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Effect of crushed sugarcane on performance, bone characteristics and intestinal morphology of free-range chicken lines

Efeito da cana-de-açúcar triturada sobre desempenho, características ósseas e morfologia intestinal de linhagens de frangos caipiras

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Highlights

Crushed sugarcane causes a linear increase in feed intake in free-range chicken.
Crushed sugarcane in the diet reduces feed intake without worsening feed conversion.
There are differences between strains for feed intake in free-range systems.
The decreased nutrient supply with sugarcane inclusion did not affect bone quality.
Intestinal morphology is affected by a greater availability of fiber for the birds.

Abstract

The objective of this study was to evaluate the effect of different levels of crushed sugarcane in the diet on the performance, bone characteristics and intestinal morphology of slow-growing broiler strains. A total of 448 chickens at 35 days of age were allotted to two randomized blocks in a 4×2 factorial arrangement (levels of feed replacement with crushed sugarcane, on a fresh-matter basis: 0, 15, 30, and 45%) and two strains (Pesadão and Label Rouge (LR)). Two replicates were used per block over time (containing the treatments and the strains), and 14 individuals were used per experimental unit. Weight gain and feed intake decreased linearly in response to the increasing sugarcane levels. Feed intake was higher in the Pesadão strain than in LR. Chicken foot weight was lower in Pesadão than in LR. There were no effects of sugarcane levels or

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strains on the tarsometatarsal bone weight, breaking strength, maximum load and deformation. The dry matter (DM) content of the tarsometatarsal bone was lower in LR than in Pesadão. The replacement of feed with sugarcane induced a linear increase in bone ash content and a decrease in bone phosphorus and calcium contents. Label Rouge chickens showed a greater crypt depth and lower values of villus/crypt ratio and mucosal layer thickness. The performance and intestinal morphology of broiler chickens are negatively affected by increasing sugarcane levels in the diet. In addition, the increasing intake of sugarcane changes the bone mineral composition without reducing mechanical resistance, in both the Pesadão and Label Rouge strains.
Key words: Alternative feedstuffs. Bone minerals. Gut health. Organic production. Poultry.

Resumo

O presente estudo foi realizado objetivando avaliar o desempenho, as características ósseas e a morfologia intestinal de duas linhagens de frangos tipo caipira alimentados com dietas contendo cana-de-açúcar triturada. Foram utilizados 448 frangos aos 35 dias de idade, distribuídos em dois blocos casualizados, em esquema fatorial 4x2, sendo quatro níveis de substituição (0; 15; 30 e 45%) da ração por cana-de-açúcar triturada e duas linhagens (Pesadão e Label Rouge (LR)), em duas repetições por bloco e 14 aves por unidade experimental. Foi observado redução linear com o aumento dos níveis de cana-de-açúcar sobre o ganho em peso e consumo de ração. O consumo de ração foi maior na linhagem Pesadão em relação à linhagem LR. No entanto, o peso do pé das aves foi menor para a linhagem Pesadão em comparação com a linhagem LR. Não foi observado efeitos da substituição da ração por cana-de-açúcar e das linhagens sobre o peso do osso tarsometatarso, carga de ruptura, força máxima e deformação. O teor de matéria seca do osso tarsometatarso foi menor para os animais LR em comparação com a linhagem Pesadão. A substituição da ração por cana-de-açúcar aumentou linearmente o teor de cinzas dos ossos e diminuiu o teor de fósforo e o de cálcio. A linhagem LR apresentou maior profundidade de criptas, menor relação vilosidade/cripta e espessura da camada da mucosa. O desempenho e a morfologia intestinal de frangos caipiras são afetados negativamente com o aumento dos níveis de cana-de-açúcar na dieta. Além disso, o aumento da ingestão de cana-de-açúcar altera a composição mineral óssea, sem prejudicar a resistência mecânica das linhagens Pesadão e LR.
Palavras-chave: Alimentos alternativos. Aves. Minerais ósseos. Produção orgânica. Saúde intestinal.

Introduction

New values have been attributed to food products, influencing the choice of consumers in Brazil and many other countries. The concern with food safety and production conditions, regarding whether or not the rules that provide animal welfare are applied, is among the main factors that determine the choice of chicken consumers (Ipek & Sozcu, 2017).

