Effects of the inclusion of sunflower hulls in the diet on growth performance and digestive tract traits of broilers and pullets fed a broiler diet from zero to 21 d of age. A comparative study[1]

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ABSTRACT The effects of including 3% sunflower hulls (SFH) in the diet on growth performance, nutrient retention (TTAR), and gastrointestinal tract (GIT) traits were studied in chicks from zero to 21 d of age. Four treatments that resulted from the combination of 2 chicken lines (female broilers vs. brown pullets) and 2 levels of SFH (zero vs. 3%) were used. The control diet contained 2,980 kcal AME_n/kg, 1.25% digestible Lys, and 8.7% neutral detergent fiber. The experimental diet included 3% SFH at the expense (wt:wt) of the whole diet. Growth performance, TTAR of nutrients, and the AME_n of the diet were greater (P = 0.097 to $P < 0.001$) in broilers than in pullets. In absolute terms, all the organs of the GIT were heavier ($P < 0.001$) and the small intestine and cecum were longer ($P < 0.001$) in broilers than in pullets. At 21 d of age, however, the relative weight (% BW) of all the organs of the GIT ($P < 0.001$) and the relative length (cm/kg BW) of the small intestine and cecum ($P < 0.01$) were greater in pullets. Gizzard pH ($P < 0.001$), total short chain fatty acids concentration in the cecum ($P = 0.098$), and villus height ($P < 0.001$) and crypt depth ($P < 0.05$) of the ileum mucosa were higher in broilers. The inclusion of SFH increased ($P < 0.05$) the AME_n content of the diet but did not affect bird performance, moisture content of the excreta, or the concentration and profile of fatty acids in the cecum. Dietary SFH increased gizzard weight and reduced gizzard pH ($P < 0.001$) at both ages. In conclusion, broilers had better growth performance, nutrient retention, and ileum absorptive capacity than pullets. The inclusion of 3% SFH at the expense of the control diet did not have any negative effect on chick performance and, in fact, increased gizzard weight, reduced gizzard pH, and improved the energy content of the diet.

Key words: excreta moisture, gastrointestinal tract traits, gizzard pH, insoluble fiber

INTRODUCTION

Dietary fiber (**DF**) affects feed intake and nutrient digestibility in poultry, with effects that depend to a high extent on the type and level of fiber inclusion (Mateos et al., 2002, 2012; Hetland and Svihus, 2007). In this respect, the inclusion of moderate amounts of insoluble fiber in the diet increases the retention time of the digesta in the upper part of the gastrointestinal tract (**GIT**) and improves the development and function of the gizzard and other digestive organs in broilers (Hetland and Svihus, 2001; González-Alvarado et al., 2007, 2008). In addition, DF stimulates HCl and bile acid production and improves amylase activity, which results in increased nutrient utilization (Hetland et al., 2003).

Broilers are selected for faster growth and improved feed to gain ratio (**F:G**) whereas pullets are selected for optimal future egg mass production. The divergent selection targets for both chicken lines result in birds differing in the anatomical and physiological characteristics of the GIT (Masic et al., 1974; Nir et al., 1993; Hetland et al., 2003; Rideau et al., 2014). Consequently, the response of the GIT of broilers and pullets to fiber inclusion and its effects on nutrient digestibility might differ between chicken lines (Slinger et al., 1964; Walugembe et al., 2014). Most of the research on the effects of the inclusion of a fiber source in the diet on GIT development and growth has been conducted with broilers (Hetland and Svihus, 2001; Jiménez-Moreno et al., 2009a; González-Alvarado et al., 2010; Mateos et al., 2012) but little information is available in pullets. Research comparing the effects of insoluble fiber on growth performance and GIT traits in modern strains of these 2 chicken lines could help to adapt the existing information on broilers to pullets.

The hypothesis tested in this research was that sunflower hulls (**SFH**) could affect GIT development and growth performance in both chicken lines. In addition, it was hypothesized that because of their lower rate of feed passage, limited digestive capacity, and reduced potential for voluntary feed intake, the effects could be less evident in pullets. The objective of this research was to compare the effects of including 3% SFH in the diet on growth performance, nutrient digestibility, and GIT traits of broilers and pullets fed a broiler diet from zero to 21 d of age.

MATERIALS AND METHODS

All procedures used were approved by the Animal Ethics Committee of the Polytechnic University of Madrid and were in compliance with the Spanish guidelines for the care and use of animals in research (Boletín Oficial del Estado, 2007).

Diets and Fiber Source

The control diet was based on corn and soybean meal and contained 2,980 kcal AME_n/kg, 22.2% CP, 1.25% digestible Lys, and 8.7% neutral detergent fiber (**NDF**). Celite, an acid-washed diatomaceous earth (Celite Hispánica S.A., Alicante, Spain), was included at 2% in the diet to increase the acid insoluble ash content of feeds and excreta. The experimental diet had similar ingredient composition to that of the control diet but was diluted (wt:wt) with 3% SFH. Before incorporation to the diet, the SFH was ground using a hammer mill (Model DFZC-635, Bühler AG, Uzwill, Switzerland) fitted with a 2.5 mm screen. Both diets met or exceeded the nutritional requirements of pullets as recommended by FEDNA (2008). The chemical analyses and physical characteristics of the SFH are shown in Table 1 and the ingredient composition and physicochemical characteristics of the experimental diets are shown in Table 2.

Husbandry and Experimental Design

In total, 280 one-day-old chicks, 140 Ross 308 female broilers with an initial BW of 46.7 ± 3.80 g and 140 Lohmann Brown pullets with an initial BW of 37.5 ± 2.84 g, were used. Birds were housed in groups of 10, according to chicken line, in 28 battery cages (0.36 m × 1.00 m; Alternative Design, Siloam Springs, AR) equipped with 2 nipple drinkers and an open trough feeder (65 cm length). Within each chicken line, all cages had similar average BW. The batteries were placed in an environmentally controlled room with an ambient temperature that was reduced gradually from 31 ± 1°C at the arrival of the chicks to 24 ± 2°C at 21 d of age. The light program consisted of 23 h of light during the first 7 d of life followed by 16 h to the end of the experiment. Feed in mash form and water were offered for ad libitum consumption. The experiment was

Table 1. Analyzed chemical composition and physical characteristics (% as-fed basis) of the sunflower hulls.

