

Behaviour	Label	Median	Mean	Optimum	Stabilised optimum
		%	%	%	%
Standing	stationary	32.6%	26.1%	17.3%	20.9%
Sitting	stationary	8.0%	12.6%	4.2%	5.1%
Walking	active	17.9%	18.3%	20.5%	20.9%
Foraging incl. Scratching	active	13.3%	14.1%	26.8%	23.0%
Dustbathing	active	1.6%	2.9%	6.4%	5.0%
Preening	active	6.9%	8.5%	13.6%	11.6%
Wing/leg stretching	active	1.3%	1.2%	1.4%	1.7%
Wing flapping	active	1.2%	0.9%	0.8%	0.9%
Drinking/Eating	stationary	17.1%	15.4%	9.1%	11.0%
<b>Sum</b>		<b>100.0%</b>	<b>100.0%</b>	<b>100.0%</b>	<b>100.0%</b>

Table 20 describes the outcome of the behavioural space model. The multiplication of the area occupied by a chicken (Appendix C, Table C.2) with the percentage of birds performing the behaviours (Table 19) can be converted into the number of birds per m<sup>2</sup>. The different models result in a range of 3.7 birds per m<sup>2</sup> (2,738 cm<sup>2</sup>/bird) in the optimum model, 3.7 birds per m<sup>2</sup> in the model using the stabilised optimum, 4.0 birds per m<sup>2</sup> (2,523 cm<sup>2</sup>/bird) in the model using the mean and 3.9 birds per m<sup>2</sup> in the model using the median (2,542 cm<sup>2</sup>/bird).

**Table 20:** This table reports the final outcome of the behavioural space model. Given are the nine selected behaviours. The total area a chicken covers in cm<sup>2</sup> is given for each behaviour selected. The frequencies that each behaviour is expressed are combined with the space needed to express this behaviour. The outcome is a space profile reflecting a high-performing laying hen in an improved environment. The sum of space needed per modelling approach is related to 1 m<sup>2</sup>. The outcome is a specific number of chickens per m<sup>2</sup>

Behaviour	Label	Total area									
		per chicken	Behaviour					Calculation			
			alpha	Median	Mean	Optimal	Stabelized optimal	Median	Mean	Optimal	Stabilised optimal
			cm <sup>2</sup>	%	%	%	%	cm <sup>2</sup>	cm <sup>2</sup>	cm <sup>2</sup>	cm <sup>2</sup>
Standing	stationary	2059.2	32.6%	26.1%	17.3%	20.9%	672.1	537.0	356.2	431.0	
Sitting/ Resting	stationary	1,468.9	8.0%	12.6%	4.2%	5.1%	116.8	185.6	61.9	74.9	
Walking	active	3,872.2	17.9%	18.3%	20.5%	20.9%	693.2	710.2	794.1	807.5	
Foraging	active	2,785.5	13.3%	14.1%	26.8%	23.0%	370.6	392.9	745.9	640.7	
Dustbathing	active	2,413.4	1.6%	2.9%	6.4%	5.0%	39.7	70.4	154.2	119.8	
Preening	active	2,494.1	6.9%	8.5%	13.6%	11.6%	172.4	212.2	338.4	289.7	
Wing/leg stretching	active	3,627.4	1.3%	1.2%	1.4%	1.7%	47.8	42.9	49.9	60.4	
Wing flapping	active	6,425.8	1.2%	0.9%	0.8%	0.9%	77.1	56.1	50.5	56.5	
Drinking/ Eating	stationary	2,057.1	17.1%	15.4%	9.1%	11.0%	352.5	316.0	186.8	226.0	

Behaviour	Label	Total area								
		per chicken	Behaviour				Calculation			
		alpha cm <sup>2</sup>	Median %	Mean %	Optimal %	Stabelized optimal %	Median cm <sup>2</sup>	Mean cm <sup>2</sup>	Optimal cm <sup>2</sup>	Stabilised optimal cm <sup>2</sup>
<b>Weighted average</b>										
<b>Total area per chicken</b>							2,542.0	2,523.2	2,738.0	2,706.7
<b>No. chicken per m<sup>2</sup></b>							<b>3.9</b>	<b>4.0</b>	<b>3.7</b>	<b>3.7</b>

Using the results obtained from the behavioural model, experts concluded that a maximum stocking density of 3.9 birds/m<sup>2</sup> (median value during day light) allows full and unconstrained expression of selected behaviours (90% certainty range from 3.5 to 4.5 birds/m<sup>2</sup>).

### Combining EKE and model results

Considering both approach EKE and behavioural model, it is concluded that the welfare consequences 'restriction of movement', 'inability to perform exploratory and foraging behaviour', 'inability to perform comfort behaviour' and 'soft tissue and integument damage' can be prevented if minimum requirements for enclosure are implemented and if a maximum stocking density of 4 laying hens or layer breeders/m<sup>2</sup> is used (66–100% certainty). This equates to 2,500 cm<sup>2</sup>/bird.

### Maximum stocking density for pullets

No EKE or behavioural space model calculation was carried out for estimating space requirements for pullets.

Chicks can be kept at high densities in the first 2 weeks of life (approximately 100–120 birds/m<sup>2</sup>) which enables them to keep warm. No differences in plumage condition were noted when pullets were reared for the first 10 days of life at 107 vs 121 birds/m<sup>2</sup> or and between 54 and 60 birds/m<sup>2</sup> to 4 weeks of age (Schwarzer et al., 2022). Beyond these first 4 weeks of life, there is some evidence of improved plumage condition in pullets reared at lower stocking densities. Bestman et al. (2009) found that risk of plumage damage increased at more than 18 pullets/m<sup>2</sup> by the end of the rearing period, while Schwarzer et al. (2022) found that the risk of plumage damage increased across the range of 11.9–31.6 pullets/m<sup>2</sup> of animal densities by end of rear. Nicol et al. (1999) found significantly more gentle feather pecking at 30 birds/m<sup>2</sup> than at lower densities of 22, 14 or 6 birds/m<sup>2</sup>. No significant effects on severe feather pecking or aggression were recorded. Reduced feather pecking behaviour has been found for pullets reared at 18 birds/m<sup>2</sup> compared with 23 birds/m<sup>2</sup> (Zepp et al., 2018), although one study reported no effects when comparing the same two densities 18 to 23 pullets/m<sup>2</sup> (Liebers et al. 2019). von Eugen et al. (2019) reported no adverse effects on the welfare of pullets housed at 20 birds/m<sup>2</sup>, and these authors found that indicators of stress were lower at 20 birds/m<sup>2</sup> than for birds housed at very low (7 birds/m<sup>2</sup>) or very high (60 birds/m<sup>2</sup>) densities. By measuring the space occupied by sitting birds of different strains and age and following the assumption that layer pullets should have at least as much additional space per bird as broiler chickens (40% more than the birds occupy when sitting), Krause and Schrader (2019) concluded that the stocking density for pullets should not exceed 15 birds/m<sup>2</sup> at the end of rearing period which is coherent with the improvement related above for densities of 18 pullets/m<sup>2</sup>. Chicks in first 2 weeks of life spend more time resting and sleeping in close proximity than older birds and so can be housed at higher stocking densities.

Assuming a maximum stocking density of 15 pullets/m<sup>2</sup> at the end of rearing (Krause and Schrader, 2019) which equates to a stocking density of 21 kg/m<sup>2</sup>, maximum animal densities for younger chicks can be extrapolated considering the estimated body weight at different ages. Therefore, the following densities per age group are expected to prevent welfare consequences 'restriction of movement', 'inability to perform exploratory and foraging behaviour', 'inability to perform comfort behaviour' and 'soft tissue and integument damage':

- 15 pullets/m<sup>2</sup> (66–100% certainty) from 10 weeks to the end of rearing;
- 25 pullets/m<sup>2</sup> (> 50–100% certainty) from 6 to 9 weeks (until day 63 of age);
- 40 pullets/m<sup>2</sup> (> 50–100% certainty) from 3 to 6 weeks (until day 42 of age);
- 100 pullets/m<sup>2</sup> (> 50–100% certainty) for first two weeks (until day 14 of age).

### 3.4.2.2. Equipment

#### *Elevated structures, platforms and perches*

#### **Motivation to be in elevated position and to perch**

Chickens are strongly motivated to perch (Olsson and Keeling, 2000; Olsson and Keeling, 2002) and roosting at night has been identified as a behavioural need for chickens (Weeks and Nicol, 2006). Domestic chickens use elevated structures for resting and other behaviours (Wood-Gush and Duncan, 1976). During their growing phase, chickens start to use elevated structures such as perches at daytime already during the first days of life (Newberry et al., 2001; Heikkilä et al., 2006; Wichman et al., 2007; Kozak et al., 2016; Malchow et al., 2019a; Malchow et al., 2019c). The use of perches at night-time starts to increase at the age of 3 weeks (Heikkilä et al., 2006; Riber et al., 2007; Malchow et al., 2019a) to approximately fourfold higher frequency compared to daytime in adult laying hens (Brendler and Schrader, 2016).

At night, the predominant behaviour of chickens on perches is resting (Blokhuis, 1984). During daytime, chickens also rest on perches but other behaviours such as preening, standing, locomotion, and exploration are shown more often than resting (Struelens et al., 2008a; Blokhuis, 1984; Braastad, 1990; Newberry et al., 2001). In housing systems where food and water is provided on elevated structures (e.g. multi-tier systems) laying hens will also use perches to change between tiers and to reach food and water facilities (Campbell et al., 2016a; Giersberg et al., 2019).

The main motivation for perching is probably seeking shelter against predators. In accordance with this anti-predator hypothesis, laying hens prefer high compared to low perches for night-time resting (roosting) in commercial aviary systems (Brendler and Schrader, 2016; Campbell et al., 2016b; Giersberg et al., 2019; Odén et al., 2002; Steinfeldt and Nielsen, 2015) which can result in overcrowding of high perches while space on lower perches remains available (Brendler and Schrader, 2016; Campbell et al., 2016b; Ali et al., 2019). The anti-predator hypothesis is also supported by the finding that perching is associated with other anti-predator related behaviours such as vigilance behaviour, fearfulness and reaction towards potential predators (Keeling, 1997; Newberry et al., 2001; Donaldson and O'Connell, 2012; Brantsæter et al., 2016a). In addition, perches or other elevated structures function to escape from or to avoid agonistic interactions with group members (Cordiner and Savory, 2001; Donaldson and O'Connell, 2012).

#### **Height of the elevated structure/perch**

Depending on the respective housing system, perches are offered at different heights and in different spatial arrangements. Within tiers of alternative systems or within furnished cages, perches often are installed at a height of about 7 cm above the grids (to allow eggs rolling below the perches). Under the grids there is either a dunging pit (e.g. single tier systems) or manure belts (e.g. multi-tier systems and furnished cages) to collect the faeces. Perches are commonly installed in parallel to the feeding troughs. A comprehensive review on perching behaviour and perch design criteria including height and position of perches within layer housings is given in EFSA AHAW Panel (2015) and in Schrader and Malchow (2020). Regarding design criteria, EFSA AHAW Panel (2015) concluded that perches should enable a stable sitting posture that can be achieved through non-slippery platforms or grids.

#### **Perching or being in elevated position**

Although chickens are capable to grasp rods, poles and branches, their feet are less adapted for grasping rather than for locomotion and scratching (Sustaita et al., 2013) and the anatomy of chickens' toes are adapted to their use on the terrestrial ground. Consequently, chickens are counted as 'ground-dwellers' (Birn-Jeffery et al., 2012; Pike and Maitland, 2004). Also, in preference studies laying hens do not show clear preferences for perches that they can grasp when compared with non-graspable grids or wire while roosting at night (reviewed in: EFSA AHAW Panel, 2015; Schrader and Malchow, 2020). In a recent study, laying hens showed a higher usage of grids compared to perches for night-time roosting when both resting sites were alternately offered (Malchow et al., 2022). However, in that study usage of resting sites at night was low compared to other studies. Thus, it is

still an open question whether it is necessary to offer perches to layers, or whether elevated platforms meet their need to perch.

The height of the resting site seems to be more important for chickens while roosting compared to the possibility to grasp (Schrader and Malchow, 2020). This is indicated by a study from Schrader and Müller (2009) in which perches and perforated elevated platforms (grids) were offered to laying hens at different heights for night-time roosting. In this study, hens preferred the elevated platforms when perches and grids were offered at the same height. However, when perches were offered in a low and grids in an elevated position, the hens preferred the elevated grids. Although it is well known that chickens prefer high compared to lower sites for night-time roosting, it is still not fully understood at which height roosting sites should be offered to meet the birds' motivation for elevated night-time roosting. This is mainly due to the uncertainty on how laying hens perceive height (Schrader and Malchow, 2020). However, in commercial multi-tier systems laying hens prefer the highest tier for night-time roosting and even if perches are arranged within tiers at different heights, they prefer the respective highest perch in a given tier (Brendler and Schrader, 2016). When perches were offered at different heights, laying hens preferentially roosted on the perches of a height above 90 cm. At lower heights there was no difference in the number of hens roosting on the perch and at the floor (Brendler et al., 2014). Based on three studies, EFSA AHAW Panel (2015) recommended that the perch height should be at least 60 cm above the ground in non-cage systems.

### Access to elevated structures

Laying hens prefer to move by walking and have a poor flying ability. Thus, perches and elevated platforms should be arranged in a way that layers can safely access and move between them. EFSA AHAW Panel (2015) and Schrader and Malchow (2020) reviewed scientific results on accessibility of perches and elevated platforms (such as tiers) for laying hens. Hens move upwards more easily than downwards and smaller horizontal distances can be covered more easily than longer horizontal distances. In case of too steep angles and too long distances between different sites of a housing system the risk of bad landings (associated, e.g. with keel bone fractures) increases. Access to elevated platforms and tiers can be facilitated by ramps allowing hens to reach the platforms or tier by walking. At angles with a slope  $> 40^\circ$  the force needed by hens to climb up increases (LeBlanc et al., 2018) and ramps with a slope  $> 40^\circ$  are less used compared to ramps with lower angles (Zheng et al., 2019). Regarding the surface of ramps, grids seem to be more suitable compared to ladders (Norman et al., 2018; Pettersson et al., 2017; Zheng et al., 2019).

Although there is only little evidence that laying hens prefer perches of certain materials and the material does not seem to significantly affect the behaviour while perching, the slip resistance of the surface of perches is strongly related to perch material and is an important perch characteristic (reviewed in: EFSA AHAW Panel, 2015; Schrader and Malchow, 2020).

### Shape and material of the perch

Studies on the shape (i.e. the cross-section) of perches are often confounded with the material and width of perches and results on effects of perch shape on behavioural and health measures are inconsistent. However, shape is strongly related to the width and the curvature of the upper side of perches. A flat curvature providing a large contact area between perch and bird and a high width of the perch increase the contact area of the keel bone and the feet while resting and are likely to be beneficial regarding the risk for keel bone deformations and fractures (review in: EFSA AHAW Panel, 2015; Schrader and Malchow, 2020). Pickel et al. (2010) systematically tested the effect of material (wood, steel, rubber coat) and diameter (27, 34, 45 mm) of round perches on perching behaviour during night-time. Neither material nor diameter of perches affected the usage. However, balance movements were lowest on perches coated with rubber and on perches with the largest diameter. In another study, Pickel et al. (2010) measured the contact area and the peak force on foot pads and on the keel bone while laying hens were sitting and standing on perches differing in width (3.4, 4.4 or 6.0 cm). In sitting hens, the peak force on foot pad was lowest and the contact area under the keel bone was largest on the widest perch (6.0 cm) compared to the smaller perches (3.4, 4.4 cm). In standing hens, the peak force on foot pad was not affected by perch width but the contact area of foot pads was highest on the widest perch compared to the smaller perches.

In addition to a non-slippery surface, perches should be wide enough and of an adequate shape allowing grasping. If perches are difficult to grasp, then birds will attempt to maintain stability with increased balance movements (Pickel et al., 2010). Soft material (softwood, rubber-covering) can decrease the risk of keel bone damages and foot pad lesions.

Although laying hens do not consistently prefer certain materials, shapes or width of perches, these properties of perches affect the footing stability while perching and probably affect the risk for keelbone fractures. Regarding perch width, EFSA AHAW Panel (2015) recommended a perch width of between 3 and 6 cm to minimise balance movements and reduce peak force under the keel bone and the foot pads.

There is a lack of knowledge on the effects of different characteristics of elevated platforms (e.g. material, mesh size of grids).

### Space needed on elevated structures

Riddle et al. (2018) investigated the space laying hens used while perching in four hybrids (Hy-Line Brown, Bovans Brown, DeKalb White, Hy-Line). The mean width of laying hens while perching ranged from 18.03 cm (DeKalb White) to 21.89 cm (Hy-Line Brown). While lying on the floor (resting on her breast with her head held up) the length of hens ranged from 29.91 cm (Hi-Line) to 33.28 cm (Bovans Brown). Assuming that the space occupied while lying on the floor and perching in a sitting position is comparable and that in a perching hen the space needed in front (head side) and behind of the perch (tail side) equals while perching, the horizontal distance between perches should be at least the length of a sitting hen, i.e. about 30 cm (15 cm head side plus 15 cm tail side). If a perch is located next to the pen wall, more space than half of the length of a hen is needed to allow entering and leaving the perch, i.e. 20 cm.

However, the minimum perch length and distance between perches needed may become larger if layer strains with larger body sizes are used.

Giersberg et al. (2017) examined the body width of brown and white layer pullets while sitting on a perch. At week 8, 12 and 19, mean body width was 10.7, 10.7 and 14.0 cm for brown and 10.5, 10.4 and 13.0 cm for white layer pullets.

Despite the preference of chickens for elevated resting site, the risk for keel bone fractures increases with an increasing three-dimensional complexity of the housing system (Käppeli et al., 2011; Heerkens et al., 2016b; Riber and Hinrichsen, 2016; Rufener and Makagon, 2020) as also described in Section 3.3.1 (Bone lesions). This is associated with an increased risk of falls and collisions (Stratmann et al., 2015). Consequently, keel bone fractures are less often observed in single-tier compared to multi-tier systems (Riber and Hinrichsen, 2016). In addition, in multi-tier systems with more than three tiers, inspection and catching of e.g. injured birds is assessed to be hampered.

### Litter

Litter materials differ in their effect to fulfil the behavioural needs to dustbath and to forage (Duncan and Kite, 1989; Hogan and Van Boxel, 1993; Guinebretière et al., 2014). For dustbathing, laying hens prefer material of a fine structure such as sand or peat compared to material with larger particle sizes such as straw or wood shavings (Petherick and Duncan, 1989; van Liere et al., 1990; Sanotra et al., 1995; Gunnarsson et al., 2000a; Shields et al., 2004; Alvino et al., 2013; Monckton et al., 2020). However, wood shavings and slightly friable materials are preferred over plain plastic or AstroTurf® mats (Scholz et al., 2010; Guinebretière et al., 2014) indicating that access to any substrate is an important external stimulus for dustbathing. Although feed has also a fine structure with particle sizes comparable to sand, laying hens prefer wood shavings for dustbathing (Scholz et al., 2010) which may be related to the lipid content in feed. Scholz et al. (2011) offered feed with different lipid contents as dustbathing material (in a experimental pen) and observed a preference for those with the lowest lipid content. This finding could be explained by the function of dustbathing to remove stale lipids from the plumage (van Liere and Bokma, 1987; Van Liere et al., 1991; van Liere, 1992; Scholz et al., 2014). Thus, material for dustbathing should have small particle size and not contain lipids.

Regarding foraging behaviour, laying hens prefer litter that contains palatable particles over litter that does not contain nutritive ingredients (Scholz et al., 2010; Guinebretière et al., 2014). They do not show clear preferences for non-nutritive litter material (de Jong et al., 2007; Monckton et al., 2020), but any litter is preferred when compared with non-littered artificial pads, e.g. plastic or AstroTurf® mats (Scholz et al., 2010; Pokharel et al., 2018) that are often used in furnished cages.

Litter area: Foraging, a combination of pecking and scratching, occupies about 40% of chickens' daylight hours (review in Monckton et al., 2020). A recent meta-analysis showed that the time spent on a substrate also correlated positively with the amount of space it covered, with hens spending more time on substrates that covered larger areas (Monckton et al., 2020). Additionally, it has been shown significant variations in space used by white and brown hens (DeKalb White, Hy-Line White, Hy-Line Brown, and Bovans Brown). Brown hens occupy more space while dustbathing than the white hens

(around 1,140 and around 960 cm for brown and white hybrids, respectively (Grebey et al., 2020)). However, white hens more likely show synchronous dustbathing, and longer dustbathing bout durations compared to the brown hens. To let the birds foraging and dustbathing simultaneously, a minimum size should be defined. However, after examination of current knowledge, it is not possible to specify a minimal surface area needed, therefore the experts agree on providing at least one third of the usable area covered with litter.

**Litter depth:** Moesta et al. (2008) compared 2 depths of litter composed of wood shavings (2 and 20 cm) under experimental conditions. Substrate depth played a small role on dustbathing behaviour of laying hens. A continuous layer of deep litter covering the full floor area is considered to allow birds to perform their foraging and comfort behaviours.

**Litter quality:** Litter has to be dry and friable to allow the performance of foraging and comfort behaviours. The litter around the drinkers is often moist or wet. Thus, management measures are necessary to always keep the litter dry and friable. In non-cage systems, depending on the humidity in the barn, the litter may become moist and clump, especially in barns with outdoor access and humid outdoor conditions (as the hens' feet and/or plumage may be wet when entering from outside).

**Age:** The litter continuously accessible from day 1 of life will stimulate the development of foraging and comfort behaviours in birds. However, in laying hen access can be restricted during morning periods until a regular nest-box use is established.

#### *Enrichment/Additional foraging opportunities*

Enrichment is a concept referring to additions and modifications to the production environment that facilitate the biological adaptation of the animals to their environment and improve their welfare (Newberry, 1995). A complex environment increases the birds' opportunities to choose among resources and stimulates locomotion (review in Estévez and Newberry, 2017; Campbell et al., 2019) with profound and long-lasting benefits for the welfare and stress adaptation of chickens. Different forms of environmental enrichment can be used to prevent or reduce problems such as injurious pecking, plumage damage (van Staaveren et al. 2021), or unwanted sexual interactions in breeding flocks (Leone and Estevez, 2008a).

Early experience of a complex environment can improve biologically relevant traits related to coping abilities. Enrichment provision may be particularly important during the early rearing, helping young pullets to respond faster to new stimuli and may act as a protective buffer against stress (Campderrich et al., 2019). Pullets having access to variation within resource types appear to be less fearful, experience less chronic stress, better able to cope with pathogenic challenges, and have an improved learning ability (Nazar et al. 2022; Yan et al., 2021). For instance, rearing young chicks under dark brooders (where the brooding heat is provided within an enclosed, dark area of the house) is effective in reducing injurious pecking at a later age which results in improved feather cover and reduced skin injuries and mortality due to cannibalism. Other welfare benefits include improved rest in dark-brooded chicks and reduced fearfulness at all ages tested (i.e. from 4 to 26 weeks) (Sirovnik and Riber, 2022). Thus, available scientific evidence suggest that complex environment should be provided since day 1 of life, and throughout the life of the bird.

Enrichments can be provided in various forms such as objects or materials that are suitable for foraging, dustbathing, exploring. Edible enrichments are of special interest for birds such as bales (of straw, or hard-pressed alfalfa), pecking blocks, sand boxes, hard surface materials that can be scratched and pecked. Pecking materials are mainly provided with the intention of avoiding or reducing injurious pecking or plumage damage. However, the results of different pecking devices are not always consistent (reviewed in Jung and Knierim (2018)), probably due to the multifactorial factors (including bird age, bird hybrid, beak trimming status and housing system, age when they are provided) impacting the efficiency of the enrichments.

It is recommended to renew regularly the enrichments that are depleted due to the pecking and scratching actions of the birds. Scatter feed items (such as grains or mealworms) can be an effective form of enrichment, as they encourage the birds to perform natural foraging behaviours (Pichova et al. (2016) and Wood et al. (2021), in broilers). Complex environment can also be achieved thanks to elevated surfaces, outdoor area, veranda, cover panels and dark brooders. Increasing the structural complexity of an enclosure with perches, dividers, and cover can increase resting and preening activity (Newberry and Shackleton, 1997).

## Nests

Pre-laying and nesting behaviour are among the highest motivated behaviours in laying hens, and both are regarded as a behavioural needs (Weeks and Nicol, 2006). During pre-laying behaviour, hens will search for a suitable nest site and perform nest building behaviour. If available, they mould substrate with their body and feet to build a nest. Pre-laying behaviour is followed by laying of an egg (oviposition). Afterwards, hens will stay in the nest for resting (Cooper and Appleby, 1996; Cooper and Appleby, 2003). Thus, to meet the behavioural needs of pre-laying and nesting behaviour the nests offered should have certain characteristics. There is a learn component on the use of the third dimension and recognition of the nest site that may limit nest use when young layers are moved into the production house, which will reflect in the so-called floor eggs, i.e. by eggs laid outside the nests in the litter or the grid floor of the housing, since jungle fowl construct breeding nests on the ground (Nicol, 2015). Therefore, it is of high importance to learn to the birds to recognise and use nest.

**Size and partitioning:** Hens are known to prefer smaller group nests (e.g. 0.43 m<sup>2</sup>) compared with larger ones (e.g. 0.86 m<sup>2</sup>) (Ringgenberg et al., 2014). The use of central partitions improves the attractiveness of group-nests (Ringgenberg et al., 2015). Considering the high nest laying in modern cage free systems, there is no evidence to question what is applied in farm in Europe: at least 1 nest for every 7 hens and for group nests at least 1 m<sup>2</sup> of nest space for a maximum of 120 hens.

**Flooring:** Hens prefer a soft nest floor substrate which they can mould with their body and feet and which allows nest-building behaviour (Duncan and Kite, 1989), such as straw, which is more suitable than plastic floor, synthetic grass or wire mesh (Huber et al., 1985; Struelens et al., 2005; Struelens et al., 2008b). In a study including only organic substrates as nesting material, straw was preferred compared to wood-shavings and peat (Clausen and Riber, 2012).

**Enclosure:** Hens prefer enclosed nests (i.e. covered from both sides, the back and the top; Appleby and McRae, 1986). Group nests are more likely to be used when they have a non-transparent front curtain, and the other three sides are enclosed. Sliced curtains allow the hens to investigate the entire length of the nest. The hens value the protection by the more closed front of the one-piece curtain, but the sliced curtains provide an easier access for nest inspections (Stämpfli et al., 2013).

**Slope:** The floor of nests is sloping, thereby allowing the eggs to roll away to the egg belt without breaking. When comparing with 18% slope, 12% is preferred by hens (Stämpfli et al., 2011). However, no data are available between 12% and 14% actually practised in Europe. Therefore, there is no scientific data against the 14% slope actually allowed.

**Nest position:** In non-cage systems for laying hens, nests often are provided in rows arranged on top of each other. In multi-tier systems, the nests of the higher row(s) are preferred compared to the lowest row (Lundberg and Keeling, 1999). In systems where hens have to choose between identical nests arranged in long rows: the preference is for nests in the corner or at the end of a row (closest to the entrance of the barn or at the ends of rows (Riber, 2010; Clausen and Riber, 2012). This so-called gregarious nesting may result in overcrowding of these nest sites (possibly leading to smothering) whereby nests in the middle of the row remain unused most of the time. repetition of what is just below Clausen and Riber (2012) offered nests that differed in their appearance. However, this heterogeneity of nests did not result in an equal use of nests but in a higher frequency of gregarious nesting in the most attractive nests. The authors suggested that the nests in the middle of rows should be made more attractive in order to reduce gregarious nesting at both ends, like using yellow colouring in the middle nests (Huber-Eicher, 2004; Zupan et al., 2007). Another factor that can affect the usage of nests in non-cage systems is their positioning within the pen. Lentfer et al. (2013) found significantly more eggs laid in nests located near the service corridor inside the building compared to nests located near the (closed) outdoor area. However, the causes for this unequal distribution remained uncertain. Practical guidelines often suggest placing nipple drinkers for the birds in front of nests in order to increase nest attractiveness. However, this could not be confirmed in a scientific study (Lentfer et al., 2013).

**Nest accessibility:** Laying hens should have easy access to nests, in particular if they are offered at a certain height in non-cage systems. Access to nests can be improved by the use of platforms in front of the nests. Compared to plastic grids, more mislaid eggs and balance movements were observed when two square wooden perches (4.5 cm in diameter) were installed in front of nests. This indicates that grids are better to enable access to nests compared to perches as used in the study (Stämpfli et al., 2013). Platforms in front of the nests or the area in front of nests that are integrated in the tiers of an aviary should offer sufficient space, because a larger number of birds may occupy this area. It is recommended to provide a platform instead of perches in front of the nest in floor housing systems

(Stämpfli et al. (2013); Jung and Knierim (2018) influencing factors decreasing feather pecking or plumage damage). In multi-tier systems, it is recommended platforms in front of the nests should be more than 30 cm in width (Lentfer et al., 2011).

### Feeders

Actual practice in Europe (Council Directive EC, 1999/74/EC) is to use a minimum feeder space of 10 and 12 cm/hen in cage and non-cage systems respectively. This is based on the width of the average bird and meant to allow all birds to feed simultaneously. When Oliveira et al. (2019) performed a small-scale experiment (60 hens in one colony cage), they found that even at the most liberal feeder space allocation (12.0 cm/hen) a maximum of 59% hens fed simultaneously (mean 32%). A larger scale experiment (Sirovnik et al. 2018, four multi-tier pens with 200 hens per feeder space allocation) found that at 10 cm/hen on average less than 10% of the hens fed simultaneously. This calls into question whether providing enough feeder space to theoretically allow all hens to feed simultaneously is a valid approach. The results could be interpreted to mean that hens do not choose to feed simultaneously. Alternatively, it could be interpreted to mean that to allow all hens to feed simultaneously they need more space at the feeder than the width of their body. In line with this, Keeling (1994) found that hens in small groups stocked at a low density (1.8 hens/m<sup>2</sup>) kept at least 24 cm from the nearest individual while foraging, suggesting that to allow birds to feed simultaneously feeder space may need to be approximately twice the width of their body.

The reason why hens may need more feeder space than the width of their body to feed comfortably is likely that close proximity during feeding leads to aggression. Aggression and jostling (i.e. displacement attempts) at the feeder increased with decreasing feeder space from 10 to 4 cm/hen in a near linear manner (Sirovnik et al., 2018). There is no scientific knowledge on the amount of synchronous feeding or aggression/jostling in non-cage systems at feeder space allowances above 10 cm/hen. Aggression at the feeder may cause birds to eat at times when there are fewer others at the feeder. In line with this, the average number of birds feeding simultaneously was found to decrease with decreasing feeder space, but only slightly: from 32% at 12 cm/hen to 29% at 6.5 cm/hen (Oliveira et al., 2019) and from 9% at 10 cm/hen to 6% at 4 cm/hen (Sirovnik et al., 2018). The two studies differ markedly in the number of birds feeding simultaneously at a specific feeder space allowance. This may in part be due to the fact that Sirovnik et al. (2018) used feeder lines that could be accessed from both sides and included the space on each side separately. While feed intake was not found to be affected by feeder space in Oliveira et al. (2019), it was greater when more feeder space was provided in Sirovnik et al. (2018). However, some of this may be due to increased spillage.

### Drinkers

Laying hens spend 2–8% of the morning and afternoon drinking (Carmichael et al. 1999; Channing et al. 2001; Ramadan and Von Borell, 2008). If this behaviour would be homogeneously spread over time, this would suggest that 1 drinking space per 12.5 hens would usually suffice (as 12.5 hens drinking 8% of the time leads to a total drinker occupancy of 100%). However, water intake is known to peak 2–3 h prior to lights-off (Xin et al. 2002) and drinking is synchronised (i.e. seeing other hens drink makes a hen more likely to drink herself, at least in small groups (Hoppitt and Laland, 2008)). This means that more drinkers are required for hens to enable them to drink freely. In line with this, the practice on the field (implementing the current Council Directive (EC, 1999/74/EC)) sets a minimum of 1 drinking nipple per 10 hens in non-cage systems (or 2.5 cm of linear water through space, or 1 cm of circular water through space per animal). The type and location of drinkers is also relevant for welfare. The use of bell-drinkers has been associated with an increased risk of feather pecking (Green et al., 2000; de Haas et al., 2014a) and the use of hanging bell drinkers and drinkers located on plastic slats has been associated with an increased risk of vent pecking (Pöttsch et al., 2001).

There is currently no scientific evidence to support that more drinking points per hen than required by the legislation would improve laying hen welfare. In fact, providing more drinking space may even affect welfare negatively: a positive association between drinking space and feather pecking was reported (Jung and Knierim, 2019), although there is a need to validate this result further before concluding. Drinker space requirements may have to be revisited as climate change gives rise to more heat stress events, as heat stress can double drinking times (Mack et al., 2013).

## Noise

Hens have sensitive hearing abilities. Their hearing depends on the frequency (tone) and the pressure level of the sound, this latter having the major influence on the subjective impression of loudness. Hens are able to detect very low-frequency tones (< 20 Hz) at pressure levels of approximately 40–80 dB, but they are most sensitive to tones between 400 and 4,000 Hz which can be detected at pressure levels below 20 dB (Temple et al., 1984; Saunders and Salvi, 1993). There is no evidence that hens can detect tones above 8,000 Hz.

Chronic exposure to sound pressures of 80 dB in young laying hens led to more resting behaviour and reduced egg production (O'Connor et al., 2011). Exposure to sounds of more than 80 dB increases stress (Campo et al., 2005) and is a risk factor for the early onset of feather pecking. Hens will learn to perform pecking tasks to turn off pure tones played at 105 dB or above, other mechanical and animal sounds played at 90 dB (MacKenzie et al., 1993) or the sounds of a commercial poultry house played at 100 dB (McAdie et al., 1993). Classical music played at 75 dB has been associated with increased fearfulness in hens (Campo et al., 2005). In studies of broiler chickens, exposure to sounds of 80 dB or 100 dB for 10 min led to a significant increase in plasma corticosterone concentration, an indicator of stress (Chloupek et al., 2009).

Average noise levels inside commercial laying hen housing have been reported as 59.7–63.4 dB (Lourenconi et al., 2015). The maximum sound pressure level recorded for flocks of pullets and hens housed in barn or free-range systems was 72 dB, with an average level of 58.3 dB (with sound levels in some flocks showing wide variation levels during the period of measurement (Gilani et al., 2013)). The probability of severe feather pecking in adult flocks was increased when pullets had been reared in environments with more variable sound levels (Gilani et al., 2013).

### 3.4.2.3. Management

#### Light

In laying hens, O'Connor et al. (2011) studied the effects of a dimmed light environment on behaviour and welfare. The study was designed to investigate whether high or low light intensity (150 lx vs 5 lx) during the critical period of coming into lay (16–24 weeks of age) influenced behaviour, physiological stress and production in laying hens. Hens in the low light pens were less active but preened and dust-bathed more than those housed in 150 lx. Ma et al. (2016) provided laying hens with a choice between compartments with different light intensities (< 1, 5, 15, 30 or 100 lx). The results show that the hens generally spent more time in lower light intensities. Specifically, the hens spent 6.4 h (45.4%) at 5 lx, 3.0 h (22.1%) at 15 lx, 3.1 h (22.2%) at 30 lx and 1.5 h (10.3%) at 100 lx under light condition and an accumulation of 10.0 h in darkness (< 1 lx) per day. Most of the eggs were laid in < 1 lx (61.9% of total). Kristensen et al. (2009) studied the effects of light intensity on social behaviour in laying hens. Social communication between pairs of either unfamiliar or familiar hens of either similar or unequal rank (highest and lowest ranking within their home groups) was assessed under light intensities of 1, 5, 20 and 100 lx with all other cues present. Only the dimmest light of 1 lx perturbed some aspects of the competition for food. Dimming the light and red light are used in practice to limit damage due to injurious pecking. Shi et al. (2019a) and Huber-Eicher et al. (2013) indeed both found that red light and low light intensity (10 lx instead of 25 lx) reduced severe feather pecking. However, dimmed or red light is not a real solution to the problem of injurious pecking and may hamper social recognition and lead to poor eye health. Therefore, dimming the light or using red light should only be used as a measure of last resort.

Birds seem to prefer specific light intensities for specific behaviours and on average spend more time in low than in high light intensities, when given the choice.

#### Natural light/day light

Rana et al. (2021) performed a preference test in cage-reared ISA Brown birds, comparing preference for indoor standard light-emitting diode (LED) white light (control) vs one of three different treatment lights: (1) visible spectrum plus infrared wavelengths (VIS); (2) visible spectrum plus UVA wavelengths (UVA); and (3) visible spectrum plus UVA and UVB wavelengths (UVA/B) presented successively at low, medium or high levels of intensity. Hens spent more time under the low intensity of the UVA/B light treatment (62%), the low intensity of VIS light (61%), medium intensities of both UVA/B light (60%) and UVA light (59%), and the high intensity of the VIS light (58%) when compared with control light. Hens spent less time feeding under all intensities of UVA light and showed more foraging, ground pecking, and preening at lower levels of UVA/B light. The study suggests that UVA/B

light (sunlight) may have positive effects for hen range use, but during peak sun intensities, hens may need additional measures (e.g. shelter) to protect themselves. Confirmation of these findings in a free-range setting is needed. Bestman et al. (2009) showed that in organic flocks, the combination of not having litter at the age of 1–4 weeks and the absence of daylight at the age of 7–17 weeks was a significant predictor of feather damage during the laying period. This indicates that organic birds would benefit from daylight during rearing, as this likely stimulates range use later in life. Indeed, Bestman et al. (2019) confirmed that a larger amount of daylight inside the house was related to a higher range use and a better plumage cover in free range hens. Also for keel bone damage, the absence of daylight was identified as a risk factor in organic laying hen flocks (Jung et al., 2019).

Especially for free-range and organic flocks, there is evidence that daylight has positive effects on range use and helps to prevent keel bone fractures and feather damage. Some studies show that birds should be given access to daylight early on to observe these positive effects in the laying period. The Rana study provides evidence that hens prefer different types of light for different activities, which advocates for a varied light environment. For multi-tier systems, this is often already the case, with higher light intensities at the upper tiers and lower light intensities below the tiers in the litter.

### 3 D navigation

Moinard et al. (2004) studied accuracy of jumps in different light sources and intensities (5, 10 or 20 lx; incandescent or high- or low-frequency fluorescent). They found no effects on accuracy. In contrast, Taylor et al. (2003) did find an effect of light intensity, comparing lower light intensities of 0.8, 1.5, 6 and 40 lx. Especially the lowest light intensity of 0.8 lx was problematic.

Very low light intensities (< 1 lx) are problematic for the birds to make accurate jumps, increasing the risk of high impact collisions and keel bone damage (mainly older studies).

Birds prefer different light intensities for different behaviours and should therefore be provided with light intensities that facilitates different functional activities.

#### *Temperature*

The influence of temperature cannot be seen separate from the effects of relative humidity and air velocity, because both have a strong impact on temperature perception (Chepete and Xin, 2004; Karaman et al., 2007; Ruzal et al., 2011). Also stocking density and thus the possibilities for the bird to lose heat are of great importance in determining the thermal zone in which a bird will not experience thermal stress (Weeks et al., 1997; Holik, 2009).

Aerts et al. (2004) indicates a thermoneutral zone of 15–26°C, with an optimal temperature for production given as 18–21°C (Al-Saffar and Rose, 2002). Exact figures are hard to give, as they are strongly dependent of relative humidity and air velocity around the animal and also depend on the hybrid, age and feather condition of the bird. Meltzer et al. (1982) indicates a thermoneutral zone for pullets older than 8 weeks of 22–27°C. They also mentioned that laying hens only start panting to if the temperature is 29°C or above. Al-Saffar and Rose (2002) calculated based on 29 publications the effect of the environmental temperature on feed intake, egg production, body weight and various egg characteristics. They found a strong decrease in egg production, feed intake and shell strength at 28°C to 30°C and indicated that from that point onwards heat stress occurs, which is in line with Van Kampen et al. (1979).

Most breed management guides (Lohmann (2015); Hy-Line (2022)) recommend that chicks reared in non-cage systems should be kept at temperatures that reduce with bird age. The first week of life is critical as chicks are unable to maintain their body temperature without external heat input. Typical recommendations are: 0–7 days 30–35°C; 8–14 days 31–33°C; 15–21 days 29–31°C; 22–28 days 26–27°C; 29–35 days 23–25°C. After the age of approximately 6 weeks, pullets have similar thermal requirements to adult hens. Humidity during the first week of life should be approximately 60% dropping to a minimum of 40% thereafter. It is important to observe the behaviour of chicks as huddling can indicate that birds are too cold, while sitting with wings spread or panting indicates they are too hot. Dark brooders are beneficial because these allow chicks to choose warmer areas for resting, and cooler areas for active behaviours (Riber and Guzman, 2016).

#### *Air quality*

##### **Dust**

Dust pollution in the pullets and layer/breeders barn can be a burden to the respiratory tract of animals and humans. Dust is divided into different particle sizes, which have a physiological subdivision (for humans). Thus, particle sizes  $\leq 100 \mu\text{m}$  are inhalable and deposit in the mouth and nose;

$\leq 10 \mu\text{m}$  are thoracic and deposit in the larynx and lungs, from 4 to  $10 \mu\text{m}$  they are tracheobrachial and deposit in the trachea and bronchi, and  $\leq 4 \mu\text{m}$  they are respirable/alveolic and deposit in the alveoli. This division mainly applies to research in mammalian lungs, with little information on how different sizes of dust particles arrive at different parts of the avian respiratory system. In terms of composition, dust can be divided into organic and inorganic dust. Inorganic dust comes from building materials or materials brought into the house by the fresh air supply (e.g. soil). Organic dust comprises feather and skin particles, feed, faecal matter, viruses, mould, bacteria, fungi and bacterial endotoxins. There are legal requirements for the air leaving the barn. In Germany, for example, no more than 0.20 kg/h or  $20 \text{ mg/m}^3$  may leave the barn as dust emissions. There is no such legal requirement for the environment inside the barn.

Dust might be difficult to measure and is bonded in exhaust systems reducing the environmental impact. But this does not lower the impact of dust on the birds inside the barn. Over those studies measuring dust concentrations in the barn, a mean of  $5.31 \text{ mg/m}^3$  total dust is reported (Carpenter, 1986; Takai et al., 1998; Redwine et al., 2002; Homidan et al., 2003; Vućemilo et al., 2008). The measured total dust content varies in the studies between approx.  $1\text{--}10 \text{ mg/m}^3$  and is therefore very variable. David et al. (2015) summarised studies reporting respirable dust rates that range from  $0.1 \text{ mg/m}^3$  in conventional cages to a maximum of  $1.19 \text{ mg/m}^3$  found in aviaries. Variability in results among studies can be due to non-standardised test procedures, and to the dependence on the activity of the animals that stir up the dust which may vary a lot from a husbandry system to another and from a flock to another. Thus, total dust is often higher in floor systems and aviaries compared to cages due to the bird's activity level, access to litter and litter quality. Furthermore, the concentration of suspended dust decreases in direct proportion to the height above the floor. As a result, floor-raised birds are exposed to the highest concentrations of dust in the room. The amount of dust also varies depending on the time of the day, with high amounts of dust being related to moments of high activity in the morning or after the lights have been turned on due to an increase in behaviours like dust-bathing.

Huang and Guo (2020) investigated air quality in layer and broiler barns showing both in cages and in loose housing systems, average aerial dust concentrations were positively correlated with indoor air temperature and negatively to relative humidity (ventilation are used to reduce humidity and will also eliminate dust).

Dust may have direct and indirect effects on health, consequently affecting welfare. High dust concentrations have a particularly negative effect on the respiratory organs. In addition, there is the burden of pathogens and resulting disease patterns, including Marek's disease. Airborne microorganisms have been found attached to dust particles, meaning that dust in a poultry house may serve as a pathogen disseminator in addition to making the animals more susceptible to normally non- or low-pathogenic microorganisms. Organic dust particles are also antigenic, causing allergic reactions, inflammation of exposed areas, hypersensitivity, impaired lung clearance reactions, depressed immune system responses to infection and respiratory disease. Michel and Huonnic (2003), compared birds at the end of laying period reared in aviaries and caged hens and find pulmonary lesions of parabronchitis which were more extensive and severe in birds in aviaries. This was thought to be a result of the different dust concentrations, with respective maximum levels of  $31.6 \text{ mg/m}^3$  and  $2.3 \text{ mg/m}^3$ .

There is no knowledge of the direct effect on welfare or behaviour. Still, as with mammals, it cannot be ruled out that birds might feel discomfort with high levels of dust. It should be taken into account that laying hens are exposed to dust for a large amount of time because of their longer lifespan compared to broilers. In poultry systems, high dust levels are a concern for the health of poultry workers.

Current knowledge gaps comprise housing design, systems for ventilation and dust removal, dust-producing litter material, management routines, and knowledge of how dust affects the health and behaviour of laying hens.

### **NH<sub>3</sub>**

According to Naseem and King (2018), the ideal concentration of NH<sub>3</sub> is below 10 ppm, but up to 25 ppm no harmful effects are found for health of humans and animals. Kristensen et al. (2000) found the threshold for becoming aversive is between 0 and 25 ppm. Jones et al. (2005) indicated that broilers find a level of 10 ppm already aversive.

Amer et al. (2004) did not find negative effects on egg production and shell quality at concentrations of 50 ppm during a period of 4 weeks, but this is not reflecting welfare of animals. At 100 ppm, egg production was reduced, as was feed and water intake and bird weight. Higher

concentrations (10 ppm) led to eye shutting and eye blinking and 20 ppm led to behaviours such as struggling, quick beak movements and escape (McKeegan et al., 2005).

## CO<sub>2</sub>

Evidence of direct effects of CO<sub>2</sub> on welfare and health is lacking. Anderson et al., (1966) found no harm on the lungs of broilers that were exposed to 5,000 ppm (0.50%) for 8 weeks. Reece and Lott (1980) found negative effects on growth in broilers only at 12,000 ppm (1.2%). However, management guides for hens (RSPCA guide to laying hens, section E 6.2) (RSPCA, 2017) and broilers (Directive 2007/74) recommend a maximum of 3,000 ppm.

### 3.4.2.4. Access to covered veranda and/or outdoor range

Outdoor access provides hens with extra space and increased opportunities to fulfil behavioural needs, and to avoid other hens. The absence of access to an outdoor area mainly contributes to the welfare consequence 'inability to perform comfort behaviour', 'inability to perform foraging and exploratory behaviour', 'group stress' and 'soft tissue and integument damage'.

