Effects of corn replacement by sorghum in broiler diets on performance and intestinal mucosa integrity

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ABSTRACT The effect of replacing corn with low-tannin sorghum on broiler performance, carcass yield, integrity of mucosa of small intestine segments, and activity of membrane enzymes of the jejunum is investigated. A total of 594 male Cobb-500 broiler chicks were randomly assigned to 3 dietary treatments: 100% corn (control), 50% corn replacement with low-tannin sorghum (low sorghum), and 100% corn replacement with low-tannin sorghum (high sorghum). Body weight gain, feed consumption, feed conversion, and carcass yield were determined at 7, 21, and 42 d, and segments of the small intestine were collected. Feed conversion and weight gain were impaired at d 42 in broilers fed the high-sorghum diet, but no differences were observed for carcass yield among the treatments ($P > 0.05$). Crypt cell mitotic index of the jejunum and ileum at d 21 and 42 was lower in broilers fed the control diet than in those fed low- and high-sorghum diets ($P < 0.05$). Aminopeptidase activity was higher in broilers fed the control diet than in those fed low- and high-sorghum diets irrespective of age ($P < 0.05$). Conversely, intestinal alkaline phosphatase activity in the small intestine did not differ among the dietary treatments ($P > 0.05$). Our results indicate that 50% corn replacement with low-tannin sorghum is suitable for broiler diets, whereas 100% corn replacement with low-tannin sorghum had negative effects on the intestinal mucosa and performance of broilers at 42 d.

Key words: aminopeptidase, broiler performance, intestinal morphometry, mitotic index, sorghum

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INTRODUCTION

Low-tannin sorghum [*Sorghum bicolor* (L.) Moench] has the potential to replace corn as an alternative poultry feed. Its nutritional value is only slightly lower than maize (Douglas et al., 1990), and given its low water demand it can be produced in semiarid areas around the world (Gualtieri and Rapaccini, 1990), and it is adapted to low-quality soils. Moreover, low-tannin sorghum has been shown to substitute corn in swine and poultry feeds without affecting their performance (Garcia et al., 2005; Campos, 2006; Bozutti, 2009). Conversely, high-tannin sorghum has been shown to adversely affect the performance of broilers (Pour-Reza and Edriss, 1997). Nevertheless, poultry farmers are reluctant to use low-tannin sorghum-based diets, especially during the first week of the broiler's life (Campos et al., 2007). The epithelial mucosa in the small intestine experiences intensive growth during this period (Uni et al., 1998), and tannins can have a negative effect on broiler performance.

Besides the negative effect on broiler performance, which is associated with lower BW gain and feed efficiency, condensed tannins found in some sorghum cultivars may increase the number of goblet cells in villi of the intestinal mucosa, or induce mucosal necrosis and subsequent villi damage (Mitjavila et al., 1977; Chang et al., 1994). Moreover, high-tannin sorghum has been shown to induce changes in ME, nitrogen utilization efficiency (Nyachoti et al., 1996), and fatty acid composition of muscle tissue (Cherian et al., 2002). However, little is known about the effect of replacing 100% corn feed with low-tannin sorghum on intestinal development and performance in newly hatched chicks.

Thus, this study aimed to evaluate the effect of corn replacement with low-tannin sorghum [*Sorghum bicolor* (L.) Moench] on broiler performance, carcass yield, intestinal development (intestinal epithelial integrity and crypt cell mitotic index), and activity of intestinal membrane enzymes.

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MATERIALS AND METHODS

Birds and Diets

Five hundred ninety-four 1-d-old male Cobb-500 broiler chicks (mean weight: 42.5 ± 0.5 g) were purchased from a commercial hatchery. The chicks were assigned to 3 dietary treatments with different corn/sorghum ratios using a completely randomized design: 100% corn (hereafter control), 50% corn and 50% sorghum (low sorghum), and 100% sorghum (high sorghum). Each treatment was replicated 6 times with 33 birds per replicate pen, totaling 18 experimental units. The experimental diets (Table 1) consisted of corn/sorghum and soybean meal and were formulated to be isonutritive and isoenergetic according to Rostagno et al. (2005). The birds were reared in 3.2 × 1.4 m pens on a litter floor (rice husk, 10 cm height) in an environmentally controlled room where ambient temperatures were maintained at thermoneutrality according to bird age (Cobb Broiler Manual). Light was provided 24 h a day, and chicks were fed ad libitum throughout the experimental period. The birds were vaccinated against infectious bursal disease (d 4 and 18) and Newcastle disease (d 7 and 18).

