ISSN 1682-8356
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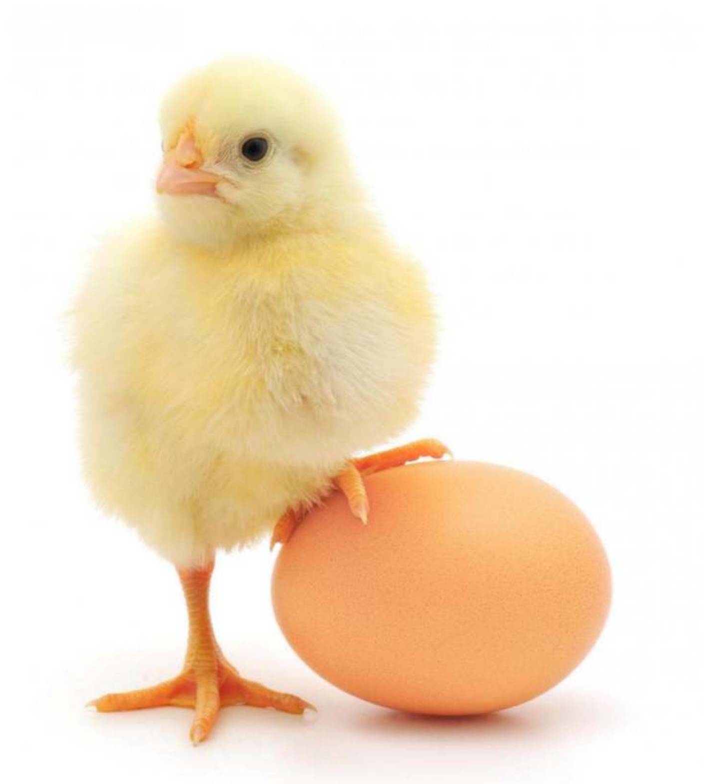

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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International Journal of Poultry Science 5 (8): 731-739, 2006
ISSN 1682-8356

Influence of Grain Particle Size and Insoluble Fiber Content on *Salmonella* Colonization and Shedding of Turkeys Fed Corn-Soybean Meal Diets[1]

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Abstract: This study aimed to determine the impact of feeding partially ground corn or insoluble fiber on intestinal development, *Salmonella* cecal colonization and fecal shedding of turkeys from 0-28d. Turkeys reared in cage-batteries were assigned to 1 of 3 diets: ground corn-SBM (GC, TRT 1), coarse ground corn-SBM (CC, TRT 2), and 4% wood shavings + ground corn-SBM (SC, TRT 3). A 3-strain cocktail of nalidixic acid-resistant *Salmonella enterica* serotypes Hadar, Javaina, and Typhimurium was orally-gavaged into each poult at placement. Cecal and fecal *Salmonella* populations, growth performance and intestinal weights and lengths were measured. The diets had no impact on *Salmonella* cecal or fecal populations. At 28d, *Salmonella* cecal populations decreased approximately 3-logs (range: 2.4-3.3 log reduction) across all treatments in comparison to 7d (P<0.0001). At 28d body weight, body gain and feed conversion ratio were not impacted by the diets. However, at 14d poults consuming the SC diet had lower feed consumption than those fed the GC and CC diets (231 vs. 243 and 252 g, P=0.001, respectively). The CC diet resulted in heavier relative gizzard weights at 28d in comparison to the GC and SC diets (30 vs. 28 and 22 g/kg, respectively, P<0.0001). Conversely, the SC treatment reduced the mass of the small intestine relative to body weight, especially the jejunum. Dietary inclusion of coarsely ground corn and wood shavings had no adverse effect on growth performance yet improved gizzard and intestinal development, which could have positive effects on intestinal health.

Key words: *Salmonella*, turkey, particle size, wood shavings, intestinal development

Introduction

Achieving the genetic potential for growth, maintaining health, and food safety at the lowest input costs are the goals of commercial poultry producers throughout the world. Whole grain feeding, dietary enzyme supplementation and pelleting are among the feed manufacturing practices used to achieve these goals. In many countries, feeding whole grains to poultry flocks has become a routine practice to reduce feed manufacturing and handling costs (Svihus *et al*., 2004), but it may also improve the feed conversion ratio (Plavnik *et al*., 2002) and nutrient digestibility (Svihus *et al*., 2004) without adversely affecting weight gain (Bennett *et al.,* 2002a,b; Svihus *et al*., 2004). Improved resistance to enteric pathogens and reduced carcass contamination due to gut breakage during evisceration may be possible by dietary inclusion of course feed particles that are retained for a longer time period in the gizzard than are finer feed particles (Ferket *et al*., 2005). Whole grain feeding also increases gizzard weight due to mechanical stimulation by the feed (Engberg *et al.,* 2002; Plavnik *et al*., 2002; Svihus *et al*., 2004), which increases reflux motility of the intestine (Duke, 1992). Some studies have also shown that whole or coarsely ground grains will be retained in the gizzard until the particles are reduced to a remarkably small and relatively homogenous size (Engberg *et al*., 2002; Svihus *et al*., 2002). Hetland *et al*. (2002) have shown that 40-70% of the feed particles entering the duodenum are smaller than 100 ìm, independent of whether ground or whole cereals have been fed. Moreover, increased gizzard activity has been associated with increased pancreatic enzyme secretion, improved feed flow regulation (Svihus *et al*., 2004), and stimulation of gastric function such as secretion of hydrochloric acid (Engberg *et al.,* 2002; Engberg *et al*., 2004). Furthermore, structural properties of feed, grain particle size, and feed formulation can influence intestinal microflora of poultry (Engberg *et al.,* 2002). The increased retention time in the gizzard and a more acidic gizzard pH may not only kill ingested enteric pathogens, but may also increase the fermentation of symbiotic bacteria in the crop that act as seed stock to colonize the lower digestive tract and competitively exclude pathogens (Engberg *et al*., 2004).