The growing demands with respect to broiler chickens have driven farmers and researchers to seek production systems that are in line with animal welfare and the environment (Castromán, Del Puerto, Ramos, Cabrera, & Saadoun, 2013). In this respect, in free-range farming, chickens have access to diverse pastures, forages, insects and worms that may be available in the rearing environment (Ipek & Sozcu, 2017). This system can provide more suitable welfare conditions thanks to the outdoor space, physical activity areas, access to alternative feed and possibility of feed selection.

In semi-intensive production, farmers must use chickens of slow-growing genetics that are adapted to the respective system (Branciari et al., 2009). However, even in broiler production, feeding accounts for the largest share of production costs, and the use of alternative ingredients such as sugarcane (Kheravii, Swick, Choct, & Wu, 2017) and other forages (Ipek & Sozcu, 2017; Zheng, Mao, Tian, Guo, & Meng, 2019) could reduce the expenditure on feeding. When used within certain limits, this approach could even improve the absorption of diet nutrients (Espósito et al., 2015).

Sugarcane is characterized by having low levels of dry matter, mineral matter and crude protein and high fiber content (Cruz et al., 2014). Conversely, it may constitute a very interesting alternative feed ingredient with great potential for use due to its broad availability (Leal et al., 2013).

In this scenario, many aspects regarding the use of efficient alternative systems in poultry production are not fully elucidated. Therefore, this study was conducted to investigate the effect of using crushed sugarcane as forage alternative, during the dry period of the year, on the performance, bone characteristics and intestinal morphology of slow-growing broiler strains.

Materials and Methods

All experimental protocols involving animals were approved by the Ethics Committee on Research Involving Animals (CEPEA) at the Federal University of São João Del Rei (UFSJ), located in São João Del Rei - MG, Brazil (approval no. 10/2011), and met the guidelines of National Council for the Control of Animal Experimentation (CONCEA).

The experiment was developed on the Caipirão do Parque farm, in Campo das Vertentes -MG. A total of 448 broiler chickens (922 ± 29 g, 35 days old) were distributed in a 4×2 factorial arrangement represented by four levels of replacement of the basal diet with sugarcane (0, 15, 30 and 45%) and two commercial strains (Label Rouge and Pesadão). A randomized-block experimental design was adopted, with four replicates and 14 chickens per experimental unit (seven males and seven females).

All chickens were housed in 16 boxes (2.5 m2) inside a shed with a ceiling height of 3.0 m. The facility was covered with fiber cement shingles, and the sides were screened with galvanized mesh and protected with plastic curtains. The floor was earthen, lined with wood shavings litter (5 cm thickness). Each box was equipped with a poultry feeder and a drinker. Each animal had access to paddocks with 45 m2 (without forage).

The diets used in the present study for the grower (35 to 60 days) and finisher (61 to 85 days) phases were composed mainly of corn and soybean meal (Table 1) and formulated to meet the nutritional requirements of medium-performance chicken (Rostagno et al., 2005). During the experimental period, feed and water were available ad libitum. The chickens received only natural light, without any use of artificial lighting. Ambient temperature data were obtained by maximum-minimum thermometers that were distributed throughout the shed. The temperature values were recorded twice daily (09h00 and 17h00) throughout the experimental period.

The experimental treatments consisted of the isometric replacement of 0, 15, 30 and 45% of the basal diet (grower and finisher) with sugarcane (stalks and leaves) that was crushed in a grinder to obtain a suitable particle size that allowed proper mixing with the feed. The sugarcane was cut and crushed daily.

Crushed sugarcane samples were collected and sent to the Animal Nutrition Laboratory of the Department Animal Science (DZO) at the Federal University of Viçosa (UFV), where they were analyzed following the methods described by Detmann et al. (2012). The sugarcane supplied to the birds had a dry matter (DM) content of 29.79%, and its chemical composition (DM basis) was 3.5% mineral matter, 52.4% neutral detergent fiber, 1.74% ether extract, 45.37% non-fiber carbohydrates, 3.01% crude protein, calcium 0.29% and 0.06% phosphorus.

The parameters of weight gain, feed intake and feed conversion were evaluated from 35 to 60 and 35 to 85 days of age. Weight gain was calculated as the difference in bird weight using the abovementioned data. Feed intake was calculated as the amount of feed supplied minus orts, divided by the number of animals in the boxes. Feed conversion was determined as the ratio between feed intake and weight gain.

