

Effect of pellet quality and biochar litter amendment on male turkey performance

K. R. Flores, A. Fahrenholz, and J. L. Grimes¹

Prestage Department of Poultry Science, North Carolina State University, Raleigh 27695-7608, USA

ABSTRACT Bedding (litter) is essential to poultry performance and health and can have an environmental impact after use in the poultry facility such as a soil amendment or as an alternative energy source. Pine shavings are the most common bedding used for turkey production. However, the increase in its price and its increasing scarcity in some areas have created new research opportunities for reusing litter as bedding. Improvement in feed pellet quality has been reported to improve poultry performance. However, the reports for turkeys are limited and dated. This study's objective was to determine how the improvement of feed pellet quality and the use of biochar added to a combination of used turkey brooder house litter and *Miscanthus* grass as bedding affects turkey performance, small intestine morphology, and ammonia production. Nicolas Select (Aviagen Turkeys, Lewisburg, WV) male poults (816) were randomly assigned to 48 concrete litter floor pens on the day of hatch. The experiment used a completely randomized block design with a 2×4 factorial arrangement of treatments: 2 levels of fines in the feed and 4 bedding treatments. The bedding treatments were

a constant level of used turkey brooder house litter combined with a varying combination of biochar and *Miscanthus* grass. Turkey's body weight (**BW**), body weight gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) were determined. Differences in treatment means were considered to be statistically significant at $P \leq 0.05$ using a mixed model in SAS 9.4. Turkeys fed the feed with improved pellet quality had a higher BW from 3 to 17 wk (17.0 ± 0.1 kg) than turkeys fed an increased abundance of fines (16.72 ± 0.1 kg). Turkeys fed feed with increased pellet quality had a lower FI (45.6 vs. 48.1 ± 0.4 kg) and improved FCR (2.20 vs. 2.31 ± 0.01) from 0 to 20 wk. Litter treatment with 20% biochar resulted in higher BW at 20 wk (20.91 ± 0.16 kg) because of increased BWG at 11 wk over the rest of the biochar levels (3.7 ± 0.1 kg). Strategies to reduce the abundance of fines in feed through feed formulation, feed manufacturing, feed transport, and in-house feed management should be considered to increase male turkeys' performance. There may be opportunities to use biochar as a litter amendment to improve turkey health and performance.

Key words: turkey, feed-fine, pellet quality, biochar, miscanthus grass

2021 Poultry Science 100:101002
<https://doi.org/10.1016/j.psj.2021.01.025>

INTRODUCTION

Animal feed starts as separate ingredients, some of which are reduced in particle size (i.e., corn grinding) at the feed mill. Ingredients are then mixed to achieve a diet that can provide for estimated animal nutritional requirements. These ingredients are processed and presented to the animal in a mash, pelleted, or crumbled form. Generally, feed that has been pelleted, or pelleted

and then crumbled, results in improved poultry performance and decreased feed and nutritional wastage when compared with animals fed mashed diets (Lanson and Smyth, 1955; Calet, 1965; Nir et al., 1995; Amerah et al., 2007a, 2007b; Zang et al., 2009; Dozier et al., 2010; Selle et al., 2010; Serrano et al., 2012, 2013). One of the benefits of pelleting is the higher density of nutrients in the pelleted feed than mashed feed (Jensen, 2000). However, this advantage could be lost when pelleted feed is degraded and the amount of fines in the feed increases. The degrading of pellets to fines can also increase feed wastage and thus a nutritional wastage (Abdollahi et al., 2013). Mechanical forces generate fines from pellets after the pellets have been formed. Fines can be generated in mill augers, during transport and bin storage, and during passage through feeder systems depending on the feed formulation and pellet mill

© 2021 Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received November 7, 2020.

Accepted January 8, 2021.

Funding: Funding sources had no role in any aspect of the preparation of this article.

¹Corresponding author: jgrimes@ncsu.edu

throughput and conditioning temperature. Therefore, pellets need to have some essential physical quality to withstand the rigors of transportation (Thomas and van der Poel, 1996). Pellet durability is considered an accepted measurement to quantify this physical quality. The higher the pellet durability, the lower the percentage of fines generated from the feed mill to the farm and the lower the abundance of fines encountered at the feeder.

Birds willprehend feed particles according to their beak size and mechanoreceptors in the beak (Moran, 1982). An increase in birds' beak size increases the particle size of the feed it canprehend. Consequently, beak conditioning (upper beak tip trimming) could significantly affect birds' feeding by decreasing the beak's size and the number of beak mechanoreceptors. The limitation would be on small feed particles that require more dexterity or a specific beak size and form. The beak conditioning of turkey poults is a common practice. As turkeys grow, their beaks also grow. The beak allows turkeys toprehend bigger pellets and whole grains. However, birds with conditioned beaks may have a more significant limitation onprehending feed presented as fines vs. pellets. Reducing feed fines is beneficial through the feed phases as the birds grow. Reducing fines in the feed is vital in the finisher feed phases where feed intake (FI) increases and pellet quality decreases because of an increase in pelleting output at the feed mill to supply the increased FI per bird. Higher feed wastage can be predicted when birds are presented with feed containing a higher abundance of fines affecting FI, body weight, and feed conversion ratio (FCR).

The negative impact of high levels of feed fines on bird performance has led to reduced feed-fines becoming an objective of feed production. The abundance of feed fines can be decreased by minipelleting or micropelleting the starter feed phase (Flores et al., 2020). Both feed milling techniques reduce the abundance of fines in the feed, which has been found to improve turkey performance at 3 wk (Flores et al., 2020). However, minipelleting feed requires particular pellet mill dies and, therefore, a monetary, time, and space investment. Other approaches can be sought to reduce the abundance of fines in the feed presented to the bird. The use of feed additives (e.g., feed binders), feed formulation changes (e.g., increased wheat in a formulation), and feed milling management (e.g., throughput and conditioning temperature of the pellet mill) are some strategies that could reduce the abundance of fines in the feed. In this study, screening the feed for specific particle sizes of crumbles and pellets was used to reduce the abundance of fines in the feed. Screening the feed was the same strategy used by Flores et al. (2020) in poults reared to 3 wk. However, data are needed to determine the effect of the abundance of feed-fines on performance of turkeys reared to market age.

Bedding provides birds with a proper medium to grow, including watering, feeding, and provision of other management practices (Monira et al., 2003). Once bedding has been used, it is generally referred to as litter, a

mixture of bedding, feces, feathers, waste feed, and so on. Consequently, bedding (and resulting litter) quality can potentially impact the performance of the bird. These include bird-to-litter interactions, moisture management, ammonia control, and management. Alternative beddings can impact subsequent use of litter, including fertilizer or energy production or even reuse as bedding for rearing a subsequent flock of birds. Amending poultry litter with alternate material may reduce bedding costs and alleviate the adverse effects of used built-up litter on bird performance. *Miscanthus* is a genus of tall and fast-growing grasses native to Southeast Asia which are being used to generate biomass-based energy (Williams and Douglas, 2011). In addition, chopped *Miscanthus giganteus* is a proven alternate bedding, and biochar has possibilities as a litter amendment (Ritz et al., 2011; Linhoss et al., 2019). *Miscanthus* grass has been used as a cosubstrate in the anaerobic digestion of chicken manure to generate energy and as a waste treatment before land applications (Li et al., 2017). It also has been tested as a total replacement for pine shavings for rearing commercial male turkeys with no negative impacts on performance (Evans et al., 2019). Biochar, a product of pyrolysis of organic matter, reduces ammonia emission when poultry litter is composted (Agyarko-Mintah et al., 2017; Janczak et al., 2017; Liu et al., 2017), and it increases soil pH, organic matter, and nutrients when applied on land (Revell et al., 2012a, 2012b; Hass et al., 2012; Abbasi and Anwar, 2015; Brantley et al., 2016). It has shown no detrimental effect on performance, foot scoring, or health of broilers while increasing the litter water retention capacity (Ritz et al., 2011; Linhoss et al., 2019).

Therefore, it was hypothesized that turkeys provided feed with reduced abundances of feed-fines (RFF) to market age would have increased body weight (BW), increased FI, and improved FCR compared with birds provided feed with increased abundance of feed fines (IFF). It was also hypothesized that litter amended with biochar would improve turkey performance and decrease litter ammonia emissions. This study's objectives were to determine the effect of feeding high-quality pellets and using biochar and *Miscanthus* grass as a litter amendment to once used turkey brooder house litter on the performance of male Large White commercial turkeys.

MATERIALS AND METHODS

Treatments and Experimental Design

The experimental design was a completely randomized block design with a 2 × 4 factorial arrangement of treatments with 2 levels of fines in the feed and 4 levels of biochar + *Miscanthus* grass in the bedding. There were 6 replicate pens of birds per individual treatment in a 48-pen house. The birds were fed the same formulations in 6 feed phases. The feeds were either screened to reduce the abundance of fines (RFF) or not screened,

resulting in an IFF. There were 4 bedding (litter) treatments with varying biochar and *Miscanthus* grass levels added to a constant level of once used turkey brooder house litter (70% of litter mix by weight). The varying biochar and grass levels consisted of biochar at 0, 5, 10, or 20% and *Miscanthus* grass at 30, 25, 20, or 10%, respectively. Therefore, the 4 litter treatments were control (70% used brooder litter, 0% biochar, and 30% *Miscanthus* grass), 5% biochar (70% used brooder litter, 5% biochar, and 25% *Miscanthus* grass), 10% biochar (70% used brooder litter, 10% biochar, and 20% *Miscanthus* grass), and 20% biochar (70% used brooder litter, 20% biochar, and 10% *Miscanthus* grass). The once used turkey brooder house litter was obtained from a local North Carolina turkey company. The *Miscanthus* grass and biochar were provided by NR3, LLC (Magnolia, NC 27408). The *Miscanthus* grass was raised locally in North Carolina and proprietarily harvested, including finely chopped during harvest after drying naturally in the field. The harvest moisture was 12 to 15%. The biochar was produced from a portion of the *Miscanthus* grass using a proprietary process including anaerobic conditions at 400°C.

