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A comparison of fluctuations of *Campylobacter* and *Escherichia coli* concentrations on broiler chicken carcasses during processing in two slaughterhouses

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ABSTRACT

The causes of differences in *Campylobacter* and *Escherichia coli* concentrations on broiler chicken carcasses after chilling between slaughterhouses are not fully identified. Therefore, it is a challenge for slaughterhouses to comply with Process Hygiene Criteria for broiler meat.

The aim of the study was to identify which processing steps contribute to increases or decreases in *Campylobacter* and *E. coli* concentrations within and between two slaughterhouses. Identifying the processing steps with variable performance could explain the differences in bacterial concentrations after chilling between slaughterhouses.

Thermotolerant *Campylobacter* and *E. coli* concentrations on carcasses during broiler processing were measured during the summer period in 21 trials after bleeding, scalding, defeathering, evisceration and chilling.

In two slaughterhouses with comparable *Campylobacter* and *E. coli* concentrations in the incoming batches (after bleeding), the mean $\log_{10}$ concentrations are found to be significantly different after chilling. *Campylobacter* concentrations decreased by 1.40 $\log_{10}$ in Slaughterhouse 1 and by 1.86 $\log_{10}$ in Slaughterhouse 2, whereas *E. coli* decreased by 2.19 $\log_{10}$ in Slaughterhouse 1 and by 2.84 $\log_{10}$ in Slaughterhouse 2. Higher concentrations of *Campylobacter* and *E. coli* on carcasses after chilling were observed in Slaughterhouse 1 in which an increase in concentrations was observed after evisceration. The effect of processing on *Campylobacter* and *E. coli* concentrations in Slaughterhouse 1 did not differ between batches. In Slaughterhouse 2, the effect of processing on the concentrations of both bacteria varied over batches. Changes in *E. coli* concentration levels during processing were similar to *Campylobacter* except for defeathering. *E. coli* concentration significantly decreased after defeathering in both slaughterhouses, whereas *Campylobacter* increased in Slaughterhouse 2 and in Slaughterhouse 1 no significant changes were observed.

The patterns of increases and decreases in bacterial concentrations during processing are specific for each slaughterhouse. Inhomogeneous patterns potentially explain the differences in concentrations after chilling between slaughterhouses. Critical processing steps should be validated in each slaughterhouse by longitudinal studies and potentially based on *E. coli*. *E. coli* has a potential to be used as an indicator of processing hygiene, because the impact of most of the studied processing steps was similar as for *Campylobacter*.

1. Introduction

Campylobacter has remained the major gastrointestinal bacterial pathogen in humans in the European Union (EU) since 2005 (European Food Safety Authority, 2014). The annual number of campylobacteriosis cases was estimated at 9 million in the European Union (Havelaar et al., 2009). Broiler meat is recognised as a major source of human infections. An estimated 20–30% of cases of campylobacteriosis in EU may be attributed to the handling, preparation and consumption of broiler meat (European Food Safety Authority, 2010a). According to risk assessment studies, the most effective reduction of human infections in the short term could be achieved by reducing *Campylobacter* numbers in

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contaminated slaughtered batches (Nauta et al., 2009). Compliance of batches sold as fresh meat with a threshold value of 1000 or 500 CFU/g of neck and breast skin would reduce the health risk by more than 50% or even 90% (European Food Safety Authority, 2011). These risk assessment results drive the initiative to establish Process Hygiene Criteria (PHC) for broiler meat to stimulate further control measures aiming at reducing carcass contamination (European Food Safety Authority, 2012a). Moreover, the PHC could be used as a tool to classify slaughterhouses according to their capability to prevent or reduce hazards and as a tool to monitor risk and verify hygiene management in slaughterhouses (European Food Safety Authority, 2012a). Compliance to the PHC is, however, a challenge for the industry. In 20% of the tested batches in the European Union, the *Campylobacter* concentrations in neck and breast skin after chilling exceeded 1000 CFU/g (European Food Safety Authority, 2010b). In The Netherlands, 30% of all produced batches would not meet the threshold of none of 5 samples per batch exceeding 1000 *Campylobacter* CFU/g of breast skin after chilling (Anonymous, 2011; Swart et al., 2013). Differences in *Campylobacter* concentrations after chilling between slaughterhouses were identified in the baseline surveys (Anonymous, 2011; European Food Safety Authority, 2010b). However, the causes of the differences were not fully identified. *Campylobacter* concentrations change along the processing line with typically a decrease after scalding and chilling, an increase after defeathering and an increase or no change after evisceration (Berrang and Dickens, 2000; Izat et al., 1988; Klein et al., 2007; Oosterom et al., 1983; Rosenquist et al., 2006; Seliwiorstow et al., 2012; Tchórzewska et al., 2013). It has not been investigated whether these changes in concentrations are maintained at similar levels between batches and between slaughterhouses. Identifying the processing steps with variable performance within and between slaughterhouses could explain the differences in *Campylobacter* concentrations after chilling and thus improve the ability of the slaughterhouses to comply with potential Process Hygiene Criteria.

Setting a PHC based on *Escherichia coli* instead of on pathogenic bacteria has been proposed (European Food Safety Authority, 2012a; European Food Safety Authority, 2012b), because indicator microorganisms are recognised to reflect better the process hygiene than pathogenic microorganisms. The advantage of using *E. coli* to monitor processing performance is also related to easier, lower-cost, omnipresent enumeration techniques and frequent occurrence of *E. coli* on the carcasses that is not impacted by seasonality as in the case of *Campylobacter* (European Food Safety Authority, 2012a; Habib et al., 2012). Furthermore, targets based on *E. coli* concentration levels on carcasses after chilling proved to be a useful tool to reduce *Campylobacter* levels on postchill carcasses (Habib et al., 2012).

The aim of the study was to identify which processing steps contribute to increases or decreases in *Campylobacter* and *E. coli* concentrations in two slaughterhouses. Moreover the purpose was to identify whether the impact of the processing steps on bacterial contamination levels varied within and between slaughterhouses and whether the impact was similar for both *Campylobacter* and *E. coli*.