Foodborne pathogen contamination of poultry and poultry products is an ongoing problem for the poultry industry which in some cases can be indirectly related to some feeding practices that have contributed to flock contamination. For example, an increase of coliform bacteria, indicating potential colonization of enteric pathogens such as *Salmonella*, was observed in the ileum of pellet-fed broiler chickens (Engberg *et al.,* 2002). The same study showed a reduction of lactose-negative enterobacteria and *Clostridium perfringens* when chickens were fed whole wheat. Results from a similar experiment showed that whole wheat feeding reduced *Salmonella* in the gizzard and ileal contents of broilers (Bjerrum *et al.,* 2005).

The structure of the feed itself can also influence the microflora of the intestinal tract of broiler chickens. Bjerrum *et al.* (2005) demonstrated that the prevalence of *Salmonella* positive gizzards decreased after whole wheat supplementation to a broiler diet. Similar results were reported by Engberg *et al.* (2002) who showed that the populations of lactose-negative bacteria decreased after whole wheat feeding. Additionally, Bennett *et al.* (2002b) evaluated the effect of feeding whole barley on turkey performance and concluded that feeding up to 20% whole barley had little or no effect on weight gain and feed efficiency. The authors also observed an improvement in skeletal health and livability; however, the intestinal microflora were not characterized. Although the duration of production is considerably longer for turkeys than broilers and their nutrition and management requirements are different, we hypothesize that particle size and structure of feed components could also influence *Salmonella* colonization in turkeys. Therefore, the main objective of this study was to explore the effect of corn particle size (coarse vs. fine) and insoluble fiber content of the diet (addition of wood shavings to a finely ground corn diet) on *Salmonella enterica* colonization, gastrointestinal tract development, and growth performance of turkey toms from 1 to 28 d of age.

Materials and Methods

Bird husbandry: Six-hundred and twenty-four 1 day-old commercial Nicholas[2] male turkeys obtained from a commercial hatchery[3] were weighed, neck-tagged and orally gavaged with 1 ml (6×10^9 colony forming units – cfu) of a cocktail of *Salmonella enterica*, as described below, before being randomly assigned to 24 experimental cages of two Alternative Design Batteries[4] with 26 birds in each cage. Each cage was 55 cm wide, 66 cm long and 45 cm high. During the experiment the room temperature was regulated continuously, with a starting temperature of 37°C and a final temperature of 24°C at 28 days. Feed and water were given *ad libitum*.

Experimental design and diets: The experimental design consisted of three dietary treatments, each with eight replicate pens of 26 turkey poults. Over the entire experimental period (1-28 d), all turkeys were fed a corn-based diet (Table 1) in mash form. The experimental diets were formulated using least-cost linear programming to meet or exceed NRC (1994) nutrient requirements. The corn included in the feed formulas was either ground fine for experimental treatments GC (ground corn) and SC (wood shavings, ground corn), respectively, or ground coarse for experimental treatment CC. The SC treatment also included 4% (w/w) particles of soft pine shavings. The corn used to prepare the fine mash diet was ground in a hammermill[5] equipped with a 3-mm screen and had a final average particle size of 560µ. The corn used in the coarse mash diet was prepared by grinding through a 4-mm screen with subsequent sieving in a 60 in. separator[6] using a 16-mm sieve which resulted in a final average particle size greater than 3000µ. All other ingredients, including the wood shavings, were ground using a 3-mm screen and had a final average particle size of 600µ. The feed did not contain antimicrobials or coccidiostats.

Table 1: Nutrient composition of turkey diets containing different particle sizes of corn and supplemented with wood shavings as a source of insoluble fiber

Ingredients	GC (TRT 1)[1]	CC (TRT 2)[2]	SC (TRT3)[3]
	------------------ % ------------------		
Corn, Grain	52.72	52.72	46.48
SBM – 48%	30.00	30.00	30.00
Gluten Meal	5.00	5.00	5.00
Soybean Oil	0.00	0.00	2.18
Poultry Meal	8.00	8.00	8.00
Wood Shavings	0.00	0.00	4.00
DL-Methionine	0.08	0.08	0.09
L-Lysine HCl	0.42	0.42	0.44
Limestone	1.26	1.26	1.24
Vit/Min Premix	2.53	2.53	2.57
TOTAL	100	100	100
	------- Calculated Analysis -------		
Kcal ME/g	2.92	2.92	2.90
Crude Protein (%)	27.53	27.53	27.01
Fat (%)	3.47	3.47	5.42
Crude Fiber (%)	2.55	2.55	6.34
Calcium (%)	1.20	1.20	1.20
Available P (%)	0.60	0.60	0.60
Sodium (%)	0.17	0.17	0.17
Lysine (%)	1.60	1.60	1.60
Methionine (%)	0.55	0.55	0.55
Cysteine (%)	0.42	0.42	0.41
	-------- Chemical Analysis[4] --------		
Dry Matter (%)	92.55	92.55	92.83
Crude Protein (%)	27.51	27.51	28.37
Gross Energy (kcal/kg)	4271.23	4271.23	4326.51
Fat (%)	2.65	2.65	4.57
Ash (%)	7.35	7.35	7.72
Fiber Total (%)	25.02	25.02	27.23
Insoluble (%)	9.02	9.02	15.95
Soluble (%)	16.00	16.00	11.28

