JOURNAL OF ANIMAL SCIENCE
The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Moisture content in broiler excreta is influenced by excreta nutrient contents

E. van der Hoeven-Hangoor, N. D. Paton, I. B. van de Linde, M. W. A. Verstegen and W. H. Hendriks

J ANIM SCI 2013, 91:5705-5713.
doi: 10.2527/jas.2013-6573 originally published online October 14, 2013

Moisture content in broiler excreta is influenced by excreta nutrient contents[1]

E. van der Hoeven-Hangoor,*[2] N. D. Paton,† I. B. van de Linde,* M. W. A. Verstegen,‡ and W. H. Hendriks‡§

*Cargill Animal Nutrition, Veilingweg 23, NL-5334 LD Velddriel, the Netherlands; †Cargill Animal Nutrition, 10 Nutrition Way, Brookville, OH 45309; ‡Animal Nutrition Group, Wageningen University, P.O. Box 338, NL-6700 AH Wageningen, the Netherlands; and §Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, NL-3584 CL Utrecht, the Netherlands

ABSTRACT: High litter moisture content, often referred to as wet litter, is a major problem in poultry production. Wet litter is often related to poor management, diseases, and digestive problems. In this experiment, the objective was to study the relationship between nutrient content and the moisture content of the excreta of broilers. A dataset containing 351 observations was built and contained the nutrient contents data including moisture content of excreta samples collected in 8 different broiler feeding trials. A biological based model approach was used to create a model with 10 and another one with 14 variables that may explain the excreta moisture level response. Subsequently, these models were compared with a statistical model that was built automatically and adjusted only if this improved the biological model. The R^2 of the 10 variable model was 0.54, in which Zn content and the interaction of NDF × K and Ca × P content were negatively associated with excreta moisture. Sodium, P, and Ca content and the interaction between content of NDF × Na were positively associated with excreta moisture. The R^2 of the 14 variable model was 0.58, in which Zn and K content and the interaction of NDF × protein and Ca × P content were negatively associated with excreta moisture, and Na, protein, P, and Ca content and the interactions in contents of NDF × Na, NDF × Zn, and K × Cu were positively associated with excreta moisture content. In conclusion, the models confirmed the effect of Na, protein, P, and Ca on excreta moisture content. Furthermore, hitherto unknown nutrient interactions that contribute to excreta moisture level were identified. As excreta levels of most nutrients can be manipulated by adjusting dietary nutrient levels, dietary formulation can be adjusted with the findings of this analysis to change levels of excreted nutrients and, consequently, also moisture output.

Key words: broilers, excreta moisture, general linear mixed model, nutrient excretion, wet litter

J. Anim. Sci. 2013.91:5705–5713
doi:10.2527/jas2013-6573

INTRODUCTION

Broilers are housed on litter, composed of bedding material mixed with feed, feathers, and excreta (Cook et al., 2011). Above a certain litter moisture content, "wet litter" can occur causing more ammonia to be produced and emitted into the air (Groot Koerkamp, 1994), which can negatively affect animal production and welfare (Kristensen and Wathes, 2000).

Fresh poultry droppings contain approximately 80% moisture (Henuk and Dingle, 2003) and significantly contribute to litter moisture content (Groot Koerkamp et al., 1999). Dietary nutrient composition can affect excreta moisture content through nutrient digestibility and passage rate of digesta through the gastrointestinal tract (**GIT**). Dietary mineral levels and ratios between minerals play an important role in excreta moisture content (Ziaei et al., 2008; Jankowski et al., 2011). Inconsistent effects of trace minerals on excreta moisture have, however, been reported by Nishimuta et al. (2006) and Zhong et al. (2007). A high dietary protein content (Collett, 2012) or insoluble fibers in the diet, which can have a high water binding capacity in the GIT (Chaplin, 2003), can increase the water content of droppings.

The effect of single dietary nutrients on excreta moisture level in poultry has been previously studied (Ferguson et al., 1998b; Smith et al., 2000; Namroud et al., 2009). Comparing the effects of various nutrients

---

[1] We gratefully acknowledge the staff of the Cargill Innovation Center Velddriel (NL-5334 LD, Velddriel, the Netherlands) for the accuracy and dedication to their work during the course of the experiments.

[2] Corresponding author: Evelien_Hangoor@Cargill.com

Received April 11, 2013.
Accepted October 2, 2013.

and nutrient interactions in a single statistical analysis on excreta moisture content may provide new insights into the multifactorial problem of wet litter in poultry production. The current study was undertaken to investigate relationships between moisture and nutrient content (CP, NDF, and minerals) of broiler excreta using a linear mixed model analysis to identify hitherto undocumented associations that could potentially be used in dietary strategies for wet litter prevention.

MATERIALS AND METHODS

The experimental methods were approved by the Ethical Committee of the Animal Science Group of Wageningen University and Research Center, Lelystad, the Netherlands.

Animal Experiments

The studies reported here were conducted during 2008 and 2009 at the research facilities of the Cargill Innovation Center Velddriel, Velddriel, the Netherlands. Birds were housed in 2 barns. Barn 1 had cages (100 by 110 cm) with a raised wire floor on top of which a rubber plate was placed and covered with a 2 cm layer of wood shavings. Each cage housed 18 birds and was equipped with 2 adjustable cup drinkers and a feeder that was positioned inside the cage during the first 14 d. From 15 d onward, feed was supplied via a feed trough in front of the cage. Birds were housed in this barn until 35 d of age. Continuous artificial lighting was maintained for 23 h/d throughout the experiments. Temperature, relative humidity, and ventilation were computer controlled with the temperature gradually decreasing by 2.5°C per week, from 34.0°C on the day of arrival (1-d-old chicks) to a final temperature of 20.0°C at the end of the experiment (35 d). Relative humidity was set at 50% throughout the experiments. Barn 2 had cages of 50 by 50 cm, each with 6 birds. Each cage was equipped with 2 adjustable nipple drinkers. During the first 3 d, a feeder was positioned inside the cage. From 4 d onward feed was supplied via a feeder trough in front of the cage with adjustable access opening and placement height. Birds in this barn were grown until 17 d of age. Continuous artificial lighting was maintained for 23 h/d for the first 3 d of the experiments, 20 h/d between 4 and 7 d, and 18 h/d for the remainder of the experiments. Temperature, relative humidity, and ventilation were computer controlled with the temperature gradually decreasing by 0.5°C per day, from 33.0°C on the day of arrival (1-d-old chicks) to a final temperature of 26.1°C at the end of the experiment (17 d). Room temperature was recorded continuously using data loggers, and relative humidity was set at 50% throughout the experiments.