More specifically, the outdoor range forms a preferred environment for foraging and dustbathing (Campbell et al., 2017a) and increases comfort behaviour while decreasing agonistic behaviour (Sokołowicz et al., 2020). It is important to note that the extent to which outdoor access affects hen welfare depends on how much hens use the outdoor area. Increased use of the outdoor area is associated with a reduction in injurious pecking and better feather cover (Bestman et al., 2017; Lambton et al., 2010; Bari et al., 2020). Range use is also associated with a lower prevalence of keel bone fractures (Richards et al., 2012) and reduced fearfulness (Campbell et al., 2016a; Hartcher et al., 2016). However, for keel bone fractures and fearfulness it is often difficult to determine if those are the cause or the effect of low use of the outdoor range. Individuals that spent more time outdoors when young (18–21 weeks) were: 2.4 times more likely to survive to the end of lay than hens that spent little time outdoors and had a lower occurrence of fatty liver syndrome (Sibanda et al., 2020).

Less is known about the welfare consequence of not having access to a covered veranda, although this is likely to contribute to 'group stress' and 'soft tissue and integument damage' as withholding veranda access increases the risk of feather pecking (Jung and Knierim, 2019). Furthermore, a high percentage of hens using the veranda is associated with lower mortality (Jung et al., 2020). The beneficial effect of the veranda may be due to reduced stocking densities inside the house, more effective withdrawal from unwanted social contacts, availability of an area combining good air quality with shelter, increase free-range use and/or improvement hygienic conditions and litter quality in the hen house by forming an intermediate area to the free range (Jung et al., 2020).

Pop-holes situated on just one side of a multi-tier house are not necessarily accessible to all birds. Birds that roost on the 'far' side of the multi-tier structures are significantly less likely to use the pop-holes and outdoor range than birds that roost on the same side as the pop-holes (Pettersson et al., 2018).

The number of hens using the range increases with greater pop-hole width per bird (Gilani et al., 2014). It is reasonable to estimate that a hen will occupy approximate 20 cm width of a pop-hole when exiting (hens are approximately 12 cm wide but may exit or enter at an angle). Studies of tagged birds show that some birds exit in less than 10 s, while others sit on the pop-hole for many minutes (Richards et al., 2011).

A 1-m wide opening would therefore allow 5 hens to pass every minute; 300 per hour (with 150 exiting, and 150 entering on average). One metre for 500 birds would permit each bird to exit 2 or 3 times during daytime hours. An opening width of less than 1 m for 500 birds could reduce the ability of hens to access veranda or range areas.

#### Veranda/Range Management Plan

Early experience in accessing the free-range determine the birds' frequency and proportion of the area used later in production (Rodriguez-Aurrekoetxea and Estevez, 2016). There are large individual variations in range use among hens within flocks with some individuals never accessing the veranda or the free-range area while others remain as heavy users (Gebhardt-Henrich et al. 2014; Rodriguez-Aurrekoetxea and Estevez, 2016). Interindividual differences may be affected by their genetic background (Icken et al., 2008), but mainly by their experience as they become less frightened with increasing familiarity of the outdoor area (Grigor et al., 1995). Thus, early range experience has been shown experimentally (Grigor et al., 1995), and under commercial conditions (Bestman and Wagenaar, 2003) to increase range use. Early range experience can be facilitated by early placement

of the young layers at the production farms, ideally the birds should arrive at the farms at least 2 weeks before onset of lay and get access to outdoor range within these 2 weeks.

The quality of the range can greatly influence its usage and the health of the birds (Maurer et al., 2013). Even range use greatly reduces the risk of helminth infection (Thapa et al., 2015).

Most studies describe that multiple effects of weather conditions, bird management, quality of the range can influence the use of the range, including the space available per bird in the range at a constant flock size (Pettersson et al., 2016; Campbell et al., 2017a). Pettersson et al. (2016) found that % range use at any time rarely exceeds 50% and is sometimes below 10%, but steps can be taken to increase range use.

Weather has a large influence on range use with studies indicating up to 4 times more ranging under optimal weather conditions compared with poor weather conditions. (Keeling et al., 1988; Nicol et al., 2003; Hegelund et al., 2005; Richards et al., 2011). In areas with less extreme weather conditions use of the range may be more stable (Rodriguez-Aurrekoetxea and Estevez, 2016).

Cover (such as shelters, bushes or other vegetation) is known to have a positive effect on the percentage of the flock using the range (e.g. Nicol et al., 2003; Harlander-Matauschek, 2006; Sherwin et al., 2013), and will also increase the % of birds that will range at a distance from the house (Hegelund et al., 2005; Gilani et al., 2014). Dawkins et al. (2003) indicated that chickens actively select areas of the range with trees, and avoid bright light, which is to be expected as descendants of the red jungle fowl which inhabit dry forest (Collias and Collias, 1967). Although artificial shelters may produce similar effects to the birds as natural shelters, a vegetative cover has a wider range of benefits for the birds, for the biodiversity of the ecosystem and as an active mitigation measure to counteract climate change (Adrizal et al., 2008). Enrichment, such as bales of alfalfa, can also be used in outdoor production systems to enhance use of the range by animals (Bonnefous et al., 2022).

Although systematic studies on the extents of its benefits are currently lacking, access to a covered veranda of 20% of the floor area (not included in the usable area) will benefit the welfare of pullets, laying hens and layers breeders. It provides choice for different light and temperature conditions and provides more opportunities to perform natural behaviour as compared to indoor housing only. Access can be provided from 6 to 8 weeks of age onwards for pullets and 2 weeks before lay for layers and breeders, for at least 8 h/day.

If it is impossible to have a covered veranda attached to the barn (e.g. because of the absence of space with existing buildings, impossibility of installations due to conflicts with ventilation systems in areas where heat stress is a problem), some of the advantages of the covered veranda in terms of bird welfare, in particular the additional space, should be provided by giving the laying hens 20% additional space indoors with reference to the minimum space allowance.

### **3.5. Welfare of hens in furnished cages, and risks associated with rearing of animals non-beak trimmed (Specific ToR 2)**

#### **3.5.1. Injurious pecking**

##### **3.5.1.1. Description of different types of injurious pecking**

Some forms of inter-bird pecking are far more damaging than others. Gentle feather pecking occurs during both rearing and laying periods and can be socially motivated or repetitive in nature (Rodenburg et al., 2004; Nicol, 2019). However, feather damage associated with gentle feather pecking is generally minor and does not appear to be closely related to other more damaging forms of pecking. In contrast, severe feather pecking involves the pulling, removal (and sometimes the ingestion) of feathers (Harlander-Matauschek and Bessei, 2005). Severe feather pecking causes feather loss and pain. It can occur during the rearing period, but often increases as hens reach sexual maturity. Severe feather pecking can be associated with vent pecking (cloacal) pecking. Early signs of severe feather pecking include the disappearance of loose feathers (which are ingested), pain vocalisations and general restlessness. Vent pecking occurs more often in flocks with a high prevalence of feather pecking and cannibalism (Pötzsch et al., 2001; Lambton et al., 2015) and can result in serious injury and death. Toe pecking can be a self-directed behaviour or a form of inter-bird pecking, directed at toes, leading to wounds and in serious cases the loss of toes (Brandsæter et al., 2017). It can be seen in both pullets and adult birds (Sethy et al., 2018), and more often in white hybrids than brown (Riber and Hinrichsen, 2016). Aggressive pecking is directed towards the head and comb of conspecifics and may lead to small, punctiform comb wounds (Welfare Quality, 2009) or, in serious cases, to lesions at the back of the head.

Forms of pecking that cause injuries (vent pecking, tissue pecking directed at toes or areas of denuded skin, aggressive pecking directed to the head or comb) are sometimes grouped as injurious pecking despite their differing causations. The term cannibalism is used to group the forms of pecking that involve tissue ingestion (Rodenburg et al., 2013).

These different forms of injurious pecking often result in denuded overall plumage, skin damage, increased risk of poor thermoregulation, increased risk of infection and increased mortality (Green et al., 2000; Lambton et al., 2010; Nicol et al., 2013; Rodenburg et al., 2013).

### 3.5.1.2. Prevalence of injurious pecking

The prevalence of injurious pecking can be measured by the percentage of flocks affected within a population, or by the percentage of birds affected within a flock (Nicol et al., 2013).

Generally, injurious pecking is seen in all housing systems. The prevalence of injurious pecking in furnished cages was estimated as 33% (at 70 weeks of age) while it was observed at 24% in free-range housing system (at 61 weeks of age). However, the cannibalism prevalence was estimated 2.5% in furnished cages where it was 8.8% in free-range (Coton et al., 2019). Several studies have reported no significant differences in the prevalence of injurious pecking between caged and non-caged flocks (Sherwin et al., 2010; Decina et al., 2019a,b) but a very high variation in prevalence observed both within and between flocks in both system may explain the absence of significant differences.

One study of furnished cages (Morrissey et al., 2016) observed bird-to-bird pecking in only 0.12% of scans, and most of these pecks were gentle feather pecks. In a review of many published studies, van Staaveren and Harlander (2020) presented reports of the percentage of flocks affected ranging from 8 to 65% in different countries and husbandry conditions. When injurious pecking does occur within a flock, then the percentage of birds affected within a flock is also variable, ranging from 15% to 95% (van Staaveren and Harlander, 2020).

There is little information on the specific frequency of toe pecking, though one study found that 14% of farmers reported seeing this behaviour in their flocks (Brantsæter et al., 2017).

### 3.5.1.3. Causes of injurious pecking

Injurious pecking has many contributing causes. severe feather pecking is thought to be a form of redirected pecking that arises when birds are frustrated in their normal foraging, comfort and exploratory activities (Weeks and Nicol, 2006; van Staaveren and Harlander, 2020). An inverse relationship has been shown between foraging and feather pecking which supports this hypothesis (Blokhuys, 1986; Lindberg and Nicol, 1994). Thus, the 'inability to perform exploratory and foraging behaviour' and the 'inability to perform comfort behaviour' are the most important underlying causes of injurious pecking. When feed is presented in pelleted form and when litter substrates are not available or suitable to support foraging behaviour, hens appear to redirect their feeding and foraging motivation by pecking at conspecifics. More recent evidence suggests that some forms of gentle feather pecking and severe feather pecking are dysfunctional behaviours that arise when early life stress and inappropriate rearing environments cause neurobiological changes (van Staaveren and Harlander, 2020). Many other management factors increase the risk of injurious pecking, including high light intensities (Drake et al., 2010), poor air quality (Drake et al., 2010; Decina et al., 2019), use of bell drinkers rather than nipple drinkers (Green et al., 2000), infection with red mite (Heerkens et al., 2015), and hence 'skin disorders (other than tissue lesions)', all increase the risk. Poor air quality can be more of a problem in single-tier systems because, with lower numbers of birds in a house than in multi-tier, the house temperature can drop making it harder to keep litter dry and friable. Also, farmers can reduce ventilation in cold periods to try to maintain reasonable temperatures, but this further reduces air quality.

Vent pecking is somewhat related to severe feather pecking and shares some causal factors (Pötzsch et al., 2001; Lambton et al., 2015) but it is also associated with egg laying, especially if hens lay outside the nest boxes, or sit at elevated positions where their cloaca region is visible to other hens. Also, the onset of lay before 20 weeks of age elevates the risk of vent pecking (Pötzsch et al., 2001). The causes of toe pecking are not yet known but the hybrid seems to be relevant as it is observed more frequently in white laying hen hybrids and not in brown (Riber and Hinrichsen, 2016). Aggression results primarily from competition for resources, including competition for females in breeder flocks. In breeding birds, the 'inability to avoid unwanted sexual behaviour' can be a cause of injurious pecking. In small flocks of pullets or hens, aggression occurs during the formation of social dominance relationships based on individual recognition, and aggression reduces once these relationships are established, and birds are familiar (Duncan and Wood-Gush, 1971). When hens are

housed in large groups aggression is often low as birds assess their relative dominance through status signalling rather than aggression (D'Eath and Keeling, 2003).

#### 3.5.1.4. Welfare consequences associated with injurious pecking

Injurious pecking in both beak-trimmed, and non-beak-trimmed birds results in the welfare consequences 'soft tissue lesions and integument damage' (Figure 19) and 'group stress'.

Although feather pecking can originate in young birds, it does not usually result in pronounced injury at this early stage, so soft tissue lesions and integument damage is not considered a highly relevant welfare consequence for pullets.

Due to feedback effects, plumage damage, wounds and group stress can stimulate further injurious pecking, particularly feather pecking, vent pecking and aggression, which can result in increased mortality.

The prevalence of the welfare consequence 'soft tissue lesions and integument damage' is high as injurious pecking is a behaviour that can affect up to 65% of flocks and up to 95% of birds within affected flocks (van Staaveren and Harlander, 2020).



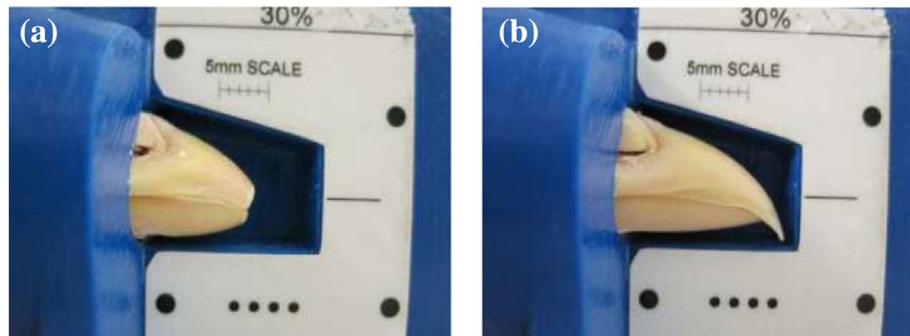
**Figure 19:** Consequences of damage to skin or vents caused by injurious pecking (Photo Credit: M. Guinebretière, ANSES)

### 3.5.2. Beak trimming

Because the welfare consequences associated with injurious pecking can be severe in most of the currently used husbandry systems, beak trimming is employed as a widespread management practice intended to reduce the prevalence of injurious pecking (particularly the number of birds affected within flocks), and the severity of damage caused by injurious pecking. The practice of beak trimming varies greatly between Member States. Some Member States have long-standing bans on the practice, others are moving towards a ban while in other Member States beak trimming is performed almost routinely, except in organic or other specialised production.

#### 3.5.2.1. Description of the process of beak trimming

Beak trimming is carried out in the first 10 days of age to remove the beak tip by infrared procedure, usually conducted on chicks prior to leaving the hatchery. Chicks are placed by the head in a carousel, and infrared radiation is directed towards the beak tip until it penetrates the underlying tissues which shows a whitened appearance. The beak tip softens in the days after treatment and the sharp beak tip is either eroded with use (Glatz and Underwood, 2021), or subsequently becomes necrotic and falls off (Damme and Urselmans, 2013) by 10–14 days after treatment (Figure 20).



**Figure 20:** A beak after standard infrared trimming (a) and an untreated beak for comparison (b) from Struthers et al., 2019a

The machinery is calibrated by altering the size of the guide-plate according to chick size and adjusting the intensity of infrared energy applied. Generally, infrared trimming is a more precise method than hot-blade trimming, with fewer birds showing beak shape abnormalities when growing (Carruthers et al., 2012). However, calibration and machine adjustments are made per flock and so a uniform flock is important to prevent mistreatment of smaller or larger chicks (Fiks-van Niekerk et al., 2006). Infrared trimming appears to offer greater protection against feather damage (Dennis et al., 2009).

The infrared method has largely replaced a previous hot-blade method which involves manual application of a guillotine-type blade heated to above 650°C that cuts and cauterises beak tissue. This hot-blade method is rarely applied in the EU, due to scientific evidence that it causes both short- and long-term pain (Chow and Hogan, 2005; Gilani et al., 2013; de Haas et al., 2014b) and has other adverse effects described below.

In some countries, the hot-blade method is used for top-up trims (Glatz and Underwood, 2021). Generally, there is only limited beak regrowth as the underlying germ-layer tissue is also destroyed during the trimming process. However, Glatz and Underwood (2021) suggested that beaks can regrow if less than half of the beak tip has been exposed to infrared. Removal of 3 mm of the upper and 2.5 mm of the lower beak is considered as removal of half of the beak tip in 1-day-old chicks. Removal of 4.5 mm of the upper and 4 mm of the lower beak is considered as removal of half of the beak tip in 10-day-old chicks (Glatz and Underwood, 2021). Due to concerns for beak regrowth in for example the USA and Australia, birds that have been infrared trimmed at the hatchery may be subsequently subjected to hot-blade trimming later in life (Glatz and Underwood, 2021). The European Directive 74/1999 dictates that trimming, if allowed, should be done before 10 days of age, but even prior to this Directive a second trim was not common practice in Europe. However, in Europe, hot-blade trimming can sometimes be conducted on adult birds in the event of a serious outbreak of injurious pecking.

### 3.5.2.2. Welfare consequences associated with beak trimming

The welfare consequences associated with beak trimming arise because the beak is highly sensitive and innervated, containing thermoreceptors, nociceptors and mechanoreceptors (Gentle, 1989) and magnetoreceptors (Freire et al., 2011). Thus, beak-trimming causes the welfare consequence 'soft tissue lesions and integument damage' (due to the mutilation itself), resulting in pain and loss of function. It also contributes to the other welfare consequences 'inability to perform exploratory or foraging behaviour', 'inability to perform comfort behaviour' and 'skin disorders (other than soft tissue damage)'.

#### *Welfare consequence 'soft tissue lesions and integument damage' as associated with beak trimming*

Beak-trimming always results in the welfare consequence 'soft tissue lesions and integument damage' related to the removal of the beak tip. The soft tissue damage caused by hot-blade trimming results in pain, stress and compromised immune function in pullets and laying hens (Nicol et al., 1999; Zimmerman et al., 2006; Steinfeldt and Nielsen, 2015) and can lead to neuroma formation adjacent to scar tissue (Lunam et al., 1996; Lunam, 2005). Neuromas are abnormal growths of nerve cells at the site of a nerve injury and are associated with chronic pain, and episodes of recurrent acute pain. If birds are hot-blade trimmed before 10 days of age, the neuromas may resolve, provided not more than 50% of the tip is removed (Kuenzel, 2007). Neuromas do not appear to resolve in birds subjected

to hot-blade trimming at older ages. Birds that have been hot-blade trimmed also show increased beak sensitivity to heat and pressure (Jongman et al., 2008) and reduced feed intake relative to untrimmed controls until 4 weeks of age (Marchant-Forde et al., 2008), both observations are suggestive of pain (Jongman et al., 2008). Despite this, hot-blade trimmed birds initially peck with greater force than untrimmed controls due to loss of mechanoreception and normal sensory feedback (Freire et al., 2011).

Hot-blade trimming has also been associated with increased plasma corticosterone concentrations 2 h post-trim in 6-day-old birds, and for up to 5 weeks in birds trimmed at older ages (Davis et al., 2004). Birds that were hot-blade trimmed at 7 days of age had elevated adrenocorticotrophic (ACTH) hormone concentrations, reduced spleen weight, higher levels of interleukins IL-1 and IL-6 and altered proportions of lymphocytes in comparison with birds that were provided with GABA supplementation to mitigate these effects of stress (Xie et al., 2013). These studies support the general proposition that the welfare consequences of beak trimming are generally more severe if the procedure is applied on birds at older ages (Freire et al., 2008; Janczak and Riber, 2015).

There is less research on the welfare consequences associated with the most commonly used infrared method. Neuromas have not been detected in birds subjected to infrared beak trimming as day-old chicks at the hatchery (McKeegan and Philbey, 2012; Struthers et al., 2019b) and birds that were infrared trimmed did not show changes in beak sensitivity (McKeegan and Philbey, 2012) or reductions in pecking force (Struthers et al., 2019b). McKeegan and Philbey (2012) thus suggested that there was only a low risk of long-term pain associated with the procedure. The procedure may be more painful if beak trimming machinery is poorly calibrated and more tissue is removed.

There is no information on the relationship between infrared trimming and measures of stress and immune function. However, infrared trimmed birds show reduced feed intake relative to untrimmed controls for up to 4 weeks of age (Marchant-Forde et al., 2008), with reduced bodyweight up to 8 (Angevaere et al., 2012) or 14 weeks of age (Honaker and Ruszler, 2004). However more recent studies suggest that early-life reductions in feed intake, feed efficiency and body weight are no longer apparent after 2–4 weeks of age (García et al., 2019; Struthers et al., 2019b).

The prevalence of the welfare consequence 'soft tissue lesions and integument damage' is high as beak trimming is widely applied.

ABMs to assess the extent of 'soft tissue lesions and integument damage' linked to beak trimming are described in Table 21.

**Table 21:** ABMs for the assessment of 'soft tissue lesions and integument damage'

ABM	NOTES
<b>Beak shape and length</b>	
Definition	Trimmed beaks are identified by blunt mandible tips, a reduction of the upper mandible overhang, and shortening of both mandibles. The extent of shortening depends on the trimming method employed and the age of the birds when trimmed.
Measurement	<p>Directly observable: Beak length and shape of a representative sample of birds can be compared to photos of birds of the same breed with intact beaks (e.g. photos provided in Struthers et al., 2019a).</p> <p>Beak length can be measured from the point on top of the beak, level with the front of the nares, to the tip of the upper beak, using callipers (Gentle et al., 1997; Baker et al., 2022; DEFRA, 2005). Average beak length (and a measure of variation within the flock) can be recorded.</p> <p>The side of the beak can be measured from the front of the naris on one side, to the tip of the beak using callipers (DEFRA, 2005) (Figure 21).</p>
	
	<p><b>Figure 21:</b> Diagram showing how top and side length of a beak can be measured with callipers (from Baker et al. (2022)</p> <p>The extension of one mandible over the other, e.g. overhang or under-extension can be measured using callipers (DEFRA, 2005). Abnormalities such as beak cracks, and asymmetries can be visually assessed (Carruthers et al., 2012). The proportion of birds affected can be recorded.</p>
Interpretation	<p>Beak trimming causes damage to nervous tissue. Considerable loss of the beak (compared to Figure 22) that is seen in all three pictures will be associated with reduced function which may cause discomfort and distress and increased short- or long-term pain. Compared with the standard trimmed beak shown in Section 3.5.2.1, the birds in the photos below have been trimmed too severely.</p>
	
	<p><b>Figure 22:</b> Considerable loss of the beak (Photo Credit: M. Guinebrière, ANSES)</p>

ABM	NOTES
Sensitivity and specificity	Sensitivity is high. If soft tissue lesions due to beak trimming is present, a change in beak structure (at least length and sharpness) will be present. Specificity is high. If soft tissue lesion due to beak trimming is absent, only a few other beak abnormalities would be expected.

#### *Welfare consequence 'inability to perform exploratory or foraging behaviour' as associated with beak trimming*

Hot-blade trimmed birds are less active than untrimmed controls (Marchant-Forde et al., 2008) and do less pecking (Freire et al., 2011). Infrared trimmed birds also show reduced exploratory behaviour (gentle feather and object pecking) compared to untrimmed controls (Struthers et al., 2019a) but to a lesser degree. For example, birds subject to infrared trimming at 1-day-old showed more walking, drinking and pecking at 5 weeks of age than birds that had been hot-blade trimmed at 7 days (Dennis and Cheng, 2012). However, greater reductions in activity are observed when a greater proportion of the beak is removed by infrared trimming (Marchant-Forde et al., 2008; Dennis and Cheng, 2012).

Hot-blade trimming also reduces navigational ability due to a loss of magnetoreception (Freire et al., 2008; Freire et al., 2011), which would compromise exploratory behaviour. There is no information on the relationship between infrared trimming and navigation. The variation in beak morphology that can arise after infrared trimming also has minor effects on behaviour (Hughes et al., 2020; Struthers et al., 2019c).

#### *Welfare consequences 'inability to perform comfort behaviour' and 'skin disorders (other than soft tissue lesions)' as associated with beak trimming*

Hot-blade trimming reduces the ability of birds to perform effective preening behaviour (Mullens et al., 2010; Chen et al., 2011). When experimentally infected with either body lice or northern fowl mites, birds that had been hot-blade trimmed at 10 days of age were significantly less able to remove these ectoparasites resulting in far higher rates of infestation than for untrimmed controls (Vezzoli et al., 2015). If, because of impaired preening ability, blood-sucking parasite levels increase, there may even be a potential welfare consequence of 'metabolic disorder' due to anaemia (Tomley and Sparagano, 2018). Birds with beaks trimmed using the infrared method are more able to use their beaks in functional preening behaviour than birds trimmed by hot-blade beak trimming (Murillo and Mullens, 2016), but there are no studies comparing red mite infestation rates between beak-trimmed birds and untrimmed controls.

#### *Mitigating Measures of the welfare consequences associated with beak trimming*

The welfare consequences associated with beak trimming are lower if birds are trimmed at a younger age. In the EU, infrared trimming is usually conducted at the hatchery and so there is no further potential to reduce welfare consequences by conducting the procedure on younger birds. There may be some potential to reduce welfare consequences by reducing the intensity of the infrared energy applied, by improved staff training, by removing a smaller proportion of the beak, but these adjustments may also reduce the effectiveness of beak trimming. Further research on improved operating procedures could provide another route to mitigation. Another way to reduce welfare consequences is to adapt the trimming process correctly to the average size of the beak between flocks.

### **3.5.3. Risks associated with raising non-beak-trimmed birds**

#### **3.5.3.1. Laying hens**

When beak trimming is not performed there are no welfare consequence related to beak condition, but other welfare consequences related to injurious pecking may worsen. Intact beaks are sharper and there is greater potential for birds to damage each other by pecking. The severity and the proportion of birds showing the welfare consequences linked to injurious pecking are usually greater in non-beak-trimmed birds.

Prevalence of injurious pecking in non-beak-trimmed hens: in adult birds housed in cage-free systems, injurious pecking is less prevalent within beak-trimmed flocks and performed at lower rates when compared with intact-beak flocks (Lambton et al., 2013; Hartcher et al., 2015a). This is because

the greater damage caused by sharp beaks, exposing frayed feathers or bare patches of skin, encourages further pecking. This is the case, even when other management procedures are adopted to try to reduce injurious pecking.

**Mortality in non-beak-trimmed hens:** Most studies have reported higher mortality in non-beak-trimmed hens in both cages (Guesdon et al., 2006; Struthers et al., 2019a) and cage-free systems (Mertens et al., 2009; DEFRA, 2015). A quantitative analysis of mortality data compiled from over 1,000 cage-free flocks from the UK, the Netherlands and Sweden (Weeks et al., 2016) showed the protective effect of beak trimming on the end of lay mortality to be significant in a model that included estimates for breed, housing system, flock size and time of year. The calculated estimate for the most commonly used breed, kept in a free-range system, was an end of lay mortality for intact-beak flocks of 8.3% compared with 7.2% for beak trimmed flocks. Riber and Hinrichsen (2017) found end of lay mortality for intact-beak flocks kept in barn systems in Denmark was 14.2% compared with 8.6% for beak-trimmed flocks. However, Schuck-Paim et al. (2021) found no effect of beak trimming status on mortality in a meta-analysis of indoor systems.

**Plumage condition in non-beak-trimmed hens:** There is strong evidence that the plumage condition of non-beak-trimmed adult hens is worse than that of beak-trimmed hens (Staack et al., 2007; Lambton et al., 2010; Lambton et al., 2013; Sepeur et al., 2015; Riber and Hinrichsen, 2017; van Staaveren et al., 2021). Hartcher et al. (2015b) found that 72.9% of birds within free-range intact-beak flocks in Australia showed evidence of feather damage or wounds compared with just 5.2% of birds from beak-trimmed flocks.

### 3.5.3.2. Pullets

Evidence about the occurrence of injurious pecking in non-beak-trimmed pullets is contrasting. Staack et al. (2007) reported increased plumage damage in beak-trimmed pullets (53% vs 30%) but Gilani et al. (2013) found that the probability of severe feather pecking during rearing was 2.7 times greater in non-beak-trimmed organic rearing flocks than in beak-trimmed barn or free-range flocks.

## 3.5.4. Preventive measures to avoid injurious pecking in non-beak-trimmed hens

### 3.5.4.1. Management strategies

#### *For laying hens, pullets and layer breeders in cage systems*

Preventing injurious pecking of birds with intact beaks in furnished cages can be difficult as there are not many measures that can be taken to prevent the onset of this behaviour in this husbandry system. Providing pecking blocks or other abrasive materials will serve as abrasives to blunt beaks. They reduce the risk of injuries due to pecking. However, attempts to reduce the welfare consequence due to injurious pecking in cage systems by adding environmental enrichment have had variable success in adult laying hens. There is no information for pullets or layer breeders.

Morrissey et al. (2016) looked into feather damage in laying hens in furnished cages by studying three main factors (i.e. breed (Hyaline Brown, Lohmann Classic), beak treatment (yes, no) and extra environmental enrichment (yes, no)). The extra enrichment consisted of polypropylene ropes, pecking mats and beak blunting boards (abrasive paste). The pecking mats comprised of a combination of compressed wood chips and biodegradable glue on a plastic mesh backing. In relation to feather cover, the study showed a significant increased feather damage due to age and breed. In addition, the feather cover in most sites worsened more quickly with age for layers that were beak trimmed compared to those that were not beak trimmed. Enrichment resulted in reduced inter-bird pecking but there was no overall effect of enrichment on plumage condition or mortality. There was no overall effect of enrichment. Better wing feather quality, but poorer thigh feather quality with no differences for neck, tail, or the rest were observed. The extra enrichment did not affect feather scores for beak-trimmed birds and, as a matter of some concern, appeared to have a negative impact on the plumage condition of non-beak-trimmed birds. In contrast, Guinebretière et al. (2020) showed that an artificial turf mat placed on the furnished cage floor and different objects hooked onto the cage façade (10 small mobile and coloured objects, plastic transparent pots filled with coloured rings and plastic chain links for a cage housing 60 hens) reduced feather cover damage.

### *For pullets in non-cage systems*

Rearing chicks and pullets with low stocking densities, lower and less variable background noise (Drake et al., 2010; Gilani et al., 2013) and, especially, the continuous provision of attractive foraging and exploratory substrates (Martins et al., 2000; Lambton et al., 2010; De Jong et al., 2013a; De Jong et al., 2013b; Gilani et al., 2013; de Haas et al., 2014b) have all been shown to reduce the risk of inter-bird pecking developing during early life. This is important because, once inter-bird pecking has developed it is likely to spread within the flock and it may also become more severe and damaging in form. Hazards for injurious pecking in rearing flocks include higher stocking density and bright light (Kjaer and Sørensen, 2002).

Careful consideration of diet for young birds is also important, particularly providing sufficient fibre (Mens et al., 2020; Van Krimpen et al., 2005), reducing the number of diet changes experienced during the rearing period (Gilani et al., 2013) and the early provision of perches (Huber-Eicher and Audige, 1999) which reduces the risk of vent pecking (Gunnarsson, 1999).

Rearing young chicks under dark brooders (where the brooding heat is provided within an enclosed, dark area of the house) is effective in reducing injurious pecking during both the rearing period and extending into adult life (Jensen et al., 2006; Gilani et al., 2012; Riber and Guzman, 2017). One reason may be because active chicks outside the brooder are separated from resting chicks who are most often the target of injurious pecking (Riber and Forkman, 2007). However, such systems have not been widely adopted on commercial farms despite their additional benefits in reducing energy usage.

It is also important to allow pullets time to adapt to the adult housing system before the onset of lay and to ensure a smooth transition to the laying house. This can be achieved by matching rearing and laying-house facilities to improve adaptation of pullets to the laying system (Janczak and Riber, 2015). Keeping rearing and laying flocks on the same farm, or earlier transfer of birds to laying accommodation can help to reduce injurious pecking as birds are able to adapt to the adult system before the onset of lay (Nicol, 2018).

### *For adult hens in non-cage systems*

For adult hens, the importance of providing substrates that enable performance of foraging and exploratory behaviour remains paramount and this is supported by a large evidence base (Rodenburg et al., 2013; Nicol et al., 2013): for example, feeding mash rather than pellets (Lambton et al., 2010; Lambton et al., 2015), increased availability and quality of foraging substrates (Chow and Hogan, 2005; Gilani et al., 2013; de Haas et al., 2014b; Decina et al., 2019).

As for pullets, it is also important to ensure that an appropriate diet with sufficient fibre is provided (van Krimpen, 2009; Qaisrani et al., 2013; Patt et al., 2022), the number of diet changes during the laying period is minimised (Green et al., 2000), and that the nutritional quality and palatability of a new diet is equal or higher than that of the previous diet (Nicol, 2019).

Additional measures to encourage harmless foraging and pecking behaviours, including the provision of environmental enrichment such as hay bales, pecking objects and blocks have small, but positive and cumulative effects. A recent meta-analysis (of data from 23 publications) examined the effect of environmental enrichment on injurious pecking and feather damage in laying hens (van Staaveren et al., 2021). Overall, the analysis confirmed that environmental enrichment was an effective management practice, but the effect was surprisingly small, reducing recorded feather damage by just 0.14 points on a 1–4 scale. In commercial flocks, enrichment is sometimes added as a response to injurious pecking rather than as a preventive tool and this may explain why Decina et al. (2019) found a positive association between injurious pecking and enrichment provision. Thus, the provision of pecking enrichments should be seen as an addition to the provision of a quality foraging substrate, which remains the most important protective factor (e.g. Mason et al., 2010).

The choice of housing system itself can influence the risk of injurious pecking. A benefit of the multi-tier system compared to the single-tier system is that birds can more easily avoid or move away from feather peckers. A benefit of providing a veranda or outdoor access is that stocking density is reduced and additional foraging and exploratory opportunities are provided. A further protective factor is good usage of an outdoor range (Heerkens et al., 2015; Pettersson et al., 2016). During the early stages of lay hens are often excluded from litter or outdoor range areas during early phases of lay to encourage nest-box use. But this increases the risk for injurious pecking and early access to outdoor range is however associated with improved plumage condition (Petek et al., 2015).

The provision of housing features such as perches, ramps and clearly differentiated functional zones for nesting, foraging and resting can reduce the risk of injurious pecking (van Niekerk, 2019). However, stocking density has inconsistent effects, with higher stocking density sometimes increasing the risk of injurious pecking (Nicol et al., 1999; Zimmerman et al., 2006; Steinfeldt and Nielsen, 2015) but not systematically, and sometimes associated with lower injurious pecking (Nicol et al., 2006; Zimmerman et al., 2006). Tailored management strategies can be employed to avoid specific forms of injurious pecking. For example, perches must be at an appropriate height to avoid problems with vent pecking (Lambton et al., 2015). The use of bell drinkers increases the risk of both feather pecking and vent pecking (Pötzsch et al., 2001; Lambton et al., 2015), possibly because of water spillage reducing litter quality. Also elevated perches, pelleted feed and changes in feed composition may increase the risk of vent pecking (Lambton et al., 2015). Aggression is often lower in multi-tier systems, as birds can easily escape from each other by moving one floor up or down. Systems with functional zones (e.g. distinct foraging, nesting and resting areas) may also reduce aggression, because lower ranked birds can separate themselves from higher ranked birds.

The complex management influences on injurious pecking are best considered holistically with priority given to those that improve welfare overall, such as the provision of foraging materials and enrichments rather than lower light intensity. Management packages (e.g. FeatherWel)<sup>3</sup> that encourage a holistic approach work better than single element solutions (Lambton et al., 2013; Pettersson et al., 2017).

Pettersson et al. (2017) showed that farms that kept flocks under conditions where severe feather pecking levels were low, were able to further reduce pecking by adopting a selection of management strategies, including additional shelters to encourage range use, pecking pans and other enrichments. Lambton et al. (2013) considered 46 potential management strategies and found that the more of the strategies that were employed on commercial farms, the lower was the level of plumage damage, severe feather pecking, mortality at 40 weeks and risk of vent pecking. However, even though the bespoke management packages applied to commercial farms were effective, injurious pecking was rarely eliminated, and it remained a significant problem on many farms.

Other considerations relate to the importance of farmer experience. Increased familiarity and knowledge associated with keeping cage-free flocks can reduce overall flock mortality (Schuck-Paim et al., 2021). The welfare outcomes for birds with intact beaks have also been found to improve with farmer experience (Nicol, 2015). Overall, studies suggest that gradual and planned changes can lead to good bird welfare (Spoelstra et al., 2013). The welfare outcomes for birds are therefore likely to be better if farmers are able to gain experience of cage-free systems before making a further transition to managing flocks with intact beaks.

#### *For layer breeders*

Layer breeders are commonly kept in single-tier systems at relatively high stocking densities. de Haas et al. (2013) measured indicators of fear and stress and feather damage in commercial white and brown layer breeders. They found differences between genotypes, with white breeders being more fearful and having more feather damage than brown breeders. There was also considerable variation in basal corticosterone levels in plasma. Stress in the layer breeders also influenced injurious pecking in the offspring of these flocks. In the white genotype, pullets from breeders with poor plumage condition and high basal corticosterone levels were more likely to already develop severe feather pecking in the first week of life (de Haas et al., 2014b). This indicates that improved housing and management of layer breeders could not only benefit the breeders directly but could also help to prevent the development of injurious pecking in the offspring. Indeed, an early onset of severe feather pecking in the pullets (< 5 weeks of age), was shown to be one of the risk factors for feather damage in the same birds when they were adult laying hens (de Haas et al., 2014a).

#### **3.5.4.2. Genetic strategies**

Some breeds have a lower tendency to show injurious pecking than others, providing an important element of choice for producers. There has for example been a tendency to switch to white breeds in countries where beak trimming is prohibited because these breeds are perceived to have a lower risk of injurious pecking (Ferryhough et al., 2020). Differences are also detected between strains of the same colour (e.g. Coton et al., 2019).

<sup>3</sup> [www.featherwel.org](http://www.featherwel.org)

Advances in technology mean that it is increasingly possible for genetic producer companies to select directly for reduced injurious pecking behaviour (Ellen et al., 2014; Ellen et al., 2019). Harlander-Matauschek and Bessei (2005) found genetic factors associated with feather eating and crop size. However, challenges in using genetic selection to reduce the risk of injurious pecking include limited selection intensities (partly because of the high environmental influences on pecking behaviour and bird survival), predicting the behaviour of birds when housed in large groups, low heritability as mortality decreases and potential trade-offs between production efficiency and welfare (Fernyhough et al., 2020).

### 3.5.4.3. Alternative strategies to obtain shortened or blunted beaks

An alternative approach to reduce beak length and sharpness is to breed or maintain birds with naturally shortened or blunt beaks.

In recent years, work has increased on the use of hard materials as pecking substrates, to reduce inter-bird pecking by redirecting the pecking behaviour but also to shorten or blunten beak tips during the course of normal exploratory behaviour. There have been a few small-scale studies on the effectiveness of beak blunting in hens (DEFRA, 2005), and some commercial innovations (e.g. the 'pickpuck' Big Dutchman, 2016; the 'pecking pan' Vencomatic). A recent study (Baker et al., 2022) found that such materials resulted in shorter beak lengths in young pullets, with some effects persisting to 15 weeks in non-beak-trimmed flocks.

Some new feeder designs (Roxell) incorporate a rugous metal plate at the bottom that when use from a young age allow to achieve a natural beak blunting effect as birds eat as shown by a recent study (Struthers et al., 2022). The use of natural beak smoothing feeders from 2 weeks of age onwards significantly reduced beak sharpness and overbite length with no impact on growth. The challenge is to reach a uniform effect on all the birds.

Other studies suggest that the provision of pecking stones, pecking blocks or other beak-blunting materials may have beneficial effects in reducing injurious pecking. However, in many cases, the provision of these materials is part of an overall management package where other enrichments are also provided (e.g. Morrissey et al., 2016; Pettersson et al., 2017) or other changes such as reduced stocking density simultaneously implemented (Zepp et al., 2018). Further research in this area, to look at unconfounded effects of the effects of hard material provision would be helpful as this method of beak blunting would largely avoid the welfare consequences outlined above. It would also be important to assess whether flocks with naturally blunted beaks have sustained improvements in plumage condition.

These strategies can also be applied in beak-trimmed hens to reduce regrowth of beaks.

An alternative future approach would be the genetic selection of birds with naturally blunter beaks (Icken et al., 2017). Ongoing research is evaluating the natural variation in beak morphology in laying hens which could form the basis for genetic selection, but birds with less damaging beaks are not yet commercially available.<sup>4</sup>

### 3.5.5. Mitigating measures to avoid injurious pecking in non-beak-trimmed hens

All of the strategies mentioned above will have mitigating effects on injurious pecking in beak-trimmed and non-beak-trimmed hens. In addition, the provision of perches, elevated structures and appropriately placed barriers and ramps should allow birds to avoid or escape from aggressive or feather pecking companions (Estévez and Newberry, 2017).

In case of a major outbreak of injurious pecking, the light intensity is sometimes lowered but a very low light intensity might have negative consequences for eye health and limits social behaviour.

## 3.6. The assessment of animal-based measures collected in slaughterhouses to monitor the level of welfare on laying hen farms (Specific ToR 3)

This Specific ToR considers laying hens at the end of the production cycle that will be sent to the slaughterhouse ('end-of-lay' or 'spent' hens). It aims at listing ABMs that can be assessed and collected at slaughter to monitor the level of on-farm welfare in laying hens across populations or regions/countries. The ABMs that help to identify more than one welfare consequence are commonly referred to as 'iceberg indicators' (EFSA, 2022).

<sup>4</sup> E.g. <https://pure.sruc.ac.uk/en/projects/blunting-the-blows-using-naturally-occurring-variation-in-beak-mo/publications/>

Recording ABMs at slaughter can provide information for assessment and benchmarking of laying hens welfare on farm (Grafl et al., 2017; Jung et al., 2022).

The full methodology and the starting list of ABMs considered potentially relevant for measurement at slaughter in laying hens is presented in Appendix D.

The outcome of the ABM selection exercise is presented in Table 22 where the scoring of the specific criteria used to select the ABMs at slaughter are reported. Total mortality (on-farm), plumage damage, wounds, keel bone fracture and carcass condemnation were selected as the most useful ABMs indicative of laying hen on farm welfare. Dirtiness and dead-on-arrival were not considered as relevant ABMs to assess on-farm welfare as they are affected mainly by the transport conditions.

**Table 22:** Selection of ABMs for laying hens on the basis of the four criteria. Scores from 0 = absence to 4 = high. The weight of each criterion is in brackets. The ABMs that were selected are highlighted in grey

ABM	Assessment	Welfare consequence (weight = 7.5)	Technology readiness (weight = 1.5)	Already measured at slaughter (weight = 0.5)	Importance rated by the network (weight = 0.5)	Weighted score
<b>Total mortality (on-farm)</b>	Ante-mortem	4	2	3	3	<b>3.6</b>
<b>Plumage damage</b>	Post-mortem	4	1	2	3	<b>3.4</b>
<b>Wounds</b>	Post-mortem	3	3	3	3	<b>3.0</b>
<b>Keel bone fracture</b>	Post-mortem	3	2	1	3	<b>2.8</b>
<b>Carcass condemnation</b>	Post-mortem	2	0	4	3	<b>1.9</b>
<b>Keel bone prominence</b>	Post-mortem	2	0	3	1	1.7
<b>Foot injuries</b>	Post-mortem	2	0	0	2	1.6

In the following sections, each ABM is described with its definition, interpretation, means of assessment and arguments for the selection (linking the scores and the scientific evidence in the literature).

### 3.6.1. Mortality (on farm)

#### 3.6.1.1. Description

**Definition:** *Total mortality, also referred to cumulative mortality, is the number of birds that die or are culled over a period of time, expressed as a percentage of the total number of birds that were alive at the start of that period.*

In the case of laying hens, it is usually expressed over the period from when the birds are transferred to the laying house until depopulation. Since the age of the birds when depopulated varies, this should be taken into consideration. For example, there is some evidence of a more rapid increase in cumulative mortality after about 30–35 weeks of age (Weeks et al., 2016). Farmers usually record mortality daily and could report cumulative mortality weekly or monthly. However, it is generally not considered worthwhile to report this variation, even if it would give information on the pattern of mortality. Distinguishing between the number of birds found dead and those that are culled is important. One way to do this is to report the total mortality and the total number of birds culled separately. Culling birds for welfare reasons is a way to reduce suffering.

Reporting the causes of death is informative. Mortality can be caused by injuries, e.g. injurious pecking, accidental injuries from the house system or unwanted sexual behaviour in breeding birds. It can also be attributed to sickness and disease. In free-ranging flocks of birds, predation can also be a cause of mortality and it is recommended that missing birds are reported in addition to mortality. If piling of birds occurs the number of birds that died by suffocation should be recorded as a specific type of mortality.

### 3.6.1.2. Interpretation

The monitoring of mortality in laying hens is commonly used to estimate flock welfare and has been considered by some experts as one of the most important indicators of health for laying hens Rodenburg et al. (2008). The higher the mortality the worse the welfare consequence associated with it, and even if a bird survives the consequences, it may still be necessary to euthanise it. The potential pain of the bird before dying is of welfare concern.

Weeks et al. (2016) modelled raw data from 10 published and unpublished studies (3,851 flocks from European producer records for commercial flocks kept in different housing systems) to identify variation in levels of mortality in laying hens. They found that cumulative mortality increased with age; decreased with flock size; was highest in non-beak-trimmed and organic flocks; and varied with both housing system and breed, even if not all differences were robust. In their final model which accounted for 84.9% of the variation in the data set, cumulative mortality varied significantly with housing system, with predicted means for free range being consistently higher than for conventional cage systems (Gonzales and Elbers, 2018 found higher mortality in free range compared to indoor alternative systems), even if actual cumulative mortality in each system varied according to season. There was also more variation in mortality in free-range flocks, which the authors interpreted to mean with good management there is scope for free-range flocks to have low mortality. They highlight the importance to determine the causes of mortality in different housing systems and which differences between hybrids are associated with the various risks for mortality.

Schuck-Paim et al. (2021) conducted a review and meta-analysis of existing evidence on laying hen mortality in commercial flocks raised in cage and indoor non-cage systems using data from 16 countries and 6,040 flocks. Like many others they refer to the fact that information on mortality is not systematically collated across the industry, leading to inconsistent results and to interactions, e.g. where differences in mortality across housing systems were found, they became non-significant when the confounding effect of beak trimming status was controlled (Weeks et al. 2016). In this meta-analysis, the authors were able to evaluate the degree of maturity of production systems. They found that except for conventional cages, mortality gradually drops as experience with each system builds up. Since 2000, they report that each year of experience with non-cage systems was associated with, on average, a decrease of 0.35–0.65% in cumulative mortality. No difference in mortality was observed between cage and non-cage systems during the more recent years. It is reasonable to expect that newly adopted systems will initially experience higher mortality. They refer to the need to adapt diets, lighting schemes, rearing and management practices, and the design of the housing structure itself as well as the genetics of the birds.

### 3.6.1.3. Assessment

#### Timing of assessment: *ante-mortem*

Since this reflects the cumulative mortality over the whole laying period on the farm, it is ante-mortem, in the same that it is before slaughtering.

#### Current use of this ABM

EFSA working group experts consider this ABM of the cumulative mortality, as it is already recorded in the majority of the EU countries for broilers in accordance with Council Directive 2007/43/EC and so could also be recorded in laying hens. The extent to which total mortality figures are currently reported to the slaughterhouse or the competent authority is unclear. Most producers will record mortality; however the total mortality may not be separated into those birds found dead and those culled. It is probably not divided into the causes of the mortality or, if it is, this is very unlikely to be standardised according to predetermined categories.