Sorghum tannin content was determined according to the method of Price et al. (1978) and expressed as milligrams of catechin per 100-g sample. The sorghum used in this study had low tannin content (5.64 mg of catechin/g, 0.56%).

The experiment was approved by the Animal Ethics Committee of the College of Agricultural and Veterinary Science, São Paulo State University (CEUA-Protocol Number 006669/10), Brazil.

Performance and Carcass Yield

Body weight and feed consumption were measured at 7, 21, and 42 d and used to calculate the performance variables (feed intake, weight gain, and feed conversion). At the end of the experiment (42 d), 2 birds from each replicate were selected and submitted to an 11-h fast to empty the intestine and avoid fecal contamination of carcasses (Mendes, 2001). After fasting, the birds were stunned and killed by cervical dislocation, and carcass yield was determined (whole carcass, breast, thigh + drumstick). Whole carcass weight was expressed as percentage of live BW, whereas carcass parts were expressed as a percentage of absolute carcass weight.

Intestinal Morphometry

At d 7, 21, and 42, two birds from each replicate were fasted for 5 h to empty the intestine. Segments measuring 3 cm in length were removed from the duodenum (distal region of duodenal loop), jejunum (region immediately anterior to Meckel's diverticulum), and ileum (region immediately anterior to the cecal junction). The

Table 1. Ingredient and nutritional composition of experimental diets

	Starter (1 to 21 d)			Grower (22 to 42 d)		
Item	0%	50%	100%	0%	50%	100%
Ingredient (%)						
Yellow corn	51.333	25.666	—	58.666	29.333	—
Sorghum[1]	—	25.666	51.333	—	29.333	58.666
Soybean meal	40.276	39.606	38.937	32.211	31.454	30.689
Soybean oil	3.839	4.531	5.222	5.078	5.866	6.656
Dicalcium phosphate	1.873	1.865	1.858	1.606	1.597	1.589
Limestone	0.912	0.921	0.930	0.827	0.837	0.848
Salt (NaCl)	0.511	0.511	0.511	0.466	0.466	0.466
DL-Methionine	0.295	0.319	0.342	0.226	0.253	0.280
L-Lysine	0.180	0.215	0.249	0.165	0.204	0.244
Premix[2]	0.100	0.100	0.100	0.100	0.100	0.100
Choline chloride	0.070	0.070	0.070	0.070	0.070	0.070
L-Threonine	0.061	0.074	0.088	0.035	0.050	0.066
Coxistac 12	0.050	0.050	0.050	0.050	0.050	0.050
Kaolin	0.500	0.406	0.310	0.500	0.386	0.277
Total	100	100	100	100	100	100
Calculated analysis						
ME (kcal/kg)	3,005	3,005	3,005	3,175	3,175	3,175
CP (%)	22.87	22.87	22.87	19.75	19.75	19.75
Calcium (%)	0.92	0.92	0.92	0.81	0.81	0.81
P available (%)	0.46	0.46	0.46	0.40	0.40	0.40
Sodium (%)	0.22	0.22	0.22	0.20	0.20	0.20
Digestible lysine %	1.28	1.28	1.28	1.07	1.07	1.07
Digestible methionine + cysteine (%)	0.91	0.91	0.91	0.77	0.77	0.77
Digestible threonine (%)	0.83	0.83	0.83	0.70	0.70	0.70

[1]Condensed tannin concentration (0.564%) determined using the method of Price et al. (1978).

