Diet structure, butyric acid, and fermentable carbohydrates influence growth performance, gut morphology, and cecal fermentation characteristics in broilers

S. N. Qaisrani,[*,†,1] M. M. van Krimpen,[‡] R. P. Kwakkel,[*] M. W. A. Verstegen,[*]and W. H. Hendriks[*]

[*]*Animal Nutrition Group, Department of Animal Sciences, Wageningen University, PO Box 338, NL-6700 AH Wageningen, the Netherlands;* [†]*University of Veterinary and Animal Sciences, Lahore, Pakistan; and* [‡]*Wageningen UR Livestock Research, PO Box 65, NL-8200 AB Lelystad, the Netherlands*

ABSTRACT An experiment with 288 male (Ross 308) 1-d-old broilers was conducted to test the hypothesis that a coarse diet supplemented with butyric acid (BA) and fermentable carbohydrates (FC) improves performance of broilers with a poorly digestible protein source. The interaction effects of diet structure (fine or coarse), FC supplementation (with or without), and BA supplementation (with or without) in a poorly digestible diet based on rapeseed meal (RSM) were tested in a factorial arrangement of 8 ($2 \times 2 \times 2$) dietary treatments. The coarseness of the diet affected feed intake (FI) ($P < 0.001$), BW gain ($P = 0.001$), and the feed conversion ratio (FCR) ($P = 0.001$) positively. Broilers fed the coarse diets had, on average, 14% heavier gizzards and 11, 7, 5, and 6% lower relative empty weights of the crop, duodenum, jejunum, and ileum, respectively, compared with those fed the fine diets. Dietary coarseness resulted in, on average, 6% greater ileal protein digestibility, 20% lower gizzard pH, 19% greater villus height, 18% lower crypt depth, and 23% reduced cecal branched chain fatty acids (BCFA) compared with chickens fed the fine diets. Broilers fed BA-supplemented diets had an improved FCR ($P = 0.004$) and decreased crypt depth ($P < 0.001$) compared with those fed diets without BA. Fermentable carbohydrate supplementation did not influence growth performance, gut development, or contents of total BCFA and total biogenic amines in the cecal digesta ($P > 0.05$). Supplementation with FC, however, decreased the cecal concentration of spermine by approximately 31% compared with broilers fed diets without FC ($P = 0.002$). In conclusion, feeding a coarse diet supplemented with BA improved performance of broilers fed a diet containing a poorly digestible protein source. The negative effects of a poorly digestible protein source can thus be partly counterbalanced by coarse grinding and BA supplementation in the diet.

Key words: diet structure, butyric acid, cecal fermentation, broilers, fermentable carbohydrates

2015 Poultry Science 94:2152–2164
http://dx.doi.org/10.3382/ps/pev003

INTRODUCTION

Feeding diets with a poorly digestible protein source results in more protein reaching the hindgut (Wiernusz et al., 1995), potentially resulting in increased protein fermentation. The latter results in the production of ammonia, amines, phenols, indoles, sulphide, branched-chain fatty acids (**BCFA**), volatile fatty acids (**VFA**), and gases (hydrogen, carbon dioxide, and methane) as well as intermediate products such as lactate and succinate (Macfarlane et al., 1992). Some of these compounds may have detrimental effects on the host's health and growth performance (Bikker et al., 2007).

Feeding coarse particles may be an effective nutritional strategy to enhance ileal protein digestibility in poultry. Coarse particles are ground into finer particles in the gizzard before they enter the duodenum, thus prolonging retention time in the gizzard (Amerah et al., 2007). This extended retention time enhances the effectiveness of hydrochloric acid and digestive enzymes that are secreted from the proventriculus into the gizzard (González-Alvarado et al., 2008); it has been shown to have positive effects on gizzard function and development and results in improved protein denaturation and digestion (Liu et al., 2013; Pacheco et al., 2013). It can be assumed, therefore, that diet structure can decrease the amount of undigested protein reaching the hindgut and reduce the potential detrimental effects of hindgut protein fermentation.

Organic acids, such as butyric acid (**BA**), supplemented as a feed additive may be another way to

Received June 17, 2014.
Accepted October 31, 2014.

[1]Corresponding author: shafqat.qaisrani@wur.nl

improve ileal protein digestibility of poorly digestible protein sources. Butyric acid is a readily available energy source for gut epithelial cells and stimulates their multiplication and differentiation (Dalmasso et al., 2008), consequently improving feed efficiency (Adil et al., 2010). Butyric acid also stimulates the development of gut-associated lymphoid tissue and the functional development of the gastrointestinal tract (**GIT**) in terms of digestion and absorption of nutrients, and it increases peptide production in the distal GIT (Cox et al., 2009). It may also reduce hindgut protein fermentation because it suppresses protein-fermenting microbiota, especially the gram-negative population in broilers (Gunal et al., 2006), by disrupting their energy metabolism (Ricke, 2003) and decreasing hindgut pH. In addition, BA decreases bacterial colonization of the intestinal wall (Langhout et al., 1999) and as a consequence, less toxic compounds are produced by pathogenic microbiota, resulting in less damage to the epithelial cells (Antongiovanni et al., 2009).

The provision of sufficient fermentable carbohydrates shifts microbial proteolytic fermentation into more carbohydrate fermentation (Awati et al., 2006) because cecal resident microbiota prefer carbohydrates as their main energy source (Rehman et al., 2008). Thus, supplementing diets with fermentable carbohydrates (potato starch is only 38% digestible in the ileum; Weurding, 2002) could decrease hindgut protein fermentation, improve gut health, and promote beneficial microbiota, all of which may positively affect growth performance.

It was hypothesized, therefore, that supplementing a diet containing a poorly digestible protein source with coarse particles combined with butyric acid and/or fermentable carbohydrates would counterbalance the negative consequences on overall broiler performance. The aim of this study was to investigate the interacting effects of diet structure, butyric acid, and fermentable carbohydrate supplementation on performance, gut morphology, and cecal fermentation characteristics in broilers.

MATERIALS AND METHODS

Animal Ethics

Experimental procedures were in accordance with Wageningen University and Netherlands Animal Experimental Committee guidelines and code of practice. Ethical approval was granted before the study was conducted.

Bird Husbandry and Diets

In total, 288 male (Ross 308) 1-d-old broilers (obtained from a commercial hatchery) were individually weighed, steel wing-banded, and randomly assigned to 1 of 36 floor pens in 3 identical environmentally controlled rooms (9 treatments × 4 replicate pens per treatment with 8 birds per pen). A 2 × 2 × 2 factorial arrangement of 8 treatments was employed to test the interaction effects of particle size (fine or coarse), fermentable carbohydrate (**FC**) supplementation (with or without), and BA supplementation (with or without) in rapeseed meal (**RSM**)–based diets. A finely ground positive control diet with soybean meal (**SBM**) as the main protein source was an additional treatment. The 4 replicates were allocated blockwise in the 3 rooms in such a way that there was at least one replicate in each room. Each pen (1.15 × 1.75 × 0.80 m; L × W × H) had a perch and 3 drinking nipples with a cup underneath connected to a water tank of 10 L capacity. Pens were separated by solid walls to prevent contact between broilers from different treatment groups. Soft Cells (Ten Damme Meddo, Winterswijk, the Netherlands) fiberless bedding was used as litter material. Each dietary treatment was randomly applied to 4 replicate pens, and broilers were allowed ad libitum access to feed and water. For the first 3 d, broilers were exposed to a 23L:1D cycle, which was reduced thereafter to 16L:8D with approx. 20 lx intensity at bird level throughout the experiment. The temperature was set at 32°C for the first 3 d, was gradually decreased to a constant 22°C by wk 4, and was maintained at 22°C until the end of the experiment.

To obtain the coarse diets, all maize and wheat were preprocessed using a roller mill with a roller distance of 1.6 mm before they were incorporated into the final coarse pellets. For the fine diets, maize, wheat, SBM, and RSM were processed with a Netzsch-condux hammer mill (Hanau, Germany) with an opening screen of 3.0 mm before they were incorporated into in the final fine pellets. The high fermentable carbohydrate level was obtained by substituting maize starch for potato starch. Butyric acid, as a nonprotected sodium butyrate (Adimix, INVE Nutri-Ad, Kasterlee, Belgium), was mixed with the feed at 2 kg/ton on top. All experimental diets (Table 1) were formulated to meet or exceed recommendations for broiler diets (CVB, 2007). The diets were offered as 2.5 mm pellets for the starter diet (0–7 d) and as 4 mm pellets for the grower diet (8-35 d). The diets did not contain antimicrobial growth promoters or coccidiostats.