#### Considerations for use a standard method

Since the total mortality is recorded by the farmer there may be mistakes or intentional misrepresentation of figures. Strategies could perhaps be implemented to minimise this, e.g. independent confirmation of the number of birds delivered to the farms to be compared with the number of birds delivered to the slaughterhouse. However, in some circumstances spent laying hens are killed on the farm and then destroyed. In loose housing systems dead birds may be missed because the presence of equipment of the system make it difficult to see them. High stocking densities or low light levels mean also that birds may be missed or only found at a later date, and in systems with access to outdoors, birds may be removed by predators and so the carcass never found.

Total mortality on farm has multifactorial causation (see previous section). The score received from the consensus exercise (score 4) in Table 22 reflects that this ABM is a good iceberg indicator because it is related to many welfare consequences on farm. However, it is important to specify the cause of death into different standardised categories and to distinguish between birds found dead and birds culled. It is also important to note that high mortality will alert on the welfare and health state but low mortality does not mean that the welfare is guarantee on the farm.

### Possibilities for automation

While in theory some of the developments to automatically record dead broilers (e.g. Liu et al. (2021)) might be adapted to record mortality in laying hens, the many different housing systems and the level of equipment in these systems will make automatic recording difficult. It is therefore assumed that recording will be done manually by the farmer as part of the routine inspection of the birds. However, if entered into a database, this can enhance traceability and facilitate aggregation of data and its use for animal welfare purposes.

## 3.6.2. Plumage damage

### 3.6.2.1. Description

Definition: Feather loss (i.e. presence of denuded areas) and damage.

Deterioration or loss of plumage due to the action of other birds or by erosion caused by the rubbing with the enclosure elements, or birds. Includes damaged feathers or feather loss (Section 3.4.1 Scientific Opinion, Table 15).

### 3.6.2.2. Interpretation

Plumage damage increases with 'inability to perform exploratory and foraging behaviour', as a consequence of injurious pecking. Plumage damage also increases with 'group stress' and is an ABM for 'soft tissue lesions and integument damages'. Flocks with a poor plumage condition can also have more wounds, but this is not always the case.

### 3.6.2.3. Assessment

**Timing of assessment:** post-mortem.

Currently, assessment of plumage condition is mainly done on-farm, for instance using the Welfare Quality protocol for Poultry version 2.0 (2019), the Tauson et al. (2005) method or the Bilcik and Keeling (1999) method but in this context, it can be carried out post-mortem but pre-scalding.

### Current use of this ABM

During the EFSA Animal welfare network meeting this ABM was recorded by half of the respondents (EFSA, 2021b). However, the number of respondents was very low and that explains the low weight given to criterion 3 and criterion 4 in this exercise. This ABM is frequently assessed on-farm, but to date it is not assessed at slaughter.

### Considerations for use a standard method

A key aspect that is important for using a scoring method is that the correct body areas are assessed. In the Welfare Quality protocol (2019), the neck area, the back/rump area and the belly area were identified as the main areas of interest, as damage in these areas can be most clearly linked to injurious pecking. Any manual or automated scoring method should allow for a clear identification of specific body areas.

A second key aspect to consider is the difference in feathering and feather colour between white and brown birds. Tauson et al. (2005) provides a guide with pictures from each scoring category for both brown and white feathered birds. This allows the scorer to score consistently across genotypes.

### Possibility for automation

At slaughter, especially a method that would involve image analysis of the birds on the slaughter line would be a practical option. Recent studies indicate that such an approach would be feasible (Pichova et al., 2017; Doehring et al., 2020; Lamping et al., 2022).

Based on the results from Lamping et al. (2022), it should be feasible to develop a method for automatic assessment of RBG images from the slaughter line (before defeathering) that can be used to assess plumage condition. Based on existing camera systems for the assessment of food pad lesions

and carcass deviations in laying hens and broilers, development of such a system for automatic assessment of plumage condition should be feasible.

### 3.6.3. Wounds

#### 3.6.3.1. Description

**Definition:** *Soft tissue damage with rupture of the skin or not. Wounds comprise of all lesions to the skin, ranging from minor superficial punctiform spots to scratches to large open wounds that go deeper than the skin (Network, 2019). It also includes bruises. It does not include bone lesion, wing fractures, beak issues or foot injuries.*

The animal experiences negative affective states such as pain, discomfort and/or distress due to physical damage to the integument or underlying tissues (e.g. single or simultaneous occurrence of scars, multiple scratches, open or scabbed wounds, hematomas, swelling, scar and muscle damage).

Wounds are highly relevant for the welfare for broiler laying hens, due to the length of time they are kept and therefore the longer duration of the ABM.

#### 3.6.3.2. Interpretation

Wounds likely result from aggressive interactions between birds (Network, 2019). In the event of feather pecking there are also wounds in the skin that might induce cannibalism. Wounds on the back of the neck and head in females, caused by males when mounting to mate, are seen in flocks with natural mating.

For use at a slaughterhouse, the determination of the age of the lesion is important to discriminate between those produced on farm and those caused by the transport or slaughter practices. The age of the lesion might be possible to be estimated based on its colour (EURCAW-Poultry, 2020). Wounds older than 24 h (and likely to occur on farm) are light green, yellow-green and light-yellow coloured whereas bruises caused earlier than 12 h and caused during catching and transportation are from intense dark red to purple colour (Gregory et al., 1992). Additionally, bruises caused at the slaughterhouse during shackling can be distinguished for being bright red in colour (Bremner and Johnston, 1996).

Nevertheless, the assessment of the age of the bruises by the colour might have its limitation. While wing and leg bruises become lighter with the time, breast bruises become darker (Northcutt et al., 2000). Furthermore, the visual perception of yellow colour changes between observers leading to a low reliability and accuracy for estimating bruise age older than 48 h (Hughes et al., 2004). Therefore, Bruises from light purple to yellow are most likely to be caused at the farm level (EURCAW-Poultry, 2020).

#### 3.6.3.3. Assessment

**Timing of assessment:** post-mortem.

Assessment of wounds post-mortem after scalding is more reliable than ante-mortem, as there are important limitations associated with ante-mortem assessment. During ante-mortem inspection some wounds may not be visible when the animal is alive due to the presence of feathers that cover the skin and impair visibility (EURCAW-Poultry-SFA, 2020). Furthermore, it can be difficult to inspect each animal, especially when birds are in a crate. Moreover, cleanliness of the birds affects the visibility of the lesions. Variable environmental conditions (e.g. poor lighting and dust) also limit the ante-mortem assessment. The visibility of the lesions is improved by cleaned carcasses being presented to the observer in a standardised way, allowing assessment of wounds in the entire batch. In either case, the presence of artefacts (e.g. wounds originating from waterbath stunning or processing, i.e. post-mortem) and visual limitations shows that it is important to adjust any assessment protocols to the practical situation of a slaughterhouse.

#### Current use of this ABM

According to the EFSA 2021b report, the majority of the MS who responded the question recorded this ABM at slaughter (5 out of 6). Unfortunately, the number of respondents was very low and that explains the low weight given to C3 and C4 in selection exercise (see Section 2.2.2 methodologies). Moreover, data on wounds are currently not often used for assessing animal welfare conditions on the farm but for meat quality and safety purposes.

#### Considerations for use as a standard method

Wounds assessment post-mortem can be performed. Artefacts such as damage due to carcass procedures need to be recognised by the observer in order to minimise any potential bias of the results.

Therefore, staff need to be trained to distinguish artefacts (i.e. lesions produced after slaughter) from wounds. Lesions can also be due to transport, however, they can be differentiated from older wounds, so the ABM is still valid for assessing welfare on farm. IKB-Kip (2021) In some Dutch slaughterhouses in the Netherlands, the percentage of dressed carcasses with scratches wounds are scored. Carcasses are assessed on the breast side and considered injured when dark red large (diffuse) haemorrhages from three centimetres (diameter) on the wing or leg are present (IKB-Kip, 2021). In conclusion, there is a need for a harmonised scoring system to monitor wounds in laying hens.

### Possibility for automation

At the slaughterhouse, wounds can be assessed in the carcass by computer vision analysis. There is already a prototype, Vetinspector, made by IHFood in Denmark ([www.ihfood.dk](http://www.ihfood.dk)), for the post-mortem inspection of lesions in chickens (Sandberg et al., 2022). It consists of two camera-stations that detect lesions on the outside of the carcass and on the viscera. The image-analysis algorithm is a neural network/artificial intelligence model, where the ability to classify lesions correctly are improved with every new carcass photographed. The model is capable of analysing pictures of carcasses at any slaughter speed. However, the technique still needs validation for the identification of the different types of lesions and the relationship with on-farm animal welfare.

A second option is the IRIS system from Marel. The Intelligent Reporting, Inspection & Selection system (IRIS; Marel) consists of a digital camera, LED lighting and advanced recognition software. It uses shape, colour and texture to detect wounds (<https://marel.com/en/products/iris-gdrael>) in the carcass. This system is in use commercially.

### 3.6.4. Keel bone fracture

#### 3.6.4.1. Description

Keel bone fractures are defined as complete or partial breaks of the keel bone, causing negative affective states such as pain, discomfort and/or distress. They may or may not have healed, resulting in callus (Section 3.4.1.1. of the opinion, Table 4). Keel bone fractures are the only type of fractures included within the welfare consequence 'bone lesions' in this opinion.

#### 3.6.4.2. Interpretation

The severity of keel bone fractures is considered to be high. Compared to birds without fractures, laying hens with keel bone fractures show differences in time spent on highly motivated behaviours (e.g. perching, nest use, locomotion), indicating reduced mobility and potentially negative affective states including pain, discomfort and distress (Riber et al., 2018). Furthermore, keel bone fractures are associated with inflammation and decreased feed intake. The duration of the welfare consequence varies as bone lesions can occur at any time during the laying period. At slaughter, also new fractures due to catching, transport and shackling may be detected. These can be distinguished by a lack of callus formation and signs of bruising in the surrounding tissue (Gregory et al., 1990).

#### 3.6.4.3. Assessment

**Timing of assessment:** post-mortem.

The occurrence of healed or new keel bone fractures can be assessed by palpation, computed tomography, ultrasound, radiography or an automated 3D camera system (Rufener and Makagon, 2020; Jung et al., 2021). Importantly, the assessment method affects the percentage of fractures that are detected as these methods vary in sensitivity (Rufener and Makagon, 2020; Jung et al., 2021). The commonly used palpation method is mostly focused on detecting healed fractures (Casey-Trott et al., 2015) although some authors have concluded that new fractures can also be identified (which is usually only possible for complete fracture of the keel bone).

Large-scale collection is considered more feasible post-mortem, after scalding.

### Current use of this ABM

According to the EFSA (2021b) report, the majority of the MSs who responded to the question recorded these injuries at slaughter (5 out of 6). Unfortunately, the number of respondents was very low and that explains the low weight given to this C3 and C4 in this exercise. However, currently, keel bone damage is not systematically used to assess animal welfare at slaughter nor on-farm.

## Considerations for use a standard method

According to the EFSA 2021b report, keel bone fractures were considered feasible to be assessed by visual observation, but it emerged that it is difficult to entirely assess fractures without palpation. Moreover, palpation can easily assess severe recent fractures, while highly trained inspectors are needed to recognise old or less severe fractures (Wilkins et al., 2004; Casey-Trott et al., 2015).

In summary, visual assessment of keel bone fractures post-mortem seems feasible as a standard method (e.g. ultrasound, radiography or an automated 3D camera system; Rufener and Makagon, 2020; Jung et al., 2021). This method allows the detection of severe keel bone fractures and callus formation on the outside of the keel bone. It is however important to realise that minor fractures and caudal fractures on the inside of the keel bone may be missed using visual assessment. To detect these, radiographic methods or post-mortem palpation of the inside of the keel bone is required.

## Possibility for automation

Jung et al. (2021) conducted an assessment of keel bone fractures post-mortem (after defeathering), based on a 3D camera-based assessment system. It remains to be determined whether a 3D camera-based assessment system is needed or whether a normal camera capturing light in red, green and blue wavelengths (RGB camera) can be used. The latter could have the advantage that several traits can be recorded by the same camera system. Given the fact that plumage may cover (part of) the keel bone, placement of the camera system after defeathering would be optimal. This is demonstrated in the study by Jung et al. (2021).

### 3.6.5. Carcass condemnations

#### 3.6.5.1. Description

Definition: Carcasses that are unfit for use as food described as: number of birds, weight of the carcass or waste per group. Condemnations may occur for many other reasons, e.g. due to septicaemia, hepatitis, pericarditis, abscess, arthritis, emaciation.

According to the Commission Implementing Regulation (EU) 2019/627, all slaughterhouses throughout the EU record carcass condemnations (including the part of the carcass condemned if only partially condemned in the case of slow growing broilers). The reason for condemnation is recorded primarily for food hygiene and meat inspection purposes. Apart from carcass contamination (e.g. with stomach contents during evisceration) during the slaughter process, most of the reasons for condemning a carcass can be also linked to welfare relevant conditions. These conditions including hematoma, bruises, breast blister, ascites, dermatitis and emaciation are undoubtedly associated with impaired animal welfare (Ellerbroek, 2019).

#### 3.6.5.2. Interpretation

During slaughter, meat inspection, laying hens carcasses may be condemned upon detection of disease or lesions that reflect animal welfare in any of the stages prior to slaughter (i.e. including on-farm and transport, lairage). Carcass condemnations is considered to be related to many welfare consequences in laying hens such as prolonged hunger, gastroenteric disorders, or skin disorders. The carcasses that are condemned are generally severely affected meaning that only the most severe cases are recorded. Clearly, other less severe conditions that do not require condemnation are not recorded.

The higher the rates of condemnation the more likely it is that there is a welfare issue on farm. Carcass condemnation is considered an iceberg indicator because it is related to many welfare consequences on farm, although further investigation on it is necessary if the actual hazard is to be identified for prevention.

#### 3.6.5.3. Assessment

**Timing of assessment:** post-mortem.

#### Current use of this ABM

All Food Business Operators must adhere to food safety legislation therefore all slaughterhouses already record this ABM for food hygiene purposes. There is little available information on the use of condemnation data for animal welfare purposes, though many projects are underway (EFSA, 2021b).

## Considerations for use a standard method

Carcass condemnations is expressed as the number of carcasses condemned, as the weight of the condemned carcasses.

There is a large variation in the recording of carcass condemnations throughout the EU. Differences reported are due to the terminology used, the type, number, and use of codes of classification and the use of electronic databases. Additionally, there is large variation between meat inspectors in the recording of the reasons for condemnation. Hence, it is difficult to compare data between different countries and/or slaughterhouses. Carcass condemnations due to other reasons than health and welfare (e.g. because of improper handling or of carcass contamination) must be excluded from the final score. There is a need for harmonisation of the criteria, both in and between MSs.

The data are recorded in all EU member states and are already available, but their use needs to be developed for welfare purposes. High levels of carcass condemnation can be used as a proxy of poor welfare, assuming that severe lesions or poor body condition (i.e. emaciation) had caused negative affective states such as pain and/or discomfort. However, as condemnation assessment addresses only the cases that are relevant for food human safety, low or expected levels of carcass condemnation might not necessarily reflect good welfare on farm.

## Possibility for automation

Decisions on carcass condemnation is made after inspection by the Official Veterinary officers.

Currently, there is a prototype vision system (IRIS GDR), mainly used for carcass classification and resulting logistics. It measures weight, yield, of whole chickens and parts and decides whether if condemnation affects the whole chicken or parts (Marel). This system supports the official veterinarian in identify condemnation by measuring bruises, fractures, scratches, etc. Other systems have been reported for poultry (BuRO, 2022). However, information on the validation of these tools with expert inspectors are not publicly available BuRO (2022).

In Germany there is also a prototype camera to detect deep dermatitis on the carcass and ascites in broilers to support Official Veterinary officers (Mr Schulte-Landwehr, CLK GmbH, Germany, personal communication, 6 September 2022, email).

Moreover, automated and harmonised indicators, can result in a valid tool to enhance the traceability and to support animal welfare monitoring schemes across different slaughterhouses/countries.

## 4. Conclusions and recommendations

### 4.1. Answers to general ToRs

The conclusions and recommendations in this Section 4.1 concern only the highly relevant welfare consequences identified and described in the Sections 3.3. The total number of welfare consequences that were identified as highly relevant per system does not reflect the overall level of welfare in that system (This section focusses on conclusions on welfare consequences, their ABMs, hazards and general points about prevention and mitigation. The recommendations about hazards (quantitative figures) that are common for different welfare consequences are described in the discussion of the Specific ToRs (Sections 3.4.1, 3.4.2 and 3.4.3; and 4.2, 4.3, and 4.4)).

The certainty level of the conclusions is provided between brackets except for those with a certainty > 90% in which no certainty range is indicated.

#### 4.1.1. Bone lesions

##### 4.1.1.1. Conclusions

- Bone lesions were identified as a highly relevant welfare consequence in laying hens and layer breeders in all husbandry systems.
- Keel bone fractures are the most common type of bone lesions during the laying period, with the majority located at the caudal part of the keel bone. Keel bone fractures are therefore identified as an ABM for bone lesions.
- While non-caudal keel bone fractures are associated with a reduction in mobility combined with negative affective state such as pain, the welfare impact of fractures typically found in the caudal tip of the keel bone (which are highly common) is considered a gap of knowledge.

- Keel bone fractures are identified by palpation (mostly used on farm) and radiography (mostly used for experimental studies), respectively. Sensitivity of palpation is considered moderate to high for the main part of the keel and low for the caudal part and specificity is considered moderate.
- Hazards for bone lesions in non-cage systems are high impact collisions and falls, and poor bone quality. Collisions and falls can be caused by inappropriate design of perches or poor accessibility to elevated structures, but also by low perch space per bird and crowded perches, as well as low light intensity impairing navigation of birds.
- In furnished cages, the main hazard for bone lesions is poor bone quality. The presence of perches is also a hazard (> 50–100% certainty) although the exact reason for this is unknown.
- When pullets are raised without elevated structures, the risk of falls and collisions is increased when they reach egg production age.
- Hens with poor bone quality due to genetic background, lack of exercise or diets leading to suboptimal bone strength, are at a higher risk of bone lesions.
- It is considered as likely as not (33–66% certainty) that large egg size at early onset of lay (20–22 weeks of age) constitutes an additional hazard for fracture of the caudal tip of the keel bone.
- The prevalence of keel bone fractures in laying hens and layer breeders housed in non-cage systems is reduced by: (i) improving bone strength by feeding appropriate diets, (ii) rearing of pullets in systems with elevated structures and (iii) reducing the risk of falls and collisions by providing easy access to perches and other elevated structures and adequate light.

#### 4.1.1.2. Recommendations

- It is recommended to improve layers' and layer breeders' resistance to keel bone fractures by genetic selection, rearing of pullets in systems with elevated structures and by developing feeding strategies to achieve greater bone strength.
- Within non-cage systems it is recommended to further reduce the risk of keel bone fractures due to collisions by:
  - stimulating the development of spatial and motor abilities during rearing through provision of enriched housing conditions (e.g. elevated structures, objects to interact with, space that promotes locomotory agility).
  - providing sufficient light to facilitate layers' and layer breeders' navigation in complex environments.
  - reducing the need for steep aerial descents (< 45° from vertical) by providing ramps or platforms between levels and providing sufficient free horizontal space.
  - providing sufficient space on safely accessible perches consisting of material that can dampen collision forces.
- Research is recommended on the:
  - effect of age and egg-size at onset of lay on the occurrence of caudal keel bone fractures,
  - effect of perches on keel bone fractures,
  - causes of caudal keel bone fractures and their impact on bird welfare.

#### 4.1.2. Group stress

##### 4.1.2.1. Conclusions

- Group stress was identified as a highly relevant welfare consequence in all categories of birds kept in groups (collective housing). The severity of group stress depends on the availability of resources, and the dynamics of the social group.
- The ABMs for group stress are: 'aggressive interactions', 'fear response', 'injurious pecking', 'physiological stress indicators', 'piling behaviour' and 'plumage damage'. Most of these can be cause and consequence of group stress. 'Aggressive interactions' is a highly specific and sensitive ABM.
- The ABMs 'fear response', 'injurious pecking', 'physiological stress indicators' and 'plumage damage' are considered iceberg indicators.
- The ABM 'piling behaviour' has a moderate specificity but a low sensitivity ABM.

- High stocking density is a hazard for group stress in laying hens, pullets and layer breeders (> 50–100% certainty).
- Hazards for group stress in all concerned animal categories are: uneven/insufficient resource allocation (increasing competition), the inaccessibility or lack of structures (e.g. perches) to escape from conflict situations, and social conflicts.
- Group stress results from a high incidence of aversive social interactions (aggressive behaviours, injurious pecking, piling, smothering) leading to negative affective states such as pain, fear or frustration that may exacerbate both individual and group level of stress. Piling can lead to smothering which is an issue of importance given its reported occurrence in non-cage housing systems in pullets, layers and layer breeders.
- In the case of layer breeders, group stress between males and females can also originate from the females' inability to avoid unwanted sexual behaviour.
- Group stress can also result from non-social welfare consequences experienced by individual birds (predation stress or sensory overstimulation) subsequently transmitted socially throughout the flock.
- Preventive measures that are likely to prevent group stress are: avoiding situations that cause fear or pain, reducing fearfulness in birds by choosing hybrids that are less prone to group stress, using dark brooders for chicks.
- Strategies to prevent injurious pecking also prevent group stress to some extent. Strategies include sufficient provision of resources to minimise competition in addition to elevated structures and cover panels to give the birds the opportunity to escape from a conflictive situation.
- Partitioning groups of laying hens, pullets and layer breeders in single- and multi-tier housing systems, by appropriate use of partitions reduce the risk of panic reactions and smothering.

#### 4.1.2.2. Recommendations

- High stocking density should be avoided (refer to Section 4.2).
- Predation stress or sensory overstimulation should be avoided in order to prevent group stress.
- It is recommended to provide resources, e.g. litter, perches, in sufficient quantity to prevent competition in laying hens, pullets and layer breeders. Some resources, e.g. nests, might be available only during a limited period of the day, but, when offered, should be provided in sufficient quantity to prevent competition.
- It is recommended to provide possibilities to escape from conflict situations by offering, e.g. perches, panels, platforms or tiers which allows laying hens, pullets and layer breeders to avoid unwanted social interactions.
- For laying hens, pullets and layer breeders in single- and multi-tier housing systems, separating birds into sub-flocks (colonies) by appropriate use of partitions is recommended to reduce the risk of panic reactions and smothering.
- Cage systems and floor systems without elevated structures should be transitioned to non-cage systems with elevated structures and one or multiple tiers for laying hens, pullets and layer breeders.
- It is recommended to perform further research on the relationships between group size, stocking density and group stress and on risk factors of piling and smothering behaviours in layers, pullets and layer breeders.

#### 4.1.3. Inability to avoid unwanted sexual behaviour

##### 4.1.3.1. Conclusions

- Inability to avoid unwanted sexual behaviour has been identified as a highly relevant welfare consequence for layer breeders in all husbandry systems except individual cages and can further result in the welfare consequence 'soft tissue lesions and integument damage'.
- ABMs to assess this welfare consequence are 'avoiding the litter area' and 'forced copulations' as well as the iceberg indicators 'plumage damage' and 'wounds to skin'.
- The ABMs 'avoidance of the litter by females' and 'forced copulation' are both sensitive to assess the presence of unwanted sexual behaviour, but only 'forced copulation' is highly specific.
- Hazards for 'inability to avoid unwanted sexual behaviour' are sexual development asynchronicity (i.e. males being sexually mature before females), a ratio of males/females

above 1/10 and the lack of elevated structures or cover panels which females can use to escape from male sexual behaviour and aggression.

- The structure of the multi-tier environment allows more effective avoidance of males by females and reduce unwanted sexual behaviours, as compared to single-tier systems (> 50–100% certainty).

#### 4.1.3.2. Recommendations

- In breeding flocks, it is recommended to maintain a ratio of males/females below 1/10 to ensure that hens can avoid unwanted sexual behaviour, by an even distribution of males and females on slatted floor and litter area.
- Claw shortening devices should be provided to reduce sharpness of the males' spurs and claws and reduce injuries made to females by males.
- Spatial partitions in the litter area may help to reduce negative interactions between males and between males and females, thus reducing the risk for unwanted sexual behaviour.
- Breeding scheme should select on more gentle mating behaviour in the males.
- Breeding flocks must be specially monitored in the late afternoon to detect occurrence of unwanted sexual behaviour and react as soon as possible to prevent the welfare consequence from happening again.

#### 4.1.4. Inability to perform comfort behaviour

##### 4.1.4.1. Conclusions

- Inability to perform comfort behaviour was identified as a highly relevant welfare consequence for laying hens, pullets and layer breeders housed in cages. This will last for the entire life span, at least for layers and breeders.
- Inability to perform comfort behaviour results in poor plumage condition, impairs normal muscle function and can lead to frustration.
- The ABMs for this welfare consequence are: 'dustbathing', 'preening', 'wing and leg stretching' and 'wing flapping'. 'Dustbathing' is a highly sensitive and highly specific ABM for assessing this welfare consequence. 'Wing and leg stretching' is both moderately sensitive and specific, and 'wing flapping' is moderately sensitive and highly specific, while 'preening' is moderately sensitive and of low specificity.
- Comfort behaviour requires space: up to 3,300 cm<sup>2</sup> for wing flapping, around 1,000 cm<sup>2</sup> for wing and leg stretching, approximately 1,100 cm<sup>2</sup> for preening and for dustbathing. Space provided in most current cage systems physically limits wing and leg stretching and wing flapping, and can shorten preening bout duration.
- The main hazard for the inability to perform complete dustbathing behaviour in collective cages for laying hens, pullets and layer breeders is the absence of an adequate pecking and scratching area. This area can be inadequate in terms of insufficient suitable space, inappropriate litter material (quality – e.g. unfriable characteristic or feed proposed as a substrate), insufficient litter (quantity – small and rare quantities of litter delivered) and/or inappropriate light.
- The main hazards in collective cages for all animal categories are: the absence of a solid floor and lack of permanently accessible litter leading to a lower frequency and duration of dustbathing behaviour, and higher levels of incomplete or sham dustbathing.
- When substrate is provided in furnished cages for laying hens, it is most of the time not adequate and restricts comfort behaviour.
- Compared to cage systems, non-cage housing for laying hens, pullets and layer breeders are more effective in allowing comfort behaviours as these systems have the possibility to provide friable substrate in a sufficient amount together with sufficient space.

##### 4.1.4.2. Recommendations

- It is recommended to use non-cage systems to prevent restriction of comfort behaviour, such as described in section (minimal enclosure).
- To allow dustbathing of birds, an appropriate dry and friable substrate should be permanently available for all birds in a sufficient quantity.

#### 4.1.5. Inability to perform exploratory or foraging behaviour

##### 4.1.5.1. Conclusions

- Inability to perform exploratory and foraging behaviours has been identified as a highly relevant welfare consequence for laying hens, pullets or layer breeders housed in cages. This will last for the entire life span, at least for layers and breeders.
- Severe limitation in opportunities to forage and explore has a negative impact on the birds' welfare, increasing the level of stress and leading to negative affective states such as frustration.
- The ABMs related to the inability to perform exploratory or foraging behaviour are: 'injurious pecking', 'plumage damage' and 'walking, scratching and pecking'. 'Walking, scratching and pecking' is highly sensitive and moderately specific to assess this welfare consequence. 'Injurious pecking' and 'Plumage damage' are iceberg indicators.
- Insufficient or absent friable material in cages for breeders and pullets or restricted access to friable litter are the main hazards for the inability to perform foraging behaviour across animal categories when they are held in cages.
- Limitation of space and elevated structures are the main hazards for the inability to perform exploration.
- Even when a substrate for adult laying hens is provided in furnished cages, the high stocking density and small cage size restricts exploratory and foraging behaviour.
- Layer breeders in single cages are severely restricted from performing exploratory and foraging behaviours due to lack of physical space and unavailability of foraging substrates.
- Provision of friable litter in sufficient amounts and frequency to prevent the welfare consequence is only effective in non-cage systems.

##### 4.1.5.2. Recommendations

- It is recommended that laying hens, pullets and layer breeders are housed in non-cage systems and provided with adequate, dry and friable substrate. The substrate should be permanently available, from day 1.
- It is recommended to provide a sufficiently spacious and enriched environment with elevated structures to allow exploratory and foraging behaviour for laying hens, pullets and layer breeders.

#### 4.1.6. Isolation stress

##### 4.1.6.1. Conclusions

- Isolation stress has been identified as a highly relevant welfare consequence for laying hen breeders in individual cages.
- Main hazards include insufficient physical space to permit the development of normal behaviour patterns and the absence of conspecifics.
- Single-bird cages severely restrict the hens' access to social interactions, inhibiting positive social behaviour and behavioural synchronisation, and leading to stress and negative affective states such as boredom, fear and frustration.
- ABMs to assess isolation stress for birds that are kept in individual cages allowing them to see and hear conspecifics are 'pacing', which is highly sensitive and moderately specific, and 'fear response' and 'physiological stress indicators' which are both considered iceberg indicators.
- Isolation stress cannot be prevented if birds are housed in single-bird cages.

##### 4.1.6.2. Recommendations

- To prevent isolation stress, single-bird cages should not be used.
- Group housing systems for breeders that allow egg collection from individual birds should be developed further to avoid isolation stress and subsequently implemented.

#### 4.1.7. Predation stress

##### 4.1.7.1. Conclusions

- Predation is a main reason for mortality in flocks with outdoor access. Even if not killed or attacked, laying hens can experience stress as a result of (perceived) predator threats, which

also affects their behaviour in a way that can cause further welfare consequences, measured by collisions and smothering.

- Predation stress can be assessed by the following ABMs: the percentage of birds in a flock that are found dead or survive with marks of predation, the surplus of missing birds above recorded mortality and the percentage of hens that use the free-range. The first two ABMs are moderately sensitive and specific, whereas sensitivity is unknown for the last ABM and its specificity is low.
- ABMs 'fear responses' and 'physiological stress responses', considered iceberg indicators, can be also used to assess predation stress.
- The main hazards for predation stress are: predator access to the free range and/or house, inappropriate responses of birds to predator presence and insufficient cover on the range.

#### 4.1.7.2. Recommendations

- It is recommended that houses for laying hens and pullets are completely closed at night, as many predators are nocturnal. All hens with free-range access should be brought into the house (or veranda) before closing the pop-holes.
- It is recommended that outdoor range is surrounded by appropriate fencing to deter terrestrial predators from entering and causing predation stress to birds with outdoor access.
- It is recommended that at least 50% of the outdoor area is covered by high vegetation, e.g. bushes and trees to reduce predation stress and possibly predation itself.
- Further research is recommended on the effectiveness of current practice such as keeping large animals (e.g. alpacas or dogs) on the range to deter predators from entering.
- Further research is recommended on strategies to prevent predation (e.g. improved range design) and on the reasons for excessive or insufficient anti-predator responses in birds (aiming to avoid problems).

#### 4.1.8. Resting problems

##### 4.1.8.1. Conclusions

- Resting problems were identified as highly relevant for all animal categories, in all housing systems that do not offer or that restrict access to perches or other elevated resting sites for day and night time rest.
- Restricted access to perches or other elevated resting sites is caused by restricted space availability on these, inappropriate design or by the birds' having difficulty accessing the resting sites.
- Resting problems can be assessed by the ABMs 'balance movement' with high sensitivity and specificity, and 'resting birds on elevated structures' (in particular during night-time nearly all birds will roost), with moderate sensitivity and high specificity.
- The ABM 'pushing and jostling behaviours' is considered an iceberg indicator relevant for restricted access to perches or other elevated resting sites and other welfare consequences.
- The main hazard for resting problems is the absence of, or inappropriate design or accessibility of, perches or other elevated sites.
- Offering adequate elevated structures, such as perches or platforms, prevents resting problems. This requires, in all animal categories and any housing system, sufficient space on the elevated structures, and between the elevated structure and the ceiling, allowing the birds easy access to the elevated structure and a normal position while resting (see minimal enclosure).

##### 4.1.8.2. Recommendations

- Sufficient perches or other elevated resting spaces should be provided in any system, matched to the age and body size of the birds (laying hens, pullets, or layer breeders).
- Perches should be designed in such a way that the perch provides a comfortable resting place and that the bodies of the birds are well supported.
- High stocking densities should be avoided to allow all birds access to elevated resting sites and to prevent disturbance of resting birds by other birds.
- It is strongly recommended to raise pullets in an environment with elevated structures that are comparable to what they will face in the laying hen housing.
- It is recommended to house pullets in a single-tier or multi-tier system with perches or other elevated resting sites starting at least after the third week of life.

- A low height of perches is recommended for (young) pullets. However, ramps to access elevated structures are recommended for all animal categories.
- The properties of the perches (e.g. diameter, shape, surface) should be adapted to pullets (e.g. adapted to smaller feet) but further research on this aspect is required.

#### 4.1.9. Restriction of movement

##### 4.1.9.1. Conclusions

- Restriction of movement has been identified as a highly relevant welfare consequence for laying hens, pullets and layer breeders housed in cages. This will last for the entire life span, at least for layers and breeders.
- Restriction of movement results in reduced behavioural activities leading to, physiological stress, reduced bone and muscle strength, poor plumage condition, negative affective states such as frustration and increased mortality.
- The ABMs for this welfare consequence are: 'bone quality', 'locomotor behaviours, e.g. jumping, flying, walking, running', 'plumage damage, and 'pushing and jostling behaviours'. 'Locomotor behaviours' and 'bone quality' are highly sensitive but of low specificity to assess this welfare consequence. The ABMs 'plumage damage' and 'pushing and jostling behaviours' are considered iceberg indicators.
- Main hazards include: insufficient space allowance per bird, insufficient total area of the enclosure, insufficient height of the enclosure, absence of elevated structures and unsuitable resource distribution (places of nest, perches, pecking and scratching area, feeders in the cage limiting any free space).
- The severity of restriction depends on cage size and stocking density, and is more severe in systems without elevated structures for all animal categories.
- In non-cage systems, elevated structures stimulate flying which is beneficial provided the risk of collisions is managed by appropriate design.
- In cage systems, unsuitable furniture arrangement and insufficient cage height can reduce the frequency of locomotor behaviours and impact bone quality.
- Individual cages severely restrict movement in layer breeders due to very limited space and lack of perches.

##### 4.1.9.2. Recommendations

- It is recommended that laying hens, pullets and layer breeders are housed in non-cage systems with elevated structures, because provision of sufficient space to avoid restriction of movement is extremely challenging in any cage system.

#### 4.1.10. Skin disorders (other than soft tissue lesions and integument damage)

##### 4.1.10.1. Conclusions

- In laying hens, skin disorders have been identified as a highly relevant welfare consequence in all systems.
- ABM identified is 'restlessness', which has low sensitivity and moderate specificity.
- Skin disorders are caused by ectoparasites (66–100% certainty), such as mites. The most common in Europe is the poultry red mite *Dermanyssus gallinae*.
- Application of Integrated Pest Management (being a combined set of preventative measures, monitoring and treatments) can keep mite infestations to a minimum and prevent the welfare consequence 'skin disorders'.
- Thorough cleaning of the house before a new flock arrives and preventing re-introduction of mites from, e.g. manure storage or from the outdoor range reduce the risk of infestation.
- Heat treatments of the empty house between flocks reduce the incidence of mites, as this not only kills adult mites, but also mite eggs.

##### 4.1.10.2. Recommendations

- In all housing systems, frequent (e.g. weekly) monitoring is recommended to detect possible mite infestations at an early stage (from birds, eggs and the environment). This should be followed by appropriate actions to reduce mite infestations if needed.

- Integrated Pest Management combined with a set of preventative measures, monitoring and treatments is recommended to keep mite infestations to a minimum.
- Barn should be cleaned and disinfected before a new flock arrives.
- Heat treatments of the empty house between flocks is recommended to reduce the incidence of mites.

#### 4.1.11. Soft tissue lesions and integument damage

##### 4.1.11.1. Conclusions

- Soft tissue lesions and integument damage were identified as a highly relevant welfare consequence in laying hens and layer breeders, in all systems, except breeders in individual cages.
- The ABMs for 'soft tissue lesions and integument damage' are: 'bruises' with moderate sensitivity and high specificity, as well as the iceberg indicators 'plumage damage' and 'wounds'. The ABM related to soft tissue damage due to beak trimming is 'beak shape and length'.
- Hazards for soft tissue lesions and integument damage are:
  - mutilations: the majority of pullets, laying hens and layer breeders in the EU are beak trimmed. Comb dubbing is a procedure carried out on male breeders of white strains.
  - aggressive interactions, injurious pecking, forced mating (in layer breeders) or any lesion inflicted by other birds or by the bird itself.
  - the housing system (birds getting trapped or wounded by sharp edges).

##### 4.1.11.2. Recommendations

- The design of the system should be so that birds cannot be trapped with toes, wings or combs. Additionally, no sharp edges should be present.
- Genetic selection for smaller comb should be performed, specifically for white male birds to avoid the need for comb dubbing.
- Preventative measures against group stress, injurious pecking, unwanted sexual behaviour should be implemented to reduce the occurrence of soft tissue lesions and integument damage. (Recommendations concerning beak trimming and prevention of injurious pecking are presented in Section 4.3)

#### 4.1.12. Welfare of male chicks of the layer breed

##### 4.1.12.1. Conclusions

- The behaviour of male chicks is comparable to pullets and they have comparable requirements. The provision of elevated structures is especially important to escape from aggressive encounters as male layer chicks may show aggressive behaviour from 10 weeks on. However, male chicks may not need a multi-tier system since they do not need to be prepared for a laying period.

##### 4.1.12.2. Recommendations

- More research is needed about current conditions of rearing of these birds and about their needs in terms of husbandry systems and associated management.

#### 4.1.13. Welfare consequences linked to feed restriction

##### 4.1.13.1. Conclusion

- Laying hens experience prolonged hunger and stress when feed is removed or when nutrient levels are substantially reduced.

##### 4.1.13.2. Recommendation:

- All birds must have access to a full daily feed ration that fulfils their physiological needs. Feed should be withdrawn for the shortest time possible before slaughter and should not be removed for more than 10 h.
- Research to establish the prevalence of birds experiencing prolonged hunger and stress due to feed withdrawal or low quality feed is recommended.

#### 4.1.14. The impact of laying hen breeding and genetics on welfare

##### 4.1.14.1. Conclusions

The genetics of laying hens can influence their welfare in several ways:

- Genetic disorders or malformations may appear in offspring, but chicks with malformations are usually culled at hatch.
- Variation may exist in behaviour and welfare traits between different genetic lines and hybrids, which are therefore more or less adapted to some husbandry systems.

Conflicts can occur between production efficiency and welfare traits:

- An extended laying period (up to 100 weeks of age) leads to a more sustainable egg production, but it decreases bone quality of older hens, and also possibly leading to bone lesions.
- Early onset of egg production is linked to an increased risk of feather pecking and possibly also caudal keel bone fractures.
- More mobile and flighty birds are more adapted to use the three-dimensional space but also are more sensitive to stressful events and human disturbance.

##### 4.1.14.2. Recommendations

- There should be a match between the hybrid and the housing/management used in specific farms.
- It is recommended to promote welfare traits (plumage condition, keel bone damage, behavioural traits related to use of space), that allow a balance between production efficiency and welfare. In this context, efficient tools to measure the impact of selection on welfare traits should be developed.
- It is recommended to select males with more gentle mating behaviours.
- It is recommended to promote research on relations between genetics and behaviour of laying hens, such as fear response (ABM piling, smothering) in order to incorporate their prevention in the breeding programs.
- It is recommended to promote research on the relationship between injurious pecking and phenotypic homogeneity of the group.
- It is recommended that information about welfare traits for each commercial hybrid (e.g. skeletal strength, plumage condition by end of lay) is published openly.
- It is recommended to perform independent science-based assessment of welfare outcomes of the different breeds under comparable conditions.
- More research is needed on the impact of production of bigger eggs (compared to hen body size) and age at onset of lay on the occurrence of caudal keel bone fractures.

#### 4.2. Answers to Specific ToR 1: The welfare of laying hens and the risks associated with alternative systems (organic, free range and barn) compared to the cage system currently allowed

##### 4.2.1. Conclusions

###### Part 1: comparing cages with non-cage systems

- In cage systems, laying hens experience the highly relevant welfare consequences 'inability to perform comfort behaviour', 'inability to perform exploratory or foraging behaviour' and 'restriction of movement'. In non-cage systems, these welfare consequences were ranked slightly relevant.
- Covered verandas and outdoor range areas facilitate the performance of some behavioural needs such as comfort behaviours (such as dustbathing), locomotion, exploratory and foraging behaviours to a greater extent than indoor non-cage systems.

## Part 2: Specification for the minimum enclosure characteristics for laying hens, pullets and layer breeders

### Environment

- Increasing group size per se does not lead to an increase in negative welfare consequences (e.g. 'group stress', 'soft tissue lesions and integument damage') provided that access to resources and management conditions are adequate (> 50–100% certainty).
- The minimum group size preventing isolation stress is two birds.
- For laying hens, layer breeders and pullets aged 6 weeks and older, and with group size larger than 30 birds, the minimum surface areas required to prevent the negative welfare consequences that arise from restriction of space (i.e. 'group stress', 'inability to avoid sexual behaviour', 'inability to perform comfort behaviour', 'inability to perform exploratory or foraging behaviour', 'resting problems', 'restriction of movement', 'soft tissue lesion and integument damage') is estimated to be 80 m<sup>2</sup>. If group size is less than 30 birds, it is judged to be 25 m<sup>2</sup> (> 50–100% certainty).
- The welfare consequences 'restriction of movement', 'inability to perform exploratory and foraging behaviour', 'inability to perform comfort behaviour' and 'soft tissue and integument damage' can be prevented if minimum requirements for enclosures are implemented (all other factors fulfilling the minimal requirement described in Section 4.2) and a maximum stocking density of 4 laying hens or layer breeders/m<sup>2</sup> is ensured (66–100% certainty).
- For pullets, the maximum stocking densities listed below will help preventing negative welfare consequences 'restriction of movement', 'inability to perform exploratory and foraging behaviour', 'inability to perform comfort behaviour' and 'soft tissue and integument damage':
  - 15 pullets/m<sup>2</sup> (66–100% certainty) from 10 weeks to the end of rearing
  - 25 pullets/m<sup>2</sup> (> 50–100% certainty) from 6 to 9 weeks (until day 63 of age)
  - 40 pullets/m<sup>2</sup> (> 50–100% certainty) from 3 to 6 weeks (until day 42 of age)
  - 100 pullets/m<sup>2</sup> (> 50–100% certainty) for first 2 weeks (until day 14 of age).

### Equipment

- Access to either perches or elevated platforms is necessary to fulfil the behavioural need for night time roosting in laying hens, layer breeders and pullets from 3 weeks of age.
- For perches and elevated platforms, increasing accessibility by adjustment of height and/or provision of ramps increases day and night time usage by birds of all categories.
- The use of elevated structures with a horizontal distance of less than 80 cm between them and angles below 45°, or below 40° for ramps to access the elevated structures, results in an increase in the number of birds reaching these elevated structures safely and a decrease in the collisions for laying hens, layer breeders and pullets aged 6 weeks and older (66–100% certainty).
- The presence of perches covered with a non-slippery surface increases the number of birds reaching the elevated structures safely and the duration of comfortable resting there for laying hens, layer breeders and pullets aged 6 weeks and older, when compared to non-covered perches (66–100% certainty).
- A minimum perch length of 18 cm per layer hen and per layer breeder, and 14 cm for pullets, increases the number of birds reaching the elevated structures safely and the duration of comfortable resting there for laying hens, layer breeders and pullets aged 6 weeks and older, compared to shorter perch length per bird (66–100% certainty).
- An horizontal distance of at least 30 cm between perches of the same height and of at least 20 cm between a perch and a surrounding of the pen (e.g. a wall) increases the number of birds resting without disturbances from birds resting on opposite perches or an opposite surrounding of the pen. This is valid for laying hens, layer breeders and pullets aged 6 weeks and older, compared to smaller distances (66–100% certainty).
- The minimum vertical space in any part of the system ('usable area', perches and tiers) allowing the bird to perform all natural behaviours is, at least:
  - 55 cm for adult laying hens,
  - 45 cm for pullets over 6 weeks,
  - 77 cm for layer breeders

- The presence of perches with a width of between 3 and 6 cm increases the number of birds reaching the elevated structures safely and the duration of comfortable resting there for laying hens, layer breeders and pullets aged 6 weeks and older, compared to widths smaller than 3 cm (> 50–100% certainty).
- The provision of litter reduces the negative welfare consequence inability to perform comfort, exploration and foraging behaviours in laying hens, layer breeders and pullets, provided that the litter meets all following criteria: dry and friable material is available on the entire litter area and at all times from the moment the birds enter the enclosure (chicks may be kept on paper or other solid substrates from days 1 to 6 or until feeding behaviour is established).
- Providing at least one third of the usable area with dry and friable litter (litter area) prevents the negative welfare consequences inability to perform comfort and exploratory or foraging behaviour, compared to when a smaller proportion of the usable area is littered (66–100% certainty).
- The permanent provision of enrichment and foraging material prevents the negative welfare consequence inability to perform exploratory or foraging behaviour (and then prevents other welfare consequences such as group stress and soft tissues lesions) in laying hens, layer breeders and pullets, if enrichment meets the following criteria:
  - Enrichment additional to the litter, with small particle size substrate for dustbathing (sand-like or peat-like substances but not faecal material);
  - Edible enrichment materials which are renewed as soon as they are depleted;
  - Enrichment materials are accessible at all times from the moment the birds enter the enclosure (chicks may be kept on paper or other solid substrates from days 1 to 6, until feeding behaviour is established).
- From field experience in Europe, nests as they are implemented seem to fulfil the need of the hens in terms of laying behaviour when all nests are equally accessible. Because nests are offered in all commercial housing systems for laying hens and layer breeders, nests have not been identified as a hazard for highly relevant welfare consequences for laying hens. However, there is scientific evidence that availability and quality of nests is important for the welfare of laying hens. The following characteristics were identified as sufficient to fulfil the behavioural need of laying hens and layer breeders for nesting behaviour.
  - The nest should contain soft, manipulable material as nest floor allowing nestbuilding behaviour;
  - The nest should be enclosed (opaque top, sides and back to achieve a secluded, safe atmosphere for the egg-laying bird).
  - Nests should be compartmented, for example no more than 0.5 m<sup>2</sup> (> 50–100% certainty) and allowing enough space/bird, for example, at least 1 individual nest for every 7 hens (> 50–100% certainty) and 1 m<sup>2</sup> nest space for a maximum 120 hens in case of group nests (> 50–100% certainty).
- A linear feeder space allowance of at least 10 cm/laying hen and breeder, or 5 cm/pullet, and circular feeders of at least 4 cm/hen and breeders, prevent the negative welfare consequences of 'group stress' that may arise in case of restricted feeder access, if the same quality of feed is provided to all birds (> 50–100% certainty).
- From field experience in Europe, a linear drinker space (e.g. nipple) allowance of at least 1 space/10 laying hens, breeders and pullets does not seem to bring specific negative welfare consequences associated with restricted access to drinkers.
- Bell drinkers may be associated with feather and vent pecking.
- Background sound pressure levels above 75 dB will impair welfare of laying hens, layer breeders and pullets (> 50–100% certainty).