Item	%
Chemical analyses	
Dry matter	93.0
Gross energy (kcal/kg)	4,423
AME_n[1] (kcal/kg)	340
Crude protein	4.7
Ether extract	3.6
Total ash	2.8
Crude fiber	50.7
Neutral detergent fiber	70.0
Acid detergent fiber	51.3
Acid detergent lignin	13.1
Total dietary fiber	86.8
Soluble fiber	2.0
Insoluble fiber	84.8
Physical characteristics	
Particle size distribution, sieve screen[2] (μm)	
>2,500	0.4
1,250	4.6
630	60.1
315	24.4
160	10.3
<80	0.2
GMD[3] ± GSD[4] (μm)	675 ± 1.68
WHC[5] ± SD (mL/g DM)	6.88 ± 0.35
SWC[6] ± SD (mL/g DM)	3.85 ± 0.19

[1]Calculated value (FEDNA, 2010).
[2]The percentage of particles bigger than 5,000 or smaller than 40 μm was negligible.
[3]Geometric mean diameter.
[4]Log geometric standard deviation.
[5]Water-holding capacity.
[6]Swelling-water capacity.

conducted as a completely randomized design with 4 treatments arranged as a 2 × 2 factorial with 2 chicken lines (female broilers vs. pullets) and 2 levels of SFH (0 vs. 3%). Each treatment was replicated 7 times and the experimental unit was a cage with 10 birds.

Growth Performance and Total Tract Apparent Retention of Nutrients

Body weight of the chicks and feed disappearance were determined by cage at 9 and 21 d of age and the data used to estimate ADFI, ADG, and F:G by period (zero to 9 d and 10 to 21 d) and cumulatively (zero to 21 d). In addition, energy intake (**EI;** kcal AME_n/day) and energy conversion ratio (**ECR;** kcal AME_n ingested/g BW gain) were estimated at the same ages. Total tract apparent retention (**TTAR**) of DM, organic matter, N, and GE was determined by the indigestible marker method and the AME_n of the diets as indicated by De Coca-Sinova et al. (2011). Briefly, clean plastic pans were placed beneath the cages at 7 and 19 d of age, and representative samples of the excreta were collected daily for 2 days. The samples were dried in oven at 60°C for 72 h, ground with a laboratory mill (model Z-I, Retsch, Stuttgart, Germany) fitted with a 1-mm screen, mixed by replicate, and stored at 4°C until further analyses. At 9 and 21 d of age, after the collection of the excreta for TTAR determination, the trays placed beneath the cages were cleaned and the excreta produced for the next 3 h was collected and

Table 2. Ingredient composition and calculated and analyzed chemical composition of the experimental diets (%, as-fed basis, unless otherwise indicated).

	Sunflower hulls	
	0%	3%
Ingredient		
Corn	34.0	33.0
Wheat	20.0	19.4
Sunflower hulls	0.0	3.0
Soy oil	1.31	1.27
Soybean meal (47% CP)	21.0	20.4
Full fat soybeans	14.8	14.3
Fish meal (60% CP)	3.00	2.92
Calcium carbonate	1.35	1.32
Monocalcium phosphate	1.20	1.16
Sodium chloride	0.31	0.30
L-Lysine (78%)	0.27	0.26
DL-Methionine (99%)	0.32	0.31
L-Threonine (99%)	0.11	0.10
L-Valine (99%)	0.03	0.03
Celite	2.00	1.94
Vitamin and mineral premix[1]	0.30	0.29
Analysed chemical composition		
Dry matter	88.4	88.7
Crude protein	22.2	21.7
Ether extract	5.8	6.1
Ash	6.3	6.2
Crude fiber	3.0	4.4
Neutral detergent fiber	8.7	10.3
Acid detergent fiber	3.9	5.7
Acid detergent lignin	1.5	1.8
Total dietary fiber	16.8	19.8
GMD[2] ± GSD[3] (μm)	975 ± 2.07	884 ± 2.07
Initial pH	5.82	5.88
Calculated chemical composition[4]		
AME_n (kcal/kg)	2,980	2,900
Digestible amino acid		
Arg	1.33	1.29
Lys	1.25	1.22
Met	0.63	0.61
Met + Cys	0.92	0.89
Thr	0.81	0.78
Trp	0.23	0.22
Calcium	1.02	0.99
Available phosphorus	0.45	0.44

[1]Provided the following (per kilogram of diet): vitamin A (trans-retinyl acetate), 10,000 IU; vitamin D3, (cholecalciferol), 3,000 IU; vitamin E (all-rac-tocopherol acetate), 30 IU; vitamin K (bisulphate menadione complex), 3 mg; riboflavin, 5 mg; pantothenic acid (D-Ca pantothenate), 10 mg; nicotinic acid, 30 mg; pyridoxine (pyridoxine, HCl), 3 mg; thiamin (thiamin-mononitrate), 1 mg; vitamin B_{12} (cyanocobalamin), 12 μg; D-biotin, 0.15 mg; choline (choline chloride), 300 mg; folic acid, 0.5 mg; Fe ($FeSO_4$;$7H_2O$), 30 mg; Zn (ZnO), 100 mg; Mn ($MnSO_4$; H_2O), 100 mg; Se (Na_2SeO_3), 0.2 mg; I (KI), 2.0 mg; Cu ($CuSO_4$; $5H_2O$), 10 mg; phytase (EC 3.1.3.26), 0.06% Natuphos (BASF Española, S.A., Tarragona, Spain); [(Endo-1,3 (4)-ß-glucanase (EC 3.2.1.6), 150 IU; Endo-1,4-ß-xylanase (EC 3.2.1.8) 150 IU; (Endofeed, GNC Bioferm, Saskatchewan, SK, Canada)]; and ethoxyquin, 110 mg.

[2]Geometric mean diameter.

[3]Log geometric standard deviation.

[4]According to FEDNA (2010).

immediately oven dried (60°C for 72 h) for determination of the DM content.

Gastrointestinal Tract Traits

After each BW determination, 2 birds per replicate were randomly selected, weighed individually, and euthanized by CO_2 asphyxiation. The GIT was excised and divided into 7 portions: crop, proventriculus, gizzard, duodenum (from the gizzard junction to the entry of the bile and pancreatic ducts), jejunum (from end of the duodenum to Meckel's diverticulum), ileum (from Meckel's diverticulum to the ileocecal junction), and cecum. The segments of the GIT were clamped to avoid bolus contamination and the pH of all organs was measured in situ using a digital pH meter (Model 507, Crison Instruments S.A., Barcelona, Spain) as indicated by Jiménez-Moreno et al. (2009b). The mean value of 2 measurements taken in each of the 2 birds was used for further evaluation.

In addition, 2 and 4 extra birds per replicate were slaughtered at 9 d and at 21 d of age, respectively, and the full GIT, from the post-crop to the cloaca, without the annex organs (liver, pancreas, and spleen), was excised, dried with desiccant paper, and weighed. The weight of the full proventriculus and gizzard, and that of the liver and pancreas also were measured in these birds and expressed in absolute (g) and in relative (% BW) terms. The gizzard of all these birds was opened and the contents were weighed. The weight of the empty gizzard was calculated by difference between the weight of the full organ and its content. The DM of the digesta of the gizzard was also determined by drying the contents at 60°C for 72 h. In addition, the absolute (cm) and relative (cm/kg BW) length of the small intestine (**SI**; duodenum, jejunum, and ileum) and of the 2 cecum were measured in all these birds using a flexible ruler with a precision of 1 mm.

