A comparative study on the effects of dietary sunflower hulls on growth performance and digestive tract traits of broilers and pullets fed a pullet diet from 0 to 21 days of age

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ABSTRACT

The effects of the inclusion of sunflower hulls (SFH) in the diet on growth performance, coefficient of total tract apparent retention (CITTAR) of nutrients, gastrointestinal tract (GIT) traits and short chain fatty acid (SCFA) concentration in the cecum were studied in chicks from 0 to 21 d of age. Treatments consisted in the combination of two chicken lines (female broilers vs. pullets) and two levels of SFH inclusion (0 vs. 30 g/kg). The control diet contained 11.94 MJ AME_n, 191 g crude protein, 9.8 g digestible Lys and 101 g neutral detergent fiber/kg. The experimental diet included 30 g SFH/kg at the expense (wt:wt) of the control diet. Growth performance was better ($P < 0.001$) in broilers than in pullets at all ages. Moisture content of the excreta was lower ($P < 0.001$) in pullets than in broilers at 21 d of age but an opposite effect was observed at 9 d. At 9 d of age, nutrient retention ($P < 0.001$) and AME_n of the diet ($P < 0.01$) were higher in broilers than in pullets but at 21 d of age the only difference detected was for N retention ($P < 0.001$). In absolute terms (g or cm) broilers had heavier ($P < 0.001$) digestive organs and longer small intestine (SI) and cecum than pullets. In relative terms, however, the GIT ($P = 0.062$), proventriculus ($P < 0.001$), gizzard ($P < 0.001$) and pancreas ($P < 0.01$) were heavier at 21 d, and the SI and the cecum were longer ($P < 0.001$) at both ages in pullets. SFH inclusion did not affect growth performance or organ weights at any age but increased the absolute length of the SI and cecum and the AME_n of the diet at 21 d of age ($P < 0.05$). At 21 d of age, fiber inclusion reduced moisture content of the excreta in broilers but no effects were observed in pullets ($P < 0.05$ for interaction). The pH of the GIT and the concentration of SCFA in the cecum were not affected by chicken line or SFH inclusion. Villus height and crypt depth were higher ($P < 0.001$) in broilers than in pullets but were not affected by SFH inclusion. In summary, broilers showed greater growth performance and nutrient retention at 9 d of age and better ileum absorptive capacity at 21 d than pullets. The dilution of the diet with 30 g SFH/kg did not affect chick performance and in fact, improved the AME_n of the diet in both chicken lines at 21 d of age.

Abbreviations: ADFI, average daily feed intake; AME_n, apparent metabolisable energy nitrogen corrected; BW, body weight; CD, crypt depth; CP, crude protein; CITTAR, coefficient of total tract apparent retention; DDGS, distiller's dried grains with solubles; DM, dry matter; ECR, energy conversion ratio; EI, energy intake; F:G, feed to gain ratio; GE, gross energy; GIT, gastrointestinal tract; N, nitrogen; NDF, neutral detergent fiber; OH, oat hulls; OM, organic matter; SCFA, short chain fatty acid; SI, small intestine; SFH, sunflower hulls; VH, villus height

1. Introduction

Current genetic selection programs for poultry aim to improve growth rate, feed to gain ratio (F:G) and meat yield in broilers and to maximize future egg production in pullets. Consequently, the two chicken lines differ not only in voluntary feed intake and body weight (BW) gain but also in basal metabolism (Kuenzel and Kuenzel, 1977), protein turnover rate (Jones et al., 1986; Saunderson and Leslie, 1988) and organ development (Lilburn and Loeffler, 2015; Kimiaetalab et al., 2017).

Under commercial conditions, the inclusion of fibrous ingredients is limited in diets for young birds because of its negative impact on feed intake and nutrient utilization (Sklan et al., 2003; Rougiere and Carre, 2010). Recent researches, however, have shown that moderate amounts of certain insoluble fiber sources, such as oat hulls (OH) and sunflower hulls (SFH), stimulates the development and health of the gastrointestinal tract (GIT) in broilers (González-Alvarado et al., 2008; Svihus, 2011; Sacranie et al., 2012). However, few researches are available on the effects of fiber on growth performance, GIT traits and nutrient digestibility in pullets. Kimiaetalab et al. (2017) reported that SFH inclusion had similar effects on growth, nutrient retention and villus development in both chicken lines. Walugembe et al. (2014), however, observed that the inclusion of 160 g/kg of a mixture of a wheat distiller's dried grains with solubles (DDGS) and wheat bran, reduced feed intake and BW gain in broilers but had no effects in pullets. The reasons for these discrepancies, however, are not well understood.

In commercial practice, pullet diets contain less energy and crude protein (CP) and more neutral detergent fiber (NDF) than broiler diets. Moreover, because of their lower energy requirements, pullets are fed often diets based on wheat and barley with limited use of maize. As a consequence, differences in chick growth and in the characteristics and ingredient composition of the diets, the two chicken lines might respond in different ways to additional fiber. The aim of this experiment was to compare the effects of the inclusion of moderate amount of an insoluble fiber source in the diet, on growth performance, nutrient retention and GIT development of broilers and pullets fed a pullet diet in mash form from 0 to 21 d of age. A second objective was to compare the data of the current research, using a pullet diet based on wheat and barley, with data of a previous research (Kimiaetalab et al., 2017) in which both chicken lines were fed a broiler diet based on maize.