Turkey Source, Housing, and Management

This study was conducted in a curtain-sided house with 48 concrete floor pens (5.946 m² per pen) with 17 birds per pen. Nicholas Select (Aviagen Turkeys, Lewisburg, WV) male poults (816) were obtained from a commercial hatchery and were placed on the day of hatch.

Poults received typical commercial poult services at the hatchery, including beak tip conditioning, and were weighed by pen group at placement, 14, and 21 d. Turkeys were weighed individually at 5, 8, 11, 14, 17, and 20 wk. The weight of each pen of birds plus culls and mortalities was used to determine the FCR. The birds were fed and watered ad libitum throughout the study. All animal-handling procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee.

Feed Manufacturing

The North Carolina State University Feed Mill Educational Unit manufactured all feed rations and feed treatments. One standard basal formulation was used per feed phase (Tables 1 and 2) for the 2 feed treatments with nutrient analyses provided in Table 3. Both feed treatments were from the same batch mixes and expected to have the same nutrient composition before screening. The starter 1 feed was blended in a counterpoise ribbon mixer (Model TRDB1260960; Hayes & Stolz, Fort Worth, TX), conditioned in a single pass conditioner (Model C18LL4/F6; California Pellet Mill, Crawfordsville, IN), pelleted in a 30-horsepower pellet mill (Model PM1112-2; Crawfordsville, IN) using a 11/64" × 13/8" pellet mill die. Feed pellets were cooled in a counterflow cooler (Model VK09X09 KL; Geelen counterflow USA, INC, Orlando, FL) and crumbled by using a Roskamp crumbler (Model 624S; Roskamp Champion, Waterloo, IA). Half of the batch (RFF) was screened

Table 1. Experimental feed program¹: feed composition (% by weight).

Ingredient	Starter 1	Starter 2	Grower 1	Grower 2	Finisher 1	Finisher 2
Corn	18.60	22.00	26.20	34.30	40.70	44.50
Wheat	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal	38.00	35.00	30.00	22.50	17.00	13.40
Poultry meal	10.00	10.00	10.00	10.00	10.00	10.00
Fat	7.03	7.03	8.09	8.08	8.05	8.08
Limestone	1.80	1.65	1.58	1.45	1.10	1.08
Monocalcium ²	2.55	2.35	2.25	2.00	1.50	1.35
Salt	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix ³	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin mix ⁴	0.20	0.20	0.20	0.20	0.20	0.20
Selenium mix	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20
Lysine ⁵	0.45	0.44	0.41	0.33	0.33	0.30
Methionine ⁶	0.45	0.43	0.38	0.30	0.28	0.25
Sodium bicarbonate	0.13	0.13	0.13	0.13	0.13	0.13
Threonine	0.15	0.13	0.13	0.08	0.08	0.08
Ingredient total	100.00	100.00	100.00	100.00	100.00	100.00
Feed per bird (kg) ⁷	2.73	5.45	8.18	11.36	13.64	15.91

¹Both reduced abundances of feed-fines (RFF) and increased abundance of feed fines (IFF) feeds shared the same ingredient composition.

²Monocalcium phosphate.

³Mineral premix provided the following per kg of diet: 120 mg manganese; 120 mg zinc; 80 mg iron; 10 mg copper; 2.4 mg iodine; 1 mg cobalt.

⁴Donated by DSM Nutritional Products; Vitamin premix provided the following per kg of diet: 26,455 IU vitamin A; 7937 IU vitamin D3; 132 IU vitamin E; 0.08 mg vitamin B12; 0.51 mg biotin; 8 mg menadione; 8 mg thiamine; 26 mg riboflavin; 44 mg pantothenic acid; 16 mg vitamin B6; 220 mg niacin; 4 mg folic acid.

⁵Ajinomoto North America.

⁶Evonik North America.

⁷Feed provided to birds in kg per feed phase. Birds fed *ad libitum*.

Table 2. Experimental feed program¹ calculated nutrient composition (%).

Nutrient composition	Starter 1	Starter 2	Grower 1	Grower 2	Finisher 1	Finisher 2
Crude protein	30.70	29.50	27.20	23.90	21.60	20.00
AMEn (kcal/Kg) ²	3,080	3,120	3,225	3,311	3,392	3,432
Crude fat	9.80	9.80	10.9	11.00	11.10	11.20
Lysine	1.89	1.80	1.65	1.39	1.25	1.140
Methionine	0.84	0.81	0.73	0.62	0.58	0.53
Methionine + cysteine	1.23	1.18	1.08	0.93	0.87	0.80
Tryptophan	0.33	0.31	0.28	0.24	0.21	0.19
Threonine	1.19	1.12	1.04	0.87	0.79	0.73
Arginine	1.89	1.80	1.64	1.42	1.25	1.15
Valine	1.31	1.25	1.15	1.01	0.91	0.84
Calcium	1.50	1.41	1.35	1.25	1.03	0.99
Av. phosphorus ³	0.75	0.71	0.68	0.63	0.52	0.49
Sodium	0.19	0.19	0.18	0.18	0.18	0.18
Chloride	0.18	0.18	0.18	0.18	0.18	0.19

¹Both reduced and increased feed fine treatments were formulated to achieve the same nutrient composition.

²Metabolized energy in kilocalories per kilogram.

³Calculated available phosphorus per feed phase.

with U.S. #6 and U.S. #8 screens (3.36- and 2.38-mm openings, respectively) in a pellet screener (Model 2 × 4 two-decker general Rotoshacker; Andritz Sprout-Bauer, INC, Muncy, PA), and the other half was kept as the original crumble (IFF treatment). Both diets were treated with a post-fat application in the counterpoise ribbon mixer. The rest of the feed program phases (Table 1) were mixed in the counterpoise ribbon mixer, pelleted in a 10-ton/h pellet mill (Model 60-130; Bliss Industries, LLC, Ponca City, OK), using a 11/64" × 13/8" pellet mill die. Pellets were cooled by using a 10-ton counter flow cooler (Model VK19X19 KL; Geelen Counterflow USA, INC, Orlando, FL) and fines removed by a pellet screener (U.S. # screen, 4.75 mm, Model 3 5/7 Roto-Shack; Andritz Sprout-Bauer, INC, Muncy, PA). Half of the screened feed was designated as the RFF treatment. The IFF treatment was manufactured by using 30% of the other half of the batch to produce fines. That 30% was sent to the crumbler (Model PC-83311855; California Pellet Mill, Crawfordsville, IN) or roller mill (Model C128889; RMS, Sea, SD) to generate fines. Then those fines were added to the remaining 70% of the second half of the screened pellets and labeled IFF. Thus, the IFF from manufacturing had at least a 30% fines content in all feed phases. Fines were created with pellets that were already coated with fat. This

process allowed both treatments to have the same fat content in both the pellets and fines (Table 4).

Nutritional Analyses

Feed samples were obtained when loading the feed truck and kept immediately afterward at -20°C until laboratory analysis. Proximate analysis (250 g) for samples of each dietary treatment feed was outsourced to quantify dry matter, crude protein, crude fat, minerals, and total lysine (Carolina Analytical Services, Bear Creek, NC), with results presented in Table 3. The FOSS near-infrared spectrophotometry (NIRS) (DS 2500; FOSS, Hilleroed, Denmark) was used to measure the moisture, crude protein, and fat levels of the fines and pellet portion in a composite sample obtained from each dietary treatment feed within each feed phase after the feed was delivered to the rearing facility (Table 4). The NIRS was set up to calculate standard deviation based on 10 replicates of each composite sample. This was accomplished to observe and estimate if birds received different nutrition based on fines vs. pellets within treatment diets. The NIRS feed nutrient measurements were accomplished using the Adisseo's Precision Nutrition Evaluation platform (Adisseo, Antony, France).

Table 3. Nutrient analyses (%) of reduced feed fines (RFF) and increased feed fines (IFF) experimental diets fed to turkeys.¹

Nutrient	Starter 1		Starter 2		Grower 1		Grower 2		Finisher 1		Finisher 2	
	RFF ²	IFF ³	RFF ²	IFF ³	RFF ²	IFF ³	RFF ²	IFF ³	RFF ²	IFF ³	RFF ²	IFF ³
Moisture	10.95	11.20	11.37	11.10	11.18	10.79	11.30	10.62	11.57	11.62	11.03	10.76
Fat	9.91	9.40	7.61	7.48	10.81	11.50	9.53	10.07	10.90	10.53	11.16	11.26
Protein	29.95	29.08	29.71	29.48	26.01	26.28	23.72	24.19	20.91	20.59	18.52	18.67
Ash	7.43	7.65	7.36	7.50	6.55	7.14	6.32	6.41	5.08	5.13	4.31	4.22
Phosphorus ⁴	1.01	1.02	1.12	1.02	1.02	1.09	1.08	1.06	1.00	0.91	0.74	0.65
Calcium	1.55	1.61	1.74	1.56	1.61	1.53	1.61	1.60	1.43	1.21	1.01	1.02
Sodium	0.18	0.19	0.18	0.18	0.19	0.15	0.15	0.15	0.18	0.20	0.15	0.20

¹Feed analysis was performed by Carolina Analytical Services (17,570 NC Highway 902, Bear Creek, NC 27207).