Wet Sieve Analysis

The diets' particle size distribution was analyzed using the wet sieve method described by Goelema et al. (1999) with minor modifications. Weighed samples of each diet were subdivided into 2 subsamples. One subsample of each diet was dried overnight at 70°C in an oven until constant weight to determine the air DM content. The other subsample of each diet was suspended in 500 mL water for 45 min to ensure adequate hydration before being washed through a set of sieves with decreasing mesh sizes of 2.5, 1.25, 0.63, 0.315, 0.160, and 0.071 mm. The contents of each sieve were subsequently collected and dried overnight at 70°C in an

Table 1. Dietary ingredients and calculated nutrients of the diets (g/kg as-fed basis).

Items	Basal RSM diet	FC-RSM diet	SBM diet
Maize starch	92.1	32.1	117.5
Maize	300.0	300.0	300.0
Wheat	100.0	100.0	100.0
Fish meal	63.0	63.0	25.0
SBM	0.0	0.0	350.0
RSM	350.0	350.0	0.0
Potato protein	30.0	30.0	10.0
Potato starch (dry)	0.0	50.0	0.0
Vegetable oil	40.0	50.0	30.0
Binding material	0.0	0.0	30.0
Premix[1]	5.0	5.0	5.0
Limestone	8.0	8.0	12.5
Monocalcium phosphate	7.0	7.0	12.0
Salt	1.2	1.2	2.3
Sodium bicarbonate	2.3	2.3	2.1
DL-Methionine	0.2	0.2	2.0
L-Threonine	0.0	0.0	0.9
L-Valine	0.0	0.0	0.7
L-Arginine-HCl	1.2	1.2	0.0
Total	1000	1000	1000
Calculated contents			
ME (kcal/kg)	2681	2680	2671
CP	225	225	215
CP (analyzed)	208	206	209
Digestible protein[2]	179	179	184
Indigestible protein	46.2	46.0	31.0
NSP[3]	178	182	159
Crude fiber	51.1	51.0	35.3
Digestible Lys[2]	10.6	10.6	10.6
Digestible Met + Cys[2]	7.8	7.8	7.7
Digestible Thr[2]	7.8	7.8	7.8
Digestible Trp[2]	2.2	2.2	2.2

RSM = rapeseed meal, FC = fermentable carbohydrates, SBM = soybean meal.

[1]Premix composition (per kg of diet): 12,000 IE retinol, 2,400 IE cholecalciferol, 50 mg dl-a-tocopherol, 1.5 mg menadione, 2.0 mg thiamine, 7.5 mg riboflavin, 3.5 mg pyridoxine, 20 mcg cyanocobalamins, 35 mg niacin, 12 mg D-pantothenic acid, 460 mg choline chloride, 1.0 mg folic acid, 0.2 mg biotin, 80 mg iron, 12 mg copper, 85 mg manganese, 60 mg zinc, 0.40 mg cobalt, 0.8 mg iodine, 0.1 mg selenium, 125 mg anti-oxidant mixture.

[2]Based on data from CVB (2007).

[3]NSP = Nonstarch polysaccharides, calculated by subtracting the CP, fat, starch, sugar, and ash content from the DM content.

oven to constant air DM weight. The dried weights of particles retained by each sieve and of the fines remaining in the bottom pan were expressed as percentages of total air DM recovered. The average particle size of the diets was calculated as (fraction $<$ 0.071 mm $\times$ 0.035) + (fraction 0.071 $-$ 0.16 mm $\times$ 0.115) + (fraction 0.16 $-$ 0.315 mm $\times$ 0.237) + (fraction 0.315 $-$ 0.630 mm $\times$ 0.472) + (fraction 0.630 $-$ 1.25 mm $\times$ 0.940) + (fraction 1.25 $-$ 2.50 mm $\times$ 1.65) + (fraction $>$ 2.50 mm $\times$ 3.50)/100. The particle size distribution of the fine and coarse diets is shown in Figure 1. For the fine and coarse RSM diets, the average particle size was 190 and 368 μm, respectively, whereas for the SBM (control) diet, it was 279 μm.

Pellet Durability

The pellet durability index was determined using a Holmen Pellet Tester (New Holmen Pellet Tester, TekPro Ltd., Norfolk, UK) by the method described by Svihus et al. (2004). The pellet samples (100 g) were circulated pneumatically through a closed pipe for 30 s. These samples were thereafter passed through a 3 mm sieve. The pellet durability index was calculated as the relative proportion of pellets retained on the 3 mm sieve. The pellet durability index values varied between 33.5 and 62.7% for the fine and 17.8 and 20.3% for the coarse diets (Figure 2).

Traits Measured

Feed intake (**FI**), water intake (**WI**), water to feed (WF) ratio, and BW gain per pen were recorded at 7, 14, 21, 28, and 34 d of age, and mortality was recorded daily. The feed conversion ratio (**FCR**) was calculated by dividing total FI by weight gain of live plus dead birds. At the end of the experiment (d 35 and 36; all treatments spread over the two days), 6 of the 8 birds from each replicate pen were randomly selected and euthanized by an intravenous injection of T-61 (0.5 mL; a watery solution containing a combination of embutramide, mebezoniumiodide, and tetracainehydrochloride; Hoechst Holland, Amsterdam, the Netherlands). Then the abdominal cavity was opened. On the dissection day, all the birds had access to feed until the moment of euthanization. The different segments of the GIT, i.e., crop, proventriculus, gizzard, duodenum (from pyloric junction to pancreo-billiary duct), jejunum (from pancreo-billiary duct to Meckle's diverticulum), ileum (from Meckle's diverticulum to ileo-cecal junction), cecum (from ostium), and colon, were segmented. The digesta from each segment were immediately removed by gentle squeezing, and the empty segments were weighed. The terminal ileum, the segment of the GIT from 15 to 2 cm anterior to the ileo-cecal junction, was clipped to avoid contamination. The ileal digesta were immediately collected from birds within a pen by gently squeezing them into a plastic container. These digesta were pooled and frozen at -20°C in air-tight containers until further chemical analysis. The ceca content of birds within a pen was quantitatively pooled and mixed. The pH was determined using a calibrated pH meter before the samples were freeze dried at -20°C pending VFA, biogenic amine, and ammonia analyses.

Tissue Collection and Histological Measurements

For intestinal morphological examination, duodenal samples (approximately 2 cm in length) from the middle of the duodenum were collected, rinsed with cold physiological saline (0.9% saline), and immediately placed in Bouin's fluid. The samples were then transferred to 70% ethanol within 24 h, embedded in paraffin, and sectioned at 5 μm thickness. For histological examination, 6 cross-sections per bird were processed using standard haematoxylin and eosin methods as described by Owusu-Asiedu et al. (2002). Villus height (the

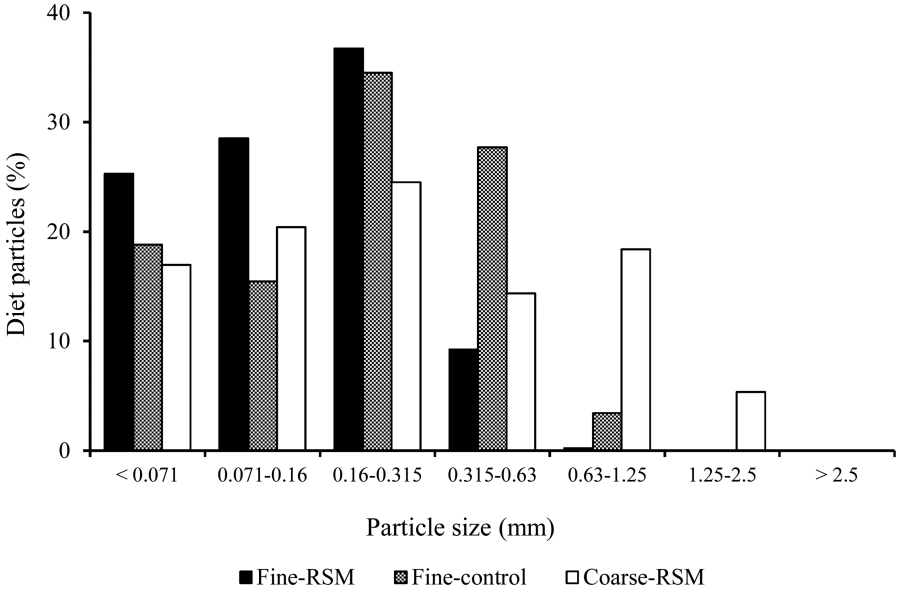

Figure 1. Particle size distribution of the control soybean meal (SBM) and experimental rapeseed meal (RSM) coarse and fine grower diets.