At 21 d of age, the concentration (μmol/g fresh digesta) of acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate and total SCFA was determined in the cecum of the 2 birds used for pH determination, and the proportion of each individual fatty acid (% total SCFA) was calculated. In addition, ileum tissue samples of approximately 3 cm in length were taken from the midpoint of the ileum in these 2 birds and fixed in a buffer solution with 10% formalin. Villus height (**VH**), from the tip of the villi to the villus crypt junction, and the associated crypt depth (**CD**), defined as the depth of the invagination between adjacent villi of the samples, were measured as described by Jiménez-Moreno et al. (2011). Two representative cross sections from each bird were viewed at 40× magnification using an Olympus BX-40 light microscope. Images were digitally captured (Soft Imaging System, version 3.2 C4040Z, Olympus, GmbH, Hamburg, Germany) and the average of at least 30 measurements taken from cross-sectional samples was used for further analysis.

Laboratory Analysis

Sunflower hulls, diets, and excreta were analyzed for moisture by oven-drying (method 930.15), total ash by muffle furnace (method 942.05), and nitrogen (**N**) by Dumas (method 968.06), using a LECO analyzer (Model FP-528, Leco Corporation, St. Joseph, MI) as described by AOAC International (2000). Gross energy was measured using an isoperibol bomb calorimeter (model 1356, Parr Instrument Company, Moline, IL) and ether extract was determined, after 3 *N* HCl acid hydrolysis, using an Ankom XT10 extraction system (Ankom Technology Corp. Macedon, NY) by method Am 5–04 of the AOCS (2004). Neutral detergent fiber, acid detergent fiber, and acid detergent lignin of the fiber source and diets were determined sequentially using a filter bag system (Ankom Technology Corp. Macedon, NY) and expressed on an ash-free basis (van Soest et al., 1991). Also, total DF (method 985.29) and the insoluble fraction of the DF (method 991.43) were analyzed as proposed by the AOAC International (2005). The soluble fraction was calculated by difference between total and insoluble DF. The pH of the drinking water and diets was measured as described by Giger-Reverdin et al. (2002) using a portable pH meter (Model 507, Crison Instruments S.A., Barcelona, Spain). Particle size distribution and mean particle size of the SFH and diets, expressed as geometric mean diameter ± log geometric standard deviation (**GMD ± GSD**), were determined in 100 g samples using a Retsch shaker (Retsch, Stuttgart, Germany) provided with 8 sieves ranging in mesh from 40 to 5,000 μm, as described by the ASAE (1995). The water-holding (WHC, mL/g DM) and swelling (SWC, mL/g DM) capacity of the SFH was determined as indicated by Jiménez-Moreno et al. (2009b). Acid-insoluble ash was analyzed as proposed by De Coca-Sinova et al. (2011). The concentration of total and of the individual short chain fatty acids (**SCFA**) was determined in cecum samples by gas chromatography, using a Perkin Elmer Autosystem XL (Perkin Elmer Inc., Waltham, MA) equipped with a flame-ionization detector and a TRB-FFAP capillary column (30 m × 0.53 mm × 1 μm; Supelco, Barcelona, Spain), as described by Carro et al. (1992). Briefly, a 1.5 g sample of fresh cecal contents was mixed with 1.5 mL of 0.5 *M* HCl and frozen at −20°C. Before analyses, samples were thawed at 4°C, homogenized, and centrifuged (13,000 × *g*) for 15 min at 4°C. One mL of the supernatant was mixed with 0.5 mL of a deproteinized solution consisting of a mixture of 20 g metaphosphoric acid and 0.6 g of crotonic acid/L and left overnight at 4°C.

Statistical Analysis

All data were analyzed as a completely randomized design with 4 treatments arranged as a 2 × 2 factorial using the GLM program of SAS (1990). The model included the chicken line (female broilers vs. pullets) and the level of SFH inclusion (zero and 3%) as main effects as well as the interaction. The experimental unit was the cage with 10 (zero to 9 d) or 6 (10 to 21 d) birds for growth performance and TTAR data, 2 (9 d) and 4 (21 d) birds for GIT measurements, and 2 birds for pH and ileum mucosa morphology. The Tukey test was used to detect differences among treatment means and the differences were considered significant at $P < 0.05$.

RESULTS

The analytical values of the SFH batch were within the range reported by other authors (Cancalon, 1971; FEDNA, 2010; Guzmán et al., 2015a). The NDF content was 70.0% and the GMD ± GSD was 675 ± 1.68 μm. The chemical composition of the experimental diets was close to expected values, confirming that the ingredients were mixed correctly.

Growth Performance and Total Tract Apparent Retention of Nutrients

Mortality was low (1.0%) and not related to any of the treatments (data not shown). No interactions between main effects were detected for any of the traits studied and, therefore, only main effects are presented.

Effects of Chicken Line. Feed intake and ADG were greater ($P < 0.001$) and F:G ratio and ECR were better ($P < 0.001$) for broilers than for pullets at all ages (Table 3). The moisture content of the excreta was higher ($P < 0.01$) in broilers than in pullets at 21 d of age but an opposite effect ($P < 0.05$) was observed at 9 d of age (Table 4).

The TTAR of all nutrients and the AME_n content of the diets were higher ($P = 0.097$ to $P < 0.001$) for

Table 3. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on growth performance of the chicks from zero to 21 d of age.

	Line		SFH			*P*-value[7]	
	Broiler	Pullet	0%	3%	RSD[1]	Line	SFH
0 to 9 d							
ADG[2]	21.3	6.3	13.9	13.7	0.86	0.001	0.679
ADFI[3]	26.0	11.5	18.8	18.8	0.73	0.001	0.898
F:G[4]	1.223	1.814	1.511	1.526	0.049	0.001	0.425
EI[5]	77.1	34.2	55.1	56.2	2.18	0.001	0.217
ECR[6]	3.63	5.28	4.41	4.50	0.145	0.001	0.085
10 to 21 d							
ADG	40.9	9.8	25.4	25.3	1.40	0.001	0.749
ADFI	58.5	18.4	38.2	38.6	1.63	0.001	0.576
F:G	1.430	1.876	1.644	1.662	0.035	0.001	0.183
EI	179.6	55.7	116.3	119.0	5.02	0.001	0.166
ECR	4.39	5.72	5.00	5.12	0.109	0.001	0.006
0 to 21 d							
ADG	34.5	8.7	21.6	21.6	1.01	0.001	0.956
ADFI	47.6	16.1	31.7	32.0	1.17	0.001	0.598
F:G	1.387	1.860	1.615	1.632	0.033	0.001	0.192
EI	135.7	46.4	90.1	92.1	3.21	0.001	0.113
ECR	4.18	5.58	4.82	4.94	0.10	0.001	0.005

[1]Residual standard deviation (n = 7 for each treatment and n = 14 for main effects). The experimental unit was a cage with 10 birds at 9 d and 6 birds at 21 d of age.
[2]Average daily gain (g).
[3]Average daily feed intake (g).
[4]Feed to gain ratio.
[5]Energy intake (kcal AME_n per bird/d).
[6]Energy conversion ratio (kcal AME_n ingested/g BW gain).
[7]The interactions between chicken line and SFH inclusion were not significant ($P > 0.05$).