²Screened feed that yielded reduced abundance of feed fines treatments.

³Added fines feed that yielded increased abundance of feed fines treatment.

⁴Total phosphorus in diet treatments.

Table 4. Nutritional analysis by NIRS¹ of pellet and fine portions within each dietary treatment.

Nutrients ²	RFF ³			IFF ⁴		
	Pellets ⁵	Fines ⁶	SD ⁷	Pellets ⁵	Fines ⁶	SD ⁷
	Grower 1					
Moisture	10.09	10.95	0.16	11.26	11.04	0.25
Fat	10.99	11.63	0.61	11.00	11.79	0.46
Crude protein	24.48	23.66	0.57	24.51	24.43	1.52
	Grower 2					
Moisture	10.34	11.04	0.20	10.18	11.02	0.14
Fat	11.01	11.76	0.39	11.81	11.38	0.52
Crude protein	22.02	21.08	1.07	22.68	22.46	0.82
	Finisher 1					
Moisture	10.91	11.28	0.25	11.92	12.67	0.20
Fat	12.37	11.83	0.53	10.04	10.21	0.36
Crude protein	19.73	19.79	0.89	18.52	18.71	0.82
	Finisher 2					
Moisture	11.04	11.63	0.27	10.13	11.06	0.27
Fat	11.72	12.19	5.93	12.18	11.93	0.63
Crude protein	16.76	17.4	7.4	16.06	17.16	1.01

¹Foss DS 2500 Near-Infrared Spectroscopy (DS 2500, FOSS, Nils Foss Allé, 1 DK-3400 Hilleroed, Denmark) using the Adisseo's Precision Nutrition Evaluation platform (Adisseo, Antony, France).

²Nutrient composition of a composite feed sample collected after farm bin extraction and before it was fed to the birds.

³Screened feed that yielded reduced abundance of feed fines (RFF) treatments.

⁴Added fines feed that yielded increased abundance of feed fines (IFF) treatment.

⁵Pellets determined as feed under US sieve number 5.

⁶Fines determined as feed over US sieve number 5.

⁷Average standard deviation of both pellets and fines parts, each with 10 readings per sample.

Particle Size Determination

Feed samples were collected at 3 separate feed handling steps for determination of particle size distribution and percentage fines for both treatments for each feed phase, whether crumbles or pellets. Samples were collected at the loading of the feed truck (before delivery to the bin at the poultry house), filling the feeding box in the poultry house (used for manual feeding of tube feeders in each pen), and at the feeder (in each pen of birds). Sampling the feed at the 3 handling locations allowed for the measurement of fines abundance in the feed and for the documentation of the degradation (increase in percentage fines) during handling until the feed was presented to the bird. Particle size variation procedures and calculations conformed to the American Society of Agricultural and Biological Engineers standard 319.4 (ASABE, 2008). Screen size for pellets and crumbles was selected following the ASAE S269.5 standard (ASABE, 2012) with a pellet diameter of 4.4 mm. For the starter 1 feed, pellets were determined by the feed retained in #4 U.S. screen (4.76 mm), crumbles were determined by the feed retained in #6 and #12 U.S. screens (3.36 mm and 1.68 mm, respectively), and fines were determined by the feed retained between a #14 U.S. screen (1.41 mm) and the collection pan. For the rest of the feed program phases, pellets were determined as the feed collected above #6 U.S. screen (3.36 mm), with fines being all feed passing through the screen.

Pellet Durability

The Holmen method pellet durability test (durability tester Model NHP100; Holmen Group, Stockholm, Sweden) was used to determine pellet durability. Pellets were screened using a U.S. #5 screen, and 4 representative 100-g samples from each feed phase and treatment (RFF and IFF) were analyzed for 30 s (2 samples) or 60 s (2 samples) to determine the Pellet Durability Index (PDI). The pellets remaining after the test period were weighed, and PDI was calculated as the percentage of pellets (by weight) left after the test.

Feeding Program

The birds were fed 6 feed phases (starter 1, starter 2, grower 1, grower 2, finisher 1, and finisher 2) on a kg of feed per bird feeding program (Tables 1 and 2). Feed weight was recorded when added to feeders. At 2, 3, 5, 8, 11, 14, 17, and 20 wk, feeders plus unconsumed feed was weighed to calculate feed usage, presented as intake (FI), and FCR.

Duodenum, Jejunum, Ileum, and Ceca Morphology

On day 21, small intestine and ceca samples were collected from one randomly chosen bird per pen. Samples were taken from the duodenal loop, jejunum, ileum, and ceca and then submerged in neutral buffered formalin. Samples were trimmed, stained, and mounted on slides at North Carolina State University College of Veterinary Medicine. Tissue samples (1 replicates/bird) were observed under a microscope and photographed at 4× magnification with an AmScope MIJ1000 microscope digital camera (AmScope, Irvine, CA). Villus height, crypt depth, and muscular thickness were measured using the AmScope software (version 3.7; AmScope, Irvine, CA) calibrated at 4× magnification, 10 subsamples per field.

Litter Ammonia Production

At 14 wk, ammonia emissions from the litter were measured using a Honeywell Gas Alert Extreme Ammonia gas monitor (Model GAXT-A2-DL; Honeywell Analytics, INC, Lincolnshire, IL). A 1-gallon Pyrex bowl with a diameter of 26.67 cm and an area of 558.65 cm² (Item#6001043; World Kitchen, LLC, Rosemont, IL) was placed in the middle of each pen, avoiding wet litter areas. The ammonia monitor was placed inside the enclosed space, and the ammonia level (ppm) was recorded at 1 min. The rate of production was calculated in ppm/s. Upon completion of the measurements, litter underneath the bowl was collected to determine its moisture content.

Litter samples were collected from all corners and the center of the pen at 20 wk. The sampling occurred 20 h after all the turkeys were removed from the pens. The samples were later homogenized with a woodchipper,

and 100 g was placed in a 500-mL glass jar to determine the rate of production ammonia using the same ammonia monitor. Ammonia production was determined from the time required for the ammonia level to reach 25 ppm. The subsample rate of production of ammonia was calculated in ppm/s. A subsample of the litter was taken to determine the moisture content of the litter. All samples were dried for 48 h at 80°C in a Blue M drying oven (General Signal, Blue Island, IL) to determine the litter's moisture content before and after weight measurements.

Chemical Composition of Litter

Litter samples were taken before the placement of poults and at 20 wk for chemical analysis. These samples were collected from different tote bags and pooled to one sample per treatment at placement and 4 samples per treatment at 20 wk. Analyses for total nitrogen (Kjeldahl method), total phosphorus, ammonia-nitrogen, nitrate-nitrogen, pH, total carbon, sodium, potassium, calcium, magnesium, and copper were conducted by the North Carolina State University Environmental Analysis Lab (Raleigh, NC).

Bacteria Levels in Litter

Before placing poults in the pens and at 20 wk after the birds were removed, litter samples were collected and analyzed for general coliform forming units and salmonella assays. Litter collection and sample analyses were accomplished as described by Walker et al. (2018). All collecting materials were autoclaved at 250°F, 22 PSI, and at least 45 min. Circular bandages (socks) (Item Tubigrip #1448; Mölnlycke Health Care, Norcross, GA) were soaked with 25 mL of buffered peptone water at 1% (BPW) in a sterile stomacher bag. The socks later were used to pick up the litter by rolling them on a PVC pipe attached to a paint roller to obtain whole pens sample (avoiding wet litter areas). The coliform forming units was estimated by the average of litter picked by the sock. No restricted traffic flow through pens was implemented during the study.

Statistical Analysis

A complete randomized block design was used. The water content of litter was included as a covariate in the models of litter ammonia production rate. Data were analyzed using a mixed model. Significant differences in main effects and interactions were separated using LS Means (Tukey HSD test) with $P \leq 0.05$. Where no first-order interactions were observed ($P \geq 0.05$), these interaction means were not included in tables. The data analysis was conducted using SAS software, version 9.4 of the SAS System for Windows (Copyright 2013 SAS Institute Inc., Cary, NC).

RESULTS

Feed Quality

Nutritional Analyses Results for wet analysis of the treatment diets within each phase are presented in Table 3. There were no observed differences between treatment diets within phases. Results comparing the fines and pellet portion of each treatment within each poststarter phase are presented in Table 4. Based on the NIR analysis, there were no apparent or appreciable differences observed in fines vs. pellets within each dietary treatment feed for moisture, fat, or crude protein based on the NIR measurements.

Particle Size For the starter 1 feed phase, the RFF diet had a statistically significant higher geometric mean diameter of particle size by mass (254- μ m difference), higher crumbles content (16% difference), higher geometric standard deviation, and lower content of pellets (10% difference) than the IFF diet (Table 5). No data were collected for the transportation effect on this feed phase.