[1]Finely ground corn. [2]Coarsely ground corn. [3]Wood shavings + finely ground corn. [4]Chemical analysis (dry matter basis): (1) Crude protein determined using Kjeldahl automatic analyzer (Kjeltec Auto 1030 Analyser, Tecator, Sweden), (2) Gross energy determined using bomb calorimetry (IKA Calorimeter System C5000 control, IKA Werke Labortechnik, Staufen, Germany), (3) Fat determined by ether extraction (Labconco Corporation, Kansas City, MO) method, (4) Ash determined by muffle oven (Thermolyne, Sybron Corporation, Dubuque, IA) method, and (5) Dietary fiber analysis was conducted by Intertek Agri Services Food Agricultural Testing Laboratory (St. Rose) using the AOAC method for soluble, insoluble and total dietary fiber analysis.

Bacterial strains and inoculum: A cocktail of *Salmonella enterica* subspecies *enterica* serovar Typhimurium, Hadar and Javaina was used as the inoculum. For the purpose of this document, serovars of *Salmonella enterica* subsp. *enterica* will be referred to as *Salmonella* accompanied by the serovar name (i.e. *Salmonella* Typhimurium or *S.* Typhimurium). The strains had been previously isolated from turkey feces (Hadar and Javaina) and chicken cecal content (Typhimurium) and were found to be naturally resistant to nalidixic acid (NAR). These *Salmonella* isolates were serotyped by the NVSL (National Veterinary Service Laboratories, Animal and Plant Health Inspection Services, United States Department of Agriculture, Ames, IA). To prepare the inoculum, the 3 strains were grown separately overnight at 37°C in brain-heart infusion (BHI) broth[7] supplemented with nalidixic acid[8] (NA) at a final concentration of 1000 ppm. The cultures were then mixed together and serially diluted in buffered peptone water[7] (BPW) to a final concentration of 6×10^9 cfu/ml. The cell count was determined by direct plating on BHI agar plates supplemented with 1000 ppm NA, after overnight incubation at 37°C. Negative controls were used for all plating procedures to ensure that the media had been properly sterilized.

Data collection: At each sampling time, 2 poults were randomly chosen from each cage, weighed and euthanized by cervical dislocation. The abdomen was opened and the proventriculus, gizzard, small intestine (duodenum, jejunum and ileum) and ceca were collected. The contents of the gizzards and proventriculus were flushed with sterile deionized (DI) water for pH determination and subsequently the weight of both organs was recorded separately. After gizzard weights were recorded, gizzards were stored in icy water for 24 hours and then the glycocalyx lining was peeled from each gizzard and weighed. Ceca were aseptically removed immediately after the poults were euthanized, weighed, placed in sterile filtered stomacher bags[9] and stored on ice for ca. 30 minutes before being cultivated for *Salmonella* enumeration and isolation. Cultivation for *Salmonella* was conducted immediately after sampling. The small intestine segments (duodenum, jejunum and ileum) and ceca from a third bird were collected and weights and lengths measured. Samples were taken at day 7, 14, 21, and 28. Gizzard lining weight was not determined at day 7. Fecal samples were collected at day 1, 14 and 28 and cultivated for *Salmonella* to assure that turkeys were not only colonized by *Salmonella* but were also shedding the bacterium. Ten grams of fresh fecal samples were collected beneath each cage. Twelve hours before fecal collection the pens were cleaned and all old fecal material removed. Turkeys were inspected daily and birds with visual health problems or poor body condition were removed, weighed and euthanized by cervical dislocation. All mortality was weighed so that an appropriated adjustment of feed conversion ratio (FCR) could be made. Feed consumption by cage and individual bird body weight (BW) were recorded at 1, 7, 14, 21, and 28 days of age.

Analytical methods: At day of placement, meconium samples from all poults were collected and examined for salmonellae using the most probable number procedure described by Santos *et al.* (2005). Meconium samples were also processed using the direct plating method to isolate any nalidixic acid resistant (NAR) bacteria. The samples were placed in separate 7 x 12 inch (17.78 x 30.48 cm) sterile filtered stomacher bags[9] followed by the addition of BPW to each bag at a 1:10 dilution. The bags were then homogenized for one minute using a stomacher[10]. After homogenization, samples were serially diluted in BPW and direct plated onto BHI+NA plates to assure the isolation of only NAR bacteria. Preliminary findings indicated that non-inoculated poults were free of NAR intestinal bacteria. Plates were incubated for 24 hours at 37°C. Fecal samples collected thereafter and pooled cecal samples were processed for NAR-*Salmonella* isolation using the direct plating method as described above.