2. Materials and methods

2.1. Sunflower hulls and diets

The physico-chemical characteristics of the batch of the SFH were reported by Kimiaetalab et al. (2017). Briefly, SFH contained 47 g CP and 700 g NDF/kg and had a geometric mean diameter $\pm$ geometric standard deviation of $675 \pm 1.68 \mu\text{m}$.

The control diet used in this experiment contained 11.94 MJ AME_n, 191 g crude protein, 9.8 g digestible Lys and 101 g NDF per kilogram, and included 20 g/kg of celite, an acid-washed diatomaceous earth (Celite Hispanica S.A., Alicante, Spain), to increase the acid insoluble ash content of feeds and excreta. The fiber containing diet was similar to the control diet but was diluted (wt:wt) with 30 g SFH/kg. Consequently, the experimental diet had less AME_n and CP and more NDF than the control diet (Table 1). Both diets, however, met or exceeded the nutritional requirements of pullets (FEDNA (Fundación Española Desarrollo Nutrición Animal), 2008).

2.2. Husbandry and experimental design

The husbandry and care of the chicks were similar to those reported by Kimiaetalab et al. (2017). Briefly, 280 one day-old chicks, 140 Ross 308 female broilers and 140 Lohmann Brown Classic pullets were used. The birds were housed at random in groups of 10 according to chicken line, in 28 battery cages. The experiment was conducted as a completely randomized design with 4 treatments arranged as a 2×2 factorial with two chicken lines (female broilers vs. pullets) and two levels of dietary SFH (0 vs. 30 g/kg). Each treatment was replicated seven times and the experimental unit was a cage with 10 birds.

2.3. Growth performance and coefficients of total tract apparent retention of nutrients

Average daily feed intake (ADFI), BW gain and feed to gain ratio (F:G) were determined by period (0–9 d and 10–21 d) and for the entire experiment (0–21 d). The coefficient of total tract apparent retention (CTTAR) of dry matter (DM), organic matter (OM), nitrogen (N) and gross energy (GE) of the diets were determined at the end of the two experimental periods. 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 (Association of Official Analytical Chemist Internatinal) (2000). Gross energy was measured using an isoperibol bomb calorimeter (model 1356, Parr Instrument Company, Moline, IL). Energy intake (EI; KJ AME_n/day) and energy conversion ratio (ECR; KJ AME_n ingested/g BW gain) were estimated, using the average AME_n content of the diets determined at 9 and 21 d of age 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 3 d. 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.

2.4. Gastrointestinal tract traits

Details on the procedures used for measuring the GIT traits were presented by Kimiaetalab et al. (2017). Briefly, two birds per

Table 1

Ingredient composition and calculated and determined chemical analysis of the experimental diets (g/kg as-fed basis).

	Sunflower hulls (g/kg)	
	0	30
Ingredient		
Wheat	440	427
Barley	201	195
Soybean meal, 470 g CP/kg	276	268
Lard	28.3	27.5
Sunflower hulls	0.0	30.0
Calcium carbonate	9.8	9.5
Dicalcium phosphate	15.2	14.7
Sodium chloride	3.4	3.3
<i>L</i> -Lysine HCL, 780 g/kg	1.0	1.0
Methionine-OH, 880 g/kg	2.3	2.2
Celite ^a	20.0	19.4
Vitamin and mineral premix ^b	3.0	2.9
Calculated analyses ^c		
AME _n (MJ/kg)	11.94	11.62
Crude fiber	32	46
Calcium	10	9.7
Available phosphorous	4.4	4.3
Digestible amino acids		
Arginine	12.1	11.7
Lysine	9.8	9.5
Methionine	4.9	4.8
Methionine + Cysteine	8.0	7.8
Threonine	6.3	6.1
Tryptophan	2.2	2.1
Determined analyses		
Dry matter	919	920
Gross energy, kcal/kg	3,941	3,872
Total ash	57	59
Crude protein	191	187
Ether extract	39	41
Neutral detergent fiber	101	121
Acid detergent fiber	51	69
Acid detergent lignin	14	16
Dietary fiber	163	193
Particle size distribution (µm)		
> 2500	41	33
1250	376	290
630	348	393
315	148	173
160	86	111
< 80	1	2
GMD ± GSD ^d (µm)	962 ± 2.01	867 ± 2.01

^a Acid washed diatomaceous earth (Celite Hispánica, S.A., Alicante, Spain).