Excreta collection during the trials was achieved using an excreta collection box, which was placed on top of the litter and facilitated pure excreta sampling without litter. At the end of the experiment litter from the cages was removed and, subsequently, excreta were collected from metal plates that had been placed underneath the cages. All excreta were collected on a cage basis and feathers and feed particles were carefully removed. The excreta samples were collected during 8 different trials that are briefly described in Table 1.

Experimental Feedstuffs

A total of 110 different dietary formulations were fed throughout the 8 trials. Replications differed per dietary formulation and ranged from 1 to 12. However, as treatment was not an effect of interest, this was not considered a limitation. In all trials feed and water were provided ad

Table 1. Overview of the 8 trials from which excreta samples were collected and analyzed

Trial	Year	Trial objective	Bird age at collection	No. of diets	Distinguishing dietary factors	Relation to excreta quality
1	2009	Emulsifiers and cereal inclusion in relation to digestibility	17 d	24	Additives	Changed nutrient digestibility
2	2009	Feedstuff in relation to microbiota	6 and 35 d	12	Crude fat, crude fiber, NDF, P, and K	Relation of microbiota with excreta quality
3	2008	Additives and cereal in relation to excreta quality	35 d	12	Crude fat, crude fiber, NDF, and K	Ammonia binding additives and fibers
4	2008	Dietary protein level in relation to growth	35 d	6	CP, crude fat, crude fiber, NDF, Ca, P, K, and Cl	Dietary protein content
5	2008	Feedstuff form and protein quality in relation to growth	35 d	6	CP, crude fat, Ca, P, Na, K, and Cl	Protein quality and GIT[1] stimulation due to structure
6	2008	Starch resistance in relation to feed efficiency	35 d	14	Crude fat and starch	Starch fermentation by increased resistance
7	2008	Feedstuff form and NSP[2] enzymes in relation to feed efficiency	35 d	12	CP, crude fat, crude fiber, NDF, P, and K	Changed nutrient digestibility and GIT stimulation due to structure
8	2009	Mineral levels in relation to excreta quality	14, 28, and 35 d	24	Range of Ca, P, Na, and Cl	Mineral level and ratios

[1] GIT = gastrointestinal tract.

[2] NSP = nonstarch polysaccharides.

libitum. There was a wide range of dietary nutrient compositions tested. Table 2 shows the minimum, maximum, median, and average value of the dietary nutrients fed.

Excreta Database

To analyze the data from the trials, a database was developed consisting of the wet chemically analyses of the excreta samples. The samples were analyzed for DM (Gravimetry, 6496, ISO, 1993; Memmert UNB 500; Memmert GmbH, Schwabach, Germany), Ca, Cu, Fe, K, Mg, Mn, Na, Zn, and P (Inductively coupled plasma atomic emission spectroscopy, 27085:2009; ISO, 1993) (Thermo Iris Intrepid II XSP Duo; Thermo Scientific Inc., Waltham, MA), CP (Combustion, 16634; ISO, 1993) (Rapid N Cube; Elementar GmbH, Hanau, Germany), and NDF (ANKOM Model 200, filter bag technique "method 6" with filter bag type 57; ANKOM Technology, Macedon, NY). Nondetergent fiber measures lignin, hemicellulose, and cellulose as major components (Van Soest et al., 1991) and was used as an indicator of insoluble fiber content. The content of soluble fibers has not been analyzed in the samples. Before statistical analysis, all excreta nutrient content were expressed on a DM basis.

The database contained 351 observations originating from 2008 (n = 178) and 2009 (n = 173) collected from barn 1 (n = 327) and barn 2 (n = 24). The samples were collected at 6 (n = 6), 17 (n = 24), 28 (n = 36), and 35 d of age (n = 285). Most samples originated from Ross 308 male birds (n = 291) although some samples were obtained from Cobb 500 (n = 24) and Hubbard Flex (n = 36) male birds. All birds originated from the same commercial hatchery (Lunteren, the Netherlands). Each trial and treatment combination was numbered with a unique code. Table 3 presents the number of observations and the minimum, maximum, median, and average value of the excreta nutrients.

Table 2. Range of dietary nutrients fed over the 8 different trials where excreta was collected (n = 354)

Item	n	Mean	Minimum	Maximum	Median
AME[1] (broiler), kcal/kg	354	2,868	2,750	3,246	2,850
CP, g/kg	354	194	175	245	194
Fat, g/kg	354	78	28	115	80
Fiber, g/kg	354	30	22	60	29
NDF, g/kg	330	102	79	180	100
Ash, g/kg	354	57	41	81	56
DM, g/kg	354	881	875	894	880
Ca, g/kg	354	8.3	4.3	15	8.0
Cl, g/kg	354	1.8	1.5	4.4	1.6
Cu, mg/kg	330	20	15	27	20
Fe, mg/kg	330	170	124	347	166
K, g/kg	354	8.2	6.5	9.7	8.3
Mg, g/kg	330	1.5	1.3	2.7	1.5
Mn, mg/kg	330	84	75	101	84
Na, g/kg	354	1.5	1.4	3.3	1.4
P, g/kg	354	6.6	4.7	10	6.5
Zn, mg/kg	330	81	75	102	80

[1] AME = apparent metabolizable energy. Calculated according to Centraal Veevoeder Bureau (2006).