For pelleted feed phases, degradation of the feed upon passage through the feeding system was determined. The abundance of feed fines increased from loading into the feed truck, filling the feed box, and placement into the feeders. Both feed treatments had a decreased particle size when the feed was sampled at the feeder; however, the RFF diet always had an overall statistically lower geometric standard deviation, higher content of pellets, and a lower content of fines than IFF. For RFF, the overall mean for all the phases was 29% fines vs. 57% for the IFF. The starter 2 and grower phase feeds averaged 21% fines for RFF vs. 54% for IFF. The finishers averaged 43% for RFF vs. 58% for IFF (Table 6).

Pellet Durability Index The RFF grower 2 had higher pellet durability when tested in the Holmen equipment at 30 and 60 s. The finisher 1 RFF diet had a higher PDI than IFF when tested at 60 s. No differences in PDI were found between RFF and IFF for the rest of the feed phases (Table 7).

Table 5. Starter 1 feed physical characteristics.

Diet	DGW ¹ (μ m)	SGW ² (μ m)	Fines (%) ³	Crumbles (%) ⁴	Pellets (%) ⁵
RFF ⁶	2.375 ^a	1.77 ^b	0.14 ^b	0.86 ^a	0.00 ^b
IFF ⁷	2.121 ^a	2.20 ^a	0.20 ^a	0.70 ^b	0.10 ^a
SEM ⁸	23	0.02	0.03	0.02	0.01
P value	0.001	0.0002	0.04	0.0004	0.01

^{a,b}Means within a column lacking a common superscript differ ($P \leq 0.05$).

¹D_{gw}: the geometric mean of particle diameter by mass.

²S_{gw}: the geometric standard deviation of particle diameter by mass, measure of variation in particle size.

³Fines determined as feed captured by US sieve number 12.

⁴Crumbles determined as feed between US sieve numbers 4 and 12.

⁵Pellets determined as feed under US sieve number 4.

⁶Screened feed that yielded reduced abundance of feed fines treatments.

⁷Added fines feed that yielded increased abundance of feed fines treatment.

⁸The standard error of the mean (SEM), n = 3 samples per treatment.

Table 6. Effect of feed transportation on physical characteristics of feed.

Diet	DGW ¹	SGW ²	Fines ³	Pellets ⁴
	—(μm)—		—(%)—	
Starter 2				
RFF ⁵ at truck	4,122 ^a	1.77 ^c	3.77 ^d	96.78 ^a
IFF ⁶ at truck	2,195 ^c	2.87 ^b	43.67 ^a	56.33 ^d
RFF ⁵ after bin	3,152 ^b	2.63 ^b	16.00 ^c	84.00 ^b
IFF ⁶ after bin	1,862 ^c	3.23 ^a	54.33 ^a	48.67 ^d
RFF ⁵ at feeder	2,877 ^b	2.63 ^b	25.67 ^b	74.33 ^c
IFF ⁶ at feeder	1,978 ^c	2.97 ^{a,b}	50.00 ^a	50.00 ^d
SEM ⁷	88	0.06	1.72	1.73
P value	<0.0001	<0.0001	<0.0001	<0.0001
Grower 1				
RFF ⁵ at truck	4,265 ^a	1.70 ^b	2.67 ^c	97.33 ^a
IFF ⁶ at truck	2,338 ^{b,c}	2.63 ^a	40.33 ^{a,b}	59.67 ^{b,c}
RFF ⁵ after bin	3,912 ^a	1.93 ^b	7.67 ^c	92.33 ^a
IFF ⁶ after bin	2,140 ^c	2.67 ^a	45.33 ^a	54.67 ^c
RFF ⁵ at feeder	2,921 ^b	2.47 ^a	26.67 ^b	73.33 ^b
IFF ⁶ at feeder	1,631 ^c	2.67 ^a	62.33 ^a	37.67 ^c
SEM ⁷	161	0.07	3.76	3.76
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Grower 2				
RFF ⁵ at truck	4,130 ^a	1.83 ^b	4.00 ^c	96.33 ^a
IFF ⁶ at truck	2,648 ^c	2.73 ^a	30.67 ^b	69.33 ^b
RFF ⁵ after bin	3,545 ^b	2.27 ^b	12.00 ^c	88.00 ^a
IFF ⁶ after bin	2,223 ^{c,d}	2.83 ^a	40.33 ^{a,b}	59.67 ^c
RFF ⁵ at feeder	4,103 ^a	1.83 ^b	9.00 ^c	91.00 ^a
IFF ⁶ at feeder	1,890 ^d	2.87 ^a	50.00 ^a	50.00 ^d
SEM ⁷	92	0.10	2.60	0.26
P value	<0.0001	<0.0001	<0.0001	<0.0001
Finisher 1				
RFF ⁵ at truck	3,930 ^a	1.97 ^c	5.00 ^d	95.00 ^a
IFF ⁶ at truck	2,404 ^b	2.67 ^a	34.33 ^c	65.67 ^b
RFF ⁵ after bin	3,532 ^a	2.20 ^{b,c}	12.33 ^d	87.67 ^a
IFF ⁶ after bin	1,808 ^b	2.90 ^a	50.00 ^b	50.00 ^c
RFF ⁵ at feeder	2,229 ^b	2.67 ^a	41.67 ^{b,c}	58.33 ^{b,c}
IFF ⁶ at feeder	1,233 ^c	2.53 ^{a,b}	75.33 ^a	24.67 ^d
SEM ⁷	104	0.09	2.42	2.42
P value	<0.0001	<0.0001	<0.0001	<0.0001
Finisher 2				
RFF ⁵ at truck	4,006 ^a	1.80 ^c	6.33 ^c	93.67 ^a
IFF ⁶ at truck	2,095 ^c	2.87 ^a	40.33 ^b	59.67 ^b
RFF ⁵ after bin	3,204 ^b	2.37 ^b	19.00 ^c	81.00 ^a
IFF ⁶ after bin	2,225 ^c	2.77 ^{a,b}	37.67 ^b	62.33 ^b
RFF ⁵ at feeder	2,073 ^c	2.93 ^a	44.67 ^b	55.33 ^b
IFF ⁶ at feeder	1,175 ^d	2.70 ^{a,b}	73.00 ^a	27.00 ^c
SEM ⁷	152	0.10	3.27	3.27
P value	<0.0001	<0.0001	<0.0001	<0.0001

^{a-d}Means within a column within a feed phase lacking a common superscript differ ($P \leq 0.05$).

¹D_{gw}: the geometric mean of particle diameter by mass.

²S_{gw}: the geometric standard deviation of particle diameter by mass.

³Fines determined in pellets as feed captured by US sieve number 6.

⁴Pellets determined as feed under US sieve number 6.

⁵Screened feed that yielded Reduced abundance of Feed Fines treatments.

⁶Added fines feed that yielded Increased abundance of Feed Fines treatment.

⁷The standard error of the mean (SEM) n = 3 samples per treatment.

Turkeys

Performance Birds fed the RFF diets had a higher BW from 2 to 17 wk than those fed the IFF diet (Table 8). However, at 20 wk, the effect of fines in the feed on the birds' BW was no longer significant. At 2, 3, and 8 wk, body weight gain (BWG) was higher when birds were fed RFF vs. IFF diets. At 20 wk, birds fed the IFF diets

had a higher BWG. A higher FI was observed for birds fed the RFF diet at 3, 5, and 8 wk; however, they had a lower FI at 17 and 20 wk than those fed the IFF diet. The FCR was improved for the birds fed the RFF throughout the experiment (Table 8).

The 10 and 20% biochar treatments resulted in a higher FI of approximately 2 kg at 20 wk than for birds fed the control treatment, while the 5% biochar treatment resulted in an intermediate effect (Table 9). Litter treatment affected FCR as early as 2 wk. The 20-week-old birds reared on the 20% biochar treatment had an improved FCR compared with birds reared on the rest of the treatments. At 11 wk of age, the litter with 20% biochar resulted in an improved bird FCR compared with birds reared on the control bedding treatment. Also at 11 wk, birds reared on 20% biochar were heavier than those reared on the control bedding treatment. The other 2 bedding treatments resulted in intermediate BW. This litter treatment effect on BW persisted to 20 wk.

Duodenum, Jejunum, Ileum, and Ceca Morphology

The abundance of fines in the feed affected some aspects of the morphology of the duodenum. The muscular thickness of the duodenum of birds at 21 d was 10% thicker when fed RFF vs. IFF (144 μm vs. 159 μm ± 4.8 with a P value of 0.03). However, this effect was not consistent, and no other part of the small intestines or ceca were affected by the feed's abundance of fines.

Litter

Ammonia Production For the 2 periods measured (14 and 20 wk), ammonia production rates were not affected by any treatment, neither feed nor litter. However, an increase in litter ammonia levels from 14 to 20 wk was observed. The production of ammonia at 14 wk was 0.43 ppm/s on average for both treatments using the bowl method. The average ammonia production was 2.86 ppm/s at 20 wk for both treatments using the jar method with 100 g of litter.

Chemical Composition of Litter Increasing the biochar percentage in the litter increased phosphorus levels, sodium, potassium, calcium, magnesium, copper, pH, and total Kindjal nitrogen at placement. All litter treatments resulted in approximately double the total Kindjal nitrogen after the trial at 20 wk. Phosphorus content increased in all treatments, with the 20% biochar resulting in an average of 3.4× the initial content of phosphorus. Nitrogen ammonia was increased in all treatments by an average of 14.15× the initial content at placement. Nitrate nitrogen, necessary for plant nutrition, was increased at an average of 6.50× in all treatments. Other nutrients followed the same trend of increased levels at the end of the trial. However, the carbon and magnesium levels were reduced in all treatments by an average of 45 and 12%, respectively (Table 10).