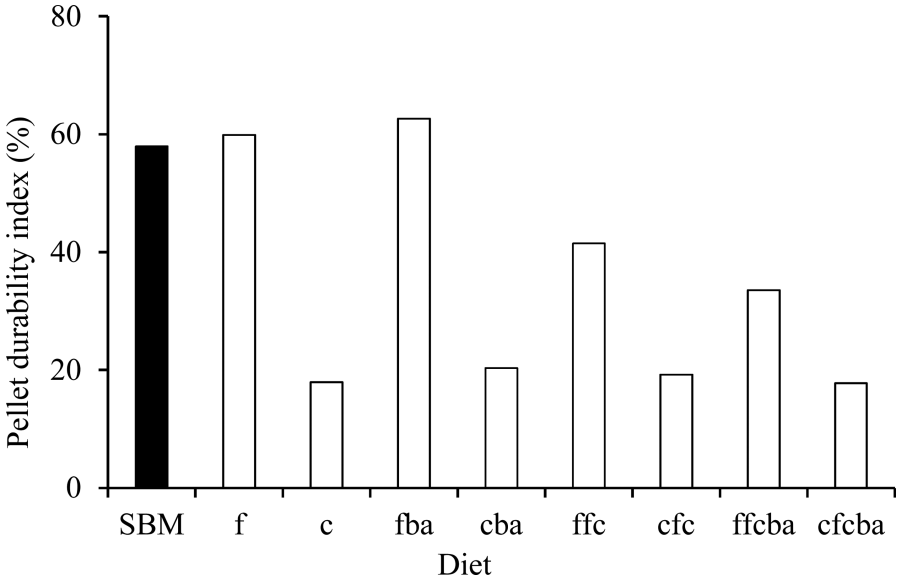

Figure 2. Pellet durability index of the control and experimental grower diets. SBM = soybean meal diet, f = fine diets, c = coarse diets, fba = fine diet supplemented with butyric acid, cba = coarse diet supplemented with butyric acid, ffc = fine diet supplemented with fermentable carbohydrates, cfc = coarse diet supplemented with fermentable carbohydrates, ffcba = fine diet supplemented with fermentable carbohydrates and butyric acid, and cfcba = coarse diet supplemented with fermentable carbohydrates and butyric acid. Fine: Ingredients passed through an opening screen of 3.0 mm in a hammer mill. Coarse: Ingredients passed through an opening screen of 1.6 mm in a roller mill.

distance from the apex of the villus to the junction of the villus and crypt) and crypt depth (the distance from the junction to the basement membrane of the epithelial cells at the bottom of the crypt) were measured on 10 intact, well-oriented villi (from the 2 cm section in the middle of the duodenum) per bird using a compound light microscope equipped with a video camera.

Chemical Analysis

Dry matter (AOAC International, 1998), organic matter (AOAC International, 2002), and nitrogen (**N**) (AOAC International, 1997) contents in the experimental diets were measured according to the standard methods. The ammonia in cecal digesta was measured by the indoles phenol-blue method (Novozamsky et al., 1974). The samples were deprotonated by adding 10% (w/v) trichloroacetic acid solution followed by centrifugation. The ammonium was transformed by phenol and hypochlorite in an alkaline solution into blue colored indoles phenol-blue by the Berthelot reaction. The ammonia content was measured spectroscopically at 623 nm.

For determination of VFAs, 5 g cecal samples and 5 mL 0.1 M phosphoric acid were shaken at 100 rpm for 30 min at room temperature before being centrifuged at 7,000 × g for 10 min at room temperature. Residues were collected, and the supernatants again were centrifuged at 20,817 × g for 10 min. Afterward, 600 μL

supernatant was transferred to a crimp vial and mixed with 600 μL phosphoric acid containing isocapronic acid (2.29 g/L concentration) as an internal standard. Volatile fatty acids were separated by gas chromatography using an EM-1000 (30 m × 0.53 mm) column from Alltech (Deerfield, IL), helium was used as a carrier gas, and VFAs were detected with a flame ionization detector (250°C). A gradient program was used with an initial temperature of 110°C (for 2 min) with an increase of 18°C/min until the temperature reached 200°C (at which temperature it was held for 2 min). Quantification of VFAs was based on a chemical standard solution (Merck) after internal standard correction.

Biogenic amines (histamine, putrescine, cadaverine, spermidine, spermine, and tyramine) in cecal digesta were determined by the method described by Meyer et al. (2013) with slight modifications. Briefly, 50 mg freeze-dried bullet mill sample was added with 20 mg sulphosalicylic acid and 1 mL 0.1 N hydrochloric acid. The samples were shaken for 15 min at a speed of 2,000 × g. Thereafter, the samples were placed in an ice bath for 15 min, vortexed, and transferred to a 1.5 mL Eppendorf tube. The supernatants were taken out, the tubes were centrifuged at 20,000 × g for 5 min, and the contents were filtered over a 0.2 μm filter. A 20 μL was injected on the column (Cation separation column LCAK17/K 4.6 × 30 mm) eluted with a combination of 0.4 N potassium citrate buffer (pH 5.75) and 2.5 N potassium citrate buffer (pH 8.4) followed by a post-column ortho-phthalaldehyde fluorescent excitation at 350 nm and emission at 425 nm.

Protease Activity

The extraction and dilution was conducted following the method described by Khoa (2007), with analysis performed following the method described by OJEC (1972). Briefly, the proventriculus was weighed, thawed, and placed in a 250 mL beaker. Thereafter, 3 mL 10 mM phosphate buffer (PBS, pH 7) per gram of proventriculus tissue was added, homogenized with ultra turrax at 0°C (the 250 mL beaker containing the proventriculus was placed in a 1 L beaker filled with ice and water), and centrifuged at 12,400 × g for 60 min at 4°C. The supernatants were extracted with a Pasteur pipette and transferred into a 2.5 mL Eppendorf cup. Thereafter, 1 mL supernatant was transferred into a plastic tube, and 4 mL 10 mM PBS (pH 7) was added. The two were mixed with a vortex and stored at −20°C. Upon chemical analysis, two samples of diluted proventriculus extract were brought to room temperature, 100 μL was mixed with 4 mL ice-cold acidified hemoglobin solution (2 g hemoglobin dissolved in 0.06 N HCl), and after 5 or 10 min, proteolytic degradation was stopped with 6.8 mL 5% (w/v) trichloroacetic acid. The contents were filtered on a Whatman paper filter, and 2.5 mL filtrate was pipetted in a 12 mL tube. Next, 5 mL sodium hydroxide solution and 3 mL 2× diluted Folin-Ciocalteu reagent (HC389020; Merck KGaA, Darmstadt, Germany) were added, and the tube was vortexed. In 5 and 10 min samples, absorbances were measured at 750 nm against water, and the difference in absorbance between the samples was indicative of the amount (μmole) of tyrosine released per minute per gram of tissue.

Apparent Ileal Digestibility of Crude Protein

Crude protein contents in the diet and ileal digesta were calculated as N × 6.25, with N being measured by the Kjeldahl method with $CuSO_4$ as a catalyst (ISO 5983). Apparent ileal CP digestibility (AID) was calculated using the following equation (Stein et al., 2001):

$$\text{AID} = [100 - (\text{Crude protein}_\text{d}/\text{Crude protein}_\text{f}) \times (\text{Ti}_\text{f}/\text{Ti}_\text{d})] \times 100$$

where crude protein$_\text{d}$ and Ti$_\text{d}$ were the concentrations of CP and titanium in the ileal digesta, respectively, and crude protein$_\text{f}$ and Ti$_\text{f}$ were the concentrations of the same dietary components in the feed, respectively, all expressed on a DM basis.

Statistical Analysis

The repeated statement within PROC MIXED of SAS (version 9.2; SAS Inst. Inc., Cary, NC) was used for data analysis. The following statistical model was used for the factorial analysis of the eight treatments:

$$\text{Y}_{ijkl} = \mu + \text{ST}_i + \text{BA}_j + \text{FC}_k + \text{ST}_i \times \text{BA}_j + \text{ST}_i \times \text{FC}_k + \text{BA}_j \times \text{FC}_k + \text{ST}_i \times \text{BA}_j \times \text{FC}_k + \text{e}_{ijkl}$$

Where:Y_{ijkl} = Measured response μ = Overall mean effect ST_i = i^{th} fixed diet structure effect (i = fine or coarse) BAj = j^{th} fixed BA supplementation effect (j = with or without BA) FC_k = k^{th} fixed FC supplementation effect (k = with or without FC) $\text{ST}_i \times \text{BA}_j$ = Interaction between diet structure and BAST$_i \times \text{FC}_k$ = Interaction between diet structure FC, $\text{BA}_j \times \text{FC}_k$ = Interaction between BA and FC supplementation, STi × BAj × FC_k = Interaction between diet structure, BA supplementation, and FC supplementation $e_{ijkl} \sim$ NID (0, σ2e) Differences were considered significant at a probability level of 5%. The interactions were ignored if the main effect for a particular trait was found to be nonsignificant. Analysis of the data showed that there were no 3-way interactions except for FI. It was decided, therefore, not to report 3-way interactions in the results section. Room effect was also tested statistically, and no significant effect was observed. Therefore, this element was omitted from statistical analysis. The SBM diet was added as the 9th treatment, was used as a

positive control group, and was disregarded from statistical analysis.

