Evaluation of free water and water activity measurements as functional alternatives to total moisture content in broiler excreta and litter samples

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ABSTRACT Litter moisture contents vary greatly between and within practical poultry barns. The current experiment was designed to measure the effects of 8 different dietary characteristics on litter and excreta moisture content. Additionally, free water content and water activity of the excreta and litter were evaluated as additional quality measures. The dietary treatments consisted of nonstarch polysaccharide content (NSP; corn vs. wheat), particle size of insoluble fiber (coarse vs. finely ground oat hulls), viscosity of a nonfermentable fiber (low- and high-viscosity carboxymethyl cellulose), inclusion of a clay mineral (sepiolite), and inclusion of a laxative electrolyte ($MgSO_4$). The 8 treatments were randomly assigned to cages within blocks, resulting in 12 replicates per treatment with 6 birds per replicate. Limited effects of the dietary treatments were noted on excreta and litter water activity, and indications were observed that this measurement is limited in high-moisture samples. Increasing dietary NSP content by feeding a corn-based diet (low NSP) compared with a wheat-based diet (high NSP) increased water intake, excreta moisture and free water, and litter moisture content. Adding insoluble fibers to the wheat-based diet reduced excreta and litter moisture content, as well as litter water activity. Fine grinding of the oat hulls diminished the effect on litter moisture and water activity. However, excreta moisture and free water content were similar when fed finely or coarsely ground oat hulls. The effects of changing viscosity and adding a clay mineral or laxative deviated from results observed in previous studies. Findings of the current experiment indicate a potential for excreta free water measurement as an additional parameter to assess excreta quality besides total moisture. The exact implication of this parameter warrants further investigation.

Key words: broiler, feed composition, litter moisture, excreta moisture, water activity

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INTRODUCTION

Litter moisture content in poultry production can vary greatly, ranging from 15 to 45% (Groot Koerkamp, 1994; Hayes et al., 2000; Miles et al., 2011b). The main input of water to the litter is via excreta, which is high in moisture and N (Nahm, 2003), although other inputs, such as water spillage, also contribute. Organic N present in the litter can be transformed to ammonia by bacteria and fungi (Carlile, 1984; Cook et al., 2011), where higher litter moisture levels can be accompanied by an increased ammonia production (Groot Koerkamp, 1994; Miles et al., 2011a,b,c). High levels of ammonia may impair the health of birds (Kristensen and Wathes, 2000). Water in litter is used for dissolution of solid urea and subsequent urea hydrolysis (Nahm, 2003). Additionally, microbes need water for growth (Brown, 1976). However, water in litter can be bound to solutes (protein, fibers, and electrolytes) or be present in a free form. The strength of the water binding is dependent on the type of bond (e.g., ionic, covalent, hydrogen) or enclosure in capillaries (Chaplin, 2003). As more functional alternatives to total moisture content, free water (van der Hoeven-Hangoor et al., 2013) and water activity ($\mathbf{A_w}$; Payne et al., 2007) may be used to assess the state of water in excreta and litter. Free water is defined here as the fraction of water that can be removed from the solution by a specific centrifuging force. Free water indicates the mechanical properties of water in a sample and includes the water entrapped in capillaries. Although, depending on the g-force applied during centrifuging, some water that was bound by weak hydrogen bonds can be included. Therefore, the value of free

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water can vary depending on the centrifuging speed applied. Water activity is a measure for the thermodynamics of water in a sample, related to the escaping tendency of water (Agoda-Tandjawa et al., 2013). Water activity at a given temperature is the ratio of equilibrium partial vapor pressure of water in the system to the equilibrium partial vapor pressure of pure liquid water at the same temperature (Reid, 2007). Water activity indicates the available water component (Opara et al., 1992) and correlates well with microbial growth (Scott, 1957). Therefore, A_w may be a better measure to assess excreta and litter quality than total moisture or free water content. Data on A_w in broiler litter and broiler excreta are mainly related to pathogen growth (e.g., reduced *Salmonella* growth was found at excreta A_w levels below approximately 0.85; Himathongkham et al., 1999; Payne et al., 2007).

Food ingredients (e.g., protein, starch, and cellulose) are known to differ in A_w characteristics (Labuza and Altunakar, 2007), providing potential to manipulate poultry excreta and litter A_w by dietary ingredients. Additionally, a strong relationship exists between moisture and A_w (Himathongkham et al., 1999), making ingredients that affect excreta moisture possible candidates to change excreta and litter A_w. Previous research in broilers showed that the water content of excreta can be changed by adding Mg as a laxative to the diet (van der Hoeven-Hangoor et al., 2013). However, A_w was not determined in the latter study. In contrast, clay minerals are often used as antidiarrheaics in human medicine (Carretero and Pozo, 2010). Clay minerals have a high-water-sorption capacity (i.e., absorption plus adsorption) due to their large surface area and the presence of micropores and channels (Galan, 1996). Sepiolite has been shown to reduce digesta viscosity and improved excreta scores in broilers fed high nonstarch polysaccharide (**NSP**) diets (Ouhida et al., 2000) and may be a potential candidate to change litter free water and A_w values.

Soluble NSP increase excreta and litter moisture due to an increased digesta viscosity (Ouhida et al., 2000; Jiménez-Moreno et al., 2013a) and concomitant higher water intake by the birds (Langhout et al., 2000). However, it is uncertain how dietary NSP content and viscosity of the digesta affect excreta and litter A_w, as soluble NSP are potent water binders. Conversely, insoluble NSP have a high water-holding capacity (Chaplin, 2003). Furthermore, they increase gizzard activity and subsequent digesta passage rate (Hetland et al., 2004). The effects of insoluble NSP on digestion seems to depend on particle size. Coarse oat hulls increased digesta passage rate compared with finely ground hulls (Hetland and Svihus, 2001), although coarse particles reduced excreta moisture content (Amerah et al., 2009; Jiménez-Moreno et al., 2013b). A high gizzard activity as a result of oat hull inclusion improved digestibility of starch (Amerah et al., 2009), CP, and DM (Jiménez-Moreno et al., 2013a). It is hypothesized that as a consequence of a reduced nutrient load in the hindgut, osmolality and water reabsorption are affected as well. The aim of the current study was to evaluate the effects of dietary characteristics, including NSP content (corn versus wheat), particle size of insoluble fiber (coarse versus fine grinded oat hulls), viscosity of a nonfermentable fiber (low- and high-viscous carboxymethyl cellulose), inclusion of a clay mineral (sepiolite), and inclusion of a laxative ($MgSO_4$), on moisture content and measurements of free water content and A_w of broiler excreta and litter.