Statistical Analysis

Data from the 8 trials were assembled and organized in such a way that for each sample the data for similar independent and dependent variables were listed in columns and trial identification was retained. Additionally, squared terms for all predictor variables were calculated as were all first order interaction terms. The final dataset of potential predictor variables included all main effects and their squared terms and first order interactions that were considered nutritionally relevant. The total number of variables offered for inclusion in the final biological models was 78. This master dataset, containing all 78 potential variables, was used for a first screening of potential variables for the statistical models. A subset of 48 potential predictor variables was subsequently used in the construction of the statistical linear models—the reduced dataset.

For the biological models, general linear mixed models, using the PROC MIXED procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC), were defined following the general form $Y = \mathbf{X\beta} + \mathbf{Z}u + \mathbf{e}$, where $\mathbf{X}$ is the known fixed effects design matrix, $\mathbf{ß}$ is the vector of unknown fixed effects parameters, $\mathbf{Z}$ is the known random effects design matrix, and $\mathbf{u}$ and $\mathbf{e}$ are random effect vectors. Investigation of the 78 variables to be included in the final design matrix of fixed effects ($\mathbf{X}$) was performed by identifying their specific contribution to the performance of the prediction equation on the basis of several factors (Hocking, 1976). These factors included 1) the significance of the independent variable in the mixed model ($P <$ 0.10), 2) the contribution of the variable to the log likelihood coefficient of determination as defined by Kramer

Table 3. Range in excreta nutrients included in the database, corrected for DM content of the sample (n = 354)

Component, % of DM	n	Mean	Minimum	Maximum	Median
DM	348	26	17	35	27
Moisture	348	74	65	83	73
CP	350	30	22	43	30
NDF	351	23	6.4	36	25
Ca	349	1.9	0.7	4.1	1.8
K	351	2.3	1.6	2.8	2.4
Mg	351	0.5	0.4	0.8	0.5
Na	350	0.3	0.2	1.1	0.1
P	351	1.5	0.9	2.8	1.4
Cu	350	0.006	0.004	0.010	0.006
Fe	351	0.068	0.045	0.110	0.065
Mn	351	0.030	0.022	0.052	0.029
Zn	350	0.063	0.020	0.181	0.060

(2005) for a mixed model, 3) the biological importance of the variable as defined by current nutritional understanding, and 4) the relationship of the factor to others already included in the reduced model. A cut-off value of $P < 0.10$ was used to not exclude any trends that may be biologically still relevant. To prevent overfitting of the reduced model (Babyak, 2004) and limit exposure to problems arising from the inclusion of too many variables a cap on the total number of predictor variables was arbitrary set at 10 and a second at 14 based on R^2 improvement as shown in Fig. 1. The principal objective was to provide a reduced model that permitted a clear understanding of relevant nutritional and biological relationships and interactions. The model, however, should not be overly complex. As is the case when any such predictor models are constructed, the inclusion or exclusion of certain predictor variables is somewhat subjective especially when observing limits placed on the total number of terms to be included in the final models.

A third model that was constructed purely on an objective basis of factor significance ($P < 0.05$) and the maximization of the coefficient of determination was completed after the approach detailed by Tilea et al. (2012). In this procedure all potential independent variables were recursively tested for their contribution to a reduced model with 1 to $n - 1$ fixed effect predictor variables. The full model (all 48 predictor variables) was also constructed and this model provided a reference to which all reduced models were compared. The objective recursive models were subsequently used in a comparison to the subjective reduced models to rationalize the process of subjective independent variable selection and check for omissions of variables that had significant contributions as a predictive factor and were ranked according to the variation they explained in the data. As such the final reduced models presented are both statistically meaningful and have biological relevance.

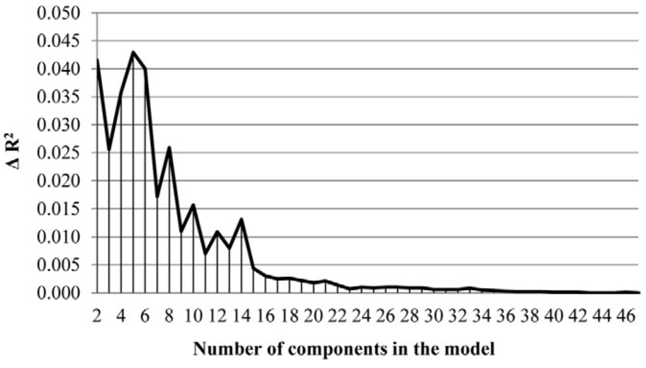

Figure 1. The R^2 improvement per addition of 1 predictor variable to the statistical model explaining excreta moisture.

RESULTS

Best Ten-Variable Biological Model

The least square estimates (the solution to the minimized sum of the squared deviations between predicted and observed values) for the optimal model with 10 variables are presented in Table 4. Random effect variables included in this model were year, trial number, and treatment. The R^2 of the model was 0.542. The results show that Zn content and the interactions between contents of NDF × K and Ca × P were negatively associated with excreta moisture, where greater levels of these components resulted in lower excreta moisture levels. Sodium, P, and Ca content and the interaction between contents of NDF × Na were positively associated with excreta moisture, where greater levels of these components resulted in greater excreta moisture levels.