Gizzard and proventriculus digesta pH were measured separately immediately following sampling. After being flushed with DI water (ca. 1:10 dilution, content:water ratio) into sterile Whirl-Pak bags[11], suspensions of proventriculus and gizzard contents were manually mixed by shaking for 1 minute. A pH probe[11] was then inserted direct into each bag and the pH recorded. The probe was washed using sterile DI water between readings.

The length of the small intestine was measured for each segment as defined by duodenum (from the gizzard to pancreatic and bile duct), jejunum (from the bile duct to Meckel's diverticulum), ileum (from the Meckel's diverticulum to ileo-cecal-colonic junction), and ceca (Samanya and Yamauchi, 2002). Each segment was placed on a ruler and the length (centimeters) recorded. Intestinal segment weights (grams) were recorded after its digesta content had been manually removed. Intestinal weights and lengths were calculated relative to live bird body weight (kilograms) (Bjerrum *et al.,* 2005).

Statistical analysis: Statistical analysis of the results was accomplished using the general linear model procedure (Proc GLM) of SAS (SAS Institute, 1996) according to the following general model: $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + E_{ijk}$, where Y_{ijk} was the observed dependent variable; μ the overall mean; α_i the dietary treatment effect; β_j the block effect; $(\alpha\beta)_{ij}$ the interaction between dietary treatment and block; and E_{ijk} the random error.

Table 2: Effect of grain particle size and level of insoluble dietary fiber on body weight of turkeys fed corn/SBM-based diets

Treatment	7 d	14 d	21 d	28 d
	(g)			
GC[1]	119.2	264.8	501.0	829.3
CC[2]	114.5	269.1	501.3	794.0
SC[3]	117.8	259.3	498.3	823.6
P-Value	0.2132	0.5245	0.9415	0.5831
SEM (12)[4]	1.801	5.940	6.795	25.224

[1]GC: finely ground corn. [2]CC: coarsely ground corn. [3]SC: wood shavings + finely ground corn. [4]SEM (12): standard error of the mean with 12 degrees of freedom.

Replicate pens of 26 birds served as experimental units. When treatment effects were identified to be significant by the F-test ($P < 0.05$), the treatment means were separated by the least-square-means (lsmeans) function of SAS with a confidence of $P < 0.05$. Before statistical analysis, all cell count (cfu) data were transformed to the base-10 logarithm and the intestinal measurement data were transformed relative to body weights. Correlations between *Salmonella* cecal population, relative ceca weight, and gizzard and proventriculus pH were accomplished using the correlation procedure of SAS (SAS Institute, 1996) and the results were expressed as r values.

Animal ethics: The experiments reported herein were conducted according to the guidelines of the Institutional Animal Care and Use Committee at North Carolina State University. All husbandry and euthanasia practices were performed with full consideration of animal welfare.

Results

Body weights, which ranged from 794 g (CC) to 829 g (GC) at 28 d, were not affected by the dietary treatments throughout the trial (Table 2). From 7 to 14 d, supplementation of insoluble fiber to the diet (SC) resulted in the lowest feed intake and the CC diet had the highest (169.3 and 191.7 g, $P < 0.01$, Table 3). Although there was no significant treatment effect observed for feed consumption between 1 and 7 d, dietary inclusion of either 160 g of insoluble fiber as wood shaving per kg of corn-based diet (SC) or coarsely ground corn (CC) resulted in significantly better feed conversion than finely ground corn (GC) (1.05, 1.08 vs. 1.16 g/g, $P < 0.001$, respectively) during the first 7 days post-hatch (Table 4). Furthermore, contrast analysis revealed that turkeys fed the diet supplemented with wood fiber had a significantly better 1 to 28 d FCR than those not supplemented (1.34 vs. 1.45 g/g, $P = 0.0443$). Particle size of the corn and inclusion of shavings into the diet had a highly significant impact on relative gizzard weights but not on gizzard digesta pH which averaged 3.3 across treatments. At day 7, the birds fed diets containing coarse ground corn (CC) and wood shavings (SC) had about 18.1% greater gizzard mass relative to body weight than those fed the diet containing the finely ground corn (GC). Moreover, this difference in relative gizzard weight between GC and CC or SC increased to 26.2% and 24.7% by 14 d and 21 d, respectively. However, by day 28 the relative gizzard weights of birds fed CC and SC diets were 36% and 20.5% greater than those fed the GC diet, respectively. As observed with relative gizzard size, poults fed the CC diet had significantly larger gizzard lining weights relative to body weight at day 28 as compared to those fed the SC and GC diets (4.4 vs. 3.9 and 3.8 g/kg, respectively; $P < 0.05$). Interestingly, when gizzard lining weights were expressed relative to the total gizzard weight (Table 6), lining weights were not significantly different between the three dietary treatments ($P > 0.05$). This finding indicates that both lining and muscle developed at a similar rate. Relative proventriculus weights and pH averaged 6.56 g/kg and 3.4, respectively, regardless of dietary treatment (data not shown).