^b Provided the following per kilogram of diet: vitamin A (trans-retinyl acetate), 11,500 IU; vitamin D3 (cholecalciferol), 2800 IU; vitamin E (all-rac-tocopherol-acetate), 40 IU; vitamin B₁, 2 mg; vitamin B₂, 8 mg; vitamin B₆, 4 mg; vitamin B₁₂ (cyanocobalamin), 0.025 mg; vitamin K₃ (bisulphate menadione complex), 3 mg; choline (choline chloride), 300 mg; nicotinic acid, 60 mg; pantothenic acid (D-calcium pantothenate), 15 mg; folic acid, 1.5 mg; D-biotin, 150 µg; zinc (ZnO), 80 mg; manganese (MnO), 70 mg; iron (FeCO₃), 60 mg; copper (CuSO₄ · 5H₂O), 8 mg; iodine (KI), 2 mg; selenium (Na₂SeO₃), 0.2 mg; Roxazyme, 200 mg [1600 U endo-1,4-β-glucanase (EC 3.2.1.4), 3600 U endo-1,3 (4)-β-glucanase (EC 3.2.1.6) and 5200 U endo-1,4-β-xylanase (EC 3.2.1.8)] supplied by DSM, S.A., Madrid, Spain; and Natuphos 5000, 60 mg [300 Phytase units (FTU)/kg] supplied by BASF Española S.A., Tarragona, Spain.

^c According to FEDNA (Fundación Española Desarrollo Nutrición Animal) (2010).^d Geometric mean diameter ± log geometric standard deviation.

replicate were euthanized by CO₂ asphyxiation after each growth performance control. The GIT was excised and divided into seven portions (crop, proventriculus, gizzard, duodenum, jejunum, ileum, and cecum) and the pH of all these segments was measured *in situ* as indicated by nez-Moreno et al. (2009a,b); . In addition, two extra birds at 9 d and four extra birds at 21 d of age were slaughtered, and the full GIT, from the post-crop to the cloaca, was excised and weighed. The weight of the annex organs (liver, spleen and pancreas) were not included in the determination of the weight of the full GIT. The weights of the full proventriculus, full gizzard, liver and pancreas were measured also in these birds. In addition, the weight of the empty gizzard and its contents, and the DM of the gizzard digesta were determined. Because of the extreme differences in BW and ADFI between broilers and pullets, data on

organ weight were expressed in absolute (g) and relative (g/kg BW) terms. The length of the small intestine (SI; duodenum, jejunum and ileum) and of the two cecum was measured also in all these birds and the results expressed in absolute (cm) and relative (cm/kg BW) terms. The concentration SCFA in cecum samples ($\mu\text{mol/g}$ fresh digesta) was measured at 21 d of age in two of the birds used for pH determination, as indicated by [Kimiaceitalab et al. \(2017\)](#). Briefly, 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 (13,000g) 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 . Also, 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 ileum tissue were measured at 21 d of age in two birds used for pH determination as described by [Jiménez-Moreno et al. \(2011\)](#). Two representative cross sections from each bird were viewed at $40\times$ 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 were used for further analysis.

2.5. Statistical analysis

Data were analyzed as a completely randomized design with four treatments arranged as a 2×2 factorial using the GLM program of [SAS Institute \(1990\)](#). The model included the chicken line (female broilers vs. pullets) and SFH inclusion (0 vs. 30 g/kg) as main effects as well as their interactions. The experimental unit was the cage for all traits. Differences between treatment means were considered significant at $P < 0.05$ and tendencies at $0.05 \leq P \leq 0.10$.

3. Results

The determined analyses of the experimental diets were close to expected values, confirming that the feeds were manufactured correctly. The mean particle size of the control and the SFH containing diet, expressed as the geometric mean diameter $\pm$ geometric standard deviation was 962 ± 2.01 and $867 \pm 2.01 \mu\text{m}$, respectively.

3.1. Growth performance and coefficient of total tract apparent retention of nutrients

Mortality was low (1.0%) and not related to any of the treatments (data not shown). The only interaction detected between chicken line and inclusion of SFH in the diet was for moisture content of the excreta at 21 d of age ([Fig. 1](#)).

3.1.1. Effects of chicken line

Feed intake and BW gain were greater ($P < 0.001$) and F:G was better ($P < 0.001$) in broilers than in pullets at all ages ([Table 2](#)). From 0 to 21 d of age, ECR was better ($P < 0.001$) for broilers than for pullets ([Table 3](#)).

Moisture content of the excreta was higher ($P < 0.001$) in broilers than in pullets at 9 d of age but no differences were detected at 21 d of age ([Table 4](#)). The CTTAR of all nutrients increased with age and were higher ($P < 0.01$ – $P < 0.001$) for broilers than for pullets at 9 d of age. At 21 d of age, however, the only difference detected was for N that was higher ($P < 0.001$) in broilers ([Table 5](#)).

3.1.2. Effects of sunflower hulls inclusion

The dilution of the diet with 30 g SFH/kg did not affect chick performance or CTTAR of nutrients at any age. The AME_n of the diet increased ($P < 0.05$) with SFH inclusion at 21 d of age but no differences were detected at 9 d ([Table 5](#)). Sunflower hulls did not affect moisture content of the excreta at 9 d of age. At 21 d of age, however, SFH inclusion reduced the moisture content of the excreta in broilers but had no effects in pullets ($P < 0.05$ for the interaction) ([Fig. 1](#)).

Fig. 1. Interaction between chicken line and SFH inclusion in the diet on moisture content of the excreta at 21 d of age.

Table 2

Influence of the chicken line and the inclusion of sunflower hulls (SFH) in the diet on growth performance from 0 to 21 days of age.