RESULTS

Bird Performance

The effects of the dietary treatments on broiler performance are presented in Table 2. Over the entire experimental period (0 to 34 d of age), broilers fed the 8 experimental (RSM) diets had, on average, 13, 26, 9, 15, and 3% lower FI, BW gain, FCR, WI, and water to feed (**WF**) ratio, respectively, compared with those fed the SBM diet (not statistically tested).

Broilers fed the coarse RSM diets had, on average, 6% greater FI, 11% greater BW gain, 5% improved FCR, and 7% lower WF ratio over the 34 d experimental period compared with those fed the fine RSM diets. Body weight gain and FCR were affected by BA supplementation during the starter period (0 to 14 d of age) as well as during the entire 34 d experimental period. Broilers fed BA-supplemented diets showed 7 and 4% greater BW gain and 5 and 3% improved FCR during both periods, respectively, compared with those fed the diets without BA. No other parameters were affected by BA supplementation. The WF ratio was affected by FC supplementation during the starter period as well as over the entire experimental period. Broilers fed FC-supplemented diets showed, on average, a 9 and 3% lower WF ratio during both periods, respectively, compared to those fed the diets without FC supplementation. During the 34 d experimental period, an interaction between diet structure (**ST**) and BA was observed for FI ($P = 0.032$), indicating that broilers fed the coarse diets supplemented with BA increased FI, whereas in broilers fed the fine diets, FI did not increase with BA supplementation. For both periods (0 to 14 and 0 to 34 d of age), interactions between ST and BA were observed for WI ($P = 0.012$ and $P = 0.013$) and the WF ratio ($P = 0.003$ and $P = 0.003$), indicating that broilers fed the coarse diets supplemented with BA had greater WI and WF ratio, whereas broilers fed the fine diets supplemented with BA had lower WI and WF ratio. During the entire experimental period, interactions between ST and FC were observed for FI ($P = 0.009$) and BW gain ($P = 0.009$), indicating that in broilers fed the coarse diets supplemented with FC, FI and BW gain decreased, whereas in broilers fed the fine diets supplemented with FC, FI and BW gain increased.

Over the entire experimental period, interactions between BA and FC were observed for FI ($P = 0.037$), FCR ($P = 0.038$), and WI ($P = 0.014$), indicating that for broilers fed a BA-supplemented diet, FC supplementation did not influence FI, whereas FC supplementation increased FI in broilers fed diets without BA.

Table 2. Effects of diet structure (ST), butyric acid (BA), and fermentable carbohydrates (FC) on performance parameters in broilers from 0 to 14 and 0 to 34 d of age.

	Observations[1]									
Effects	FI (g/bird/d)		BW gain (g/bird/d)		FCR (g/g)		WI (ml/bird/d)		WF ratio (mL/bird/d)	
Age (d) →	0–14	0–34	0–14	0–34	0–14	0–34	0–14	0–34	0–14	0–34
Control group[2] (SBM[3] diet)	38.2	117.2	31.6	84.5	1.23^{y}	1.40^{y}	97.1	224.7	2.59^{x}	1.92
Experimental groups[2] (RSM[3] diet)	35.3	102.5	27.4	67.3	1.30^{x}	1.53^{x}	81.3	190.7	2.28^{y}	1.86
Factorial analysis of the experimental RSM diets										
ST: Fine										
With BA										
With FC	35.4	98.4^{c}	$27.9^{a,b,c}$	$65.8^{c,d}$	$1.31^{a,b}$	$1.50^{b,c}$	$76.6^{c,d,e}$	176.1^{b}	$2.16^{b,c,d}$	1.79^{b}
Without FC	34.7	99.4^{c}	$26.3^{c,d}$	$63.6^{d,e}$	1.38^{a}	$1.56^{a,b}$	$85.2^{a,b,c}$	$196.2^{a,b}$	$2.40^{a,b,c}$	1.97^{a}
Without BA										
With FC	36.4	$104.1^{a,b}$	$26.9^{c,d}$	$65.1^{c,d}$	$1.36^{a,b}$	1.60^{a}	90.9^{a}	202.3^{a}	$2.46^{a,b}$	$1.94^{a,b}$
Without FC	33.1	96.8^{c}	25.4^{d}	60.5^{e}	$1.33^{a,b}$	1.60^{a}	$87.5^{a,b,c}$	$194.7^{a,b}$	2.60^{a}	2.01^{a}
ST: Coarse										
With BA										
With FC	35.7	$106.2^{a,b}$	$29.2^{a,b}$	72.6^{a}	1.23^{b}	1.46^{c}	$75.3^{c,d,e}$	$187.7^{a,b}$	$2.08^{c,d}$	1.77^{c}
Without FC	35.5	107.2^{a}	29.6^{a}	71.8^{a}	1.21^{b}	$1.49^{b,c}$	$88.1^{a,b}$	205.6^{a}	$2.48^{a,b}$	1.92^{b}
Without BA										
With FC	36.4	103.5^{b}	$27.1^{b,c,d}$	$67.7^{b,c}$	1.35^{a}	$1.53^{b,c}$	72.4^{e}	$186.1^{a,b}$	1.96^{d}	1.82^{c}
Without FC	35.9	$105.4^{a,b}$	$26.5^{c,d}$	$71.1^{a,b}$	1.36^{a}	$1.48^{b,c}$	$74.0^{d,e}$	176.9^{b}	2.04^{d}	1.68^{d}
Pooled SE	0.86	1.19	0.78	1.19	0.04	0.02	4.35	7.37	0.11	0.07
P-value										
ST	0.11	<0.001	0.014	0.001	0.87	0.001	0.021	0.54	0.003	0.004
BA	0.86	0.72	0.003	0.009	0.006	0.004	0.98	0.79	0.82	0.89
FC	0.063	0.35	0.15	0.22	0.81	0.597	0.13	0.32	0.013	0.020
ST × BA	0.51	0.032	0.12	0.58	0.005	0.379	0.012	0.013	0.003	0.003
ST × FC	0.17	0.009	0.21	0.009	0.60	0.269	0.46	0.86	0.77	0.35
BA × FC	0.25	0.037	0.67	0.59	0.47	0.038	0.071	0.014	0.19	0.065

[x,y,a–e]Means without a common superscript within a column significantly differ($P < 0.05$).

[1]FI = feed intake, BW = body weight, FCR = feed conversion ratio, WI = water intake, WF ratio = water to feed ratio.

[2]Control group, n = 4 replicates; experimental groups, n = 32 replicates; for each replicate in all the treatments, n = 8 birds.

[3]SBM = soybean meal, [3]RSM = rapeseed meal.

Fermentable carbohydrate supplementation improved the FCR in broilers fed BA-supplemented diets, whereas the FCR was decreased by FC supplementation in broilers fed the diet without BA. Water intake was decreased in broilers fed FC in BA-supplemented diets, whereas FC supplementation increased WI in broilers fed diets without BA.

Digestive Tract Measurements

The effects of dietary treatments on relative empty weights of the GIT segments are presented in Table 3. Broilers fed the 8 experimental (RSM) diets had, on average, 14% heavier gizzards and 11% lower relative weights of the colon compared with broilers fed the SBM diet. Other GIT segments were similar between broilers fed the 8 RSM diets and the SBM diet. Broilers fed the coarse RSM diets had, on average, 11, 7, 5, and 6% lower relative empty weights of the crop, duodenum, jejunum, and ileum, respectively, compared with those fed the fine RSM diets ($P < 0.05$). The gizzard was, however, on average 12% heavier in broilers fed the coarse diets compared with those fed the fine diets ($P < 0.001$). An interaction between ST and BA was observed for the empty relative weight of the gizzard ($P = 0.021$), indicating that in the broilers fed the coarse diet , BA supplementation resulted in a lower gizzard weight, whereas in the broilers fed the fine diets, BA supplementation increased gizzard weight.