General Coliform Unit Count and Salmonella All pens were negative for salmonella. Initial coliform units

Table 7. RFF¹ and IFF² feeds PDI³ tested with the Holmen method at 30 and 60 s (%).

Diet	Starter 2	Grower 1	Grower 2	Finisher 1	Finisher 2
30 s					
RFF	87.58	90.26	89.62 ^a	89.9	82.1
IFF	86.88	90.97	85.97 ^b	90.7	84.6
SEM ⁴	1.0300	0.23	0.21	0.34	0.52
P value	0.6700	0.19	0.0064	0.25	0.08
60 s					
RFF	68.90	73.68	77.1 ^a	76.95 ^a	56.71
IFF	66.87	78.18	64.3 ^b	70.93 ^b	62.81
SEM ⁴	0.8100	1.44	1.01	0.79	2.1
P value	0.2200	0.16	0.01	0.0327	0.18

^{a,b}Means within a column within a time period lacking a common superscript differ ($P \leq 0.05$).

¹Screened feed that yielded reduced abundance of feed fines treatment.

²Added fines feed that yielded increased abundance of feed fines treatment.

³Pellet durability index.

⁴The standard error of the mean (SEM) n = 2 samples per treatment.

of litter for the control, 5% biochar, 10% biochar, and 20% biochar treatments were 5.0, 5.2, 5.1, and 4.8 log CFU per gram, respectively, with no statistical difference due to treatment.

The litter with 20% biochar had a lower amount of coliforms than the control litter at 20 wk (7.85 vs. 8.2 Log CFU/g \pm 0.07, $P = 0.009$). Litter containing 5 and 10% biochar had an intermediate effect on the coliform load (8.08 and 8.09 Log CFU/g \pm 0.07, respectively).

DISCUSSION

Feed particle size selection increases as the birds' beak size increases with age (Moran, 1982). Therefore, beak conditioning could impact birds' capacity to preach spe-

cific particle sizes from the feed. The increase in turkey FI and FCR observed at 5 wk with the decrease in the abundance of feed-fines between IFF and RFF can explain the higher BW of RFF-fed birds vs. IFF-fed birds. Screening the crumbles increased the starter's textural characteristics and reduced the levels of fines that are detrimental to FCR and BW (Proudfoot and Hulan, 1982). These results are consistent with those of previous studies on the effects of RFF crumbles and mini-pellets on turkey poults (Favero et al., 2009; Flores et al., 2020) where the inclusion of mini-pellets with a similar particle size to the crumbles used herein increased BW at the end of the starter period.

Changing the feed form by screening the feed increased the average particle size of feed by reducing the abundance of fines in crumbles and pellets from 57% for IFF to 29% fines for RFF (overall mean for all the phases). The fines generated from fully pelleted feed in this study had all the nutritional thermal processing benefits, and nutrition, of a fully formed pellet. However, those fines did not have the physical form benefit of a fully formed pellet, which might impact performance (Jensen, 2000). The form of the crumbles and pellets was altered by selecting for a more substantial feed particle size. Feed form has been categorized as having a significant influence on poultry performance (Quentin et al., 2004) with increased pellet quality leading to improved performance. Screening the feeds increased the textural characteristics and reduced the levels of fines detrimental to BWG and FCR (Proudfoot and Hulan, 1982). Feeding RFF vs. IFF pellets increased bird BW until 17 wk, decreased FI, and improved FCR until 20 wk. The RFF crumbles, having a smaller gap in particle size to pellets, could decrease the stress

Table 8. Effect of feed fines abundance on male turkey performance.

Feed	Turkey age in weeks								
	0	2	3	5	8	11	14	17	20
Bodyweight (kg/bird)									
RFF ¹	0.065	0.47 ^a	0.88 ^a	2.29 ^a	5.28 ^a	8.88 ^a	13.40 ^a	17.00 ^a	20.59
IFF ²	0.065	0.44 ^b	0.87 ^b	2.16 ^b	5.00 ^b	8.60 ^b	13.14 ^b	16.72 ^b	20.68
SEM ³	0.003	0.01	0.01	0.03	0.04	0.06	0.06	0.09	0.12
P value	0.600	<0.01	<0.01	<0.01	<0.01	<0.01	0.006	0.03	0.51
Bodyweight gain (kg/bird)									
RFF ¹	NA ⁴	0.40 ^a	0.82 ^a	1.34	3.00 ^a	3.6	4.53	3.59	3.68 ^b
IFF ²	NA ⁴	0.38 ^b	0.75 ^b	1.41	2.84 ^b	3.6	4.54	3.59	4.10 ^a
SEM ³	NA ⁴	0.004	0.01	0.025	0.03	0.033	0.04	0.06	0.08
P-value	NA ⁴	0.0002	<0.0001	0.083	0.0002	0.94	0.79	0.94	0.004
Feed intake (kg/bird)									
RFF ¹	NA ⁴	0.52	1.08 ^a	3.18 ^a	8.03 ^a	15.31	27.64	34.53 ^b	45.58 ^b
IFF ²	NA ⁴	0.51	1.04 ^b	3.05 ^b	7.85 ^b	15.14	28.07	35.32 ^a	48.10 ^a
SEM ³	NA ⁴	0.01	0.01	0.03	0.07	0.13	0.2	0.25	0.37
P-value	NA ⁴	0.25	0.02	0.01	0.07	0.35	0.14	0.03	<0.01
Feed conversion ratio									
RFF ¹	NA ⁴	1.119 ^b	1.231 ^b	1.393	1.520 ^b	1.725 ^b	2.062 ^b	2.032 ^b	2.205 ^b
IFF ²	NA ⁴	1.159 ^a	1.285 ^a	1.416	1.572 ^a	1.763 ^a	2.137 ^a	2.113 ^a	2.311 ^a
SEM ³	NA ⁴	0.01	0.009	0.015	0.008	0.012	0.011	0.012	0.014
P value	NA ⁴	<0.01	<0.01	0.284	<0.01	0.027	<0.01	<0.01	<0.01

^{a,b}Means within a column within a performance section lacking a common superscript differ ($P \leq 0.05$).

¹Screened feed that yielded reduced abundance of feed fines treatment.

²Added fines feed that yielded increased abundance of feed fines treatment.

³The standard error of the mean (SEM) n = 24 pens with 17 birds per treatment.

⁴Nonapplicable.

Table 9. Effect of biochar¹ inclusion on male turkey performance.

Litter mix ²	Turkey age in weeks								
	0	2	3	5	8	11	14	17	20
	Bodyweight (kg/bird)								
0% Char	0.065	0.45	0.84	2.18	5.07	8.56 ^b	13.00 ^b	16.54 ^b	20.29 ^b
5% Char	0.065	0.44	0.83	2.14	5.05	8.66 ^{a,b}	13.22 ^{a,b}	16.84 ^{a,b}	20.52 ^{a,b}
10% Char	0.065	0.46	0.87	2.29	5.22	8.81 ^{a,b}	13.33 ^{a,b}	16.94 ^{a,b}	20.82 ^{a,b}
20% Char	0.065	0.46	0.86	2.27	5.22	8.92 ^a	13.53 ^a	17.13 ^a	20.91 ^a
SEM ³	0.004	0.01	0.02	0.04	0.06	0.09	0.09	0.13	0.16
<i>P</i> value	0.842	0.24	0.21	0.07	0.11	0.04	0.002	0.012	0.02
	Bodyweight gain (kg/bird)								
0% Char	NA ⁴	0.38	0.77	1.35	2.89	3.49 ^b	4.44	3.54	3.92
5% Char	NA ⁴	0.38	0.76	1.32	2.90	3.61 ^{a,b}	4.56	3.62	3.76
10% Char	NA ⁴	0.39	0.80	1.42	2.93	3.59 ^{a,b}	4.52	3.61	4.07
20% Char	NA ⁴	0.40	0.80	1.41	2.94	3.70 ^a	4.61	3.59	3.78
SEM ³	NA ⁴	0.01	0.01	0.04	0.04	0.05	0.06	0.09	0.11
<i>P</i> value	NA ⁴	0.25	0.18	0.12	0.72	0.02	0.19	0.93	0.17
	Feed intake (kg/bird)								
0% Char	NA ⁴	0.52	1.06	3.07	7.86	15.09	27.43	34.63	45.71 ^b
5% Char	NA ⁴	0.51	1.05	3.07	7.82	15.04	27.56	34.55	46.36 ^{a,b}
10% Char	NA ⁴	0.53	1.09	3.19	8.14	15.63	28.36	35.26	47.75 ^a
20% Char	NA ⁴	0.51	1.06	3.11	7.94	15.14	28.08	35.26	47.52 ^a
SEM ³	NA ⁴	0.01	0.02	0.05	0.10	0.18	0.28	0.35	0.48
<i>P</i> value	NA ⁴	0.32	0.20	0.22	0.10	0.09	0.08	0.30	0.00
	Feed conversion ratio								
0% Char	NA ⁴	1.147 ^a	1.267	1.412	1.551	1.764 ^a	2.110	2.094	2.234
5% Char	NA ⁴	1.154 ^a	1.269	1.432	1.549	1.737 ^{a,b}	2.085	2.052	2.251
10% Char	NA ⁴	1.159 ^a	1.267	1.399	1.560	1.775 ^a	2.129	2.084	2.275
20% Char	NA ⁴	1.095 ^b	1.229	1.387	1.523	1.698 ^b	2.075	2.059	2.273
SEM ³	NA ⁴	0.014	0.012	0.021	0.012	0.017	0.016	0.017	0.020
<i>P</i> value	NA ⁴	0.012	0.074	0.275	0.160	0.0098	0.077	0.281	0.426

^{a,b}Means within a column within a performance section lacking a common superscript differ ($P \leq 0.05$).