[2]Warranty levels per kilogram of product: vitamin A, 7,000,000 IU; vitamin D, 3,000,000 IU; vitamin E, 25,000 IU; vitamin K, 980 mg; vitamin B_1, 1,780 mg; vitamin B_2, 9,600 mg; vitamin B_6, 3,465 mg; vitamin B_{12}, 10,000 μg; biotin, 160 mg; calcium pantothenate, 9,500 mg; niacin, 34,650 mg; Mn, 76,260 mg; Zn, 91,250 mg; Cu, 10,000 mg; and antioxidant, 100 mg.

intestinal segments were opened longitudinally, washed with PBS at pH 7.4 and 4°C, and fixed with 10% formaldehyde at pH 7.4 for 48 h, dehydrated, diaphanized, and embedded in paraffin. Then, 5-μm-thick slices were prepared and stained with periodic acid-Schiff stain (**PAS**; McManus and Mowry, 1960). Villus height (tip of the villus to the villus crypt junction) of 20 random villi was measured per slice, and mean values were calculated. The number of goblet cells (PAS+) was determined by counting the number of cells over 200 μm in 10 random villi per sample. Images were captured at 200× magnification and analyzed using Image J software (Rasband, 2004).

Crypt Cell Mitotic Index

Segments measuring 3 cm in length were removed from the duodenum, jejunum, and ileum, opened longitudinally, washed with PBS at pH 7.4 and 4°C, and fixed with 10% buffered formalin for 48 h. Immunohistochemistry was performed to detect proliferating cell nuclear antigen protein (PCNA Clone PC10, mouse monoclonal antibody, Biocare Medical, Concord, CA) using the avidin-biotin peroxidase complex (Starr Trek Universal HRP detection kit, Biocare Medical) according to the method of Miranda et al. (2009). Controls were included for each immunohistochemistry system. Section images (400× magnification) of 10 crypts per sample for cells with brown-staining $PCNA^+$ nuclei and blue-staining $PCNA^-$ nuclei were captured over 100 μm from the crypt base using an image analyzer (DM2500 Microscopy, DFC280 digital camera, QWin Software Leica, AOTEC, São Paulo, Brazil). Crypt depth was measured from the base of the crypt to the crypt-villus transition region in 15 crypts per sample.

Intestinal Epithelium Integrity

Segments measuring 1.5 cm in length were collected from each small intestine segment (duodenum, jejunum, and ileum), processed, and observed under a scanning electron microscope (Jeol model JSM 25SII, Jeol, São Paulo, Brazil) operating at 15 kv. Photomicrographs of 3 sections per segment totaling 1.368 mm^2 in mucosal surface were analyzed for epithelial loss using lesion scores adapted from Gomide et al. (2004). Briefly, mucosa damage was ranked in ascending order (1 to 5) from villus without apparent epithelial loss to lack of epithelium in the entire villus.

Activity of Intestinal Membrane Enzymes

At d 7, 21, and 42, jejunum samples were removed, opened lengthwise, and washed with PBS at pH 7.4 and 4°C. Brush border membrane vesicles (**BBMV**) were prepared based on the method of Louvard et al. (1973). The enrichment factor of BBMV was calculated for aminopeptidase and intestinal alkaline phosphatase as the ratio of final to initial specific activity. The enrichment factors for aminopeptidase and intestinal alkaline phosphatase were 4.7 and 11.5, respectively. Aminopeptidase (EC 3.4.11.2) activity was determined according to Rueda et al. (2007), and intestinal alkaline phosphatase (EC 3.1.3.1) according to Pizauro et al. (1995). The substrates used were *p*-nitroaniline and *p*-nitrophenol for aminopeptidase and alkaline phosphatase, respectively. Specific activity was expressed as nanomoles of substrate/minute per milligram of protein. All enzyme assays were performed in triplicate. Total protein content in the samples was determined according to the method of Hartree (1972), using bovine serum albumin as the standard.

Statistical Analysis

Data were analyzed in completely randomized design using the GLM procedure of SAS Institute Inc. (2003), with percentage of corn replacement with sorghum (2 df), broiler age (2 df), and interactions (4 df) as fixed effects, and the random error. Broiler age and interaction effects were not included in performance models. Data were analyzed for the presence of outliers (box-and-whisker plot), normal distribution of Studentized errors (Cramer-Von-Mises test), and homogeneity of variances (Brown-Forsythe; Littell et al., 2002). Differences among treatment means were checked for significance using Duncan's multiple-range test ($P < 0.05$).