2. Materials and methods

2.1 . Slaughterhouses

The samples were taken in two commercial broiler chicken slaughterhouses. Slaughterhouse 1 is located in Germany and daily processes 130 000 broilers, whereas Slaughterhouse 2 is situated in The Netherlands and daily processes 240 000 broilers. The slaughterhouses were selected because of similarities in the processing equipment applied. During the study, the stunning, scalding and defeathering equipment was modernised in Slaughterhouse 1 prior to trials in 2013. The processing parameters remained the same.

2.2. Collection and preparation of samples

Thermotolerant *Campylobacter* and *E. coli* concentrations during broiler processing were measured in 21 trials. Eleven trials were performed in Slaughterhouse 1 (trials' ID: A, B, C, I, K, L, N, O, Q, R, U). Ten trials were performed in Slaughterhouse 2 (trials' ID: D, E, F, G, H, J, M, P, S, T). The trials were performed between June–October 2012 (trials A–M) and June–October 2013 (trials N–U). This sampling period was chosen in order to increase the probability of *Campylobacter* positive flocks (European Food Safety Authority, 2010c). Each trial was performed on a different day and included one batch, defined as a group of chickens raised together in one shed (European Food Safety Authority, 2011).

The *Campylobacter* status of the batch was ascertained by the slaughterhouses. In Slaughterhouse 1, bootswabs (in 2012 and 2013) and cloacal swabs (in 2012) at farms were taken 2–3 days prior to sampling in the slaughterhouse. The bootswabs were enriched in Campy Food broth (bioMérieux SA, Marcy l'Etoile, France), the cloacal swabs in Preston broth (prepared according to manufacturer guidelines — Oxoid). From the enrichment broth 1 ml was taken for further analysis and the positivity was checked by PCR with a detection limit of 100 CFU/ml. In Slaughterhouse 2, faecal droppings were collected at farms one week prior to sampling in the slaughterhouse. The faecal droppings were streaked on mCCDA, incubated and confirmed according to a Dutch national method (Anonymous, 2010). The limit of detection was 100 CFU/g.

During the trials, the first samples were collected after at least 1000 carcasses of the investigated batch had passed through the line, in order to avoid potential cross-contamination from the previously slaughtered batch. Samples were collected after the following processing steps: 1) just after bleeding, 2) just after scalding, 3) just after defeathering, 4) after evisceration and evisceration spraying cabinet but before inside and outside washing and 5) just after chilling. These steps were chosen, because the most dynamic changes in bacterial contamination levels were reported after these steps (Rosenquist et al., 2006).

The sampling plan to collect quantitative data is presented in Table 1. Whole carcass rinse was performed as described previously (Pacholewicz et al., 2013). The carcasses were removed from the line after selected processing steps. The cloacae of the non-eviscerated carcasses were plugged with a fibre tampon to prevent faecal and intestinal leakage while rinsing. Prevention of leakage of faecal material as a result of plugging was compared to results from rinsing the carcasses that were plugged and sealed (results not shown). Plugging and sealing of the vent were previously reported to prevent the faecal leakage (Berrang et al., 2001). After placing the carcasses into sterile plastic bags (Hevel, Zaandam, The Netherlands), 500 ml of peptone saline was added and the carcasses were shaken by hands for 60 s (Anonymous, 2000; Nauta et al., 2007; Reich et al., 2008). The same volume of the rinse was used for carcasses with feathers, defeathered or eviscerated carcasses to overcome differences in bacterial recovery, because removal rate was reported to differ with different volumes of rinse fluid (Williams et al., 2010).

In addition positivity of caeca from carcasses sampled after evisceration was checked in trials J–U. Caecal material was plated on Campy Food Agar and in case of no growth, enrichment was done according to the Dutch national method (Anonymous, 2010).

Breast skin samples after chilling were additionally collected during trials in 2013. The purpose of collecting the breast skin samples was to compare results from rinse and breast skin. The skin samples were collected as previously described (Anonymous, 2011). In short: 25 g (+/− 5 g) of skin from breast corpus was cut and placed in a stomacher bag.

Samples collected after different processing steps do not correspond to the same carcass, except for the caecal samples. These samples were collected from the same eviscerated carcasses that were sampled by the whole carcass rinse method.

The number of samples collected per trial for each sampling location was different (Table 1). In trials A–D — 3 samples were collected, in trials E–G — 4 and in trials H–U — 8. The difference in the number of

Table 1
Sampling plan presents processing steps after which the samples were collected, type of collected samples, number of samples collected in that particular trial (A–U) and enumeration threshold.

Processing step	Sample type	Unit	Enumeration threshold CFU/ml or CFU/g *Campylobacter*/*E. coli*	Trials 2012													Trials 2013								Sum
				A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	
				Slaughterhouse 1 or Slaughterhouse 2																					
				1	1	1	2	2	2	2	2	1	2	1	1	2	1	1	2	1	1	2	2	1	
After bleeding	Whole carcass rinse	CFU/ml	10/10	3	3	3	0	4	4	4	8	8	8	8	8	8	8	8	8	8	8	8	8	8	133
After scalding	Whole carcass rinse	CFU/ml	1/10	3	3	3	3	4	4	4	8	8	8	8	8	8	8	8	8	8	8	8	8	8	136
After defeathering	Whole carcass rinse	CFU/ml	1/10	3	3	3	3	4	4	4	8	8	8	8	8	8	8	8	8	8	8	8	8	8	136
After evisceration	Whole carcass rinse	CFU/ml	1/10	3	3	3	3	4	4	4	8	8	8	8	8	8	8	8	8	8	8	8	8	8	136
After chilling	Whole carcass rinse	CFU/ml	1/1	3	3	3	3	4	4	4	8	8	8	8	8	8	8	8	8	8	8	8	8	8	136
	Breast skin sample	CFU/g	10/10	0	0	0	0	0	0	0	0	0	0	0	0	0	8	8	8	8	8	8	8	8	64
Total analysed samples				15	15	15	12	20	20	20	40	40	40	40	40	40	48	48	48	48	48	48	48	48	741
Number of samples below *Campylobacter* enumeration threshold				8	0	0	0	0	0	1	1	19	0	1	0	0	0	7	0	1	0	7	1	0	
Number of samples below *E. coli* enumeration threshold				0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	

samples between the trials was related to the fact that samples collected during trials A–G were not only analysed by the culture method but also by the PMA-qPCR method (Pacholewicz et al., 2013). The purpose was to validate the PMA-qPCR method for quantifying viable *Campylobacter* cells, as an alternative for the culture method. It was not feasible to analyse more samples per location with both methods. As the PMA-qPCR method was not validated, the culture method only was used in trials H–U and more samples could be accommodated.