¹Biochar is abbreviated as Char in this table.

²All litter combinations contained 70% used brooder litter and a complementary level of miscanthus grass to the biochar percentage to achieve 100% mix.

³The standard error of the mean (SEM) $n = 24$ pens with 17 birds per treatment.

⁴Nonapplicable.

attributed to a feed particle size change (Lecuelle et al., 2010). The smaller shift in particle size of RFF vs. IFF crumbles and pellets could decrease the stress attributed to a feed particle size change (Lecuelle et al., 2010) between the starter and grower feed phases.

Altering fines in the treatment feeds in the study herein by screening one treatment and adding to the other did not affect feed nutrient content as measured in feed after being augered from the feed bin at the rearing facility. However, De Jong et al. (2017) reported that fines were generated from pellets during broiler feed manufacture, handling, and transport and that nutrient content can differ with fines having less protein than pellets. In addition, De Jong et al. (2017) compared the nutrient content of fines vs. pellets as feed was delivered to the birds via a feed auger line. In the study herein, all feed was screened from fines before postpelleting fat application. Fines in the IFF were generated from finished screened pellets already coated with fat. It is also worth noting that in the current experiment, feed was sampled after feed manufacture and after the feed was augered from the rearing facility feed bin into feed box immediately before delivery of feed into each experimental pen. At that location, nutrient content of fines compared with pellets with each treatment was not appreciably different based on an NIR composite sample. Also, all batches of feed were delivered manually

and “in total” to the birds in each pen which accessed the feed in tube feeders. The feed was not delivered to pens via a feed auger line.

The increase in the physical feed wastage, and more importantly, a nutritional feed wastage, generated by not using the nutrients of the feed, should be considered (Abdollahi et al., 2013). Although not measured during the study, birds were observed to waste more fines than pellets. Also, pellets that were dropped to the litter floor were observed to be consumed by the birds. The fines that were dropped to the litter floor were not observed to be recovered by the birds. Therefore, the authors suggest that the differences in performance due to feed treatment in this study were due to increased feed wastage of feed fines as the birds consumed the feed.

The effect of pelleted feed on bird performance has been extensively studied and reviewed (Lanson and Smyth, 1955; Calet, 1965; Nir et al., 1995; Amerah et al., 2007a, 2007b; Zang et al., 2009; Dozier et al., 2010; Selle et al., 2010; Serrano et al., 2012, 2013); however, there have been few studies investigating the influence of pellet quality on turkeys. Pellet quality, as defined by the fines content, is critical because of mechanical receptors present in the beak (Moran, 1982), which could be disturbed by the beak conditioning of turkeys (Favero et al., 2009) and may override the chemical sense (Nir et al., 1995) resulting in the level of feed-

Table 10. Effect of biochar on the chemical composition of the litter in mg/kg.

Litter mix ¹²	Litter chemical composition										
	TKN ¹	TP ²	NH ₃ N ³	NO ₃ N ⁴	pH ⁵	TC ⁶	Na ⁷	K ⁸	Ca ⁹	Mg ¹⁰	Cu ¹¹
	Before placement										
0% Biochar	12,140	3,810	366	61	7.82	392200	938	5,923	5,777	1,068	127
5% Biochar	16,220	4,170	370	47	8.25	41,5500	1,769	9,428	7,315	1,617	195
10% Biochar	14,460	4,580	367	44	8.47	34,1900	2,327	11,324	8,221	2,357	229
20% Biochar	19,800	12,500	327	53	9.34	35,5000	7,050	29,456	29,087	6,799	631
	At 20 wk										
0% Biochar	22,527	14,769	4,975	557	8.53	19,5600	3,957	14,627	23,062	2,108	87
5% Biochar	21,670	13,569	4,870	330	8.67	20,5800	4,110	15,207	22,689	2,077	121
10% Biochar	24,947	14,664	4,975	306	8.61	22,1600	4,326	15,596	21,978	2,276	132
20% Biochar	24,419	14,129	5,410	134	8.49	21,0800	4,988	18,416	24,321	2,693	170

¹Total Kjeldah nitrogen.²Total phosphorous.³Ammoniacal nitrogen.⁴Nitrate nitrogen.⁵pH (-log of hydrogen concentration).⁶Total carbon.⁷Sodium.⁸Potassium.⁹Calcium.¹⁰Magnesium.¹¹Copper.¹²All litter combinations contained 70% used brooder litter and a complementary level of miscanthus grass to the biochar percentage to achieve 100% mix.

fines a significant factor for bird performance. The results herein are similar to those in a broiler study by Dozier et al. (2010), in which feeding higher quality pellets, with reduced fines, resulted in improved performance.

It is critical to measure the feed pellet quality at the feeder and not only at the feed mill. A substantial increase in feed fines while transporting and handling was observed in this study. As an example, there is an increase in approximately 38% of feed fines in the RFF finishers. Reducing the abundance of fines in the crumbled and pelleted feed generated at the feed mill reduced the abundance of fines present at the feeder. It may be postulated that the lower abundance of fines in the product when shipped will result in fewer fines in the feed when finally presented to the bird. Hence, the abundance of fines will be cumulative for each stage of handling and transport. These observations on feed quality and the effects of transportation agree with those of Thomas and van der Poel (1996), who reported that pellets need to have a basic form of physical quality in durability to withstand the rigors of transportation.

Significant differences in the duodenum, ileum, and ceca morphology at 21 d were observed because of RFF treatments. The increase of the birds' duodenum muscularis thickness due to RFF contradicts previous experiments that were carried out in this laboratory (Flores et al., 2020).

Biochar is one of the products of pyrolysis of organic matter, a process involving high temperature under anaerobic conditions (USDA, 2017). Biochar has been produced from numerous and varied agricultural wastes including *Miscanthus* (Ioannidou and Zabaniotou, 2007). Pyrolysis of biomass while producing biochar (solid) also produces bio-oil (liquid) and syngas (gas). Linhoss et al. (2019) reported that the addition of bio-

char to pine shavings increased water capacity up to 20% inclusion rate and then again at 75% inclusion rate. In the experiment herein, *Miscanthus* grass was not supplemented at 100% of the litter. It was mixed with biochar to amend once-used turkey brooder litter. The use of the grass allowed the use of once-used turkey litter at a constant rate. Therefore, varying the amount of biochar did not result in a concentration or dilution of the used turkey litter. Therefore, the levels of biochar selected for the experiment herein (0 to 20% biochar) were used to generate a range of data that could possibly identify the minimum level of biochar inclusion which may affect turkey production and performance.

The inclusion of 20% biochar in the litter resulted in increased BW, BWG, and improved FCR at 11 wk. This is the only week when BWG was affected; however, the effect on BW persisted to 20 wk for birds reared on 20% biochar. The effect of biochar in the litter on turkey performance might be explained by potential stress caused when removing caking fecal material from the pens. At 10 wk, litter in pens was observed to have a layer of cake on top that could affect animal welfare such as foot pad dermatitis and other performance issues. The litter cake was manually removed from all pens to reduce the potential adverse effects of this cake litter. The cake-removing activity caused some flighty behavior in the birds which might have increased stress experienced by the birds. It was observed, but not quantitated, that litter in pens with 20% biochar did not contain as much cake as the other pens resulting in reduced human activity in those pens during litter cake removal. The lack of statistically significant differences in FI, but a difference in BW and FCR, could suggest an energy expenditure by a stress factor that could have been reduced with lower amounts of cake, management activity, and bird stress in those pens. Linhoss

et al. (2019) reported that biochar added at 10% and 20% increased the water-holding capacity of pine shavings by 21.6 and 32.2%, respectively. The addition of 20% biochar to litter may have reduced the amount of litter cake in those pens resulting in less time in working in the pens and reduced stress experienced by the birds. In turn, birds that experienced less stress could be expected to have better and more efficient growth (Bartz et al., 2018).

At 20 wk, there were no differences observed in ammonia emission from the litter or litter moisture because of treatment, indicating that the litter's biochar did not affect the pen's final environmental conditions. Ritz et al. (2011) reported that when biochar amendments were not acidified, ammonia levels were not reduced by biochar litter amendments. Nonetheless, based on chemical analysis comparisons of the litter, before placement and at 20 wk, increasing the levels of biochar in the litter increased the litter's nitrogen adsorption levels. The house hallway had a maximum 7-ppm level measured at the height of 1.5 m from the floor. These ammonia levels could have originated from any treatment. Enclosed ventilated rooms with ammonia readers installed in each pen at bird level could be a more effective way to measure daily ammonia produced by the litter treatments. A reduction of pathogen loads could be found in the litter with higher biochar levels at 20 wk. The CFU count has been related to the pathogen load of beddings; lower counts of CFU could be related to lower loads of intestinal pathogens (Hartel et al., 2000). Salmonella was not present in any of the treatments. The absence of salmonella could be due to the lack of the organism's presence in the feed, whole house, or farm. Although no gut microbiome analysis was performed in the experiment herein to infer biochar's action in the gut, further work is justified to gather more information about the effects of biochar on *Salmonella* and *E. coli* in the digestive tract and the environment.