Best Fourteen-Variable Biological Model

The least square estimates for the optimal model with 14 variables are presented in Table 5. Random effect variables included in this model were trial number and treatment. The R^2 of the model was 0.583. The results show that Zn and K content and the interactions between NDF × CP and Ca × P contents were negatively associated with excreta moisture, where greater levels of the components resulted in lower excreta moisture levels, and Na, CP, P, and Ca content and the interactions between NDF × Na, NDF × Zn, and K × Cu content were positively associated with excreta moisture content.

Table 4. Least squares estimates and corresponding significance levels for the nutrients explaining the excreta moisture content in the excreta from the 10-variable biological linear mixed model

Component	Least square estimate	SE	*P*-value
Age			<0.001
6 d*	0		
17 d	7.38	4.56	
28 d	–2.12	1.50	
35 d	0.40	1.42	
Zn	171.95	25.56	<0.001
Na^2	17.34	4.46	<0.001
P	6.62	1.08	<0.001
Ca	4.27	0.82	<0.001
NDF × Na	0.74	0.19	<0.001
NDF × K	–0.05	0.03	0.049
Ca × P	–2.07	0.47	<0.001
Na	–32.56	5.54	<0.001
Zn^2	–721.06	136.49	<0.001

*d = days of age.

Best Ten-Variable Automated Statistical Model

The least square estimates for the automated statistical model with 10 variables are presented in Table 6. Because of limitations in the calculation method used by Tilea et al. (2012), no random effect variables could be included in this model. The R^2 of the model was 0.399. Results show that Zn content and the interactions between NDF × CP, NDF × K, Ca × P, and Na × P content were negatively associated with excreta moisture, where greater levels of the components resulted in lower excreta moisture levels. Crude protein and P^2 content and the interactions between Ca × Mg, NDF × Na, and NDF × Zn content were positively associated with excreta moisture, where greater levels of the components resulted in greater excreta moisture levels.

Best Fourteen-Variable Automated Statistical Model

The least square estimates for the automated statistical model with 14 variables are presented in Table 7. No random effect variables could be included in this model because of limitations in the calculation method used by Tilea et al. (2012). The R^2 of the model was 0.438. Results show that Zn, K, Cu^2, and Mg content and the interactions between NDF × CP, Ca × P, and Na × P content were negatively associated with excreta moisture, where greater levels of the components resulted in lower excreta moisture levels. Calcium, CP, and P^2 content and the interactions in contents of NDF × Na, NDF × Zn, and K × Cu were positively associated with excreta moisture content.

Table 5. Least squares estimates and corresponding significance levels for the nutrients explaining the excreta moisture content in the excreta resulting from the 14-variable biological linear mixed model

Component	Least square estimate	SE	*P*-value
Age			<0.001
6 d*	0		
17 d	3.68	2.51	
28 d	–6.55	1.78	
35 d	–4.04	1.73	
K × Cu	140.93	75.26	0.062
Zn	80.48	49.17	0.104
Na^2	15.87	4.34	<0.001
P	5.74	1.04	<0.001
Ca	3.81	0.77	<0.001
NDF × Zn	3.39	1.61	0.036
NDF × Na	1.44	0.27	<0.001
CP	0.60	0.13	<0.001
NDF × CP	–0.02	0.01	<0.001
Ca × P	–1.84	0.44	<0.001
K	–2.13	0.96	0.026
Na	–48.79	6.96	<0.001
Zn^2	–665.42	134.45	<0.001

*d = days of age.

DISCUSSION

Two linear models were used in the present study to describe the relationships between excreta composition (CP, NDF, and minerals) and excreta moisture. An automated objective statistical approach including all single variables, squared variables, and relevant interactions was compared with a biologically inspired method based on our current knowledge from the literature. As such, the biological approach may be perceived as subjective. In the automated model procedure an iterative process was used to derive an optimal solution, all combinations of variables were recursively fitted, and the fit statistics were recorded. For the models containing 14 variables the comparison of the automated statistical model with the biologically compiled model showed that the 2 models had 9 components in common. These were Zn, Ca, CP, and K content and the interactions between Ca × P, K × Cu, NDF × Na, NDF × CP, and NDF × Zn content. Consequently, 5 components were unique for each of the 2 models. The automated statistical model contained 8 variables that were originally included in the biological model with 6 variables remaining, which potentially could mathematically improve the biological model. Of these, one (the interaction of K × Cu) was selected for inclusion in the biological model to improve its performance, resulting in an improvement in R^2 from 0.581 to 0.583. No other changes indicated by the automated statistical model improved the explained fraction of data variance and inclusion of some even reduced the R^2.

For the models containing 10 variables the differences between the automated and biological models were large, with only 4 variables in common to both models. The final biological model included K and CP content and not the interactions of NDF × Na and NFD × K content. Replacing K and CP by these 2 interactions as indicated by the automated statistical model improved the model R^2 from 0.530 to 0.542. Other changes reduced the explained

Table 6. Least squares estimates and corresponding significance levels for the nutrients explaining the excreta moisture content in the excreta resulting from the 10-variable automated statistical linear model

Component	Least square estimate	SE	*P*-value
Ca × Mg	7.76	1.06	<0.001
NDF × Zn	5.72	1.26	<0.001
P^2	3.95	0.41	<0.001
NDF × Na	1.89	0.22	<0.001
CP	0.64	0.11	<0.001
NDF × CP	–0.02	<0.01	<0.001
NDF × K	–0.11	0.03	<0.001
Ca × P	–2.27	0.37	<0.001
Na × P	–27.77	3.85	<0.001
Zn	–134.30	29.40	<0.001

fraction of the variance by the model. Adding additional parameters to the model can potentially improve the variance explained by the model. However, this can lead to an overly optimistic model (the problem of overfitting of the model), where excreta components are added without significantly improving model performance. This approach does not necessarily yield an improved predictive model and may result in models that cannot be replicated with other datasets (Babyak, 2004). An approach was chosen to define the number of components based on change of R^2. Subsequent to the 14 original excreta predictor variables, the addition of a single or pair of predictor variables resulted in only a minor (<0.01) improvement in the model R^2. This approach was supported by the change in R^2 values in the automated model, which showed peaks at 10 and 14 predictor components in the model with little subsequent improvement (Fig. 1).