All collected samples were stored at 3 °C (+/− 2 °C), as specified in ISO 10272-2 (Anonymous, 2006), until laboratory analysis. In the case of 15 trials (A, B, D–G, I, J, L, N, O, Q, R, T, U) the samples were stored overnight. Due to the slaughter time of the batches and travelling time from the slaughterhouses to the laboratory, it was not feasible to perform the analytical work on the same day. Overnight storage of the rinse samples did not affect the number of recovered *Campylobacter* (results not shown). In the case of the remaining 6 trials (C, H, K, M, P, S) the analytical work was done on the day on which the samples were collected.

2.3. Analytical methods

The serial dilutions of whole carcass rinse samples were done in Butterfield's Buffer (3M The Netherlands, Zoeterwoude, product number BPPFV9BF) or peptone saline. No differences in counts were observed while using both media (results not shown). The breast skin samples were homogenised with 9 volumes of peptone saline. Serial dilutions of the homogenate were done in Butterfield's Buffer. *Campylobacter* enumeration was done on Campy Food Agar (bioMérieux SA, Marcy l'Etoile, France, product number 43471), according to ISO 10272-2 (Anonymous, 2006). Five *Campylobacter* presumptive colonies per sample were confirmed by microscopic observation after Gram staining. The *Campylobacter* isolates were not identified at species level. The enumeration threshold for the rinse samples was 10 CFU/ml for samples after bleeding, because higher concentrations were expected in the samples after bleeding than after the following steps or 1 CFU/ml for samples after scalding, defeathering, evisceration and chilling. The enumeration threshold for breast skin samples was 10 CFU/g.

E. coli enumeration was done on Petri films (3M™ Petrifilm™ from 3M, The Netherlands, Zoeterwoude, products numbers for *E. coli* 64140). Blue colonies with associated gas bubbles were counted with the 3M™ Petrifilm™ Plate Reader (Model 6499, 3M, Germany) after 24 hour incubation at 37 °C. The *E. coli* enumeration threshold for the rinse samples was 10 CFU/ml (samples after bleeding, scalding, defeathering, evisceration) and 1 CFU/ml (samples after chilling). The enumeration threshold for breast skin samples was 10 CFU/g.

2.4. Statistical analysis

Several analyses were performed in order to identify, on a per slaughterhouse basis (1), whether trends (i.e. the whole pattern of increases and decreases over the processing steps) were different between the trials, and (2) which processing steps significantly increased or decreased the mean numbers of organisms on the carcasses. Furthermore, for each of these questions, it was analysed (3) whether the effect differed between the slaughterhouses. The analysis was performed separately for *Campylobacter* and *E. coli*.

The *Campylobacter* and *E. coli* concentrations (per ml of rinse sample and per g of breast skin sample) were transformed to the $\log_{10}$ scale. The normality and homoscedasticity of the residuals were checked by diagnostic plots. Counts below the enumeration threshold were replaced by half the threshold according to Rosenquist et al., 2006. Sensitivity analysis was carried out to investigate the different replacements of the enumeration threshold. There was no effect of various replacements of the enumeration threshold on the results. Trials with many samples below enumeration threshold (around 50%) or negative results from caeca were not included in the analysis. For statistical analysis we used trials that had consistent types of samples collected.

The models used to analyse the data did not accommodate zeros; therefore, 100% of prevalence in a positive batch was assumed. Data analysis was performed in R software, package lme4 (3.0.3, 2014, R development Core Team).

2.4.1. Models developed for the identification of critical processing steps in a slaughterhouse

Two linear mixed effect models were prepared to identify whether the impact of the processing steps varied between trials (Fig. 1). The first model had processing step as an explanatory factor and trial as a random effect (model 1). In model 1, the intercept (β_0 = mean concentration level after bleeding) varied over trials (b_0 = random effect over trials with mean 0) and the effect of the processing step (slope) was the same for each trial (β indicates a fixed effect), the residual error (ε) varied over carcasses. The "Concentration" is the mean concentration ($\log_{10}$ CFU/ml) in samples collected after a particular step ("Scalding",

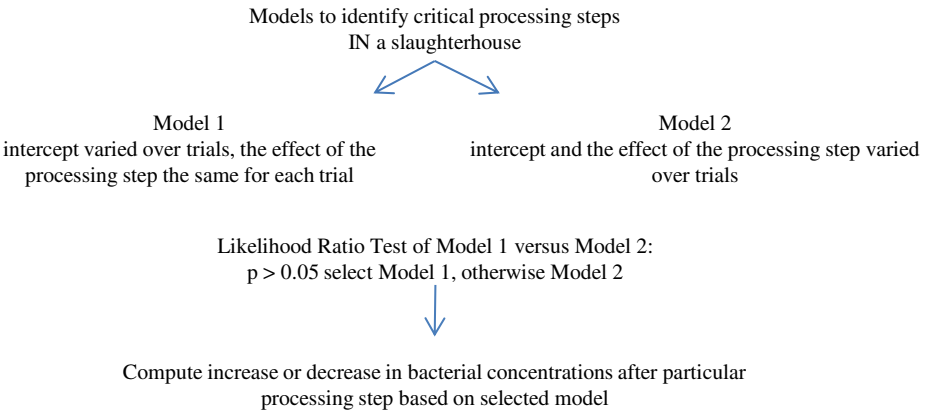

Fig. 1. Overview of selection of a model to identify the critical processing steps in a slaughterhouse.