The effects of dietary treatments on duodenal villus height, crypt depth, and villus height to crypt depth ratio (**VCR**) are presented in Table 4. Broilers fed the 8 RSM diets had, on average, 11, 49, and 42% lower villus height, crypt depth, and VCR, respectively, compared to broilers fed the SBM diet. Villus height, crypt depth, and VCR were affected by diet structure, as broilers fed the coarse RSM diets had, on average, 19% greater villus height, 18% deeper crypt, and 48% greater VCR compared to broilers fed the fine RSM diets. Villus height was not affected by BA supplementation. Crypt depth and VCR were affected by BA supplementation, whereas broilers fed BA-supplemented diets showed, on average, 10% deeper crypts and 14% greater VCR compared to broilers fed diets without BA supplementation. Fermentable carbohydrate supplementation resulted in 5 and 6% lower villus heights and deeper crypts, respectively, compared with those fed diets without FC. Interactions between ST and BA were observed for villus height ($P = 0.045$), crypt depth ($P = 0.041$), and VCR ($P < 0.001$), indicating that in broilers fed the coarse diets, BA supplementation did not influence villus height, whereas in those fed the fine diets, BA supplementation decreased villus height. Crypt depth was decreased and VCR was increased in broilers fed BA-supplemented coarse diets; in those fed the fine diets, both parameters remained unaffected by BA supplementation. An interaction between ST and FC for crypt depth was observed ($P = 0.041$), indicating that FC supplementation did not affect crypt depth in broilers fed the coarse diets, whereas crypt depth was decreased in those fed the fine diets supplemented with FC. Interactions were observed between BA and FC for crypt depth

Table 3. Effects of diet structure (ST), butyric acid (BA), and fermentable carbohydrates (FC) on mean relative weights (g/100 g BW) of empty gastrointestinal segments in broilers at 35 and 36 d of age.

Effects	Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Ceca	Colon
Control group[1] (SBM[2] diet)	0.32	0.62	1.00^{y}	0.91	1.23	1.10	0.37	0.18^{y}
Experimental groups[1] (RSM[2] diet)	0.32	0.60	1.14^{x}	0.94	1.25	1.12	0.36	0.20^{x}
Factorial analysis of the experimental RSM diets								
ST: Fine								
With BA								
With FC	0.34^{a,b}	0.68	1.08^{d,e}	1.00^{a,b}	1.32^{a,b}	1.13^{a,b}	0.37	0.20
Without FC	0.34^{a,b}	0.65	1.13^{b,c,d}	0.96^{a,b}	1.24^{a,b}	1.15^{a,b}	0.37	0.22
Without BA								
With FC	0.32^{a,b}	0.56	0.98^{e}	0.93^{a,b}	1.26^{a,b}	1.19^{a}	0.35	0.20
Without FC	0.36^{a}	0.58	1.07^{d,e}	1.02^{a}	1.34^{a}	1.15^{a,b}	0.39	0.21
ST: Coarse								
With BA								
With FC	0.31^{a,b}	0.55	1.24^{a,b}	0.91^{a,b}	1.22^{a,b}	1.09^{b,c}	0.38	0.20
Without FC	0.28^{b}	0.63	1.13^{b,c,d}	0.87^{b}	1.19^{b}	1.04^{c}	0.33	0.19
Without BA								
With FC	0.31^{a,b}	0.56	1.25^{a}	0.92^{a,b}	1.22^{a,b}	1.10^{b,c}	0.36	0.21
Without FC	0.31^{a,b}	0.56	1.25^{a}	0.91^{a,b}	1.23^{a,b}	1.11^{b}	0.35	0.19
Pooled SE	0.01	0.05	0.04	0.05	0.05	0.03	0.02	0.01
P-value								
ST	0.001	0.21	<0.001	0.029	0.047	0.006	0.15	0.064
BA	0.62	0.098	0.80	0.75	0.61	0.19	0.70	0.67
FC	0.76	0.68	0.89	0.95	0.90	0.57	0.68	0.43
ST × BA	0.55	0.38	0.021	0.59	0.92	0.77	0.88	0.42
ST × FC	0.15	0.53	0.052	0.44	0.86	0.69	0.038	0.019
BA × FC	0.15	0.83	0.19	0.19	0.16	0.95	0.17	0.89

[x,y,a–e]Means without a common superscript within a column significantly differ ($P < 0.05$).

[1]Control group, n = 4 replicates; experimental groups, n = 32 replicates; for each replicate in all the treatments, n = 6 birds.

[2]SBM = soybean meal, [2]RSM = rapeseed meal.

Table 4. Effects of diet structure (ST), butyric acid (BA), and fermentable carbohydrates (FC) on duodenal villus height, crypt depth, and villus height to crypt depths ratio (VCR) in broilers at 35 and 36 d of age.

Effects	Villus height (μm)	Crypt depth (μm)	VCR
Control group[1] (SBM[2] diet)	1,413^{x}	164^{y}	9.59^{x}
Experimental groups[1] (RSM[2] diet)	1,260^{y}	244^{x}	5.56^{y}
Factorial analysis of the experimental RSM diets			
ST: Fine			
With BA			
With FC	1,028^{c}	259^{b}	4.11^{d}
Without FC	1,197^{b}	267^{b}	4.62^{d}
Without BA			
With FC	1,147^{b,c}	255^{b}	4.66^{d}
Without FC	1,205^{b}	299^{a}	4.30^{d}
ST: Coarse			
With BA			
With FC	1,380^{a}	208^{d}	6.92^{b}
Without FC	1,397^{a}	199^{d}	7.64^{a}
Without BA			
With FC	1,335^{a}	232^{c}	5.94^{c}
Without FC	1,332^{a}	248^{b,c}	5.62^{c}
Pooled SE	41.8	8.04	0.25
P-value			
ST	<0.001	<0.001	<0.001
BA	0.89	<0.001	0.002
FC	0.041	0.006	0.45
ST × BA	0.045	0.041	<0.001
ST × FC	0.070	0.041	0.72
BA × FC	0.27	0.006	0.010

$^{x,y,a–d}$Means without a common superscript within a column significantly differ($P < 0.05$).

[1]Control group, n = 4 replicates; experimental groups, n = 32 replicates.

[2]SBM = soybean meal, [2]RSM = rapeseed meal.

($P = 0.006$) and VCR ($P = 0.010$), indicating that FC supplementation in broilers fed BA-supplemented diets did not influence crypt depth, whereas FC supplementation decreased crypt depth in broilers fed diets without BA. The FC diet supplemented with BA decreased VCR, compared to the diet without BA supplementation.

Digesta Characteristics

The effects of dietary treatments on digesta characteristics are presented in Table 5. Broilers fed the 8 RSM diets had, on average, 19, 6, and 26% lower gizzard pH, cecal pH, and cecal ammonia, respectively, compared to broilers fed the SBM diet. As seen in the factorial analysis of the experimental groups, broilers fed the coarse diets had, on average, 20% more acidic gizzard pH and 6% improved ileal apparent protein digestibility compared with those fed the fine diets. No interactions were found between these traits. Dietary treatments did not influence protease activity in the proventriculus ($P > 0.05$). There was a trend for BA supplementation to increase protease activity in the proventriculus ($P = 0.056$).

The effects of dietary treatments on cecal VFA concentrations are presented in Table 6. Broilers fed the SBM diet had, on average, 9% greater total VFA concentrations. Percentages of propionic acid (7.0 and 5.5%), total BCFA (2.66 and 1.78%), and valeric acid (1.63 and 2.05%) relative to total VFA differed between the broilers fed the SBM and RSM diets, respectively. Broilers fed the coarse RSM diets had a lower percentage of isovaleric acid ($P < 0.026$) and total BCFA ($P < 0.037$) in the total VFAs measured compared with broilers fed the fine RSM diets (1.56 vs. 2.01%).

The effects of dietary treatments on cecal biogenic amines are presented in Table 7. Broilers fed the SBM diet had approximately 16% greater total biogenic amine concentrations in the cecal digesta compared with those fed the RSM diets. Neither diet structure nor BA supplementation affected ($P > 0.05$) cecal biogenic amine concentrations. A trend, however, for BA supplementation to decrease cadaverine concentration in the cecal digesta was observed ($P = 0.055$). Broilers fed FC-supplemented diets had, on average, a 31% lower concentration of spermine in the cecal digesta compared with those fed diets without FC ($P < 0.02$).

DISCUSSION

The present study was conducted to investigate the impact of diet structure, BA supplementation, and FC supplementation on performance, gut morphology, and hindgut fermentation characteristics in broilers. It was hypothesized that broilers' poor performance due to a poorly digestible protein source could be counterbalanced by feeding a coarsely ground diet supplemented with BA and FC. Performance, GIT development, and cecal digesta characteristics were therefore studied as explanatory variables. Increased villus height and

Table 5. Effects of diet structure (ST), butyric acid (BA), and fermentable carbohydrates (FC) on gizzard and cecal pH, cecal NH_3, apparent ileal protein digestibility, and protease activity in the proventriculus in broilers at 35 and 36 d of age.