At 85 days of age, two birds (males) whose weight was close to the average of the box were slaughtered per experimental unit, totaling 32 chickens. The chickens were stunned by cervical dislocation, which was followed by the procedures of bleeding, scalding (59 ºC for 120 s), plucking and evisceration. The hot carcasses were weighed and their respective abdominal fat removed.

The chicken feet were weighed and immersed in boiling water for five minutes to soften the narrow layer of skin, collagen and cartilage in the region around the tarsometatarsal bone. After the cleaning procedure, the fresh bones were weighed. Subsequently, the tarsometatarsal bones were pre-dried in a forced-air oven at 65 °C. Breaking strength was analyzed using a SHIMADZU instrument (AGX PLUS 100 kN; Shimadzu of Brazil, São Paulo - Brazil) at a temperature of 23 °C. This procedure allows obtaining the parameters of weight, breaking strength, maximum load and deformation.

Subsequently, the final drying procedure was performed in the oven at 105 °C for 24 h. Ash was determined in muffle at 600 °C. One gram (1 g) of dry (and ground) matter was placed in digestion tubes, to which 25 mL nitric-perchloric solution (1:1) were added at 160 °C, until the sample became colorless. The chemical analyses were carried out at the Animal Nutrition Laboratory at DZO/UFV. The calcium content was determined by atomic absorption spectrophotometry and phosphorus by colorimetry (Detmann et al., 2012).

Segments approximately 1.0-cm long were collected from the mid-portion of the duodenum of each individual. These portions were opened by the mesenteric border, extended by the serosa and fixed in Bouin´s solution for 24 h. These samples were subsequently washed and stored in alcohol for analysis. The small intestine segments were dehydrated in alcohol, cleared in xylol and paraffin-embedded at the Histopathology Laboratory of the Department of Veterinary Medicine (DMV) at UFV. A 7-μm section was extracted and stained by the

Table 1
Centesimal and calculated compositions of the basal diet used in the grower and finisher phases

Ingredient	Grower (35 to 60 days old)	Finisher (61 to 85 days old)
Corn	64.61	73.51
Soybean meal 45%	30.612	22.534
Soybean oil	1.440	1.321
Common salt	0.330	0.255
Mineral supplement[1]	0.050	0.050
Vitamin supplement[2]	0.100	0.100
DL-methionine (99%)	0.168	0.108
L-lysine HCl (78%)	0.117	0.041
Choline chloride 70%	0.050	0.050
Dicalcium phosphate	1.322	1.222
Calcitic limestone	1.209	0.809
Total	100.000	100.000
Calculated composition		
Crude protein (%)	19.375	16.343
Metabolizable energy (kcal kg^{-1})	3.000	3.100
Calcium (%)	0.881	0.686
Available phosphate (%)	0.351	0.325
Sodium (%)	0.15	0.12
Digestible methionine + cystine (%)	0.716	0.597
Digestible lysine (%)	1.006	0.760
Digestible threonine (%)	0.654	0.551
Digestible arginine (%)	1.212	0.984
Digestible tryptophan (%)	0.21	0.17
Digestible valine (%)	0.815	0.690

[1] Per kg of feed: manganese - 75 mg; iron - 50 mg; zinc - 70 mg; copper - 8.50 mg; cobalt - 2 mg; iodine - 1.5 mg; and excipient q.s. - 1,000 g.
[2] Per kg of feed: vit. A -12,000 IU; vit. D3 - 2,200 IU; vit. E - 30 IU; vit. B1 - 2.2 mg; vit. B2 - 6 mg; vit. B6 - 3.3 mg; vit. B12 - 16 mg; pantothenic acid - 13 mg; vit. K3 - 2.5 mg; folic acid - 1 mg; selenium - 0.12 mg; antioxidant -10 mg; and excipient q.s.

hematoxylin and eosin (HE) technique. The pre-established parameters were measured using a microscope coupled to an image analyzer (40x) at the Laboratory of Biology and Animal Ecology of the Department of Natural Sciences (DCNAT) at the Federal University of São João Del Rei (UFSJ). Twenty readings were performed per sample to measure villus height, crypt depth and the thickness of the mucosal, submucosal, circular muscle and longitudinal muscle layers. The absorption surface (AS) was calculated by the following formula: AS (mm^2) = villus height (VH) (mm) × width at half-height of the villus (mm).