Table 4. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on moisture content of the excreta.

	Line		SFH			P-value[2]	
	Broiler	Pullet	0%	3%	RSD[1]	Line	SFH
9 d	79.1	80.4	79.8	79.7	1.10	0.004	0.865
21 d	75.1	73.7	74.3	74.5	1.79	0.043	0.876

[1]Residual standard deviation (n = 7 for each treatment and n = 14 for main effects). The experimental unit was a cage with 10 birds at 9 d and 6 birds at 21 d of age.

[2]The interactions between chicken line and SFH inclusion were not significant ($P > 0.05$).

broilers than for pullets at both ages with the most pronounced differences observed for N retention (Table 5).

Effects of Sunflower Hulls Inclusion. The inclusion of SFH in the diet did not affect growth performance of the birds, but increased ECR ($P < 0.01$) (Table 3). Sunflower hulls did not affect moisture content of the excreta (Table 4) or TTAR of the nutrients at any age, but improved the AME_n of the diets at 9 ($P < 0.05$) and 21 ($P < 0.01$) d of age (Table 5).

Gastrointestinal Tract Traits

Effects of Chicken Line. In absolute terms, all the organs of the GIT were heavier ($P < 0.001$) in broilers than in pullets (Table 6). Significant interactions between chicken line and SFH inclusion were observed for the absolute weight (g) of the proventriculus at 21 d of age ($P < 0.05$) and of the gizzard at 9 d ($P < 0.05$) and 21 d ($P < 0.001$) of age (Figure 1) and for the empty weight and fresh digesta content of the gizzard at both ages (Figure 2). In relative terms (% BW), however, no interactions between main effects were detected for any organ. Moreover, at 21 d of age, all the digestive organs were heavier ($P < 0.001$) in pullets than in broilers. Fresh digesta content (g) of the gizzard was greater in broilers than in pullets ($P < 0.001$) at both ages but the DM was not affected (Table 7).

The SI and the cecum were longer ($P < 0.001$) in absolute terms (cm) but shorter ($P < 0.001$) in relative terms (cm/kg BW) in broilers than in pullets at both ages (Table 8). The pH of the gizzard was higher in broilers ($P < 0.001$) at both ages (Table 9).

Effects of Sunflower Hulls Inclusion. The inclusion of SFH in the diet had little effect on the absolute weight of the different organs of the GIT, except for the full proventriculus at 21 d of age that was reduced ($P < 0.05$) and the full gizzard at both ages ($P < 0.001$) that was increased (Table 6). In relative terms, the only differences observed were for the weight of the GIT at 21 d of age ($P < 0.05$) and of the gizzard at both ages ($P < 0.001$) that increased with SFH inclusion.

The empty weight ($P < 0.001$) and the fresh digesta content (g) of the gizzard at 9 ($P < 0.01$) and 21 ($P < 0.001$) d of age increased with SFH inclusion (Table 7). Sunflower hulls did not affect the absolute or relative length of the SI and cecum (Table 8) but decreased the pH of the gizzard ($P < 0.001$) and cecum ($P < 0.05$ to $P < 0.01$) at both ages (Table 9).

Short Chain Fatty Acid Concentration and Ileum Morphology

No interactions between chicken line and SFH inclusion were detected for any of the traits studied and, therefore, only main effects are presented.

Effects of Chicken Line. Broilers tended to have higher concentration of total SCFA ($P = 0.098$) and higher proportion of acetate ($P = 0.077$) but lower of valerate ($P = 0.052$) than pullets (Table 10). Chicken line affected ($P < 0.05$) the mucosa structure of the ileum, with broilers showing higher VH and CD than pullets (Table 11).

Effects of Sunflower Hulls Inclusion. The inclusion of SFH in the diet did not affect the concentration

Table 5. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on the total tract apparent retention (TTAR, %) of the nutrients and AME_n content of the diets.

	Line		SFH			P-value[2]	
	Broiler	Pullet	0%	3%	RSD[1]	Line	SFH
At 9 d of age							
Dry matter	67.5	63.9	65.5	65.8	1.28	0.001	0.559
Organic matter	71.2	67.1	69.4	69.0	1.44	0.001	0.367
Nitrogen	62.5	50.5	55.8	57.1	2.60	0.001	0.185
Gross energy	72.6	71.3	71.8	72.1	1.26	0.009	0.461
AME_n (kcal/kg)	2,965	2,912	2,918	2,959	51.3	0.013	0.049
At 21 d of age							
Dry matter	68.9	67.3	67.8	68.4	1.06	0.001	0.152
Organic matter	73.0	71.6	72.3	72.4	1.01	0.001	0.669
Nitrogen	64.1	55.0	58.7	60.5	2.75	0.001	0.103
Gross energy	75.3	74.7	74.8	75.2	0.78	0.072	0.239
AME_n (kcal/kg)	3,072	3,052	3,041	3,083	31.5	0.097	0.002

[1]Residual standard deviation (n = 7 for each treatment and n = 14 for main effects). The experimental unit was a cage with 10 birds at 9 d and 6 birds at 21 d of age.

[2]The interactions between chicken line and SFH inclusion were not significant ($P > 0.05$).

Table 6. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on the absolute (g) and relative (% BW) weight of the organs of the gastrointestinal tract (GIT) of the chicks.