MATERIALS AND METHODS

Birds and Housing

The experiment was performed in a broiler unit consisting of 1 room with 96 cages, divided into 6 rows of 16 cages. Treatments were allocated into blocks, resulting in a total of 12 blocks with 8 cages each. Five hundred seventy-six 1-d-old male Ross 308 chicks, derived from 40-wk-old broiler breeders, were purchased from a commercial hatchery (Lunteren, the Netherlands) and allocated to 8 dietary treatments across 96 cages so that each treatment was represented in each block. Each cage housed 6 birds with an initial individual chick weight of 41.0 ± 1.3 g.

The cages (50 × 50 cm) had a raised wire floor with a metal plate on top, covered with a 2-cm layer of wood shavings. Litter was prevented from spreading to adjacent cages by placing vertical plastic plates of 20 cm (in height) between the cages. Each cage was equipped with 2 adjustable nipple drinkers and a feeder that was positioned inside the cage for the first 3 d. From 3 d onward, feed was supplied via a feeder trough in front of the cage. Both feed and water were provided ad libitum throughout the study. Continuous artificial lighting was maintained for 23 h/d for the first 3 d of the experiment, 20 h/d between 4 and 7 d, and 18 h/d for the remainder of the experiment. Temperature, RH, 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 RH was set at 50% throughout the experiment. The study was approved by the Ethical Committee of the Animal Science Group of Wageningen University and Research Center, Lelystad, the Netherlands.

Experimental Diets

The 8 diets were formulated to meet the nutrient requirements of broilers (CVB, 2006) and to contain similar contents of ME (2,750 AME/kg) and apparent fecal digestible Lys, Met + Cys, Thr, and Val (Table 1). In advance of diet formulation, batches of wheat, corn, and soybean meal were reserved and analyzed for CP (Combustion, ISO 16634, Rapid N Cube, Elementar GmbH, Hanau, Germany) and Ca and P content (ICP-

Table 1. Ingredient and nutritional composition of the experimental diets

Item	Corn diet	Wheat diet
Ingredient composition, g/kg		
Corn	612.1	—
Wheat	—	606.1
Soybean meal (>48% CP)	321.9	294.2
Soya oil	16.8	50.4
Premix starter[1]	10.0	10.0
Limestone	16.7	17.2
Monocalcium phosphate	14.0	14.0
Sodiumbicarbonate	3.02	2.61
NaCl	1.80	1.95
DL-Met	2.13	1.88
L-Lys HCl	1.36	1.33
L-Thr	0.09	0.28
Calculated chemical composition, g/kg (unless otherwise noted)		
CP	205	213
Crude fat	45	64
Crude fiber	27	24
DM	878	869
Crude ash	61	61
AME_n[2] (poultry), kcal/kg	2,907	2,980
AME_n (broiler), kcal/kg	2,750	2,750
Lys	11.92	11.60
Met	5.22	4.90
Met + Cys	8.52	8.47
Thr	7.79	7.71
Trp	2.37	2.68
Ile	8.65	8.71
Arg	13.66	13.73
Val	9.61	9.61
AFD Lys[3]	10.20	10.20
AFD Met	4.83	4.52
AFD Met + Cys	7.45	7.45
AFD Thr	6.43	6.43
AFD Trp	2.07	2.33
AFD Ile	7.51	7.55
AFD Arg	12.11	12.08
AFD Val	8.16	8.16
Linoleic acid	21.73	31.01
Ca	9.68	9.68
P	6.43	6.44
Na	1.60	1.60
K	9.27	9.12
Cl	1.80	1.80
Dietary electrolyte balance, mEq	256	252

[1]Contributed per kilogram of diet: riboflavin, 4.5 mg; niacinamide, 40 mg; D-pantothenic acid, 9 mg; choline chloride, 500 mg; cyanocobalamin, 20 µg; vitamin E (DL-α-tocopheryl acetate), 30 mg; menadione, 2.3 mg; vitamin A (retinyl-acetate), 12,500 IU; cholecalciferol, 5,000 IU; biotin, 0.1 mg; folic acid, 0.5 mg; $FeSO_4 \cdot H_2O$, 147 mg; MnO_2, 100 mg; $CuSO_4 \cdot 5H_2O$, 40 mg; $ZnSO_4 \cdot H_2O$, 143 mg; Na_2SeO_3, 0.5 mg; KI, 2 mg; antioxidant (oxytrap PXN), 125 mg.

[2]Calculated according to CVB (2006).

[3]AFD = apparent fecal digestible, calculated according to CVB (2006).

AES, ISO 27085:2009, Thermo Iris Intrepid II XSP Duo, Thermo Scientific Inc., Waltham, MA). Near-infrared reflectance spectroscopy analysis (Bruker MPA, ISO 12099, Bruker Optik GmbH, Ettlingen, Germany) was used to estimate DM, crude fat, crude fiber, and crude ash content.

