

## Correlation Between Excreta Dry Matter and Nutrients in Broilers by Principal Component Analysis

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### PCA of broiler excreta composition

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Preventing wet litter is a major focus in today's poultry industry, as it can result in animal welfare problems, production losses and increased costs to negate its impact. However, this multifactorial problem is usually subjectively assessed using litter score to estimate the dry matter content of excreta. Wet litter problems occur below 65-75% dry matter and can be accurately estimated using Near Infrared Reflectance Spectrometry. Dietary factors affecting wet litter include protein content, NDF (insoluble fibre), mineral levels and ratios of various nutrients. The objective of the present study was to determine the principle components in broiler excreta "nutrient" analysis correlated to excreta DM. Two hundred eighty eight broiler excreta samples were collected during 2008 and 2009 and analysed for moisture, nitrogen (N), NDF, Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn. Excreta nutrient data were subjected to ProcGLM (SAS institute) to correct for trial variation; thereafter the residuals were subjected to Principal Component Analysis (PCA; SAS institute). Both the first ( $R^2=-0.16$ ) and fifth ( $R^2=-0.39$ ) factor had a significant negative correlation with excreta DM. Nutrients with the highest loadings from the first factor were Fe, K, Mg, Mn, NDF and Zn and in the fifth factor P and Na had the highest loading. When using a generalized linear model, results indicated that Fe and NDF increase excreta DM, whereas Ca, Na, P and Zn reduce excreta DM. Protein or nitrogen may play a less important role in explaining excreta DM than currently thought.

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### Introduction

Wet litter continues to be a major problem in the broiler (and other poultry) industry as it negatively affects bird health (e.g. Necrotic Enteritis, footpad dermatitis, coccidiosis (Francesch and Brufau, 2004), welfare and performance of poultry (Van Der Klis and Lensing, 2007). Litter is considered "wet" when the dry matter content is too low (Collet, 2006), due to cumulative moisture from urine, faeces and water spillage exceeding the amount of water that removed by evaporation. The range for accepted levels of dry matter lies between 65% (Eichner *et al.*, 2007) and 75% (Collet, 2006). A litter score is usually applied as a subjective assessment of the dry matter content of excreta. However, excreta dry matter content can be accurately estimated using Near Infrared Reflectance Spectrometry (NIRS) (De Oliveira *et al.*, 2009; Hangoor *et al.*, 2009). The term "wet litter" is not only used to describe the status of the litter in the broiler house, but is also used to describe non-specific diseases of the gastro-intestinal or urinary tract in broilers that disturb water balance and the digestion of nutrients (Collet, 2006).

The main factors affecting prevalence of wet litter are related to management and housing of birds (e.g. water and climate management, density (Mitran *et al.*, 2008; Weaver Jr and Meijerhof, 1991), disease challenge, dietary factors (Francesch and Brufau, 2004) and gut microbiota (Collet, 2006). High dietary protein levels are associated with higher moisture content of the litter (Ferguson *et al.*, 1998). When birds are fed excessive dietary protein, it is necessary for the bird to catabolise the excess protein, resulting in high levels of nitrogen excretion via the kidneys. Consequently, the water intake of broilers will increase and decrease excreta dry matter content (Elwinger and Svensson, 1996). Furthermore, rapidly fermentable dietary fibres provide substrates for bacteria to form short-chain fatty acids (SCFA) (Jamroz *et al.*, 2002), whereas slow or incomplete fermented dietary fibre reduce transit time through the gastrointestinal tract (GIT), increase faecal weight and improves laxation (Dikeman and Fahey Jr, 2006). Insoluble fibres reduce transit time through the gastro intestinal tract (GIT), improve water-holding capacity and assist in bulking of the faeces (Montagne *et al.*, 2003). In contrast, soluble fibres, such as gums, pectins, psyllium and  $\beta$ -glucans, are considered to be viscous dietary fibres and have antinutritive activities in broiler chickens (Jamroz *et al.*, 2002). Also minerals (in diet and/or water) can affect excreta moisture; sodium (Na) and potassium (K) stimulate water intake and consequently increase excreta moisture. A bird's ability to tolerate excess K is greater than for Na (Ahmad and Sarwar, 2006). The relation of Na to excreta moisture has been previously reviewed by (Francesch and Brufau, 2004). Potassium (associated with current feeding of vegetarian diets) is absorbed early in the small intestine and excreted mainly via the kidney. When feeding high protein diets, the endogenous acid production can increase acid-base balance (Ahmad and Sarwar, 2006).