Student-Newman-Keuls' test was applied to compare the means of the parameters between the chicken strains and regression analysis was carried out to evaluate the parameters as a function of the levels of sugarcane inclusion. This procedure was performed adopting the significance level of 5% and using the linear and quadratic functions of the System for Statistical Analysis (Sistemas para Análise Estatística e Genéticas [SAEG], 2007) version 9.1, developed at UFV.

Results and Discussion

The average minimum (16.2±3.18 ºC) and maximum (25.12±4.65 ºC) temperatures as well as average relative humidity (76.46 ± 13.88%) throughout the experimental period remained within the recommended ranges for chicken farming.

There was no significant interaction effect between strains and sugarcane inclusion levels on weight gain (WG), feed intake (FI) or feed conversion ratio (FC). In the period from 35 to 60 days of age, the increasing sugarcane inclusion levels caused a linear decrease ($P<0.05$) in WG (Y=1505.06 - 7.29X, R^2 = 0.99) and FI (Y= 5286.44- 28.76X, R^2 = 0.95) (Table 2). From 35 to 85 days, there was also a linear decrease ($P<0.05$) in WG (Y= 2635.80-94.06X, R^2 = 0.99) and FI (Y=11186.8 - 50.06X, R^2 = 0.93). In the interval between 35 and 85 days, FI was higher ($P<0.05$) in the Pesadão strain than in Label Rouge (LR). However, the sugarcane inclusion levels did not affect ($P>0.05$) the FC of the individuals.

Label Rouge chickens showed a lower FI, as also described by Madeira et al. (2010). These authors compared this strain with Ross 308, Master Griss and Vermelhão Pesado (Pesadão). Savino, Coelho, Rosario and Silva (2007) compared different chicken genotypes, replacing the feed with corn, and found significant changes in performance regarding WG and FC, suggesting that the choice of the chicken strain is a relevant aspect to be analyzed in alternative production systems.

The results show that the reduction in FI and WG was in response to the increased sugarcane intake. The same trend was noted by Espósito et al. (2015), who observed a decrease in FI and WG with increasing levels of crushed sugarcane in the diet of free-range chickens. This worse result in animal performance is related to the nutritional imbalance originated by the inclusion of sugarcane, considering that its chemical and nutritional composition tends to limit the contribution of protein and amino acid fractions, thereby restricting the increase in nitrogen compounds required for physical development. In this respect, Amaefule, Ukpanah and Ibok (2011) found that methionine and lysine supplementation was necessary in diets containing 30 and 40% pigeon pea, respectively, for the chicken performance to be similar to that of the birds that were not fed sugarcane.

Table 2

Performance of slow-growing broiler strains (Label Rouge and Pesadão) fed different levels of sugarcane (0, 15, 30 and 45%) at 35-60 and 35-85 days of age

Parameter	Strain	Feed replacement with sugarcane					CV (%)
		0%	15%	30%	45%	Mean	
35-60 days							
Feed intake (g)	Label Rouge	5056	4886	4545	3729	4554	
	Pesadão	5303	5037	4518	4041	4725	
	Mean (*L)	5179	4961	4531	3885	4639	7.69
Weight gain (g)	Label Rouge	1445	1378	1335	1157	1329	
	Pesadão	1499	1495	1273	1145	1353	
	Mean (*L)	1472	1437	1304	1151	1341	6.55
Feed conversion	Label Rouge	3.52	3.55	3.4	3.26	3.43	
	Pesadão	3.52	2.37	3.59	3.55	3.26	
	Mean	3.52	3.46	3.51	3.40	3.47	7.71
Sugarcane intake (g)[1]	Label Rouge	0	296	662	1011	656	
	Pesadão	0	301	662	1093	685	
	Mean (*L)	0	299	662	1052	671	13.93
35-85 days							
Feed intake (g)	Label Rouge	10810	10263	9796	8489	9840b	
	Pesadão	11189	10872	10173	8892	10270a	
	Mean (*L)	10999	10559	9984	8690	10058	6.43
Weight gain (g)	Label Rouge	2592	2438	2403	2201	2409	
	Pesadão	2648	2576	2353	2183	2440	
	Mean (*L)	2620	2507	2378	2192	2424	5.52
Feed conversion	Label Rouge	4.19	4.22	4.1	3.88	4.10	
	Pesadão	4.2	4.21	4.33	4.07	4.20	
	Mean	4.2	4.21	4.22	3.98	4.15	6.04
Sugarcane intake (g)[1]	Label Rouge	0	604	1397	2290	1430	
	Pesadão	0	652	1459	2392	1501	
	Mean (*L)	0	628	1428	2341	1466	13.39