The first experimental diet (corn) was based on 61.2% corn, which can be considered a good digestible diet for broilers due to a low NSP content. The second experimental diet (wheat) contained 60.6% wheat to obtain a higher level of NSP. Dietary contents were adjusted with SBM, soya oil, mineral sources, and crystalline amino acids to ensure that the diets were isonutritious and isocaloric on an AME basis. Other diets had test products added to the wheat diet: whole and hammer-milled oat hulls (Dhuyvetter BvBa, Kruishoutem, Belgium) were included at 2.5% to create a coarse oat hull (**cOH**) and a fine oat hull diet (**fOH**). Particle size distribution for the coarse oat hulls was: >5.6 mm, 0%; >2.8 mm, 55%; >2.5 mm, 1%; >2.0 mm, 9%; >1.7 mm, 11%; >1.4 mm, 7%; >1.0 mm, 10%; >0.5 mm, 4%; and >0 mm, 3%. For the fine oat hulls, particle distribution was: >2.8 mm, 0%; >2.5 mm, 0%; >1.4 mm, 1%; >0.8 mm, 25%; >0.5 mm, 25%; and >0 mm, 49%, as determined by dry sieving. Furthermore, 1% carboxymethylcellulose sodium salt (**CMC**; Sigma-Aldrich, St. Louis, MO) with a low viscosity (50–200 mPa·s, 4% in H_2O; **lCMC**) and with a high viscosity (1,500–3,000 mPa·s, 1% in H_2O; **hCMC**) were added. Carboxymethylcellulose inclusion levels were as reported by Smits et al. (1997) and contained around 80 g of Na/kg. Dietary Na level was not corrected in the lCMC and hCMC diet, as it is uncertain how much of the Na from CMC is available for absorption in the GIT. Finally, 1% sepiolite (**SEP**; $Mg_2H_2Si_3O_9 \cdot xH_2O$; Sigma-Aldrich), was added as a water-adsorbent clay mineral, and, based on previous results (van der Hoeven-Hangoor et al., 2013), 1.03% magnesium sulfate ($MgSO_4 \cdot H_2O$, 20.2% Mg; **$MgSO_4$**) was added as a laxative. It was decided not to use an inert filler, as these ingredients are similar to clay minerals, one of the test components. Therefore, test products were added by exchanging wheat on an equal weight basis, without any further correction.

The ingredient composition of the experimental corn and wheat diets is presented in Table 1. Diets were pelleted with steam addition (approximately 80°C) at 2.5 mm. After production, all diets were analyzed for CP (Combustion, ISO 16634, Rapid N Cube, Elementar GmbH), crude fiber (AOCS Ba 6a-05, Ankom A 200, Ankom Technology, Macedon, NY), DM (Gavimetry, ISO 6496, Memmert UNB 500, Memmert GmbH, Schwabach, Germany), and Ca and P (ICP-AES, ISO 27085:2009. Thermo Iris Intrepid II XSP Duo, Thermo Scientific Inc.) content. Near-infrared reflectance spectroscopy analysis (Bruker MPA, ISO 12099, Bruker Optik GmbH, Ettlingen, Germany) was applied to estimate crude fat content. Chemical composition of the experimental diets is provided in Table 2.

Data Collection

Bird weights were recorded per pen at the start of the experiment (d 0) and for individual birds at 3 and 14 d of age. In addition, feed consumption for each pen was recorded on the same days as the birds were weighed. Based on BW gain and feed consumption, feed conversion ratio (**FCR**; kilograms of feed consumed per kilograms of weight gain) was calculated. Water intake was measured per cage using load cells continuously recording water bucket weights throughout the experiment. Water intake data are reported as values during the total period.

At 15 d of age, a litter sample (approximately 100 g) was taken from a spot in the middle of each cage, removing the full depth of the litter layer. Subsequently, the sample was mixed and divided into 2 subsamples for analysis of moisture content and A_w. Free water measurements were not suitable for litter samples, probably due to the relatively low moisture fraction. To facilitate excreta sampling, the litter and the rubber plate were removed from all cages, leaving the birds on the wire floor from 15 d of age onwards. On the morning of d 16, collection plates were placed underneath each cage and excreta (approximately 100 g) were collected with feathers and feed particles removed in 3 or 4 intervals of 2 h each, depending on the excreta production. Excreta were refrigerated (4°C) in between intervals, pooled per cage, weighed, and thoroughly mixed. Homogenized excreta were divided into 2 subsamples for analysis of moisture content and A_w. In addition, free water content was determined using the method described previously by van der Hoeven-Hangoor et al. (2013).

At 17 d of age, 5 birds per cage were weighed before being killed by cervical dislocation. From each bird, the jejunum (from the end of the duodenum to Meckel's diverticulum) and colon (from the ileo-cecal junction to the beginning of the cloaca) digesta contents were gently expelled by hand, collected, and pooled per segment and per cage. Digesta samples were frozen at 20°C for later DM analysis. In accordance with Kocher et al. (2000), part of the jejunum digesta was subjected to centrifugation at 12,000 × g at 4°C for 10 min, and the supernatants stored overnight at 4°C. Subsequently, viscosity was analyzed using a Brookfield DV-I+ viscometer (Brookfield Engineering Laboratories, Inc., Middleborough, MA) with a CP 40 cone at 25°C. Each sample was measured in triplicate. When shear-thinning (i.e., when viscosity decreased with increasing shear rates instead of staying the same) occurred, the sample was measured at 4 to 5 different RPM to calculate viscosity at 100 RPM. Colon digesta osmolality was measured in duplicate with an Advanced Model

Table 2. Chemical composition of the experimental diets after production

Analyzed composition, g/kg	Corn	Wheat	cOH[1]	fOH[2]	lCMC[3]	hCMC[4]	SEP[5]	$MgSO_4$[6]
CP	216	227	227	222	220	223	222	226
Crude fat	46	65	64	64	63	63	63	63
Crude fiber	20	22	29	26	22	21	22	22
DM	882	865	865	868	867	867	867	864
Ca	10.0	10.0	9.9	10.5	10.3	10.1	10.6	10.2
P	7.0	6.9	7.1	7.5	7.5	6.8	7.5	7.3

[1]cOH = coarse oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >5.6 mm, 0%; >2.8 mm, 55%; >2.5 mm, 1%; >2.0 mm, 9%; >1.7 mm, 11%; >1.4 mm, 7%; >1.0 mm, 10%; >0.5 mm, 4%; and >0 mm, 3%.