The dietary treatments had no effect on relative intestinal lengths throughout the experiment. At day 28, the mean lengths were 26.5, 56.0, 61.7, 19.6 cm/kg of body weight for duodenum, jejunum, ileum and ceca, respectively (Table 7). In contrast, jejunum weight was affected by the dietary treatments (Table 8). At day 14, birds fed the CC diet had significantly greater jejunum weights than those fed GC or SC diets ($P = 0.01$). By day 28, however, relative jejunum weight of birds fed the SC diet were significantly ($P = 0.01$) lower than those fed either GC or CC. Similar treatment effects were observed with respect to the relative size of the whole small intestine (sum of the relative weights of duodenum, jejunum and ileum) (Table 9). Poults fed the diet containing wood shavings (SC) had lower relative small intestinal weights at 28d than those fed the GC or CC diets (7.6 to 9.0 g/kg lighter, respectively, $P = 0.01$).

The results of the *Salmonella* population estimates in the cecal and fecal samples are summarized in Table 10. Pooled meconium samples collected from 3 individual cages (one cage per treatment) were positive for *Salmonella* at day of placement. These *Salmonella* isolates were further tested and found to be sensitive to nalidixic acid. Moreover, there was no significant difference in the population of NAR-*Salmonella* organisms detected between the three treatment groups. Although not influenced by dietary treatment, average cecal *Salmonella enterica* populations in the poults decreased 3-logs from day 7 to 28 (6.29 vs. 3.48 log/g, $P < 0.0001$). Fecal *Salmonella* populations were not significantly affected by the dietary treatments and averaged log 4.7 cfu/g from day 7 to 28.

Relative ceca weight was inversely correlated to cecal *Salmonella* populations (Table 11). Additionally, although not very strong, the positive correlation between cecal populations and proventriculus pH ($r = 0.34$, $P =$

Table 3: Effect of grain particle size and level of insoluble dietary fiber on periodic feed consumption of turkeys fed corn/SBM-based diets

Treatment	1 to 7 d	7 to 14 d	14 to 21 d	21 to 28 d	1 to 28 d
	(g)				
GC[1]	69.5	173.7[b]	304.4	530.5	1078.1
CC[2]	59.9	191.7[a]	321.4	510.4	1083.4
SC[3]	61.6	169.3[c]	296.7	488.0	1015.5
P-value	0.1170	0.0023	0.3802	0.2431	0.1739
SEM(12)[4]	2.674	3.668	12.327	16.840	26.514

[1]GC: finely ground corn. [2]CC: coarsely ground corn. [3]SC: wood shavings + finely ground corn. [4]SEM(12): standard error of the mean with 12 degrees of freedom. [a,b, c]Means with different superscripts within a column differ significantly (P < 0.05).

Table 4: Effect of grain particle size and level of insoluble dietary fiber on periodic feed conversion ratio of turkeys fed corn/SBM-based diets

Treatment	1 to 7 d	7 to 14 d	14 to 21 d	21 to 28 d	1 to 28 d
	(g/g)				
GC[1]	1.16[a]	1.20	1.29	1.68	1.41
CC[2]	1.08[b]	1.25	1.38	1.76	1.48
SC[3]	1.05[b]	1.16	1.20	1.53	1.34
P-VALUE	0.0008	0.7511	0.1880	0.3181	0.0695
SEM (12)[4]	0.015	0.052	0.049	0.105	0.039

[1]GC: finely ground corn. [2]CC: coarsely ground corn. [3]SC: wood shavings + finely ground corn. [4] SEM(12): standard error of the mean with 12 degrees of freedom. [a,b]Means with different superscripts within a column differ significantly (P < 0.05).

0.001) suggest that acid exposure in the proventriculus may be an important inhibitor of *Salmonella* colonization in the ceca. Although there was no significant correlation observed between cecal *Salmonella* population and gizzard pH (P > 0.05), a positive correlation was found between the digesta pH in the proventriculus and the digesta pH in the gizzard (r = 0.44, P < 0.0001).

Discussion

Feeding coarsely ground corn or increasing the insoluble fiber content of the diet by 4% had a greater effect on growth performance of turkeys during the starting phase of the trial (1-14 d) than during the last two weeks of the trial (14-28 d). Feeding coarse ground corn or wood shavings improved the FCR during the critical 7 d post-hatch period (Table 4, P = 0.001). The positive effect of the SC diet continued through day 14 as the SC poults consumed less feed than birds on the other two treatments, without adversely affecting body weight (Table 2, P = 0.001). By the end of the experiment (28 d) the SC fed birds had about 8% lower cumulative FCR than the birds fed non-supplemented diets. These findings agree with the work reported by Yasar (2003) who showed that broiler chickens fed whole or coarsely ground wheat from 1 to 28 days of age had better growth performance when compared to birds fed finely ground wheat. Development of the intestinal tract is probably one of the main reasons for the better performance of the birds fed diets with large particle size and insoluble fiber content in comparison to the ground corn/SBM treatment group (Jones and Taylor, 2001).

Feeding coarsely ground corn and wood shavings also increased the relative gizzard weights by day 28 by 35% and 25%, respectively. Similar results have been observed for turkey toms fed whole barley (Bennett *et al.*, 2002a) and broiler chickens (Bennett *et al.*, 2002b) fed whole wheat, where gizzard weights increased 34% and 37%, respectively. Other studies also reported increased gizzard weights when broilers were fed whole wheat (Svihus and Hetland, 2001; Svihus *et al.*, 2002; Plavnik *et al.*, 2002).