[1] Dry matter basis; * (P<0.01); L = linear effect; CV = coefficient of variation.

Furthermore, according to Amerah, Ravindran and Lentle (2009), the effects of insoluble fiber on the digestive tract development and performance of broilers differ depending on the source and coarseness of fiber. Thus, the high fiber fraction of sugarcane could negatively affect FI and the digestion and absorption processes associated with the diet nutrients, reducing growth performance.

The similar FC between the sugarcane inclusion levels, despite its antinutritional or low-density nutritional factors, may be related to the higher tolerance or adaptation of the chicken to this circumstance (of low forage availability) associated with the longer time available for compensatory gain to take place. Similarly, Faria et al. (2011) evaluated the inclusion of 10% cassava leaf meal, leucaena meal and rice meal and reported that growth performance was not changed in comparison with diets based on corn and soybean meal. The similar performance between the strains evaluated in the present study denotes that they are equivalent and would exhibit the same effects when subjected to diets containing sugarcane as an alternative feedstuff.

There was no interaction effect (P>0.05) between strains and sugarcane inclusion levels for any of the physical or chemical parameters of the tarsometatarsal bone. Foot weight was lower (P<0.05) in the Pesadão strain than in LR (Table 3). However, neither the strains nor the sugarcane levels in the diet influenced the weight, breaking strength, maximum strength or deformation of the tarsometatarsal bone.

Table 3
Weight and physical analysis of the tarsometatarsal bones of slow-growing broiler strains (Label Rouge and Pesadão) fed different levels of sugarcane (0, 15, 30 and 45%) and slaughtered at 85 days of age

Parameter	Strain	Feed replacement with sugarcane					CV (%)
		0%	15%	30%	45%	Mean	
Foot weight (g)	Label Rouge	56.63	56.50	58.38	52.25	55.94a	
	Pesadão	53.13	52.88	51.38	51.38	52.19b	
	Mean	54.88	54.69	54.88	51.81	54.07	6.05
Tarsometatarsal bone weight (g)[1]	Label Rouge	6.06	5.94	6.35	5.66	6.00	
	Pesadão	6.13	6.15	6.26	5.66	6.05	
	Mean	6.09	6.05	6.30	5.66	6.03	9.78
Breaking strength (kgf)	Label Rouge	0.98	1.09	1.58	1.10	1.19	
	Pesadão	1.04	1.62	1.97	0.47	1.28	
	Mean	1.01	1.35	1.78	0.79	1.23	73.92
Maximum load (kgf)	Label Rouge	21.14	18.90	20.48	20.03	20.14	
	Pesadão	22.57	20.25	20.50	20.96	21.07	
	Mean	21.85	19.57	20.49	20.49	20.60	13.91
Deformation (mm)	Label Rouge	0.41	1.31	0.76	2.95	1.36	
	Pesadão	0.55	0.84	1.86	1.22	1.12	
	Mean	0.48	1.07	1.31	2.09	1.24	95.23

Means followed by different lowercase letters in the column differ by the Newman-Keuls test (P<0.05); 1Dry matter basis; CV = coefficient of variation.

The dry matter (DM) content of the tarsometatarsal bone was lower (P<0.05) in the LR individuals than in the Pesadão chickens (Table 4). The replacement of feed with sugarcane caused a linear increase (P<0.05) in bone ash content (Y=60.95 +0.0086X, R^2 = 0.96) and a linear decrease in the bone phosphorus (Y=5.414 - 0.0110X, R^2 = 0.69) and calcium (Y=24.569 - 0.0456X, R^2 = 0.69) contents.