[2]fOH = milled oat hulls. Dhuyvetter BvBa. Particle size distribution: >2.8 mm, 0%; >2.5 mm, 0%; >1.4 mm, 1%; >0.8 mm, 25%; >0.5 mm, 25%; and >0 mm, 49%.

[3]lCMC = 1% carboxymethylcellulose sodium salt with a low viscosity: 50–200 mPa·s, 4% in H_2O. Sigma-Aldrich, St. Louis, MO.

[4]hCMC = 1% carboxymethylcellulose sodium salt with a high viscosity: 1,500–3,000 mPa·s, 1% in H_2O. Sigma-Aldrich.

[5]SEP = 1% sepiolite: $Mg_2H_2Si_3O_9 \cdot H_2O$. Sigma-Aldrich.

[6]$MgSO_4$ = 1.03% magnesium sulfate $MgSO_4.H_2O$ (monohydrate): 20.2% Mg. K+S Kali GmbH, Kassel, Germany.

3320 Micro-Osmometer (Advanced Instruments Inc., Norwood, MA). Osmolality was determined for the $MgSO_4$ treatment and for comparison also the corn and the wheat diet. Viscosity and A_w had to be measured as soon as possible after sampling. Due to availability of the measuring device and the time required per sample, a reduced number of replications were analyzed.

Moisture and A_w Analysis

To determine litter and excreta moisture content, samples were oven-dried at 70°C for 16 h, then subsequently ground and dried at 103°C for another 4 h. Jejunum and colon digesta were freeze-dried to determine moisture content. For both oven and freeze drying, the difference in weight before and after drying was expressed relative to total sample weight. Fresh litter and excreta A_w were measured with a HygroPalm HP23 Hand-held Indicator (Rotronic AG, Bassersdorf, Switzerland). The device uses an algorithm provided by the manufacturer to accelerate the A_w measurement and provide a result within approximately 5 min. As specified by the manufacturer, the A_w value measured with this method deviates less than 0.005 from the full equilibration measurement. A fixed volume (40 mL) for litter samples and a fixed weight (30 g) for excreta samples were placed in the sensor chamber.

Statistical Analysis

For comparison of means among the different treatments, all data were subjected to mixed model analysis using the PROC MIXED procedure in SAS (version 9.2, 2008, SAS Institute Inc., Cary, NC) according to the general linear model

$$Y_{ijk} = \mu + \tau_i + B_j + R_k + \varepsilon_{ijk},$$

where Y_{ijk} = specific response measured for each experimental unit; μ = overall mean for the specific response; τ_i = fixed effect of treatment (i = I, II, III, ..., VIII); B_j = random block effect (j = A, B, C, ..., F); R_k = random row effect (k = X or Y); and ε_{ijk} = residual error term. Pen was the experimental unit. Preplanned contrasts were used to determine significant relationships for (1) corn versus wheat, (2) wheat versus coarse oat hulls, (3) fine versus coarse oat hulls, (4) low versus high viscous CMC, (5) wheat versus SEP, and (6) wheat versus $MgSO_4$. Specific a priori identification of these contrasts negated the need to conduct exhaustive all-pairwise comparisons among treatment means, reducing exposure of the results to the associated rise in experiment-wise error rates.

Nineteen of the 48 litter A_w data points exceeded the theoretical limit of 1.0, due to variation in precision of the sensor. Therefore, values for all data points were decreased by the difference between the highest measured value and 1.0 (i.e., 0.007). Distributions of the means and residuals were examined to assess normality and homogeneity of the data (Ott and Longnecker, 2001). Litter and excreta A_w data and jejunum viscosity data were found to be non-normally distributed. Water activity data were subsequently normalized using an arc-sin square root transformation; jejunum viscosity was normalized by a natural logarithm transformation. Following transformation, it was evident that the transformed data had an improved normality profile that was sufficiently normalized, permitting further analysis (Shapiro and Wilk, 1965). For litter and excreta, A_w sample temperature was added as a covariate to the model, as A_w is dependent on the temperature at the time of measurement (Roos, 2007). For jejunum, viscosity-measuring day was added as a random variable to the model to account for variation created by measuring on 2 subsequent days.

RESULTS

Excreta and Litter Characteristics

Excreta moisture content was higher ($P = 0.044$) in birds fed the corn diet compared with birds fed the wheat diet (82.9 vs. 80.9%; Table 3). Replacing 2.5% wheat with coarse oat hulls resulted in a lower ($P = 0.035$) excreta moisture content (78.8 vs. 80.9%). Feeding the lCMC diet resulted in a higher ($P = 0.049$) excreta moisture content compared with feeding the hCMC diet (83.1 vs. 81.1%). None of the other treatments showed a significant effect on excreta moisture content.

Excreta free water content of birds fed the corn diet was higher ($P < 0.0001$) compared with birds fed the wheat diet (30.8 vs. 18.3%, Table 3). Feeding the lCMC diet resulted in a higher ($P = 0.001$) excreta free water content compared with feeding the hCMC diet (29.9 vs. 19.9%). Replacing 1.03% wheat by $MgSO_4$ resulted in a higher ($P = 0.015$) excreta free water content (25.8 vs. 18.3%). None of the other treatments showed a significant effect on excreta free water content. Excreta A_w was not affected by the dietary treatments tested (Table 4).

Litter moisture content was higher ($P = 0.0004$) in birds fed the corn diet compared with birds fed the wheat diet (56.9 vs. 44.8%; Table 3). Replacing 2.5% wheat with coarse oat hulls numerically resulted in a lower ($P = 0.069$) litter moisture (38.8 vs. 44.8%). Fine grinding of the oat hulls resulted in a higher ($P = 0.017$) litter moisture content compared with coarse grinding of oat hulls (46.8 vs. 38.8%). None of the other treatments significantly affected litter moisture content.