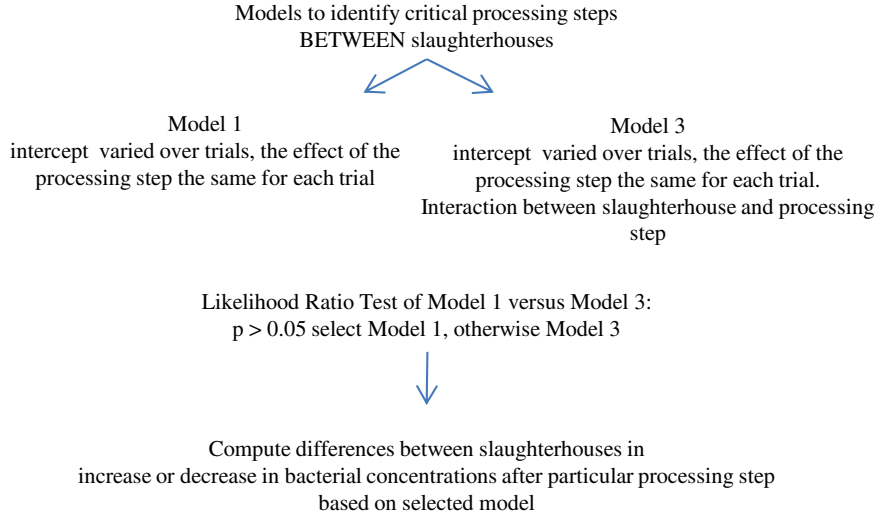

Fig. 2. Overview of selection of a model to identify the critical processing steps between the slaughterhouses.

"Defeathering", "Evisceration", "Chilling", dummy variables with 1 for the respective process step and 0 otherwise).

$$\text{Concentration} = b_0 + \beta_0 + \beta_1\text{Scalding} + \beta_2\text{Defeathering} + \beta_3\text{Evisceration} + \beta_4\text{Chilling} + \varepsilon \quad (1)$$

In the second model, not only the intercept (initial concentration level after bleeding) varied over trials, but also the impact of the processing step (slope). The random effects b_i were added to each processing step.

$$\text{Concentration} = b_0 + \beta_0 + (b_1 + \beta_1)\text{Scalding} + (b_2 + \beta_2)\text{Defeathering} + (b_3 + \beta_3)\text{Evisceration} + (b_4 + \beta_4)\text{Chilling} + \varepsilon \quad (2)$$

Since model 1 and model 2 are nested models, the comparison was done using a likelihood ratio test. Based on the selected model (model 1 or model 2), an increase or decrease in bacterial concentration after particular processing steps (effect of a processing step) was computed at each slaughterhouse. The effects of the processing steps were calculated as e.g. the predicted concentration after defeathering minus the predicted concentration after scalding.

2.4.2. Models developed for the identification of critical processing steps between slaughterhouses

In the following analyses it was computed whether, and if so, how the impact of processing steps differed between slaughterhouses (Fig. 2). Model 1 was run fitting the data from both slaughterhouses together. It was analysed whether the impact of the processing steps was the same at both slaughterhouses.

Another model was prepared (model 3) to address the question whether the patterns of the impact of the subsequent processing steps differ between the slaughterhouses. An interaction formula between slaughterhouses and processing step was added to the model 3.

$$\begin{aligned}\text{Concentration} = {} & b_0 + \beta_0 + \beta_1\text{Scalding} + \beta_2\text{Defeathering} + \beta_3\text{Evisceration} \\ & + \beta_4\text{Chilling} + \beta_5\text{Slaughterhouse} + \beta_6\text{Scalding} * \text{Slaughterhouse} \\ & + \beta_7\text{Defeathering} * \text{Slaughterhouse} + \beta_8\text{Evisceration} * \text{Slaughterhouse} \\ & + \beta_9\text{Chilling} * \text{Slaughterhouse} + \varepsilon\end{aligned} \quad (3)$$

Models 1 and 3 were nested and, thus, compared by the likelihood ratio test. The significance of any coefficients β_6 to β_9 indicated a difference between the slaughterhouses for the respective processing step. The differences in the effect at processing step were calculated with Slaughterhouse 1 as the reference, as e.g. predicted concentration after defeathering minus the predicted concentration after scalding in Slaughterhouse 2 subtracted from the predicted concentration after

Table 2
Comparison of models used to identify critical processing steps within and between slaughterhouses. P values determine selection of the models, if p value < 0.05 Model 2 was selected to identify critical processing steps within a slaughterhouse or Model 3 was selected to identify critical processing steps between slaughterhouses.

	Campylobacter						*E. coli*					
	Slaughterhouse 1		Slaughterhouse 2		Both slaughterhouses		Slaughterhouse 1		Slaughterhouse 2		Both slaughterhouses	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 3	Model 1	Model 2	Model 1	Model 2	Model 1	Model 3
	Run 1	Run 1	Run 2	Run 2	Run 5	Run 1	Run 3	Run 3	Run 4	Run 4	Run 6	Run 2
df	7	21	7	21	7	12	7	21	7	21	7	12
AIC	650.6693	662.7846	777.0657	777.1634	1442.054	1426.995	577.3792	593.1349	621.8779	613.3394	1246.27	1209.512
logLik	−318.3346	−310.3923	−381.5329	−367.5817	−714.027	−701.4973	−281.6896	−275.5674	−303.939	−285.6697	−616.1486	−592.7561
Chi-square	13.791		26.251		33.491		11.344		35.851		58.903	
p value	0.465		0.024		3e−06		0.659		0.001		2e−11	

defeathering minus the predicted concentration after scalding in Slaughterhouse 1.

2.4.3. Additional analysis

Two additional analyses were performed, 1) an ANOVA to analyse whether concentrations after chilling at each slaughterhouse differed between batches for both *Campylobacter* and *E. coli*; and 2) a linear regression model (model 4) to analyse the effect of sample type (rinse and skin), organism (*Campylobacter* and *E. coli*), slaughterhouse (Slaughterhouse 1 or Slaughterhouse 2) and interaction of organism and slaughterhouse on concentrations after chilling (Concentration).

$$\text{Concentration} = \beta_0 + \beta_1 \text{Type} + \beta_2\ \text{Organism} + \beta_3 \text{Slaughterhouse} + \beta_4\ \text{Organism} * \text{Slaughterhouse} + \varepsilon \quad (4)$$