RESULTS

Performance and Carcass Yield

Weight gain and feed conversion at 42 d of age was better in birds fed low sorghum diet than in those fed high sorghum diet ($P \leq 0.05$) and not significantly different from the control diet (Table 2). Interestingly, no significant differences in performance parameters ($P > 0.05$) were found among the treatments during the period of 1 to 21 d of age, suggesting that 100% corn replacement with sorghum after hatching did not negatively affect broiler performance during the initial development period. There were no significant differences among the treatments ($P > 0.05$) in whole carcass or carcass part weights of broiler chickens at 42 d of age (data not shown).

Morphometric Variables

Morphometric parameters (villus height, crypt depth, and number of goblet cells) in the small intestinal mucosa segments were not affected by diet, except for villus height in the duodenum ($P < 0.05$), which was smaller in 7-d-old broilers fed the low-sorghum diet

Table 2. Feed intake, weight gain, and feed conversion in broiler chicks assigned 3 dietary treatments (0, 50, and 100% corn replacement with low-tannin sorghum) at 1 to 7, 1 to 21, and 1 to 42 d of age

	Corn replacement with sorghum in the diet (%)					
Parameter	0	50	100	SEM	*P*-value	CV (%)
Feed intake (g)						
1 to 7 d	166	165	162	2	0.63	3.43
1 to 21 d	1,233	1,232	1,213	14	0.58	2.95
1 to 42 d	4,643	4,649	4,634	29	0.94	1.60
Weight gain (g)						
1 to 7 d	146	142	142	2	0.35	3.77
1 to 21 d	955	932	938	10	0.30	2.81
1 to 42 d	2,765^{ab}	2,804^{a}	2,729^{b}	19	0.05	1.76
Feed conversion (g/g)						
1 to 7 d	1.13	1.16	1.17	0.01	0.18	2.77
1 to 21 d	1.29	1.32	1.29	0.02	0.25	2.86
1 to 42 d	1.68^{ab}	1.66^{b}	1.70^{a}	0.01	0.04	1.46

^{a,b}Means followed by different superscripts in the same row are significantly different by Duncan's multiple-range test ($P < 0.05$). Data are means of 6 replicates per treatment.

than in broilers fed high-sorghum or control diets. Villus height and crypt depth were significantly affected by age in all small intestine segments ($P < 0.05$) irrespective of diet (data not shown).

Mitotic Index

Cell proliferation in duodenum crypts was not affected by diet or broiler age ($P > 0.05$). Conversely,

Table 3. Crypt cell mitotic index (%) of the duodenum, jejunum, and ileum from broiler chicks assigned 3 dietary treatments (0, 50, and 100% corn replacement with low-tannin sorghum) in a 42-d feeding trial

	Corn replacement with sorghum in the diet (%)				
Item	0	50	100	Mean	SEM
Duodenum					
7 d	55.5	55.3	57.2	56.2	2.54
21 d	47.3	51.6	57.6	52.1	1.89
42 d	54.3	57.2	65.1	58.1	3.26
Mean	52.0	54.7	59.1		
SEM	3.45	1.78	2.22		
P-value					
Diet	0.1516				
Age	0.2257				
Diet × age	0.8667				
CV (%)	13.86				
Jejunum					
7 d	42.2^{xb}	65.3^{xa}	55.6^{yab}	54.5	3.86
21 d	56.3^{xa}	46.7^{xa}	59.7^{ya}	54.6	2.87
42 d	56.6^{xa}	59.0^{xa}	70.8^{xa}	61.3	3.31
Mean	51.1	56.7	60.3		
SEM	3.28	4.70	2.14		
P-value					
Diet	0.0475				
Age	0.1128				
Diet × age	0.0269				
CV (%)	13.81				
Ileum					
7 d	51.9	56.9	52.6	53.5	1.87
21 d	47.5	46.0	55.7	49.2	2.61
42 d	43.8	65.3	62.9	55.0	4.27
Mean	47.7^{b}	56.2^{a}	56.7^{a}		
SEM	2.58	3.42	2.16		
P-value					
Diet	0.0187				
Age	0.1509				
Diet × age	0.0638				
CV (%)	13.87				

