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Simultaneous inclusion of sorghum and cottonseed meal or millet in broiler diets: effects on performance and nutrient digestibility

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Two experiments were conducted to investigate the use of sorghum, cottonseed meal and millet in broiler diets and their interaction when they are used simultaneously. In Experiment 1, a corn-soybean meal control diet was compared with eight experimental treatments based on low tannin sorghum (S30, S45 and S60), cottonseed meal (CM15, CM40) or both ingredients included in the same diet (S30/CM40, S45/CM25 and S60CM15). Results showed that BW gain was not affected by the inclusion of sorghum or cottonseed meal. However, feed intake tended to be affected by the cereal type with the highest values with sorghum-based diets. Feed conversion ratio increased ($P < 0.001$*) with sorghum-based diets compared with the control diet, whereas a combination of cottonseed meal and sorghum in the same diet did not affect the feed conversion ratio. Significant differences (*$P < 0.001$*) were observed in apparent ileal digestibility (%) of protein and energy with the cottonseed meal and sorghum/cottonseed meal-based diets having lower protein and energy digestibility compared with corn-based diets. In Experiment 2, a control diet was compared with six diets in which corn was substituted at 60%, 80% or 100% by either sorghum or millet and other three diets with simultaneous inclusion of these two ingredients (S30/M30, S40/M40, S50/M50). Single or combined inclusion of sorghum and millet resulted in similar feed intake and growth performance as the control diet. Apparent ileal digestibility of protein and energy was higher with millet-based diets (*$P < 0.001$*). Total tract digestibility of protein in sorghum and millet-based diets tended to decrease linearly with the increasing level of substitution. Sorghum-based diets resulted in lower total tract digestibility of fat compared with millet and sorghum/millet-based diets (*$P < 0.001$*). Higher total tract digestibility of starch were obtained with the control diet and millet-based diets compared with the sorghum-based treatments. Results of the two experiments suggest that broiler growth performance was not affected by the dietary level of sorghum, millet or cottonseed meal. Nutrient digestion can, however, be affected by these feed ingredients.*

Keywords: broiler, sorghum, millet, cottonseed meal, digestibility

Implications

Sorghum, millet and cottonseed meal can be used in poultry nutrition to replace corn and soybean meal. Results showed that the overall performance is not affected when high quality ingredients (low tannin, low gossypol) are used. Some effects on feed intake and feed efficiency were identified and discussed. The present study provides additional knowledge to the use of sorghum, millet and cottonseed meal in commercial poultry production.

Introduction

The worldwide competitiveness of the poultry industry requires finding alternative to major feed ingredients used in broiler feeding to reduce the cost of feeding. Sorghum (*Sorghum bicolor*) and millet (*Pennisetum glaucum*, *Panicum miliaceum*) have been widely investigated in broiler nutrition as substitutes to corn. Cottonseed meal (*Gossypium* spp.) has also been examined as an alternative to soybean meal. Results indicated that similar (Davis *et al.*, 2003; Hidalgo *et al.*, 2004) or improved performance (Baurhoo *et al.*, 2011) was obtained with millet-based diets compared with corn-based diets. Whereas for sorghum and cottonseed meal, a reduction in feed efficiency (Jacobs and Parsons, 2013) or equivalent performance as the control diet were obtained (Jacob *et al.*, 1996; Azman and Yilmaz, 2005).

In a meta-analysis on the use of these feed ingredients (Batonon-Alavo *et al.*, 2015), we observed that millet-based diets gave similar performance as the corn-based diets while cottonseed meal-based diets increased feed intake. Average daily gain was reduced with both sorghum- and cottonseed

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meal-based diets. However, there was no significant dose–response effect with millet and cottonseed meal. Growth performance decreased with the increasing level of substitution of corn with sorghum.