Age	Line		SFH (g/kg)		SEM ^a	P-value ^b	
	Broiler	Pullet	0	30		Line	SFH
0–9 d							
BWG ^c	15.7	4.5	10.1	10.0	0.08	0.001	0.479
ADFI ^d	21.5	10.2	15.8	15.9	0.07	0.001	0.270
F:G ^e	1.371	2.287	1.819	1.839	0.0146	0.001	0.360
10–21 d							
BWG	30.7	7.7	19.4	19.1	0.33	0.001	0.422
ADFI	52.3	17.9	35.2	35.0	0.49	0.001	0.675
F:G	1.704	2.307	2.000	2.012	0.0133	0.001	0.487
0–21 d							
BWG	24.3	6.3	15.4	15.2	0.20	0.001	0.366
ADFI	39.1	14.5	26.9	26.8	0.28	0.001	0.788
F:G	1.611	2.301	1.949	1.963	0.0116	0.001	0.379

^a Standard error of means (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. The initial body weight of the chicks was 46.7, 37.5, 42.1 and 42.1 for broiler, pullet, 0% SFH and 3% SFH, respectively.

^b The interactions between chicken line and SFH inclusion were not significant (P > 0.05) for all variables.

^c BW gain per day (g/d).

^d Average daily feed intake (g).

^e Feed to gain ratio.

Table 3Influence of the chicken line and the inclusion of sunflower hulls (SFH) in the diet on calculated and determined energy intake (EI, KJ AME_n/d) and energy conversion ratio (ECR, KJ AME_n ingested/g BW gain) from 0 to 21 days of age.

	Line		SFH (g/kg)		SEM ^a	P-value ^b	
	Broiler	Pullet	0	30		Line	SFH
Calculated							
EI	461	171	321	311	3.3	0.001	0.044
ECR	19.0	27.1	23.3	22.8	0.14	0.001	0.031
Determined ^c							
EI	457	165	311	311	3.3	0.001	0.848
ECR	18.8	26.1	22.3	22.6	0.13	0.001	0.130

^a Standard error of means (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.

^b The interactions between chicken line and SFH inclusion were not significant for all variables (P > 0.05).

^c The AME_n used for estimation was the average of values determined at 9 and 21 d of age from 10 to 21 d of age (See Table 5).

Table 4

Influence of chicken line and the inclusion of sunflower hulls (SFH) in the diet on moisture content (g/kg) of the excreta.

Age	Line		SFH (g/kg)		SEM ^a	P-value ^b	
	Broiler	Pullet	0	30		Line	SFH
9 d	798	822	811	809	4.3	0.001	0.687
21 d	746	730	741	735	2.5	0.001	0.104

^a Standard error of means (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.

^b At 21 d of age, a significant (P = 0.034) interaction between chicken line and SFH inclusion was detected (Fig. 1).

3.2. Gastrointestinal tract traits

3.2.1. 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). In relative terms, however, an opposite effect was observed in some of the organs. In this respect, at 9 d of age the relative weights of the full proventriculus (P = 0.06) and full and empty gizzard (P < 0.001) were lower in broilers than in pullets, but an opposite effect was observed for the full GIT (P < 0.001), pancreas (P < 0.05) and liver (P < 0.05). At 21 d of age, pullets had heavier (g/kg BW) full GIT (P = 0.062), full proventriculus (P < 0.001), full and empty gizzard (P < 0.001) and pancreas (P < 0.01) than broilers (Tables 6 and 7). In absolute and relative terms, the fresh digesta content of the gizzard was greater (P < 0.001) in broilers at both

Table 5

Influence of chicken line and the inclusion of sunflower hulls (SFH) in the diet on the coefficient of total tract apparent retention (CTTAR) of the nutrients and AME_n content of the diets.

	Line		SFH (g/kg)			P-value ^b	
CTTAR	Broiler	Pullet	0	30	SEM ^a	Line	SFH
At 9 d of age							
Dry matter	0.695	0.645	0.673	0.666	0.0071	0.001	0.484
Organic matter	0.717	0.665	0.694	0.687	0.0067	0.001	0.465
Nitrogen	0.569	0.472	0.510	0.531	0.0109	0.001	0.196
Gross energy	0.716	0.680	0.700	0.695	0.0072	0.002	0.612
AME _n (MJ/kg)	11.44	10.92	11.16	11.20	0.112	0.003	0.800
At 21 d of age							
Dry matter	0.723	0.721	0.721	0.724	0.0039	0.734	0.658
Organic matter	0.748	0.746	0.748	0.747	0.0044	0.761	0.866
Nitrogen	0.615	0.545	0.576	0.585	0.0094	0.001	0.509
Gross energy	0.755	0.756	0.756	0.756	0.0034	0.883	0.906
AME _n (MJ/kg)	12.07	12.13	12.02	12.18	0.052	0.424	0.044

^a Standard error of means (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.

^b The interactions between chicken line and SFH inclusion were not significant (P > 0.05).