3. Results

3.1. Critical processing steps within slaughterhouses

Different models fitted the data best at different slaughterhouses. Model 1 fitted the data collected in Slaughterhouse 1 best for *Campylobacter* ($p = 0.465$) and for *E. coli* ($p = 0.659$) (Table 2). Thus, in Slaughterhouse 1, the *Campylobacter* concentration levels as shown in Fig. 3 and *E. coli* concentration levels as shown in Fig. 4 varied with respect to the initial external contamination of the batches (concentration in rinse from carcasses collected after bleeding). The impact of the processing steps did not vary over batches in Slaughterhouse 1 (Figs. 3 and 4). *Campylobacter* concentration on broilers after bleeding in Slaughterhouse 1 varied widely from 2.3 to 6.4 $\log_{10}$ CFU/ml (Fig. 3) whereas *E. coli* concentrations varied from 3.1 to 5.7 $\log_{10}$ CFU/ml (Fig. 4). In Slaughterhouse 2, model 2 fitted the data best for *Campylobacter* ($p = 0.024$) and for *E. coli* ($p = 0.001$) (Table 2). Thus, in Slaughterhouse 2, *Campylobacter* concentration levels as shown in Fig. 5 and *E. coli* concentration levels as shown in Fig. 6 varied with respect to the initial external contamination. In addition, as shown in Figs. 5 and 6, the impact of processing steps varied between batches. *Campylobacter* concentration on broilers after bleeding in Slaughterhouse 2 varied widely from 1.6 to 6.1 $\log_{10}$ CFU/ml (Fig. 5) whereas *E. coli* concentrations varied from 3.6 to 6.4 $\log_{10}$ CFU/ml (Fig. 6). Based on the selected models (Model 1 in Slaughterhouse 1 and Model 2 in Slaughterhouse 2) the changes in the *Campylobacter* and *E. coli* concentrations in each slaughterhouse were computed as the average increase or decrease per processing step (Table 3). In both slaughterhouses, the concentrations decreased significantly after scalding for *Campylobacter* by 1.17 $\log_{10}$ in Slaughterhouse 1 and by 1.58 $\log_{10}$ in Slaughterhouse 2 and for *E. coli* by 0.64 $\log_{10}$ in Slaughterhouse 1 and by 1.29 $\log_{10}$ in Slaughterhouse 2 (Table 3). Defeathering contributed to a significant increase in the *Campylobacter* concentration by 0.41 $\log_{10}$ in Slaughterhouse 2 ($p = 0.01$). No significant differences were found in Slaughterhouse 1 after defeathering in comparison to the concentrations after scalding ($p = 0.92$). A significant decrease in *E. coli*

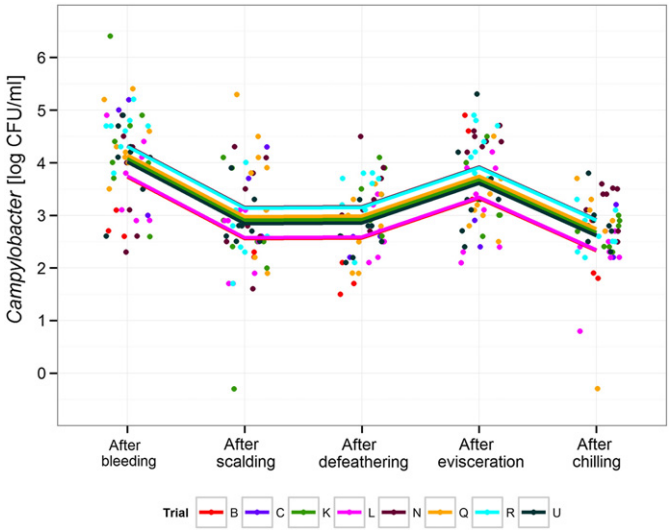

Fig. 3. *Campylobacter* concentrations in whole broiler carcass rinse samples (log CFU/ml) after selected processing steps in Slaughterhouse 1. The lines indicate the concentrations per sampled batch (trial), based on the selected model (Table 2); the points indicate the concentrations in the individual samples.

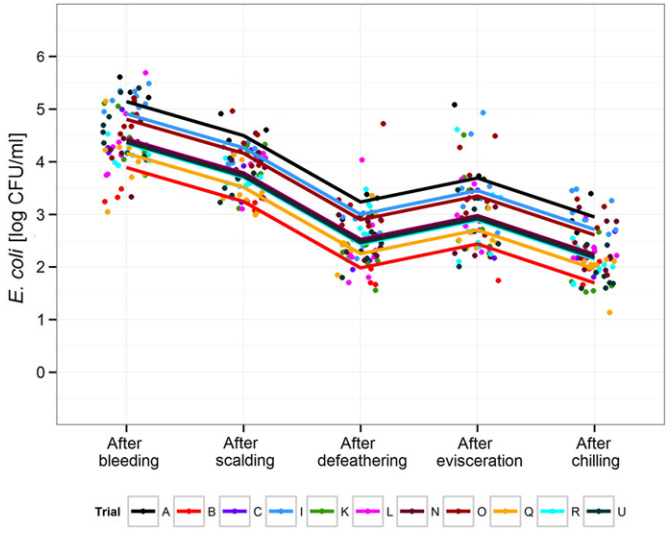

Fig. 4. *E. coli* concentrations in whole broiler carcass rinse samples (log CFU/ml) after selected processing steps in Slaughterhouse 1. The lines indicate the concentrations per sampled batch (trial), based on the selected model (Table 2); the points indicate the concentrations in the individual samples.

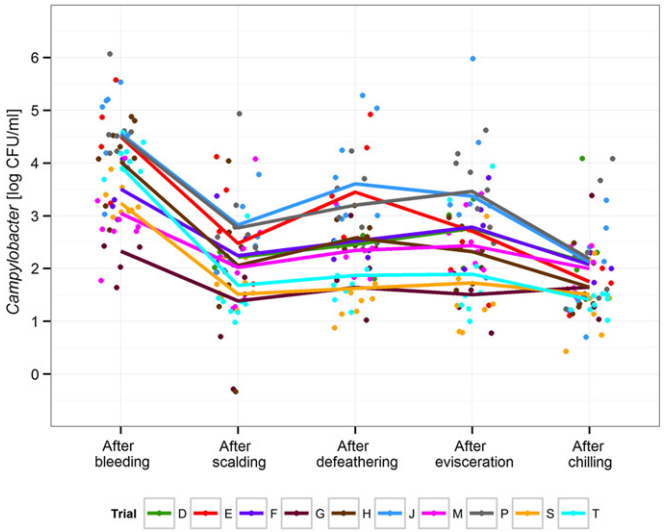

Fig. 5. *Campylobacter* concentrations in whole broiler carcass rinse samples (log CFU/ml) after selected processing steps in Slaughterhouse 2. The lines indicate the concentrations per sampled batch (trial), based on the selected model (Table 2); the points indicate the concentrations in the individual samples.