This reduction in growth performance obtained in sorghum and cottonseed meal-based diets may be related to their content of anti-nutritional factors. According to several authors, tannins and phytate in sorghum form complexes with protein and carbohydrates, particularly with starch, thereby reducing nitrogen and starch digestibility (Selle *et al.*, 2010; Mahmood *et al.*, 2014). Birds fed high tannin diets suffered from a severe decrease of growth compared with low tannin or control fed birds (Flores *et al.*, 1994; Mahmood *et al.*, 2006). In addition, Kafirin has been examined in sorghum by Selle *et al.* (2010). Its presence in sorghum is associated with a poor amino acid digestibility. In cottonseed meal, free gossypol decreases lysine digestibility and inhibits the activity of pepsin and trypsin, thus reducing growth in broilers (Nagalakshmi *et al.*, 2007).

For more flexibility in the type of ingredients used in the least cost feed formulation, it could be hypothesized that simultaneous utilization of more than one of these alternative feed ingredients in poultry diets will become commonplace. Consequently, the question arises whether simultaneous inclusion of sorghum and cottonseed meal in broiler feeding would result in additive effect or a synergy of the anti-nutritional factors. Besides, the protein content of millet is higher than corn and the essential amino acid profile is more balanced in millet than in corn (Heuzé and Tran, 2012). The high essential amino acid concentrations, the high digestibility of these amino acids (Adeola and Orban, 1995; Yin *et al.*, 2002) and the changes in the small intestine mucosa morphology were reported to be the factors leading to a better feed efficiency with millet (Baurhoo *et al.*, 2011; Goodarzi Boroojeni *et al.*, 2011). It could therefore be assumed that bird's nutrient digestibility increases when millet is used in sorghum-based diets.

This work was, therefore, designed to evaluate the effects of partial or total substitution of corn with sorghum and millet, and partial replacement of soybean meal with cottonseed meal on performance and nutrient digestibility in broiler. It is the aim of this study to investigate the interactions that might be induced on broiler performance when sorghum and cottonseed meal are simultaneously included in the diet or when sorghum and millet totally replaced corn in the diet.

Material and methods

General

Two trials were conducted: the first experiment combined sorghum and cottonseed meal and the second one used sorghum and millet. Nutritional composition of these feed ingredients are described in Table 1. The same batch of sorghum (*S. bicolor*) originating from France was used in the two experiments. It was a sorghum with red pericarp, but without testa layer, and therefore had low condensed tannin content (1.80 g/kg catechin equivalents) and low total phenolics (3.5 g/kg tannic acid equivalent). Millet (*P. miliaceum*) originated from France. Prepressed solvent extracted cottonseed meal (*Gossypium* spp., bought in France) having 402 g/kg CP and 1.03 g/kg free gossypol content was used in Experiment 1. Birds were reared in wire-floored battery cages (83 cm wide × 52 cm height × 75 cm depth) in an environmentally controlled house. The environmental temperature was maintained at 32°C during the 1st week and decreased to 23°C until the end of the experiment according to Ross guidelines (Aviagen, 2007). Experimental procedures and animal care were carried out according to French legislation at the time of the study (2013) and were approved by the local ethical committee.

Table 1 *Analyzed composition of sorghum (S), cottonseed meal (CM) and millet (M)*

	Sorghum	Cottonseed meal	Millet
Analyzed composition (g/kg, as fed)			
DM	864	920	869
CP	82.8	402	116
Crude fat	35.2	25.0	45.2
Starch	631	8.70	582
Total sugars	3.80	68.6	1.20
NDF	138.6	206.6	151.9
ADF	50.8	119.1	84.2
ADL	17.3	29.9	20.0
Condensed tannins	1.80	nd	nd
Free gossypol	nd	1.03	nd
ME (MJ/kg)[1]	13.33	7.69	13.19

nd = not determined; ME = metabolizable energy; DM = dry matter; EE = ether extract; CF = crude fibre; NFE = nitrogen free extract.
[1]ME content was estimated using European energy calculation (Janssen, 1989). S (tannin < 4 g) $MEn = 31.02 \times CP + 77.03 \times EE + 37.67 \times NFE$; CM expeller or solvent, $MEn = 21.36 \times DM + 47.13 \times EE - 30.55 \times CF$; M (corn equation), $MEn = 36.21 \times CP + 85.44 \times EE + 37.26 \times NFE$; with DM, EE, CF and NFE = DM − (EE + CP + Ash + CF).