The gizzard is an important organ in the digestion process and greatly influences the normal intestinal motility (Duke, 1992). It influences digestion and absorption of nutrients by increasing digesta retention via periodic reverse peristalsis (Moran, 1982). Furthermore, the rate of the cloaca-ceca reflux, a low amplitude colonic anti-peristalsis that conveys the urethral secretion along the epithelial surface of the rectum into the ceca where uric acid and nitrogen is converted into microbial biomass, is dependent upon the activity of the upper-intestine motility, particularly of the gizzard (Duke, 1992). Therefore, an improvement in gizzard activity would have a beneficial effect in general bird digestion and absorption of nutrients.

The use of whole grains also contribute to the optimization of enteric health and digestive capacity in broilers by improving the development and function of the foregut organs such as the crop, proventriculus and gizzard (Taylor and Jones, 2004). For example, whole grain feeding not only increases the size of the gizzard musculature because of greater grinding activity, but it also increases the volume and retention time of ingesta in the organ. Coarse particles, such as whole wheat or whole barley, increase gizzard motility (Bennett *et al.*, 2002a; Bennett *et al.*, 2002b; Plavnik *et al.*, 2002; Svihus *et al.*, 2002). Retention time of feed in the gizzard is also increased after feeding coarser rations (Svihus *et al.*, 2002) because feed particles need to be reduced to a small and homogenous size that average 100 ìm before

Table 5: Effect of grain particle size and level of insoluble dietary fiber on relative gizzard and gizzard lining weight of turkeys fed corn/SBM-based diets

Treatment	7 d	14 d		21 d		28 d	
	RGW[5]	RGW	RGLW[6]	RGW	RGLW	RGW	RGLW
	(g/kg)						
GC[1]	45.16^{b}	28.73^{b}	4.55^{b}	27.26^{b}	4.93	22.03^{c}	3.77^{b}
CC[2]	54.57^{a}	36.48^{a}	5.55^{a}	34.11^{a}	4.93	29.93^{a}	4.43^{a}
SC[3]	52.15^{a}	36.04^{a}	5.40^{a}	33.88^{a}	5.22	27.57^{b}	3.93^{b}
P-value	0.0135	<0.0001	0.0066	<0.0001	0.9651	<0.0001	0.0161
SEM (36)[4]	2.2140	0.8586	0.2226	0.7832	0.1896	0.6465	0.1223

[1]GC: finely ground corn. [2]CC: coarsely ground corn. [3] SC: wood shavings + finely ground corn. [4] SEM(36): Standard error of the mean with 36 degrees of freedom. [5] RGW: relative gizzard weight (grams of tissue per kilogram of live body weight). [6] RGLW: relative gizzard lining weight (grams of tissue per kilogram of body weight). [a,b, c] Means with different superscripts within a column differ significantly (P < 0.05).

Table 6: Effect of grain particle size and level of insoluble dietary fiber on gizzard lining weight relative to total gizzard weight[1] of turkeys fed corn/SBM-based diets

Treatment	14 d	21 d	28 d
		(g/g)	
GC[2]	0.14	0.16	0.15
CC[3]	0.15	0.16	0.15
SC[4]	0.17	0.16	0.16
P-Value	0.3684	0.5941	0.4622
SEM (36)[5]	0.0061	0.0059	0.0051

[1]Relative gizzard lining weight: gizzard lining weight/gizzard weight. [2]GC: finely ground corn. [3]CC: coarsely ground corn. [4]SC: wood shavings + finely ground corn. [5]SEM(36): standard error of the mean with 36 degrees of freedom. [a,b]Means with different superscripts within a column differ significantly (P < 0.05).

exiting the gizzard (Hetland *et al.*, 2002). Increasing retention time in the gizzard exposes the contents to greater peptic digestion which is particularly important for enhancing protein digestion efficiency in the small intestine. Bjerrum *et al.* (2005) reported that feeding whole grains to broiler chickens caused a significant reduction in the gizzard content pH, probably due to mechanical stimulation of the proventriculus which leads to increased hydrochloric acid production. Each time the gizzard contracts, some of its contents are refluxed back into the proventriculus for further exposure to peptic secretions of pepsin and hydrochloric acid. Pepsin is an endopeptidase that cleaves proteins into multiple peptide fragments. These peptide fragments are then further digested by pancreatic exopeptidases such as trypsin and chymotrypsin. Whole grain feeding also increases pancreas and liver secretions (Svihus *et al.*, 2004). Therefore, the benefit of enhanced gizzard function is better foregut digestion of protein and fat resulting in less available nutrients in the hindgut for use by pathogenic organisms such as salmonellae and clostridia.

In addition to improving the efficiency and capacity of digestion, dietary inclusion of coarse materials that promote gizzard activity may influence the composition and populations of intestinal microflora by suppressing the colonization of enteric pathogens which compete for the same nutrients as the host and promoting the colonization of symbiotic organisms. For instance, increased gizzard retention discourages the colonization of ingested pathogens sensitive to acidic conditions. Conversely, increased gizzard retention may promote the growth of fermentative microorganisms in the crop (e.g. lactobacilli) which are more resistant to the lower pH in the gizzard environment, thereby seeding the intestine with symbiotic bacteria. However, Svihus *et al.* (2002) did not detect any changes in the passage rate of feed through the gizzard after feeding whole grains. Thus, the inverse relationship of dietary particle size and intestinal colonization of microbial pathogens is less likely attributed to prolonged exposure to hydrochloric acid in the gizzard and more likely due to improved protein and fat digestibility in the foregut.