## Management

- Light intensities above 5 lx encourage activity and, therefore, improve welfare in laying hens, pullets and layer breeders (66–100% certainty).
- From field experience in Europe, a light spectrum being near-natural including UV and light period of 16 h light and 8 h dark does not impair bird welfare. A flicker frequency above 95 Hz is more appropriate for birds (> 50–100% certainty) than lower ones. Additionally, instead of light being uniform in housing, functional areas of different light intensities and spectra and

natural light help to maintain good welfare. Periods of dusk and dawn help the good distribution of the hens in the system (e.g. finding a roosting place), and help the navigation in the system and therefore improve bird welfare.

- Laying hens, layer breeders and pullets, are prevented from heat and cold stress when they are maintained in the following temperature ranges:
  - Laying hens and layer breeders: 15–26°C (depending on the hybrid).
  - Pullets:
    - Age day 1 to day 7 at 30–32°C;
    - Age of week 2 at 28–30°C;
    - Age week 3 at 26–28°C;
    - Age week 4 at 24–26°C;
    - Age week 5 at 22–24°C;
    - From age week 6 at 20–24°C.
- The use of dark brooders will allow young pullet to rest in warmer areas and to perform activities in cooler zones and will prevent injurious pecking in adults.
- To limit the formation of dust, the choice of litter material and how it is provided and managed is crucial. In general, using de-dusted litter is a major advantage which is not generally applied in practice, but ventilation can also be increased to capture the dust through filtering. However, at the moment maximum recommended dust concentrations are only available for human health.
- A clear relationship between laying hens' welfare and dust concentration in air has not been established and constitutes a gap in knowledge, even though we know that high concentration of total dust (> 30 mg/m<sup>2</sup>) induces respiratory disorders in laying hens.
- When ammonia concentration is higher than 10 ppm, laying hens show increasing signs of aversion.

Access to covered verandas and outdoor range:

- Pop-holes of at least 45 cm high for pullets, 55 cm for laying hens and 77 cm for breeders will allow birds to exit and enter easily.
- Pop-holes can be raised to protect the house environment, but the opening should not be higher than 25 cm from ground-level to allow the birds to see the outdoor range (which improves range use) and to make it easy for birds to jump through the pop hole.
- From field experience in Europe, at least 2 m linear of pop holes/1000 birds allows bird to easily enter and exit the barn.
- The presence of a covered veranda and eventually in combination with outdoor range will allow birds to access different climatic and light conditions and to explore and forage more in extra-space providing new opportunities.
- Ensuring provision of outdoor range covered with at least 50% natural vegetation such as bushes and trees will encourage the birds to use the range to perform more exploration, foraging and comfort behaviours.

#### 4.2.2. Recommendations:

The following characteristics are recommended for a minimal enclosure to house laying hens, pullets and breeders, each recommendation if valid when all other parameters are following the minimum enclosure characteristics:

- Group size: a minimum of 2 birds is necessary, but no clear cut off for maximum group size has been found.
- It is recommended to carry out research on the effect of group size on welfare of laying hens in commercial settings including the transition to non-beak-trimmed birds.
- Ratio male/female: In breeders, it should not be more than one male for 10 females. The number of males should be lowered if there are signs of inability to avoid unwanted sexual behaviour for females.
- Minimum area should be 80 m<sup>2</sup> for group sizes of more than 30 birds for laying hens and layer breeders and 25 m<sup>2</sup> for groups of less than 30 birds.
- Stocking density should not exceed 4 birds/m<sup>2</sup>

- Perches should be non-slippery and between 3 and 6 cm in diameter, with horizontal distance of less than 80 cm between them and angles below 45° (below 40° for ramps to access the elevated structures). A minimum perch length of 18 cm per layer hen and per layer breeder, and 14 cm for pullets, and the distance of at least 30 cm between perches of the same height and of at least 20 cm between a perch and a surrounding of the pen are recommended.
- Elevated platforms should be available for all birds from 3 weeks of age.
- The minimum height in any part of the system (above the usable area tiers and perches) should be at least 55 cm for laying hens, at least 77 cm for laying breeders and at least 45 cm for pullets over 6 weeks of age.
- The total height of an enclosure (including covered veranda), from floor to ceiling should be at least 2 m to allow humans to enter the system and check the birds.
- Dry and friable litter should be accessible at all times, from day 1 and onwards.
- In addition to litter, edible enrichment should be accessible at all times from day 1 and onwards
- Nests should be available for laying hens and female layer breeders and should be closed. The nest floor should be made of soft manipulable material. Platforms of at least 30 cm width should be available in front of the nest, rather than perches. Middle of nest rows should be made more attractive by, e.g. partitioning the outside area in front of nest, colour marking or use of attractive material.
- Feed should be accessible at all times. Sufficient feeder space should be provided to prevent the negative welfare consequences of 'group stress' that may arise in case of restricted access to feeders: at least 10 cm of linear feeder space/laying hen or breeder, or 5 cm/pullet, or at least 4 cm/hen or breeder if circular feeders are used and 2 cm for pullets.
- A linear drinker space (e.g. nipple) allowance of at least 1 space/10 laying hens, breeders and pullets is recommended.
- Noise levels should not exceed 75 dB.
- Light from 5 lx and higher should be provided during the day.
- Natural light should be provided in addition to artificial light. Eight hours of continuous darkness (artificial light turned off) per day should be provided to laying hens and breeders. Periods of dusk and dawn should be provided.
- Temperature in the barn should be between 15 and 26°C for laying hens and laying breeders and for pullets between 32°C and 20°C, depending on age.
- The maximum concentration of NH<sub>3</sub> should not be higher than 10 ppm and maximum concentration of total dust should never be higher than 30 mg/m<sup>3</sup> and more research is needed to determine the maximum dust and CO<sub>2</sub> concentration not leading to negative welfare consequences.
- The use of dark brooders is recommended for young pullets.
- The presence of a covered veranda is recommended for all categories of birds (20% additional space minimum, in addition to usable area), accessible and closable. If combined with outdoor range, the latter should be directly accessible either from the house or through the veranda and ideally through both lateral sides of the facility.
- Outdoor range is encouraged. When it is provided, it should be accessible during daylight hours and should be covered at 50% with bushes and trees.

A covered veranda should always be available:

- Provide a surface of at least 20% of the usable area in the barn.
- Provide protection from dominant winds, wild birds, and some pathogens by windbreaks.
- Having one longer side open (with net or grid) letting entering natural light.
- Provide protection from rain with provision of a solid roof.
- Have a minimum height of 2 m to allow veranda to be checked by farm staff daily.
- Be covered by dry, friable litter.
- Have at least one side (on the longer side) that is non-solid (e.g. wire mesh or simply open) to provide natural light and fresh air, with the possibility to close this side off in case of adverse weather conditions.
- Provide enrichments such as hay bales, foraging materials and other pecking substrates.
- Have good drainage.
- Be accessible during daylight hours; and in any case when indoor lights are on.

Outdoor range should:

- Be directly accessible either from the house or via the veranda and ideally through both lateral (longer) sides of the facility.
- Be directly accessible via pop holes exiting from the barn or the veranda to the outdoor range.
- Comprise a unified area and birds should not have to move through narrow outdoor fenced corridors or passages to reach any part of the range.
- Be available during daylight hours. Birds should be encouraged back into the house at dusk to reduce the risk of predation.
- Be covered with vegetation that should provide a canopy at a height of > 50 cm from ground level. The vegetal cover should be provided at a distance of no more than 20 m from the pop-holes and be available at all times when hens have access to the range. Vegetation cover should be distributed appropriately to encourage full range use and at least 50% being covered by high vegetation, e.g. bushes and trees.

Management requirements for systems with outdoor access

- Pullets that will move to free-range systems should be provided with access to a veranda and, ideally, an outdoor range from 8 to 10 weeks of age depending on hybrids and climatic conditions.
- Layers should be introduced to and encouraged to use the veranda and range areas within 2 weeks after arrival to the production facility. Where a veranda is present, birds should be introduced to this before being introduced to the range, as its use will facilitate the transition to the free-range area.
- Additional measures should be taken as necessary to –exclude or discourage predators (e.g. use of electric fencing) and to reduce direct contact between chickens and wild birds (e.g. ensuring no pools of water to attract wild birds).
- A management plan should be devised to minimise build-up of parasites and other pathogenic agents to maintain veranda litter and range areas in good, dry condition, and to encourage birds to move away from pop-holes to use the full area. The use of large rocks in the area adjacent to the pop-holes can allow good drainage and encourage birds to move to areas of natural vegetation.

#### 4.3. Answers to Specific ToR 2: welfare of hens in furnished cages, and risks associated with rearing of animals non-beak trimmed

##### 4.3.1. Conclusions

- Beak trimming is conducted to reduce the prevalence and severity of pecking (e.g. injurious pecking and vent pecking) in laying hens and laying breeders, which might be required under current husbandry systems. Injurious pecking results in serious welfare consequences. The damage incurred by injurious pecking is more severe in non-beak-trimmed flocks than infrared beak trimming in beak-trimmed flocks.
- The process of beak trimming itself causes welfare consequences. There is variation in the amount of tissue removed during infrared trimming and the welfare consequences are greater when more tissue is removed. Infrared trimming incurs reduced welfare consequences compared with hot-blade trimming but adverse effects are still apparent compared to untrimmed birds.
- Injurious pecking occurs at a similar level in all types of housing systems, with great variation in prevalence between flocks.
- Injurious pecking is harder to control in non-cage systems because of the larger group size, but there is also far greater potential to adopt preventive management in non-cage systems, e.g.; provision of a variety of enrichments.
- Injurious pecking can be reduced by management and by choice of hybrids but can still occur even in well-managed flocks.
- Alternative approaches to reduce the sharpness of the beak without trimming consist of (i) breeding birds with short or blunt beaks; (ii) achieving and maintaining blunt beaks by using hard, abrasive surface materials that shorten the beak as birds eat (e.g. abrasive feeders); (iii) the use of hard materials as pecking substrates (e.g. pecking stones) to reduce inter-bird

pecking by redirecting the pecking behaviour towards the environment. The challenge with these alternatives is to reach a uniform effect on all the birds.

- Damage to skin and integument associated with injurious pecking is likely to be reduced in birds that have naturally shortened or blunted beaks (66–100% certainty).

#### 4.3.2. Recommendations

- The practice of beak trimming should not be necessary if good management practices are implemented, such as: use of dark brooders during rearing young pullets, maintaining dry, friable and accessible litter at all times, providing mash diets with sufficient fibre and proteins, minimising the number of diet changes, providing appropriate distribution of light in the activity areas, keeping temperature and humidity within recommended intervals, keeping ammonia concentrations below 10 ppm, using a covered veranda, and reduce stocking density.
- All husbandry and management practices described above to prevent severe pecking should be optimised, before beak trimming is phased out. Keeping birds with intact beaks will prevent the negative welfare consequences associated with soft tissue damage from this procedure.
- Where outdoor access is provided, allow early usage from arrival at laying house, encourage even use of whole area by the birds, provide shelter, and ensure that the house does not become cold, wet or windy by shielding pop-holes, and avoid mud in vicinity of house.
- Hybrids that are adapted to non-cage systems and less prone to group stress and injurious pecking should be used, as these will have a lower risk of injury when kept with intact beaks.
- Further research and interaction with geneticists should be undertaken to promote the development of hybrids with beak shapes that are less likely to cause skin damage (but still allowing functionality for feeding and exploratory behaviours) when birds are kept with intact beaks.
- Farmers should inspect birds on a daily basis and take immediate action if early signs of injurious pecking are detected.
- The application of methods to reduce the sharpness of the beak without trimming it (e.g. abrasive feeders, pecking stones) is recommended to reduce the risk of injuries in flocks with sharp beaks, and at the same time avoid the lesions due to beak trimming. It should be used in combination with prevention of injurious pecking.
- Research on the best methods, location and timings of provision of beak blunting materials is urgently needed, particularly in commercial flocks.
- Farmers making a transition to cage-free systems should first gain experience with these systems before making a further transition to managing flocks with intact beaks.

#### 4.4. Answers to Specific ToR 3: the assessment of animal-based measures collected in slaughterhouses to monitor the level of welfare on laying hen farms

##### 4.4.1. Conclusions

- Total mortality, plumage damage, wounds, keelbone fractures and carcass condemnations are the most promising ABMs for collection at slaughterhouses to monitor the level of laying hen welfare on farm. However, the readiness for automation is different for the different ABMs.
- The ABMs collected at the slaughterhouse lead to underestimations of the prevalence of the welfare consequences on farms, as they do not include the hens that die or were culled on farm.
- Currently there is a lot of variation in the method used for measuring each ABM and only a few are validated. This impairs standardisation within and between EU member states and subsequently comparison of the resulting data.
- Unified and standardised scoring systems and protocols across different regions/countries are necessary to monitor and benchmark the welfare of laying.
- The Technology Readiness Level (TRL) of automated monitoring of the ABMs at slaughterhouses currently varies between the proposed ABMs. Methods for wounds and carcass condemnations are the most advanced, to our knowledge.

#### 4.4.2. Recommendations

- Monitoring total mortality, plumage damage, wounds, keelbone fractures and carcass condemnations should be implemented to identify flocks of hens with diverse welfare consequences, thereby potentially enabling the implementation of target inspection
- The cumulative daily mortality rate and cumulative daily culled rate should be calculated together with the rates at the time of slaughter to provide information on the pattern of mortality in the flock.
- Harmonised assessment methods and scoring systems should be developed and implemented for the identified ABMs.
- Databases should be used to enable animal welfare benchmarking between and within Member States and risk assessment exercises.
- Systems for automatic and continuous assessment of ABMs and data recording should be concordant with the standardised manual method. For the assessment of plumage damage, wounds, keel bone fractures and carcass condemnation, automated technologies in the slaughter line should be fully automated and validated and then finally implemented.

#### 4.5. Main recommendations concerning more urgent welfare issues in egg production

Considering the above conclusions and recommendations, the following recommendations are considered the most impactful to improve laying hens, layer breeders and pullets welfare (the quantitative recommendations can be found Section 4.2):

- House all birds in non-cage systems.
- Implement protocols to define welfare trait information (e.g. keel bone fractures and plumage condition) for all commercial hybrids to encourage further progress in genetic selection and to enable producers to choose strains with a reduced risk of bone lesions and soft tissue lesions and integument damage.
- Provide dry and friable litter, available at all times, supplemented by the provision of new litter material and other enrichments that support comfort and exploratory behaviour, naturally shorten beaks and reduce the risk of injurious pecking.
- Implementing all preventive measures against injurious pecking should be done to facilitate a phasing out of beak trimming.
- House flocks with easily accessible, elevated platforms and/or perches to permit simultaneous resting by all birds, and to enable birds to avoid or escape each other.
- Provide a covered veranda for all birds to reduce effective/local stocking density during daytime periods when birds are most active, and permit birds to choose between temperatures, light conditions and substrate quality. This would reduce the risk of the welfare consequences inability to perform foraging, exploratory and comfort behaviour. Compared to an outdoor range, the risk of predation stress, gastroenteric disorders and other infectious diseases in case of outbreaks in the MS will be reduced. In climates where a covered veranda cannot be provided, provide extra space to birds.
- Implement harmonised assessment methods and scoring systems for monitoring mortality on farm and wounds, plumage damage, keel bone fractures and carcass condemnation at slaughter. Such tools can be used to monitor welfare level across farms in Europe.
- Rear pullets with dark brooders to reduce fearfulness during rearing and the subsequent laying period. Rear them in a system allowing the development of navigation skills.
- In layer breeders: reduce male aggression to females e.g. by reducing proportion of males included in flocks (below 1:10), selecting male birds for reduced aggression, include partition panel to allow female to escape males and ensure synchrony in sexual maturity of both sexes.

## References

- Abeyesinghe SM, Drewe JA, Asher L, Wathes CM and Collins LM, 2013. Do hens have friends? *Applied Animal Behaviour Science*, 143, 61–66. <https://doi.org/10.1016/j.applanim.2012.12.003>
- Adrizal, Patterson PH, Hulet RM, Bates RM, Despot DA, Wheeler EF, Topper PA, Anderson DA and Thompson JR, 2008. The potential for plants to trap emissions from farms with laying hens: 2. Ammonia and dust. *Journal of Applied Poultry Research*, 17, 398–411, ISSN 1056-6171. <https://doi.org/10.3382/japr.2007-00104>

- Aerts JM, Wathes CM and Berckmans D(PG), 2004. Environmental management for laying hens. Cabi Publishing, Cambridge.
- Agnvall B and Jensen P, 2016. Effects of divergent selection for fear of humans on behaviour in red junglefowl. *PLoS One*, 11, e0166075. <https://doi.org/10.1371/journal.pone.0166075>
- Albentosa MJ and Cooper J, 2004. Effects of cage height and stocking density on the frequency of comfort behaviours performed by laying hens housed in furnished cages. *Animal Welfare*, 13, 419–424. <https://doi.org/10.1017/S0962728600028670>
- Albentosa MJ, Cooper JJ, Luddem T, Redgate SE, Elson HA and Walker AW, 2007. Evaluation of the effects of cage height and stocking density on the behavior of laying hens in furnished cages. *British Poultry Science*, 48, 1–11. <https://doi.org/10.1080/00071660601156479>
- Ali ABA, Campbell DLM, Karcher DM and Siegford JM, 2019. Nighttime roosting substrate type and height among 4 strains of laying hens in an aviary system1. *Poultry Science*, 98, 1935–1946. <https://doi.org/10.3382/ps/pey574>
- Al-Rawi B and Craig JV, 1975. Agonistic behavior of caged chickens related to group size and area per bird. *Applied Animal Ethology*, 2, 69–80. [https://doi.org/10.1016/0304-3762\(75\)90066-8](https://doi.org/10.1016/0304-3762(75)90066-8)
- Al-Saffar AA and Rose SP, 2002. Ambient temperature and the egg laying characteristics of laying fowl. *World's Poultry Science Journal*, 58, 317–331. <https://doi.org/10.1079/wps20020025>
- Alvino GM, Tucker CB, Archer GS and Mench JA, 2013. Astro turf as a dustbathing substrate for laying hens. *Applied Animal Behaviour Science*, 146, 88–95. <https://doi.org/10.1016/j.applanim.2013.03.006>
- Amer A, Pingel H, Hillig J, Soltan M and Von Borell E, 2004. Impact of atmospheric ammonia on laying performance and egg shell strength of hens housed in climatic chambers. *Archiv Fur Geflugelkunde*, 68, 120–124.
- Anderson DP, Beard CW and Hanson RP, 1966. Influence of poultry house dust, ammonia, and carbon dioxide on the resistance of chickens to Newcastle disease virus. *Avian Diseases*, 10, 177–188.
- Angevaere MJ, Prins S, van der Staay FJ and Nordquist RE, 2012. The effect of maternal care and infrared beak trimming on development, performance and behavior of Silver Nick hens. *Applied Animal Behaviour Science*, 140, 70–84.
- Appleby MC, 2004. What causes crowding? Effects of space, facilities and group size on behaviour, with particular reference to furnished cages for hens. *Animal Welfare*, 13, 313–320.
- Appleby MC and McRae HE, 1986. The individual nest box as a super-stimulus for domestic hens. *Applied Animal Behaviour Science*, 15, 169–176. [https://doi.org/10.1016/0168-1591\(86\)90062-6](https://doi.org/10.1016/0168-1591(86)90062-6)
- Appleby MC, Hughes BO and Hogarth GS, 1989. Behaviour of laying hens in a deep litter house. *British Poultry Science*, 30, 545–553. <https://doi.org/10.1080/00071668908417178>
- Armstrong EA, Richards-Rios P, Addison L, Sandilands V, Guy JH, Wigley P, Boswell T and Smulders TV, 2022. Poor body condition is associated with lower hippocampal plasticity and higher gut methanogen abundance in adult laying hens from two housing systems. *Scientific Reports*, 12, 15505. <https://doi.org/10.1038/s41598-022-18504-1>
- Arnould C, Frayssse V and Mirabito L, 2001. Use of pen space by broiler chickens reared in commercial conditions: access to feeders and drinkers. *British Poultry Science*, 42, 281–296.
- Asher L, Collins LM, Pfeiffer DU and Nicol CJ, 2013. Flocking for food or flockmates? *Applied Animal Behaviour Science*, 147, 94–103.
- Baker SL, Robison CI, Karcher DM, Toscano MJ and Makagon MM, 2020. Keel impacts and associated behaviors in laying hens. *Applied Animal Behaviour Science*, 222, 104886.
- Baker PE, Nicol CJ and Weeks CA, 2022. The effect of hard pecking enrichment during rear on feather cover, feather pecking behaviour and beak length in beak-trimmed and intact-beak laying hen pullets. *Animals*, 12, 674.
- Banks EM, Wood-Gush DG, Hughes BO and Mankovich NJ, 1979. Social rank and priority of access to resources in domestic fowl. *Behavioural Processes*, 4, 197–209. [https://doi.org/10.1016/0376-6357\(79\)90001-9](https://doi.org/10.1016/0376-6357(79)90001-9)
- Bari MS, Laurenson YCSM, Cohen-Barnhouse AM, Walkden-Brown SW and Campbell DLM, 2020. Effects of outdoor ranging on external and internal health parameters for hens from different rearing enrichments. *PeerJ*, 8, e8720. <https://doi.org/10.7717/peerj.8720>
- Barrett J, Rayner A, Gill R, Willings T and Bright A, 2014. Smothering in UK free-range flocks. Part 1: incidence, location, timing and management.
- Bateson M and Martin P, 2021. *Measuring behaviour: an introductory guide*. Cambridge university press.
- Bennett DC, Yee A, Rhee YJ and Cheng KM, 2011. Effect of diatomaceous earth on parasite load, egg production, and egg quality of free-range organic laying hens. *Poultry Science*, 90, 1416–1426. <https://doi.org/10.3382/ps.2010-01256>
- Bestman M and Wagenaar JP, 2014. Health and welfare in Dutch organic laying hens. *Animals (Basel)*, 4, 374–390. <https://doi.org/10.3390/ani4020374>
- Bestman M and Bikker-Ouwejan J, 2020. Predation in organic and free-range egg production. *Animals*, 10, 177.
- Bestman M, Koene P and Wagenaar J-P, 2009. Influence of farm factors on the occurrence of feather pecking in organic reared hens and their predictability for feather pecking in the laying period. *Applied Animal Behaviour Science*, 121, 120–125. <https://doi.org/10.1016/j.applanim.2009.09.007>

- Bestman M, Verwer C, Brenninkmeyer C, Willett A, Hinrichsen LK, Smajlhodzic F, Heerkens JLT, Gunnarsson S and Ferrante V, 2017. Feather-pecking and injurious pecking in organic laying hens in 107 flocks from eight European countries. *Animal Welfare*, 26, 355–363.
- Bestman M, Verwer C, van Niekerk T, Leenstra F, Reuvekamp B, Amsler-Kepalaite Z and Maurer V, 2019. Factors related to free-range use in commercial laying hens. *Applied Animal Behaviour Science*, 214, 57–63.
- Bestman MWP, 2022. Welfare and health aspects of free ranges for laying hens. Utrecht University.
- Bestman MWP and Wagenaar JP, 2003. Farm level factors associated with feather pecking in organic laying hens. *Livestock Production Science*, 80, 133–140. [https://doi.org/10.1016/S0301-6226\(02\)00314-7](https://doi.org/10.1016/S0301-6226(02)00314-7)
- Bhagwat AL and Craig JV, 1979. Effects of male presence on agonistic behavior and productivity of White Leghorn hens. *Applied Animal Ethology*, 5, 267–282. [https://doi.org/10.1016/0304-3762\(79\)90061-0](https://doi.org/10.1016/0304-3762(79)90061-0)
- Bilcik B and Estevez I, 2005. Impact of male–male competition and morphological traits on mating strategies and reproductive success in broiler breeders. *Applied Animal Behaviour Science*, 92, 307–323. <https://doi.org/10.1016/j.applanim.2004.11.007>
- Bilcik B and Keeling LJ, 1999. Changes in feather condition in relation to feather pecking and aggressive behaviour in laying hens. *British Poultry Science*, 40, 444–451. <https://doi.org/10.1080/00071669987188>
- Birn-Jeffery AV, Miller CE, Naish D, Rayfield EJ and Hone DW, 2012. Pedal claw curvature in birds, lizards and Mesozoic dinosaurs—complicated categories and compensating for mass-specific and phylogenetic control. *PLoS One*, 7, e50555.
- Black AJ and Hughes BO, 1974. Patterns of comfort behaviour and activity in domestic fowls: a comparison between cages and pens. *British Veterinary Journal*, 130, 23–33. [https://doi.org/10.1016/S0007-1935\(17\)35987-0](https://doi.org/10.1016/S0007-1935(17)35987-0)
- Blokhuis HJ, 1984. Rest in poultry. *Applied Animal Behaviour Science*, 12, 289–303. [https://doi.org/10.1016/0168-1591\(84\)90121-7](https://doi.org/10.1016/0168-1591(84)90121-7)
- Blokhuis HJ, 1989. The effect of a sudden change in floor type on pecking behaviour in chicks. *Applied Animal Behaviour Science*, 22, 65–73, ISSN 0168-1591. [https://doi.org/10.1016/0168-1591\(89\)90080-4](https://doi.org/10.1016/0168-1591(89)90080-4)
- Blokhuis HJ, 1986. Feather-pecking in poultry: Its relation with ground-pecking. *Applied Animal Behaviour Science*, 16, 63–67. [https://doi.org/10.1016/0168-1591\(86\)90040-7](https://doi.org/10.1016/0168-1591(86)90040-7)
- Bonnefous C, Collin A, Guilloteau LA, Guesdon V, Filliat C, Réhault-Godbert S, Rodenburg TB, Tuytens FAM, Warin L, Steinfeldt S, Baldinger L, Re M, Ponzio R, Zuliani A, Venezia P, Väre M, Parrott P, Walley K, Niemi JK and Lettieri C, 2022. Welfare issues and potential solutions for laying hens in free range and organic production systems: A review based on literature and interviews. *Frontiers in Veterinary Science*, 9, 952922. <https://doi.org/10.3389/fvets.2022.952922>
- Borchelt PL and Overmann SR, 1974. Development of dustbathing in bobwhite quail. I. Effects of age, experience, texture of dust, strain, and social facilitation. *Developmental Psychobiology*, 7, 305–313. <https://doi.org/10.1002/dev.420070407>
- Bortolotti GR, Marchant TA, Blas J and German T, 2008. Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. *Functional Ecology*, 22, 494–500.
- Bouba I, Visser B, Kemp B, Rodenburg TB and van den Brand H, 2021. Predicting hatchability of layer breeders and identifying effects of animal related and environmental factors. *Poultry Science*, 100, 101394. <https://doi.org/10.1016/j.psj.2021.101394>
- Bouwstra R, Gonzales JL, de Wit S, Stahl J, Fouchier RAM and Elbers ARW, 2017. Risk for low pathogenicity avian influenza virus on poultry farms, the Netherlands, 2007–2013. *Emerging Infectious Diseases*, 23, 1510–1516. <https://doi.org/10.3201/eid2309.170276>
- Bozkurt Z, Bayram İ, Türkmenoğlu İ and Aktepe O, 2006. Effects of cage density and cage position on performance of commercial layer pullets from four genotypes. *Turkish Journal of Veterinary and Animal Sciences*, 30, 17–28.
- Braastad BO, 1990. Effects on behaviour and plumage of a key-stimuli floor and a perch in triple cages for laying hens. *Applied Animal Behaviour Science*, 27, 127–139. [https://doi.org/10.1016/0168-1591\(90\)90012-3](https://doi.org/10.1016/0168-1591(90)90012-3)
- Brantsæter M, Nordgreen J, Rodenburg TB, Tahamtani FM, Popova A and Janczak AM, 2016a. Exposure to increased environmental complexity during rearing reduces fearfulness and increases use of three-dimensional space in laying hens (*Gallus gallus domesticus*). *Frontiers in Veterinary Science*, 3, 14. <https://doi.org/10.3389/fvets.2016.00014>
- Brantsæter M, Tahamtani FM, Moe RO, Hansen TB, Orritt R, Nicol C and Janczak AM, 2016b. Rearing laying hens in aviaries reduces fearfulness following transfer to furnished cages. *Frontiers in Veterinary Science*, 3, 13. <https://doi.org/10.3389/fvets.2016.00013>
- Brantsæter M, Nordgreen J, Hansen TB, Muri K, Nødtvedt A, Moe RO and Janczak AM, 2018. Problem behaviors in adult laying hens—identifying risk factors during rearing and egg production. *Poultry Science*, 97, 2–16.
- Bremner A and Johnston M, 1996. *Poultry meat hygiene and inspection*. WB Saunders Company Ltd.
- Brendler C, Kipper S and Schrader L, 2014. Vigilance and roosting behaviour of laying hens on different perch heights. *Applied Animal Behaviour Science*, 157, 93–99. <https://doi.org/10.1016/j.applanim.2014.06.004>
- Brendler C and Schrader L, 2016. Perch use by laying hens in aviary systems. *Applied Animal Behaviour Science*, 182, 9–14. <https://doi.org/10.1016/j.applanim.2016.06.002>

- Bright A, Jones TA and Dawkins MS, 2006. A non-intrusive method of assessing plumage condition in commercial flocks of laying hens. *Animal Welfare*, 15, 113–118.
- Bright A and Johnson EA, 2011. Short communications: Smothering in commercial free-range laying hens: A preliminary investigation. *Veterinary Record*, 168, 512. <https://doi.org/10.1136/vr.c7462>
- Buijs S, Heerkens JLT, Ampe B, Delezie E, Rodenburg TB and Tuytens FAM, 2019. Assessing keel bone damage in laying hens by palpation: effects of assessor experience on accuracy, inter-rater agreement and intra-rater consistency. *Poultry Science*, 98, 514–521. <https://doi.org/10.3382/ps/pey326>
- Buijs S, Nicol CJ, Booth F, Richards G and Tarlton JF, 2020. Light-based monitoring devices to assess range use by laying hens. *Animal*, 14, 814–823. <https://doi.org/10.1017/S1751731119002830>
- BuRO, 2022. Available online: <https://english.nvwa.nl/documents/animal/welfare/buro/documents/advice-from-buro-on-the-use-of-sensor-technology-to-promote-animal-welfare-in-slaughterhouses>
- Bush SE and Clayton DH, 2018. Anti-parasite behaviour of birds. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, 373, 20170196.
- Cameron AR and Baldock FC, 1998. A new probability formula for surveys to substantiate freedom from disease. *Preventive Veterinary Medicine*, 34, 1–17. [https://doi.org/10.1016/S0167-5877\(97\)00081-0](https://doi.org/10.1016/S0167-5877(97)00081-0)
- Campbell D, 2020. Skeletal health of layers across all housing systems and future research directions for Australia. *Animal Production Science*, 61, 883–892.
- Campbell DLM, Bari MS and Rault JL, 2021. Free-range egg production: its implications for hen welfare. *Animal Production Science*, 61, 848–855.
- Campbell DLM, de Haas EN and Lee C, 2019. A review of environmental enrichment for laying hens during rearing in relation to their behavioral and physiological development. *Poultry Science*, 98, 9–28. <https://doi.org/10.3382/ps/pey319>
- Campbell DLM, Hinch GN, Downing JA and Lee C, 2016a. Fear and coping styles of outdoor-preferring, moderate-outdoor and indoor-preferring free-range laying hens. *Applied Animal Behaviour Science*, 185, 73–77. <https://doi.org/10.1016/j.applanim.2016.09.004>
- Campbell DLM, Makagon MM, Swanson JC and Siegford JM, 2016b. Litter use by laying hens in a commercial aviary: dust bathing and piling. *Poultry Science*, 95, 164–175. <https://doi.org/10.3382/ps/pev183>
- Campbell DL, Goodwin SL, Makagon MM, Swanson JC and Siegford JM, 2016c. Failed landings after laying hen flight in a commercial aviary over two flock cycles. *Poultry Science*, 95, 188–197. <https://doi.org/10.3382/ps/pev270>
- Campbell DLM, Hinch GN, Downing JA and Lee C, 2017a. Outdoor stocking density in free-range laying hens: effects on behaviour and welfare. *Animal*, 11, 1036–1045. <https://doi.org/10.1017/S1751731116002342>
- Campbell DLM, Hinch GN, Dyal TR, Warin L, Little BA and Lee C, 2017b. Outdoor stocking density in free-range laying hens: radio-frequency identification of impacts on range use. *Animal*, 11, 121–130. <https://doi.org/10.1017/S1751731116001154>
- Campderrich I, Liste G and Estevez I, 2017. The looks matter; aggression escalation from changes on phenotypic appearance in the domestic fowl. *PLoS One*, 12, e0188931. <https://doi.org/10.1371/journal.pone.0188931>
- Campderrich I, Nazar FN, Wichman A, Marin RH, Estevez I and Keeling LJ, 2019. Environmental complexity: a buffer against stress in the domestic chick. *PLoS One*, 14, e0210270. <https://doi.org/10.1371/journal.pone.0210270>
- Campo JL, Gil MG and Dávila SG, 2005. Effects of specific noise and music stimuli on stress and fear levels of laying hens of several breeds. *Applied Animal Behaviour Science*, 91, 75–84. <https://doi.org/10.1016/j.applanim.2004.08.028>
- Candelotto L, Stadelmann M, Gebhardt-Henrich SG, Stratmann A, van de Braak TGH, Guggisberg D, Zysset P and Toscano MJ, 2020. Genetic variation of keel and long bone skeletal properties for 5 lines of laying hens. *Journal of Applied Poultry Research*, 29, 937–946. <https://doi.org/10.1016/j.japr.2020.09.004>
- Candelotto L, Stratmann A, Gebhardt-Henrich SG, Rufener C, van de Braak T and Toscano MJ, 2017. Susceptibility to keel bone fractures in laying hens and the role of genetic variation. *Poultry Science*, 96, 3517–3528. <https://doi.org/10.3382/ps/pex146>
- Carleial R, McDonald GC and Pizzari T, 2020. Dynamic phenotypic correlates of social status and mating effort in male and female red junglefowl, *Gallus gallus*. *Journal of Evolutionary Biology*, 33, 22–40.
- Carmichael NL, Walker W and Hughes BO, 1999. Laying hens in large flocks in a perchery system: Influence of stocking density on location, use of resources and behaviour. *British Poultry Science*, 40, 165–176. <https://doi.org/10.1080/00071669987566>
- Carpenter GA, 1986. Dust in livestock buildings—review of some aspects. *Journal of Agricultural Engineering Research*, 33, 227–241. [https://doi.org/10.1016/S0021-8634\(86\)80038-5](https://doi.org/10.1016/S0021-8634(86)80038-5)
- Carruthers C, Gabrush T, Schwean-Lardner K, Knezacek TD, Classen HL and Bennett C, 2012. On-farm survey of beak characteristics in White Leghorns as a result of hot blade trimming or infrared beak treatment. *Journal of Applied Poultry Research*, 21, 645–650. <https://doi.org/10.3382/japr.2011-00433>
- Casey-Trott T, Heerkens JLT, Petrik M, Regmi P, Schrader L, Toscano MJ and Widowski T, 2015. Methods for assessment of keel bone damage in poultry. *Poultry Science*, 94, 2339–2350. <https://doi.org/10.3382/ps/pev223>

- Casey-Trott TM, Guerin MT, Sandilands V, Torrey S and Widowski TM, 2017. Rearing system affects prevalence of keel-bone damage in laying hens: a longitudinal study of four consecutive flocks. *Poultry Science*, 96, 2029–2039. <https://doi.org/10.3382/ps/pex026>
- Caulfield MP and Padula MP, 2020. HPLC MS-MS analysis shows measurement of corticosterone in egg albumen is not a valid indicator of chicken welfare. *Animals*, 10, 821. <https://doi.org/10.3390/ani10050821>
- Channing CE, Hughes BO and Walker AW, 2001. Spatial distribution and behaviour of laying hens housed in an alternative system. *Applied Animal Behaviour Science*, 72, 335–345.
- Chen BL, Haith KL and Mullens BA, 2011. Beak condition drives abundance and grooming-mediated competitive asymmetry in a poultry ectoparasite community. *Parasitology*, 138, 748–757. <https://doi.org/10.1017/S0031182011000229>
- Chen S, Xiang H, Zhang H, Zhu X, Wang D, Wang J, Yin T, Liu L, Kong M, Li H and Zhao X, 2019. Rearing system causes changes of behavior, microbiome, and gene expression of chickens. *Poultry Science*, 98, 3365–3376. <https://doi.org/10.3382/ps/pez140>
- Cheng KM, Burns JT and Shoffner RN, 1985. Mating behaviour and fertility in domestic chickens. I. Inbreeding. *Applied Animal Behaviour Science*, 13, 371–381. [https://doi.org/10.1016/0168-1591\(85\)90016-4](https://doi.org/10.1016/0168-1591(85)90016-4)
- Chepete HJ and Xin H, 2004. Ventilation rates of a laying hen house based on new vs. old heat and moisture production data. *Applied Engineering in Agriculture*, 20, 835–842.
- Chielo LI, Pike T and Cooper J, 2016. Ranging behaviour of commercial free-range laying hens. *Animals*, 6, 28. <https://doi.org/10.3390/ani6050028>
- Chloupek P, Voslařová E, Chloupek J, Bedáňová I, Pištěková V and Večerek V, 2009. Stress in broiler chickens due to acute noise exposure. *Acta Veterinaria Brno*, 78, 93–98.
- Chow A and Hogan JA, 2005. The development of feather pecking in Burmese red junglefowl: the influence of early experience with exploratory-rich environments. *Applied Animal Behaviour Science*, 93, 283–294. <https://doi.org/10.1016/j.applanim.2005.01.004>
- Clark CEF, Akter Y, Hungerford A, Thomson P, Islam MR, Groves PJ and O'Shea CJ, 2019. The intake pattern and feed preference of layer hens selected for high or low feed conversion ratio. *PLoS One*, 14, e0222304. <https://doi.org/10.1371/journal.pone.0222304>
- Clausen T and Riber AB, 2012. Effect of heterogeneity of nest boxes on occurrence of gregarious nesting in laying hens. *Applied Animal Behaviour Science*, 142, 168–175. <https://doi.org/10.1016/j.applanim.2012.10.005>
- Cloutier S and Newberry RC, 2000. Recent social experience, body weight and initial patterns of attack predict the social status attained by unfamiliar hens in a new group. *Behaviour*, 137, 705–726.
- Cloutier S, Newberry RC, Forster CT and Girsberger KM, 2000. Does pecking at inanimate stimuli predict cannibalistic behaviour in domestic fowl? *Applied Animal Behaviour Science*, 66, 119–133. [https://doi.org/10.1016/S0168-1591\(99\)00068-4](https://doi.org/10.1016/S0168-1591(99)00068-4)
- Collias NE and Collias EC, 1967. A field study of the red jungle fowl in north-central India. *The Condor*, 69, 360–386.
- Collias NE and Collias EC, 1996. Social organization of a red junglefowl, *Gallus gallus*, population related to evolution theory. *Animal Behaviour*, 51, 1337–1354. <https://doi.org/10.1006/anbe.1996.0137>
- Collins LM, Asher L, Pfeiffer DU, Browne WJ and Nicol CJ, 2011. Clustering and synchrony in laying hens: the effect of environmental resources on social dynamics. *Applied Animal Behaviour Science*, 129, 43–53. <https://doi.org/10.1016/j.applanim.2010.10.007>
- Colson S, Arnould C and Michel V, 2008. Influence of rearing conditions of pullets on space use and performance of hens placed in aviaries at the beginning of the laying period. *Applied Animal Behaviour Science*, 111, 286–300. <https://doi.org/10.1016/j.applanim.2007.06.012>
- Compston J, 2006. Bone quality: what is it and how is it measured? *Arquivos Brasileiros de Endocrinologia e Metabologia*, 50, 579–585.
- Cooper JJ and Albentosa MJ, 2003. Behavioural priorities of laying hens. *Avian and Poultry Biology Reviews*, 14, 127–149.
- Cooper JJ and Appleby MC, 1996. Demand for nest boxes in laying hens. *Behavioural Processes*, 36, 171–182. [https://doi.org/10.1016/0376-6357\(95\)00027-5](https://doi.org/10.1016/0376-6357(95)00027-5)
- Cooper JJ and Appleby MC, 2003. The value of environmental resources to domestic hens: a comparison of the work-rate for food and for nests as a function of time. *Animal Welfare*, 12, 39–52.
- Cordiner LS and Savory CJ, 2001. Use of perches and nestboxes by laying hens in relation to social status, based on examination of consistency of ranking orders and frequency of interaction. *Applied Animal Behaviour Science*, 71, 305–317. [https://doi.org/10.1016/S0168-1591\(00\)00186-6](https://doi.org/10.1016/S0168-1591(00)00186-6)
- Cornetto T, Estevez I and Douglass LW, 2002. Using artificial cover to reduce aggression and disturbances in domestic fowl. *Applied Animal Behaviour Science*, 75, 325–336. [https://doi.org/10.1016/S0168-1591\(01\)00195-2](https://doi.org/10.1016/S0168-1591(01)00195-2)
- Coton J, Guinebretière M, Guesdon V, Chiron G, Mindus C, Laravoire A, Pauthier G, Balaine L, Descamps M, Bignon L, Huneau-Salaün A and Michel V, 2019. Feather pecking in laying hens housed in free-range or furnished-cage systems on French farms. *British Poultry Science*, 60, 617–627. <https://doi.org/10.1080/00071668.2019.1639137>
- Cotter PF, 2015. An examination of the utility of heterophil-lymphocyte ratios in assessing stress of caged hens. *Poultry Science*, 94, 512–517. <https://doi.org/10.3382/ps/peu009>

- Craig JV and Bhagwat AL, 1974. Agonistic and mating behavior of adult chickens modified by social and physical environments. *Applied Animal Ethology*, 1, 57–65. [https://doi.org/10.1016/0304-3762\(74\)90008-X](https://doi.org/10.1016/0304-3762(74)90008-X)
- Creel S, 2001. Social dominance and stress hormones. *Trends in Ecology & Evolution*, 16, 491–497, ISSN 0169-5347. [https://doi.org/10.1016/S0169-5347\(01\)02227-3](https://doi.org/10.1016/S0169-5347(01)02227-3)
- Damme K, 2017. Daten und Fakten zur Mast von Hahnenküken aus Legelinien. *Bioland*, 2.
- D'Eath RB and Keeling LJ, 2003. Social discrimination and aggression by laying hens in large groups: from peck orders to social tolerance. *Applied Animal Behaviour Science*, 84, 197–212. <https://doi.org/10.1016/j.applanim.2003.08.010>
- Damme K and Urselmans S, 2013. Infrared beak treatment—a temporary solution? *Lohmann Information*, 48, 36–44.
- David B, Oppermann Moe R, Michel V, Lund V and Mejdell C, 2015. Air quality in alternative housing systems may have an impact on laying hen welfare. Part I—Dust. *Animals*, 5, 495–511.
- Davis GS, Anderson KE and Jones DR, 2004. The effects of different beak trimming techniques on plasma corticosterone and performance criteria in single comb white leghorn hens. *Poultry Science*, 83, 1624–1628. <https://doi.org/10.1093/ps/83.10.1624>
- Dawkins MS, 1985. Cage height preference and use in battery-kept hens. *The Veterinary Record*, 116, 345–347. <https://doi.org/10.1136/vr.116.13.345>
- Dawkins MS, 1989. Time budgets in Red Junglefowl as a baseline for the assessment of welfare in domestic fowl. *Applied Animal Behaviour Science*, 24, 77–80. [https://doi.org/10.1016/0168-1591\(89\)90126-3](https://doi.org/10.1016/0168-1591(89)90126-3)
- Dawkins MS, 2003. Behaviour as a tool in the assessment of animal welfare. *Zoology*, 106, 383–387.
- Dawson W, 2000. Regulation of body temperature. In: GC Di dalam Whittow (ed). *Sturkie's Avian Physiology*. Ed ke-5. Academic Press, San Diego.
- Decina C, Berke O, van Staaveren N, Baes CF, Widowski TM and Harlander-Matauschek A, 2019a. A cross-sectional study on feather cover damage in Canadian laying hens in non-cage housing systems. *BMC Veterinary Research*, 15, 1–9.
- Decina C, Berke O, van Staaveren N, Baes CF, Widowski TM and Harlander-Matauschek A, 2019b. An investigation of associations between management and feather damage in Canadian laying hens housed in furnished cages. *Animals*, 9, 135.
- de Haas EN, 2020. Opportunities to improve the welfare of young chickens. *Understanding the Behaviour and Improving the Welfare of Chickens*. pp. 261–312.
- de Haas EN, Bolhuis JE, de Jong IC, Kemp B, Janczak AM and Rodenburg TB, 2014a. Predicting feather damage in laying hens during the laying period. Is it the past or is it the present? *Applied Animal Behaviour Science*, 160, 75–85. <https://doi.org/10.1016/j.applanim.2014.08.009>
- de Haas EN, Bolhuis JE, Kemp B, Groothuis TGG and Rodenburg TB, 2014b. Parents and early life environment affect behavioral development of laying hen chickens. *PLoS One*, 9, e90577. <https://doi.org/10.1371/journal.pone.0090577>
- de Haas EN, Kemp B, Bolhuis JE, Groothuis T and Rodenburg TB, 2013. Fear, stress, and feather pecking in commercial white and brown laying hen parent-stock flocks and their relationships with production parameters. *Poultry Science*, 92, 2259–2269. <https://doi.org/10.3382/ps.2012-02996>
- De Haas EN, Newberry RC, Edgar J, Riber AB, Estevez I, Ferrante V, Hernandez CE, Kjaer JB, Ozkan S, Dimitrov I, Rodenburg TB and Janczak AM, 2021. Prenatal and early postnatal behavioural programming in laying hens, with possible implications for the development of injurious pecking. *Frontiers in Veterinary Science*, 8, 678500. <https://doi.org/10.3389/fvets.2021.678500>
- De Jong I, Reuvekamp B and Gunnink H, 2013a. Can substrate in early rearing prevent feather pecking in adult laying hens? *Animal Welfare*, 22, 305–314.
- De Jong IC, Gunnink H, Rommers JM and Bracke M, 2013b. Effect of substrate during early rearing on floor-and feather pecking behaviour in young and adult laying hens. *Archiv Für Geflügelkunde*, 77, 15–22.
- de Jong IC, Wolthuis-Fillerup M and van Reenen CG, 2007. Strength of preference for dustbathing and foraging substrates in laying hens. *Applied Animal Behaviour Science*, 104, 24–36. <https://doi.org/10.1016/j.applanim.2006.04.027>
- DEFRA (Department for Environment Food and Rural Affairs), 2005. The effectiveness of beak blunting as an alternative to beak trimming - AW1129. United Kingdom. 25 pp. Available online: <http://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=10926&FromSearch=Y&Publisher=1&SearchText=beak&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description>
- DEFRA (Department for Environment, Food & Rural Affairs), 2015. A study to test the effectiveness of management strategies in reducing injurious pecking of laying hens with intact beaks in non cage systems. London, United Kingdom. Available online: <https://sciencesearch.defra.gov.uk/ProjectDetails?ProjectID=18160&FromSearch=Y&Publisher=1&SearchText=beak&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description>
- Dennis RL and Cheng HW, 2012. Effects of different infrared beak treatment protocols on chicken welfare and physiology. *Poultry Science*, 91, 1499–1505. <https://doi.org/10.3382/ps.2011-01651>
- Dennis RL, Fahey AG and Cheng HW, 2009. Infrared beak treatment method compared with conventional hot-blade trimming in laying hens. *Poultry Science*, 88, 38–43. <https://doi.org/10.3382/ps.2008-00227>