The biological model contained both fixed and random effect variables whereas a limitation of the automated model macro (Tilea et al., 2012) confined variables to include solely fixed effects. This resulted in different outcomes for the 2 models. The full model predictor variable set used by the automated model macro included 48 predictor variables and explained less variation with an R^2 of 0.468 compared with the mixed model with 14 variables and an R^2 of 0.583. When using Kramer's (2005) method to calculate R^2, error variance associated with random effects is partitioned from the true error reducing the true error value and increasing the R^2 value. These findings indicate that the inclusion of random effects (mixed model) resulted in an improved model compared with including fixed effects only. Furthermore, the biological model was composed based on biological relevance and statistical contribution whereas the automated model was assembled using only a statistical algorithm.

Table 7. Least squares estimates and corresponding significance levels for the nutrients explaining the excreta moisture content in the excreta resulting from the 14-variable automated statistical linear model

Component	Least square estimate	SE	*P*-value
K × Cu	1,889.00	529.30	<0.001
Mg	135.15	34.49	<0.001
Ca	4.69	0.80	<0.001
NDF × Zn	4.69	1.27	<0.001
P^2	3.84	0.42	<0.001
NDF × Na	1.75	0.22	<0.001
CP	0.68	0.10	<0.001
NDF × CP	–0.03	<0.01	<0.001
Ca × P	–2.49	0.46	<0.001
K	–14.45	3.24	<0.001
Na × P	–25.53	3.91	<0.001
Mg^2	–107.72	29.79	<0.001
Zn	–108.60	29.70	<0.001
Cu^2	–318,000.00	93,608.00	<0.001

The models indicate that mineral output makes an important contribution to excreta moisture output. Included in the model were Na, P, Ca, and K content, where increased excretion of Na, P, and Ca resulted in greater moisture excretion whereas increased K excretion resulted in lower excreta moisture. The greater excreta moisture or litter wetness scores with elevated dietary Na has been found by various authors (Murakami et al., 1997; Smith et al., 2000; Oviedo-Rondón et al., 2001; Borges et al., 2003b; Jankowski et al., 2011). This finding is related to a linear increase in water intake with increasing dietary Na levels (Smith et al., 2000; Ahmad et al., 2009) or increased osmolality of the digesta, which prevents water reabsorption in the hindgut. In feces of pigs, minerals have been reported to be the main osmotic particles (Etheridge et al., 1984). Excess in dietary Na levels increased the absorption of this mineral and hence increased osmolality of the blood, resulting in an increased Na excretion by the kidneys (Vena et al., 1990). Dietary salt content affects the osmotic pressure of the blood when absorbed, which is a thirst regulating mechanism (Borges et al., 2003a). As a result, birds will consume more water and if the digesta has a greater osmotic value, this will result in additional water output via the excreta (Vena et al., 1990; Smith et al., 2000).

Increased dietary P content resulted in a linear increase in water intake and subsequent greater excreta moisture in laying hens (Smith et al., 2000). The results of P in our model were in agreement with these findings. Decreasing dietary P levels reduces excretion of P, indicating the ability to manipulate excreta P content via diet (Ziaei et al., 2008). Increased dietary K content in laying hens (Smith et al., 2000) or reduced K retention in broilers (Ziaei et al., 2007) resulted in greater excreta moisture. In contrast, a greater excreta K level reduced excreta moisture level in our model even though the range of dietary K levels tested was small compared with the range tested by Smith et al. (2000). However, these authors did not measure the output of the minerals in the excreta, which may be a factor explaining the difference. Additionally, differences in strain or age of the laying hens may have caused variability in the response. Indeed, an increased litter moisture with increasing broiler age was demonstrated by Eichner et al. (2007) and was related to an increased water to feed ratio with age (Ziaei et al., 2008). Also, the model presented here included age as a factor affecting excreta moisture level, indicating an age dependent effect on excreta moisture. However, the effect was contradictory to Eichner et al. (2007), as in the present study increasing age reduced excreta moisture. The age data in our analysis may have been confounded by the effect of barn, where all samples collected at 17 d of age were collected from barn 2. As barn was included as a random effect variable in the models, this interaction effect was not testable.

Crude protein was included in the model and an increased nitrogen excretion was related to greater excreta moisture content. This is in line with results reported by Ferguson et al. (1998a) and Namroud et al. (2008). Elevated dietary protein content (overformulation of diets) or an imbalanced amino acid profile has been identified as a cause of wet litter (Collet, 2006; Namroud et al., 2009). The excess protein supply is catabolized and used as an energy source and the nitrogen is excreted as uric acid by birds. Increasing the dietary CP level from 17 to 23% has been shown to result in an increased amount of uric acid, nitrogen, and moisture excreted on an excreta DM basis (Namroud et al., 2009). Unabsorbed dietary nutrients (e.g., protein and carbohydrates) at the end of the small intestine are a potential substrate for the microbiota in the distal GIT (distal ileum and ceca) (Apajalahti et al., 2004). Fermentation of protein produces metabolites that are harmful for the host and increase digesta pH (Apajalahti, 2005). Increased protein content of the digesta in the hindgut can, therefore, affect microbial composition in the hindgut and affect water reabsorption. However, in a previous experiment no relation of microbial composition and excreta moisture content was observed (van der Hoeven-Hangoor et al., 2013b).