Feeding birds the cOH diet resulted in a lower litter A_w ($P = 0.001$) compared with feeding the wheat diet (0.973 vs. 0.994; Table 4). Grinding of the oat hulls changed litter A_w, where feeding birds the cOH diet resulted in a lower ($P = 0.043$) litter A_w compared with feeding the fOH diet (0.973 vs. 0.987). None of the other treatments showed a significant effect on litter A_w.

Table 3. Fifteen-day-old litter moisture content and excreta moisture and free water content of 16-d-old broiler chickens fed various diets[1]

Item	Excreta, %		Litter moisture, %
	Moisture	Free water	
Diet			
Corn	82.9	30.8	56.9
Wheat	80.9	18.3	44.8
cOH[2]	78.8	16.2	38.8
fOH[3]	79.4	20.9	46.8
lCMC[4]	83.1	29.9	51.9
hCMC[5]	81.1	19.9	46.6
SEP[6]	79.9	16.8	46.0
$MgSO_4$[7]	81.1	25.8	47.3
Pooled SEM	0.75	2.15	2.42
Treatment *P*-value	0.0001	<0.0001	<0.0001
P-values for preplanned contrasts			
Corn vs. wheat	0.044	<0.0001	0.0004
Wheat vs. coarse OH	0.035	0.501	0.069
Fine vs. coarse OH	0.561	0.123	0.017
Low vs. high viscous CMC	0.049	0.001	0.114
Wheat vs. sepiolite	0.285	0.645	0.725
Wheat vs. $MgSO_4$	0.851	0.015	0.457

[1]n = 12 replicates for all treatments.

[2]cOH = coarse oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >5.6 mm, 0%; >2.8 mm, 55%; >2.5 mm, 1%; >2.0 mm, 9%; >1.7 mm, 11%; >1.4 mm, 7%; >1.0 mm, 10%; >0.5 mm, 4%; and >0 mm, 3%.

[3]fOH = milled oat hulls. Dhuyvetter BvBa. Particle size distribution: >2.8 mm, 0%; >2.5 mm, 0%; >1.4 mm, 1%; >0.8 mm, 25%; >0.5 mm, 25%; and >0 mm, 49%.

[4]lCMC = 1% carboxymethylcellulose sodium salt with a low viscosity: 50–200 mPa·s, 4% in H_2O. Sigma-Aldrich, St. Louis, MO.

[5]hCMC = 1% carboxymethylcellulose sodium salt with a high viscosity: 1,500–3,000 mPa·s, 1% in H_2O. Sigma-Aldrich.

[6]SEP = 1% sepiolite: $Mg_2H_2Si_3O_9 \cdot H_2O$. Sigma-Aldrich.

[7]$MgSO_4$ = 1.03% magnesium sulfate $MgSO_4 \cdot H_2O$ (monohydrate): 20.2% Mg. K+S Kali GmbH, Kassel, Germany.

Dissection Results

Feeding the cOH diet increased relative gizzard weight by 0.46% ($P = 0.003$) compared with feeding the wheat diet, and by 0.40% ($P = 0.009$) compared with the fOH diet (Table 5). None of the other treatments showed significant effects on relative gizzard weight.

Digesta moisture content of the jejunum was lower ($P < 0.0001$) when the corn diet was fed compared with the wheat diet (81.4 vs. 83.3%; Table 5). Replacing wheat with 2.5% coarse oat hulls or with 1% SEP increased jejunum digesta moisture content by 1.2 ($P = 0.0002$) and 0.7% ($P = 0.045$), respectively. Feeding the fOH diet resulted in a lower ($P < 0.0001$) jejunum digesta moisture content compared with feeding the cOH diet (83.2 vs. 84.5%).

Feeding birds the fOH diet resulted in a lower ($P = 0.024$) colon digesta moisture content compared with feeding the cOH diet (81.6 vs. 82.5%; Table 5). Birds fed the $MgSO_4$ diet had a higher ($P = 0.001$) colon digesta moisture content compared with birds fed the wheat diet (83.5 vs. 82.0%). None of the other treatments showed significant effects on digesta moisture content of the jejunum or colon.

Jejunum digesta viscosity was higher ($P < 0.0001$) in birds fed the wheat diet compared with birds fed the corn diet (2.56 and 1.89 mPa·s; Table 5). Feeding the hCMC diet resulted in a higher ($P < 0.0001$) jejunum digesta viscosity compared with feeding the lCMC diet (3.87 vs. 3.16 mPa·s). None of the other treatments showed significant effects on jejunum digesta viscosity.

Osmolality of the colon supernatant was not affected ($P = 0.42$) by feeding the wheat compared with the corn diet (Table 6). Feeding the $MgSO_4$ diet numerically resulted in a higher ($P = 0.099$) colon osmolality compared with feeding the wheat diet (397 vs. 372 mOsm/kg).

Production Performance

Mortality rate was low, at 0.35% (data not shown). Average BW at 14 d of age (442 g) was in line with Aviagen Ross 308 performance objectives (481 g). Production performance results are presented in Table 7.

Feeding the corn diet increased ADG by 20.8% ($P < 0.0001$), ADFI by 18.7% ($P < 0.0001$), and reduced FCR by 1.7% ($P = 0.0005$) compared with feeding the wheat diet. Replacing part of the wheat with coarse oat hulls, SEP, or $MgSO_4$ had no effect on production performance. Feeding the cOH diet numerically deteriorated ($P = 0.090$) FCR by 0.9% compared with the fOH diet. Birds fed the hCMC diet had a 7.1% higher ($P = 0.010$) ADG and 7.8% higher ($P = 0.006$) ADFI compared with birds fed the lCMC diet.