- Dennis RL, Newberry RC, Cheng HW and Estevez I, 2008. Appearance matters: artificial marking alters aggression and stress. *Poultry Science*, 87, 1939–1946. <https://doi.org/10.3382/ps.2007-00311>
- Dixon LM, 2008. Feather pecking behaviour and associated welfare issues in laying hens. *Avian Biology Research*, 1, 73–87.
- Donaldson CJ and O'Connell NE, 2012. The influence of access to aerial perches on fearfulness, social behaviour and production parameters in free-range laying hens. *Applied Animal Behaviour Science*, 142, 51–60. <https://doi.org/10.1016/j.applanim.2012.08.003>
- Downing JA and Bryden WL, 2008. Determination of corticosterone concentrations in egg albumen: a non-invasive indicator of stress in laying hens. *Physiology and Behavior*, 95, 381–387. <https://doi.org/10.1016/j.physbeh.2008.07.001>
- Döhring S, Jung L and Andersson R, 2020. Plumage damage in laying hens-automated detection in practical test-technical note. *European Poultry Science*, 84.
- Drake KA, Donnelly CA and Dawkins MS, 2010. Influence of rearing and lay risk factors on propensity for feather damage in laying hens. *British Poultry Science*, 51, 725–733. <https://doi.org/10.1080/00071668.2010.528751>
- Duncan I, 2009. Mating behaviour and fertility. *Proceedings of the Biology of Breeding Poultry. Poultry Science Symposium Series*. pp. 111–132.
- Duncan IJH and Wood-Gush DGM, 1971. Frustration and aggression in the domestic fowl. *Animal Behaviour*, 19, 500–504.
- Duncan IJH and Kite VG, 1989. Nest site selection and nest-building behaviour in domestic fowl. *Animal Behaviour*, 37, 215–231. [https://doi.org/10.1016/0003-3472\(89\)90112-7](https://doi.org/10.1016/0003-3472(89)90112-7)
- Duncan IJH, Widowski TM, Malleau AE, Lindberg AC and Petherick JC, 1998. External factors and causation of dustbathing in domestic hens. *Behavioural Processes*, 43, 219–228.
- Dunn IC, De Koning D-J, McCormack HA, Fleming RH, Wilson PW, Andersson B, Schmutz M, Benavides C, Dominguez-Gasca N and Sanchez-Rodriguez E, 2021. No evidence that selection for egg production persistency causes loss of bone quality in laying hens. *Genetics Selection Evolution*, 53, 1–13.
- EC, 2021. Available online: [https://ec.europa.eu/info/sites/default/files/food-farming-fisheries/farming/documents/eggs-dashboard\\_en.pdf](https://ec.europa.eu/info/sites/default/files/food-farming-fisheries/farming/documents/eggs-dashboard_en.pdf)
- Edgar J, Held S, Jones C and Troisi C, 2016. Influences of maternal care on chicken welfare. *Animals*, 6, 2.
- Edgar J, Held S, Paul E, Pettersson I, I'Anson Price R and Nicol C, 2015. Social buffering in a bird. *Animal Behaviour*, 105, 11–19. <https://doi.org/10.1016/j.anbehav.2015.04.007>
- Edgar JL and Nicol CJ, 2018. Socially-mediated arousal and contagion within domestic chick broods. *Scientific Reports*, 8, 10509. <https://doi.org/10.1038/s41598-018-28923-8>
- Edwards LE and Hemsworth PH, 2021. The impact of management, husbandry and stockperson decisions on the welfare of laying hens in Australia. *Animal Production Science*, 61, 944–967. <https://doi.org/10.1071/AN19664>
- EFSA (European Food Safety Authority), 2005. Welfare aspects of various systems for keeping laying hens, 143 pp.
- EFSA (European Food Safety Authority), Hart, A, Maxim, L, Siegrist, M, Von Goetz, N, da Cruz, C, Merten, C, Mosbach-Schulz, O, Lahaniatis, M, Smith, A and Hardy, A, 2019. Guidance on Communication of Uncertainty in Scientific Assessments. *EFSA Journal* 2019;17(1):5520, 73 pp. <https://doi.org/10.2903/j.efsa.2019.5520>
- EFSA (European Food Safety Authority), 2021. The use of animal-based measures at slaughter for assessing the welfare of laying hen on farm: scientific NCPs Network exercise. *EFSA supporting publication* 2021;18(12):EN-7055, 22 pp. <https://doi.org/10.2903/sp.efsa.2021.EN-7055>
- EFSA (European Food Safety Authority), 2022. Annual report of the EFSA Networks on Animal Welfare 2022. *EFSA supporting publication* 2022;19(12):EN-7764, 43 pp. <https://doi.org/10.2903/sp.efsa.2022.EN-7764>
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2005. Opinion of the Scientific Panel on Animal Health and Welfare (AHAW) on a request from the Commission related to the welfare aspects of various systems of keeping laying hens. *EFSA Journal* 2005;3(3):197, 174 pp. <https://doi.org/10.2903/j.efsa.2005.197>
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2012. Guidance on risk assessment for animal welfare. *EFSA Journal* 2012;10(1):2513. 30 pp. <https://doi.org/10.2903/j.efsa.2012.2513>
- EFSA AHAW Panel (EFSA Panel on Animal Health and Animal Welfare), 2015. Scientific Opinion on welfare aspects of the use of perches for laying hens. *EFSA Journal* 2015;13(6):4131, 70 pp. <https://doi.org/10.2903/j.efsa.2015.4131>
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen, SS, Alvarez, J, Bicout, DJ, Calistri, P, Canali, E, Drewe, JA, Garin-Bastuji, B, Gonzales Rojas, JL, Gortázar Schmidt, C, Herskin, M, Michel, V, Miranda Chueca, MA, Padalino, B, Roberts, HC, Spooler, H, Stahl, K, Viltrop, A, Winckler, C, Mitchell, M, James Vinco, L, Voslarova, E, Candiani, D, Mosbach-Schulz, O, Van der Stede, Y and Velarde, A, 2022a. Scientific Opinion on the welfare of domestic birds and rabbits transported in containers. *EFSA Journal* 2022;20(9):7441, 188 pp. <https://doi.org/10.2903/j.efsa.2022.7441>

- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen, SS, Alvarez, J, Bicout, DJ, Calistri, P, Canali, E, Drewe, JA, Garin-Bastuji, B, Gonzales Rojas, JL, Gortazar Schmidt, C, Herskin, M, Miranda MA, Chueca, MV, Padalino, B, Pasquali, P, Roberts, HC, Spoolder, H, Stahl, K, Velarde, A, Viltrop, A, Edwards, S, Ashe, S, Candiani, D, Fabris, C, Lima, E, Mosbach-Schulz, O, Gimeno, CR, Van der Stede, Y, Vitali, M and Winckler, C, 2022b. Scientific Opinion on the methodological guidance for the development of animal welfare mandates in the context of the Farm to Fork Strategy. *EFSA Journal* 2022;20(7):7403, 29 pp. <https://doi.org/10.2903/j.efsa.2022.7403>
- EFSA Scientific Committee, Benford, D, Halldorsson, T, Jeger, MJ, Knutsen, HK, More, S, Naegeli, H, Noteborn, H, Ockleford, C, Ricci, A, Rychen, G, Schlatter, JR, Silano, V, Solecki, R, Turck, D, Younes, M, Craig, P, Hart, A, Von Goetz, N, Koutsoumanis, K, Mortensen, A, Ossendorp, B, Martino, L, Merten, C, Mosbach-Schulz, O and Hardy, A, 2018. Guidance on uncertainty analysis in scientific assessments. *EFSA Journal* 2018;16(1):5123, 39 pp. <https://doi.org/10.2903/j.efsa.2018.5123>
- Elbers ARW and Gonzales JL, 2021. Efficacy of an automated laser for reducing wild bird visits to the free range area of a poultry farm. *Scientific Reports*, 11, 1–11. <https://doi.org/10.1038/s41598-021-92267-z>
- Ellen ED, Rodenburg TB, Albers GAA, Bolhuis JE, Camerlink I, Duijvesteijn N, Knol EF, Muir WM, Peeters K, Reimert I, Sell-Kubiak E, van Arendonk JAM, Visscher J and Bijma P, 2014. The prospects of selection for social genetic effects to improve welfare and productivity in livestock. *Frontiers in Genetics*, 5, 377. <https://doi.org/10.3389/fgene.2014.00377>
- Ellen ED, Van Der Sluis M, Siegford J, Guzha O, Toscano MJ, Bennewitz J, van der Zande LE, van der Eijk JAJ, de Haas EN, Norton T, Piette D, Tetens J, de Klerk B, Visser B and Rodenburg TB, 2019. Review of sensor technologies in animal breeding: phenotyping behaviors of laying hens to select against feather pecking. *Animals*, 9, 108.
- Ellerbroek LI, 2019. Does chicken become healthier? An inventory on the basis of the rates and reasons for condemnation of poultry meat from 2002 to 2017 in German slaughterhouses. *Journal of Food Safety and Food Quality*, 70, 128–134.
- Elson, A, 2004. The laying hen: systems of egg production. *Proceedings of the WPSA 27th Poultry Science Symposium on the Welfare of the Laying Hen*. Bristol, pp. 67–80
- Engel JM, 2016. The effects of floor space allowance and nest box access on the welfare of caged laying hens (*Gallus gallus domesticus*). University of Melbourne, Faculty of Veterinary and Agricultural Sciences.
- Engel JM, Widowski T, Tilbrook AJ and Hemsworth PH, 2011. Further investigation of non-invasive measures of stress in laying hens. *Proceedings of the Australian Poultry Science Symposium*, 126–129 pp.
- Engel JM, Widowski TM, Tilbrook AJ, Butler KL and Hemsworth PH, 2019. The effects of floor space and nest box access on the physiology and behavior of caged laying hens. *Poultry Science*, 98, 533–547. <https://doi.org/10.3382/ps/pey378>
- Enquist M and Leimar O, 1990. Enquist & Leimar: evolution of fatal fighting. *Animal Behaviour*, 39, 1–9.
- Estevez I, 2009. Behaviour and environmental enrichment in broiler breeders. *Biology of breeding poultry*. CABI, Wallingford. pp. 261–283.
- Estevez I, 2020. Understanding poultry social behaviour and its impact on animal welfare. *Understanding the behaviour and improving the welfare of chickens*, Burleigh Dodds Science Publishing. pp. 117–148.
- Estévez I and Newberry R, 2017. The contribution of environmental enrichment to sustainable poultry production. In: T Applegate (ed). *Achieving Sustainable Production of Poultry Meat*. 1st edn. Burleigh Dodds Science Publishing, London, UK. pp. 247–271.
- Estevez I, Keeling LJ and Newberry RC, 2003. Decreasing aggression with increasing group size in young domestic fowl. *Applied Animal Behaviour Science*, 84, 213–218. <https://doi.org/10.1016/j.applanim.2003.08.006>
- Estevez I, Newberry RC and Keeling LJ, 2002. Dynamics of aggression in the domestic fowl. *Applied Animal Behaviour Science*, 76, 307–325. [https://doi.org/10.1016/S0168-1591\(02\)00013-8](https://doi.org/10.1016/S0168-1591(02)00013-8)
- Estevez I, Newberry RC and De Reyna LA, 1997. Broiler chickens: a tolerant social system. *Etología*, 5, 19–29.
- Estevez I and Christman MC, 2006. Analysis of the movement and use of space of animals in confinement: the effect of sampling effort. *Applied Animal Behaviour Science*, 97, 221–240.
- Estevez I, Mallapur A, Miller C and Christman MC, 2010. Short-and long-term movement patterns in complex confined environments in broiler chickens: the effects of distribution of cover panels and food resources. *Poultry Science*, 89, 643–650.
- EURCAW-Poultry-SFA (European Union Reference Centre for Animal Welfare for Poultry and other small farmed animals), 2020. Question to the Centre (Query 004-2020). Available online: <https://sitesv2.anses.fr/en/system/files/Answer-004-2020-ENG.pdf>
- EURCAW-Poultry-SFA, 2022. Question to EURCAW-Poultry-SFA (Q2E-Poultry-SFA-2021-004). 17 pp. Available online: [https://sitesv2.anses.fr/en/system/files/Answer\\_QE2-EURCAW-Poultry-SFA-2021-004.pdf](https://sitesv2.anses.fr/en/system/files/Answer_QE2-EURCAW-Poultry-SFA-2021-004.pdf)
- Eusemann BK, Patt A, Schrader L, Weigend S, Thöne-Reineke C and Petow S, 2020. The role of egg production in the etiology of keel bone damage in laying hens. *Frontiers in Veterinary Science*, 7, 81. <https://doi.org/10.3389/fvets.2020.00081>
- EU, 2020. Commission Implementing Regulation (EU) 2020/464 of 26 March 2020 laying down certain rules for the application of Regulation (EU) 2018/848 of the European Parliament and of the Council as regards the documents needed for the retroactive recognition of periods for the purpose of conversion, the production of organic products and information to be provided by Member States (Text with EEA relevance). *OJ L* 98, 31.3.2020, p. 2–25.

- Fawcett DL, Casey-Trott TM, Jensen L, Caston LJ and Widowski TM, 2020. Strain differences and effects of different stocking densities during rearing on the musculoskeletal development of pullets. *Poultry Science*, 99, 4153–4161. <https://doi.org/10.1016/j.psj.2020.05.046>
- Farm Animal Welfare Council, 2009. *Animal Welfare in Great Britain: Past, Present and Future*. FAWC, 17 Smith Square, London. <http://www.fawc.org.uk>. Available online: [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment\\_data/file/319292/Farm\\_Animal\\_Welfare\\_in\\_Great\\_Britain\\_-\\_Past\\_Present\\_and\\_Future.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/319292/Farm_Animal_Welfare_in_Great_Britain_-_Past_Present_and_Future.pdf)
- Feltenstein MW, Ford NG, Freeman KB and Sufka KJ, 2002. Dissociation of stress behaviors in the chick social-separation-stress procedure. *Physiology & Behavior*, 75, 675–679. [https://doi.org/10.1016/S0031-9384\(02\)00660-1](https://doi.org/10.1016/S0031-9384(02)00660-1)
- Fernyhough M, Nicol CJ, van de Braak T, Toscano MJ and Tønnessen M, 2020. The ethics of laying hen genetics. *Journal of Agricultural and Environmental Ethics*, 33, 15–36. <https://doi.org/10.1007/s10806-019-09810-2>
- Fiks-van Niekerk T, de Jong I, Veldkamp T, van Emous R and van Middelkoop J (Animal Sciences Group), 2006. Literatuurstudie ingrepen bij pluimvee = Literature study mutilations poultry. pp. 1570–8624.
- Fiks-van Niekerk TGCM, Reuvekamp BFJ and van Emous RA, 2002. Experiences with different models of furnished cages. *Proceedings of the Poultry Welfare Symposium*, 21–22 September 2002, Seville, Spain.
- Fleming R, McCormack H, McTeir L and Whitehead C, 1998. Medullary bone and humeral breaking strength in laying hens. *Research in Veterinary Science*, 64, 63–67.
- Forkman B, Boissy A, Meunier-Salaün MC, Canali E and Jones RB, 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & Behavior*, 92, 340–374. <https://doi.org/10.1016/j.physbeh.2007.03.016>
- Fossum O, Jansson DS, Etterlin PE and Vågsholm I, 2009. Causes of mortality in laying hens in different housing systems in 2001 to 2004. *Acta Veterinaria Scandinavica*, 51(1), 1–9.
- Frankenhuis MT, Vertommen MH and Hemminga H, 1991. Influence of claw clipping, stocking density and feeding space on the incidence of scabby hips in broilers. *British Poultry Science*, 32, 227–230.
- Fraser D, Duncan IJH, Edwards SA, Grandin T, Gregory NG, Guyonnet V, Hemsworth PH, Huertas SM, Huzzey JM, Mellor DJ, Mench JA, Špinková M and Whay HR, 2013. *General Principles for the welfare of animals in production systems: the underlying science and its application*. *The Veterinary Journal*, 198, 19–27. <https://doi.org/10.1016/j.tvjl.2013.06.028>
- Freire R, Eastwood MA and Joyce M, 2011. Minor beak trimming in chickens leads to loss of mechanoreception and magnetoreception. *Journal of Animal Science*, 89, 1201–1206. <https://doi.org/10.2527/jas.2010-3129>
- Freire R, Glatz PC and Hinch G, 2008. Self-administration of an analgesic does not alleviate pain in beak trimmed chickens. *Asian-Australasian Journal of Animal Sciences*, 21, 443–448. <https://doi.org/10.5713/ajas.2008.70039>
- Freire R, Wilkins LJ, Short F and Nicol CJ, 2003. Behaviour and welfare of individual laying hens in a non-cage system. *British Poultry Science*, 44(1), 22–29. <https://doi.org/10.1080/0007166031000085391>
- Fulton RM, 2017. Causes of normal mortality in commercial egg-laying chickens. *Avian Diseases*, 61, 289–295. <https://doi.org/10.1637/11556-120816-RegR>
- García J, Mandalawi HA, Fondevila G and Mateos GG, 2019. Influence of beak trimming and inclusion of sodium butyrate in the diet on growth performance and digestive tract traits of brown-egg pullets differing in initial body weight. *Poultry Science*, 98, 3937–3949.
- Gauly M, Duss C and Erhardt G, 2007. Influence of *Ascaridia galli* infections and anthelmintic treatments on the behaviour and social ranks of laying hens (*Gallus gallus domesticus*). *Veterinary Parasitology*, 146, 271–280. <https://doi.org/10.1016/j.vetpar.2007.03.005>
- Gebhardt-Henrich SG, Fröhlich EK, Burose F, Fleurent J, Gantner M and Zähler M, 2014. Individual tracking of laying hens with an RFID-System. *Landtechnik*, 69, 301–307.
- Gebhardt-Henrich SG, Pfulg A, Fröhlich EK, Käppli S, Guggisberg D, Liesegang A and Stoffel MH, 2017. Limited associations between keel bone damage and bone properties measured with computer tomography, three-point bending test, and analysis of minerals in Swiss laying hens. *Frontiers in Veterinary Science*, 4, 128. <https://doi.org/10.3389/fvets.2017.00128>
- Gentle MJ, 1989. Cutaneous sensory afferents recorded from the nervus intramandibularis of *Gallus gallus var domesticus*. *Journal of Comparative Physiology A*, 164, 763–774. <https://doi.org/10.1007/BF00616748>
- Gentle MJ and Hunter LN, 1991. Physiological and behavioural responses associated with feather removal in *Gallus gallus var domesticus*. *Research in Veterinary Science*, 50, 95–101. [https://doi.org/10.1016/0034-5288\(91\)90060-2](https://doi.org/10.1016/0034-5288(91)90060-2)
- Gentle MJ, Hughes BO, Fox A and Waddington D, 1997. Behavioural and anatomical consequences of two beak trimming methods in 1- and 10-d-old domestic chicks. *British Poultry Science*, 38, 453–463.
- Giersberg MF, Kemper N, Hartung J, Schrader L and Spindler B, 2017. Determination of body width in brown and white layer pullets by image analyses. *British Poultry Science*, 58, 230–235.
- Giersberg MF and Kemper N, 2018. Rearing male layer chickens: a German perspective. *Agriculture*, 8, 176.
- Giersberg MF, Spindler B and Kemper N, 2019. Linear space requirements and perch use of conventional layer hybrids and dual-purpose hens in an aviary system. *Frontiers in Veterinary Science*, 6, 231. <https://doi.org/10.3389/fvets.2019.00231>

- Gilani A-M, Knowles TG and Nicol CJ, 2012. The effect of dark brooders on feather pecking on commercial farms. *Applied Animal Behaviour Science*, 142, 42–50. <https://doi.org/10.1016/j.applanim.2012.09.006>
- Gilani A-M, Knowles TG and Nicol CJ, 2013. The effect of rearing environment on feather pecking in young and adult laying hens. *Applied Animal Behaviour Science*, 148, 54–63. <https://doi.org/10.1016/j.applanim.2013.07.014>
- Gilani AM, Knowles TG and Nicol CJ, 2014. Factors affecting ranging behaviour in young and adult laying hens. *British Poultry Science*, 55, 127–135. <https://doi.org/10.1080/00071668.2014.889279>
- Giraldo J, Acevedo C, Gutiérrez D, Galeano L, Zapata N and Cerón-Muñoz M, 2014. Caracterización del comportamiento de las gallinas (*Gallus gallus domesticus*) sometidas a sistemas de producción de huevo en jaula. *Livestock Research for Rural Development*, 26, 134.
- Glatz PC and Underwood G, 2021. Current methods and techniques of beak trimming laying hens, welfare issues and alternative approaches. *Animal Production Science*, 61, 968–989. <https://doi.org/10.1071/AN19673>
- Gnauk S, 2020. Ein Geschäft mit Potenzial. *DGS Magazin*, 2.
- Gonzales JL and Elbers AR, 2018. Effective thresholds for reporting suspicions and improve early detection of avian influenza outbreaks in layer chickens. *Scientific Reports*, 8, 1–9.
- Grafl B, Polster S, Sulejmanovic T, Pürner B, Guggenberger B and Hess M, 2017. Assessment of health and welfare of Austrian laying hens at slaughter demonstrates influence of husbandry system and season. *British Poultry Science*, 58, 209–215. <https://doi.org/10.1080/00071668.2017.1280723>
- Gray H, Davies R, Bright A, Rayner A and Asher L, 2020. Why do hens pile? Hypothesizing the causes and consequences. *Frontiers in Veterinary Science*, 7, 616836. <https://doi.org/10.3389/fvets.2020.616836>
- Grebe TC, Ali ABA, Swanson JC, Widowski TM and Siegford JM, 2020. Dust bathing in laying hens: strain, proximity to, and number of conspecifics matter. *Poultry Science*, 99, 4103–4112. <https://doi.org/10.1016/j.psj.2020.04.032>
- Green LE, Lewis K, Kimpton A and Nicol CJ, 2000. Cross-sectional study of the prevalence of feather pecking in laying hens in alternative systems and its associations with management and disease. *Veterinary Record*, 147, 233–238. <https://doi.org/10.1136/vr.147.9.233>
- Gregory NG, Payne SR, Devine CD and Cook CJ, 2009. Effect of lipopolysaccharide on sickness behaviour in hens kept in cage and free range environments. *Research in Veterinary Science*, 87, 167–170. <https://doi.org/10.1016/j.rvsc.2009.01.003>
- Gregory NG, Wilkins LJ, Austin SD, Belyavin CG, Alvey DM and Tucker SA, 1992. Effect of catching method on the prevalence of broken bones in end of lay hens. *Avian Pathology*, 21, 717–722. <https://doi.org/10.1080/03079459208418894>
- Gregory NG, Wilkins LJ, Eleperuma SD, Ballantyne AJ and Overfield ND, 1990. Broken bones in domestic fowls: effect of husbandry system and stunning method in end-of-lay hens. *British Poultry Science*, 31, 59–69. <https://doi.org/10.1080/00071669008417231>
- Grigor PN, Hughes BO and Appleby MC, 1995. Effects of regular handling and exposure to an outside area on subsequent fearfulness and dispersal in domestic hens. *Applied Animal Behaviour Science*, 44, 47–55.
- Groves PJ, 2021. Non-infectious diseases and laying hen welfare. *Animal Production Science*, 61, 1013–1017.
- Guesdon V, Ahmed AMH, Mallet S, Faure JM and Nys Y, 2006. Effects of beak trimming and cage design on laying hen performance and egg quality. *British Poultry Science*, 47, 1–12.
- Guhl AM and Ortman LL, 1953. Visual patterns in the recognition of individuals among chickens. *The Condor*, 55, 287–298. <https://doi.org/10.2307/1365008>
- Guinebretière M, 2017. Comment aménager les cages de poules pondeuses afin d'enrichir leur comportement, tout en préservant les performances zootechniques et l'hygiène de la cage - Etude focalisée sur la taille de groupe et les solutions pour aménager l'aire de grattage et le nid. Tours, France, Ecole doctorale Santé, Sciences Biologiques, Chimie du Vivant - discipline/spécialité: Sciences de la Vie. 221 pp.
- Guinebretière M, Beyer H, Arnould C and Michel V, 2014. The choice of litter material to promote pecking, scratching and dustbathing behaviours in laying hens housed in furnished cages. *Applied Animal Behaviour Science*, 155, 56–65. <https://doi.org/10.1016/j.applanim.2014.02.013>
- Guinebretière M, Michel V and Arnould C, 2015. Dustbathing, pecking and scratching behaviours of laying hens in furnished cages are enhanced by the presence of rubber mats and litter distribution. *Applied Animal Behaviour Science*, 171, 128–137. <https://doi.org/10.1016/j.applanim.2015.08.017>
- Guinebretière M, Mika A, Michel V, Balaine L, Thomas R, Keïta A and Pol F, 2020. Effects of management strategies on non-beak-trimmed laying hens in furnished cages that were reared in a non-cage system. *Animals*, 10, 399.
- Gunnarsson S, 1999. Effect of rearing factors on the prevalence of floor eggs, cloacal cannibalism and feather pecking in commercial flocks of loose housed laying hens. *British Poultry Science*, 40, 12–18. <https://doi.org/10.1080/00071669987773>
- Gunnarsson S, Matthews LR, Foster TM and Temple W, 2000a. The demand for straw and feathers as litter substrates by laying hens. *Applied Animal Behaviour Science*, 65, 321–330. [https://doi.org/10.1016/S0168-1591\(99\)00065-9](https://doi.org/10.1016/S0168-1591(99)00065-9)
- Gunnarsson S, Yngvesson J, Keeling LJ and Forkman B, 2000b. Rearing without early access to perches impairs the spatial skills of laying hens. *Applied Animal Behaviour Science*, 67, 217–228. [https://doi.org/10.1016/S0168-1591\(99\)00125-2](https://doi.org/10.1016/S0168-1591(99)00125-2)

- Harlander-Matauschek A and Bessei W, 2005. Feather eating and crop filling in laying hens. *Archiv Fur Geflugelkunde*, 69, 241.
- Harlander-Matauschek A, Rodenburg TB, Sandilands V, Tobalske BW and Toscano MJ, 2015. Causes of keel bone damage and their solutions in laying hens. *World's Poultry Science Journal*, 71, 461–472. <https://doi.org/10.1017/S0043933915002135>
- Harlander-Matauschek A, Felsenstein K, Niebuhr K and Troxler J, 2006. Influence of pop hole dimensions on the number of laying hens outside on the range. *British Poultry Science*, 47, 131–134.
- Hartcher KM, Hickey KA, Hemsworth PH, Cronin GM, Wilkinson SJ and Singh M, 2016. Relationships between range access as monitored by radio frequency identification technology, fearfulness, and plumage damage in free-range laying hens. *Animal*, 10, 847–853. <https://doi.org/10.1017/S1751731115002463>
- Hartcher KM, Tran KTN, Wilkinson SJ, Hemsworth PH, Thomson PC and Cronin GM, 2015a. The effects of environmental enrichment and beak-trimming during the rearing period on subsequent feather damage due to feather-pecking in laying hens. *Poultry Science*, 94, 852–859. <https://doi.org/10.3382/ps/pev061>
- Hartcher KM, Tran MKTN, Wilkinson SJ, Hemsworth PH, Thomson PC and Cronin GM, 2015b. Plumage damage in free-range laying hens: Behavioural characteristics in the rearing period and the effects of environmental enrichment and beak-trimming. *Applied Animal Behaviour Science*, 164, 64–72. <https://doi.org/10.1016/j.applanim.2014.12.011>
- Heerkens JLT, Delezie E, Ampe B, Rodenburg TB and Tuytens FAM, 2016a. Ramps and hybrid effects on keel bone and foot pad disorders in modified aviaries for laying hens. *Poultry Science*, 95, 2479–2488. <https://doi.org/10.3382/ps/pew157>
- Heerkens JLT, Delezie E, Kempen I, Zoons J, Ampe B, Rodenburg TB and Tuytens FAM, 2015. Specific characteristics of the aviary housing system affect plumage condition, mortality and production in laying hens. *Poultry Science*, 94, 2008–2017. <https://doi.org/10.3382/ps/pev187>
- Heerkens JLT, Delezie E, Rodenburg TB, Kempen I, Zoons J, Ampe B and Tuytens FAM, 2016b. Risk factors associated with keel bone and foot pad disorders in laying hens housed in aviary systems. *Poultry Science*, 95, 482–488. <https://doi.org/10.3382/ps/pev339>
- Hegelund L, Sorensen JT, Kjaer JB and Kristensen IS, 2005. Use of the range area in organic egg production systems: effect of climatic factors, flock size, age and artificial cover. *British Poultry Science*, 46, 1–8.
- Heikkilä M, Wichman A, Gunnarsson S and Valros A, 2006. Development of perching behaviour in chicks reared in enriched environment. *Applied Animal Behaviour Science*, 99, 145–156. <https://doi.org/10.1016/j.applanim.2005.09.013>
- Hemsworth PH and Edwards LE, 2021. Natural behaviours, their drivers and their implications for laying hen welfare. *Animal Production Science*, 61, 915–930. <https://doi.org/10.1071/AN19630>
- Herbert GT, Redfearn WD, Brass E, Dalton HA, Gill R, Brass D, Smith C, Rayner AC and Asher L, 2021. Extreme crowding in laying hens during a recurrent smothering outbreak. *Veterinary Record*, 188, e245.
- Hester PY, 2014. The effect of perches installed in cages on laying hens. *World's Poultry Science Journal*, 70, 247–264. <https://doi.org/10.1017/S0043933914000270>
- Hester PY, Enneking SA, Haley BK, Cheng HW, Einstein ME and Rubin DA, 2013. The effect of perch availability during pullet rearing and egg laying on musculoskeletal health of caged White Leghorn hens. *Poultry Science*, 92, 1972–1980. <https://doi.org/10.3382/ps.2013-03008>
- Hewlett SE and Nordquist RE, 2019. Effects of maternal care during rearing in White Leghorn and Brown Nick layer hens on cognition, sociality and fear. *Animals*, 9, 454.
- Hillemacher, S., Tiemann, I., 2018. Marktpotential für Geflügelprodukte aus Hahnenfleisch von Legehybrid-, Zweinutzungshybridlinien und Zweinutzungsrassen, Forschungsbericht 189, Landwirtschaftliche Fakultät der Universität Bonn, p. 47.
- Hinrichsen LK, Labouriau R, Engberg RM, Knierim U and Sørensen JT, 2016. Helminth infection is associated with hen mortality in Danish organic egg production. *The Veterinary Record*, 179, 196. <https://doi.org/10.1136/vr.103614>
- Hoerning B, 2004. Welfare of laying hens in furnished cages. *Welfare of laying hens in Europe*. Tierhaltung, Bd, 28.
- Hofmann T, Schmucker S, Grashorn M and Stefanski V, 2021. Short- and long-term consequences of stocking density during rearing on the immune system and welfare of laying hens. *Poultry Science*, 100, 101243. <https://doi.org/10.1016/j.psj.2021.101243>
- Hogan JA and Van Boxel F, 1993. Causal factors controlling dustbathing in Burmese red junglefowl: some results and a model. *Animal Behaviour*, 46, 627–635. <https://doi.org/10.1006/anbe.1993.1239>
- Holík V, 2009. Management of laying hens to minimize heat stress. *Lohmann Information*, 44, 16–29.
- Homidan AA, Robertson JF and Petchey AM, 2003. Review of the effect of ammonia and dust concentrations on broiler performance. *World's Poultry Science Journal*, 59, 340–349. <https://doi.org/10.1079/WPS20030021>
- Honaker CF and Ruszler PL, 2004. The effect of claw and beak reduction on growth parameters and fearfulness of two leghorn strains. *Poultry Science*, 83, 873–881.
- Hoppitt W, Blackburn L and Laland KN, 2007. Response facilitation in the domestic fowl. *Animal Behaviour*, 73, 229–238.
- Hoppitt W and Laland KN, 2008. Social processes affecting feeding and drinking in the domestic fowl. *Animal Behaviour*, 76, 1529–1543.

- Huang D and Guo H, 2020. Diurnal and seasonal variations of odor emissions from broiler and cage-layer barns in the Canadian Prairies. *Environmental Science and Pollution Research*, 27, 26631–26642.
- Huber-Eicher B, 2004. The effect of early colour preference and of a colour exposing procedure on the choice of nest colours in laying hens. *Applied Animal Behaviour Science*, 86, 63–76. <https://doi.org/10.1016/j.applanim.2003.12.003>
- Huber-Eicher B and Audige L, 1999. Analysis of risk factors for the occurrence of feather pecking in laying hen growers. *British Poultry Science*, 40, 599–604. <https://doi.org/10.1080/00071669986963>
- Huber HU, Fölsch DW and Stähli U, 1985. Influence of various nesting materials on nest site selection of the domestic hen. *British Poultry Science*, 26, 367–373. <https://doi.org/10.1080/00071668508416824>
- Huber-Eicher B, Suter A and Spring-Stähli P, 2013. Effects of colored light-emitting diode illumination on behavior and performance of laying hens. *Poultry Science*, 92, 869–873.
- Hughes BO, 1983. Conventional and shallow cages: a summary of research from welfare and production aspects. *World's Poultry Science Journal*, 39, 218–228. <https://doi.org/10.1079/WPS19830020>
- Hughes BO, Carmichael NL, Walker AW and Grigor PN, 1997. Low incidence of aggression in large flocks of laying hens. *Applied Animal Behaviour Science*, 54, 215–234.
- Hughes BO and Wood-Gush DGM, 1977. Agonistic behaviour in domestic hens: the influence of housing method and group size. *Animal Behaviour*, 25, 1056–1062. [https://doi.org/10.1016/0003-3472\(77\)90056-2](https://doi.org/10.1016/0003-3472(77)90056-2)
- Hughes C, Struthers S, Shynkaruk T, Gomis S, Gupta A and Schwean-Lardner K, 2020. Research Note: Beak morphology of infrared beak-treated laying hens and its impact on production and welfare. *Poultry Science*, 99, 1395–1399.
- Hughes VK, Ellis PS and Langlois NEI, 2004. The perception of yellow in bruises. *Journal of Clinical Forensic Medicine*, 11, 257–259. <https://doi.org/10.1016/j.jcfm.2004.01.007>
- Huneau-Salaün A, Guinebretière M, Taktak A, Huonnic D and Michel V, 2011. Furnished cages for laying hens: study of the effects of group size and litter provision on laying location, zootechnical performance and egg quality. *Animal*, 5, 911–917.
- Hy-Line, 2022. Hy-line Brown Alternative Systems - Management Guide. Available online: <https://www.hyline.com/varieties/guide?productid=d3b7c322-48b3-4cdf-9b4d-14f50d5987b3&typeid=d09daefc-d0b4-439-e-b0bf-bf79e218a19b&languageid=3e6be941-6e5a-42f7-9809-09da73b29164>
- Icken W, Caverio D, Schmutz M, Thurner S, Wendl G and Preisinger R, 2008. Analysis of the free range behaviour of laying hens and the genetic and phenotypic relationships with laying performance. *British Poultry Science*, 49, 533–541.
- IKB-Kip, 2021. Annex 8 Broiler Evaluation System. Productschap Pluimvee en Eieren. Available online: <https://www.avined.nl/wp-content/uploads/8-Broiler-evaluation-system-IKB-Kip-version-6-210601.pdf>
- Jacobs L, Vezzoli G, Beerda B and Mench JA, 2019. Northern fowl mite infestation affects the nocturnal behavior of laying hens. *Applied Animal Behaviour Science*, 216, 33–37. <https://doi.org/10.1016/j.applanim.2019.04.007>
- Janczak AM and Riber AB, 2015. Review of rearing-related factors affecting the welfare of laying hens. *Poultry Science*, 94, 1454–1469. <https://doi.org/10.3382/ps/pev123>
- Jansson DS, Nyman A, Vågsholm I, Christensson D, Göransson M, Fossum O and Höglund J, 2010. Ascarid infections in laying hens kept in different housing systems. *Avian Pathology*, 39, 525–532. <https://doi.org/10.1080/03079457.2010.527923>
- Jensen P, Keeling L, Schütz K, Andersson L, Mormède P, Brändström H, Forkman B, Kerje S, Fredriksson R, Ohlsson C, Larsson S, Mallmin H and Kindmark A, 2005. Feather pecking in chickens is genetically related to behavioural and developmental traits. *Physiology & Behavior*, 86, 52–60. <https://doi.org/10.1016/j.physbeh.2005.06.029>
- Jensen AB, Palme R and Forkman B, 2006. Effect of brooders on feather pecking and cannibalism in domestic fowl (*Gallus gallus domesticus*). *Applied Animal Behaviour Science*, 99, 287–300.
- Jones BR and Waddington D, 1992. Modification of fear in domestic chicks, *Gallus gallus domesticus*, via regular handling and early environmental enrichment. *Animal Behaviour*, 43, 1021–1033. [https://doi.org/10.1016/S0003-3472\(06\)80015-1](https://doi.org/10.1016/S0003-3472(06)80015-1)
- Jones EKM, Wathes CM and Webster AJF, 2005. Avoidance of atmospheric ammonia by domestic fowl and the effect of early experience. *Applied Animal Behaviour Science*, 90, 293–308.
- Jones MEJ and Mench JA, 1991. Behavioral correlates of male mating success in a multisire flock as determined by DNA fingerprinting. *Poultry Science*, 70, 1493–1498. <https://doi.org/10.3382/ps.0701493>
- Jones RB, 1989. Chronic stressors, tonic immobility and leucocytic responses in the domestic fowl. *Physiology & Behavior*, 46, 439–442. [https://doi.org/10.1016/0031-9384\(89\)90017-6](https://doi.org/10.1016/0031-9384(89)90017-6)
- Jones RB and Harvey S, 1987. Behavioural and adrenocortical responses of domestic chicks to systematic reductions in group size and to sequential disturbance of companions by the experimenter. *Behavioural Processes*, 14, 291–303. [https://doi.org/10.1016/0376-6357\(87\)90075-1](https://doi.org/10.1016/0376-6357(87)90075-1)
- Jong I and Van Emous R, 2017. Broiler breeding flocks: management and animal welfare. pp. 211–229.
- Jongman EC, Glatz PC and Barnett JL, 2008. Changes in behaviour of laying hens following beak trimming at hatch and re-trimming at 14 weeks. *Asian-Australasian Journal of Animal Sciences*, 21, 291–298. <https://doi.org/10.5713/ajas.2008.60152>

- Jung L and Knierim U, 2018. Are practice recommendations for the prevention of feather pecking in laying hens in non-cage systems in line with the results of experimental and epidemiological studies? *Applied Animal Behaviour Science*, 200, 1–12. <https://doi.org/10.1016/j.applanim.2017.10.005>
- Jung L and Knierim U, 2019. Differences between feather pecking and non-feather pecking laying hen flocks regarding their compliance with recommendations for the prevention of feather pecking – a matched concurrent case-control design. *Applied Animal Behaviour Science*, 219, 104839. <https://doi.org/10.1016/j.applanim.2019.104839>
- Jung L, Niebuhr K, Hinrichsen LK, Gunnarsson S, Brenninkmeyer C, Bestman M, Heerkens J, Ferrari P and Knierim U, 2019. Possible risk factors for keel bone damage in organic laying hens. *Animal*, 13, 2356–2364. <https://doi.org/10.1017/S175173111900003X>
- Jung L, Brenninkmeyer C, Niebuhr K, Bestman M, Tuytens FAM, Gunnarsson S, Sørensen JT, Ferrari P and Knierim U, 2020. Husbandry conditions and welfare outcomes in organic egg production in eight European countries. *Animals*, 10, 2102.
- Jung L, Nasirahmadi A, Schulte-Landwehr J and Knierim U, 2021. Automatic assessment of keel bone damage in laying hens at the slaughter line. *Animals*, 11, 163.
- Jung L, Rufener C and Petow S, 2022. A tagged visual analog scale is a reliable method to assess keel bone deviations in laying hens from radiographs. *Frontiers in Veterinary Science*, 9, 937119. Available online: <http://europepmc.org/abstract/MED/36061110>
- Kang HK, Park SB, Kim SH and Kim CH, 2016. Effects of stock density on the laying performance, blood parameter, corticosterone, litter quality, gas emission and bone mineral density of laying hens in floor pens. *Poultry Science*, 95, 2764–2770. <https://doi.org/10.3382/ps/pew264>
- Karaman S, Tarhan S and Ergunes G, 2007. Analysis of indoor climatic data to assess the heat stress of laying hens. *International Journal of Natural and Engineering Sciences*, 1, 65–68.
- Käppeli S, Gebhardt-Henrich SG, Fröhlich E, Pfulg A and Stoffel MH, 2011. Prevalence of keel bone deformities in Swiss laying hens. *British Poultry Science*, 52, 531–536. <https://doi.org/10.1080/00071668.2011.615059>
- Kaufmann F, Daş G, Sohnrey B and Gauly M, 2011. Helminth infections in laying hens kept in organic free range systems in Germany. *Livestock Science*, 141, 182–187. <https://doi.org/10.1016/j.livsci.2011.05.015>
- Keeling L, 1997. A comparison of two basic characteristics of a perch for laying hens. *Proceedings of the 31st International Congress of the ISAE, Research Institute of Animal Production*, 13–16 pp.
- Keeling LJ and Duncan IJH, 1991. Social spacing in domestic fowl under seminatural conditions: the effect of behavioural activity and activity transitions. *Applied Animal Behaviour Science*, 32, 205–217. [https://doi.org/10.1016/S0168-1591\(05\)80044-9](https://doi.org/10.1016/S0168-1591(05)80044-9)
- Keeling LJ, 1994. Feather pecking—who in the group does it, how often and under what circumstances. In: *Proceedings of the 9th European Poultry Conference*. pp. 288–289.
- Keeling LJ, Newberry RC and Estevez I, 2017. Flock size during rearing affects pullet behavioural synchrony and spatial clustering. *Applied Animal Behaviour Science*, 194, 36–41. <https://doi.org/10.1016/j.applanim.2017.04.002>
- Keeling LJ, Hughes BO and Dun P, 1988. Performance of free-range laying hens in a polythene house and their behaviour on range. *Farm Building Progress*, 94, 21–28.
- Khanal T, Bédécarrats GY, Widowski TM and Kiarie EG, 2020. Rearing cage type and dietary limestone particle size: II, effects on egg production, eggshell, and bone quality in Lohmann selected Leghorn-Lite hens. *Poultry Science*, 99, 5763–5770. <https://doi.org/10.1016/j.psj.2020.06.079>
- Kilpinen O, Roepstorff A, Permin A, Nørgaard-Nielsen G, Lawson LG and Simonsen HB, 2005. Influence of *Dermanyssus gallinae* and *Ascaridia galli* infections on behaviour and health of laying hens (*Gallus gallus domesticus*). *British Poultry Science*, 46, 26–34. <https://doi.org/10.1080/00071660400023839>
- Kim T and Zuk M, 2000. The effects of age and previous experience on social rank in female red junglefowl, *Gallus gallus spadiceus*. *Animal Behaviour*, 60, 239–244. <https://doi.org/10.1006/anbe.2000.1469>
- Kjaer JB and Sørensen P, 2002. Feather pecking and cannibalism in free-range laying hens as affected by genotype, dietary level of methionine + cystine, light intensity during rearing and age at first access to the range area. *Applied Animal Behaviour Science*, 76, 21–39. [https://doi.org/10.1016/S0168-1591\(01\)00209-X](https://doi.org/10.1016/S0168-1591(01)00209-X)
- Koch G and Elbers ARW, 2006. Outdoor ranging of poultry: a major risk factor for the introduction and development of High-Pathogenecity Avian Influenza. *NJAS - Wageningen Journal of Life Sciences*, 54, 179–194. [https://doi.org/10.1016/S1573-5214\(06\)80021-7](https://doi.org/10.1016/S1573-5214(06)80021-7)
- Kozak M, Tobalske B, Springthorpe D, Szkotnicki B and Harlander-Matauschek A, 2016. Development of physical activity levels in laying hens in three-dimensional aviaries. *Applied Animal Behaviour Science*, 185, 66–72. <https://doi.org/10.1016/j.applanim.2016.10.004>
- Krause ET and Schrader L, 2019. Suggestions to derive maximum stocking densities for layer pullets. *Animals*, 9, 348.
- Krautwald-Junghanns ME, 2021. Haltung von Bruderhähnen - Literaturreview - Veterinärmed. Fakultät, Klinik f. Vögel u. Reptilien, Leipzig, p. 31.
- Kristensen HH, Burgess LR, Demmers TGH and Wathes CM, 2000. The preferences of laying hens for different concentrations of atmospheric ammonia. *Applied Animal Behaviour Science*, 68, 307–318. [https://doi.org/10.1016/S0168-1591\(00\)00110-6](https://doi.org/10.1016/S0168-1591(00)00110-6)
- Kristensen HH, White RP and Wathes PCM, 2009. Light intensity and social communication between hens. *British Poultry Science*, 50, 649–656. <https://doi.org/10.1080/00071660903277353>