Experiment 1: simultaneous use of sorghum and cottonseed meal (Experiment 1)

Four hundred and eighty six-day-old male Ross PM3 broilers were fed *ad libitum* from 1 to 8 days of age with a starter diet containing 12.6 MJ/kg of metabolizable energy (ME) and 225 g/kg of CP. On day 8, birds were randomly selected and divided into nine groups. Each group contained nine replicates of six birds. Birds were assigned to replicates on the basis of BW such that homogenous BW were obtained between each replicate and treatment. The experimental period ran from 8 to 28 days of birds age. Birds were given *ad libitum* access to feed and water throughout the experiment.

Nine different treatments were offered in a pelleted (steam pelleting with moisture addition) form (Table 2). The control treatment (C1) was based on corn and soybean meal as the main ingredients and contained 12.3 MJ/kg ME and 231 g/kg CP. The experiment was designed with dietary level of substitution and type of ingredient as the main effects. Three experimental diets were formulated by substituting

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Table 2 *Dietary composition and nutrient content of experimental diets given from 8 to 28 days in Experiment 1*

	Control	Sorghum-based diets			Cottonseed meal-based diets		Sorghum and cottonseed meal-based diets		
	C1	S30	S45	S60	CM15	CM40	S30/CM40	S45/CM25	S60/CM15
Ingredient (g/kg as fed)									
Wheat	60.0	60.0	73.1	80.0	60.0	60.0	54.6	71.5	99.2
Corn	513.2	385.6	304.4	230.0	513.2	514.1	385.6	314.4	222.0
S	–	165.0	243.0	320.2	–	–	165.0	230.0	290.0
Soybean meal (48%)	365.1	346.0	340.0	332.0	307.0	207.9	206.0	241.0	275.0
CM	–	–	–	–	55.0	146.0	130.0	91.2	58.0
Soybean oil	25.0	7.0	2.0	–	27.0	30.0	15.0	10.0	15.0
Limestone	4.0	4.0	4.0	4.0	4.0	3.2	4.0	4.0	4.0
Dicalcium phosphate	23.0	23.0	23.0	23.0	22.1	23.5	24.5	24.0	24.1
Salt	3.0	2.5	3.0	2.9	3.0	2.1	2.3	2.5	2.5
Trace mineral premix[1]	4.1	4.1	4.6	4.6	4.1	4.1	4.1	4.1	4.1
D,L-Methionine	1.3	1.3	1.3	1.5	1.6	2.0	1.9	1.7	1.6
L-Lysine HCl	1.1	1.3	1.4	1.6	2.2	5.0	5.0	4.1	3.3
L-Threonine	0.2	0.2	0.2	0.2	0.8	2.1	2.0	1.5	1.2
Calculated composition (g/kg unless specified)[2]									
CP	215	216	217	218	216	218	218	218	219
Crude fat	51.8	34.7	29.6	27.5	54.4	58.4	44.0	38.4	42.2
Ca	9.4	9.3	9.4	9.4	9.1	9.0	9.5	9.4	9.5
Av. P	4.8	4.6	4.6	4.5	4.5	4.5	4.5	4.5	4.6
Dig Lys	11.3	11.1	11.1	11.1	11.0	11.1	11.0	11.1	11.2
Dig Meth + Cyst	7.5	7.4	7.4	7.5	7.4	6.9	6.8	6.9	7.1
Dig Thr	7.5	7.4	7.4	7.3	7.4	7.4	7.4	7.4	7.5
Dig Trp	2.2	2.2	2.2	2.2	1.9	1.5	1.6	1.8	2.0
Dig Arg	13.4	13.0	12.9	12.7	12.2	10.3	10.1	10.8	11.5
Free gossypol (mg/kg)	–	–	–	–	5.0[3]	12.0[3]	13.0	9.0	6.0
ME (MJ/kg)[4]	12.3	12.0	11.9	11.9	12.5	12.3	11.9	11.9	12.1
Analyzed composition (g/kg)									
CP	231	224	222	227	226	217	219	215	215
Crude fat	53.4	42.3	39.0	36.6	61.6	66.6	50.0	45.9	52.0
Condensed tannins	0.47	0.60	0.63	0.77	0.66	0.81	0.89	0.78	0.68
NDF	120	118	116	120	130	131	119	126	136
ADF	33.7	37.4	39.5	41.0	39.6	49.0	44.6	46.4	48.6
ADL	5.5	6.3	7.5	7.9	8.0	5.4	1.9	2.7	13.9