concentrations by 1.26 $\log_{10}$ in Slaughterhouse 1 and by 0.44 $\log_{10}$ in Slaughterhouse 2 after defeathering was found (Table 3). Evisceration in Slaughterhouse 1 caused a significant increase in *Campylobacter* (0.75 $\log_{10}$) and in *E. coli* (0.46 $\log_{10}$) concentrations. In Slaughterhouse 2, the *Campylobacter* and *E. coli* concentration levels after evisceration were not significantly different from the levels after defeathering (Table 3). Significantly lower concentration levels in *Campylobacter* and *E. coli* were found after chilling in comparison to concentrations on carcasses collected after evisceration in both slaughterhouses (Table 3). The concentrations on carcasses after chilling, as compared by ANOVA, differed significantly ($p < 0.001$) between batches in each slaughterhouse.

Average *Campylobacter* concentration levels were significantly reduced through the processing by 1.40 $\log_{10}$ in Slaughterhouse 1, and also significantly reduced by 1.86 $\log_{10}$ CFU/ml in Slaughterhouse 2 (Table 3, Figs. 3 and 5). With respect to *E. coli*, the concentrations after chilling were significantly lower by 2.19 $\log_{10}$ in Slaughterhouse 1 and also significantly lower by 2.84 $\log_{10}$ in Slaughterhouse 2 (Table 3, Figs. 4 and 6).

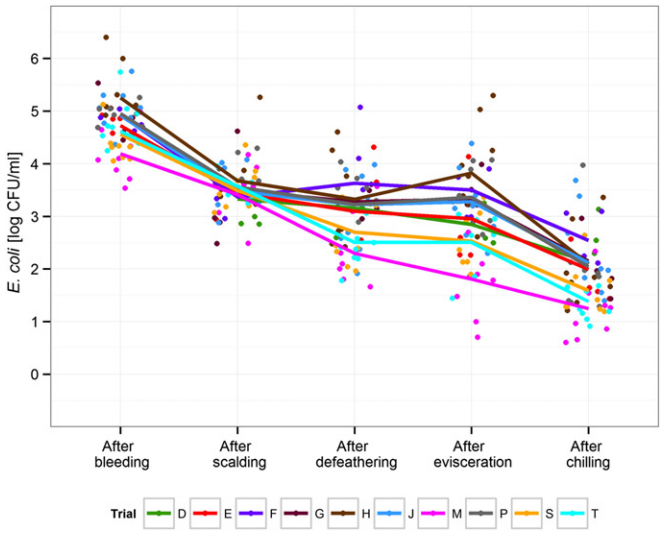

Fig. 6. *E. coli* concentrations in whole broiler carcass rinse samples (log CFU/ml) after selected processing steps in Slaughterhouse 2. The lines indicate the concentrations per sampled batch (trial), based on the selected model (Table 2); the points indicate the concentrations in the individual samples.

Table 3
Increases and decreases in *Campylobacter* and *E. coli* concentrations after selected processing steps. P values < 0.05 indicate significant increase or decrease in concentrations.

Slaughterhouse	Processing step	*Campylobacter* log10	*Campylobacter* p value	*E. coli* log10	*E. coli* p value
Slaughterhouse 1	Scalding–bleeding	−1.17	<0.01*	−0.64	<0.01*
	Defeathering–scalding	0.01	0.92	−1.26	<0.01*
	Evisceration–defeathering	0.75	<0.01*	0.46	<0.01*
	Chilling–evisceration	−1.00	<0.01*	−0.74	<0.01*
	Total decrease: chilling–bleeding	−1.40	<0.01*	−2.19	<0.01*
Slaughterhouse 2	Scalding–bleeding	−1.58	<0.01*	−1.29	<0.01*
	Defeathering–scalding	0.41	0.01*	−0.44	0.01*
	Evisceration–defeathering	−0.03	0.86	−0.05	0.72
	Chilling–evisceration	−0.65	<0.01*	−1.06	<0.01*
	Total decrease: chilling–bleeding	−1.86	<0.01*	−2.84	<0.01*

Significant p values are marked with asterisks.

3.2. Difference in the effect of processing steps between slaughterhouses

The impact of slaughterhouse on bacterial concentrations at the processing steps was best described by model 3 for *Campylobacter* ($p = 3e-06$) (Table 2) and for *E. coli* ($p = 2e-11$) (Table 2). No significant differences were observed between the slaughterhouses with respect to the *Campylobacter* and *E. coli* mean initial contamination level (after bleeding) (Table 4). The average *Campylobacter* concentration after bleeding in Slaughterhouse 1 was 4.08 $\log_{10}$ CFU/ml, and 3.80 $\log_{10}$ CFU/ml in Slaughterhouse 2. The average *E. coli* concentration after bleeding in Slaughterhouse 1 was 4.48 $\log_{10}$ CFU/ml, and 4.77 $\log_{10}$ CFU/ml in Slaughterhouse 2.

With respect to *Campylobacter* concentrations, significant differences in the impact of the processing steps between slaughterhouses were observed after scalding and evisceration. Reduction after scalding was 0.51 $\log_{10}$ higher in Slaughterhouse 2 than in Slaughterhouse 1. Evisceration in Slaughterhouse 1 contributed to an increase in concentrations that was 0.78 $\log_{10}$ higher than in Slaughterhouse 2 (Table 4). Increase in *Campylobacter* concentrations after defeathering was higher by 0.39 $\log_{10}$ in Slaughterhouse 2 than in Slaughterhouse 1; however, the difference was barely significant ($p = 0.05$).

With respect to *E. coli* concentrations, significant differences in the impact of the processing steps between slaughterhouses were observed after scalding, defeathering, evisceration and chilling. Reduction after scalding was 0.62 $\log_{10}$ higher in Slaughterhouse 2 than in Slaughterhouse 1 (Table 4). Reduction after defeathering in *E. coli* concentrations

Table 4
Outcome of Model 3: comparison of the increases and decreases in *Campylobacter* and *E. coli* concentration levels at selected processing steps between Slaughterhouses 1 and 2. P values < 0.05 indicate significant difference in the impact of the processing step between slaughterhouses.