	Line		SFH			*P*-value[6]	
	Broiler	Pullet	0%	3%	RSD[5]	Line	SFH
At 9 d of age							
BW[1] (g)	197	82	141	139	16.9	0.001	0.735
Absolute weight (g)[2]							
GIT	39.3	15.6	27.2	27.6	3.43	0.001	0.637
Proventriculus[3]	2.16	1.01	1.59	1.58	0.22	0.001	0.764
Gizzard[4]	11.3	5.5	7.69	9.08	1.33	0.001	0.001
Pancreas	0.87	0.35	0.64	0.58	0.16	0.001	0.160
Liver	8.74	3.56	6.18	6.11	0.76	0.001	0.726
Relative weight (% BW)							
GIT	20.0	19.0	19.2	19.9	2.41	0.131	0.326
Proventriculus	1.10	1.24	1.17	1.17	0.16	0.002	1.000
Gizzard	5.74	6.72	5.74	6.72	0.97	0.001	0.001
Pancreas	0.44	0.43	0.46	0.41	0.10	0.481	0.088
Liver	4.44	4.35	4.41	4.38	0.58	0.584	0.887
At 21 d of age							
BW (g)	786	224	510	500	60.9	0.001	0.353
Absolute weight (g)[2]							
GIT	92.1	31.0	61.3	61.9	7.89	0.001	0.716
Proventriculus	4.50	1.87	3.31	3.07	0.62	0.001	0.045
Gizzard	24.2	10.1	14.6	19.7	4.00	0.001	0.001
Pancreas	2.74	0.96	1.83	1.86	0.44	0.001	0.733
Liver	27.6	9.11	18.8	17.9	2.97	0.001	0.129
Relative weight (% BW)							
GIT	11.8	13.9	12.6	13.1	1.18	0.001	0.027
Proventriculus	0.57	0.84	0.72	0.70	0.10	0.001	0.300
Gizzard	3.09	4.51	3.26	4.35	0.59	0.001	0.001
Pancreas	0.35	0.43	0.38	0.40	0.08	0.001	0.208
Liver	3.52	4.07	3.84	3.75	0.40	0.001	0.276

[1]Average of 2 and 4 birds at 9 and 21 d of age, respectively.
[2]Full gastrointestinal tract without the annex organs (pancreas, spleen, and the liver).
[3]Full proventriculus.
[4]Full gizzard.
[5]Residual standard deviation (n = 7 for each treatment and n = 14 for main effects). The experimental unit was formed by 2 and 4 birds per replicate at 9 and 21 d of age, respectively.
[6]The interactions between chicken line and SFH inclusion were not significant for the relative weight of all the organs ($P > 0.05$).

of total SCFA or the FA profile in the cecum (Table 10). Similarly, ileum mucosa structure was not affected by SFH inclusion (Table 11).

DISCUSSION

Significant interactions between main effects were observed for the absolute weight of the full proventriculus at 21 d and gizzard at 9 and 21 d of age and for the empty weight and fresh digesta content of the gizzard at both ages. The interactions detected showed a similar trend, with SFH showing more pronounced effects in broilers than in pullets (Figures 1 and 2). Consequently, the discussion will focus mostly on main effects.

Growth Performance and Total Tract Apparent Retention of Nutrients

Effects of Chicken Line. Growth performance was greater for broilers than for pullets at all ages. From zero to 21 d of age, ADFI and ADG were 3 and 4 times greater and F:G ratio was 25% better for broilers, consistent with the objectives of the current programs of selection for poultry based on fast growth rate and feed efficiency for broilers and harmonic growth of body tissues during the rearing phase for pullets. In the current research, female broilers grew 15.4% less than expected based on the Ross (2014) guidelines whereas pullets grew 16.0% faster than indicated in the Lohmann (2014) guidelines. The lower than expected ADG observed in broilers was caused primarily by the feeding of mash diets as reported by Serrano et al. (2012) and Jiménez-Moreno et al. (2016). On the other hand, the higher than expected ADG reported for pullets fed a broiler diet suggests that modern layer strains might respond to increases in the nutrient content of the starter diet with improved growth.

Nutrient retention and AME_n of the diet increased with age, irrespective of chicken line, consistent with most published research in broilers (Batal and Parsons, 2002; Gracia et al., 2009; Kim and Corzo, 2012). In the current research, nutrient retention was higher in broilers than in pullets, with the most pronounced effects observed for N. In contrast, Walugembe et al. (2014) reported that the AME_n of 2 isonutritive diets that varied widely in NDF content was similar for both chicken lines. Modern broiler strains are more voracious and

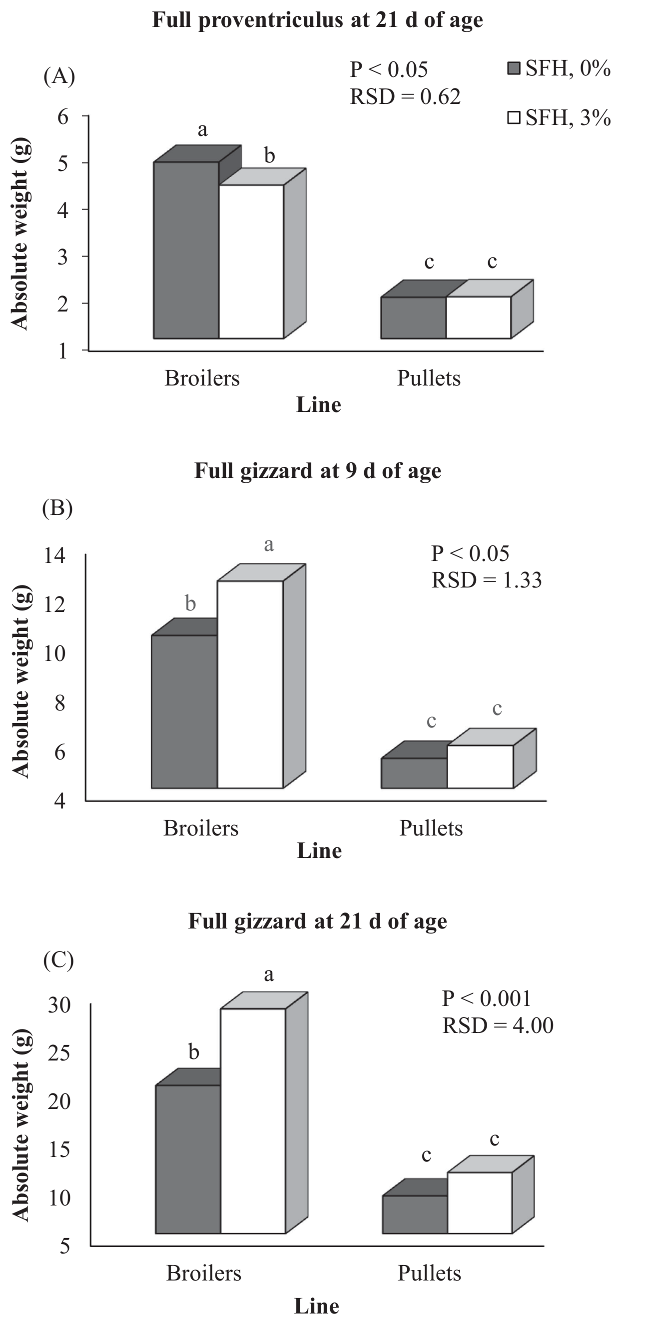

Figure 1. Interaction between chicken line (Line) and sunflower hulls (SFH) inclusion on the absolute weight of the full proventriculus at 21 d (A) and full gizzard at 9 d (B) and 21 d (C) of age.