In the present study, cecal colonization and fecal shedding of NAR-*Salmonella* were not affected by the dietary treatments. Other recently completed studies documented that feed particle size did not influence cecal *Salmonella* colonization of broilers fed whole wheat (Bjerrum *et al.*, 2005) or coarsely ground corn (Huang *et al.*, 2006). Mikkelsen *et al.* (2004) also observed no significant effects of feed particle size and feed form on *Salmonella* death rate in the ceca of pigs. Several factors may have influenced the outcome of the present study including the use of a high inoculation dose (6×10^{9} cfu/bird) and the young age at which the poults were challenged. The poults received a very high dose of *Salmonella* at a very young age before introduction and adaptation to the dietary treatments. In addition, the duration of the experiment may have been insufficient to detect a positive response. Although there was no significant dietary effect on gizzard and proventriculus digesta pH, cecal *Salmonella* populations were significantly correlated to the proventriculus pH (r = 0.34, P = 0.001), showing that acid exposure in the proventriculus may interfere with the colonization of the ceca by *Salmonella*. Santos *et al.* (2005) reported a significant linear correlation between *Salmonella* population and pH in litter collected from commercial turkey houses. Similarly, Bjerrum *et al.* (2005) reported a reduction of *Salmonella* populations in the gizzard

Table 7: Mean relative intestinal weight[1] and length[2] as a function of body weights of small intestine segments and ceca of turkeys fed corn/SBM-based diets[3]

Age	Duodenum	Jejunum	Ileum	CECA
	(g/kg)			
7	17.54	29.88	25.48	9.03
14	15.74	21.96	22.61	10.81
21	13.19	18.25	17.86	11.76
28	11.96	17.059	17.86	13.06
	(cm/kg)			
7	109.113	251.526	249.881	71.370
14	61.882	128.477	139.750	42.719
21	39.533	83.271	93.686	26.445
28	26.521	56.004	61.738	19.622

[1]Relative intestinal weight: grams of tissue/kg of body weight. [2]Relative intestinal length: cm of tissue/kg of body weight.
[3]Average means across dietary treatments, since no significant treatment effects were detected (P > 0.05).

Table 8: Effect of grain particle size and level of insoluble dietary fiber on relative jejunum weight (g of tissue/kg of body weight) of turkeys fed corn/SBM-based diets

Treatment	7 d	14 d	21 d	28 d
	(g/kg)			
GC[1]	29.13	20.81[b]	18.47	18.28[a]
CC[2]	31.67	24.34[a]	19.03	17.72[a]
SC[3]	28.85	20.73[b]	17.24	15.18[b]
P-value	0.4976	0.0112	0.1031	0.0110
SEM (36)[4]	1.2119	0.9127	0.5857	0.7286

[1]GC: finely ground corn. [2]CC: coarsely ground corn. [3]SC: wood shavings + finely ground corn. [4]SEM (36): Standard error of the mean with 36 degrees of freedom. [a,b]Means with different superscripts within a column differ significantly (P < 0.05).

Table 9: Effect of grain particle size and level of insoluble dietary fiber on relative small intestine weight[1] (g of tissue/kg of body weight) of turkeys fed corn/SBM-based diets

Treatment	7 d	14 d	21 d	28 d
	(g/kg)			
GC[2]	71.55	57.90	49.78	50.36[a]
CC[3]	75.47	64.84	51.21	48.98[a]
SC[4]	71.73	58.16	46.87	41.31[b]
P-value	0.3767	0.1873	0.3009	0.0116
SEM (36)[5]	1.9494	1.8914	1.4578	1.4957

[1] Small intestine weight is the sum of the duodenum, jejunum and ileum weights. [2] GC: finely ground corn. [3] CC: coarsely ground corn. [4]SC: wood shavings + finely ground corn. [5]SEM(36): Standard error of the mean with 36 degrees of freedom. [a,b]Means with different superscripts within a column differ significantly (P< 0.05).

contents of broilers when the pH was lowered by feeding whole wheat. Even though the correlation between *Salmonella* cecal population and gizzard pH was not significant, a positive relationship was detected between the pH of the gizzard and proventriculus digesta (r = 0.44, P < 0.0001).

A significant inverse correlation between cecal *Salmonella* population and relative ceca weight was observed (r = -0.34, P = 0.001). Similar results were reported by Santos (2006) who found that turkeys fed diets high in non-starch polysaccharides (NSP) had lower cecal *Salmonella* populations and increased relative ceca weight. The author suggested that high dietary NSP (fiber) content may have increased commensal microbial fermentation in the ceca thus increasing short-chain volatile fatty acid (SCFA) production which may have suppressed *Salmonella* colonization. SCFA decrease cecal pH which creates a stressful environment for the growth of some pathogenic microorganisms like *Salmonella*. Furthermore, SCFA produced by increased microbial fermentation in the lower intestinal tract have important metabolic functions that control microbial populations in the gastrointestinal tract. SCFA stimulate intraepithelial lymphocytes and natural killer cells (Ishizuka *et al*., 2004) which enhances the immunocompetence of the host animal (Lan, 2004) and suppresses the colonization of pathogens (Bertschinger *et al*., 1978; Lowry *et al*., 2005). SCFA-producing commensal bacteria also compete with pathogens for available nutrients and attachment sites in the intestinal tract (Simon *et al*., 2004), therefore competitively excluding *Salmonella*.