Table 6

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

	Line		SFH (g/kg)			P-value ^b	
	Broiler	Pullet	0	30	SEM ^a	Line	SFH
At 9 d							
BW ^c (g)	158	71	113	116	1.8	0.001	0.263
Absolute weight (g)							
GIT ^d	37.5	15.1	25.9	26.7	0.59	0.001	0.330
Proventriculus ^e	1.69	0.84	1.29	1.24	0.033	0.001	0.254
Gizzard ^f	11.1	5.71	8.17	8.63	0.195	0.001	0.111
Pancreas	0.87	0.35	0.63	0.59	0.029	0.001	0.319
Liver	7.28	3.10	5.15	5.23	0.120	0.001	0.654
Relative weight (g/kg BW)							
GIT	239	212	226	226	4.2	0.001	0.941
Proventriculus	10.9	11.8	11.6	11.0	0.32	0.060	0.209
Gizzard	70.5	80.0	74.6	75.9	1.681	0.001	0.579
Pancreas	5.60	4.84	5.42	5.02	0.243	0.037	0.262
Liver	46.0	43.5	45.2	44.3	0.65	0.014	0.304
At 21 d							
BW ^g (g)	615	181	388	408	10.2	0.001	0.180
Absolute weight (g)							
GIT	103	32.0	67.3	68.1	1.13	0.001	0.606
Proventriculus	3.99	1.51	2.69	2.82	0.080	0.001	0.260
Gizzard	23.2	9.71	16.3	16.7	0.49	0.001	0.567
Pancreas	2.57	0.86	1.66	1.78	0.062	0.001	0.180
Liver	23.3	6.85	15.0	15.2	0.57	0.001	0.813
Relative weight (g/kg BW)							
GIT	171	179	176	174	2.7	0.062	0.610
Proventriculus	6.59	8.45	7.53	7.51	0.182	0.001	0.956
Gizzard	38.6	54.7	47.5	45.8	1.00	0.001	0.215
Pancreas	4.23	4.79	4.47	4.55	0.133	0.007	0.657
Liver	38.2	38.1	38.9	37.5	0.81	0.951	0.251

^a Standard error of means (n = 7 for each treatment and n = 14 for main effects). The experimental unit was formed by 2 or 4 birds per replicate at 9 and 21 d of age, respectively.

^b The interactions between chicken line and SFH inclusion were not significant (P > 0.05).

^c Average of 2 birds chosen at random per replicate.

^d Full gastrointestinal tract without the annex organs (pancreas, spleen, and the liver).

^e Full proventriculus.

^f Full gizzard.

^g Average of 4 birds chosen at random per replicate.

Table 7

Influence of chicken line and the inclusion of sunflower hulls (SFH) in the diet on the absolute (g) and relative (g/kg BW) empty gizzard weight and gizzard digesta characteristics.^a

	Line		SFH (g/kg)			P-value ^c	
	Broiler	Pullet	0	30	SEM ^b	Line	SFH
At 9 d							
Absolute weight	7.03	3.82	5.33	5.53	0.13	0.001	0.278
Relative weight	44.6	53.7	49.0	49.3	1.08	0.001	0.839
Fresh digesta content (g)	4.04	1.87	2.87	3.04	0.075	0.001	0.119
Absolute empty:full gizzard	0.63	0.67	0.66	0.65	0.006	0.001	0.278
Digesta dry matter (g/kg)	363	362	360	366	5.6	0.925	0.484
At 21 d							
Absolute weight	14.7	6.80	10.6	11.0	0.31	0.001	0.374
Relative weight	24.6	38.4	31.9	31.0	0.70	0.001	0.369
Fresh digesta content (g)	8.51	2.91	5.70	5.72	0.215	0.001	0.944
Absolute empty:full gizzard	0.64	0.70	0.67	0.63	0.006	0.001	0.453
Digesta dry matter (g/kg)	373	369	370	373	4.1	0.558	0.709

^a The average BW of the chicks are shown in Table 6.

^b Standard error of means (n = 7 for each treatment and n = 14 for main effects). The experimental unit was formed by 2 or 4 birds per replicate at 9 and 21 d of age, respectively.

^c The interactions between chicken line and SFH inclusion were not significant (P < 0.05).

ages but the DM content of the digesta was not affected by chicken line (Table 7).

In absolute terms, the SI and cecum were longer (P < 0.001) in broilers than in pullets but in relative terms an opposite effect (P < 0.001) was observed (Table 8). The pH of the different organs of the GIT were not affected by chicken line (Table 9).

3.2.2. Effects of sunflower hulls inclusion

Sunflower hulls inclusion did not affect the characteristics of the gizzard or the weight (g or g/kg BW) and the pH of the organs of the GIT at any age (Tables 6, 7 and 9). Sunflower hulls increased the absolute length of the GIT, with differences that were significant for the SI (P < 0.05) at 21 d and for the cecum (P ≤ 0.01) at both ages. In relative terms, an opposite effect was observed for the cecum (P < 0.05) at 9 d of age (Table 8).

Table 8

Influence of chicken 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.^a

	Line		SFH (g/kg)			P-value ^c	
Age	Broiler	Pullet	0	30	SEM ^b	Line	SFH
At 9 d							
Absolute length							
SI ^d	112	70.1	89.2	93.1	1.70	0.001	0.115
Cecum ^e	9.08	6.35	7.40	8.03	0.158	0.001	0.010
Relative length							
SI	727	991	854	863	18.3	0.001	0.737
Cecum	58.5	89.2	71.6	76.2	1.46	0.001	0.035
At 21 d							
Absolute length							
SI	150	99	118	132	3.9	0.001	0.022
Cecum	15.3	10.1	11.9	13.6	0.51	0.001	0.028
Relative length							
SI	252	561	390	423	17.6	0.001	0.209
Cecum	25.6	57.2	39.5	43.3	1.80	0.001	0.158

^a The average of the birds used is shown in Table 6.