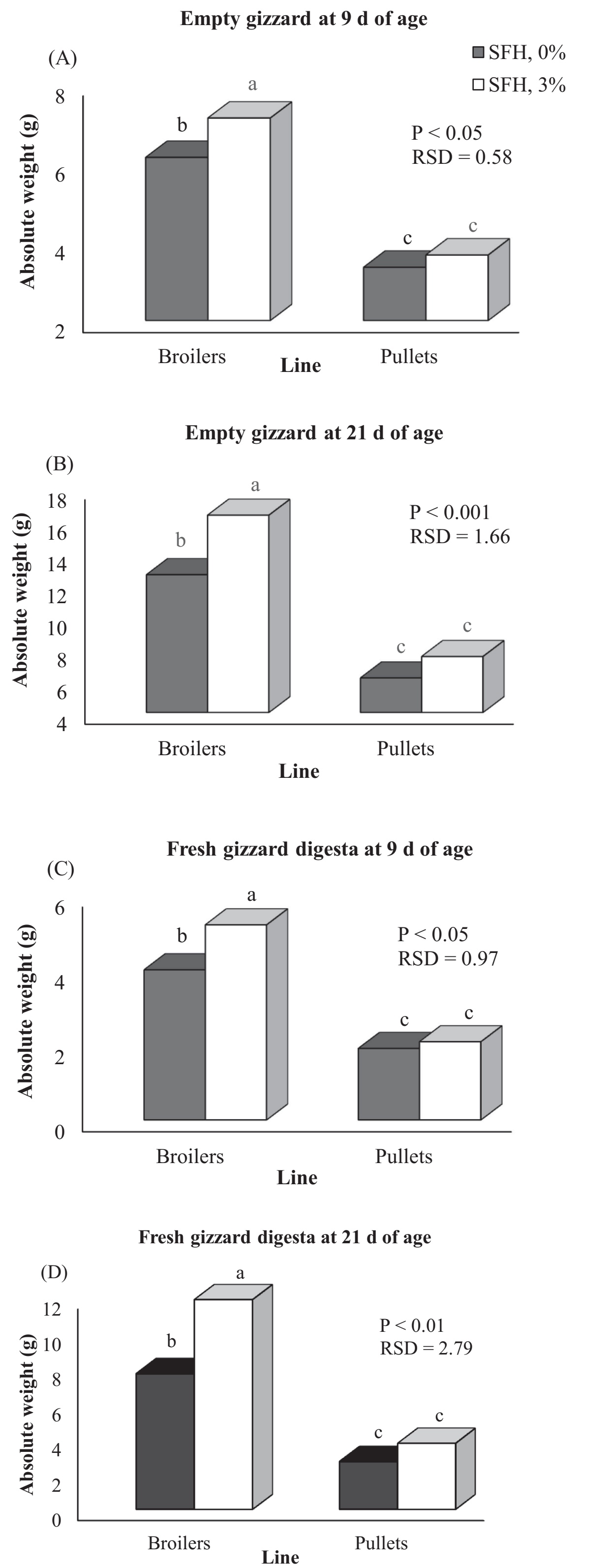

Figure 2. Interaction between chicken line (Line) and sunflower hull (SFH) inclusion in the diet on the absolute weight of the empty gizzard at 9 d (A) and 21 d (B) of age, and on the fresh digesta content of the gizzard at 9 d (C) and 21 d (D) of age.

had shorter retention time of the digesta in the proximal part of the GIT than pullets, which may have a negative effect on nutrient digestibility. Kaminiska (1979) reported that at the same age, pullets utilized feed energy more efficiently than broilers, suggesting that the longer SI and reduced rate of digesta passage through the GIT was the main reason for the improvement observed. Uni et al. (1995), however, observed that at 4 d of age broilers secreted more amylase, trypsin, and lipase per gram of feed and had better villus development and higher nutrient digestibility than pullets. The greater enzyme production and absorption capacity of broilers may counteract the negative effects of a faster rate of passage and the final outcome might depend on factors such as the age of the experimental birds and the physico-chemical characteristics (e.g. mash vs. pellets) of the experimental diets. In this respect, little effect

Tabl e 7. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on the absolute (g) and relative (% BW) empty gizzard weight and gizzard digesta characteristics of the chicks.[1]

	Line		SFH			P-value[3]		
	Broiler	Pullet	0%	3%	RSD[2]	Line	SFH	Line × SFH
At 9 d of age								
Absolute weight	6.63	3.50	4.74	5.39	0.58	0.001	0.001	0.031
Relative weight	3.39	4.28	3.58	4.09	0.50	0.001	0.001	0.821
Fresh digesta content (g)	4.61	2.00	2.96	3.65	0.97	0.001	0.009	0.053
Absolute empty:full gizzard weight	59.7	64.1	62.8	60.9	5.78	0.007	0.236	0.501
Digesta DM (%)	36.2	36.8	36.4	36.5	1.94	0.288	0.913	0.805
At 21 d of age								
Absolute weight	14.4	6.83	9.39	11.9	1.66	0.001	0.001	0.001
Relative weight[2]	1.85	3.06	2.17	2.73	0.29	0.001	0.001	0.238
Fresh digesta content (g)	9.81	3.26	5.23	7.83	2.79	0.001	0.001	0.004
Absolute empty:full gizzard weight	61.4	68.3	67.3	62.4	6.91	0.001	0.001	0.176
Digesta DM (%)	36.5	37.1	36.7	36.9	1.71	0.363	0.824	0.877

[1]The average BW of the birds used is shown in Table 6.
[2]Residual standard deviation (n = 7 for each treatment and n = 14 for main effects). The experimental unit was formed by 2 and 4 birds per replicate at 9 and 21 d of age, respectively.
[3]The interactions between chicken line and SFH inclusion were significant ($P > 0.05$) for the absolute weight of the gizzard at 9 d ($P < 0.05$) and 21 d ($P < 0.001$) and for the fresh digesta content at 9 d ($P = 0.053$). See Figures 1 and 2, respectively.