- Kuenzel WJ, 2007. Neurobiological basis of sensory perception: welfare implications of beak trimming. *Poultry Science*, 86, 1273–1282. <https://doi.org/10.1093/ps/86.6.1273>
- Lagadic H and Faure JM, 1987. Preferences of domestic hens for cage size and floor types as measured by operant conditioning. *Applied Animal Behaviour Science*, 19, 147–155.
- Lambton S, Knowles T, Yorke C and Nicol C, 2015. The risk factors affecting the development of vent pecking and cannibalism in free-range and organic laying hens. *Animal Welfare*, 24, 101–111.
- Lambton SL, Knowles TG, Yorke C and Nicol CJ, 2010. The risk factors affecting the development of gentle and severe feather pecking in loose housed laying hens. *Applied Animal Behaviour Science*, 123, 32–42. <https://doi.org/10.1016/j.applanim.2009.12.010>
- Lambton SL, Nicol CJ, Friel M, Main DC, McKinstry JL, Sherwin CM, Walton J and Weeks CA, 2013. A bespoke management package can reduce levels of injurious pecking in loose-housed laying hen flocks. *Veterinary Record*, 172, 423.
- Lamping C, Derks M, Koerkamp PG and Kootstra G, 2022. ChickenNet-an end-to-end approach for plumage condition assessment of laying hens in commercial farms using computer vision. *Computers and Electronics in Agriculture*, 194, 106695.
- Lara LJ and Rostagno MH, 2013. Impact of heat stress on poultry production. *Animals*, 3, 356–369.
- Larsen H, Cronin GM, Gebhardt-Henrich SG, Smith CL, Hemsworth PH and Rault JL, 2017. Individual ranging behaviour patterns in commercial free-range layers as observed through RFID tracking. *Animals (Basel)*, 7, 21. <https://doi.org/10.3390/ani7030021>
- Larsen H, Hemsworth P, Cronin G, Gebhardt-Henrich S, Smith C and Rault J-L, 2018. Relationship between welfare and individual ranging behaviour in commercial free-range laying hens. *Animal*, 12, 2356–2364.
- LayWel, 2006. Welfare implications of changes in production systems for laying hens. Available online: [www.laywel.eu](http://www.laywel.eu)
- LeBlanc C, Tobalske B, Szkotnicki B and Harlander-Matuschek A, 2018. Locomotor behavior of chickens anticipating incline Walking. *Frontiers in Veterinary Science*, 4, 233.
- Lentfer TL, Gebhardt-Henrich SG, Fröhlich EKF and von Borell E, 2011. Influence of nest site on the behaviour of laying hens. *Applied Animal Behaviour Science*, 135, 70–77. <https://doi.org/10.1016/j.applanim.2011.08.016>
- Lentfer TL, Gebhardt-Henrich SG, Fröhlich EKF and von Borell E, 2013. Nest use is influenced by the positions of nests and drinkers in aviaries. *Poultry Science*, 92, 1433–1442. <https://doi.org/10.3382/ps.2012-02718>
- Leone EH, Estevez I and Christman MC, 2007. Environmental complexity and group size: Immediate effects on use of space by domestic fowl. *Applied Animal Behaviour Science*. 102, p. 39–52.
- Leone EH and Estévez I, 2008a. Use of space in the domestic fowl: separating the effects of enclosure size, group size and density. *Animal Behaviour*, 76, 1673–1682. <https://doi.org/10.1016/j.anbehav.2008.08.004>
- Leone EH and Estévez I, 2008b. Space use according to the distribution of resources and level of competition. *Poultry Science*, 87, 3–13. <https://doi.org/10.3382/ps.2007-00026>
- Li G, Zhao Y, Porter Z and Purswell JL, 2021. Automated measurement of broiler stretching behaviors under four stocking densities via faster region-based convolutional neural network. *Animal*, 15, 100059. <https://doi.org/10.1016/j.animal.2020.100059>
- Li X, Chen D, Li J and Bao J, 2016. Effects of furnished cage type on behavior and welfare of laying hens. *Asian-Australasian Journal of Animal Sciences*, 29, 887–894. <https://doi.org/10.5713/ajas.15.0576>
- Lichovnikova M, Hampel D, Nedomová Š, Kupčíková L and Anderle V, 2017. The effect of genotype on the behaviour of free range chickens. *Journal of Central European Agriculture*, 18, 632–645.
- Liebers CJ, Schwarzer A, Erhard M, Schmidt P and Louton H, 2019. The influence of environmental enrichment and stocking density on the plumage and health conditions of laying hen pullets. *Poultry Science*, 98, 2474–2488. <https://doi.org/10.3382/ps/pez024>
- Lindberg AC and Nicol CJ, 1997. Dustbathing in modified battery cages: is sham dustbathing an adequate substitute? *Applied Animal Behaviour Science*, 55, 113–128. [https://doi.org/10.1016/S0168-1591\(97\)00030-0](https://doi.org/10.1016/S0168-1591(97)00030-0)
- Lindberg AC and Nicol CJ, 1994. An evaluation of the effect of operant feeders on welfare of hens maintained on litter. *Applied Animal Behaviour Science*, 41, 211–227.
- Liste G, Campderrich I, de Heredia IB and Estevez I, 2015. The relevance of variations in group size and phenotypic appearance on the behaviour and movement patterns of young domestic fowl. *Applied Animal Behaviour Science*, 163, 144–157. <https://doi.org/10.1016/j.applanim.2014.11.013>
- Liu H-W, Chen C-H, Tsai Y-C, Hsieh K-W and Lin H-T, 2021. Identifying images of dead chickens with a chicken removal system integrated with a deep learning algorithm. *Sensors*, 21, 3579.
- Lohmann, 2015. Layer type pullet rearing - Managing the first days. Available online: <https://lohmann-breeders.com/layer-type-pullet-rearing/> [Accessed: 05.12.2022].
- Lourenconi D, Yanagi Junior T, Oliveira DD, Campos AT and Lima RR, 2015. Environmental conditions in a conventional screened poultry shed. *Engenharia Agrícola*, 35, 1–10.
- Lundberg A and Keeling LJ, 1999. The impact of social factors on nesting in laying hens (*Gallus gallus domesticus*). *Applied Animal Behaviour Science*, 64, 57–69. [https://doi.org/10.1016/S0168-1591\(99\)00020-9](https://doi.org/10.1016/S0168-1591(99)00020-9)

- Lundberg AS and Keeling LJ, 2003. Social effects on dustbathing behaviour in laying hens: using video images to investigate effect of rank. *Applied Animal Behaviour Science*, 81, 43–57. [https://doi.org/10.1016/S0168-1591\(02\)00239-3](https://doi.org/10.1016/S0168-1591(02)00239-3)
- Lundén G, Oscarsson R, Hedlund L, Gjølén J and Jensen P, 2022. Play ontogeny in young chickens is affected by domestication and early stress. *Scientific Reports*, 12, 13576. <https://doi.org/10.1038/s41598-022-17617-x>
- Lunam CA, Glatz PC and Hsu YJ, 1996. The absence of neuromas in beaks of adult hens after conservative trimming at hatch. *Australian Veterinary Journal*, 74, 46–49.
- Lunam CA, 2005. The anatomy and innervation of the chicken beak: effects of trimming and re-trimming. *Poultry Welfare Issues: Beak Trimming*, 51–68.
- Ma H, Xin H, Zhao Y, Li B, Shepherd TA and Alvarez I, 2016. Assessment of lighting needs by W-36 laying hens via preference test. *Animal*, 10, 671–680.
- Mack LA, Felver-Gant JN, Dennis RL and Cheng HW, 2013. Genetic variations alter production and behavioral responses following heat stress in 2 strains of laying hens. *Poultry Science*, 92, 285–294.
- MacKenzie JG, Foster TM and Temple W, 1993. Sound avoidance by hens. *Behavioural Processes*, 30, 143–156. [https://doi.org/10.1016/0376-6357\(93\)90004-B](https://doi.org/10.1016/0376-6357(93)90004-B)
- MacLachlan SS, Ali ABA, Toscano MJ and Siegford JM, 2020. Influence of later exposure to perches and nests on flock level distribution of hens in an aviary system during lay. *Poultry Science*, 99, 30–38. <https://doi.org/10.3382/ps/pez524>
- Main DC, Mullan S, Atkinson C, Bond A, Cooper M, Fraser A and Browne W, 2012. Welfare outcomes assessment in laying hen farm assurance schemes. *Animal Welfare*, 21, 389–396.
- Malchow J, Berk J, Puppe B and Schrader L, 2019a. Perches or grids? What do rearing chickens differing in growth performance prefer for roosting? *Poultry Science*, 98, 29–38. <https://doi.org/10.3382/ps/pey320>
- Malchow J, Dudde A, Berk J, Krause E, Sanders O, Puppe B and Schrader L, 2019b. Is the rotarod test an objective alternative to the gait score for evaluating walking ability in chickens? *Animal Welfare*, 28, 261–269.
- Malchow J, Eusemann BK, Petow S, Krause ET and Schrader L, 2022. Productive performance, perching behavior, keel bone and other health aspects in dual-purpose compared to conventional laying hens. *Poultry Science*, 101, 102095. <https://doi.org/10.1016/j.psj.2022.102095>
- Malchow J, Puppe B, Berk J and Schrader L, 2019c. Effects of elevated grids on growing male chickens differing in growth performance. *Frontiers in Veterinary Science*, 6, 203. <https://doi.org/10.3389/fvets.2019.00203>
- Malchow J, 1995. Technology readiness levels. *White Paper*, April, 6
- Marchant-Forde RM, Fahey AG and Cheng HW, 2008. Comparative effects of infrared and one-third hot-blade trimming on beak topography, behavior, and growth. *Poultry Science*, 87, 1474–1483. <https://doi.org/10.3382/ps.2006-00360>
- Marchewka J, Estevez I, Vezzoli G, Ferrante V and Makagon MM, 2015. The transect method: a novel approach to on-farm welfare assessment of commercial turkeys. *Poultry Science*, 94, 7–16. <https://doi.org/10.3382/ps/peu026>
- Marchewka J, Watanabe TTN, Ferrante V and Estevez I, 2013. Welfare assessment in broiler farms: transect walks versus individual scoring. *Poultry Science*, 92, 2588–2599. <https://doi.org/10.3382/ps.2013-03229>
- Martins SIFS, Jongen WMF and van Boekel MAJS, 2000. A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science & Technology*, 11, 364–373. [https://doi.org/10.1016/S0924-2244\(01\)00022-X](https://doi.org/10.1016/S0924-2244(01)00022-X)
- Mason G, Dixon L and Duncan I, 2010. The effects of four types of enrichment on feather pecking behaviour in laying hens housed in barren environments.
- Mathis GF, Newman LJ, Fitz-Coy S, Lumpkins B, Charette R and Fuller L, 2018. Comparison of breeder/layer coccidiosis vaccines: part 1-precocity and pathogenicity. *Journal of Applied Poultry Research*, 27, 33–37.
- Matur E, Akyazi İ, Eraslan E, Ergül Ekiz E, Eseceli H, Keten M, Metiner K and Aktaran Bala D, 2016. The effects of environmental enrichment and transport stress on the weights of lymphoid organs, cell-mediated immune response, heterophil functions and antibody production in laying hens. *Animal Science Journal*, 87, 284–292.
- McArdie TM, Foster TM, Temple W and Matthews LR, 1993. A method for measuring the aversiveness of sounds to domestic hens. *Applied Animal Behaviour Science*, 37, 223–238. [https://doi.org/10.1016/0168-1591\(93\)90113-4](https://doi.org/10.1016/0168-1591(93)90113-4)
- McDonald GC, Spurgin LG, Fairfield EA, Richardson DS and Pizzari T, 2017. Pre- and postcopulatory sexual selection favor aggressive, young males in polyandrous groups of red junglefowl. *Evolution*, 71, 1653–1669.
- McDonald GC, Spurgin LG, Fairfield EA, Richardson DS and Pizzari T, 2019. Differential female sociality is linked with the fine-scale structure of sexual interactions in replicate groups of red junglefowl, *Gallus gallus*. *Proceedings of the Royal Society B: Biological Sciences*, 286, 20191734. <https://doi.org/10.1098/rspb.2019.1734>
- McGary S, Estevez I and Bakst MR, 2003a. Potential relationships between physical traits and male broiler breeder fertility. *Poultry Science*, 82, 328–337. <https://doi.org/10.1093/ps/82.2.328>
- McGary S, Estevez I and Russek-Cohen E, 2003b. Reproductive and aggressive behavior in male broiler breeders with varying fertility levels. *Applied Animal Behaviour Science*, 82, 29–44. [https://doi.org/10.1016/S0168-1591\(03\)00038-8](https://doi.org/10.1016/S0168-1591(03)00038-8)
- McKeegan DEF, Smith FS, Demmers TGM, Wathes CM and Jones RB, 2005. Behavioral correlates of olfactory and trigeminal gaseous stimulation in chickens, *Gallus domesticus*. *Physiology & Behavior*, 84, 761–768.

- McKeegan D and Philbey A, 2012. Chronic neurophysiological and anatomical changes associated with infrared beak treatment and their implications for laying hen welfare. *Animal Welfare*, 21, 207–217.
- Meltzer A, Goodman G and Fistool J, 1982. Thermoneutral zone and resting metabolic rate of growing white leghorn-type chickens. *British Poultry Science*, 23, 383–391. <https://doi.org/10.1080/00071688208447972>
- Mench J, 1993. Problems associated with broiler breeder management. *Proceedings of the Proceedings of the Fourth European Symposium on Poultry Welfare*, 195–207.
- Mench JA and Blatchford RA, 2014. Determination of space use by laying hens using kinematic analysis. *Poultry Science*, 93, 794–798.
- Mench JA, van Tienhoven A, Marsh JA, McCormick CC, Cunningham DL and Baker RC, 1986. Effects of cage and floor pen management on behavior, production, and physiological stress responses of laying hens. *Poultry Science*, 65, 1058–1069. <https://doi.org/10.3382/ps.0651058>
- Mens AJW, Van Krimpen MM and Kwakkel RP, 2020. Nutritional approaches to reduce or prevent feather pecking in laying hens: any potential to intervene during rearing? *World's Poultry Science Journal*, 76, 591–610.
- Merrill RJN, Cooper JJ, Albentosa MJ and Nicol CJ, 2006. The preferences of laying hens for perforated astroturf over conventional wire as a dust-bathing substrate in furnished cages. *Animal Welfare*, 15, 173–178.
- Mertens K, Löffel J, De Baere K, Zoons J, De Baerdemaeker J, Decuyper E and De Ketelaere B, 2009. Layers in aviary system: Effects of beak trimming and alternative feed formulation on technical results and egg quality. *Journal of Applied Poultry Research*, 18, 90–102. <https://doi.org/10.3382/japr.2008-00025>
- Meuser V, Weinhold L, Hillemacher S and Tiemann I, 2021. Welfare-related behaviors in chickens: characterization of fear and exploration in local and commercial chicken strains. *Animals: An Open Access Journal from MDPI*, 11, 679. <https://doi.org/10.3390/ani11030679>
- Michel V and Huonnic D, 2003. A comparison of welfare, health and production performance of laying hens reared in cages or in aviaries. *British Poultry Science*, 44, 775–776.
- Millman ST and Duncan IJH, 2000a. Effect of male-to-male aggressiveness and feed-restriction during rearing on sexual behaviour and aggressiveness towards females by male domestic fowl. *Applied Animal Behaviour Science*, 70, 63–82. [https://doi.org/10.1016/S0168-1591\(00\)00141-6](https://doi.org/10.1016/S0168-1591(00)00141-6)
- Millman ST and Duncan IJH, 2000b. Strain differences in aggressiveness of male domestic fowl in response to a male model. *Applied Animal Behaviour Science*, 66, 217–233. [https://doi.org/10.1016/S0168-1591\(99\)00082-9](https://doi.org/10.1016/S0168-1591(99)00082-9)
- Mishra A, Koene P, Schouten W, Spruijt B, van Beek P and Metz JH, 2005. Temporal and sequential structure of behavior and facility usage of laying hens in an enriched environment. *Poultry Science*, 84(7), 979–991. <https://doi.org/10.1093/ps/84.7.979>
- Moe RO, Nordgreen J, Janczak AM, Bakken M, Spruijt BM and Jensen P, 2014. Anticipatory and foraging behaviors in response to palatable food reward in chickens: effects of dopamine D2 receptor blockade and domestication. *Physiology & Behavior*, 133, 170–177. <https://doi.org/10.1016/j.physbeh.2014.05.023>
- Moesta A, Knierim U, Briese A and Hartung J, 2008. The effect of litter condition and depth on the suitability of wood shavings for dustbathing behaviour. *Applied Animal Behaviour Science*, 115, 160–170. <https://doi.org/10.1016/j.applanim.2008.06.005>
- Moinard C, Rutherford KMD, Haskell MJ, McCorquodale C, Jones RB and Green PR, 2005. Effects of obstructed take-off and landing perches on the flight accuracy of laying hens. *Applied Animal Behaviour Science*, 93, 81–95. <https://doi.org/10.1016/j.applanim.2004.11.016>
- Moinard C, Statham P, Haskell MJ, McCorquodale C, Jones RB and Green PR, 2004. Accuracy of laying hens in jumping upwards and downwards between perches in different light environments. *Applied Animal Behaviour Science*, 85, 77–92. <https://doi.org/10.1016/j.applanim.2003.08.008>
- Mollenhorst H, Rodenburg TB, Bokkers EAM, Koene P and de Boer IJM, 2005. On-farm assessment of laying hen welfare: a comparison of one environment-based and two animal-based methods. *Applied Animal Behaviour Science*, 90, 277–291. <https://doi.org/10.1016/j.applanim.2004.08.011>
- Møller AP and Erritzøe J, 1992. Acquisition of breeding coloration depends on badge size in male house sparrows *passer domesticus*. *Behavioral Ecology and Sociobiology*, 31, 271–277. <https://doi.org/10.1007/BF00171682>
- Monckton V, Ellis JL and Harlander-Matauschek A, 2020. Floor substrate preferences of chickens: a meta-analysis. *Frontiers in Veterinary Science*, 7, 584162. <https://doi.org/10.3389/fvets.2020.584162>
- Moroki Y and Tanaka T, 2016. A pecking device as an environmental enrichment for caged laying hens. *Animal Science Journal*, 87, 1055–1062. <https://doi.org/10.1111/asj.12525>
- Morrissey KLH, Brocklehurst S, Baker L, Widowski TM and Sandilands V, 2016. Can non-beak treated hens be kept in commercial furnished cages? Exploring the effects of strain and extra environmental enrichment on behaviour, feather cover, and mortality. *Animals*, 6, 17.
- Moyle JR, Yoho DE, Harper RS and Bramwell RK, 2010. Mating behavior in commercial broiler breeders: female effects. *Journal of Applied Poultry Research*, 19, 24–29. <https://doi.org/10.3382/japr.2009-00061>
- Mul MF, van Vugt SMA, Goselink YSM and van den Brand H, 2020. Effects of heating laying hen houses between consecutive laying cycles on the survival of the poultry red mite *Dermanyssus gallinae*. *Veterinary Parasitology*, 288, 109307. <https://doi.org/10.1016/j.vetpar.2020.109307>

- Mullens BA, Chen BL and Owen JP, 2010. Beak condition and cage density determine abundance and spatial distribution of northern fowl mites, *Ornithonyssus sylviarum*, and chicken body lice, *Menacanthus stramineus*, on caged laying hens. *Poultry Science*, 89, 2565–2572. <https://doi.org/10.3382/ps.2010-00955>
- Murillo AC, Abdoli A, Blatchford RA, Keogh EJ and Gerry AC, 2020. Parasitic mites alter chicken behaviour and negatively impact animal welfare. *Scientific Reports*, 10, 8236. <https://doi.org/10.1038/s41598-020-65021-0>
- Murillo AC, Chappell MA, Owen JP and Mullens BA, 2016. Northern fowl mite (*Ornithonyssus sylviarum*) effects on metabolism, body temperatures, skin condition, and egg production as a function of hen MHC haplotype. *Poultry Science*, 95, 2536–2546. <https://doi.org/10.3382/ps/pew175>
- Murillo AC and Mullens BA, 2016. Timing diatomaceous earth-filled dustbox use for management of northern fowl mites (Acari: Macronyssidae) in cage-free poultry systems. *Journal of Economic Entomology*, 109, 2572–2579. <https://doi.org/10.1093/jee/tow165>
- Naseem S and King AJ, 2018. Ammonia production in poultry houses can affect health of humans, birds, and the environment—techniques for its reduction during poultry production. *Environmental Science and Pollution Research*, 25, 15269–15293. <https://doi.org/10.1007/s11356-018-2018-y>
- National Research Council (US), 2011. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edn. National Academies Press (US), Washington (DC).
- Nazar FN, Skånberg L, McCrear K and Keeling LJ, 2022. Increasing environmental complexity by providing different types of litter and perches during early rearing boosts coping abilities in domestic fowl chicks. *Animals*, 12, 1969.
- Nelson JR, Settar P, Berger E, Wolc A, O'Sullivan N and Archer GS, 2020. Brown and white egg-layer strain differences in fearfulness and stress measures. *Applied Animal Behaviour Science*, 231, 105087. <https://doi.org/10.1016/j.applanim.2020.105087>
- Network WQ, 2019. Welfare Quality assessment protocol for laying hens Version 2.0. Welfare Quality Network.
- Newberry R, 1999. Exploratory behaviour of young domestic fowl. *Applied Animal Behaviour Science*, 63, 311–321. [https://doi.org/10.1016/S0168-1591\(99\)00016-7](https://doi.org/10.1016/S0168-1591(99)00016-7)
- Newberry RC, Estevez I and Keeling LJ, 2001. Group size and perching behaviour in young domestic fowl. *Applied Animal Behaviour Science*, 73, 117–129. [https://doi.org/10.1016/S0168-1591\(01\)00135-6](https://doi.org/10.1016/S0168-1591(01)00135-6)
- Newberry RC, 1995. Environmental enrichment: Increasing the biological relevance of captive environments. *Applied Animal Behaviour Science*, 44, 229–243.
- Newberry RC, Keeling LJ, Estevez I and Bilčík B, 2007. Behaviour when young as a predictor of severe feather pecking in adult laying hens: the redirected foraging hypothesis revisited. *Applied Animal Behaviour Science*, 107, 262–274. <https://doi.org/10.1016/j.applanim.2006.10.010>
- Newberry RC and Shackleton DM, 1997. Use of visual cover by domestic fowl: a venetian blind effect? *Animal Behaviour*, 54, 387–395. <https://doi.org/10.1006/anbe.1996.0421>
- Nicol C, 2007. Space, time, and unassuming animals. *Journal of Veterinary Behavior: Clinical Applications and Research*, 2, 188–192. <https://doi.org/10.1016/j.jveb.2007.10.002>
- Nicol CJ, 1987a. Behavioural responses of laying hens following a period of spatial restriction. *Animal Behaviour*, 35, 1709–1719. [https://doi.org/10.1016/S0003-3472\(87\)80063-5](https://doi.org/10.1016/S0003-3472(87)80063-5)
- Nicol CJ, 1987b. Effect of cage height and area on the behaviour of hens housed in battery cages. *British Poultry Science*, 28, 327–335. <https://doi.org/10.1080/00071668708416965>
- Nicol C, 2015. A study to test the effectiveness of management strategies in reducing injurious pecking of laying hens with intact beaks in non cage systems. Defra Final Report. Department for Environment, Food & Rural Affairs, London. Available online: [sciencesearch.defra.gov.uk/Document.aspx](https://sciencesearch.defra.gov.uk/Document.aspx)
- Nicol CJ, 2019. Feather pecking in laying hens: why they do it, and welfare implications. *Poultry Feathers and Skin: The Poultry Integument in Health and Welfare*. pp. 31–46.
- Nicol CJ, Bouwsema J, Caplen G, Davies AC, Hockenhull J, Lambton SL, Lines JA, Mullan S and Weeks CA, 2017. Farmed Bird Welfare Science Review. Department of Economic Development, Jobs, Transport and Resources.
- Nicol CJ, Bestman M, Gilani AM, De Haas EN, De Jong IC, Lambton S, Wagenaar JP, Weeks CA and Rodenburg TB, 2013. The prevention and control of feather pecking: application to commercial systems. *World's Poultry Science Journal*, 69, 775–788. <https://doi.org/10.1017/S0043933913000809>
- Nicol CJ, Brown SN, Glen E, Pope SJ, Short FJ, Warriss PD, Zimmerman PH and Wilkins LJ, 2006. Effects of stocking density, flock size and management on the welfare of laying hens in single-tier aviaries. *British Poultry Science*, 47, 135–146.
- Nicol CJ, Caplen G, Edgar J and Browne WJ, 2009. Associations between welfare indicators and environmental choice in laying hens. *Animal Behaviour*, 78, 413–424. <https://doi.org/10.1016/j.anbehav.2009.05.016>
- Nicol CJ, Caplen G, Statham P and Browne WJ, 2011. Decisions about foraging and risk trade-offs in chickens are associated with individual somatic response profiles. *Animal Behaviour*, 82, 255–262. <https://doi.org/10.1016/j.anbehav.2011.04.022>
- Nicol CJ, Gregory NG, Knowles TG, Parkman ID and Wilkins LJ, 1999. Differential effects of increased stocking density, mediated by increased flock size, on feather pecking and aggression in laying hens. *Applied Animal Behaviour Science*, 65, 137–152. [https://doi.org/10.1016/S0168-1591\(99\)00057-X](https://doi.org/10.1016/S0168-1591(99)00057-X)
- Nicol CJ and Guilford T, 1991. Exploratory activity as a measure of motivation in deprived hens. *Animal Behaviour*, 41, 333–341. [https://doi.org/10.1016/S0003-3472\(05\)80485-3](https://doi.org/10.1016/S0003-3472(05)80485-3)

- Nicol CJ, Pötzsch C, Lewis K and Green LE, 2003. Matched concurrent case-control study of risk factors for feather pecking in hens on free-range commercial farms in the UK. *British Poultry Science*, 44, 515–523.
- Nicol C, 2018. Feather pecking and cannibalism: Can we really stop beak trimming?. *Advances in Poultry Welfare*. Woodhead Publishing, 197. pp. 175.
- Nordenfors H, Höglund J and Uggla A, 1999. Effects of temperature and humidity on oviposition, molting, and longevity of *Dermanyssus gallinae* (Acari: Dermanyssidae). *Journal of Medical Entomology*, 36, 68–72. <https://doi.org/10.1093/jmedent/36.1.68>
- Norman KI, Weeks CA, Pettersson IC and Nicol CJ, 2018. The effect of experience of ramps at rear on the subsequent ability of layer pullets to negotiate a ramp transition. *Applied Animal Behaviour Science*, 208, 92–99. <https://doi.org/10.1016/j.applanim.2018.08.007>
- Norman KI, Weeks CA, Tarlton JF and Nicol CJ, 2021. Rearing experience with ramps improves specific learning and behaviour and welfare on a commercial laying farm. *Scientific Reports*, 11, 8860. <https://doi.org/10.1038/s41598-021-88347-9>
- Northcutt JK, Buhr RJ and Rowland GN, 2000. Relationship of broiler bruise age to appearance and tissue histological characteristics. *Journal of Applied Poultry Research*, 9, 13–20. <https://doi.org/10.1093/japr/9.1.13>
- O'Connor EA, Parker MO, Davey EL, Grist H, Owen RC, Szladovits B, Demmers TGM, Wathes CM and Abeyesinghe SM, 2011. Effect of low light and high noise on behavioural activity, physiological indicators of stress and production in laying hens. *British Poultry Science*, 52, 666–674. <https://doi.org/10.1080/00071668.2011.639342>
- Odén K, Keeling LJ and Algers B, 2002. Behaviour of laying hens in two types of aviary systems on 25 commercial farms in Sweden. *British Poultry Science*, 43, 169–181. <https://doi.org/10.1080/00071660120121364>
- Oliveira JL, Xin H and Wu H, 2019. Impact of feeder space on laying hen feeding behavior and production performance in enriched colony housing. *Animal*, 13, 374–383.
- Olsson IAS, Duncan IJH, Keeling LJ and Widowski TM, 2002. How important is social facilitation for dustbathing in laying hens? *Applied Animal Behaviour Science*, 79, 285–297. [https://doi.org/10.1016/S0168-1591\(02\)00117-X](https://doi.org/10.1016/S0168-1591(02)00117-X)
- Olsson IAS and Keeling LJ, 2000. Night-time roosting in laying hens and the effect of thwarting access to perches. *Applied Animal Behaviour Science*, 68, 243–256. [https://doi.org/10.1016/S0168-1591\(00\)00097-6](https://doi.org/10.1016/S0168-1591(00)00097-6)
- Olsson IAS and Keeling LJ, 2002. The push-door for measuring motivation in hens: laying hens are motivated to perch at night. *Animal Welfare*, 11, 11–19.
- Olsson IAS and Keeling LJ, 2005. Why in earth? Dustbathing behaviour in jungle and domestic fowl reviewed from a Tinbergian and animal welfare perspective. *Applied Animal Behaviour Science*, 93, 259–282.
- Oviedo-Rondón EO, Wineland MJ, Small J, Cutchin H, McElroy A, Barri A and Martin S, 2009. Effect of incubation temperatures and chick transportation conditions on bone development and leg health. *Journal of Applied Poultry Research*, 18, 671–678. <https://doi.org/10.3382/japr.2008-00135>
- Owen JP, Delany ME, Cardona CJ, Bickford AA and Mullens BA, 2009. Host inflammatory response governs fitness in an avian ectoparasite, the northern fowl mite (*Ornithonyssus sylviarum*). *International Journal for Parasitology*, 39, 789–799. <https://doi.org/10.1016/j.ijpara.2008.12.008>
- Patt A, Halle I, Dudde A, Olbrich A, Sieburg-Rockel J and Krause ET, 2022. Influence of different dietary fibre contents in the diet on feather pecking, locomotor activity and performance of laying hens. *British Poultry Science*, 63, 571–580.
- Paul ES, Browne WH, Mendl MT, Caplen G, Trevarthen A, Held S and Nicol CJ, 2022. Assessing animal welfare: a triangulation of preference, judgement bias and other candidate welfare indicators. *Animal Behaviour*, 186, 151–177.
- Peixoto MRLV, Karrow NA, Newman A and Widowski TM, 2020. Effects of maternal stress on measures of anxiety and fearfulness in different strains of laying hens. *Frontiers in Veterinary Science*, 7, 128. <https://doi.org/10.3389/fvets.2020.00128>
- Permin A, Bisgaard M, Frandsen F, Pearman M, Kold J and Nansen P, 1999. Prevalence of gastrointestinal helminths in different poultry production systems. *British Poultry Science*, 40, 439–443. <https://doi.org/10.1080/00071669987179>
- Petek M, Topal E and Cavusoglu E, 2015. Effects of age at first access to range area on pecking behaviour and plumage quality of free-range layer chickens. *Archives Animal Breeding*, 58, 85–91.
- Petherick JC and Duncan IJH, 1989. Behaviour of young domestic fowl directed towards different substrates. *British Poultry Science*, 30, 229–238. <https://doi.org/10.1080/00071668908417143>
- Pettersson IC, Freire R and Nicol CJ, 2016. Factors affecting ranging behaviour in commercial free-range hens. *World's Poultry Science Journal*, 72, 137–150. <https://doi.org/10.1017/S0043933915002664>
- Pettersson IC, Weeks CA and Nicol CJ, 2017. Provision of a resource package reduces feather pecking and improves ranging distribution on free-range layer farms. *Applied Animal Behaviour Science*, 195, 60–66. <https://doi.org/10.1016/j.applanim.2017.06.007>
- Pichova K, Nordgreen J, Leterrier C, Kostal L and Moe RO, 2016. The effects of food-related environmental complexity on litter directed behaviour, fear and exploration of novel stimuli in young broiler chickens. *Applied Animal Behaviour Science*, 174, 83–89. <https://doi.org/10.1016/j.applanim.2015.11.007>
- Pichová K and Bilčík B, 2017. Assessment of the effect of housing on feather damage in laying hens using IR thermography. *Animal*, 11, 661–669.

- Pickel T, Scholz B and Schrader L, 2010. Perch material and diameter affects particular perching behaviours in laying hens. *Applied Animal Behaviour Science*, 127, 37–42. <https://doi.org/10.1016/j.applanim.2010.08.005>
- Pike AVL and Maitland DP, 2004. Scaling of bird claws. *Journal of Zoology*, 262(1), 73–81.
- Pizzari T, 2016. The Wood-Gush legacy: a sociobiology perspective to fertility and welfare in chickens. *Applied Animal Behaviour Science*, 181, 12–18. <https://doi.org/10.1016/j.applanim.2016.01.025>
- Pizzari T, Biernaskie JM and Carazo P, 2015. Inclusive fitness and sexual conflict: how population structure can modulate the battle of the sexes. *BioEssays*, 37, 155–166. <https://doi.org/10.1002/bies.201400130>
- Pizzari T and Birkhead TR, 2001. For whom does the hen cackle? The function of postoviposition cackling. *Animal Behaviour*, 61, 601–607. <https://doi.org/10.1006/anbe.2000.1620>
- Pokharel BB, Boecker I, Kwon IY, Jeyachanthiran L, McBride P and Harlander-Matauschek A, 2018. How does the presence of excreta affect the behavior of laying hens on scratch pads? *Poultry Science*, 97, 743–748. <https://doi.org/10.3382/ps/pex375>
- Porter RH, Roelofsen R, Picard M and Arnould C, 2005. The temporal development and sensory mediation of social discrimination in domestic chicks. *Animal Behaviour*, 70, 359–364. <https://doi.org/10.1016/j.anbehav.2004.10.019>
- Pöttsch CJ, Lewis K, Nicol CJ and Green LE, 2001. A cross-sectional study of the prevalence of vent pecking in laying hens in alternative systems and its associations with feather pecking, management and disease. *Applied Animal Behaviour Science*, 74, 259–272. [https://doi.org/10.1016/S0168-1591\(01\)00167-8](https://doi.org/10.1016/S0168-1591(01)00167-8)
- Preisinger R, 2021. Commercial layer breeding: Review and forecast. *Zuchtungskunde*, 93, 210–228.
- Pufall A, Harlander-Matauschek A, Hunniford M and Widowski TM, 2021. Effects of rearing aviary style and genetic strain on the locomotion and musculoskeletal characteristics of layer pullets. *Animals*, 11, 634.
- Pullin AN, Temple SM, Bennett DC, Rufener CB, Blatchford RA and Makagon MM, 2020. Pullet rearing affects collisions and perch use in enriched colony cage layer housing. *Animals*, 10, 1269.
- Qaisrani SN, Van Krimpen MM and Kwakkel RP, 2013. Effects of dietary dilution source and dilution level on feather damage, performance, behavior, and litter condition in pullets. *Poultry Science*, 92, 591–602.
- Radwan LM, 2020. Genetic improvement of egg laying traits in Fayoumi chickens bred under conditions of heat stress through selection and gene expression studies. *Journal of Thermal Biology*, 89, 1–7. <https://doi.org/10.1016/j.jtherbio.2020.102546>
- Rana MS, Cohen-Barnhouse AM, Lee C and Campbell DLM, 2021. Preference testing for UV light spectrum and intensity in laying hens. *Poultry Science*, 100, 101063. <https://doi.org/10.1016/j.psj.2021.101063>
- Ramadan SGA and Von Borell E, 2008. Role of loose feathers on the development of feather pecking in laying hens. *British Poultry Science*, 49, 250–256.
- Ratner SC and Thompson RW, 1960. Immobility reactions (fear) of domestic fowl as a function of age and prior experience. *Animal Behaviour*, 8, 186–191. [https://doi.org/10.1016/0003-3472\(60\)90025-7](https://doi.org/10.1016/0003-3472(60)90025-7)
- Redwine JS, Lacey R, Mukhtar S and Carey JB, 2002. Concentration and emissions of ammonia and particulate matter in tunnel-ventilated broiler houses under summer conditions in Texas. *Transactions of ASAE*, 45, 1101. <https://doi.org/10.13031/2013.9943>
- Reece FN and Lott BD, 1980. Effect of carbon dioxide on broiler chicken performance. *Poultry Science*, 59, 2400–2402. <https://doi.org/10.3382/ps.0592400>
- Regmi P, Deland T, Steibel J, Robison sC, Haut R, Orth M and Karcher D, 2015. Effect of rearing environment on bone growth of pullets. *Poultry Science*, 94, 502–511.
- Rentsch AK, Rufener CB, Spadavecchia C, Stratmann A and Toscano MJ, 2019. Laying hen's mobility is impaired by keel bone fractures and does not improve with paracetamol treatment. *Applied Animal Behaviour Science*, 216, 19–25. <https://doi.org/10.1016/j.applanim.2019.04.015>
- Riber A and Hinrichsen L, 2016. Keel-bone damage and foot injuries in commercial laying hens in Denmark. *Animal Welfare*, 25, 179–184.
- Riber AB, 2010. Development with age of nest box use and gregarious nesting in laying hens. *Applied Animal Behaviour Science*, 123, 24–31. <https://doi.org/10.1016/j.applanim.2009.12.016>
- Riber AB, Casey-Trott TM and Herskin MS, 2018. The influence of keel bone damage on welfare of laying hens. *Frontiers in Veterinary Science*, 5. <https://doi.org/10.3389/fvets.2018.00006>
- Riber AB and Guzman DA, 2016. Effects of dark brooders on behavior and fearfulness in layers. *Animals*, 6, 3.
- Riber AB and Guzman DA, 2017. Effects of different types of dark brooders on injurious pecking damage and production-related traits at rear and lay in layers. *Poultry Science*, 96, 3529–3538. <https://doi.org/10.3382/ps/pex177>
- Riber AB, Wichman A, Braastad BO and Forkman B, 2007. Effects of broody hens on perch use, ground pecking, feather pecking and cannibalism in domestic fowl (*Gallus gallus domesticus*). *Applied Animal Behaviour Science*, 106, 39–51. <https://doi.org/10.1016/j.applanim.2006.07.012>
- Riber AB and Hinrichsen LK, 2017. Welfare consequences of omitting beak trimming in barn layers. *Frontiers in Veterinary Science*, 4, 222.
- Riber AB and Forkman B, 2007. A note on the behaviour of the chicken that receives feather pecks. *Applied Animal Behaviour Science*, 108, 337–341.
- Richards G, Nasr M, Brown S, Szamocki E, Murrell J, Barr F and Wilkins L, 2011. Use of radiography to identify keel bone fractures in laying hens and assess healing in live birds. *Veterinary Record*, 169, 279.
- Richards G, Wilkins L, Knowles T, Booth F, Toscano M, Nicol C and Brown S, 2012. Pop hole use by hens with different keel fracture status monitored throughout the laying period. *Veterinary Record*, 170, 494.

- Riddle ER, Ali ABA, Campbell DLM and Siegford JM, 2018. Space use by 4 strains of laying hens to perch, wing flap, dust bathe, stand and lie down. *PLoS One*, 13, e0190532. <https://doi.org/10.1371/journal.pone.0190532>
- Riedel A, Rieke L, Kemper N and Spindler B, 2022. A place to be? Use of winter gardens with and without automatic enrichment devices by laying hens. *Applied Sciences*, 12, 3650.
- Ringgenberg N, Fröhlich EK, Harlander-Matauschek A, Toscano MJ, Würbel H and Roth BA, 2015. Nest choice in laying hens: effects of nest partitions and social status. *Applied Animal Behaviour Science*, 169, 43–50. <https://doi.org/10.1016/j.applanim.2015.04.013>
- Ringgenberg N, Fröhlich EK, Harlander-Matauschek A, Würbel H and Roth BA, 2014. Does nest size matter to laying hens? *Applied Animal Behaviour Science*, 155, 66–73. <https://doi.org/10.1016/j.applanim.2014.02.012>
- Rodenburg T, Tuytens F, De Reu K, Herman L, Zoons J and Sonck B, 2008. Welfare assessment of laying hens in furnished cages and non-cage systems: an on-farm comparison. *Animal Welfare*, 17, 363–373.
- Rodenburg TB, Buitenhuis AJ, Ask B, Uitdehaag KA, Koene P, van der Poel JJ, van Arendonk JAM and Bovenhuis H, 2004. Genetic and phenotypic correlations between feather pecking and open-field response in laying hens at two different ages. *Behavior Genetics*, 34, 407–415. <https://doi.org/10.1023/B:BEGE.0000023646.46940.2d>
- Rodenburg TB, Tuytens FAM, Sonck B, De Reu K, Herman L and Zoons J, 2005. Welfare, health, and hygiene of laying hens housed in furnished cages and in alternative housing systems. *Journal of Applied Animal Welfare Science*, 8, 211–226.
- Rodenburg TB, van Gerwen M, Meijer E, Tobias TJ, Giersberg MF, Goerlich VC, Nordquist RE, Meijboom FLB and Arndt SS (2020). End the cage age: looking for alternatives.
- Rodenburg TB, Van Krimpen MM, De Jong IC, De Haas EN, Kops MS, Riedstra BJ, Nordquist RE, Wagenaar JP, Bestman M and Nicol CJ, 2013. The prevention and control of feather pecking in laying hens: identifying the underlying principles. *World's Poultry Science Journal*, 69, 361–374. <https://doi.org/10.1017/S0043933913000354>
- Rodriguez-Aurrekoetxea A and Estevez I, 2014. Aggressiveness in the domestic fowl: distance versus 'attitude'. *Applied Animal Behaviour Science*, 153, 68–74. <https://doi.org/10.1016/j.applanim.2014.01.007>
- Rodriguez-Aurrekoetxea A and Estevez I, 2016. Use of space and its impact on the welfare of laying hens in a commercial free-range system. *Poultry Science*, 95, 2503–2513. <https://doi.org/10.3382/ps/pew238>
- Rose P and Croft D, 2015. The potential of Social Network Analysis as a tool for the management of zoo animals. *Animal Welfare*, 24, 123–138. <https://doi.org/10.7120/09627286.24.2.123>
- RSPCA, 2017. RSPCA welfare standards for laying hens. Available online: <https://science.rspca.org.uk/documents/1494935/9042554/RSPCA+welfare+standards+for+laying+hens+2017+%28PDF+4.46MB%29.pdf/fd2c382d-1a4a-29ee-781f-158c34ca6082?t=1557668428002>
- Rufener C and Makagon MM, 2020. Keel bone fractures in laying hens: a systematic review of prevalence across age, housing systems, and strains. *Journal of Animal Science*, 98, S36–S51. <https://doi.org/10.1093/jas/skaa145>
- Rufener C, Rentsch AK, Stratmann A and Toscano MJ, 2020. Perch positioning affects both laying hen locomotion and forces experienced at the keel. *Animals*, 10, 1223.
- Rushen J, 1982. The peck orders of chickens: how do they develop and why are they linear? *Animal Behaviour*, 30, 1129–1137.
- Ruzal M, Shinder D, Malka I and Yahav S, 2011. Ventilation plays an important role in hens' egg production at high ambient temperature. *Poultry Science*, 90, 856–862. <https://doi.org/10.3382/ps.2010-00993>
- Sandberg M, Jørgensen A and Fagertun J, 2022. VetInspector - A tool for post mortem inspection of chicken. Proceedings of the 4th International Conference on Animal Health Surveillance, Copenhagen, Denmark, 21 pp. Available online: [https://icahs4.org/fileadmin/user\\_upload/ICAHS4\\_2020/final\\_abstract\\_book.pdf](https://icahs4.org/fileadmin/user_upload/ICAHS4_2020/final_abstract_book.pdf)
- Sanotra GS, Vestergaard KS, Agger JF and Lawson LG, 1995. The relative preferences for feathers, straw, wood-shavings and sand for dustbathing, pecking and scratching in domestic chicks. *Applied Animal Behaviour Science*, 43, 263–277. [https://doi.org/10.1016/0168-1591\(95\)00562-7](https://doi.org/10.1016/0168-1591(95)00562-7)
- Saunders SS and Salvi RJ, 1993. Psychoacoustics of normal adult chickens: thresholds and temporal integration. *The Journal of the Acoustical Society of America*, 94, 83–90.
- Savory CJ, Jack MC and Sandilands V, 2006. Behavioural responses to different floor space allowances in small groups of laying hens. *British Poultry Science*, 47, 120–124.
- Scanes CG, 2016. Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. *Poultry Science*, 95, 2208–2215. <https://doi.org/10.3382/ps/pew137>
- Scanes CG, Butler LD and Kidd MT, 2020. Chapter 20 - reproductive management of poultry. In: FW Bazer, GC Lamb and G Wu (eds). *Animal Agriculture*. Academic Press. pp. 349–366.
- Schiavone A, Pugliese N, Circella E and Camarda A, 2020. Association between the poultry red mite *Dermanyssus gallinae* and potential avian pathogenic *Escherichia coli* (APEC). *Veterinary Parasitology*, 284, 109198. <https://doi.org/10.1016/j.vetpar.2020.109198>
- Schütz, K., Mergenthaler, M., Wittmann, M., 2018. Marktpotential für Geflügelprodukte aus Hahnenfleisch von Lege und Zweinutzungshybriden, Forschungsberichte Nr. 45, Fachhochschule Südwestfalen Standort Soest, Fachbereich Agrarwirtschaft, p. 61.
- Scholz B, Kjaer JB, Petow S and Schrader L, 2014. Dustbathing in food particles does not remove feather lipids. *Poultry Science*, 93, 1877–1882. <https://doi.org/10.3382/ps.2013-03231>