^b Standard error of means (n = 7 for each treatment and n = 14 for main effects). The experimental unit was formed by 2 or 4 birds per replicate at 9 and 21 d of age, respectively.

^c The interactions between chicken line and SFH inclusion were not significant (P > 0.05).

^d Small intestine (duodenum, jejunum, and ileum).

^e Average length of the 2 cecum.

Table 9Influence of chicken line and the inclusion of sunflower hulls (SFH) in the diet on the pH of the organs of the gastrointestinal tract.^a

	Line		SFH (g/kg)			P-value ^c	
	Broiler	Pullet	0	30	SEM ^b	Line	SFH
At 9 d							
Crop	4.50	4.39	4.42	4.47	0.092	0.400	0.736
Proventriculus	2.82	2.98	2.91	2.89	0.182	0.556	0.920
Gizzard	2.62	2.64	2.67	2.59	0.079	0.895	0.441
Duodenum	5.77	5.83	5.78	5.82	0.064	0.513	0.719
Jejunum	5.56	5.67	5.60	5.63	0.049	0.111	0.628
Ileum	6.54	6.74	6.57	6.71	0.113	0.225	0.383
Cecum	5.84	5.75	5.77	5.81	0.098	0.524	0.763
At 21 d							
Crop	4.74	4.86	4.77	4.83	0.082	0.343	0.588
Proventriculus	2.80	2.94	2.90	2.84	0.115	0.398	0.690
Gizzard	2.28	2.21	2.26	2.23	0.042	0.235	0.540
Duodenum	5.90	5.89	5.87	5.91	0.029	0.769	0.381
Jejunum	5.66	5.71	5.67	5.69	0.032	0.230	0.685
Ileum	6.71	6.83	6.72	6.82	0.107	0.415	0.498
Cecum	5.84	5.81	5.79	5.86	0.079	0.753	0.563

^a The pH of the drinking water was 6.88.^b Standard error of means (n = 7 for each treatment and n = 14 for main effects). The experimental unit was formed by 2 birds at 9 and 21 d of age.^c The interactions between chicken line and SFH inclusion were not significant (P > 0.05).

3.3. Short chain fatty acid in the cecum and morphology of the ileum

3.3.1. Effects of chicken line

The concentration of SCFA in the cecum was 6.7 percent units higher in broilers than in pullets but the differences were not significant (Table 10). The proportions of the individual SCFA were not affected by chicken line, except for butyrate (P = 0.054) and isobutyrate (P = 0.074) that tended to be higher for broilers. Villus height and CD were greater (P < 0.001) in broilers than in pullets (Table 11).

3.3.2. Effects of sunflower hulls inclusion

SFH inclusion did not affect total SCFA concentration or the proportion of the individual SCFA in the cecum. Similarly, villus morphology of the ileum was not affected by fiber inclusion.

4. Discussion

4.1. Growth performance and coefficient of total tract apparent retention of nutrients

4.1.1. Effects of chicken line

Broilers grew 4 times faster, ate 3 times more feed and had 30% better F:G than pullets, consistent with the main objectives of the genetic selection programs of the two chicken lines. Broilers are more voracious than pullets, and at young ages, broilers secrete more pancreatic enzymes per gram of feed and have better development of the ileal mucosa than pullets, consistent with data of Uni et al.

Table 10

Influence of chicken line and the inclusion of sunflower hulls (SFH) in the diet on short chain fatty acid (SCFA) concentration (μmol/g fresh digesta) and profile (% total SCFA) in the cecum at 21 d of age.

	Line		SFH (g/kg)		SEM ^a	P-value ^b	
	Broiler	Pullet	0	30		Line	SFH
SCFA (μmol/g)	71.8	65.1	69.3	67.5	4.30	0.291	0.780
Percentage of each individual SCFA							
Acetate	73.6	76.0	75.2	74.5	1.04	0.127	0.616
Propionate	1.85	2.60	2.29	2.16	0.40	0.203	0.826
Butyrate	23.4	20.6	21.4	22.6	0.103	0.065	0.430
Isobutyrate	0.109	0.021	0.077	0.052	0.035	0.087	0.615
Isovalerate	0.621	0.480	0.690	0.410	0.177	0.599	0.274
Valerate	0.364	0.305	0.323	0.345	0.083	0.621	0.854

^a Standard error of means (n = 7 for each treatment and n = 14 for main effects). The experimental unit was formed by 2 birds per replicate.^b The interactions between chicken line and SFH inclusion were not significant (P > 0.05).

Table 11

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

	Line		SFH (g/kg)		SEM ^a	P-value ^b	
	Broiler	Pullet	0	30		Line	SFH
Villus height (µm)	1149	847	968	1028	40.2	0.001	0.307
Crypt depth (µm)	138	109	122	124	4.3	0.001	0.665
VH:CD ^c	8.38	7.88	8.00	8.26	0.339	0.310	0.596

^a Standard error of means (n = 7 for each treatment and n = 14 for main effects). The experimental unit was formed by 2 birds per replicate.^b The interactions between chicken line and SFH inclusion were not significant (P > 0.05).^c Villus height (VH) to crypt depth (CD) ratio.