The objective of the present study was to determine the principle components in broiler excreta "nutrient" analysis correlated to excreta DM.

### **Materials and Methods**

A database was selected containing the excreta data from seven different trials conducted at the Provimi research farm, The Netherlands during 2008 and 2009. The trials were conducted with Ross 308 and Hubbard Flex male broilers, and designed to test the effects of different raw materials, level of amino acids, energy and minerals, and/or the impact of enzymes on broiler performance. In total 288 broiler excreta samples were collected and analysed using standard AOAC procedures for moisture, N, NDF, Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn and expressed on a dry matter basis. Excreta nutrient data were subjected to ProcGLM (SAS institute) to correct for trial variation; corrections were made for breed (Ross or Hubbard) and housing effect. Six samples were considered outliers, based on the mean value plus or minus 2.5 times the standard deviation and removed from the data set. Subsequently, Principal Component Analysis (SAS JMP, SAS Institute Inc., Cary, North Carolina) was used to visualize the data, and to derive a small number of independent linear combinations (principal components) of the set of variables that capture as much of the variability in the original variables as possible (Jolliffe, 1986). Finally, multivariate correlations were used to explain excreta DM level using the principal components. Additionally, the direct effect of the nutrients on excreta DM were tested, using a generalized linear model (excreta DM = breed + block + Ca + Cu + Fe + K + Mg + Mn + Na + NDF + P + N + Zn).



## Results and discussion

A wide range of excreta nutrient values was available for the principal component analysis (PCA). מקור ההפניה לא נמצא. shows the mean, minimum and maximum values per nutrient component.

**Table 1:** Overview of the mean, minimum and maximum in percentage (%) or parts per million (ppm) for the analyzed excreta characteristics for broilers around 35 days of age.

Component	Mean	Minimum	Maximum
Calcium (%)	0.49	0.19	1.12
Copper (ppm)	15.8	10.4	26.8
Dry matter (%)	26.4	17.5	35.3
Iron (ppm)	181.1	110.5	287.7
Magnesium (%)	0.14	0.10	0.24
Manganese (ppm)	79.9	54.3	131.9
Neutral detergent fibre (%)	6.11	1.48	9.63
Nitrogen (%)	8.17	4.98	12.0
Phosphorus (%)	0.38	0.21	0.74
Potassium (%)	0.61	0.32	0.90
Sodium (%)	0.09	0.04	0.25
Zinc (ppm)	176.8	49.3	508.0

The first five factors extracted by PCA of the excreta characteristics together explained 74.3% of the variation in the original dataset. Following the PCA analysis, the eigenvalues of the five factors were correlated with excreta DM; both the first ( $R^2 = -0.16$ ;  $P=0.006$ ) and the fifth factor ( $R^2 = -0.39$ ;  $P<.0001$ ) had a significant negative correlation with excreta DM. The first factor explained 30.9% of the variance in the original data and the fifth factor 8.4%. Within each factor loadings of individual nutrients greater than 0.3 or lower than -0.3 were considered meaningful; from the first factor Fe (0.42), K (0.38), Mg (0.43), Mn (0.36), NDF (0.34) and Zn (0.34) and from the fifth factor P (0.77) and Na (0.55) had the highest loading and negatively affected excreta DM.

The analysis with the GLM were different to the PCA analyses and showed a negative least square estimate for Ca (-0.84;  $P=0.006$ ), Na (-5.86;  $P<.0001$ ), P (-1.64;  $P=0.001$ ) and Zn (-0.003;  $P=0.001$ ) and a positive least square estimate for Fe (0.004;  $P=0.024$ ) and NDF (0.07;  $P=0.035$ ) on excreta DM. This implies that Ca, Na, P and Zn led to wetter excreta and Fe and NDF to dryer broiler excreta.

The PCA and the GLM method were contradicting regarding the effect of Fe and NDF on excreta DM, most likely due to the modelling difference. It should be further evaluated which model fits best for the available set of data. The causal relation between Fe or Zn and excreta DM is not clear. Nitrogen had no correlation with excreta DM, even though there was a large

variation in dietary and excreta nitrogen levels. NDF is studied to a lesser extend in broilers; analysing the type of NSP fibre would further improve the dataset.

## Conclusion

The present study indicates that Fe and NDF increased excreta DM, whereas Ca, Na, P and Zn reduce excreta DM. Potassium and nitrogen may play a less important role in explaining excreta DM than currently thought.

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