Bone resistance is dependent on the calcium and phosphorus contents (Williams, Solomon, Waddington, Thorp, & Farquharson, 2000). The increasing dietary sugarcane inclusion levels resulted in a reduction of the calcium and phosphorus contents in the tarsometatarsal bone. This phenomenon indicates greater mobilization of these minerals to maintain growth, since higher sugarcane intakes caused a decrease in feed intake and greater damage to the intestinal epithelium, which resulted in decreased nutrient absorption.

Table 4
Chemical composition of the tarsometatarsal bones of different strains of slow -growing broilers (Label Rouge and Pesadão) fed different levels of sugarcane (0, 15, 30 and 45%) and slaughtered at 85 days of age

Parameter	Strain	Feed replacement with sugarcane					CV (%)
		0%	15%	30%	45%	Mean	
Dry matter	Label Rouge	59.91	60.32	57.94	61.30	59.87b	
	Pesadão	61.72	63.13	62.70	62.13	62.42a	
	Mean	60.82	61.73	60.32	61.71	61.15	5.03
Ash (%)[1]	Label Rouge	60.68	61.24	61.93	62.64	61.62	
	Pesadão	59.36	62.06	62.15	62.94	61.63	
	Mean (*L)	60.02	61.65	62.04	62.79	61.63	3.01
Phosphorus (%)[1]	Label Rouge	5.41	5.06	5.01	4.97	5.11	
	Pesadão	5.68	5.15	4.94	5.10	5.22	
	Mean (*L)	5.55	5.10	4.98	5.04	5.17	8.28
Calcium (%)[1]	Label Rouge	24.22	23.94	23.43	22.58	23.54	
	Pesadão	25.95	22.30	22.92	22.99	23.54	
	Mean (*L)	25.09	23.12	23.18	22.79	23.55	7.26

Means followed by different lowercase letters in the column differ by the Newman-Keuls test (P<0.05); 1 Dry matter basis; * (P<0.05), L = linear effect; CV = coefficient of variation.

However, sugarcane inclusion did not negatively affect the bone structure of the chickens despite the reduction in the phosphorus and calcium contents. This suggests that the calcium and phosphorus levels available in the experimental diets were sufficient to meet the requirements for bone development in the animals in this production system.

In addition, the increase in tibia phosphorus and calcium contents is accompanied by a simultaneous increase in total ash content (Browning & Cowieson, 2014). In this context, the results of the present study are very interesting, since the decreasing phosphorus and calcium levels were unexpectedly accompanied by an increase in tibia ash content.

The hypothesis to explain this phenomenon could be related to an ionic exchange, centered mainly in the cationic exchange of divalent calcium (Ca(II)) by divalent magnesium (Mg(II)), which is also found in a significant concentration in the bones (Shastak & Rodehutscord, 2015). According to Atteh and Lesson (1983), the decrease in bone calcium and phosphorus contents could be the effect of an increase in magnesium and/or silicon level and, consequently, in mineral matter. However, the mechanisms involved in this process need further investigation.

There was no interaction effect ($P>0.05$) between strains and sugarcane inclusion levels on intestinal morphology (Table 5). The LR strain showed a higher ($P<0.05$) crypt depth (CD) and lower villus/crypt ratio (VCR) and mucosal layer thickness (MLT) values than Pesadão.

There was a linear decrease ($P<0.05$), on the average of the strains, for the parameters of villus height (VH), VCR and MLT. A linear increase ($P<0.05$) was also observed in the LR for the parameters of villus width (VW), submucosal layer thickness (SMLT) and circular muscle layer thickness (CMLT). The sugarcane inclusion levels induced a quadratic effect ($P<0.05$) on CD ($Y=166.889 + 2.08607X - 0.034866X^2$, $R^2 = 0.99$) on average of the strains, and on VW ($Y=144.713 + 4.93302X - 0.09613X^2$, $R^2 = 0.84$), SMLT ($Y=77.873 + 4.93302X - 0.0961283X^2$, $R^2 = 0.84$) and CMLT ($Y=43.9828 + 1.07641X - 0.021161X^2$, $R^2 = 0.46$) in the Pesadão strain.