Besides known associations between excreta content of nutrients and moisture in broilers, hitherto undocumented associations were also observed. These included responses to excreta Zn content and several interactions between nutrient contents listed as follows: Ca × P, K × Cu, NDF × CP, NDF × Na, and NDF × Zn. The majority of experiments have measured the effect of increasing dietary levels of a single mineral on excreta moisture content or the ratio between Na, K, and Cl, expressed as the dietary electrolyte balance (**dEB**) (Ravindran et al., 2008). The dEB expresses the ability of Na^+ and K^+ to neutralize hydroxyl groups (OH^-) and Cl^- to neutralize hydrogen ions (H^+). Increasing dEB increases excreta moisture levels (Ahmad et al., 2009), because of increased Na (Ravindran et al., 2008) or K intake (Ahmad and Sarwar, 2006). An increase of dietary Ca or P levels did not show consistent effects on excreta moisture (Ferguson et al., 1998a; Smith et al., 2000; Ziaei et al., 2008). This may be related to the lower electrolytic capacity of divalent ions compared with monovalent ions or because of the presence of a relationship between Ca and P, as shown here. A relationship between Ca and P is known in relation to bone deposition, where both minerals are required for bone mineralization and a shortage of one mineral may result in losses of the other (Létourneau-Montminy et al., 2010). Fecal P content has been shown to rapidly increase when optimum tibia ash content is reached (Waldroup et al., 2000). A negative correlation between Na and K was reported in humans with diarrhea (fecal moisture above 80%) (Nishimuta et al., 2006), with lower K levels when Na increased, resulting in a greater fecal moisture content. Additionally, in laying hens, P increased excreta moisture especially at high dietary Na levels (Smith et al., 2000). In contrast, our model did not identify a direct interaction between Na and K or P. The apparently contradictory results for some interactions could be a result of anomaly in the different datasets used to study this relationship. Several interactive effects between minerals (e.g., Na and Zn) and NDF on excreta moisture were observed. These may be explained by ion-exchange properties of insoluble dietary fibers, where binding is dependent on the concentration of the minerals (Laszlo, 1987). Nondetergent fiber measures lignin, hemicellulose, and cellulose as major components (Van Soest et al., 1991) and was used as an indicator of insoluble fiber content. Available data are limited to in vitro studies and additional experiments are required to further explore these interactions.

Trace mineral (e.g., Mn, Zn, and Cu) concentrations in the excreta are closely related to dietary intake although their level does not affect excreta moisture (Zhong et al., 2007). In contrast, feeding increasing dietary Mg levels increased excreta moisture output in broilers (Van der Hoeven-Hangoor et al., 2013a), which was not confirmed in the present experiment. In the models, increasing Zn levels reduced excreta moisture. Zinc is well known as a diarrhea treatment in piglets (Fairbrother et al., 2005) and humans (Patel et al., 2011). The proposed mechanism is through inhibition of Cl secretion into the lumen and enhancement of Na absorption, thereby preventing water excretion because of osmotic differences between serum and digesta (Hoque et al., 2009). Additionally, effects of Zn on microbial composition throughout the intestinal tract in newly weaned piglets have been observed (Højberg et al., 2005), which may affect fermentation of undigested nutrients and subsequent water reabsorption. Indeed, caeca and mid-colon DM content was found to be increased (approximately 10 and 20%, respectively) when higher Zn levels were fed to the piglets (Højberg et al., 2005). Typically, trace minerals are added to the diet using a premix, without taking into account the trace mineral content of the macro ingredients, thereby supplying levels usually greater than specified. More in-depth evaluation of the effect of Zn on excreta moisture in broilers seems warranted.

Several nutrients such as excreta fat, soluble fiber content, starch, uric acid, and Cl content are missing in the database and were not taken into account by the model. They may have a relationship with excreta moisture although literature of the effects of these nutrients on excreta quality is limited. Except for the effect of soluble nonstarch polysaccharide content, which has been observed to increase digesta viscosity (Ouhida et al., 2000; Jiménez-Moreno et al., 2013) and concomitant increased water intake by the birds (Langhout et al., 2000), resulting in increased excreta and litter moisture content. Being part of the dEB calculation, Cl may be important to add to the database. However, no relation between Cl and litter wetness score (Murakami et al., 1997, 2001) or excreta moisture (Oviedo-Rondón et al., 2001) have been observed. Furthermore, diet characteristics (e.g., diet form, particle size, and pelleting temperature) could affect water intake or digesta composition in the hindgut and thereby change water reabsorption due to osmotic value.

Biological and automated statistical approaches were used to create 4 models to identify excreta nutrients that are associated with moisture content in broiler excreta. Increased excretion of Na, P, and Ca was shown to be associated with greater moisture excretion whereas increased K excretion was associated with lower excreta moisture. Several nutrients that have been identified previously were confirmed to affect excreta moisture content. Furthermore, hitherto undocumented excreta nutrient content (Zn) and interactions between excreta nutrient contents (NDF × CP, NDF × K, and Ca × P) that contribute to excreta moisture level were identified warranting further investigation to elaborate how they contribute to excreta moisture levels. As excreta levels of most nutrients can be manipulated by adjusting dietary levels, dietary formulation can be informed with the findings of this analysis permitting altered nutrient levels in the excreta.

LITERATURE CITED

Ahmad, T., T. Mushtaq, M. A. Khan, M. E. Babar, M. Yousaf, Z. U. Hasan, and Z. Kamran. 2009. Influence of varying dietary electrolyte balance on broiler performance under tropical summer conditions. J. Anim. Physiol. Anim. Nutr. (Berl.) 93:613–621.

Ahmad, T., and M. Sarwar. 2006. Dietary electrolyte balance: Implications in heat stressed broilers. Worlds Poult. Sci. J. 62:638–653.