Table 8. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on the absolute (cm) and relative length (cm/kg BW) of the small intestine (SI) and the cecum of the chicks.[1]

	Line		SFH			P-value[5]	
	Broiler	Pullet	0%	3%	RSD[4]	Line	SFH
At 9 d of age							
Absolute length							
SI[2]	114	74.0	93.2	94.3	7.49	0.001	0.607
Cecum[3]	8.93	6.39	7.50	7.82	0.97	0.001	0.221
Relative length							
SI	581	907	739	749	95.2	0.001	0.698
Cecum	44.6	75.5	58.7	61.3	10.5	0.001	0.356
At 21 d of age							
Absolute length							
SI	145	94.0	119	120	8.21	0.001	0.647
Cecum	14.1	9.43	11.7	11.8	1.35	0.001	0.889
Relative length							
SI	186	419	303	307	29.4	0.001	0.166
Cecum	18.0	41.6	29.6	30.0	4.91	0.001	0.715

[1]The average BW of the birds used is shown in Table 6.
[2]Small intestine (duodenum, jejunum, and ileum).
[3]Length of the 2 cecum.
[4]Residual standard deviation (n = 7 for each treatment and n = 14 for main effects). The experimental unit was formed by 2 and 4 birds per replicate at 9 and 21 d of age, respectively.
[5]The interactions between chicken line and SFH inclusion were not significant ($P > 0.05$).

of rate of feed passage on nutrient digestibility was expected in the current research, because the diets were fed as mash, and mash diets have a slower rate of passage through the upper part of the GIT than pelleted diets (Serrano et al., 2013; Svihus, 2014). The lower N retention observed in pullets was expected because pullets require less protein and have lower capacity for N retention than broilers. In addition, the diet used (prestarter broiler feed) contained more CP than a regular commercial pullet diet, increasing further N excretion. The data suggest that the greater enzyme production and better development of the ileum villus surface of modern broiler strains overcome the problems related to their faster rate of passage, resulting in a net increase in nutrient retention.

Excreta moisture was higher in pullets than in broilers at 9 d of age but an opposite effect was observed at 21 d, although, the differences were of small magnitude. An excess of dietary N increases uric production and eventually, water intake. Consequently, moisture content of the excreta will increase, with effects being more pronounced in pullets than in broilers, consistent with the results reported for 9-day-old chicks. Other mechanisms, however, might regulate and affect water metabolism and excretion in older birds. In this respect, van der Hoeven-Hangoor et al. (2014) reported that the moisture content of the excreta increased as feed intake increased and Shires et al. (1987) observed that broilers had a faster rate of feed passage and greater feed intake than pullets. Consequently, the moisture content of the

Table 9. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet on the pH of the organs of the gastrointestinal tract of the chicks.[1]

	Line		SFH			P-value[3]	
	Broiler	Pullet	0%	3%	RSD[2]	Line	SFH
At 9 d of age							
Crop	4.32	4.35	4.32	4.35	0.086	0.267	0.465
Proventriculus	2.43	2.41	2.43	2.42	0.077	0.454	0.645
Gizzard	2.70	2.40	2.58	2.52	0.047	0.001	0.001
Duodenum	5.76	5.79	5.77	5.79	0.059	0.128	0.271
Jejunum	5.68	5.72	5.68	5.71	0.068	0.161	0.276
Ileum	6.37	6.38	6.37	6.39	0.074	0.980	0.352
Cecum	5.93	5.88	5.94	5.87	0.080	0.133	0.018
At 21 d of age							
Crop	4.52	4.49	4.49	4.52	0.051	0.108	0.141
Proventriculus	2.46	2.47	2.47	2.46	0.050	0.418	0.340
Gizzard	2.57	2.37	2.55	2.37	0.075	0.001	0.001
Duodenum	6.07	6.06	6.05	6.09	0.063	0.635	0.198
Jejunum	5.90	5.95	5.91	5.94	0.067	0.111	0.365
Ileum	6.65	6.69	6.67	6.68	0.071	0.161	0.711
Cecum	6.01	5.96	6.05	5.93	0.091	0.148	0.002

[1]The pH of the drinking water was 6.88.
[2]Residual standard deviation (n = 7 for each treatment and n = 14 for main effects). The experimental unit was formed by 2 birds per replicate at 9 and 21 d of age.
[3]The interactions between chicken line and SFH inclusion were not significant ($P > 0.05$).

excreta should be higher in broilers than in pullets, in agreement with the results reported herein for chicks at 21 d of age.

Effects of Sunflower Hulls Inclusion. The dilution of the diet with 3% SFH did not affect chick performance at any age. The authors have not found any research comparing the effects of dietary SFH on growth performance of these 2 chicken lines. In pullets, Guzmán et al. (2015a) observed a 6.4% increase in ADG from zero to 21 d of age with the inclusion of 2% SFH in the diet. Similar results were reported by González-Alvarado et al. (2007) and Jiménez-Moreno et al. (2011, 2013a, 2016) in broilers. In contrast, Walugembe et al. (2014) observed that the inclusion of 16% of a combination of dried distiller's grains with solubles (**DDGS**) and wheat bran, in isocaloric diets based on corn and soybean meal, reduced ADG and voluntary FI in broilers but not in pullets. Probably, the high fiber content (15% NDF) of the diet used by Walugembe et al. (2014) caused the 9.3% reduction in feed intake in broilers. However, no effects of fiber on feed intake in any of the 2 chicken lines were observed in the current research in which the NDF content of the SFH diet was 10.3%.

Sunflower hulls did not affect moisture content of the excreta at any age but, visually, the appearance and consistency of the excreta improved when SFH was included in the diet (data not shown). These results agree with data of Jiménez-Moreno et al. (2016) in broilers fed diets that contained 2.5 or 5.0% SFH. Moreover, van der Hoeven-Hangoor et al. (2014) reported drier excreta when the control diet was supplemented with 2.5% OH.

In general, SFH inclusion improved nutrient retention although the differences were not significant. In fact, the AME_n of the diet increased significantly with SFH inclusion at both ages. Jiménez-Moreno et al. (2013a) and González-Alvarado et al. (2010) in broilers reported also a 2.1% and 4.4% increase in the energy content of the diet with the inclusion of 2.5 and 3.0% OH, respectively. In this respect, Hetland et al. (2003) showed that OH inclusion increased amylase activity and bile acid concentration in the jejunum of laying hens fed a corn-soybean meal diet. Moreover, Yokhana et al. (2016) showed an increase in proteolytic enzyme activity in the pancreas of 13-week-old pullets fed a diet supplemented with 1% of a fiber source with 65% crude fiber and 20% lignin (Arbocel; JRS Co. Inc., Rosenberg, Germany). Also, Kalmendal et al. (2011) reported higher ether extract digestibility when 20% sunflower meal was included in the diet, although in this research, DM digestibility and AME_n of the diet decreased. In the current experiment, the control and experimental diets contained 3.0 and 4.4% crude fiber whereas in the research of Kalmendal et al. (2011) the values were 2.6 and 8.0%, respectively. Jiménez-Moreno et al. (2013a) reported that the inclusion of up to 5% OH in a low fiber diet increased nutrient retention in broilers but that a further increase to 7.5% tended to reduce it.

Table 10. Influence of chicken line (Line) and the inclusion of sunflower hulls (SFH) in the diet short chain fatty acids (SCFA) concentration (μmol/g fresh digesta) and proportion fatty acids proportion (% SCFA) in the cecum at 21 d of age.