Another explanation for the inverse association between cecal *Salmonella* colonization and relative ceca weight might be that the natural intestinal microflora of birds having higher cecal *Salmonella* colonization may be suppressed, thereby compromising the normal function of the ceca resulting in a hypotrophy of the appendage. This is consistent with the findings of Tannock and Savage (1976) who showed that the ceca of germ-free mice challenged with *Salmonella* Typhimurium were significantly smaller (% of body weight) than the ceca of unchallenged germ-free mice. The authors also showed that once mice were either vaccinated with heat-killed *Salmonella* or were exposed to indigenous bacteria prior to challenge, the ceca were of normal size. The authors suggested that there may be a synergistic mechanism between the influence of the normal microflora and the host's immune response.

Intestinal lengths were not affected by the treatment diets; however, jejunum and total small intestine weights were lower for turkeys fed diets supplemented with 4% wood shavings. At 28 days of age, the jejunum of turkeys fed the diet supplemented with wood shavings (SC) weighed 17% less on a relative basis than the other two dietary treatments (GC and CC) (P = 0.01). The metabolic cost involved in maintaining the gastrointestinal tract accounts for about 30% of the total body metabolic rate (Aiello, 1997). Thus, larger digestive organs may compromise lean tissue growth because more protein synthesis and energy is directed toward organ growth (Lan, 2004). In agreement, the performance data in this study showed that the SC diet reduced the FCR which is likely related to the reduction in the relative mass of the small intestine.

Table 10: Effect of grain particle size and level of insoluble dietary fiber on *Salmonella* cecal colonization and fecal shedding of turkeys fed corn/SBM-based diets

Treatments	1 d	7 d	14 d		21 d	28 d	
	Fecal[5]	Cecal	Cecal	Fecal	Cecal	Cecal	Fecal
	log MPN/g[6]	log cfu/g[7]					
GC[1]	3.46	5.92	4.17	4.80	5.65	3.50	4.00
CC[2]	3.46	6.38	4.69	5.84	5.77	3.69	4.84
SC[3]	2.98	6.57	4.95	5.67	5.48	3.26	5.43
P-value	-	0.8938	0.5551	0.0895	0.4749	0.5952	0.0613
SEM(12)[4]	-	0.4122	0.5816	0.1980	0.3350	0.5456	0.1984

[1]C: finely ground corn. [2]C: coarsely ground corn. [3]SC: wood shavings + finely ground corn. [4]SEM(12): Standard error of the mean with 12 degrees of freedom. [5]*Salmonella* population results represent only one positive pen at placement day. [6]log MPN/g: base-10 logarithm of the most probable number of *Salmonella* present per gram of turkey fecal samples (meconium). [7]log cfu/g: base-10 logarithm of the colony count of *Salmonella* present per gram of turkey cecal samples.

Table 11: Pearson correlation coefficients of *Salmonella* cecal population, relative ceca weight, and pH of proventriculus and gizzard of turkey toms fed corn/SBM-based diets

	LOG C[1] × RCW[2]	LOG C × PV pH[3]	LOG C × G pH[4]	PV pH × G pH
r[5]	-0.34	+0.34	+0.13	+0.44
P-value	0.001	0.001	0.193	<0.0001

[1]LOG C: base-10 logarithm of the cell count (cfu) of *Salmonella* present per gram of turkey cecal samples. [2]RCW: relative ceca weight (grams of tissue per kilogram of body weight). [3]PV pH: proventriculus pH. [4]G pH: gizzard pH. [5]r-VALUE: Person correlation coefficients, n = 96.

In conclusion, dietary inclusion of coarse ground corn or wood shavings improved FCR during the critical 7 d post-hatch period. Additionally, the positive effect of dietary supplementation of wood shavings continued through day 14 as the SC-fed poults consumed less feed than did the poults fed the other two treatments without adversely influencing body weight. Inclusion of wood shavings also improved the 1 to 28 d FCR of turkey poults by 8%. Supplementation of wood shavings to the diet decreased the relative small intestine and jejunum weight. This weight reduction is also likely associated with the reduction in feed conversion ratio in the SC-treatment group as less energy is required to maintain the gastrointestinal tract. Diets formulated with coarse ground corn and wood shavings increased the relative gizzard weight and possibly improved the digestion and absorption of nutrients as the gizzard is an important organ in the digestion process and greatly influences the normal intestinal motility. Finally, cecal *Salmonella* populations were positively correlated to proventriculus pH indicating that a more acidic proventriculus may contribute to reduced cecal *Salmonella* colonization.

Acknowledgment

This study was supported by a USDA Initiative for Future Agriculture and Food Systems (IFAFS) grant. The authors wish to thank Annette Israel, Jamie Warner, Jean de Oliveira, Ondulla Foye, Renee Plunske, Mike Mann, Robert Neely and Gene Alborne for their technical assistance during this study. Appreciation is also extended to Carl Whisenant for helping with the preparation of the coarse ground corn and wood shavings used in this experiment and to Paul Otto for assisting with feed manufacturing.

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