$^{x,y;\ a,b}$Means followed by different superscripts in the same row (^{a,b}) or column (^{x,z}) are significantly different by Duncan's multiple-range test ($P < 0.05$). Data are means of 4 replicates per treatment.

increased cell proliferation rates were observed in the jejunum at d 42 in broilers fed the high-sorghum diet ($P < 0.05$), but no significant differences were found between broilers fed control or low-sorghum diets at any age. In addition, the highest (70.8%) and lowest (42.2%) mitotic index values were found in the jejunum in broilers fed the high-sorghum diet at d 42 and broilers fed control diet at d 7, respectively ($P < 0.05$). There were no effects of broiler age or the interaction between broiler age and diet on cell proliferation in ileum crypts ($P > 0.05$). However, overall mitotic index in the ileum was 9% higher in broilers fed low- or high-sorghum diets than in birds fed the control diet ($P < 0.05$; Table 3).

Enzymatic Activity in BBMV from Jejunal Mucosa

Aminopeptidase. Aminopeptidase activity was affected by diet and broiler age ($P < 0.05$, Table 4). Enzymatic activity in broilers fed control diet was 22% higher than in those fed the high-sorghum diet (656 vs. 514 nmol/min per mg of protein). Moreover, 7-d-old broilers exhibited 40% lower aminopeptidase activity than 21- and 42-d-old broilers, but no significant differences were observed between the latter 2 trial ages (Table 4).

Intestinal Alkaline Phosphatase. Intestinal alkaline phosphatase activity was not affected by diet ($P > 0.05$). However, lower enzymatic activity was observed at d 7 than at d 21 and 42 ($P < 0.05$), irrespective of diet (Table 4).

Degree of Epithelial Loss

Epithelial loss in small intestine segments is represented in Figure 1. Replacing corn with sorghum revealed a more severe epithelial loss at 21 and 42 d in the duodenum, reaching degrees 4 and 5. Conversely, epithelial loss caused by corn replacement was less severe in the jejunum and ileum at all studied ages. Thus, epithelial loss increased with broiler age and decreased along the small intestine.

DISCUSSION

In this study, we evaluated the effect of replacing corn with low-tannin sorghum [*Sorghum bicolor* (L.) Moench] on broiler performance, carcass yield, and intestinal development. We found no significant differences in feed intake, weight gain, and feed conversion among the dietary treatments from 1 to 21 d, indicating that all dietary corn can be replaced by low-tannin sorghum grain without significantly affecting broiler performance during the initial growth period.

Table 4. Enzymatic activity (nmol of substrate[1]/min per mg of protein) in brush-border membrane vesicles (BBMV)[2] of jejunal mucosa from broiler chicks assigned 3 dietary treatments (0, 50, and 100% corn replacement with low-tannin sorghum) in a 42-d feeding trial

	Corn replacement with sorghum in the diet (%)				
Item	0	50	100	Mean	SEM
Aminopeptidase					
7 d	424	387	430	414^{y}	17
21 d	815	699	599	717^{x}	37
42 d	728	702	542	657^{x}	42
Mean	656^{a}	596^{ab}	514^{b}		
SEM	48	49	29		
P-value					
Diet	0.0182				
Age	<0.0001				
Diet × age	0.1832				
CV (%)	21.67				
Intestinal alkaline phosphatase					
7 d	889	1,077	1,233	1,066^{y}	141
21 d	2,483	2,487	2,125	2,365^{x}	150
42 d	1,859	2,080	2,144	2,028^{x}	115
Mean	1,651	1,881	1,817		
SEM	206	186	182		
P-value					
Diet	0.7855				
Age	<0.0001				
Diet × age	0.6523				
CV (%)	32.06				

$^{x,y;\ a,b}$Means followed by different superscripts on the same row (^{a,b}) or column (^{x,z}) are significantly different by Duncan's multiple-range test ($P < 0.05$). Data are means of 6 replicates per treatment.