(1995a,b) and Kimiaetalab et al. (2017).

The CTTAR of all nutrients increased with age in agreement with data of Gracia et al. (2009) for starch and NDF and of Jiménez-Moreno et al. (2009a) for all nutrients except for soluble ash and EE. At 9 d of age, nutrient retention and AME_n of the diets were greater in broilers than in pullets, in agreement with data of Kimiaetalab et al. (2017) comparing both chicken lines. At 21 d of age, however, the only difference detected was for N. Probably, pullets of this age had the ability to obtain more energy by fermentation of the NDF than broilers, reducing the differences in nutrient retention between the two chicken lines, as reported by Walugembe et al. (2015).

Moisture content of the excreta was higher in pullets than in broilers at 9 d of age but not at 21 d. At 9 d of age, the reduced N retention of the pullets increased the need of extra water to eliminate the excess of uric acid produced through the urine. Consequently, moisture content of the excreta was higher in pullets. At 21 d of age, the difference in N retention between the two chicken lines was reduced and in fact, no differences in CTTAR were observed for any of the other nutrients. Consequently, less effect of diet on moisture content of the excreta was expected. Moreover, an interaction between chicken line and SFH inclusion was detected at this age; SFH reduced moisture content of the excreta in broilers but not in pullets. The data suggest that the response to fiber inclusion, in respect to litter quality, was more beneficial in broilers than in pullets.

4.1.2. Effects of sunflower hulls inclusion

The dilution of the diet with 30 g SFH/kg did not reduce chick performance at any age, consistent with the results of Kimiaetalab et al. (2017). Jiménez-Moreno et al. (2016) in broilers and Guzmán et al. (2015) in pullets, reported also that the inclusion of 25 or 20 g SFH/kg diet did not affect BW of the chicks from 1 to 21 d of age. In contrast, Walugembe et al. (2014) observed that the inclusion of 160 g of a mixture of DDGS and wheat bran/kg diet reduced by 10.9% BW gain in broilers but had no effects in pullets. In the research of Walugembe et al. (2014), fiber inclusion increased the NDF content of the diet from 100 to 150 g/kg and reduced ADFI in broilers by 5.9%. In the current research, however, the inclusion of 30 g SFH/kg diet increased the NDF of the diet from 101 to 121 g/kg, an increase that did not affect ADFI or BW gain in any of the two chicken lines. Similar lack of negative effects of fiber inclusion on ADFI and growth of the birds has been reported by other authors (Sacranie et al., 2012; Jiménez-Moreno et al., 2013a; Guzmán et al., 2015). Probably, as suggested by Mateos et al. (2012), the response of young birds to additional insoluble dietary fiber depends on the ingredient composition of the control diet, with effects being more pronounced when this diet is low in fiber.

Moisture content of the excreta was reduced with SFH inclusion in broilers but no effects were observed in pullets. Jiménez-Moreno et al. (2016) reported also a reduction in moisture content the excreta in broilers at same ages. Rezaei et al. (2011) reported also that the inclusion of 5 g micronized cellulose/kg diet reduced litter moisture content in broilers. All these data indicate that the inclusion of moderate levels of insoluble fiber in the diet had positive or no effects on excreta quality.

The inclusion of SFH increased the AME_n of the diet at 21 d of age but did not affect the retention of any of the other nutrients studied except N. Kimiaetalab et al. (2017) reported also that the inclusion of 30 g SFH/kg diet increased the AME_n of the diet without any significant effect on the retention of the other nutrients. The improvement in AME_n of the diet with SFH inclusion was more pronounced in the research of Kimiaetalab et al. (2017) than in the current research. The NDF of the control diet used by Kimiaetalab et al. (2017) was 87 g/kg whereas in the current research the control diet contained 101 g NDF/kg. Consequently, a greater benefit of the extra fiber on the AME_n of the diet was expected in the research of Kimiaetalab et al. (2017) than in the current research (Mateos et al., 2012). In this respect, Jiménez-Moreno et al. (2013a) reported improvements in the CTTAR of the nutrients and AME_n of the diet with the inclusion of up to 50 g OH/kg but the benefits decreased with a further increase to 75 g/kg, consistent with the results reported herein. Insoluble fiber sources are retained for longer in the upper part of the GIT, increasing gizzard function, the intensity of the antiperistaltic movements and the production of HCl, amylase and bile acids (Duke, 1986; Hetland et al., 2003; Jiménez-Moreno et al., 2009b). To notice that in the research of Kimiaetalab et al. (2017) and in the research reported herein, SFH was included at the expense of the basal diet and consequently, the calculated AME_n content of the SFH containing diet was lower than that of the control diet. Consequently, the inclusion of SFH had a considerable beneficial effect on the utilization of the energy of the non-fibrous constituents of the diet in both researches.