Processing step	*Campylobacter* Differences Slaughterhouse 1–Slaughterhouse 2 [log10]	*Campylobacter* p value	*E. coli* Differences Slaughterhouse 1–Slaughterhouse 2 [log10]	*E. coli* p value
Bleeding	0.23	0.36	−0.32	0.08
Scalding–bleeding	0.51	0.01*	0.62	<0.01*
Defeathering–scalding	−0.39	0.05	−0.74	<0.01*
Evisceration–defeathering	0.78	<0.01*	0.48	<0.01*
Chilling–evisceration	−0.32	0.11	0.36	0.01*
Bleeding–chilling	0.58	<0.01*	0.72	<0.01*

Significant p values are marked with asterisks.

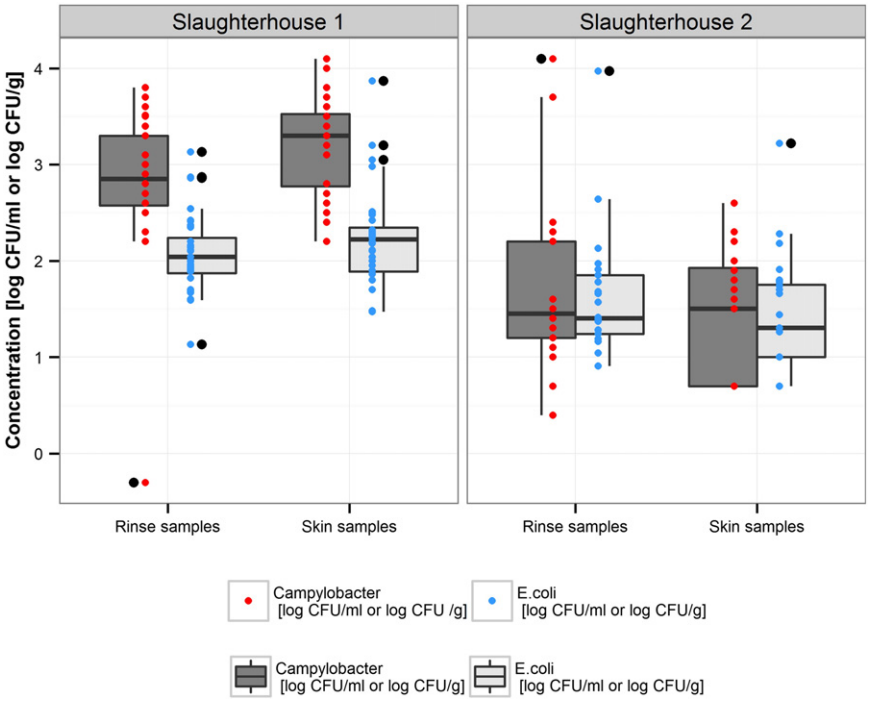

Fig. 7. *Campylobacter* and *E. coli* concentrations in whole carcass rinse (log CFU/ml) and breast skin (log CFU/g) samples collected in trials (N, Q, R, U in Slaughterhouse 1 and P, S, T in Slaughterhouse 2). The red dots indicate the Campylobacter concentration in the individual samples, whereas blue *E. coli* concentrations. The line inside each box indicates the median, the upper whiskers indicate 75th percentiles and the lower whiskers indicate 25th percentiles. The black dots indicate the outliers.

in Slaughterhouse 1 was 0.74 $\log_{10}$ higher than in Slaughterhouse 2. Evisceration in Slaughterhouse 1 contributed to an increase in concentration that was 0.48 $\log_{10}$ higher than in Slaughterhouse 2 (Table 4). Decrease in *E. coli* concentration after chilling was higher in Slaughterhouse 2 by 0.36 $\log_{10}$ than in Slaughterhouse 1 (Table 4). Overall reduction through the processing was significantly higher by 0.58 $\log_{10}$ for *Campylobacter* and by 0.72 $\log_{10}$ for *E. coli* in Slaughterhouse 2 than in Slaughterhouse 1.

Based on the linear regression model (model 4), sample type (rinse and skin samples) did not have a significant effect ($p = 0.44$) on *Campylobacter* and *E. coli* concentrations after chilling (Fig. 7). Slaughterhouse had significant effect on concentrations after chilling ($p < 2e-16$). The concentrations were significantly lower in Slaughterhouse 2 by 1.41 $\log_{10}$. Organism had significant effect on concentrations after chilling. *E. coli* concentrations were significantly lower by 0.85 $\log_{10}$ ($p = 2.01e-13$) comparing to the baseline (*Campylobacter* in rinse samples in Slaughterhouse 1). The interaction between slaughterhouse and organism was significant ($p = 2.23e-06$), with *E. coli* in Slaughterhouse 2 lower by 0.8 $\log_{10}$ as compared to the baseline.

4. Discussion

4.1. Impact of the processing steps on bacterial concentrations after chilling

Data collected in this study confirmed that certain slaughterhouses produce carcasses with lower bacterial concentrations after chilling, as previously reported (Anonymous, 2011; European Food Safety Authority, 2010c). This study revealed that, even if the *Campylobacter* and *E. coli* concentrations in the incoming batches were similar in the studied slaughterhouses, the concentrations after chilling were significantly lower in Slaughterhouse 2 (Fig. 7). The concentration levels in the incoming batches (after bleeding) were highly variable in each slaughterhouse as reported by other studies (Berrang and Dickens, 2000; Oosterom et al., 1983; Seliwiorstow et al., 2012). Our study identified that the pattern of increases and decreases in bacterial concentrations along the processing steps is specific for each slaughterhouse. The pattern was similar in Slaughterhouse 1 for all tested batches. This regularity can be potentially explained by the processing parameters applied consistently for the processed batches even after equipment modernisation. The regular peak after evisceration suggests insufficient control of this step what may have caused higher concentrations after chilling in comparison to Slaughterhouse 2. There the impact of processing steps varied between batches and *Campylobacter* concentration increased after defeathering. Irregularity in the pattern in Slaughterhouse 2 suggests that, potentially, the control of the processes was not always done in the same way. In-depth analysis of batch-related characteristics, batch handling operations, processing parameters and hygienic conditions in each slaughterhouse is needed to explain the variations in the observed patterns and its impact on contamination of the carcasses after chilling.