Apajalahti, J. 2005. Comparative gut microflora, metabolic challenges, and potential opportunities. J. Appl. Poult. Res. 14:444–453.

Apajalahti, J., A. Kettunen, and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. Worlds Poult. Sci. J. 60:223–232.

Babyak, M. A. 2004. What you see may not be what you get: A brief, nontechnical introduction to overfitting in regression-type models. Psychosom. Med. 66:411–421.

Borges, S. A., A. V. Fischer Da Silva, J. Ariki, D. M. Hooge, and K. R. Cummings. 2003a. Dietary electrolyte balance for broiler chickens exposed to thermoneutral or heat-stress environments. Poult. Sci. 82:428–435.

Borges, S. A., A. V. Fischer Da Silva, J. Ariki, D. M. Hooge, and K. R. Cummings. 2003b. Dietary electrolyte balance for broiler chickens under moderately high ambient temperatures and relative humidities. Poult. Sci. 82:301–308.

Centraal Veevoeder Bureau. 2006. Veevoedertabel (livestock feed table). Centraal Veevoeder Bureau, Lelystad, the Netherlands.

Chaplin, M. F. 2003. Fibre and water binding. Proc. Nutr. Soc. 62:223–227.

Collet, S. R. 2006. Wet litter: Its causes and prevention and the role of nutrition. In: G. C. Perry, editor, Avian gut function in health and disease. CABI Publishing, Wallingford, UK. p. 195–209.

Collett, S. R. 2012. Nutrition and wet litter problems in poultry. Anim. Feed Sci. Technol. 173:65–75.

Cook, K. L., M. J. Rothrock Jr., M. A. Eiteman, N. Lovanh, and K. Sistani. 2011. Evaluation of nitrogen retention and microbial populations in poultry litter treated with chemical, biological or adsorbent amendments. J. Environ. Manage. 92:1760–1766.

Eichner, G., S. L. Vieira, C. A. Torres, J. L. B. Coneglian, D. M. Freitas, and O. A. Oyarzabl. 2007. Litter moisture and footpad dermatitis as affected by diets formulated on an all-vegetable basis or having the inclusion of poultry by-product. J. Appl. Poult. Res. 16:344–350.

Etheridge, R. D., R. W. Seerley, and T. L. Huber. 1984. The effect of diet on fecal moisture, osmolarity of fecal extracts, products of bacterial fermentation and loss of minerals in feces of weaned pigs. J. Anim. Sci. 58:1403–1411.

Fairbrother, J. M., E. Nadeau, and C. L. Gyles. 2005. *Escherichia coli* in postweaning diarrhea in pigs: An update on bacterial types, pathogenesis, and prevention strategies. Anim. Health Res. Rev. 6:17–39.

Ferguson, N. S., R. S. Gates, J. L. Taraba, A. H. Cantor, A. J. Pescatore, M. J. Ford, and D. J. Burnham. 1998a. The effect of dietary crude protein on growth, ammonia concentration, and litter composition in broilers. Poult. Sci. 77:1481–1487.

Ferguson, N. S., R. S. Gates, J. L. Taraba, A. H. Cantor, A. J. Pescatore, M. L. Straw, M. J. Ford, and D. J. Burnham. 1998b. The effect of dietary protein and phosphorus on ammonia concentration and litter composition in broilers. Poult. Sci. 77:1085–1093.

Groot Koerkamp, P. W. G. 1994. Review on emissions of ammonia from housing systems for laying hens in relation to sources, processes, building design and manure handling. J. Agric. Eng. Res. 59:73–87.

Groot Koerkamp, P. W. G., J. H. W. Raaben, L. Speelman, and J. H. M. Metz. 1999. Litter composition and ammonia emission in aviary houses for laying hens. Part iii, water flow to the litter through fresh droppings. J. Agric. Eng. Res. 73:363–371.

Henuk, Y. L., and J. G. Dingle. 2003. Poultry manure: Source of fertilizer, fuel and feed. Worlds Poult. Sci. J. 59:350–360.

Hocking, R. R. 1976. Analysis and selection of variables in linear regression. Biometrics 32:1–49.

Højberg, O., N. Canibe, H. D. Poulsen, M. S. Hedemann, and B. B. Jensen. 2005. Influence of dietary zinc oxide and copper sulfate on the gastrointestinal ecosystem in newly weaned piglets. Appl. Environ. Microbiol. 71:2267–2277.

Hoque, K. M., R. Sarker, S. E. Guggino, and C. M. Tse. 2009. A new insight into pathophysiological mechanisms of zinc in diarrhea. In: M. Fromm and J. D. Schulzke, editors, Molecular structure and function of the tight junction: From basic mechanisms to clinical manifestations. New York Academy of Sciences, New York, NY. p. 279–284.

International Organization for Standardization (ISO). 1993. ISO Standards catalogue. Geneva, Switzerland.

Jankowski, J., Z. Zduńczyk, J. Juśkiewicz, and P. Kwieciński. 2011. The effect of different dietary sodium levels on the growth performance of broiler chickens, gastrointestinal function, excreta moisture and tibia mineralization. J. Anim. Feed Sci. 20:93–106.

Jiménez-Moreno, E., M. Frikha, A. de Coca-Sinova, J. García, and G. G. Mateos. 2013. Oat hulls and sugar beet pulp in diets for broilers 1. Effects on growth performance and nutrient digestibility. Anim. Feed Sci. Technol. 182:33–43.

Kramer, M. 2005. R^2 statistics for mixed models. In: Proc. 17th Annual Kansas State Conference on Applied Statistics in Agriculture, Manhattan, KS. p. 148–160.

Kristensen, H. H., and C. M. Wathes. 2000. Ammonia and poultry welfare: A review. Worlds Poult. Sci. J. 56:235–245.