Table 4. Water activity (A_w) in 15-d-old litter and excreta of 16-d-old broiler chickens fed various diets, including sample temperature as a covariable[1]

	Excreta water activity		Litter water activity	
Item	Mean[2]	95% CI[3]	Mean[2]	95% CI[3]
Diet				
Corn	0.979	0.96 –0.990	0.994	0.988–0.998
Wheat	0.979	0.962–0.990	0.994	0.987–0.998
cOH[4]	0.979	0.962–0.990	0.973	0.960–0.983
fOH[5]	0.978	0.961–0.990	0.987	0.978–0.994
lCMC[6]	0.979	0.964–0.991	0.992	0.985–0.997
hCMC[7]	0.979	0.962–0.991	0.989	0.980–0.995
SEP[8]	0.980	0.963–0.991	0.993	0.985–0.998
$MgSO_4$[9]	0.980	0.964–0.991	0.994	0.988–0.999
Treatment *P*-value	0.417	—	0.014	—
Sample temperature *P*-value	0.097	—	0.006	—
P-values for preplanned contrasts				
Corn vs. wheat	0.697	—	0.872	—
Wheat vs. coarse OH	0.873	—	0.001	—
Fine vs. coarse OH	0.351	—	0.043	—
Low vs. high viscous CMC	0.534	—	0.521	—
Wheat vs. sepiolite	0.343	—	0.821	—
Wheat vs. $MgSO_4$	0.121	—	0.851	—

[1]n = 6 replicates for all treatments.

[2]Backtransformed arc-sin square root least squares means.

[3]Backtransformed 95% confidence interval provided instead of SEM.

[4]cOH = coarse oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >5.6 mm, 0%; >2.8 mm, 55%; >2.5 mm, 1%; >2.0 mm, 9%; >1.7 mm, 11%; >1.4 mm, 7%; >1.0 mm, 10%; >0.5 mm, 4%; and >0 mm, 3%.

[5]fOH = milled oat hulls. Dhuyvetter BvBa. Particle size distribution: >2.8 mm, 0%; >2.5 mm, 0%; >1.4 mm, 1%; >0.8 mm, 25%; >0.5 mm, 25%; and >0 mm, 49%.

[6]lCMC = 1% carboxymethylcellulose sodium salt with a low viscosity: 50–200 mPa·s, 4% in H_2O. Sigma-Aldrich, St. Louis, MO.

[7]hCMC = 1% carboxymethylcellulose sodium salt with a high viscosity: 1,500–3,000 mPa·s, 1% in H_2O. Sigma-Aldrich.

[8]SEP = 1% sepiolite: $Mg_2H_2Si_3O_9 \cdot xH_2O$. Sigma-Aldrich.

[9]$MgSO_4$ = 1.03% magnesium sulfate $MgSO_4 \cdot H_2O$ (monohydrate): 20.2% Mg. K+S Kali GmbH, Kassel, Germany.

Average daily water intake increased ($P = 0.0001$) by 17.2% when feeding the corn diet compared with the wheat diet. None of the other treatments had significant effects on average daily water intake. The ratio of water to feed intake was not affected by the experimental diets tested.

DISCUSSION

The dietary treatments, which consisted of various factors including modulating the NSP content (corn versus wheat), particle size of insoluble fiber (coarsely versus finely ground oat hulls), clay mineral (sepiolite), viscosity of a nonfermentable fiber (low- and high-viscosity carboxymethyl cellulose), and a laxative electrolyte ($MgSO_4$), changed broiler excreta moisture, excreta free water, and litter moisture content. However, only small effects of the different treatments on excreta and litter A_w were observed. The latter is likely related to the high values of A_w measured, which were all close to the maximum A_w value of 1.0. The moisture content of excreta measured in this experiment at 16 d of age was high (mean value of 80.9%) compared with a moisture content of 63.5% measured in 18-d-old broilers by Jiménez-Moreno et al. (2013a) and 70.6% measured in 14-d-old broilers by van der Hoeven-Hangoor et al. (2013). Given the strong relationship between moisture and A_w (Himathongkham et al., 1999; Hayes et al., 2000), this probably resulted in the high excreta A_w values measured in this experiment. Also litter moisture content was high (mean value of 47.4%), which resembles values measured in caked litter areas (Sistani et al., 2003). Indeed, litter caking was observed at the end of the experiment. The high moisture content of the litter may also be related to the housing conditions. Six birds were housed per 50- × 50-cm cage, with the feeder on one side of the cage and the drinking nipples on the other side. In practice, areas around drinkers are usually higher in moisture (Miles et al., 2013) and show more frequent caking of the litter (Miles et al., 2008) compared with other parts of the barn. The design of the experimental cages may, therefore, have interfered with the litter conditions. Still, Hayes et al. (2000) also found that 74.4% of the samples had an A_w greater than 0.90 and 72.1% of the samples had litter moisture contents above 30% in 86 commercial poultry houses; only at litter moisture content below 26% did they find A_w to decrease rapidly. With the A_w values as observed in the current experiment (mean value of 0.99 for both litter and excreta), no effect on microbial

Table 5. Relative gizzard weight, jejunum and colon digesta moisture content, and jejunum digesta viscosity of 17-d-old broiler chickens fed various diets

Item	Relative gizzard weight,[1] %	Digesta moisture,[1] %		Jejunum viscosity,[2] mPa·s	
		Jejunum	Colon	Mean[3]	95% CI[4]
Diet					
Corn	1.95	81.4	82.0	1.89	1.54–2.30
Wheat	2.06	83.3	82.0	2.56	2.09–3.13
cOH[5]	2.52	84.5	82.5	2.69	2.21–3.27
fOH[6]	2.12	83.2	81.6	2.62	2.14–3.20
lCMC[7]	2.06	83.3	82.9	3.16	2.59–3.87
hCMC[8]	1.97	83.4	82.7	3.87	3.17–4.73
SEP[9]	1.95	83.9	82.2	2.44	1.99–2.98
$MgSO_4$[10]	1.91	83.7	83.5	2.42	1.98–2.96
Pooled SEM	0.11	0.27	0.37		
Treatment *P*-value	0.003	<0.0001	0.001	<0.0001	
P-values for preplanned contrasts					
Corn vs. wheat	0.465	<0.0001	0.939	<0.0001	
Wheat vs. coarse OH	0.003	0.0002	0.246	0.223	
Fine vs. coarse OH	0.009	<0.0001	0.024	0.511	
Low vs. high viscous CMC	0.582	0.635	0.748	<0.0001	
Wheat vs. sepiolite	0.457	0.045	0.653	0.227	
Wheat vs. $MgSO_4$	0.309	0.165	0.001	0.167	

[1]n = 12 replicates for all treatments.