Effects	Gizzard pH	Cecal pH	NH_3 (g/kg DM)	Protein digestibility (%)	Protease activity (μmol tyrosine released/min/g of tissue)
Control group[1] (SBM[2] diet)	3.96^{x}	6.45^{x}	3.20^{x}	72.9	84.6^{y}
Experimental groups[1] (RSM[2] diet)	3.20^{y}	6.07^{y}	2.36^{y}	70.2	97.1^{x}
Factorial analysis of the experimental RSM diets					
ST: Fine					
With BA					
With FC	3.93^{a}	5.98	2.07	70.8^{a,b}	105.5
Without FC	3.10^{b,c,d}	6.11	2.30	66.9^{b}	104.0
Without BA					
With FC	3.78^{a,b}	6.14	2.67	67.1^{b}	88.3
Without FC	3.44^{a,b,c}	6.03	2.33	68.6^{a,b}	94.4
ST: Coarse					
With BA					
With FC	3.02^{c,d}	5.98	2.48	74.9^{a}	97.2
Without FC	2.89^{c,d}	6.15	2.32	69.4^{a,b}	101.2
Without BA					
With FC	2.66^{d}	6.10	2.22	72.9^{a,b}	92.2
Without FC	2.79^{c,d}	6.08	2.49	72.0^{a,b}	94.3
Pooled SE	0.246	0.10		2.53	6.80
P-value					
ST	0.004	0.85	0.74	0.038	0.71
BA	0.71	0.67	0.24	0.85	0.056
FC	0.10	0.52	0.99	0.23	0.59
ST × BA	0.37	0.89	0.12	0.72	0.44
ST × FC	0.11	0.63	0.63	0.59	0.94
BA × FC	0.29	0.14	0.75	0.18	0.77

$^{x,y,a–d}$Means without a common superscript within a column significantly differ ($P < 0.05$).

[1]Control group, n = 4 replicates; experimental groups, n = 32 replicates; for each replicate in all the treatments, n = 6 birds.

[2]SBM = soybean meal, [2]RSM = rapeseed meal.

decreased crypt depth in the duodenum were used as indicators of intestinal health.

The observed poorer performance (lower FI, reduced BW gain, and poor FCR) of the broilers fed the RSM diets compared with those fed the SBM diet is in accordance with expectations (Montazer-Sadegh et al., 2008; Chiang et al., 2010; Saleem, 2013). This reduced performance of broilers on the RSM diets in the present study cannot be attributed to hindgut protein fermentation. Greater levels of BCFA, biogenic amines, and ammonia are considered evidence of the occurrence of protein fermentation (Macfarlane et al., 1992). These fermentation products were, however, not increased in broilers fed the RSM diets in the present study. Other factors such as the presence of antinutritional factors, glucosinolates, tannins, phytic acid, and sinapine may have resulted in the poor performance of broilers fed the RSM diets (Khajali and Slominski, 2012; Rutkowski et al., 2012; Ahmed et al., 2014). Reduced villus height, deeper crypts, and lower VCR in broilers fed RSM diets compared with those fed the SBM diet may be due to the aforementioned antinutritional factors. Greater villus height and VCR are indicative of proper digestion and absorption of nutrients (Chiang et al., 2010). Some antinutritional factors result in gut wall damage and increased endogenous protein losses (Smits et al., 1997). High inclusion levels of RSM (>20%) increased the energy needed for gut wall repair and for the liver's metabolic activities, which may result in reduced performance (Woyengo et al., 2011). Improved performance in broilers fed the coarse diets compared with those fed the fine diets is in accordance with expectations and confirms some recent broiler studies (Jacobs et al., 2010; Rodgers et al., 2012; Jacobs and Parsons, 2013; Pacheco et al., 2013). The decreased gizzard pH in broilers fed the coarse diet can be explained by the greater stimulatory activity of the gizzard, which allows more hydrochloric acid secretion. Our current study's finding of greater protein digestibility with coarse particles is also in accordance with findings of recent broiler studies (Pacheco et al., 2013; Liu et al., 2013). This improved digestibility may be attributed to a more functional gizzard and increased gastric reflux between proventriculus and gizzard that result in more time for gastric enzyme activity and even for more protease activity in the duodenum (Benedetti et al., 2011). Pacheco et al. (2013) reported greater protein digestibility in broilers fed coarsely ground corn compared with those fed fine corn (86.1 vs. 84.8%). In addition, fine particles can increase digesta viscosity (Amerah et al., 2007), which may negatively affect nutrient digestibility (Smits et al., 1997).

The lower weights of the empty crops in broilers fed the coarse diets indicate less feed accumulation in the crop compared with those fed the fine diets. Our current study's finding of greater relative gizzard weights in broilers fed the coarse diets compared with those fed the fine diets is supported by other recent research (Benedetti et al., 2011; Bhuiyan et al., 2012; Rodgers et al., 2012; Jacobs and Parsons, 2013).

Table 6. Effects of diet structure (ST), butyric acid (BA), and fermentable carbohydrates (FC) on cecal volatile fatty acids (VFA [mmol/kg DM]) concentrations in broilers at 35 and 36 d of age.

Effects	Total VFA	Acetic	Propionic acid[1]	Butyric acid[1]	Valeric acid[1]	Isobutyric acid[1]	Isovaleric acid[1]	Total BCFA[2]
Control group[3] (SBM[4] diet)	92.6	71.6	7.0^{x}	17.1	1.63^{y}	1.28	1.38^{x}	2.66^{x}
Experimental groups[3] (RSM[4] diet)	83.9	73.0	5.5^{y}	18.0	2.05^{x}	0.96	0.82^{y}	1.78^{y}
Factorial analysis of the experimental RSM diets								
ST: Fine								
With BA								
With FC	86.9	72.2	4.72	19.9	1.97	1.18	1.01^{a}	2.18^{a}
Without FC	78.6	73.8	5.75	16.9	2.16	1.07	1.06^{a}	2.12^{a,b}
Without BA								
With FC	94.4	72.8	5.71	18.2	2.00	0.95	0.79^{a,b}	1.74^{a,b}
Without FC	73.7	73.6	5.69	16.7	2.28	0.99	0.99^{a,b}	1.98^{a,b}
ST: Coarse								
With BA								
With FC	90.3	73.1	5.38	18.0	2.00	0.89	0.66^{b}	1.55^{a,b}
Without FC	87.7	72.3	6.27	17.4	2.09	1.10	0.82^{a,b}	1.92^{a,b}
Without BA								
With FC	79.9	74.3	5.15	17.6	1.95	0.72	0.59^{b}	1.31^{b}
Without FC	79.8	72.1	5.61	18.9	1.94	0.78	0.66^{b}	1.44^{a,b}
Pooled SE	8.86	1.20	0.44	1.25	0.16	0.19	0.17	0.29
P-value								
ST	0.87	0.83	0.67	0.96	0.36	0.206	0.026	0.037
BA	0.54	0.68	0.98	0.82	0.89	0.14	0.29	0.12
FC	0.22	0.89	0.072	0.29	0.23	0.71	0.33	0.42
ST × BA	0.42	0.88	0.16	0.39	0.42	0.72	0.92	0.87
ST × FC	0.30	0.13	0.79	0.14	0.41	0.52	0.95	0.71
BA × FC	0.69	0.53	0.25	0.35	0.98	0.99	0.90	0.94

$^{x,y,a-b}$Means without a common superscript within a column significantly differ ($P < 0.05$).

[1]Percentage of total VFA (acetic acid + propionic acid + butyric acid + valeric acid + isobutyric acid + isovaleric acid).

[2]BCFA = branched chain fatty acids (sum of isobutyric and isovaleric acids).

[3]Control group, n = 4 replicates; experimental groups, n = 32 replicates; for each replicate in all the treatments, n = 6 birds.

[4]SBM = soybean meal, [4]RSM = rapeseed meal.

Jacobs and Parson (2013) reported 47 and 22% heavier gizzards in broilers fed whole sorghum and coarse corn, respectively, compared to those fed fine diets. The improved gizzard weight in broilers fed the coarse diets may be due to a more functional gizzard compared with those fed the fine diets. The greater relative weights of the duodenums, jejunums, and ilea in broilers fed the fine diets can be explained by their increased activity as a result of a more poorly developed and smaller nonfunctional gizzard. Greater duodenal villus height and VCR along with a lower crypt depth in broilers fed the coarse diets suggest improved digestion of nutrients because of proper predigestion in the foregut (Pacheco et al., 2013). Greater villus heights in broilers fed coarse diets may also be due to less abrasive action by digesta in the duodenum. Correspondingly, Sogunle et al. (2013) reported 89% greater duodenal villus heights in broilers fed diets with, on average, a dietary particle size of 2 mm compared with those fed a particle size of 1 mm.