Langhout, D. J., J. B. Schutte, J. D. Jong, H. Sloetjes, W. A. Verstegen, and S. Tamminga. 2000. Effect of viscosity on digestion of nutrients in conventional and germ-free chicks. Br. J. Nutr. 83:533–540.

Laszlo, J. A. 1987. Mineral binding-properties of soy hull. Modeling mineral interactions with an insoluble dietary fiber source. J. Agric. Food Chem. 35:593–600.

Létourneau-Montminy, M. P., A. Narcy, P. Lescoat, J. F. Bernier, M. Magnin, C. Pomar, Y. Nys, D. Sauvant, and C. Jondreville. 2010. Meta-analysis of phosphorus utilisation by broilers receiving corn-soyabean meal diets: Influence of dietary calcium and microbial phytase. Animal 4:1844–1853.

Murakami, A. E., E. O. Oviedo-Rondón, E. N. Martins, M. S. Pereira, and C. Scapinello. 2001. Sodium and chloride requirements of growing broiler chickens (twenty-one to forty-two days of age) fed corn-soybean diets. Poult. Sci. 80:289–294.

Murakami, A. E., E. A. Saleh, J. A. England, D. A. Dickey, S. E. Watkins, and P. W. Waldroup. 1997. Effect of level and source of sodium on performance of male broilers to 56 days. J. Appl. Poult. Res. 6:128–136.

Namroud, N. F., M. Shivazad, and M. Zaghari. 2008. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. Poult. Sci. 87:2250–2258.

Namroud, N. F., M. Shivazad, and M. Zaghari. 2009. Impact of dietary crude protein and amino acids status on performance and some excreta characteristics of broiler chicks during 10–28 days of age. J. Anim. Physiol. Anim. Nutr. (Berl.) 94:280–286.

Nishimuta, M., N. Inoue, N. Kodama, E. Morikuni, Y. H. Yoshioka, N. Matsuzaki, M. Shimada, N. Sato, T. Iwamoto, K. Ohki, H. Takeyama, and H. Nishimuta. 2006. Moisture and mineral content of human feces-high fecal moisture is associated with increased sodium and decreased potassium content. J. Nutr. Sci. Vitaminol. (Tokyo) 52:121–126.

Ouhida, I., J. F. Pérez, J. Piedrafita, and J. Gasa. 2000. The effects of sepiolite in broiler chicken diets of high, medium and low viscosity. Productive performance and nutritive value. Anim. Feed Sci. Technol. 85:183–194.

Oviedo-Rondón, E. O., A. E. Murakami, A. C. Furlan, I. Moreira, and M. Macari. 2001. Sodium and chloride requirements of young broiler chickens fed corn-soybean diets (one to twenty-one days of age). Poult. Sci. 80:592–598.

Patel, A. B., M. Mamtani, N. Badhoniya, and H. Kulkarni. 2011. What zinc supplementation does and does not achieve in diarrhea prevention: A systematic review and meta-analysis. BMC Infect. Dis. 11:122.

Ravindran, V., A. J. Cowieson, and P. H. Selle. 2008. Influence of dietary electrolyte balance and microbial phytase on growth performance, nutrient utilization, and excreta quality of broiler chickens. Poult. Sci. 87:677–688.

Smith, A., S. P. Rose, R. G. Wells, and V. Pirgozliev. 2000. Effect of excess dietary sodium, potassium, calcium and phosphorus on excreta moisture of laying hens. Br. Poult. Sci. 41:598–607.

Tilea, A. M., P. L. Francis III, B. W. Gillespie, and R. Saran. 2012. Model selection using recursive macro: Enhancements to r^2 selection in proc reg. In: Proc. Midwest SAS Users Conference, Minneapolis, MN. Paper SA16-2012.

Van der Hoeven-Hangoor, E., I. B. Van de Linde, N. D. Paton, M. W. A. Verstegen, and W. H. Hendriks. 2013a. Effect of different magnesium sources on digesta and excreta moisture content and production performance in broiler chickens. Poult. Sci. 92:382–391.

van der Hoeven-Hangoor, E., J. M. B. M. van der Vossen, F. H. J. Schuren, M. W. A. Verstegen, J. E. de Oliveira, R. C. Montijn, and W. H. Hendriks. 2013b. Ileal microbiota composition of broilers fed various commercial diet compositions. Poult. Sci. 92:2713–2723.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.

Vena, V. E., T. H. Lac, and R. F. Wideman Jr. 1990. Dietary sodium, glomerular filtration rate autoregulation, and glomerular size distribution profiles in domestic fowl (*Gallus gallus*). J. Comp. Physiol., B 160:7–16.

Waldroup, P. W., J. H. Kersey, E. A. Saleh, C. A. Fritts, F. Yan, H. L. Stilborn, R. C. Crum, and V. Raboy. 2000. Nonphytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. Poult. Sci. 79:1451–1459.

Zhong, L. L., J. H. Yao, N. Cheng, Y. J. Sun, Y. R. Liu, Y. J. Wang, X. Q. Sun, and H. B. Xi. 2007. Effects of supplementing with single or multiple trace minerals on growth performance, fecal mineral excretion and nutrient utilization in pullets from 1 to 18 weeks of age. Asian-Australas. J. Anim. Sci. 20:976–982.

Ziaei, N., J. H. Guy, S. A. Edwards, P. J. Blanchard, J. Ward, and D. Feuerstein. 2007. Effect of gender on factors affecting excreta dry matter content of broiler chickens. J. Appl. Poult. Res. 16:226–233.

Ziaei, N., J. H. Guy, S. A. Edwards, P. J. Blanchard, J. Ward, and D. Feuerstein. 2008. Effect of reducing dietary mineral content on growth performance, water intake, excreta dry matter content and blood parameters of broilers. Br. Poult. Sci. 49:195–201.