Different patterns of increases and decreases in *Campylobacter* concentration along the processing steps were included also by different risk assessment models (Nauta et al., 2009); with a slight increase (Nauta et al., 2005) or a considerable decrease (Hartnett et al., 2001) after defeathering and small (Nauta et al., 2005) or large (Hartnett et al., 2001) increases after evisceration. Despite the different dynamics during processing predicted by various models, similar *Campylobacter* concentration levels after chilling were predicted (Nauta et al., 2009). As shown in the current study however, these levels differed both between slaughterhouses and between batches. Therefore such variability between batches and slaughterhouses can further inform risk assessment models.

Defeathering and evisceration cause an increase in bacterial concentration on carcasses. However based on data collected in our study and on the risk assessment models (Nauta et al., 2009), an increase was not always observed after these steps. The differences in the impact of defeathering and evisceration on *Campylobacter* concentrations estimated by various models were a consequence of the assumptions made on the faecal contamination during those processing steps (Nauta et al., 2009). It suggests that differences observed in our study in the impact of these processing steps between slaughterhouses could potentially be explained by the degree of control of faecal

contamination. Such contamination is the major cause of increase in *Campylobacter* concentration after defeathering and evisceration (Berrang et al., 2001; Berrang et al., 2004; Musgrove et al., 1997; Rosenquist et al., 2006). However, if the concentration on the carcasses entering these processing steps is high, the additional contamination from leaking faeces may not be observed on the log scale. Furthermore, the bacterial concentrations after defeathering and evisceration are not only impacted by faecal leakage and concentration on incoming carcasses, but also by washing. To explain the causes of the differences in the impact of defeathering and evisceration on *Campylobacter* and *E. coli* concentrations, a detailed analysis of processing parameters and factors impacting the extent to which the faecal and caecal material contaminate the carcasses is needed.

Our results also confirmed the decrease in *Campylobacter* and *E. coli* concentrations after scalding and chilling as previously reported in a review (Guerin et al., 2010). Other authors observed higher reduction in concentrations after scalding than is the case in our study (Berrang and Dickens, 2000). The concentration of *Campylobacter* decreased after chilling in the analysed slaughterhouses in agreement with other studies (Huezo et al., 2007; Rosenquist et al., 2006). Drying explains the decrease in *Campylobacter* concentration after chilling (Alter and Scherer, 2006; Murphy et al., 2006; Oosterom et al., 1983). *Enterobacteriaceae* were reported to be less sensitive to drying than *Campylob*acter (Oosterom et al., 1983). The observed decrease in *Campylobacter* and *E. coli* concentrations after chilling in comparison to concentration after evisceration can additionally be explained by washing prior to chilling.

4.2. Campylobacter and E. coli along the processing line

Changes in the concentration levels during processing were similar for both *Campylobacter* and *E. coli* except for defeathering (Table 3). In addition, models that fitted *Campylobacter* data did also fit *E. coli* data in each slaughterhouse. *E. coli* concentrations significantly decreased after defeathering in Slaughterhouse 1 by 1.26 $\log_{10}$ and in Slaughterhouse 2 by 0.44 $\log_{10}$, whereas *Campylobacter* increased in Slaughterhouse 2 by 0.41 $\log_{10}$ and in Slaughterhouse 1 no significant changes were observed. Decrease after defeathering in *Enterobacteriaceae* (Göksoy et al., 2004; Oosterom et al., 1983) and in *Coliforms* (Göksoy et al., 2004) was previously reported. The opposite impact of defeathering on *Campylobacter* and *E. coli* can be related to potential differences in their ability to attach to the skin. Differences within even one bacterial species in the attachment to inert surfaces were previously reported (Hue et al., 2011; Sulaeman et al., 2010). Good correlation of *Campylobacter* and *E. coli* during processing was reported (Duffy et al., 2014); however, the defeathering step was not investigated. On the contrary other studies reported weak correlation (Berrang and Dickens, 2000; Williams and Ebel, 2014). Based on orthogonal regression applied for our data, the *Campylobacter* and *E. coli* concentrations were not correlated (results not shown). Although there is a lack of correlation, the direction of the changes in concentration (either increase or decrease) is similar for *Campylobacter* and for *E. coli* at most of the tested processing steps, except for defeathering (Table 3). *E. coli* has thus the potential to be used as an indicator of the hygienic status of the processing and to be used to identify the critical processing steps; however, more understanding is needed of the opposite impact of defeathering on *Campylobacter*.

4.3. Sampling methods

As the sample type had neither effect on *Campylobacter* nor on *E. coli* concentrations (Fig. 7), both methods (whole carcass rinse and breast skin collected after chilling) can be used to evaluate the hygienic status during processing.

Batches sampled during trials A, I, and O although determined as positive on farms, resulted in many samples below the detection limit (50% in trials A and I) or negative caeca results in the slaughterhouse (trial O). These trials were not informative for the model; hence, they were not included in the analysis. Positive results on the farms versus negative results in the caeca collected in the slaughterhouse can be explained by potential early stage of colonisation in these batches. At the early colonisation stage not all broilers are colonised and the level is low. In future studies, it is advisable to use highly contaminated flocks in order to determine the impact of the processing steps on *Campylobacter* concentrations. Highly contaminated batches were also reported to result in the major risk for consumers (Nauta et al., 2009).

5. Conclusions

Differences in *Campylobacter* and *E. coli* concentrations on carcasses after chilling between slaughterhouses are potentially caused by specific increases and decreases in concentrations during processing. Our results confirmed that defeathering and evisceration are the most critical steps during processing, leading to increase in *Campylobacter* concentrations on carcasses. There are however slaughterhouses that are able to control increases in concentrations after these steps and to maintain the impact of all processing steps at similar levels for each slaughtered batch. Critical processing steps should be validated in each slaughterhouse individually by longitudinal studies and potentially based on *E. coli*. *E. coli* has a potential to be used as an indicator of the steps, because the impact of most studied processing steps on *E. coli* and *Campylobacter* concentrations was similar, except for defeathering.

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