4.2. Gastrointestinal tract traits

4.2.1. Effects of chicken line

In absolute terms, all the organs of the GIT were heavier and the SI and cecum were longer in broilers than in pullets, consistent with the greater GIT capacity and ADFI of broilers. [Uni et al. \(1995a\)](#) and [Kimiaeitalab et al. \(2017\)](#) reported also that the SI of young birds was 30 and 35% longer in broilers than in pullets, respectively. In relative terms, however, the differences in organ weight were less evident and in fact, the gizzard was heavier and the SI and cecum were longer in pullets, in agreement with data of [Kimiaeitalab et al. \(2017\)](#).

The pH of the different segments of the GIT were not affected by chicken line, consistent with data of [Kimiaeitalab et al. \(2017\)](#) for all organs except the gizzard that was lower in pullets. The reason for the inconsistency in gizzard pH between these two experiments, conducted under similar management conditions, are not apparent. In this respect, in the research of [Kimiaeitalab et al. \(2017\)](#) the control diet was based on maize and contained 87 g NDF/kg whereas in the current research the control diet was based on wheat and barley and contained 101 g NDF/kg. In broilers, [González-Alvarado et al. \(2007\)](#) reported that the pH of the gizzard depended on the main cereal of the diet, with greater response when fed maize than when fed rice. In pullets, [Frikha et al. \(2009\)](#) and [Saldaña et al. \(2015\)](#), however, reported similar gizzard pH with maize or wheat diets, although in these two researches the pH was measured in older birds (7 and 17 wk of age, respectively).

4.2.2. Effects of sunflower hulls inclusion

The inclusion of SFH in the diet did not affect the weight or the pH of the different organs of the GIT but increased the absolute length of the SI at 21 d and of the cecum at both ages. These results are consistent in general, with data reported by [Kimiaeitalab et al. \(2017\)](#). [Wang et al. \(2015\)](#) reported also an increase in the length of the SI of broilers with the inclusion of 60 g DDGS/kg diet.

4.3. Short chain fatty acid and ileum morphology

4.3.1. Effects of chicken line

The concentration of total SCFA in the cecum was 6.7 percent units higher in broilers than in pullets but the difference was not significant. [Walugembe et al. \(2015\)](#) and [Kimiaeitalab et al. \(2017\)](#) reported also a 10.8 and 8.7 percent units greater production of SCFA in broilers than in pullets, differences that were significant in both researches. [Józefiak et al. \(2004\)](#) and [Rehman et al. \(2009\)](#) indicated that SCFA concentration in the cecum is highly variable with a range reported in the literature from 53 to 156 $\mu\text{mol/g}$ fresh digesta. Probably, the high variability in SCFA concentration observed in the current research, precluded the detection of any significant difference between the two chicken lines. The SCFA profile in the cecum was similar for the two chicken lines, except for butyrate and isobutyrate that were higher in broilers. Butyrate stimulates the proliferation of epithelial cell and the development and growth of the intestines ([Roediger and Moore, 1981](#); [Sakata, 1987](#)), consistent with the better development of the ileum mucosa in broilers compared with pullets reported herein.

Villus height of the ileum was greater in broilers than in pullets, in agreement with data of [Kimiaeitalab et al. \(2017\)](#). An increase in VH suggests better capacity for absorption of nutrients of the small intestine ([Montagne et al., 2003](#); [Chiang et al., 2010](#)), consistent with the better F:G reported in broilers in the current research.

4.3.2. Effects of sunflower hulls inclusion

The concentration of total SCFA in the cecum and the ileal villus morphology were not affected by the inclusion of fiber in the diet, consistent with data of [Walugembe et al. \(2015\)](#) and [Kimiaeitalab et al. \(2017\)](#). Similarly, [Jiménez-Moreno et al. \(2013b\)](#) reported that the inclusion of 25 g OH/kg did not affect jejunal morphology in 18 d-old broilers. In contrast, [Rezaei et al. \(2011\)](#) observed that the inclusion of 5 g micronized cellulose/kg diet increased VH in the ileum of 42 d-old broilers. The existing data indicate that the effects of dietary fiber on villus morphology depend primarily on the level of inclusion and the physico-chemical characteristics of the fiber source. For example, [Jiménez-Moreno et al. \(2011\)](#) reported that the inclusion of 25 g pea hulls/kg but not of 75 g/kg, increased VH in 15 d-old broilers.

5. Conclusions

Broilers and pullets differ in growth performance, nutrient retention, GIT organ development and ileal villus height. Broilers grew faster and were more efficient than pullets at all ages and had better CTTAR of all nutrients at 9 d of age. In absolute terms, the gizzard was heavier and the SI and cecum were longer in broilers than in pullets but an opposite effect was observed in relative terms. The dilution of the control diet with 30 g SFH/kg did not reduce chick performance and in fact, improved the AME_n of the diet at 21 d of age. The inclusion of SFH at the expense (wt:wt) of the whole diet did not affect the weight or the pH of the organs of the GIT but increased the absolute length of the SI and the cecum.

No interactions between chicken line and SFH inclusion were detected for any of the variables studied except for moisture content of the excreta at 21 d of age that decreased in broilers with SFH inclusion. The data suggest that most of the information available on the effects of inclusion of insoluble fiber sources in broilers might be used, at some extent, in studies conducted with pullets.

Conflicts of interest

The authors confirm that there are not conflicts of interest in this research.

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