[2]n = 9 replicates for all treatments.

[3]Geometric least squares means.

[4]Backtransformed 95% CI provided instead of SEM.

[5]cOH = coarse oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >5.6 mm, 0%; >2.8 mm, 55%; >2.5 mm, 1%; >2.0 mm, 9%; >1.7 mm, 11%; >1.4 mm, 7%; >1.0 mm, 10%; >0.5 mm, 4%; and >0 mm, 3%.

[6]fOH = milled oat hulls. Dhuyvetter BvBa. Particle size distribution: >2.8 mm, 0%; >2.5 mm, 0%; >1.4 mm, 1%; >0.8 mm, 25%; >0.5 mm, 25%; and >0 mm, 49%.

[7]lCMC = 1% carboxymethylcellulose sodium salt with a low viscosity: 50–200 mPa·s, 4% in H_2O. Sigma-Aldrich, St. Louis, MO.

[8]hCMC = 1% carboxymethylcellulose sodium salt with a high viscosity: 1,500–3,000 mPa·s, 1% in H_2O. Sigma-Aldrich.

[9]SEP = 1% sepiolite: $Mg_2H_2Si_3O_9{\cdot}H_2O$. Sigma-Aldrich.

[10]$MgSO_4$ = 1.03% magnesium sulfate $MgSO_4{\cdot}H_2O$ (monohydrate): 20.2% Mg. K+S Kali GmbH, Kassel, Germany.

activity was expected. For *Salmonella*, no growth inhibition was observed by Himathongkham et al. (1999), Hayes et al. (2000), or Payne et al. (2007) at excreta A_w levels above 0.85, whereas A_w levels below 0.85 reduced growth. These findings indicate measuring A_w is less suitable for high-moisture substrates, such as excreta and litter, and had no discriminant ability in the current experimental setup.

To assess the effect of dietary NSP content, and hence the digestibility of the diet, a low-NSP, corn-based diet was compared with a higher NSP wheat-based diet.

Table 6. Colon osmolality of 17-d-old broiler chickens fed 3 experimental diets[1]

Item	Colon osmolality, mOsm/kg
Diet	
Corn	384
Wheat	372
$MgSO_4$[2]	397
Pooled SEM	12.6
Treatment *P*-value	0.246
P-values for preplanned contrasts	
Corn vs. wheat	0.420
Wheat vs. $MgSO_4$	0.099

[1]n = 9 replicates for all treatments.

[2]$MgSO_4$ = 1.03% magnesium sulfate $MgSO_4{\cdot}H_2O$ (monohydrate): 20.2% Mg. K+S Kali GmbH, Kassel, Germany.

In line with expectations, the birds fed the corn diet had superior ADG, ADFI, and FCR compared with birds fed the wheat diet (Jørgensen et al., 1996; Knudsen, 1997; Jia et al., 2009). Interestingly, however, litter moisture increased by 12.1% in the corn diet compared with the wheat diet. In line with litter moisture, excreta moisture increased by 2.0% and excreta free water content by 12.6% when the corn diet was fed compared with the wheat diet. The results from the current experiment were opposite to expectations, as increasing soluble NSP content increased fecal moisture content in dogs (Twomey et al., 2003) and in broilers (Jiménez-Moreno et al., 2013a). It is hypothesized that as a result of the high feed intake of the birds fed the corn diet, the transit time decreased, reducing the time for water reabsorption in the hindgut. The very high feed intake by the corn-fed birds resulted in a significantly higher excreta production (40.4 and 31.3 g/h for the corn and wheat diets, respectively; data not shown), which in combination with a higher excreta moisture content resulted in a higher litter moisture addition in the corn treatment. In agreement with this hypothesis, a relationship between increased passage rate and higher feed intake (Almirall and Esteve-Garcia, 1994) and a strong relationship (r = 0.83) between transit time and fecal DM weight (Wiggins, 1984) have been previously observed. These findings suggest that besides digest-

Table 7. Body weight, ADG, ADFI, average daily water intake (ADWI), feed conversion ratio (FCR), and water-to-feed ratio (WFR) of broiler chickens fed various diets[1]

Item	BW to 14 d, g	0 to 14 d of age				
		ADG, g	ADFI, g	ADWI, g	FCR	WFR
Diet						
Corn	512	33.6	39.4	112.4	1.172	2.85
Wheat	431	27.8	33.2	95.9	1.193	2.89
cOH[2]	428	27.7	33.3	91.3	1.201	2.74
fOH[3]	440	28.5	33.9	97.5	1.191	2.88
lCMC[4]	415	26.8	31.7	97.3	1.185	3.07
hCMC[5]	442	28.7	34.2	99.8	1.191	2.92
SEP[6]	429	27.8	32.9	96.9	1.186	2.94
$MgSO_4$[7]	435	28.2	33.5	97.5	1.188	2.91
Pooled SEM	9.20	0.65	0.79	3.05	0.005	0.08
Treatment *P*-value	<0.0001	<0.0001	<0.0001	0.0004	0.0008	0.169
P-values for preplanned contrasts						
Corn vs. wheat	<0.0001	<0.0001	<0.0001	0.0001	0.0005	0.689
Wheat vs. coarse OH	0.820	0.826	0.947	0.263	0.172	0.149
Fine vs. coarse OH	0.280	0.263	0.456	0.134	0.070	0.180
Low vs. high viscous CMC	0.010	0.010	0.006	0.563	0.345	0.176
Wheat vs. sepiolite	0.896	0.945	0.752	0.819	0.202	0.605
Wheat vs. $MgSO_4$	0.648	0.602	0.745	0.708	0.337	0.834

[1]n = 12 replicates for all treatments.