Broilers fed the coarse diets were expected to show decreased concentrations of cecal BCFAs and biogenic amines because of improved ileal protein digestibility due to enhanced gizzard weight. In the present work, however, coarse diets had no influence on cecal biogenic amines concentrations, but there was a decrease in total BCFA, mainly isovaleric acid, which is produced as a result of bacterial fermentation of leucine. The improved performance in broilers fed BA-supplemented diets is in accordance with previously reported broiler studies (Antongiovanni et al., 2009; Adil et al., 2010; Pouraziz et al., 2013). Pouraziz et al. (2013) reported approximately 8 and 6% improved BW and FCR, respectively, in broilers fed a diet with 0.004 g/g BA glycerides compared with broilers fed a control diet in both starter and grower phases. These positive effects of BA supplementation may be due to improved digestion and absorption of nutrients (Mansoub, 2011), as a consequence of increased pancreatic enzyme secretion, and because of their effects on gut mucosa and their antimicrobial activity (Adil et al., 2010). Improved gut morphology in broilers fed BA-supplemented diets may be due to the provision of energy to enterocytes because BA is one of the major energy sources for these cells (Czerwiński et al., 2012). Antongiovanni et al. (2009) hypothesized BA to be the main growth promoter of the gut wall in broilers. Histological changes in the intestine as a result of BA supplementation may increase the surface area for absorption of nutrients in the intestine, enhancing growth performance and thereby reducing the amount of substrate available for fermentation by microbiota in the hindgut of the broilers. This was confirmed in this study by the improved growth performance in broilers fed the diets supplemented with BA compared with those fed the diets without BA. Due to improved gut health, as illustrated by enhanced gut morphology, BA supplementation was expected to increase ileal digestibility of protein. In the

Table 7. Effects of diet structure (ST), butyric acid (BA), and fermentable carbohydrates (FC) on cecal biogenic amines (mmol/kg DM) concentration in broilers at 35 and 36 d of age.

Effects	Histamine	Putrescine	Cadaverine	Spermidine	Spermine	Tyramine	Total
Control group[1] (SBM[2] diet)	0.45	1.71	0.43	21.7[x]	0.60	1.31	27.4[x]
Experimental groups[1] (RSM[2] diet)	0.54	1.44	0.80	18.3[y]	0.86	0.84	23.1[y]
Factorial analysis of the experimental RSM diets							
ST: Fine							
With BA							
With FC	0.36	1.27	0.59	17.5	0.61[c]	0.75	24.42
Without FC	0.40	1.46	0.65	20.4	1.00[a,b]	0.85	24.76
Without BA							
With FC	0.66	1.73	1.04	17.9	0.79[b,c]	0.84	22.94
Without FC	0.37	1.33	0.87	18.1	1.00[a,b]	0.82	22.42
ST: Coarse							
With BA							
With FC	0.38	1.18	0.82	16.7	0.72[b,c]	0.92	20.72
Without FC	1.17	1.58	0.61	18.7	0.88[a,b,c]	0.91	23.88
Without BA							
With FC	0.43	1.49	0.96	17.9	0.71[b,c]	0.85	21.84
Without FC	0.55	1.46	0.91	19.1	1.24[a]	0.81	24.08
Pooled SE	0.17	0.18	0.19	1.42	0.13	0.19	2.13
P-value							
ST	0.14	0.88	0.80	0.73	0.61	0.67	0.49
BA	0.55	0.32	0.055	0.93	0.17	0.84	0.68
FC	0.19	0.75	0.50	0.13	0.002	0.96	0.38
ST × BA	0.101	0.79	0.67	0.39	0.59	0.69	0.39
ST × FC	0.026	0.25	0.79	0.98	0.71	0.82	0.35
BA × FC	0.052	0.055	0.88	0.39	0.66	0.78	0.77

[x,y,a–c]Means without a common superscript within a column significantly differ ($P < 0.05$).

[1]Control group, n = 4 replicates; experimental groups, n = 32 replicates; for each replicate in all the treatments, n = 6 birds.

[2]SBM = soybean meal, [2]RSM = rapeseed meal.

present study, however, the BA-supplemented diets did not affect ileal digestibility of protein. Therefore, the BA-supplemented diets did not affect the concentration of total cecal VFA, BCFA, and biogenic amines. A tendency toward a lower cecal cadaverine concentration in broilers fed the BA-supplemented diets can be explained by a decrease in the number of pathogenic microbiota such as *Clostridium perfringens* (Qaisrani et al., unpublished data), as *C. perfringens* seems to be involved in protein fermentation in the hindgut (Richardson et al., 2013). The decreased cecal concentration of spermine in broilers fed FC-supplemented diets may indicate a lower concentration of spermidine present in the ceca. Spermine is produced with the catabolism of spermidine, which in turn is produced with the fermentation of amino acids such as histidine, ornithine, arginine, and methionine. This indicates less fermentation of the aforementioned amino acids in the ceca of broilers fed the FC-supplemented diets.

The overall results of the present study indicate that RSM resulted in poor performance and impaired gut morphology. Inclusion of dietary coarse particles improved gut development. Heavier gizzards, enhanced gut morphology, better protein digestibility, and improved FCR as well as significantly lower concentrations of hindgut fermentation products, BCFA, were found in broilers fed the coarse diets. Supplementing with BA further improved the performance of broilers fed a coarse diet. In conclusion, feeding a coarse diet supplemented with BA improves performance of broilers fed a poorly digestible protein source.

ACKNOWLEDGMENTS

The authors gratefully acknowledge funding from the Higher Education Commission (HEC) of Pakistan for S.N. Qaisrani to pursue a doctorate degree in the Netherlands. Technicians of the animal facility Carus (Wageningen University, the Netherlands) are also thanked for their assistance in performing the trial.

REFERENCES

Adil, S., T. Banday, G. A. Bhat, M. S. Mir, and M. Rehman. 2010. Effect of dietary supplementation of organic acids on performance, intestinal histomorphology, and serum biochemistry of broiler chicken. Vet. Med. Int. 10:4061–4067.

Ahmed, A., I. Zulkifli, A. S. Farjam, N. Abdullah, and J. B. Liang. 2014. Extrusion enhances metabolizable energy and ileal amino acids digestibility of canola meal for broiler chickens. Ital. J. Anim. Sci. 13:44–47.

Amerah, A. M, V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007. Feed particle size: Implications on the digestion and performance of poultry. World's Poult. Sci. J. 63:439–455.

Antongiovanni, M., A. Buccioni, F. Petacchi, S. Leeson, S. Minieri, A. Martini, and R. Cecchi. 2009. Butyric acid glycerides in the diet of broiler chickens: Effects on gut histology and carcass composition. Ital. J. Anim. Sci. 6:19–25.

AOAC International. 2002. Animal feeding stuffs: Determination of crude ash. ISO 5984. Int. Organ. Standardization, Geneva, Switzerland.

AOAC International. 1998. Animal feeding stuffs: Determination of moisture and other volatile matter content. ISO 6496. Int. Organ. Standardization, Geneva, Switzerland.

AOAC International. 1997. Animal feeding stuffs: Determination of nitrogen content and calculation of crude protein content. Kjeldahl method. ISO 5983. Int. Organ. Standardization, Geneva, Switzerland.

Awati, A., B. A. Williams, M. W. Bosch, W. J. Gerrits, and M. W. A. Verstegen. 2006. Effect of inclusion of fermentable carbohydrates in the diet on fermentation end-product profile in feces of weanling piglets. J. Anim. Sci. 84:2133–2140.

Benedetti, M. P., J. R. Sartori, F. B. Carvalho, L. A. Pereira, V. B. Fascina, A. C. Stradiotti, A. C. Pezzato, C. Costa, and J. G. Ferreira. 2011. Corn texture and particle size in broiler diets. Rev. Bras. Cienc. Avic. 13:227–234.

Bhuiyan, M. M., F. Islam, A. J Cowieson, and P. A. Iji. 2012. Effect of source and processing on maize grain quality and nutritional value for broiler chickens. I. Heat treatment and physicochemical properties. Asian J. Poult. Sci. 6:101–116.

Bikker, P., A. Dirkzwager, J. Fledderus, P. Trevisi, I. Le Huërou-Luron, J. P. Lallès, and A. Awati. 2007. Dietary protein and fermentable carbohydrates contents influence growth performance and intestinal characteristics in newly weaned pigs. Livest. Sci. 108:194–197.

Chiang, G., W. Q. Lu, X. S. Piao, J. K. Hu, L. M. Gong, and P. A. Thacker. 2010. Effects of feeding solid-state fermented rapeseed meal on performance, nutrient digestibility, intestinal ecology and intestinal morphology of broiler chickens. Asian-Aust. J. Anim. Sci. 23:263–271.