- Scholz B, Kjaer JB, Urselmans S and Schrader L, 2011. Litter lipid content affects dustbathing behavior in laying hens. *Poultry Science*, 90, 2433–2439. <https://doi.org/10.3382/ps.2011-01480>
- Scholz B, Urselmans S, Kjaer JB and Schrader L, 2010. Food, wood, or plastic as substrates for dustbathing and foraging in laying hens: a preference test. *Poultry Science*, 89, 1584–1589. <https://doi.org/10.3382/ps.2009-00598>
- Schrader L and Malchow J, 2020. The role of perches in chicken welfare. Understanding the behaviour and improving the welfare of chickens. Burleigh Dodds Science Publishing. pp. 375–416.
- Schrader L and Müller B, 2009. Night-time roosting in the domestic fowl: the height matters. *Applied Animal Behaviour Science*, 121, 179–183. <https://doi.org/10.1016/j.applanim.2009.09.010>
- Schuck-Paim C, Negro-Calduch E and Alonso WJ, 2021. Laying hen mortality in different indoor housing systems: a meta-analysis of data from commercial farms in 16 countries. *Scientific Reports*, 11, 3052. <https://doi.org/10.1038/s41598-021-81868-3>
- Schwarzer A, Erhard M, Schmidt P, Zismann M and Louton H, 2022. Effects of stocking rate and environmental enrichment on the ontogeny of pecking behavior of laying hen pullets confined in aviary compartments during the first 4 weeks of life. *Animals*, 12, 2639.
- Schwean-Lardner K, Fancher BI and Classen HL, 2012. Impact of daylength on behavioural output in commercial broilers. *Applied Animal Behaviour Science*, 137, 43–52. <https://doi.org/10.1016/j.applanim.2012.01.015>
- Scott GB, Hughes BO, Lambe NR and Waddington D, 1999. Ability of laying hens to jump between perches: individual variation and the effects of perch separation and motivation on behaviour. *British Poultry Science*, 40, 177–184. <https://doi.org/10.1080/00071669987575>
- Scott AB, Toribio JA, Singh M, Groves P, Barnes B, Glass K, Moloney B, Black A and Hernandez-Jover M, 2018. Low pathogenic avian influenza exposure risk assessment in australian commercial chicken farms. *Frontiers in Veterinary Science*, 5, 68. <https://doi.org/10.3389/fvets.2018.00068>
- Seehuus B, Mendl M, Keeling LJ and Blokhuis H, 2013. Disrupting motivational sequences in chicks: are there affective consequences? *Applied Animal Behaviour Science*, 148, 85–92. <https://doi.org/10.1016/j.applanim.2013.07.008>
- Sepour S, Spindler B, Schulze-Bispung M, Habig C, Andersson R, Beyerbach M and Kemper N, 2015. Comparison of plumage condition of laying hens with intact and trimmed beaks kept on commercial farms. *Archiv für Geflügelkunde*, 79.
- Sethy K, Dash R, Vaidantika V, Priyadarshinee P, Dhaigude V, Dwibedy P and Adhikary PD, 2018. Cannibalism: a threat to poultry industry. *INDIAN FARMER*, 5, 357–370.
- Sharma N, Hunt PW, Hine BC and Ruhnke I, 2019. The impacts of *Ascaridia galli* on performance, health, and immune responses of laying hens: new insights into an old problem. *Poultry Science*, 98, 6517–6526. <https://doi.org/10.3382/ps/pez422>
- Sherwin CM, Nasr MAF, Gale E, Petek M, Stafford K, Turp M and Coles GC, 2013. Prevalence of nematode infection and faecal egg counts in free-range laying hens: relations to housing and husbandry. *British Poultry Science*, 54, 12–23. <https://doi.org/10.1080/00071668.2012.757577>
- Sherwin CM, Richards GJ and Nicol CJ, 2010. Comparison of the welfare of layer hens in 4 housing systems in the UK. *British Poultry Science*, 51, 488–499.
- Shi H, Li B, Tong Q, Zheng W and Feng G, 2019b. Influence of nest boxes and claw abrasive devices on feather pecking and the fear responses of layer breeders in natural mating colony cages. *Applied Animal Behaviour Science*, 220, 104842. <https://doi.org/10.1016/j.applanim.2019.104842>
- Shi H, Tong Q, Zheng W, Tu J and Li B, 2019a. Effects of nest boxes in natural mating colony cages on fear, stress, and feather damage for layer breeders. *Journal of Animal Science*, 97, 4464–4474. <https://doi.org/10.1093/jas/skz301>
- Shields SJ, Garner JP and Mench JA, 2004. Dustbathing by broiler chickens: a comparison of preference for four different substrates. *Applied Animal Behaviour Science*, 87, 69–82. <https://doi.org/10.1016/j.applanim.2004.01.003>
- Shifaw A, Feyera T, Walkden-Brown SW, Sharpe B, Elliott T and Ruhnke I, 2021. Global and regional prevalence of helminth infection in chickens over time: a systematic review and meta-analysis. *Poultry Science*, 100, 101082. <https://doi.org/10.1016/j.psj.2021.101082>
- Shimmura T, Azuma T, Hirahara S, Eguchi Y, Uetake K and Tanaka T, 2008a. Relation between social order and use of resources in small and large furnished cages for laying hens. *British Poultry Science*, 49, 516–524.
- Shimmura T, Bracke MBM, De Mol RM, Hirahara S, Uetake K and Tanaka T, 2011. Overall welfare assessment of laying hens: comparing science-based, environment-based and animal-based assessments. *Animal Science Journal*, 82, 150–160. <https://doi.org/10.1111/j.1740-0929.2010.00834.x>
- Shimmura T, Eguchi Y, Uetake K and Tanaka T, 2007. Differences of behavior, use of resources and physical conditions between dominant and subordinate hens in furnished cages. *Animal Science Journal*, 78, 307–313.
- Shimmura T, Eguchi Y, Uetake K and Tanaka T, 2008b. Effects of separation of resources on behaviour of high-, medium- and low-ranked hens in furnished cages. *Applied Animal Behaviour Science*, 113, 74–86. <https://doi.org/10.1016/j.applanim.2007.11.007>
- Shimmura T, Maruyama Y, Fujino S, Kamimura E, Uetake K and Tanaka T, 2015. Persistent effect of broody hens on behaviour of chickens. *Animal Science Journal*, 86, 214–220. <https://doi.org/10.1111/asj.12253>

- Shimmura T, Suzuki T, Hirahara S, Eguchi Y, Uetake K and Tanaka T, 2008. Pecking behaviour of laying hens in single-tiered aviaries with and without outdoor area. *British Poultry Science*, 49, 396–401.
- Sibanda TZ, O'Shea CJ, de Souza VJ, Kolakshyapati M, Welch M, Schneider D, Courtice J and Ruhnke I, 2020. Managing free-range laying hens—part B: early range users have more pathology findings at the end of lay but have a significantly higher chance of survival—an indicative study. *Animals*, 10, 1911.
- Sirovnik J and Riber AB, 2022. Why-oh-why? Dark brooders reduce injurious pecking, though are still not widely used in commercial rearing of layer pullets. *Animals*, 12, 1276.
- Sirovnik J, Stratmann A, Gebhardt-Henrich SG, Würbel H and Toscano MJ, 2018. Feeding from perches in an aviary system reduces aggression and mortality in laying hens. *Applied Animal Behaviour Science*, 202, 53–62. <https://doi.org/10.1016/j.applanim.2018.01.005>
- Skånberg L, Kjærsgaard Nielsen CB and Keeling LJ, 2021. Litter and perch type matter already from the start: exploring preferences and perch balance in laying hen chicks. *Poultry Science*, 100, 431–440. <https://doi.org/10.1016/j.psj.2020.11.041>
- Sohail SS, Bryant MM and Roland DA, 2004. Effect of reducing cage density on performance and economics of second-cycle (Force rested) commercial leghorns. *Journal of Applied Poultry Research*, 13, 401–405.
- Sokół R and Rotkiewicz T, 2010. Histopathological changes of the skin in hens infested with *Dermanyssus gallinae*. *Polish Journal of Veterinary Sciences*, 13, 385–387.
- Sokolowicz Z, Dykiel M, Topczewska J, Krawczyk J and Augustyńska-Prejsnar A, 2020. The effect of the type of non-caged housing system, genotype and age on the behaviour of laying hens. *Animals*, 10, 2450.
- Sorosh Z, Salari S, Sari M, Fayazi J and Tabatabaei S, 2019. Dietary zinc supplementation and the performance and behaviour of caged laying hens. *Animal Production Science*, 59, 331–337. <https://doi.org/10.1071/AN16706>
- Sossidou EN, Dal Bosco A, Castellini C and Grashorn MA, 2015. Effects of pasture management on poultry welfare and meat quality in organic poultry production systems. *World's Poultry Science Journal*, 71, 375–384. <https://doi.org/10.1017/S0043933915000379>
- Sparagano O, Pavličević A, Murano T, Camarda A, Sahibi H, Kilpinen O, Mul M, Emous RV, SI B and Hoel K, 2009. Prevalence and key figures for the poultry red mite *Dermanyssus gallinae* infections in poultry farm systems. *Experimental & Applied Acarology*, 48, 3–10.
- Sparagano OAE, George DR, Harrington DWJ and Giangaspero A, 2014. Significance and control of the poultry red mite, *Dermanyssus gallinae*. *Annual Review of Entomology*, 59, 447–466. <https://doi.org/10.1146/annurev-ento-011613-162101>
- Sparks NHC, Conroy MA and Sandilands V, 2008. Socio-economic drivers for UK organic pullet rearers and the implications for poultry health. *British Poultry Science*, 49, 525–532. <https://doi.org/10.1080/00071660802290416>
- Spoelstra SF, Koerkamp PG, Bos AP, Elzen B and Leenstra FR, 2013. Innovation for sustainable egg production: realigning production with societal demands in The Netherlands. *World's Poultry Science Journal*, 69, 279–298.
- Staacck M, Gruber B, Keppler C, Zaludik K, Niebuhr K and Knierim U, 2007. Importance of the rearing period for laying hens in alternative systems. *DTW. Deutsche Tierärztliche Wochenschrift*, 114, 86–90.
- Stahl P, Ruetten S and Gros L, 2002. Predation on free-ranging poultry by mammalian and avian predators: field loss estimates in a French rural area. *Mammal Review*, 32, 227–234.
- Stämpfli K, Roth BA, Buchwalder T and Fröhlich EKF, 2011. Influence of nest-floor slope on the nest choice of laying hens. *Applied Animal Behaviour Science*, 135, 286–292. <https://doi.org/10.1016/j.applanim.2011.10.008>
- Stämpfli K, Buchwalder T, Fröhlich EKF and Roth BA, 2013. Design of nest access grids and perches in front of the nests: Influence on the behavior of laying hens. *Poultry Science*, 92, 890–899. <https://doi.org/10.3382/ps.2011-02046>
- Steenfeldt S and Nielsen BL, 2015. Welfare of organic laying hens kept at different indoor stocking densities in a multi-tier aviary system. I: egg laying, and use of veranda and outdoor area. *Animal*, 9, 1509–1517. <https://doi.org/10.1017/S1751731115000713>
- Steinhaus H, 1999. *Mathematical snapshots*, Courier Corporation.
- Stratmann A, Fröhlich EKF, Gebhardt-Henrich SG, Harlander-Matauschek A, Würbel H and Toscano MJ, 2015. Modification of aviary design reduces incidence of falls, collisions and keel bone damage in laying hens. *Applied Animal Behaviour Science*, 165, 112–123. <https://doi.org/10.1016/j.applanim.2015.01.012>
- Stratmann A, Fröhlich EKF, Gebhardt-Henrich SG, Harlander-Matauschek A, Würbel H and Toscano MJ, 2016. Genetic selection to increase bone strength affects prevalence of keel bone damage and egg parameters in commercially housed laying hens. *Poultry Science*, 95, 975–984. <https://doi.org/10.3382/ps/pew026>
- Struelens E, Tuytens FAM, Duchateau L, Leroy T, Cox M, Vranken E, Buyse J, Zoons J, Berckmans D, Ödberg F and Sonck B, 2008a. Perching behaviour and perch height preference of laying hens in furnished cages varying in height. *British Poultry Science*, 49, 381–389. <https://doi.org/10.1080/00071660802158332>
- Struelens E, Tuytens FAM, Janssen A, Leroy T, Audoorn L, Vranken E, De Baere K, Ödberg F, Berckmans D, Zoons J and Sonck B, 2005. Design of laying nests in furnished cages: influence of nesting material, nest box position and seclusion. *British Poultry Science*, 46, 9–15. <https://doi.org/10.1080/00071660400024050>

- Struelens E, Van Nuffel A, Tuytens FAM, Audoom L, Vranken E, Zoons J, Berckmans D, Ödberg F, Van Dongen S and Sonck B, 2008b. Influence of nest seclusion and nesting material on pre-laying behaviour of laying hens. *Applied Animal Behaviour Science*, 112, 106–119. <https://doi.org/10.1016/j.applanim.2007.07.010>
- Struthers S, Classen HL, Gomis S and Schwean-Lardner K, 2019a. The effect of beak tissue sloughing and post-treatment beak shape on the productivity of infrared beak-treated layer pullets and hens. *Poultry Science*, 98, 3637–3646.
- Struthers S, Gupta A, Gomis S, Herwig E and Schwean-Lardner K, 2019b. Understanding how infrared beak treatment affects the beak tissue and the healing response of brown and white feathered layer pullets. *Animals*, 9, 665. <https://doi.org/10.3390/ani9090665>
- Struthers S, Classen HL, Gomis S, Crowe TG and Schwean-Lardner K, 2019c. The impact of beak tissue sloughing and beak shape variation on the behavior and welfare of infrared beak-treated layer pullets and hens. *Poultry Science*, 98, 4269–4281.
- Struthers S, Buchynski K, Chew J, Gomis S, Herwig E, Shynkaruk T and Schwean-Lardner K, 2022. Specialized beak blunting feeders and their potential as an alternative to current beak treatment methods in leghorn pullets. *Journal of Applied Poultry Research*, 31, 100285.
- Sustaita D, Pouydebat E, Manzano A, Abdala V, Hertel F and Herrel A, 2013. Getting a grip on tetrapod grasping: form, function, and evolution. *Biological Reviews*, 88, 380–405. <https://doi.org/10.1111/brv.12010>
- Tahamtani FM, Hansen TB, Orritt R, Nicol C, Moe RO and Janczak AM, 2014. Does rearing laying hens in aviaries adversely affect long-term welfare following transfer to furnished cages? *PLoS One*, 9, e107357. <https://doi.org/10.1371/journal.pone.0107357>
- Tahamtani FM, Hinrichsen LK and Riber AB, 2018. Laying hens performing gregarious nesting show less pacing behaviour during the pre-laying period. *Applied Animal Behaviour Science*, 202, 46–52. <https://doi.org/10.1016/j.applanim.2018.01.010>
- Takai H, Pedersen S, Johnsen JO, Metz JHM, Groot Koerkamp PWG, Uenk GH, Phillips VR, Holden MR, Sneath RW, Short JL, White RP, Hartung J, Seedorf J, Schröder M, Linkert KH and Wathes CM, 1998. Concentrations and emissions of airborne dust in livestock buildings in northern Europe. *Journal of Agricultural Engineering Research*, 70, 59–77. <https://doi.org/10.1006/jaer.1997.0280>
- Tao X and Xin H, 2003. Temperature-humidity-velocity index for market-size broilers. *Proceedings of the 2003 ASAE Annual Meeting*, 1 pp.
- Tauson R, Kjaer J, Maria G, Cepero R and Holm K, 2005. Applied scoring of integument and health in laying hens. *Animal Science Papers and Reports*, 23, 153–159.
- Taylor PE, Scott GB and Rose P, 2003. The ability of domestic hens to jump between horizontal perches: effects of light intensity and perch colour. *Applied Animal Behaviour Science*, 83, 99–108. [https://doi.org/10.1016/S0168-1591\(03\)00127-8](https://doi.org/10.1016/S0168-1591(03)00127-8)
- Temple W, Foster TM and O'Donnell CS, 1984. Behavioural estimates of auditory thresholds in hens. *British Poultry Science*, 25, 487–493. <https://doi.org/10.1080/00071668408454890>
- Thapa S, Hinrichsen LK, Brenninkmeyer C, Gunnarsson S, Heerkens JLT, Verwer C, Niebuhr K, Willett A, Grilli G, Thamsborg SM, Sørensen JT and Mejer H, 2015. Prevalence and magnitude of helminth infections in organic laying hens (*Gallus gallus domesticus*) across Europe. *Veterinary Parasitology*, 214, 118–124. <https://doi.org/10.1016/j.vetpar.2015.10.009>
- Thapa S, Thamsborg SM, Meyling NV, Dhakal S and Mejer H, 2017. Survival and development of chicken ascarid eggs in temperate pastures. *Parasitology*, 144, 1243–1252. <https://doi.org/10.1017/S0031182017000555>
- Thøfner I, Hougen HP, Villa C, Lynnerup N and Christensen JP, 2020. Pathological characterization of keel bone fractures in laying hens does not support external trauma as the underlying cause. *PLoS One*, 15, e0229735. <https://doi.org/10.1371/journal.pone.0229735>
- Thøfner ICN, Dahl J and Christensen JP, 2021. Keel bone fractures in Danish laying hens: prevalence and risk factors. *PLoS One*, 16, e0256105. <https://doi.org/10.1371/journal.pone.0256105>
- Tomley FM and Sparagano O, 2018. Spotlight on avian pathology: red mite, a serious emergent problem in layer hens. *Avian Pathology*, 47, 533–535. <https://doi.org/10.1080/03079457.2018.1490493>
- Toscano MJ, Dunn IC, Christensen J-P, Petow S, Kittelsen K and Ulrich R, 2020. Explanations for keel bone fractures in laying hens: are there explanations in addition to elevated egg production? *Poultry Science*, 99, 4183–4194. <https://doi.org/10.1016/j.psj.2020.05.035>
- Underwood G, Andrews D, Phung T and Edwards LE, 2021. Incubation, hatchery practice and the welfare of layer hens. *Animal Production Science*, 61, 867–875. <https://doi.org/10.1071/AN20391>
- Van Hoorebeke S, Van Immerseel F, Schulz J, Hartung J, Harisberger M, Barco L, Ricci A, Theodoropoulos G, Xylouri E, De Vylder J, Ducatelle R, Haesebrouck F, Pasmans F, de Kruif A and Dewulf J, 2010. Determination of the within and between flock prevalence and identification of risk factors for *Salmonella* infections in laying hen flocks housed in conventional and alternative systems. *Preventive Veterinary Medicine*, 94, 94–100. <https://doi.org/10.1016/j.prevetmed.2009.11.022>
- Van Kampen M, Mitchell BW and Siegel HS, 1979. Thermoneutral zone of chickens as determined by measuring heat production, respiration rate, and electromyographic and electroencephalographic activity in light and dark environments and changing ambient temperatures. *The Journal of Agricultural Science*, 92, 219–226.

- Van Krimpen MM, Kwakkel RP, Reuvekamp BFJ, Van Der Peet-Schwering CMC, Den Hartog LA and Verstegen MWA, 2005. Impact of feeding management on feather pecking in laying hens. *World's Poultry Science Journal*, 61, 663–686.
- Van Krimpen MM, Kwakkel RP, Van der Peet-Schwering CMC, Den Hartog LA and Verstegen MWA, 2009. Effects of nutrient dilution and nonstarch polysaccharide concentration in rearing and laying diets on eating behavior and feather damage of rearing and laying hens. *Poultry Science*, 88, 759–773.
- Van Liere D, Aggrey S, Brouns F and Wiepkema P, 1991. Oiling behaviour and the effect of lipids on dustbathing behaviour in laying hens *Gallus gallus domesticus*. *Behavioural Processes*, 24, 71–81.
- van Liere DW, 1992. The significance of fowls' bathing in dust. *Animal Welfare*, 1, 187–202.
- van Liere DW and Bokma S, 1987. Short-term feather maintenance as a function of dust-bathing in laying hens. *Applied Animal Behaviour Science*, 18, 197–204. [https://doi.org/10.1016/0168-1591\(87\)90193-6](https://doi.org/10.1016/0168-1591(87)90193-6)
- van Liere DW, Kooijman J and Wiepkema PR, 1990. Dustbathing behaviour of laying hens as related to quality of dustbathing material. *Applied Animal Behaviour Science*, 26, 127–141. [https://doi.org/10.1016/0168-1591\(90\)90093-S](https://doi.org/10.1016/0168-1591(90)90093-S)
- van Niekerk T, 2019. Evidence-based management of injurious pecking. *Poultry Feathers and Skin: The Poultry Integument in Health and Welfare*, 32, 57.
- van Niekerk T and Workamp J (Wageningen Livestock Research), 2022. Scenario's voor het vraagstuk "het doden van eendagshaantjes van legrassen". <https://edepot.wur.nl/574241>
- van Staaveren N, Decina C, Baes CF, Widowski TM, Berke O and Harlander-Matauschek A, 2019. Housing and management practices on 33 pullet farms in Canada. *Animals*, 9, 49.
- van Staaveren N, Ellis J, Baes CF and Harlander-Matauschek A, 2021. A meta-analysis on the effect of environmental enrichment on feather pecking and feather damage in laying hens. *Poultry Science*, 100, 397–411. <https://doi.org/10.1016/j.psj.2020.11.006>
- van Staaveren N and Harlander A, 2020. Cause and prevention of injurious pecking in chickens. *Understanding the behaviour and improving the welfare of chickens*. Burleigh Dodds Science Publishing. pp. 509–566.
- Vasdal G, Marchewka J, Newberry RC, Estevez I and Kittelsen K, 2022a. Developing a novel welfare assessment tool for loose-housed laying hens – the aviary transect method. *Poultry Science*, 101, 101533. <https://doi.org/10.1016/j.psj.2021.101533>
- Vasdal G, Muri K, Stubbsj en SM, Moe RO and Kittelsen K, 2022b. Qualitative behaviour assessment as part of a welfare assessment in flocks of laying hens. *Applied Animal Behaviour Science*, 246, 105535. <https://doi.org/10.1016/j.applanim.2021.105535>
- Vestergaard K, 1982. Dust-bathing in the domestic fowl — diurnal rhythm and dust deprivation. *Applied Animal Ethology*, 8, 487–495. [https://doi.org/10.1016/0304-3762\(82\)90061-X](https://doi.org/10.1016/0304-3762(82)90061-X)
- Vestergaard KS, Skadhauge E and Lawson LG, 1997. The stress of not being able to perform dustbathing in laying hens. *Physiology & Behavior*, 62, 413–419. [https://doi.org/10.1016/S0031-9384\(97\)00041-3](https://doi.org/10.1016/S0031-9384(97)00041-3)
- Vezzoli G, Mullens BA and Mench JA, 2015. Relationships between beak condition, preening behavior and ectoparasite infestation levels in laying hens. *Poultry Science*, 94, 1997–2007. <https://doi.org/10.3382/ps/pev171>
- von Eugen K, Nordquist RE, Zeinstra E and van der Staay FJ, 2019. Stocking density affects stress and anxious behavior in the laying hen chick during rearing. *Animals*, 9, 53.
- von Waldburg-Zeil CG, van Staaveren N and Harlander-Matauschek A, 2019. Do laying hens eat and forage in excreta from other hens? *Animal*, 13, 367–373. <https://doi.org/10.1017/S1751731118001143>
- Vu emilo M, Matkovi  K, Vinkovi  B, Macan J, Varnai V, Prester L, Grani  K and Orct T, 2008. Effect of microclimate on the airborne dust and endotoxin concentration in a broiler house. *Czech Journal of Animal Science*, 53, 83–89.
- Weeks CA, Lambton SL and Williams AG, 2016. Implications for welfare, productivity and sustainability of the variation in reported levels of mortality for laying hen flocks kept in different housing systems: a meta-analysis of ten studies. *PLoS One*, 11, e0146394.
- Weeks CA and Nicol CJ, 2006. Behavioural needs, priorities and preferences of laying hens. *World's Poultry Science Journal*, 62, 296–307. <https://doi.org/10.1079/WPS200598>
- Weeks CA, Webster AJF and Wyld HM, 1997. Vehicle design and thermal comfort of poultry in transit. *British Poultry Science*, 38, 464–474. <https://doi.org/10.1080/00071669708418023>
- Welfare Quality<sup>®</sup>, 2009. Welfare Quality<sup>®</sup> assessment protocol for poultry (broilers, laying hens). Welfare Quality<sup>®</sup> Consortium. Lelystad, Netherlands.
- Wei H, Bi Y, Xin H, Pan L, Liu R, Li X, Li J, Zhang R and Bao J, 2020. Keel fracture changed the behavior and reduced the welfare, production performance, and egg quality in laying hens housed individually in furnished cages. *Poultry Science*, 99, 3334–3342. <https://doi.org/10.1016/j.psj.2020.04.001>
- Wei H, Chen Y, Zeng X, Bi Y, Wang Y, Zhao S, Li J, Li X, Zhang R and Bao J, 2021. Keel-bone fractures are associated with bone quality differences in laying hens. *Animal Welfare*, 30, 71–80.
- Wei H, Li C, Xin H, Li S, Bi Y, Li X, Li J, Zhang R and Bao J, 2019. Keel fracture causes stress and inflammatory responses and inhibits the expression of the orexin system in laying hens. *Animals*, 9, 804.
- Whay H, Main D, Green L, Heaven G, Howell H, Morgan M, Pearson A and Webster A, 2007. Assessment of the behaviour and welfare of laying hens on free-range units. *Veterinary Record*, 161, 119–128.

- Whitehead C, 2004. Skeletal disorders in laying hens: the problem of osteoporosis and bone. *Welfare of the Laying Hen*, 27, 259.
- Whitehead CC and Fleming RH, 2000. Osteoporosis in cage layers. *Poultry Science*, 79, 1033–1041. <https://doi.org/10.1093/ps/79.7.1033>
- Wichman A, Heikkilä M, Valros A, Forkman B and Keeling LJ, 2007. Perching behaviour in chickens and its relation to spatial ability. *Applied Animal Behaviour Science*, 105, 165–179. <https://doi.org/10.1016/j.applanim.2006.05.009>
- Wichman A and Keeling LJ, 2008. Hens are motivated to dustbathe in peat irrespective of being reared with or without a suitable dustbathing substrate. *Animal Behaviour*, 75, 1525–1533. <https://doi.org/10.1016/j.anbehav.2007.10.009>
- Widowski T, Hemsworth PH, Barnett JL and Rault JL, 2016. Laying hen welfare I. Social environment and space. *World's Poultry Science Journal*, 72, 333–342. <https://doi.org/10.1017/S0043933916000027>
- Widowski TM, Caston LJ, Hunniford ME, Cooley L and Torrey S, 2017. Effect of space allowance and cage size on laying hens housed in furnished cages, part I: performance and well-being. *Poultry Science*, 96, 3805–3815. <https://doi.org/10.3382/ps/pex197>
- Widowski TM and Duncan IJH, 2000. Working for a dustbath: are hens increasing pleasure rather than reducing suffering? *Applied Animal Behaviour Science*, 68, 39–53. [https://doi.org/10.1016/S0168-1591\(00\)00088-5](https://doi.org/10.1016/S0168-1591(00)00088-5)
- Wilkins L, Brown S, Zimmerman P, Leeb C and Nicol C, 2004. Investigation of palpation as a method for determining the prevalence of keel and furculum damage in laying hens. *Veterinary Record*, 155, 547–549.
- Wilkins L, McKinstry J, Avery N, Knowles T, Brown S, Tarlton J and Nicol C, 2011. Influence of housing system and design on bone strength and keel bone fractures in laying hens. *Veterinary Record*, 169, 414.
- Winter J, Toscano MJ and Stratmann A, 2021. Piling behaviour in Swiss layer flocks: description and related factors. *Applied Animal Behaviour Science*, 236, 105272. <https://doi.org/10.1016/j.applanim.2021.105272>
- Winter J, Stratmann A, Toscano MJ, Cardwell JM and Nicol CJ, 2022. Piling behaviour in British layer flocks: observations and farmers' experiences. *Applied Animal Behaviour Science*, 253, 105686. <https://doi.org/10.1016/j.applanim.2022.105686>
- Wolff I, Klein S, Rauch E, Erhard M, Mönch J, Härtle S, Schmidt P and Louton H, 2019. Harvesting-induced stress in broilers: comparison of a manual and a mechanical harvesting method under field conditions. *Applied Animal Behaviour Science*, 221, 104877. <https://doi.org/10.1016/j.applanim.2019.104877>
- Wood-Gush DGM and Duncan IJH, 1976. Some behavioural observations on domestic fowl in the wild. *Applied Animal Ethology*, 2, 255–260. [https://doi.org/10.1016/0304-3762\(76\)90057-2](https://doi.org/10.1016/0304-3762(76)90057-2)
- Wood-Gush DGM and Vestergaard K, 1989. Exploratory behavior and the welfare of intensively kept animals. *Journal of Agricultural Ethics*, 2, 161–169. <https://doi.org/10.1007/BF01826929>
- Wood B, Rufener C, Makagon MM and Blatchford RA, 2021. The utility of scatter feeding as enrichment: do broiler chickens engage with scatter-fed items? *Animals*, 11, 3478.
- Wurtz KE, Thodberg K, Berenjjan A, Foldager L, Tahamtani FM and Riber AB, 2022. Commercial layer hybrids kept under organic conditions: a comparison of range use, welfare, and egg production in two layer strains. *Poultry Science*, 101, 102005.
- Xie W-y, Hou X-y, Yan F-b, Sun G-r, Han R-l and Kang X-t, 2013. Effect of  $\gamma$ -aminobutyric acid on growth performance and immune function in chicks under beak trimming stress. *Animal Science Journal*, 84, 121–129. <https://doi.org/10.1111/j.1740-0929.2012.01051.x>
- Xin H, Gates RS, Puma MC and Ahn DU, 2002. Drinking water temperature effects on laying hens subjected to warm cyclic environments. *Poultry Science*, 81, 608–617.
- Xu X, Wang C, Huang Y, Zhang S, Yu H, Meng J and Pan B, 2020. Evaluation of the vaccine efficacy of three digestive protease antigens from *Dermanyssus gallinae* using an in vivo rearing system. *Vaccine*, 38, 7842–7849. <https://doi.org/10.1016/j.vaccine.2020.10.010>
- Yan C, Xiao J, Chen D, Turner SP, Li Z, Liu H, Chen S and Zhao X, 2021. Feed restriction induced changes in behavior, corticosterone, and microbial programming in slow-and fast-growing chicken breeds. *Animals*, 11, 141.
- Ye X, Avendano S, Dekkers JCM and Lamont SJ, 2006. Association of twelve immune-related genes with performance of three broiler lines in two different hygiene environments. *Poultry Science*, 85, 1555–1569. <https://doi.org/10.1093/ps/85.9.1555>
- Zachar G, Tóth AS, Gerecsei LI, Zsebők S, Ádám Á and Csillag A, 2019. Valproate exposure in ovo attenuates the acquisition of social preferences of young post-hatch domestic chicks. *Frontiers in Physiology*, 10, 881. <https://doi.org/10.3389/fphys.2019.00881>
- Zeltner E and Hirt H, 2003. Effect of artificial structuring on the use of laying hen runs in a free-range system. *British Poultry Science*, 44, 533–537.
- Zeltner E and Hirt H, 2008. Factors involved in the improvement of the use of hen runs. *Applied Animal Behaviour Science*, 114, 395–408. <https://doi.org/10.1016/j.applanim.2008.04.007>
- Zepp M, Louton H, Erhard M, Schmidt P, Helmer F and Schwarzer A, 2018. The influence of stocking density and enrichment on the occurrence of feather pecking and aggressive pecking behavior in laying hen chicks. *Journal of Veterinary Behavior*, 24, 9–18. <https://doi.org/10.1016/j.jveb.2017.12.005>

- Zhang B and Coon CN, 1997. The relationship of various tibia bone measurements in hens. *Poultry Science*, 76, 1698–1701. <https://doi.org/10.1093/ps/76.12.1698>
- Zheng H, Li B, Tong Q, Chen G and Li X, 2019. Modification of perchery system: Preference for ramps rather than ladders during early adaptation period for cage-reared pullets. *International Journal of Agricultural and Biological Engineering*, 12, 34–42.
- Zimmerman PH, Buijs SAF, Bolhuis JE and Keeling LJ, 2011. Behaviour of domestic fowl in anticipation of positive and negative stimuli. *Animal Behaviour*, 81, 569–577. <https://doi.org/10.1016/j.anbehav.2010.11.028>
- Zimmerman PH, Koene P and van Hooff JARAM, 2000. Thwarting of behaviour in different contexts and the gavel-call in the laying hen. *Applied Animal Behaviour Science*, 69, 255–264. [https://doi.org/10.1016/S0168-1591\(00\)00137-4](https://doi.org/10.1016/S0168-1591(00)00137-4)
- Zimmerman PH, Lindberg AC, Pope SJ, Glen E, Bolhuis JE and Nicol CJ, 2006. The effect of stocking density, flock size and modified management on laying hen behaviour and welfare in a non-cage system. *Applied Animal Behaviour Science*, 101, 111–124. <https://doi.org/10.1016/j.applanim.2006.01.005>
- Zimmerman PH, Lundberg A, Keeling LJ and Koene P, 2003. The effect of an audience on the gavel-call and other frustration behaviours in the laying hen (*Gallus gallus domesticus*). *Animal Welfare*, 12, 315–326.
- Zulkifli I, Norazlina I, Htin NN and Juriah K, 2006. Physiological and behavioural responses of laying hens to repeated feed deprivation. *Archiv Fur Geflugelkunde*, 70, 22–27.
- Zupan M, Kruschwitz A and Huber-Eicher B, 2007. The influence of light intensity during early exposure to colours on the choice of nest colours by laying hens. *Applied Animal Behaviour Science*, 105, 154–164. <https://doi.org/10.1016/j.applanim.2006.05.011>

## Abbreviations

ABMs	animal-based measures
AHAW	EFSA Panel on Animal Health and Animal Welfare
CT	Computed tomography scan
DB	numbers of dustbathing
DEFRA	Department for Environment Food and Rural Affairs
Dexa	dual-energy X-ray absorptiometry
FAWC	Farm Animal Welfare Committee
FECs	faecal egg counts
ECI	European Citizen Initiative
EFFAB	European Forum of Farm Animal Breeders
EKE	expert knowledge elicitation
EURCAW-Poultry-SFA	European Union Reference Centre for Animal Welfare for Poultry and Other Small Farmed Animals
GABA	gamma-aminobutyric acid
IRIS system	Intelligent Reporting, Inspection & Selection system
KAT	Verein für kontrollierte alternative Tierhaltungsformen e.V.
LED	light-emitting diode
MSs	Member States
PB	proportion of each of the behavioural needs
RGB camera	red, green and blue wavelengths
ToRs	Terms of References
TRL	Technology Readiness Level
UV	ultraviolet
UVA	visible spectrum plus UVA wavelengths
UVB wavelengths	ultraviolet B rays
UVA/B	visible spectrum plus UVA and UVB wavelengths
VIS	visible spectrum plus infrared wavelengths
WQ	Welfare quality

## Appendix A – Request for data to reply to the European Commission on the scientific opinion on the welfare of laying hens

Questions from the Working Group and answers from EFFAB on layer breeders (including (great)grandparents and pure lines) kept in individual/collective cages.

<b>Question</b>	
<b>1</b>	<b>General questions on cages in pure lines and (great) grandparent flocks</b>
1.1	Are cages used in the EU?
1.2	What is the ratio between (individual/collective) caged and non-caged systems?
1.3	What is the ratio between individual and collective cage systems?
1.4	Please specify the factors determining the decision to house birds in individual or collective cages
1.5	What is the size of an individual cage? Please provide sizes of all (most) cages that are in use.
1.6	What is the size of a collective cage? Please provide sizes of all (most) cages that are in use.
1.6.1	What is the usual group size in a collective cage?
1.6.2	What is the usual bird stocking density (n birds /m <sup>2</sup> ) in a collective cage?
1.7	During which stage are birds caged (e.g., rearing, production)?
1.7.1	For how long are the birds caged?
1.8	What are the reasons for the use of individual cages for genetic selection in laying strains?
1.9	Are all cages provided with (if not all, then how many?):
1.9.1	<input type="checkbox"/> Perches?
1.9.2	<input type="checkbox"/> Scratching areas? If yes, which material is used?
1.9.3	<input type="checkbox"/> Nests? Please specify if this differs for individual and collective cages
1.10	Lighting for caged birds
	<input type="checkbox"/> Are the birds exposed to natural daylight?
	<input type="checkbox"/> If not, how many hours of artificial light per day?
	<input type="checkbox"/> Which is the light intensity (lux)?
1.11	Lightening non-caged birds
	<input type="checkbox"/> Are the birds exposed to natural day light?
	<input type="checkbox"/> If not, how many hours of artificial light per day?
1.12	Which is the normal light intensity (lux)?
1.13	Are caged birds water restricted, i.e. do not have continuous access to water.
1.13.1	If yes, for how many hours a day do they not have access to water?
<b>2</b>	<b>Breeding specific questions</b>
2.1	Are cages only used for some strains and/or certain sexes?
2.2	
2.3	How often is artificial insemination (AI) used:
2.3.1	For birds in individual cages?
2.3.2	For birds in collective cages?
2.4	Why is pair mating not used for the desired crosses?
2.5	Are brooding eggs shipped to the EU from non-EU countries?
2.5.1	Are (some) pure lines or (great) grandparents being kept in cages in non-European countries and their eggs shipped to Europe where the offspring is then kept in non-cage systems?
2.0.5.3	If so, what is the proportion of this practice overall?
<b>3</b>	<b>Questions on mutilations</b>
3.1	What proportion of layer breeders (including (great) grandparents and pure lines) are (please specify denominator (% of all birds or male birds)):
3.1.1	<input type="checkbox"/> beak-trimmed?
3.1.2	<input type="checkbox"/> Comb dubbed?
3.1.3	<input type="checkbox"/> De-spurred?
3.1.4	<input type="checkbox"/> Toe-clipped?
3.1.4.1	- if toe-clipped, which toe(s)?

3.2	At what age are the mutilations conducted?
3.2.1	- beak-trimming
3.2.2	- comb dubbing
3.2.3	- de-spurring
3.2.4	- toe-clipping
3.3	Are you aware of other mutilations practiced?
3.3.1	If so, could you provide information on each practice separately, describing the method and age of bird when applied?
3.4	Is the selection process aiming for a reduction of feather pecking and cannibalism in the commercial laying hybrids?
3.4.1	If yes, can you describe the process?
3.4.2	Which data are you collecting to assess the effect?
3.4.3	How important is this breeding goal relative to other breeding goals (e.g., more production)?
<b>4</b>	<b>Questions related to multiplication farms</b>
4.1.1	What are the main welfare and health problems for breeding birds kept in individual cages?
4.1.2	What are the main welfare and health problems for breeding birds kept in collective cages?
4.1.3	What are the main welfare and health problems for breeding birds kept in non-cage systems?
4.2	Can you provide figures on mortality for rearing and reproduction phases in each system?
4.3	What are the normal rearing densities and flock sizes in multiplication flocks?
4.4	Are foraging materials and perches normally available for breeding flocks?
4.5	Are there any issues with fertility? Are there any perceived issues with forced copulations?
4.6	What is the average prevalence of floor eggs and how is this issue handled? Are electric fences used to deter birds from certain areas of the house?
4.7	Is there any strategy on improving rearing conditions in order to reduce floor eggs in reproduction phase?
4.8	What are the most important areas for improvement on behaviour and welfare?
<b>5</b>	<b>Future without individual cages</b>
5.1	How could individual cages be phased out?
5.2	What is the industry needs for individual monitoring?
5.2.1	How could this be achieved in non-cage systems?

## Appendix B – Expert knowledge elicitation Report

This is a summary of the two Expert Knowledge Elicitation exercises.

### B.1. Part 1 – Expert Knowledge Elicitation exercise description

#### *Purpose of the elicitation*

The expert knowledge elicitation exercise aimed at estimating the effect of increasing amounts of stocking density (measured in 'number of laying hens/m<sup>2</sup> available') on the welfare of laying hens thanks to two ABMs: 'the flock average of plumage damage' and 'the average percentage of birds in the litter area that are foraging'.

#### *Evidence and uncertainty*

Ahead of and during the EKE, the Working Group gathered evidence and sources of uncertainty from peer-reviewed literature. Summaries of the evidence and the sources of uncertainty are reported for the two ABMs: in Part 2 for plumage damage and Part 3 for foraging.

#### *Definition of the populations*

The value of the two ABMs were elicited for two different populations: a 'non exposed' population' and a 'highly exposed' population.

For the purpose of the EKE, the 'non-exposed' population was defined as a hypothetical group of laying hens housed in a barn with very low stocking density (2 hens/m<sup>2</sup>). The non-exposed population of laying hens acted as a reference for the value of the ABM in laying hens with no restriction of space.

For the purposes of the EKE, the 'highly exposed' population was defined as a hypothetical group of laying hens housed in a barn with stocking density of 12 hens/m<sup>2</sup> which is what it is currently allowed in the European legislation.<sup>5</sup>

In these two hypothetical populations, the flocks were considered to be composed of at least 500 non-beak-trimmed hens aged of at least 50 weeks and the space in additional areas (e.g. outdoor area, wintergarden/veranda) was not considered. For plumage damage, an additional question was raised for beak-trimmed flocks.

#### *Attendants, roles and expertise*

The elicitation group included one or two facilitators experienced in the elicitation of expert knowledge using the Sheffield method and a rapporteur from the EFSA scientific secretariat staff. The experts of the elicitation group were the experts of the Working Group on 'Welfare of laying hens' and two hearing experts.

#### *The elicitation protocol*

For each ABM, the EKE was carried out according to the following steps:

- a) The question was reviewed and any queries were clarified.
- b) The evidence was reviewed: the relevant section of the background document was examined. Any points of clarification or differences were discussed; participants were asked to summarise any additional evidence they considered relevant.
- c) Question 1: Elicitation of the ABM for the non-exposed population: the parameter elicited was the value of the ABM in a non-exposed population (2 hens/m<sup>2</sup>). The elicitation was done in three steps:

1st step: The group of experts were asked to write down their initial judgements for the median value of the parameter, then the lower and upper limit and finally the interquartile range (1st and 3rd quartile) to express their uncertainty about their estimate. The experts were invited to reflect on the evidence provided and the important factors that can influence the value of the parameter.

2nd step: Then initial individual judgements were collected and visualised in a graph. Following a discussion, the experts could retain their initial judgement or amend it.

3rd step: A consensus judgement expressing the collective view of the group was agreed.

<sup>5</sup> Council Directive 1999/74/EC.

- d) Question 2: Elicitation of the ABM for the highly exposed population: the parameter elicited was the value of the ABM in a highly exposed population (12 hens/m<sup>2</sup>). The elicitation for the highly exposed population was done in three steps similarly to the elicitation for the non-exposed population (see c). For plumage damage, this elicitation was done for non-beak-trimmed and beak-trimmed flocks.
- e) Question 3: Elicitation of the maximum stocking density without change: the parameter elicited was the maximum stocking density resulting in no change in the value of the ABM compared to the value observed in the non-exposed population. The elicitation of the maximum stocking density without change in comparison to the non-exposed population was done in three steps similarly to the elicitation for the non-exposed population (see c). plumage damage, the value for beak-trimmed flocks was discussed.
- f) For foraging behaviour, the coefficient of variation was agreed through discussion.

## B.2. Part 2 – EKE 1 on plumage damage

### Purpose

The expert knowledge elicitation 1 (EKE 1) exercise aimed at estimating the effect of increasing amounts of stocking density (measured in 'hens/m<sup>2</sup>') on 'the flock average of plumage damage'.

### Definition of the ABM

The flock average of plumage damage was estimated based on the average score taken across a sample of individually scored birds. For each bird, three body parts are scored on a 3-point scale:

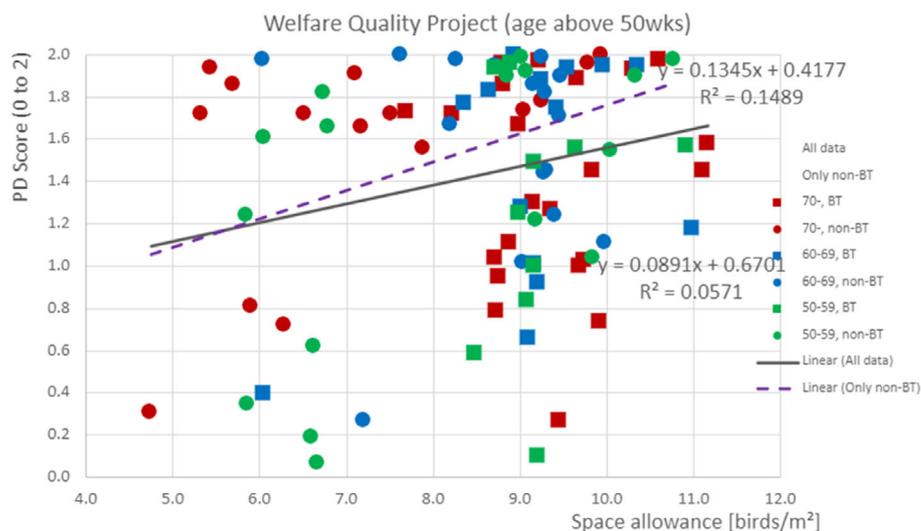
- 0 = no or slight wear, (nearly) complete feathering (only single feathers lacking);
- 1 = moderate wear, i.e. damaged feathers (worn, deformed) or one or more featherless areas < 5 cm in diameter at the largest extent;
- 2 = at least one featherless area ≥ 5 cm in diameter at the largest extent.

For the EKE, the average of the three body-part scores corresponds to the individual bird score. The individual bird scores of sampled birds are then considered to estimate the flock average of plumage damage.

### Evidence

Figure B.1 summarises the data from the literature.

### Source of uncertainty



**Figure B.1:** Scatterplot of plumage damage score and space allowance (birds/m<sup>2</sup>) and the linear relationship (from literature on beak-trimmed and non-beak-trimmed laying hens above 50 weeks of age).

The experts gathered the main factors (other than space allowance) that can influence the flock average of plumage damage:

Litter: poor litter quality can lead to inability to perform comfort behaviour which may impair plumage.

Light and temperature

Outdoor access: Increased use of the outdoor area is associated with a reduction in injurious pecking and better feather cover (Bestman et al., 2017; Lambton et al., 2010; Bari et al., 2020).

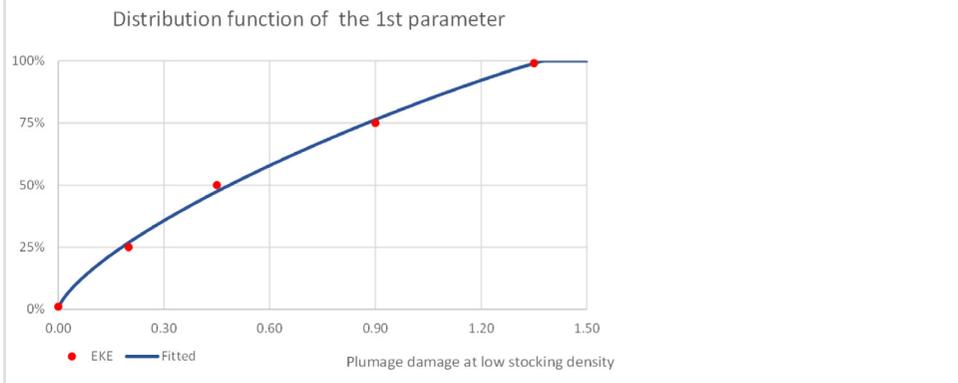
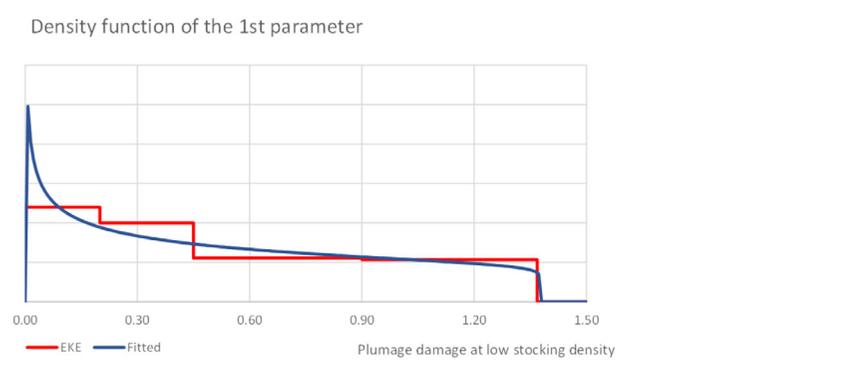
Breed: there are behavioural differences with effect on plumage damage between white and brown breeds, but this is difficult to quantify.

Number of tiers

Age

**B.2.1. Question 1: Elicitation of plumage damage at the flock level in a non-exposed population (2 hens/m<sup>2</sup>)**

Overview of the results of the Expert Knowledge Elicitation (1st EKE question)															
Parameter	Median ABM under very low stocking density														
Stratification	Laying hens (non-beak trimmed)														
Question	What is the average plumage damage score (averaged across all sampled birds and three body parts), that is shown in a flock of layers with very low stocking density (SD) (2 hens per usable area in msq) for the median flock (of the distribution of all such flocks)?														
Results	P1%	P2.5%	<b>P5%</b>	P10%	P16.7%	P25%	P33.3%	<b>P50%</b>	P66.7%	P75%	P83.3%	P90%	<b>P95%</b>	P97.5%	P99%
Elicited values	0.00					0.20		0.45		0.90					1.35
EKE results	0.0019	0.0070	0.0187	0.0496	0.102	0.181	0.272	0.486	0.737	0.877	1.03	1.16	1.26	1.31	1.35
Fitted distribution	BetaGeneral (0.71046, 1.0788, 0, 1.377)														



**Figure (a):** Comparison of elicited and fitted values/density function to describe the remaining uncertainties of the parameter

**Figure (b):** Cumulative distribution function (CDF) of the likelihood of the parameter

The coefficient of variation of plumage damage in a non-exposed population (2 hens/m<sup>2</sup>) was set at 0.70 based on the literature.