Table 5
Small intestine morphometry of slow-growing broiler strains (Label Rouge and Pesadão) fed different levels of sugarcane (0, 15, 30 and 45%) and slaughtered at 85 days of age

Parameter	Strain	Feed replacement with sugarcane					CV (%)
		0%	15%	30%	45%	Mean	
Crypt depth (µm)	Label Rouge	191.1	191.9	199.8	197.5	195.1 a	
	Pesadão	142.8	188.4	196.7	182.7	177.7 b	
	Mean (*Q)	166.9	190.2	198.3	190.1	186.4	10.22
Villus height (µm)	Label Rouge	1323.5	1179.0	1187.0	1159.9	1212.4	
	Pesadão	1266.6	1240.7	1164.1	1161.9	1208.3	
	Mean (*L)	1295.1	1209.8	1175.5	1160.9	1210.3	7.34
Villus/Crypt ratio	Label Rouge	6.9	6.2	6.0	5.9	6.2 b	
	Pesadão	8.9	6.7	5.9	6.4	7.0 a	
	Mean (*L)	7.9	6.4	6.0	6.2	6.6	10.86
Villus width (µm)	Label Rouge (*L)	164.1	184.8	190.2	209.4	187.1	
	Pesadão (*Q)	149.4	182.9	220.3	167.3	180	
	Mean	156.8	183.9	205.3	188.4	183.6	11.01
Submucosal layer thickness (µm)	Label Rouge (*L)	97.2	117.9	123.4	142.6	120.3	
	Pesadão (*Q)	82.6	116.1	153.5	100.5	113.2	
	Mean	89.9	117.0	138.4	121.5	116.7	17.31
Longitudinal muscle layer thickness (µm)	Label Rouge	277.0	227.4	232.1	239.5	244.0	
	Pesadão	241.9	227.6	233.1	226.8	232.3	
	Mean	259.4	227.5	232.6	233.1	238.2	13.37
Circular muscle layer thickness (µm)	Label Rouge (*L)	46.6	45.9	57.6	65.3	53.9	
	Pesadão (*Q)	46.5	47.8	64.8	47.1	51.5	
	Mean	46.6	46.9	61.2	56.2	52.7	12.81
Mucosal layer thickness (µm)	Label Rouge	1205.2	1183.5	1176.9	701.3	1066.7 a	
	Pesadão	1149.0	942.8	924.3	807.2	955.8 b	
	Mean (*L)	1177.1	1063.2	1050.6	754.3	1011.3	14.36

Means followed by different lowercase letters in the column differ by the Newman-Keuls test ($P<0.05$); *($P<0.05$); L = linear effect; Q = quadratic effect; CV = coefficient of variation.

The better VCR and MLT results of Pesadão suggest superior adaptability of the intestinal environment of this strain when fed diets containing sugarcane. This fact indicates greater integrity in this structure, which in turn reveals greater mucosal digestion and absorption capacity. However, this effect is not sufficient to influence the production performance of the chickens, considering that almost all parameters did not differ between the strains. Santos et al. (2015) observed that the fast-growing chicken strain (Cobb) showed a greater duodenum VH at 42 days old when compared with a slow-growing strain

(Isa Label). Nonetheless, in agreement with Verdal et al. (2010), the higher VH may simply be a physiological mechanism to compensate for the low functionality of the gastric region.

In addition, the increasing sugarcane levels in the diets negatively affected the intestinal morphological variables, as evidenced mainly by the decreased VH and VCR and increased CD. This indicates that the increasing intake of crushed sugarcane by the free-range chickens in the present study caused inflammation in the intestinal mucosa, which resulted in poor growth performance and lower calcium and phosphorus bone contents. Furthermore, from the replacement level of 25.5% of feed with sugarcane, there is a possible decrease in nutrient uptake by the crypt cells, which tends to affect the mitotic process.

Conclusions

The growth performance and intestinal morphology of slow-growing broilers are negatively affected by increasing levels of sugarcane in the diet. The increasing replacement of feed with sugarcane modifies bone mineral composition without impairing its mechanical resistance, in both evaluated strains (Label Rouge and Pesadão). However, the Pesadão strain has better intestinal morphology characteristics than Label Rouge. Although the dietary inclusion of sugarcane negatively affects the abovementioned parameters, the use of sugarcane is suitable to control animal growth as well as a means of providing forage in the periods of lower feed availability for chickens.

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