[1]Substrates used were *p*-nitroaniline for aminopeptidase and *p*-nitrophenol for intestinal alkaline phosphatase.

[2]Enrichment factors of enzymes were determined to assess the purity of BBMV. The enrichment factors for aminopeptidase and intestinal alkaline phosphatase were 4.7 and 11.5, respectively.

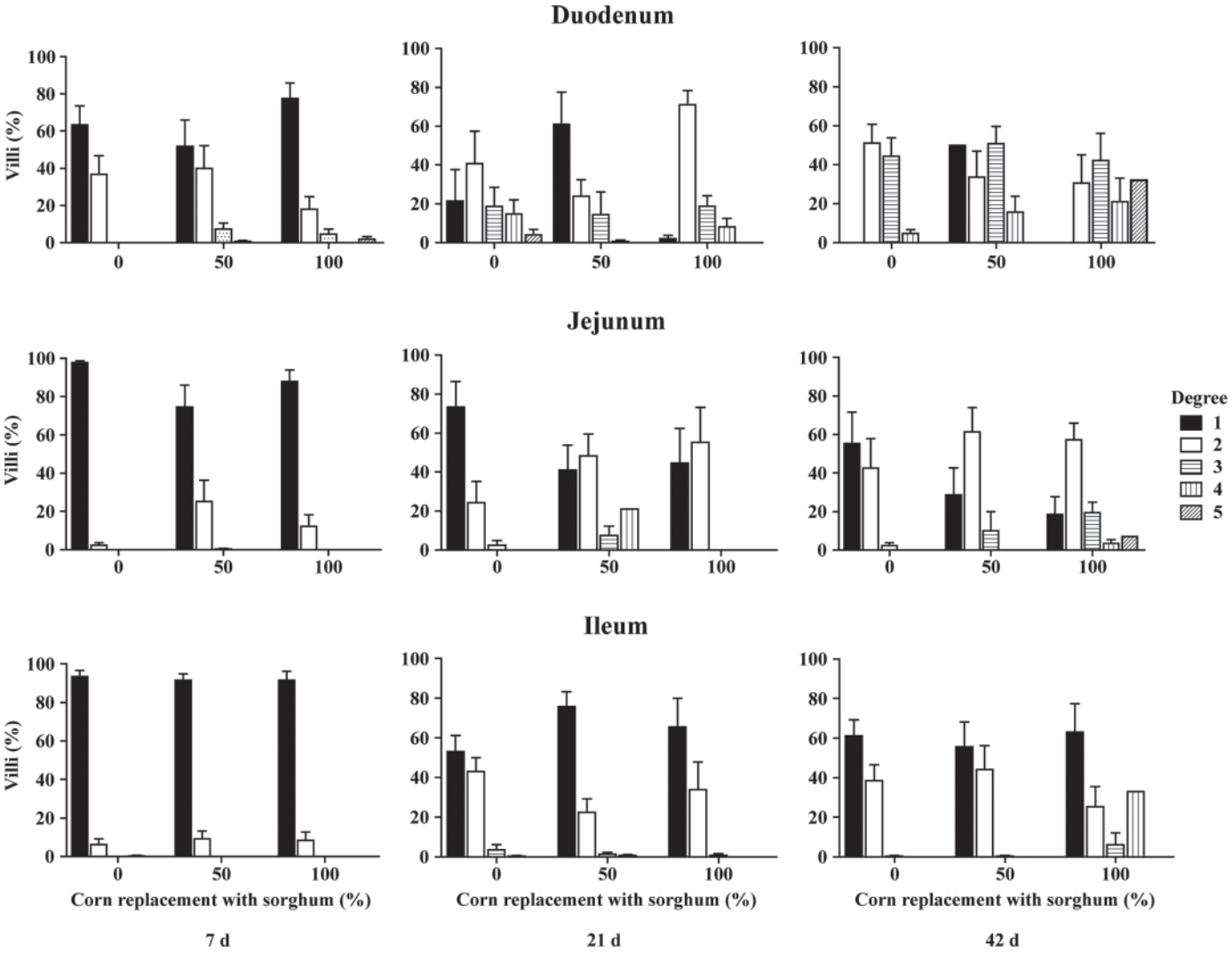

Figure 1. Epithelial loss in intestinal segments of broiler chicks assigned 3 dietary treatments (0, 50, and 100% corn replacement with low-tannin sorghum) in a 42-d feeding trial. Degrees of epithelial loss adapted from Gomide et al. (2004).