In other broiler production trials, the use of biochar as a bedding or bedding amendment has not resulted in bird performance differences. No performance differences were observed in broilers reared to 42 d when *Miscanthus* grass was used as a litter treatment (Hulet et al., 2010). Ritz et al. (2011) compared 3 biochars from 3 agriculture waste products. Two products were acidified. While the use of the acidified products resulted in reduced ammonia, none of the biochar products resulted in any broiler performance differences at 35 d. Linhoss et al. (2019) added biochar at various rates to broiler bedding. There were no 35-day broiler performance differences due to the use of the biochar.

In the study herein, no ammonia production differences were observed due to litter treatment which includes *Miscanthus* grass inclusion. The lack of effect of *Miscanthus* grass bedding on subsequent ammonia production agrees with reports on other alternative bedding materials (Grimes et al., 2002). For example, no effect on litter ammonia production was observed in a broiler

study when chopped Bermuda grass and Switchgrass were used as litter treatments (Davis et al., 2015). Also, Bermuda grass hay mixed with pine shavings as bedding did not affect turkey hen performance nor litter nitrogen content when compared with pine shavings (Grimes et al., 2002). The lack of studies with *Miscanthus* grass in poultry creates the opportunity to work with *Miscanthus* grass for its potential energy value and usage as a bedding material or litter amendment.

Since poults can consume litter or particulates in the litter, biochar could have been consumed from the litter. Out of the 36 pens with biochar, 9 birds contained biochar (data not shown) in the gizzard at 21 d. Out of those 9 birds, 6 birds came from the treatment with 20% biochar in the litter. These numbers could be higher depending on biochar's hardness; if soft, it could be easily invisible and not quantified because of a reduction of size in the gizzard. However, the birds with biochar visible in the gizzard suggested that it is possible that birds ate biochar from the litter when it was highly available, even though the biochar was not supplemented into the feed.

Miscanthus grass is also being used as a cosubstrate in the anaerobic digestion of chicken manure to generate energy and as a waste treatment before land applications (Li et al., 2017). It is also worth noting that biochar, alongside biooil and syngas, could be produced from poultry litter and tested as a litter amendment as well. In several studies, biochar from poultry litter has been reported to have a high pH (Hass et al., 2012), high phosphorus levels (Revell et al., 2012a), and high levels of toxic-heavy-metal levels (Evans et al., 2015, 2016) and can decrease the presence of specific pathogens in birds (Prasai et al., 2016). If the birds consume biochar, it could increase the birds' pH at the duodenum, decreasing the buffering solutions' need and changing its morphology. The extra phosphorus supplementation and its availability for the turkeys will depend on the litter's source and its biomass litter residue (Akpe et al., 1984). The pH could then change the microbiota and the morphology of the duodenum and ileum. Arsenic levels in biochar should be measured and taken into consideration when biochar is used. Evans et al. (2015) found negative performance in broilers when biochar was fed to the birds because of a high concentration of arsenic in the biochar used in that study. Biochar performed similarly to positive control when a low arsenic poultry litter biochar was fed to broilers (Evans et al., 2016). There were no observed bird health issues or performance loss in the study herein that could be linked to birds reared on a litter with biochar. While biochar did not influence the diversity of microorganisms in the gut when fed to layers, it did lower the number of specific pathogens (Prasai et al., 2016). The increase in villus height and surface has been previously linked with increased performance (Wu et al., 2004). The increased villus height could explain the higher performance of litter with 20% biochar in the litter when birds were stressed at 10 wk, although the small intestine

morphology data only correspond to 21 d. The change on BWG for 11 wk was enough to separate BW of birds on 20% biochar litter from the control, even though there were no other statistically changes on BWG in subsequent weeks.

Once litter has no more purpose for bird production, litter can have numerous uses, including fertilizer, a general soil amendment, or alternative energy substrate (Bolan et al., 2010). Composting the litter is crucial in reducing pathogen loads and producing an enhanced agronomic product (Mukhtar et al., 2004). The chemical values analyzed could be used as a rough estimate of the litter's nutrient values for plants. When composting, biochar will absorb ammonium and immobilize ammonia (Mandal et al., 2016), by its sorption properties facilitated by its high porosity, surface, and ion exchange (Joseph et al., 2010), thus reducing the ammonia liberated to the environment when composted. The biochar effect will depend on the rate of application of biochar (Janczak et al., 2017). The amount of ammonia and other nitrogen sources that the biochar adsorbed during the grow-out operations should also need to be considered. Once the litter with biochar has been composted, it may increase the pH, water-holding capacity, organic matter, and nutrients in the soil (Hass et al., 2012). Several studies have shown the benefits of biochar with different crops (Revell et al., 2012a; Abbasi and Anwar, 2015; Brantley et al., 2016; Mierzwa-Hersztek et al., 2016). After composting, nutrient analysis is encouraged before agricultural usage because of biochar characteristics variability (Verheijen et al., 2010) and litter composition.

In conclusion, improving pellet quality by reducing the abundance of fines in the feed can improve commercial, Large White male turkey performance reared to market age. Screening crumbles and pellets might not be a viable economic activity. However, other options could be viable to reduce the abundance of fines in the feed. Ingredient quality, feed formulation, feed milling management, feed transportation, and feed management at the farm all can impact pellet quality and feed reduce fines, which could be beneficial for the birds' performance. Ultimately the decision will rely on the specific cost of implementing particular strategies to reduce feed fines in each commercial operation. Amending litter with significant biochar levels reduced the coliform forming units in the litter while maintaining the same ammonia levels at 20 wk. The FCR was improved for birds on the 20% biochar litter during the 8- to 11-wk rearing period while BW was increased from 11 to 20 wk. Subsequent research could include testing poultry litter, with *Miscanthus* grass and biochar, for energy content and suitability for use as a biomass fuel.

DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

- Abbasi, M. K., and A. A. Anwar. 2015. Ameliorating effects of biochar derived from poultry manure and white clover residues on soil nutrient status and plant growth promotion - greenhouse experiments. *PLoS One* 10:e0131592.
- Abdollahi, M. R., V. Ravindran, and B. Svihus. 2013. Pelleting of broiler diets: an overview with emphasis on pellet quality and nutritional value. *Anim. Feed Sci. Technol.* 179:1–23.
- Agyarko-Mintah, E., A. Cowie, L. Van Zwieten, B. P. Singh, R. Smillie, S. Harden, and F. Fornasier. 2017. Biochar lowers ammonia emission and improves nitrogen retention in poultry litter composting. *Waste Manage.* 61:129–137.
- Akpe, M. P., P. E. Waibel, and R. V. Morey. 1984. Bioavailability of phosphorus in poultry litter biomass ash residues for turkeys. *Poult. Sci.* 63:2100–2102.
- Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007a. Feed particle size: Implications on the digestion and performance of poultry. *Worlds Poult. Sci. J.* 63:439–455.
- Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007b. Influence of feed particle size and feed form on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters. *Poult. Sci.* 86:2615–2623.
- ASABE. 2008. Method of determining and expressing fineness of feed materials by sieving. *Asabe s319.4*. Pages 1–7 in *ASABE Standards 2008*. American Society of Agricultural and Biological Engineers American Society of Agricultural and Biological Engineers, St. Joseph, MI.
- ASABE. 2012. Densified products for bulk handling – definitions and method. *Asabe s269.5*. Pages 1–8 in *ASABE Standards 2012*. American Society of Agricultural and Biological Engineers American Society of Agriculture and Biological Engineers, St. Joseph, MI.
- Bartz, B. M., D. R. McIntyre, and J. L. Grimes. 2018. Effects of Management Related practices on Turkey hen performance supplemented with either original XPCTM or AviCareTM. *Front. Vet. Sci.* 5:185.
- Brantley, K. E., M. C. Savin, K. R. Brye, D. E. Longer, and M. Goss. 2016. Nutrient availability and corn growth in a poultry litter biochar-amended loam soil in a greenhouse experiment. *Soil Use Manage.* 32:279–288.
- Bolan, N. S., A. A. Szogi, T. Chuasavathi, B. Seshadri, M. J. Rothrock, Jr, and P. Panneerselvam. 2020. Uses and management of poultry litter. *Poult. Sci. J.* 66:673–698.
- Calet, C. 1965. The relative value of pellets versus mash and grain in poultry nutrition. *Worlds Poult. Sci. J.* 21:23–52.
- Davis, J. D., J. L. Purswell, and A. S. Kiess. 2015. Evaluation of chopped switchgrass and chopped bermudagrass as litter materials over multiple heavy broiler flocks. *J. Appl. Poult. Res.* 24:343–351.
- De Jong, J. A., J. M. DeRouchey, M. D. Tokach, R. D. Goodband, J. C. Woodworth, S. S. Dritz, C. R. Stark, C. K. Jones, H. E. Williams, J. Erceg, B. Haberl, L. McKinney, G. Smith, D. Van Otterloo, and C. B. Paulk. 2017. Formation of pellet fines during the feed manufacturing process, transportation and feed line delivery, and their nutrient composition. *Appl. Eng. Agric.* 33:921–926.
- Dozier, W. A., K. C. Behnke, C. K. Gehring, and S. L. Branton. 2010. Effects of feed form on growth performance and processing yields of broiler chickens during a 42-day production period. *J. Appl. Poult. Res.* 19:219–226.
- Evans, A. M., J. W. Boney, and J. S. Moritz. 2016. The effect of poultry litter biochar on pellet quality, one to 21 d broiler performance, digesta viscosity, bone mineralization, and apparent ileal amino acid digestibility. *J. Appl. Poult. Res.* 26:89–98.
- Evans, C., J. Garlich, I. Barasch, C. Stark, A. Fahrenholz, and J. L. Grimes. 2019. The effects of miscanthus grass as a bedding source and the dietary inclusion of unheated, low-trypsin inhibitor soybeans on the performance of commercial tom turkeys reared to market age. *J. App. Poul. Res.* 28:982–996.
- Evans, A. M., S. A. Loop, and J. S. Moritz. 2015. Effect of poultry litter biochar diet inclusion on feed manufacture and 4- to 21-d broiler performance. *J. Appl. Poult. Res.* 24:380–386.