Cox, M. A., J. Jackson, M. Stanton, A. Rojas-Triana, L. Bober, M. Laverty, X. Yang, F. Zhu, J. Liu, S. Wang, F. Monsma, G. Vassileva, M. Maguire, E. Gustafson, M. Bayne, C. C. Chou, D. Lundell, and C. H. Jenh. 2009. Short-chain fatty acids act as antiinflammatory mediators by regulating prostaglandin and cytokines. World J. Gastroentero. 15:5549–5557.

CVB (Central Bureau for Livestock Feeding). 2007. Veevoedertabel, Centraal Veevoederbureau. Lelystad, the Netherlands.

Czerwiński, J., O. Højberg, S. Smulikowska, R. M. Engberg, and A. Mieczkowska. 2012. Effects of sodium butyrate and salinomycin upon intestinal microbiota, mucosal morphology and performance of broiler chickens. Arch. Anim. Nutr. 66:102–116.

Dalmasso, G., H. T. T. Nguyen, Y. Yan, L. Charrier-Hisamuddin, S. V. Sitaraman, and D. Merlin. 2008. Butyrate transcriptionally enhances peptide transporter PepT1 expression and activity. PLoS ONE 3:2476–2489.

Goelema, J. O., A. Smits, L. M. Vaessen, and A. Wemmers. 1999. Effects of pressure toasting, expander treatment and pelleting on in vitro and in situ parameters of protein and starch in a mixture of broken peas, lupins and faba beans. Anim. Feed Sci. Technol. 78:109–126.

González-Alvarado, J. M, E. Jiménez-Moreno, D. G. Valencia, R. Lázaro, and G. G. Mateos. 2008. Effects of fiber source and heat processing of the cereal on the development and pH of the gastrointestinal tract of broilers fed diets based on corn or rice. Poult. Sci. 87:1779–1795.

Gunal, M., G. Yayli, O. Kaya, N. Karahan, and O. Sulak. 2006. The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. Int. J. Poult. Sci. 5:149–155.

Jacobs, C. M., and C. M. Parsons. 2013. The effects of coarse ground corn, whole sorghum, and a prebiotic on growth performance, nutrient digestibility, and cecal microbial populations in broilers fed diets with and without corn distillers dried grains with solubles. Poult. Sci. 92:2347–2357.

Jacobs, C. M., P. L. Utterback, and C. M. Parsons. 2010. Effects of corn particle size on growth performance and nutrient utilization in young chicks. Poult. Sci. 89:539–544.

Khajali, F., and B. A. Slominski. 2012. Factors that affect the nutritive value of canola meal for poultry. Poult. Sci. 91:2564–2575.

Khoa, M. A. 2007. Wet and coarse diets in broiler nutrition: Development of the GI tract and performance. PhD Thesis. Wageningen University, the Netherlands.

Langhout, D. J., J. B. Schutte, P. Van Leeuwen, J. Wiebenga, and S. Tamminga. 1999. Effect of dietary high and low methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. Br. Poult. Sci. 40:340–347.

Liu, S. Y., P. H. Selle, and A. J. Cowieson. 2013. The kinetics of starch and nitrogen digestion regulate growth performance and nutrient utilisation of broilers fed coarsely ground, sorghum-based diets. Anim. Prod. Sci. 53:1033–1040.

Macfarlane, G. T., G. R. Gibson, E. Beatty, and J. H. Cummings. 1992. Estimation of short-chain fatty acid production from protein by human intestinal bacteria based on branched-chain fatty acid measurements. FEMS Microbiol. Lett. 101:81–88.

Mansoub, N. H. 2011. Comparitive effect of butyric acid, probiotic and garlic on performance and serum composition of broiler chickens. Am. Eurasian. J. Agric. Environ. Sci. 11:507–511.

Meyer, B., J. Zentek, and A. Harlander-Matauschek. 2013. Differences in intestinal microbial metabolites in laying hens with high and low levels of repetitive feather-pecking behavior. Physiol. Behav. 110:96–101.

Montazer-Sadegh, R., Y. Ebrahim-Nezhad, and N. Maberi-Sis. 2008. Replacement of different levels of rapeseed meal with soybean meal on broilers performance. Asian J. Anim. & Vet. Advan. 3:278–285.

Novozamsky, I., R. Van Eck, J. C. H. Showenburg, and F. Walinga. 1974. Total nitrogen determination in plant material by means of the indole-phenol blue method. Neth. J. Agri. Sci. 22:3–5.

OJEC (Official Journal of the European Community). 1972. Establishing community methods of analysis for the official control of feedingstuffs. Off. J. Eur. Community No. L 123/6.

Owusu-Asiedu, A., S. K. Baidoo, C. M. Nyachoti, and R. R. Marquardt. 2002. Response of early-weaned pigs to spray-dried porcine or animal plasma-based diets supplemented with egg-yolk antibodies against enterotoxigenic *Escherichia coli*. J. Anim. Sci. 80:2895–2903.

Pacheco, W. J., C. R. Stark, P. R. Ferket, and J. Brake. 2013. Evaluation of soybean meal source and particle size on broiler performance, nutrient digestibility, and gizzard development. Poult. Sci. 92:2914–2922.

Pouraziz, S., H. Aghdam Shahryar, and S. Chekani-Azar. 2013. Effects of dietary saccharomyces cerevisiae and butyric acid glycerides on performance and serum lipid level of broiler chickens. Kafkas Univ. Vet. Fak. Derg. 19:903–907.

Rehman, H., J. Böhm, and J. Zentek. 2008. Effects of differentially fermentable carbohydrates on the microbial fermentation profile of the gastrointestinal tract of broilers. J. Anim. Physiol. Anim. Nutr. 92:471–480.

Ricke, S. C. 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult. Sci. 82:632–639.

Richardson, A. J., N. McKain, and R. J. Wallace. 2013. Ammonia production by human faecal bacteria, and the enumeration, isolation and characterization of bacteria capable of growth on peptides and amino acids. BMC Microbiol. 13:6–13.

Rodgers, N. J., M. Choct, H. Hetland, F. Sundby, and B. Svihus. 2012. Extent and method of grinding of sorghum prior to inclusion in complete pelleted broiler chicken diets affects broiler gut development and performance. Anim. Feed Sci. Technol. 171:60–67.

Rutkowski, A., S. Kaczmarek, A. Szczyrkowska, and D. Józefiak. 2012. The effect of particle size of full-fat rapeseed and multicarbohydrase enzyme supplementation on nutrient digestibility and performance in broilers. J. Anim. Feed Sci. 21:324–333.

Saleem, G. 2013. Necrotic enteritis, disease induction, predisposing factors and novel biochemical markers in broilers chickens. PhD Thesis. University of Glasgow, Scotland.

Smits, C. H., A. Veldman, M. W. A. Verstegen, and A. C. Beynen. 1997. Dietary carboxymethylcellulose with high instead of low viscosity reduces macronutrient digestion in broiler chickens. J. Nutr. 127:483–487.

Sogunle, O. M., B. B. Olatoye, M. Agric, L. T. Egbeyale, A. V. Jegede, O. A. Adeyemi, D. A. Ekunseitan, and K. O. Bello. 2013. Feed forms of different particle sizes: Effects on growth performance, carcass characteristics, and intestinal villus morphology of cockerel chickens. Pac. J. Sci. Technol. 14:405–415.

Stein, H. H., S. W. Kim, T. T. Nielsen, and R. A. Easter. 2001. Standardized ileal protein and amino acid digestibility by growing pigs and sows. J. Anim. Sci. 79:2113–2122.

Svihus, B., K. H. Kløvstad, V. Perez, O. Zimonja, S. Sahlström, R. B. Schüller, W. K. Jeksrud, and E. Prestløkken. 2004. Physical and nutritional effects of pelleting of broiler chicken diets made from wheat ground to different coarsenesses by the use of roller mill and hammer mill. Anim. Feed Sci. Technol. 117: 281–293.

Weurding, R. E. 2002. Kinetics of starch digestion and performance of broiler chickens. PhD Thesis. Wageningen University, the Netherlands.

Wiernusz, C. J., R. G. J. Shields, D. J. Van Vlierbergen, P. D. Kigin, and R. Ballard. 1995. Canine nutrient digestibility and stool quality evaluation of canned diets containing various soy protein supplements. Vet. Clin. Nutr. 2: 49–56.

Woyengo, T. A., E. Kiarie, and C. M. Nyachoti. 2011. Growth performance, organ weights, and blood parameters of broilers fed diets containing expeller-extracted canola meal. Poult. Sci. 90:2520–2527.