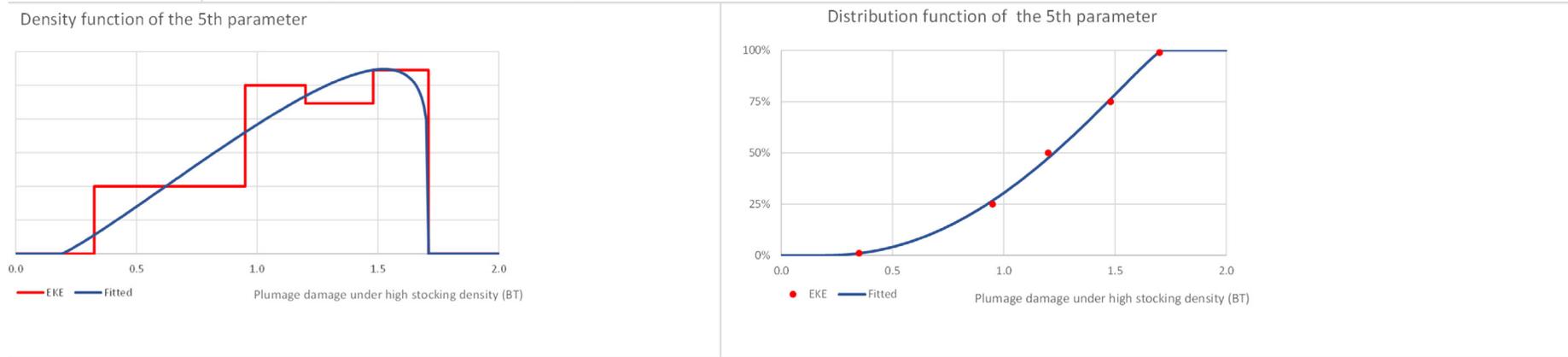
### B.2.2. Question 2: Elicitation of plumage damage at the flock level in highly exposed population (12 hens/m<sup>2</sup>)

For non-beak-trimmed laying hens

Overview of the results of the Expert Knowledge Elicitation															
Parameter	Median ABM with large stocking density														
Stratification	Laying hens (Non-beak trimmed)														
Question	What is the average plumage damage, that is shown in a flock of layers with high stocking density (12 non beak-trimmed hens per usable area in msq) for a median flock (of the distribution of all such flocks)? [-]														
Results	P1%	P2.5%	<b>P5%</b>	P10%	P16.7%	P25%	P33.3%	<b>P50%</b>	P66.7%	P75%	P83.3%	P90%	<b>P95%</b>	P97.5%	P99%
Elicited values	0.60					1.00		1.5		1.8					2.0
EKE results	0.600	0.620	0.657	0.738	0.854	1.00	1.16	1.44	1.70	1.80	1.89	1.95	1.98	1.99	2.00
Fitted distribution	BetaGeneral (0.85427, 0.64794, 0.59, 2)														
Density function of the 4th parameter								Distribution function of the 4th parameter							
<p><b>Figure (a):</b> Comparison of elicited and fitted values/density function to describe the remaining uncertainties of the parameter</p>								<p><b>Figure (b):</b> Cumulative distribution function (CDF) of the likelihood of the parameter</p>							

For beak-trimmed laying hens

Overview of the results of the Expert Knowledge Elicitation															
Parameter	Median ABM with large stocking density														
Stratification	Laying hens (beak trimmed)														
Question	What is the average plumage damage, that is shown in a flock of layers with high stocking density (12 non beak-trimmed hens per usable area in msq) for a median flock (of the distribution of all such flocks)? [-]														
Results	P1%	P2.5%	<b>P5%</b>	P10%	P16.7%	P25%	P33.3%	<b>P50%</b>	P66.7%	P75%	P83.3%	P90%	<b>P95%</b>	P97.5%	P99%
Elicited values	0.35					0.95		1.20		1.48					1.70
EKE results	0.350	0.436	0.531	0.664	0.795	0.926	1.04	1.23	1.39	1.47	1.54	1.61	1.65	1.68	1.70
Fitted distribution	BetaGeneral (2.1408, 1.1589, 0.19, 1.71)														



**Figure (a):** Comparison of elicited and fitted values/density function to describe the remaining uncertainties of the parameter

**Figure (b):** Cumulative distribution function (CDF) of the likelihood of the parameter

**B.2.3. Question 3: Elicitation of the maximum stocking density without effect on plumage damage**

For non-beak-trimmed laying hens

Overview of the results of the Expert Knowledge Elicitation (3rd EKE question)																
Parameter	Maximal stocking density without effect															
Stratification	Laying hens (Non-beak trimmed)															
Question	What is the maximal stocking density with no effect on the average plumage damage? [kg/m <sup>2</sup> ]															
Results	P1%	P2.5%	<b>P5%</b>	P10%	P16.7%	P25%	P33.3%	<b>P50%</b>	P66.7%	P75%	P83.3%	P90%	<b>P95%</b>	P97.5%	P99%	
Elicited values	2.90					3.60		4.25		5.50					7.00	
EKE results	2.91	2.93	2.99	3.11	3.29	3.53	3.79	4.37	5.04	5.42	5.85	6.25	6.62	6.84	7.01	
Fitted distribution	BetaGeneral (0.87877, 1.4241, 2.89, 7.2)															
Density function																
<b>Figure (a):</b>	Comparison of elicited and fitted values / density function to describe the remaining uncertainties of the parameter								<b>Figure (b):</b> Cumulative distribution function (CDF) of the likelihood of the parameter							

For beak-trimmed laying hens

To distinguish the maximum stocking density without effect on plumage damage between non-beak-trimmed and beak-trimmed laying hens, a simplified question was done. The experts were asked to think of what the maximum stocking density without effect on plumage damage would likely be if the hens were beak trimmed. The experts agreed that the effect on scoring for plumage damage would be lower, but the effect of stocking density would be the same whether the laying hens are beak trimmed or non-beak trimmed and the maximum stocking density without effect on plumage damage would likely stay around 4 birds/m<sup>2</sup>.

#### B.2.4. Risk assessment model for plumage damage

See Figure 17 in Section 3.4.2.1 of the scientific opinion Welfare of laying hens on farm.

### B.3. Part 3 – EKE 2 on foraging

#### Purpose

The expert knowledge elicitation 2 (EKE 2) exercise aimed at estimating the effect of increasing amounts of stocking density (measured in 'hens/m<sup>2</sup>') on foraging behaviour.

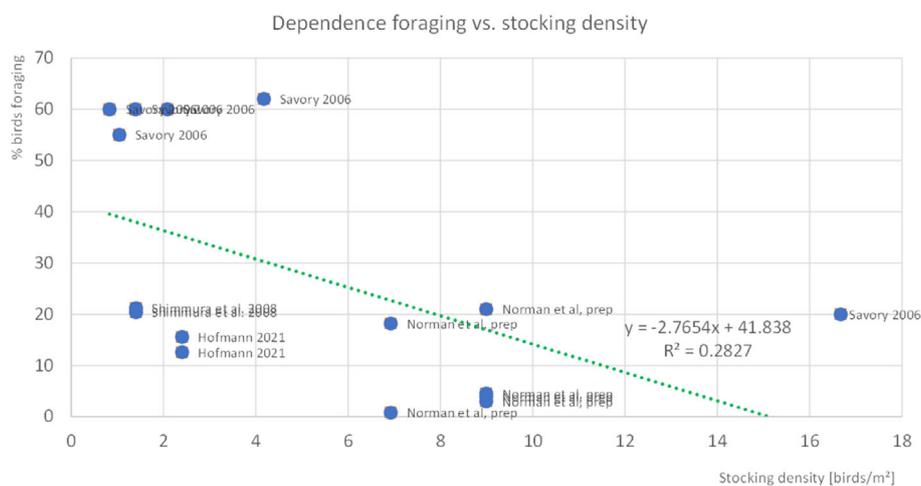
#### Definition of the ABM

Foraging behaviour includes walking, scratching and pecking. It is measured as the percentage of birds showing foraging behaviour in a screening study during day-time or time of lightning.

A typical configuration was assumed: multi-tier or single tier farms with partly litter and partly other floor types. The litter area was considered of typical commercial type and normal quality.

#### Evidence

The experts extracted data from literature on foraging behaviour in relation to stocking density. Four papers were then kept for the graph below (Figure B.2).



**Figure B.2:** Scatterplot of % birds foraging and stocking density of laying hens (birds/m<sup>2</sup>) and the linear relationship (from 4 studies: Savory et al., 2006; Shimmura, 2008; Hofmann, 2021, and Norman (in prep))

#### Source of uncertainty

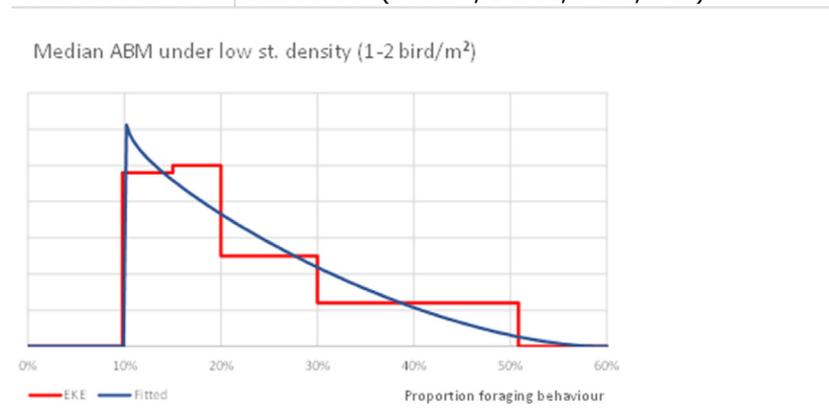
The experts gathered the main factors (other than space allowance) that can influence the percentage of layers showing foraging behaviour:

- 1) Litter quality: a non-attractive litter can decrease foraging behaviour and an attractive litter can lead to more birds foraging
- 2) Outdoor access: the diversity in the environment leads to higher values

- 3) Light and temperature
- 4) Breed: there are behavioural differences
- 5) Number of tiers
- 6) Age

### B.3.1. Question 1: Elicitation of foraging behaviour in non-exposed population (2 hens/m<sup>2</sup>)

Overview of the results of the Expert Knowledge Elicitation (1st EKE question)															
Parameter	Median ABM under very low stocking density														
Stratification	Laying hens														
Question	What is the average percentage of birds, that is showing forage behaviour in a flock of layers with very low stocking density (ABM with very low stocking density) for a median flock (median of the distribution)? [%]														
Results	P1%	P2.5%	<b>P5%</b>	P10%	P16.7%	P25%	P33.3%	<b>P50%</b>	P66.7%	P75%	P83.3%	P90%	<b>P95%</b>	P97.5%	P99%
Elicited values	10%					15%		20%		30%					50%
EKE results	10.1%	10.3%	10.7%	11.6%	12.9%	14.6%	16.5%	20.7%	26.1%	29.4%	33.6%	38.0%	42.8%	46.5%	50.2%
Fitted distribution	BetaGeneral (0.95844, 2.6432, 0.099, 0.59)														



**Figure (a):** Comparison of elicited and fitted values/density function to describe the remaining uncertainties of the parameter



**Figure (b):** Cumulative distribution function (CDF) of the likelihood of the parameter

**Summary of the evidence used for the evaluation**

- For the EKE following scenario is assumed: Observations during daytime/time of lightning; indoor farming, without access to outdoor areas; a typical configuration of multi- or single tier farms with partly litter and partly other floor types (multi-tier: ca. 1/3 litter, single-tier > 1/3 litter, not 100%); the stocking density is assumed about 1–2 birds per m<sup>2</sup> on the litter area; the litter area is considered of typical commercial type and normal quality
- References used: Hofman 2021, Savory et al. 2006, Shimmura et al. 2008

**Main uncertainties**

- Experimental setting may bias the measurement due to different behaviour than in farm settings
- The influence of specific breeds and small floc sizes is unclear
- Amount, quality and distribution of the litter areas may influence the observations
- Outdoor areas or more diversity in the environment may bias the observations

**Reasoning for a scenario which would lead to a reasonable high proportion**

The judgement on the upper limit considers that

- Farms have only parts as litter areas with attractive and easy accessible litter areas
- Breeds with less effective foraging behaviour, thus higher proportion of foraging

**Reasoning for a scenario which would lead to a reasonable low proportion**

The judgement on the lower limit considers that

- Commercial settings do not motivate for foraging
- Unattractive or less accessible litter areas
- Breeds with effective foraging, thus lower proportion of foraging

**Fair estimate as judgement on the weighted evidence**

The judgement on the median considers that

- Lower proportions of foraging is more likely in commercial setting, even under low stocking density
- Attractiveness of litter is important and judged low in commercial settings

**Precision of the judgement as description of remaining uncertainties**

The judgement on the interquartile range considers that

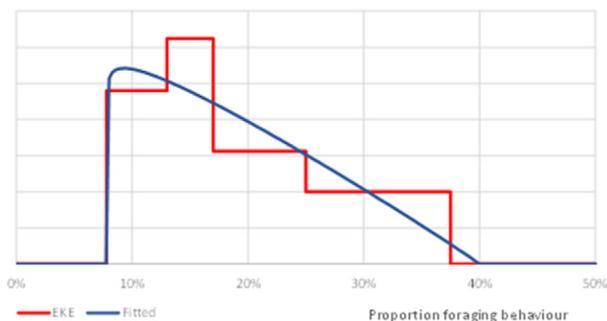
- High uncertainty on values below the median, preference for low ABM values
- Medium uncertainties on values above the median, high ABM values are less likely

### B.3.2. Question 2: Elicitation of foraging behaviour in highly exposed population (12 hens/m<sup>2</sup>)

#### Overview of the results of the Expert Knowledge Elicitation (3rd EKE question)

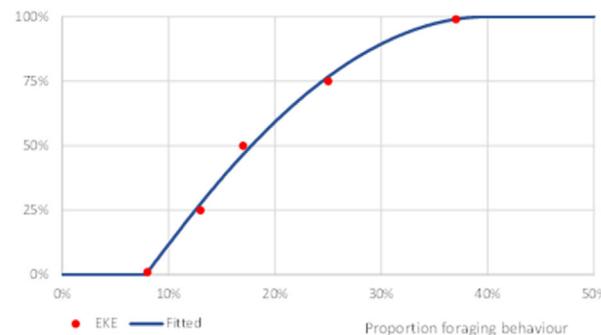
Parameter	Median ABM under high stocking density														
Stratification	Laying hens														
Question	What is the average percentage of birds, that is showing forage behaviour in a flock of layers with high stocking density (ABM with very low stocking density) for a median flock (median of the distribution)? [%]														
Results	P1%	P2.5%	<b>P5%</b>	P10%	P16.7%	P25%	P33.3%	<b>P50%</b>	P66.7%	P75%	P83.3%	P90%	<b>P95%</b>	P97.5%	P99%
Elicited values	8%					13%		17%		25%					37%
EKE results	8.00%	8.29%	8.76%	9.68%	10.9%	12.5%	14.2%	17.8%	22.0%	24.5%	27.4%	30.3%	33.2%	35.2%	37.0%
Fitted distribution	BetaGeneral (1.0468, 1.9709, 0.078, 0.4)														

Median ABM under high st. density (8-9 bird/m<sup>2</sup>)



**Figure (a):** Comparison of elicited and fitted values / density function to describe the remaining uncertainties of the parameter

Median ABM under high st. density (8-9 bird/m<sup>2</sup>)



**Figure (b):** Cumulative distribution function (CDF) of the likelihood of the parameter

**Summary of the evidence used for the evaluation**

- For the EKE following scenario is assumed: Observations during daytime/time of lightning; indoor farming, without access to outdoor areas; a typical configuration of multi- or single-tier farms with partly litter and partly other floor types (multi-tier: ca. 1/3 litter, single-tier > 1/3 litter, not 100%); the stocking density is assumed about 8–9 birds per m<sup>2</sup> on the litter area; the litter area is considered of typical commercial type and normal quality
- References used: Norman et al. In prep., Savory et al. 2006
- The proportion of litter area and the litter quality is lower in flocs with higher stocking density
- Higher stocking density implies more perches, etc.

**Main uncertainties**

- Experimental setting may bias the measurement due to different behaviour than in farm settings
- The influence of specific breeds and small floc sizes is unclear
- Outdoor areas or more diversity in the environment may bias the observations
- The distribution of the total floc, if homogeneous or clustered, is unclear
- The influence of group behaviour is unclear

<b>Reasoning for a scenario which would lead to a reasonable high proportion</b>	<b>Reasoning for a scenario which would lead to a reasonable high proportion</b> <ul style="list-style-type: none"> <li>• Clustered animals, which allow foraging behaviour for the individual hens</li> <li>• Groups are motivating common foraging behaviour</li> </ul>
<b>Reasoning for a scenario which would lead to a reasonable low proportion</b>	<b>Reasoning for a scenario which would lead to a reasonable low proportion</b> <ul style="list-style-type: none"> <li>• Small space inhibits foraging behaviour and lowers the reachability of litter areas for individual hens</li> </ul>
<b>Fair estimate as judgement on the weighted evidence</b>	<b>Fair estimate as judgement on the weighted evidence</b> <ul style="list-style-type: none"> <li>• Compared to low stocking density is the foraging behaviour further reduced</li> </ul>
<b>Precision of the judgement as description of remaining uncertainties</b>	<b>Precision of the judgement as description of remaining uncertainties</b> <ul style="list-style-type: none"> <li>• High uncertainty on values below the median, preference for low ABM values</li> <li>• Low uncertainties on values above the median, high ABM values are less likely</li> </ul>

**B.3.3. Agreement on the coefficient of variation of % of birds foraging in a non-exposed population (2 hens/m<sup>2</sup>)**

The experts were asked what the % of birds foraging would likely be in a well-designed farm (easy access, large litter area, good quality of litter and enrichment) and in a poor average farm (difficult access, minimal litter area, poor quality of litter and no enrichment) at low stocking density (2 hens/m<sup>2</sup>). Afterwards, the experts agreed on a median coefficient of variation of 20%.

The design of the farm is a driving factor for the % of birds foraging.

There is a stocking density effect, but it is not a driving factor which explains why there is a huge uncertainty about the judgements.

**B.3.4. Risk assessment model for foraging behaviour**

See Figure 18 in Section 3.4.2.1 of the scientific opinion Welfare of laying hens on farm.

## Appendix C – Behavioural space model

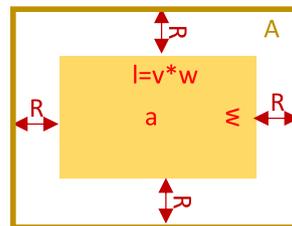
### C.1. Rectangular model as an alternative to circular model for the calculation of the space required per individual, based on the space needed to perform selected behaviours

Alternatively to the circular model, we could assume that the space that an individual chicken needs, is approximately shaped like a rectangular with a form ratio ' $v = \text{length}:\text{width} = l/w$ ' (Figure C.1). This can be reasoned as proxy of the real body shape, or by a directed behaviour (all chickens perform the behaviour in one direction). The rectangular model assumes that all chickens are synchronised in the same direction.



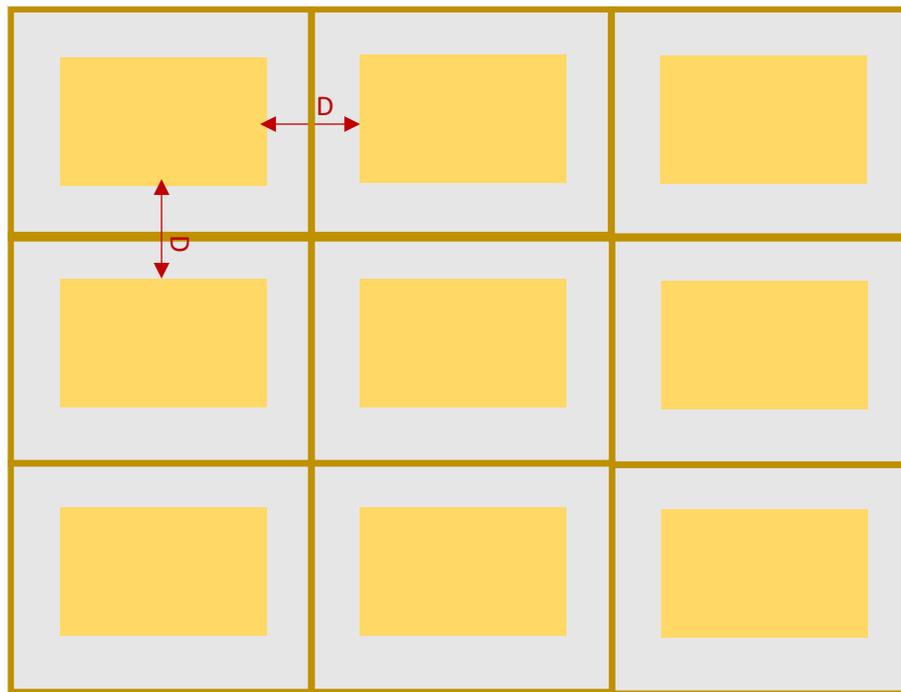
**Figure C.1:** Picture of 'Ross 308' (Photo Credit: Sonja Hillemacher)

Starting with an average area ' $a$ ' occupied by one average chicken with specific behaviour. This area can be expressed as a rectangular with form ratio ' $v = l/w$ ' and ' $b = \text{SQRT}(a/v)$ '. The distance model assumes a minimal distance ' $D$ ' between chicken of same behaviour. It is interpreted as an additional band of with ' $R = D/2$ ' around the rectangle (Figure C.2). Individual chicken model:



**Figure C.2:** Rectangular model of an individual chicken

The larger area with buffer zone around the chicken is ' $A = (b + 2R) * (v * b + 2R)$ '.



**Figure C.3:** Rectangular model for a flock

Assuming that the chicken will optimally distribute in an area, we assume no additional area between the chicken (Figure C.3). The rectangular model assumes that all chickens are synchronised in the same direction. The rectangular model allows most chicken per  $m^2$  for  $v = 1$ , but less than the circular model. When the shape of the chicken becomes more elongate, the necessary space between the chickens increases, and finally the number of chickens per  $m^2$  decreases.

## C.2. Results of model calculations

**Calculation of minimum, stabilised minimum, median, mean, stabilised maximum, maximum, optimum, stabilised optimum of proportion of birds showing the selected behaviours.**

**Table C.1:** For each behaviour, the label and the following values are given; minimum, stabilised minimum (median of the two lowest values), median, mean, maximum, stabilised maximum (median of two highest values) are given. Based on these values, in the optimum approach the median of the neutral behaviours was combined with the maximum of the positive behaviours, whereas in the stabilised optimum, the median of the neutral behaviours was combined with the stabilised maximum of the positive behaviours

Behaviour	Label	Min	Stabelized Min	Median	Mean	Max	Stabelized Max	Optimum (neg = min, neutral = median, pos = max)	Stabilised Optimum (neg = min, neutral = median, pos = max)
		%	%	%	%	%	%	%	%
<b>Standing</b>	stationary	7.5	10.4	24.6	22.0	36.3	32.2	24.6	24.6
<b>Sitting</b>	stationary	3.1	3.9	6.0	10.6	27.7	19.6	6.0	6.0
<b>Walking</b>	active	3.8	7.3	13.5	15.4	29.2	24.5	29.2	24.5
<b>Foraging incl. Scratching</b>	active	1.0	2.1	10.0	11.9	38.1	27.1	38.1	27.1
<b>Dustbathing</b>	active	0.1	0.4	1.2	2.5	9.1	5.8	9.1	5.8
<b>Preening</b>	active	2.0	2.6	5.2	7.2	19.3	13.7	19.3	13.7
<b>Wing/leg stretching</b>	active	0.0	0.0	1.0	1.0	2.0	2.0	2.0	2.0
<b>Wing flapping</b>	active	0.0	0.4	0.9	0.7	1.1	1.0	1.1	1.0
<b>Drinking/Eating</b>	stationary	7.5	10.2	12.9	12.9	18.4	15.7	12.9	12.9

#### Data extraction space covered

**Table C.2:** The table reports the extracted and recalculated data for the space covered of an animal. Given is the behaviour and the corresponding label and the extracted data from four different references. In addition (grey-lettered cells), assumptions were made as these data could not be retrieved from references (Walking = 2 \* standing; drinking/eating = 1 \* standing)

Behaviour	Label	Spindler et al., 2016 (LSL)	Spindler et al., 2016 (LB)	Mench & Blatchford, 2014	Dawkins & Hardie, 1989	Riddle et al. 2018	Q2E-Poultry-SFA-2021-004	Data points
		cm <sup>2</sup>	cm <sup>2</sup>	cm <sup>2</sup>	cm <sup>2</sup>	cm <sup>2</sup>	cm <sup>2</sup>	N
<b>Standing</b>	stationary	397.0	457.1	563.0	475.0	615.0	524.0	6
<b>Sitting/Resting</b>	stationary	412.8	486.7			590.0	599.0	4
<b>Walking</b>	active				2*Standing			0
<b>Foraging</b>	active				856.0		856.0	2
<b>Dustbathing</b>	active					1,101.0	1,095.0	2
<b>Preening</b>	active				1,150.0		1,150.0	2
<b>Wing/leg stretching</b>	active				893.0		893.0	2
<b>Wing flapping</b>	active			1,693.0		3,109.0	2,361.5	3
<b>Drinking/Eating</b>	stationary				1*Standing			0

## Calculation of the mean occupied by a laying hen performing a specific behaviour

### 'The circular model' and calculation of r and d

#### Data extraction of interindividual distance

**Table C.3:** The table reports on the selected behaviours and their label and presents the circular approach as well as the interindividual distance which are part of the behavioural space model. Regarding the calculation of the circle reflecting the chicken, area a represents the mean space covered per behaviour in cm<sup>2</sup>, d is the diameter and r the radius after converting the space into a circle (see section 2.2.2.1 for details). To this circle (space covered by a chicken expressing a specific behaviour), the interindividual distance was added. From one reference, interindividual distances for each behaviour were extracted and converted into a radius. As not all distances could be extracted, assumptions were made on sitting/resting and dustbathing reflecting a preening bird, wing/leg stretching and wing flapping reflecting a walking bird, and drinking/eating a standing bird

Behaviour	Label	Chicken-circle			Distance-circle		
		area	diameter	radius	Distance	Radius	Area
		a (Mean)	d	r	D Keeling, 1994	R	A
		cm <sup>2</sup>	cm	cm	cm	cm	cm <sup>2</sup>
<b>Standing</b>	stationary	505.2	25.4	12.7	23.4	11.7	1867.5
<b>Sitting / Resting</b>	stationary	522.1	25.8	12.9	15.4	7.7	1,332.1
<b>Walking</b>	active	1,010.4	35.9	17.9	31.0	15.5	3,511.7
<b>Foraging</b>	active	856.0	33.0	16.5	23.7	11.9	2,526.2
<b>Dustbathing</b>	active	1,098.0	37.4	18.7	15.4	7.7	2,188.7
<b>Preening</b>	active	1,150.0	38.3	19.1	15.4	7.7	2,261.9
<b>Wing/leg stretching</b>	active	893.0	33.7	16.9	31.0	15.5	3,289.7
<b>Wing flapping</b>	active	2,387.8	55.1	27.6	31.0	15.5	5,827.6
<b>Drinking/Eating</b>	stationary	505.2	25.4	12.7	23.4	11.7	1,865.6

#### Derivations

Based on the stabilised optimal model, further pictures can be generated showing how many birds can show a certain behaviour on 1 m<sup>2</sup> at the same time (excluding further space for additional animals) and how many birds would show a behaviour under scan sampling in the case of 9 animals/m<sup>2</sup>.

For example, given 1 m<sup>2</sup> of space, 1.6 birds could flap their wings at the same time giving no additional space for any other birds.

This picture does not take into account that behaviours are often socially facilitated and synchronised.

**Table C.4:** Additional pictures are given to show how many birds simultaneously could perform a behaviour or how a random scan sample of flock in  $m^2$  with a stocking density of 9 animals would look alike vs. a random scan sample of a 'optimum stabilised' flock with a stocking density of 3.7 animals

<b>Behaviour</b>	<b>How many birds could simultaneously perform the behaviour in 1 <math>m^2</math> (excluding any other birds)?</b>	<b>How many birds would perform the behaviour given a stocking density of 9 animals/<math>m^2</math> (scan sample of flock)?</b>	<b>How many birds would perform the behaviour given a stocking density of 3.7 animals/<math>m^2</math> (scan sample of flock)?</b>
<b>Standing</b>	4.9	1.9	0.77
<b>Sitting / Resting</b>	6.8	0.5	0.19
<b>Walking</b>	2.6	1.9	0.77
<b>Foraging</b>	3.6	2.1	0.85
<b>Dustbathing</b>	4.1	0.4	0.18
<b>Preening</b>	4.0	1.0	0.43
<b>Wing/leg stretching</b>	2.8	0.1	0.06
<b>Wing flapping</b>	1.6	0.1	0.03
<b>Drinking/ Eating</b>	4.9	1.0	0.41
<b>Sum of animals</b>		<b>9</b>	<b>3.7</b>

## Appendix D – Assessment of ABMs collected in slaughterhouses to monitor the level of welfare on laying hen farms

The starting point was a list of seven ABMs as potentially relevant for measurement at slaughter in laying hens. These ABMs and their descriptions were identified by EFSA experts on the basis of existing literature (Welfare Quality<sup>®</sup>, 2009; EFSA AHAW Panel, 2005) and for each ABM the preferred time of assessment (i.e. ante- and/or post-mortem) was also proposed. To gather information on their use in practice, the seven ABMs were discussed by the EFSA scientific National Contact Points (NCPs) network meeting (2021),<sup>6</sup> in the context of an exercise during the annual Network meeting (for the list of ABMs, their description, full details on methodology and results of this exercise, see EFSA, 2021).

In addition to the initial list of seven ABMs, on the basis of EFSA expert opinion, 'dirtiness', 'plumage damage', 'dead on arrival', 'wounds' and 'total mortality on farm' were added in the ante-mortem assessment; and 'foot injuries' in the post-mortem assessment. For the complete list of ABMs assessed under this Specific ToR see Table 62.

**Table D.1:** List of ABMs potentially relevant to collect in slaughterhouses for monitoring the level of welfare on broilers farms produced by the EFSA's experts, and indication of the preferred time of assessment (*ante-* or *post-mortem*). Descriptions of ABMs are available in Section 3.6

ABMs in laying hens			
ante-mortem		post-mortem	
1	Dirtiness <sup>(a)</sup>	1	Keel bone fracture
2	Plumage damage <sup>(a)</sup>	2	Plumage damage
3	Dead on arrival <sup>(a)</sup>	3	Indicator used for carcass condemnation on account of poor health
4	Wounds <sup>(a)</sup>	4	Wounds
5	Total mortality (on farm) <sup>(a)</sup>	5	Bruise
		6	Red or inflamed skin
		7	Keel bone prominence
		8	Foot injuries <sup>(a)</sup>

(a): Added by EFSA experts after the EFSA sNCP Network meeting 2021.

From the ABMs listed in Table D.1 a semi-quantitative consensus exercise was carried out to identify those ABMs that could best represent the overall animal welfare conditions in the farm. The exercise consisted of two steps: (i) Screening of ABMs; (ii) Selection of ABMs (see Figure D.1).

The Screening was carried through an Experts' opinion exercise on the initial list of ABMs, on the basis of four (screening) criteria (i.e. questions to answer with a Yes/No option):

- 1) Relevance to animal welfare: *Is the ABM relevant to the welfare consequences defined in this opinion, and not only to production and meat quality aspects?*
- 2) Relationship with the farm (and not transport or lairage): *Is the ABM indicative of a welfare consequence of the farm and not caused or masked by transport, lairage and slaughter?*
- 3) Existing data in literature: *Do scientific publications describe the ABM detailing methodologies, prevalence and the relation with on-farm welfare consequences?*
- 4) Feasibility for large scale collection: *Is the ABM already routinely collected or there is evidence that it could be collected in a national program?*

As precautionary principle, if consensus was not reached, the criterion was considered a 'Yes'. Only ABMs that received a 'Yes' for all criteria passed to the second step (Selection).

The Selection step consisted of a ranking of the ABMs based on four criteria presented below. This was followed by expert's selection of those with the highest ranking.

<sup>6</sup> The scientific NCPs Network is composed by the EU MS (including EFTA Countries) National Contact Points that provide scientific support under Art 20 of Reg. (EC) 1,099/2009 on the protection of the animals at the time of killing (scientific NCPs).

The four criteria were:

- 1) Welfare consequences (C1): the experts identified which welfare consequences on farm (from the list in Section 2.2.2.1) could be associated with the selected ABM. They scored the ABM according to the number of different welfare consequences selected.
- 2) Technology readiness (C2): each ABM was evaluated for the known level of readiness of an automated system to be adopted by the market, based on the technology readiness scale (Mankins, 1995).
- 3) Already used at slaughter (C3): the ABMs were scored according to the answers received from the exercises of the scientific NCPs Network (EFSA, 2021b).
- 4) Priority given by the Network (C4): the ABMs were scored according to the answer received from the scientific NCPs Network exercise (EFSA, 2021b).

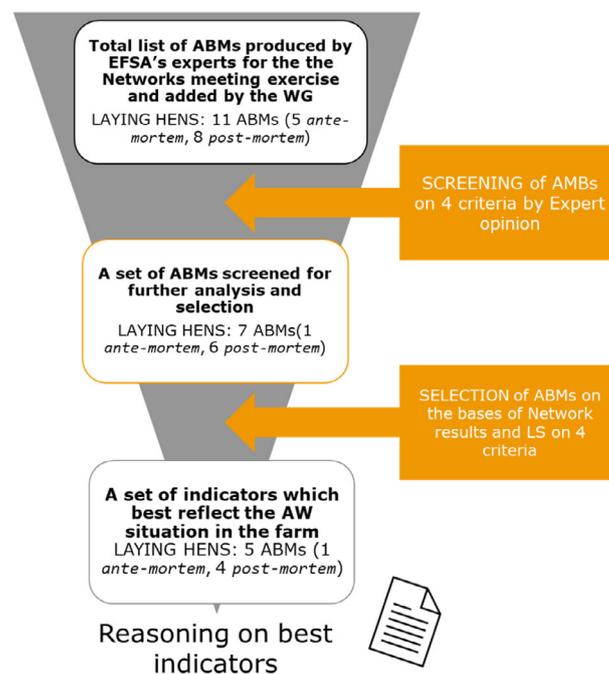
For each of these criteria, the EFSA experts agreed on a score from 0 to 4, where '0' means absence and '4' the highest score.

Finally, a weight was attributed by expert consensus to each criterion according to its importance in answering the request of the mandate. The allocated weights were: C1 = 7.5; C2 = 1.5; C3 = 1.5; C4 = 0.5.

A final score (weighted score) was calculated following the formula below:

$$\text{Weighted score} = \frac{(\text{score}_{C1} * \text{weight}_{C1}) + (\text{score}_{C2} * \text{weight}_{C2}) + (\text{score}_{C3} * \text{weight}_{C3}) + (\text{score}_{C4} * \text{weight}_{C4})}{\sum_{C1}^{C4} \text{weights}}$$

The full process leading to the final list of ABMs that were selected is summarised in Figure D.1



**Figure D.1:** Flow chart of the process leading to the selection of the ABMs that were considered to best reflect animal welfare in laying hen farms

## Appendix E – Uncertainty analysis

**Table E.1:** Overview of the approaches followed by EFSA experts to assess the mandate Specific ToRs

Specific ToR #	Exposure variable	Approach/type of assessment	Section in this Scientific opinion/source
1	The welfare of laying hens and the risks associated with alternative systems (organic, free range and barn) compared to the cage system currently allowed;	Space allowance/ Stocking density  ABMs: plumage damage and % walking	Quantitative assessment (EKE)  Section 2.2.2.1 and Section 3.4.2.4
		Space allowance/stocking density  ABMs: neutral and positive behaviours	Quantitative assessment – Circular model  Section 2.2.2.1 and Section 3.4.2.4
		Group size; minimum size area; elevated structures; litter; enrichment; nests; feeders; drinkers; noise; light; rtemperature; air quality; access to covered veranda or outdoor range	Narrative assessment  Section 3.4.2
2	Welfare of hens in furnished cages, and risks associated with rearing of animals non-beak trimmed	Mutilations	Narrative assessment  Section 3.5
3	The assessment of animal-based measures collected in slaughterhouses to monitor the level of welfare on laying hen farms	NA	Semi-quantitative assessment  Section 3.6

**Table E.2:** Sources of uncertainty associated with the assessment methodology and inputs (broad literature search, ELS, expert opinions) for the identification and assessment of the welfare consequences, and related ABMs

Source of Uncertainty	Nature or cause of the uncertainty	Impact of the uncertainty on the assessment
<b>Literature search - Language</b>	The search was performed exclusively in English (at least the abstract for other languages). More studies could have been identified by including references with abstracts in languages other than English.	The number of relevant welfare consequences, ABMs and/or hazards may have been underestimated.
<b>Literature search – Publication type</b>	Studies considered included primary research studies identified through the extensive literature search and grey literature (factsheets, guidelines, conference papers, EU reports, book chapters, etc.) known to the EFSA Experts, but an extensive search of the grey literature was not conducted. Therefore, there may be reports and other guidance documents on animal	Underestimation of the published relevant papers. The number of relevant welfare consequences, ABMs and/or hazards may have been underestimated.

Source of Uncertainty	Nature or cause of the uncertainty	Impact of the uncertainty on the assessment
	welfare of which the EFSA Experts were not aware of.	
<b>Literature search – Search strings</b>	Although the search criteria were thoroughly discussed, some synonyms may have not been used in the search strings, and thus less hits might have been retrieved	The number of highly relevant welfare consequences, ABMs and/or hazards may have been underestimated.
<b>Literature search – Source of studies</b>	The search was limited to Web of Science all databases. Although the search was complemented by internet searches and manual searches of the publicly available literature, no data were retrieved from other sources (e.g. industry data). More information could have been retrieved by applying different searches and/or methods (e.g. public call for data).	The number of highly relevant welfare consequences, ABMs and/or hazards may have been underestimated.
<b>Literature search – inclusion and exclusion criteria</b>	The screening phase might have led to the exclusion of certain studies that could have included relevant information.	Underestimation of the published relevant papers. The number of relevant welfare consequence, ABMs and/or hazards may have been underestimated.
<b>Expert group – number and type of experts</b>	A limited number (7–9) of experts were selected based on their knowledge on animal welfare in the different laying hen categories (pullets, laying hens, laying breeders) and related husbandry systems. They also had to show they have no conflict of interest. This may have resulted in reduced level of technical knowledge derived from the field practice.	The number and variety of highly relevant welfare consequence, ABMs and/or hazards may have been over or underestimated.
<b>Laying hen categories (Day-old chicks &amp; pullets and laying hens and laying breeders) considered in the studies retrieved in the extensive literature search</b>	The animals used in the studies retrieved might not be the breeds/strains or categories currently used in the EU to study welfare consequences and ABMs, thus requiring an extrapolation exercise from the experts.	Under- or overestimation of the level of magnitude of the welfare consequences and related ABMs.
<b>Farming conditions and practices in the studies retrieved in the extensive literature search</b>	The studies retrieved through the ELS could have been performed anywhere in the world, and thus may consider laying hen farming conditions different from those currently allowed in the EU, also regarding animal welfare. Thus, experts had to extrapolate findings to the EU relevant conditions in some cases.	Under- or overestimation of the level of magnitude of the welfare consequences and related ABMs.
<b>Time allocation</b>	The time allocated to this opinion were limited and additional time for reflection would have facilitated a more in-depth discussion of some of the aspects.	Under- or overestimation of the level of magnitude of the welfare consequences and related ABMs.

Source of Uncertainty	Nature or cause of the uncertainty	Impact of the uncertainty on the assessment
<b>Lack of data on ABMs</b>	Although many ABMs have been successfully developed and applied in the scientific literature, for many the amount of data is still too limited to draw quantitative conclusions and conclusions relied largely on expert opinion.	Under or overestimation of the effect of exposure variables and husbandry circumstances on welfare consequences.
<b>Approach/Type of assessment</b>	The approach used to assess the exposure variables of Specific ToRs (EKE, semi-quantitative, qualitative (y/n) or narrative) might have led to different representation of the results, enhancing, or limiting the understanding of findings.	Under or overestimation of the effect of exposure variables and ABMs on welfare consequences.

## Appendix F – Measuring welfare consequences

Behaviours can be measured in terms of time budget dedicated to each activity (e.g. time spent doing a certain activity), or in terms of frequency of the behaviour in a group of animals, expressed either as % of animals performing the behaviour per time unit or frequency per individual per time unit.

Measuring behaviour requires the division of a continuous stream of movements into different categories (discrete behaviours) that should be clearly defined, using clear and unambiguous criteria. In studies of laying hens, functional categories are most often used to define behaviours, based on previous work that has established the adaptive role of the actions shown (e.g. preening, feeding or nesting). Sometimes, structural categories are used where the function of the behaviour is less clear (e.g. alert posture, head shake).

Measurable characteristics of a behaviour include its frequency (number of occurrences per time unit) and its duration (the duration of time a behavioural state is performed before a transition to a anotherone). Some behaviours have a very brief duration, and these are often described as 'events' and only their frequency is recorded. Other behaviours, such as resting, standing or perching, have relatively long durations and can be described as 'states' where frequency, total duration and mean bout length can be recorded (Bateson and Martin, 2021).

Measurement strategies tend to vary depending on group size. In small groups it may be possible to observe individual animals for a specified duration of time, a technique called focal (animal) sampling. Usually, a continuous record from each individual is obtained allowing calculation of frequencies and durations of each behavioural element and/or state performed. In larger groups, there are practical difficulties in observing individual birds, including marking, identification and tracking of fast-moving birds in a complex house. The use of Radio frequency identity (RFID) tags and FRID-readers allow focal animal information to be obtained on bird movement and location and, if combined with other technologies such as accelerometry, it is possible to obtain information on individual behaviour, even within large flocks (Gebhardt-Henrich et al., 2014).

In larger flocks of hens, scan sampling is typically used (Shimmura et al., 2011). A randomly or systematically selected sample of the flock, is scanned rapidly, and the number of animals performing each behaviour recorded, alongside a note of the total number of animals present in the chosen area at the time of the scan. This approach allows an estimation of behavioural frequencies, but it is not sensitive enough to quantify the occurrence of sporadic behaviours or those of very brief duration (Vasdal et al., 2022b).

Occasionally, an observer may want to focus on just one specific behaviour, for example a specific type of vocalisation such as a gavel call. In this case, every occurrence of the behaviour is recorded from the group or flock under consideration, using a technique called (all occurrence) behaviour sampling (Zimmerman et al., 2000).

Behaviour is usually recorded either by direct observation by trained individuals, by sensor, by video recording and subsequent analysis, both methods being time consuming. An advantage of the direct observation technique is that the observer can adjust position to keep track of birds that for example are hidden behind others. However, the presence of an observer can itself affect bird behaviour, and can sometimes be inefficient (e.g. if birds are all resting for a prolonged period the observer cannot fast-forward). Video recordings can be used instead but have opposing advantages (lack of effect on bird behaviour, higher (?) efficiency) and disadvantages (immobile).

To assess the prevalence of an ABM or a mean score of a flock, the two most common approaches are: observing all animals of the flock or use a flock sample. The first option is challenging if the number of animals in the group is large. The second approach, using a representative sample provides an estimation of the true result in the population. The obtained estimation is reliable if sampling requirements have been validated.

The representativeness of a sample is ensured by the sampling method. The selection of animals should be randomised (e.g. when walking in a barn, pick up one animal every five) and could rely on some stratification (e.g. if 20% of animals are counted in the outdoor range, randomly sample 20% outside and 80% in barn).

The number of animals composing the sample gives the precision of the result, and although larger samples should provide a more precise result the relationship normally is not linear.

Depending on the ABM and of the objective of the sampling (Cameron and Baldock, 1998), two situations may arise:

Situation 1: The objective is to assess the prevalence of birds in a flock with a specific ABM (e.g. cannibalism wound, foot lesion). The following data are needed to calculate the sample size:

- The population size: total number of birds in the flock,
- The expected prevalence. If not known, rather pick up a low value.
- The relative precision: the accuracy to achieve.
- Confidence level: usually used at 95%

As an example, if the objective is to assess, in a flock of 20,000 laying hens, an expected prevalence of foot lesions of 1% with 30% relative precision (it means that the result will be something like  $1\% \pm 0.3\%$ ) and 95% confidence level, the minimum sample size is 4,226 animals. If the objective is to assess the level of keel bone fracture that is expected to be around 20%, with a relative precision of 50% ( $20\% \pm 10\%$ ), the minimum sample size is 62 birds.

**Table F.1:** Minimum sample size depending on expected prevalence and relative precision (example for a population of 20,000 birds and assuming a sensitivity and specificity of 100%)

Precision, %	Expected prevalence, %								
	1	2	3	4	5	10	15	20	25
10	38,032	18,824	12,422	9,220	7,300	3,458	2,177	1,537	1,153
20	9,508	4,706	3,106	2,305	1,825	865	545	385	289
30	<b>4,226</b>	2,092	1,381	1,025	812	385	242	171	129
40	2,377	1,177	777	577	457	217	137	97	73
50	1,522	753	497	369	292	139	88	62	47
60	1,057	523	346	257	203	97	61	43	33

Situation 2: The objective is to detect if the prevalence in the flock is above or below a certain 'level' called design prevalence (e.g. check if loss of plumage in pullets do not exceed 1% of animals).

Then to calculate the sample size, the following data will be needed:

- The population size: total number of birds in the flock
- The prevalence threshold
- The confidence interval: usually used at 95%

For example: the number of birds in the flock is approx. 10,000 and the objective is to determine if the prevalence of pullets with a white patch in the plumage is over 1% or not. In this case, 294 animals will have to be scored and if no animal shows a white patch the prevalence in the flock of pullets with plumage defect is below 1%.

**Table F.2:** Minimum sample size depending on prevalence threshold and flock size (assuming a sensitivity and specificity of 100%)

Prevalence threshold, %	Total number of birds in the flock					
	200	500	1,000	5,000	10,000	20,000
0.5	190	349	450	564	581	589
1	155	225	258	290	<b>294</b>	296
2	105	129	138	147	148	148
3	78	90	94	98	98	99
4	62	69	71	73	74	74
5	51	56	57	59	59	59
10	27	28	29	29	29	29