- Favero, A., A. Maiorka, F. Dahlke, R. F. P. Meurer, R. S. Oliveira, and R. F. Sens. 2009. Influence of feed form and corn particle size on the live performance and digestive tract development of turkeys. *J. Appl. Poult. Res.* 18:772–779.
- Flores, K. R., J. L. Grimes, and A. Fahrenholz. 2020. Effect of feed form, soybean meal protein content, and rovbio® advance on poul live performance to 3 weeks of age. *Poult. Sci.* 99:6705–6714.
- Grimes, Jesse L., Jody Smith, and C. Michael Williams. 2002. Some alternative litter materials used for growing broilers and turkeys. *World's Poult. Sci. J.* 58:515–526.
- Hartel, P. G., W. I. Segars, J. D. Summer, J. V. Collins, A. T. Phillips, and E. Whittle. 2000. Survival of fecal coliforms in fresh and stacked broiler litter. *J. Appl. Poult. Res.* 9:505–512.
- Hass, A., J. M. Gonzalez, I. M. Lima, H. W. Godwin, J. J. Halvorson, and D. G. Boyer. 2012. Chicken manure biochar as liming and nutrient source for acid appalachian soil. *J. Environ. Qual.* 41:1096–1106.
- Hulet, R. M., P. H. Patterson, T. L. Cravener, and T. A. Volk. 2010. Alternative bedding for broilers: from vegetative buffers to fuels. *Poult. Sci.* 89:42.
- Ioannidou, O., and A. Zabaniotou. 2007. Agricultural residues as precursors for activated carbon production - a review. *Renew. Sustain. Energy Rev.* 11:1966–2005.
- Janczak, D., K. Malińska, W. Czekala, R. Cáceres, A. Lewicki, and J. Dach. 2017. Biochar to reduce ammonia emissions in gaseous and liquid phase during composting of poultry manure with wheat straw. *Waste Manage.* 66:36–45.
- Jensen, L. S. 2000. Influence of pelleting on the nutritional needs of poultry. *Asian-Australas J. Anim. Sci.* 13:35–46.
- Joseph, S. D., M. Camps-Arbestain, Y. Lin, P. Munroe, C. H. Chia, J. Hook, L. Van Zwieten, S. Kimber, A. Cowie, and B. P. Singh. 2010. An investigation into the reactions of biochar in soil. *Soil Res.* 48:501–515.
- Lanson, R. K., and J. R. Smyth. 1955. Pellets vs. Mash plus pellets vs. Mash for broiler feeding. *Poult. Sci.* 34:234–235.
- Lecuella, S., I. Bouvarel, A.-M. Chagneau, P. Lescoat, F. Laviron, and C. Leterrier. 2010. Feeding behaviour in turkeys with a change-over from crumbs to pellets. *Appl. Anim. Behav. Sci.* 125:132–142.
- Li, C., S. Strömberg, G. Liu, I. A. Nges, and J. Liu. 2017. Assessment of regional biomass as co-substrate in the anaerobic digestion of chicken manure: impact of co-digestion with chicken processing waste, seagrass and miscanthus. *Biochem. Eng. J.* 118:1–10.
- Linross, J. E., J. L. Purswell, J. T. Street, and M. R. Rowland. 2019. Evaluation of biochar as a litter amendment for commercial broiler production. *J. Appl. Poult. Res.* 28:1089–1098.
- Liu, N., J. Zhou, L. Han, S. Ma, X. Sun, and G. Huang. 2017. Role and multi-scale characterization of bamboo biochar during poultry manure aerobic composting. *Bioresour. Technol.* 241:190–199.
- Mandal, S., R. Thangarajan, N. S. Bolan, B. Sarkar, N. Khan, Y. S. Ok, and R. Naidu. 2016. Biochar-induced concomitant decrease in ammonia volatilization and increase in nitrogen use efficiency by wheat. *Chemosphere.* 142:120–127.
- Mierzwa-Hersztek, M., K. Gondek, and A. Baran. 2016. Effect of poultry litter biochar on soil enzymatic activity, ecotoxicity and plant growth. *Appl. Soil Ecol.* 105:144–150.
- Monira, K. N., M. A. Islam, M. J. Alam, and M. A. Wahid. 2003. Effect of litter materials on broiler performance and evaluation of manure value of used litter in late autumn. *Asian-australas J. Anim. Sci.* 16:555–557.
- Moran, E. T. 1982. *Comparative Nutrition of Fowl & Swine: The Gastrointestinal Systems.* University of Guelph, Ontario, Canada.
- Mukhtar, S., J. L. Ullman, J. B. Carey, and R. E. Lacey. 2004. A review of literature concerning odors, ammonia, and dust from broiler production facilities: 3. Land application, processing, and storage of broiler litter. *J. Appl. Poult. Res.* 13:514–520.
- Nir, I., R. Hillel, I. Ptichi, and G. Shefet. 1995. Effect of particle size on performance. 3. Grinding pelleting interactions. *Poult. Sci.* 74:771–783.
- Prasai, T. P., K. B. Walsh, S. P. Bhattacharai, D. J. Midmore, T. T. H. Van, R. J. Moore, and D. Stanley. 2016. Biochar, bentonite and zeolite supplemented feeding of layer chickens alters intestinal microbiota and reduces campylobacter load. *PLoS One* 11:e0154061.
- Proudfoot, F. G., and H. W. Hulan. 1982. Feed texture effects on the performance of Turkey broilers. *Poult. Sci.* 61:327–330.
- Quentin, M., I. Bouvarel, and M. Picard. 2004. Short- and long-term effects of feed form on fast- and slow-growing broilers. *J. Appl. Poult. Res.* 13:540–548.
- Revell, K. T., R. O. Maguire, and F. A. Agblevor. 2012a. Field trials with poultry litter biochar and its effect on forages, green peppers, and soil properties. *Soil Sci.* 177:573.
- Revell, K. T., R. O. Maguire, and F. A. Agblevor. 2012b. Influence of poultry litter biochar on soil properties and plant growth. *Soil Sci.* 177:402.
- Ritz, C. W., A. S. Tasistro, D. E. Kissel, and B. D. Fairchild. 2011. Evaluation of surface-applied char on the reduction of ammonia volatilization from broiler litter. *J. Appl. Poult. Res.* 20:240–245.
- Selle, P. H., D. J. Cadogan, X. Li, and W. L. Bryden. 2010. Implications of sorghum in broiler chicken nutrition. *Anim. Feed Sci. Technol.* 156:57–74.
- Serrano, M. P., M. Frikha, J. Corchero, and G. G. Mateos. 2013. Influence of feed form and source of soybean meal on growth performance, nutrient retention, and digestive organ size of broilers. 2. Battery study. *Poult. Sci.* 92:693–708.
- Serrano, M. P., D. G. Valencia, J. Méndez, and G. G. Mateos. 2012. Influence of feed form and source of soybean meal of the diet on growth performance of broilers from 1 to 42 days of age. 1. Floor pen study. *Poult. Sci.* 91:2838–2844.
- Thomas, M., and A. F. B. van der Poel. 1996. Physical quality of pelleted animal feed 1. Criteria for pellet quality. *Anim. Feed Sci. Technol.* 61:89–112.
- USDA. 2017. What is pyrolysis?. Accessed Dec. 2020. <https://www.ars.usda.gov/northeast-area/wyndmoor-pa/eastern-regional-research-center/docs/biomass-pyrolysis-research-1/what-is-pyrolysis/>.
- Verheijen, F., S. Jeffery, A. C. Bastos, M. Van der Velde, and I. Diafas. 2010. Biochar application to soils. A critical scientific review of effects on soil properties, processes, and functions. *EUR* 24099:162.
- Walker, G. K., S. Jalukar, and J. Brake. 2018. The effect of refined functional carbohydrates from enzymatically hydrolyzed yeast on the transmission of environmental salmonella senftenberg among broilers and proliferation in broiler housing. *Poult. Sci.* 97:1469.
- Williams, M., and J. Douglas. 2011. *Planting and Managing Giant Miscanthus as a Biomass Energy Crop.* US Department of Agriculture, Washington DC. Technical Note No. 4.
- Wu, Y. B., V. Ravindran, D. G. Thomas, M. J. Birtles, and W. H. Hendriks. 2004. Influence of phytase and xylanase, individually or in combination, on performance, apparent metabolizable energy, digestive tract measurements and gut morphology in broilers fed wheat-based diets containing adequate level of phosphorus. *Br. Poult. Sci.* 45:76–84.
- Zang, J. J., X. S. Piao, D. S. Huang, J. J. Wang, X. Ma, and Y. X. Ma. 2009. Effects of feed particle size and feed form on growth performance, nutrient metabolizability and intestinal morphology in broiler chickens. *Asian Austral. J. Anim. Sci.* 22:107–112.