Similarly to our findings, Pour-Reza and Edriss (1997) reported that tannins did not significantly affect broiler performance when dietary tannin concentration did not exceed 2.6 g/kg. However, feed conversion and weight gain were impaired in broilers receiving a high-sorghum diet at the end of the feeding period (1 to 42 d), even though no effects on feed intake were observed. Carcass yield was not affected ($P > 0.05$) by replacement of corn with low-tannin sorghum in the diet. This result is consistent with that of Kumar et al. (2005), who found that the yield of cut-up parts, especially breast, was not affected by different tannin levels in red sorghum.

The intestine, along with the other splanchnic organs, accounts for 20 to 35% of whole-body protein turnover and energy expenditure for growth, function, and maintenance of intestinal mucosa (Stoll and Burrin, 2006). During the experimental period we observed significant changes in the intestinal mucosa of broilers fed sorghum diets, such as higher mitotic index and epithelial loss. These results could explain the reduced aminopeptidase activity in the jejunum mucosa. This enzyme is responsible for almost all peptidase activity in the brush-border membrane of the jejunum and ileum. A similar reduction in membrane enzyme activity has already been observed in chickens fed sorghum-based diets. For instance, Nyamambi et al. (2007) observed reduced sucrase activity in broilers chicks fed sorghum. These negative effects on enzyme activity have been attributed to the ability of tannins to bind, coagulate, and precipitate protein including digestive enzymes (Hagerman and Butler, 1981). Moreover, the peripheral region of the endosperm in sorghum grains is constituted by proteins that form a resistant layer impairing physical and enzymatic degradation of starch (Rooney and Pflugfelder, 1986). Antunes et al. (2006) found lower apparent and true ME values in sorghum grain genotypes with hard endosperm texture than in grains with intermediate or soft endosperm texture. Therefore, sorghum endosperm texture should also be considered when using sorghum in poultry feed.

Epithelial integrity and membrane enzymes are also fundamental to secure digestion and absorption of nutrients from the intestinal lumen, as changes in the intestinal mucosa could affect nutrient digestion and absorption. Nyamambi et al. (2007) observed that duodenal villus height and crypt depth were reduced with increasing tannin levels in the diet. In this study, villus height and crypt depth were not affected in the low-tannin sorghum treatment from 1 to 21 d, even though we observed higher epithelial loss and mitotic index in the duodenum and jejunum, respectively. However, these changes in intestinal mucosa were not severe enough to affect the amount of absorbed nutrients, as there were no effects on feed intake, weight gain, and feed conversion. These results indicate that nutritive value of feed may affect the intestinal mucosa during the initial growth period without significantly affecting broiler performance.

Despite the very low tannin concentration in the starter and grower diets (0.30% and 0.34%, respectively), the effect on intestinal mucosa could happen along the age range (1 to 42 d), affecting some productive parameters (worse feed conversion and weight gain). The effects of tannins on the mucosa induced higher energy expenditure (epithelial loss), which affected broiler feed efficiency. Additionally, due to the increase in energy requirements during the broiler growing stage (22 to 42 d), more cereals were included in the diets, resulting in a higher proportion of sorghum in formulations. Therefore, the inclusion of antinutritional factors present in cereal grains can cause deleterious effects on the intestinal mucosa.

In conclusion, 50% corn replacement with low-tannin sorghum is suitable for broiler diets, whereas 100% corn replacement with low-tannin sorghum upon hatching had negative effects on the intestinal mucosa and performance of broilers at 42 d.

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