[2]cOH = coarse oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >5.6 mm, 0%; >2.8 mm, 55%; >2.5 mm, 1%; >2.0 mm, 9%; >1.7 mm, 11%; >1.4 mm, 7%; >1.0 mm, 10%; >0.5 mm, 4%; and >0 mm, 3%.

[3]fOH = milled oat hulls. Dhuyvetter BvBa, Kruishoutem, Belgium. Particle size distribution: >2.8 mm, 0%; >2.5 mm, 0%; >1.4 mm, 1%; >0.8 mm, 25%; >0.5 mm, 25%; and >0 mm, 49%.

[4]lCMC = 1% carboxymethylcellulose sodium salt with a low viscosity: 50–200 mPa·s, 4% in H_2O. Sigma-Aldrich, St. Louis, MO.

[5]hCMC = 1% carboxymethylcellulose sodium salt with a high viscosity: 1,500–3,000 mPa·s, 1% in H_2O. Sigma-Aldrich.

[6]SEP = 1% sepiolite: $Mg_2H_2Si_3O_9.H_2O$. Sigma-Aldrich.

[7]$MgSO_4$ = 1.03% magnesium sulfate $MgSO_4{\cdot}H_2O$ (monohydrate): 20.2% Mg. K+S Kali GmbH, Kassel, Germany.

ibility, transit time may be important also for excreta moisture output.

The effect of adding insoluble fibers with different fiber particle sizes was evaluated by replacing 2.5% wheat in the wheat diet by coarse oat hulls. The cOH diet reduced litter A_w and numerically reduced litter moisture content by 6.0% compared with feeding the wheat diet. Additionally, in line with Jiménez-Moreno et al. (2013a), feeding the cOH diet reduced excreta moisture by 2.1% compared with the wheat diet, whereas excreta free water was not affected. Nonetheless, the absolute A_w value of 0.973 for the cOH diet is still too high to expect any effect on microbial growth in the litter. In line with findings by Amerah et al. (2009), the effect of the cOH diet on litter moisture content and litter A_w disappeared when the oat hulls were finely ground. Conversely, no effect of particle size of the oat hulls was observed on excreta moisture or excreta free water content. Birds fed the fOH diet had similar excreta water content compared with the cOH diet. Litter moisture observations were more consistent and indicate a potential for coarse oat hulls to improve litter quality parameters.

Our aim was to change digesta viscosity by feeding a high- or low-viscous CMC and measure its effect on excreta and litter quality parameters. Higher digesta viscosity increases moisture content of the excreta (van der Klis et al., 1993). In line with findings by Ouhida et al. (2000), van der Klis et al. (1993), and Smits et al. (1997), a small but significant increase from 3.16 to 3.87 mPa·s in jejunum viscosity was found between birds fed the low- or high-viscous CMC. Changing digesta viscosity had no effect on litter moisture content or on litter A_w. Although, feeding the lCMC diet increased excreta moisture content by 2.0% and excreta free water content by 10.0% compared with feeding the hCMC diet. The lower feed intake of the lCMC birds lowered excreta production compared with the hCMC birds (30.0 vs 32.0 g/h, data not shown), resulting in a lower total moisture addition to the litter compared with the hCMC diet. The lower excreta free water content of birds fed the hCMC is potentially related to water being trapped in a gel matrix in the more viscous digesta (Hetland et al., 2004). This limits release of the water after applying a centrifugal force. Similarly, in line with Ouhida et al. (2000) and Jia et al. (2009), lower excreta free water content was observed in birds fed the wheat diet compared with the corn diet and coincided with a higher jejunum digesta viscosity in the wheat-fed birds. These results show that higher digesta viscosity reduces excreta free water content, although the exact mechanism is not fully understood at this point. Our findings support the hypothesis that excreta free water determination provides a different quality measurement compared with total excreta water content.

In contrast to observations by Ouhida et al. (2000), adding 1.0% clay mineral with a high-water absorbent capacity (SEP) to the wheat-based diet had no effect on litter or excreta moisture content and A_w, or excreta free water content. No clear explanation for this discrepancy is apparent. Also in contrast with previous observations (Lee and Britton, 1987; Ikarashi et al., 2011; van der Hoeven-Hangoor et al., 2013) was the absence of an effect on excreta or litter moisture of adding a laxative ($MgSO_4$). The absence may have been related to the high average moisture content in the current experiment (80.9%), which was higher compared with that of our previous experiment (70.6%), and may have limited the effect of the laxative. However, in line with previous observations (van der Hoeven-Hangoor et al., 2013), $MgSO_4$ increased excreta free water content by 7.6% and colon digesta moisture content by 1.4%. No change in water intake or WFR was observed, although a numerically higher colon digesta osmolality was measured in the $MgSO_4$ fed birds compared with the wheat-fed birds. The latter results are most likely indicative for reduced water reabsorption in the hindgut. An inverse linear relationship between osmolality and net fluid absorption in the small intestine of pigs has been observed by Kiers et al. (2006). Again, the difference in response between excreta moisture and excreta free water results is indicative of free water determination being a different quality measurement to assess excreta quality.

In summary, the results of the current experiment show limited effects of dietary NSP content, particle size of insoluble fiber, viscosity, clay mineral inclusion, or laxative capacity on excreta or litter A_w. Additionally, limitations of A_w measurements in high-moisture samples are shown. Excreta and litter moisture content increased when a corn-based diet (low NSP) compared with a wheat-based diet (high NSP) was fed, which indicates that transit time affects moisture intake and excretion. The effect of transit time on water reabsorption warrants further investigation. Coarse oat hulls reduced excreta and litter moisture content and litter A_w compared with feeding a wheat-based diet. The importance of particle size was confirmed for litter moisture and A_w, as fine grinding of the oat hulls diminished this effect. Free water was affected by changing viscosity and by adding a laxative, supporting the hypothesis that free water determination provides a different quality measurement to assess excreta quality. The relevance of this measurement in practical situations warrants additional investigation.

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