	Line		SFH			P-value[2]	
	Broiler	Pullet	0%	3%	RSD[1]	Line	SFH
SCFA (μmol/g)	83.03	74.32	78.17	79.18	13.46	0.098	0.845
Fatty acid (% of total SCFA)							
Acetate	78.2	75.9	76.2	77.9	3.33	0.077	0.215
Propionate	4.95	5.66	4.89	5.72	1.87	0.322	0.254
Butyrate	14.81	15.46	16.07	14.20	3.40	0.619	0.158
Isobutyrate	0.397	0.391	0.368	0.420	0.357	0.964	0.704
Isovalerate	0.717	1.438	1.388	0.767	1.417	0.175	0.347
Valerate	0.926	1.151	1.084	0.993	0.291	0.052	0.413

[1]Residual standard deviation (n = 7 for each treatment and n = 14 for main effects). The experimental unit was formed by 2 birds per replicate.
[2]The interactions between chicken line and SFH inclusion were not significant ($P > 0.05$).

Table 11. Influence of chicken line (Line) and inclusion of 3% sunflower hulls (SFH) in the diet on ileal mucosa morphology at 21 d of age.

	Line		SFH			P-value[3]	
	Broiler	Pullet	0%	3%	RSD[1]	Line	SFH
Villus height (μm)	1,075	931	1,001	999	82.2	0.001	0.775
Crypt depth (μm)	132	122	127	127	9.04	0.012	0.791
VH:CD[2]	8.14	7.64	7.86	7.90	0.508	0.030	0.942

[1]Residual standard deviation (n = 7 for each treatment and n = 14 for main effects). The experimental unit was formed by 2 birds per replicate.
[2]Villus height (VH) to crypt depth (CD) ratio (μm/μm).
[3]The interactions between chicken line and SFH inclusion were not significant ($P > 0.05$).

Gastrointestinal Tract Traits

Effects of Chicken Line. In absolute terms, the GIT was heavier and the SI and cecum were longer in broilers than in pullets, in agreement with most published reports (Nir et al., 1993; Mahagna and Nir, 1996; Zavarize et al., 2012). In relative terms, however, an opposite effect was observed in most cases. In fact, at 21 d of age, the relative weight of all the organs of the GIT, and the relative length of the small intestine and cecum, were significantly greater in pullets. Similar data have been reported by Mahagna and Nir (1996) and Zavarize et al. (2012) comparing broilers and pullets at young ages. The data suggest that when comparing chicken lines with marked differences in rate of growth, the interest of comparing in relative terms the weight and length of the GIT organs should be taken cautiously (Buzala et al., 2015).

Chicken line did not affect the pH of the different segments of the GIT, except for gizzard pH that was reduced at both ages. Shires et al. (1987) observed that the retention time of the digesta in the upper part (proventriculus and gizzard) of the GIT was longer in 86-day-old pullets than in 44-day-old broilers (86 vs. 54 min). A longer retention time of the digesta in the gizzard increases HCl concentration and reduces pH (Jiménez-Moreno et al., 2009a), consistent with the results reported herein. In addition, the digesta content was proportionally lower in pullets than in broilers, which results in a higher proportion of HCl per gram of digesta and, consequently, in a reduction in pH.

Effects of Sunflower Hulls Inclusion. Fiber inclusion had little effect on the development of the GIT in both chicken lines, except for the gizzard that was heavier and had higher fresh digesta content and lower pH with additional SFH. In pullets, Guzmán et al. (2015b) reported also a 8.7% increase in gizzard weight at 35 d of age with the inclusion of 4% straw in the diet. Moreover, González-Alvarado et al. (2008) observed that the inclusion of 3% OH increased gizzard weight by 32% in 22-day-old broilers, in agreement with the data reported herein.

Most available data in broilers (González-Alvarado et al., 2007; Jiménez-Moreno et al., 2009b; 2013b; Sacranie et al., 2012) and pullets (Guzmán et al., 2015b) observed a reduction in gizzard pH with the inclusion of insoluble fiber sources in the diet. Highly lignified insoluble fiber sources, such as OH and SFH are retained longer in the gizzard, resulting in an increase in HCl production and, consequently, in a reduction in gizzard pH (Mateos et al. 2012).

Short Chain Fatty Acid in the Cecum and Ileal Morphology

Effects of Chicken Line. Substrates entering the cecum are a mixture of digesta fractions that escaped from digestion in the upper part of the GIT, with SCFA being the major end products. In the current research, the concentration of total SCFA was higher in broilers than in pullets. Walugembe et al. (2015) suggested that the higher feed intake in broilers as compared with pullets provided more nutrients to the microorganisms of the cecum, resulting in higher SCFA production. In this respect, the cecum was longer in broilers than in pullets, which may be indicative of a higher potential for fermentation of the digesta.

The proportion of valerate and isovalerate in the cecum was higher in pullets than in broilers. Most SCFA produced in the gut results from the fermentation of complex carbohydrates, but proteins and peptides are also net contributors. In fact, these SCFA originate primarily from the oxidative deamination and decarboxylation of the branched chain amino acids (Allison, 1978; Andries et al., 1987). The higher proportion of these two FA reported for pullets was consistent with the excess of protein of the diets as compared with their requirements, with more N compounds available for fermentation in the cecum.

The VH and CD of the ileum mucosa were higher in broilers than in pullets, in agreement with data of Uni et al. (1995) and consistent with the improved nutrient digestibility observed for broilers. Zavarize et al. (2012) reported also higher VH and CD in broilers than in pullets at 7 d of age, although in this research the difference in CD was not significant.

Effects of Sunflower Hulls Inclusion. The inclusion of SFH in the diet did not affect the concentration of total SCFA in the cecum, in agreement with the results of Walugembe et al. (2015). Sunflower hulls did not affect the structure of the ileal mucosa. In contrast, Kalmendal et al. (2011) reported a linear decrease in VH as the level of sunflower meal increased from zero to 30%. In the research of Kalmendal et al. (2011), the crude fiber content of the diet increased from 2.3 to 11.0% with sunflower meal inclusion, whereas in the current research, the increase was from 3.0 to 4.4%. In this respect, Jiménez-Moreno et al. (2011) reported that the inclusion of 2.5% pea hulls in the diet tended to increase VH in the jejunum mucosa but that a further increase to 7.5% had an opposite effect.

In summary, broilers grew faster and had greater nutrient digestibility and ileum absorption capacity than pullets. The inclusion of 3% SFH at the expense of the whole diet (wt:wt) reduced the pH of the gizzard and improved the AME_n content of the diet, without showing any negative effect on chick performance. Broilers and pullets responded in a similar way to an increase in the insoluble fiber content of the diet. The lack of interactions between chicken lines and SFH inclusion for most of the variables studied suggests that the information available on the inclusion of insoluble fiber sources in broiler diets might be used, to some extent, in research conducted with pullets.

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