C1 = control diet; S = sorghum; CM = cottonseed meal; ME = metabolizable energy.
The number represents the level of substitution of corn for S-based diets or soybean meal for CM-based diets.
[1]Premix composition (per kg): vitamin A, 1 100 000 UI; vitamin D_3, 300 000 UI; vitamin E, 4000 UI; vitamin B_1, 250 mg; vitamin B_2, 800 mg; vitamin B_6, 500 mg; vitamin B_{12}, 2.5 mg; vitamin PP, 5000 mg; vitamin C, 10 000 mg; vitamin K_3, 300 mg; Zn, 5400 mg; Cu, 3000 mg; Fe, 6000 mg; I, 124 mg; Se, 29.7 mg; Mn, 8000 mg; Ca, 0.17 mg; Co, 60 mg; Mg, 0.08 g; folic acid, 150 mg; choline chloride, 50 004 mg; biotin, 15 mcg. Premix also supplied titanium 1.0 g/kg of diet and Avatec, 0.6 g/kg of diet.
[2]Nutritional values were calculated, based on Sauvant *et al.* (2004).
[3]Analyzed composition.
[4]ME content estimated using European energy equation (Fisher and McNab, 1987).

30%, 45% or 60% of the corn by sorghum (S) and two other diets were similarly formulated by replacing 15% or 40% of the soybean meal with cottonseed meal (CM). To test the effect of simultaneous inclusion of both S and CM, three other diets combined the level of substitution of sorghum and cottonseed meal as follows: 30% S and 40% CM, 45% S and 25% CM; 60% S and 15% CM. Diets were formulated to meet Ross broiler nutrient specifications (Aviagen, 2007) and included 1 g/kg titanium dioxide (TiO_2) as digestibility marker. All diets were formulated to be isocaloric and isonitrogenous by adjusting soybean oil, wheat and amino acids supply to avoid any essential amino acids as limiting factor.

Experiment 2: total corn substitution by millet and sorghum in broiler diets (Experiment 2)

Four hundred and eighty six-day-old male Ross PM3 broilers were fed *ad libitum* with the same starter feed as in Experiment 1 from 1 to 8 days. On day 8, nine replicates (of six chicks per cage) of similar BW were constituted per treatment. The experimental period ran from 8 to 27 days of age. Birds were given one of the 10 treatments: a control (C2) complete feed and nine experimental diets designed as a factorial arrangement (3 × 3) with dietary level of substitution and type of ingredient as the main effects (Table 3). All diets were fed as pellets.

The aim of this experiment was to establish whether it would be possible to substitute corn with sorghum beyond

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Table 3 *Dietary composition and nutrient content of experimental diets given from 8 to 27 days in Experiment 2*

	Control	Sorghum-based diets			Millet-based diets			Sorghum and millet-based diets		
	C2	S60	S80	S100	M60	M80	M100	S30/M30	S40/M40	S50/M50
Ingredient (g/kg as fed)										
Wheat	61.8	50.0	60.4	71.0	70.1	75.9	105.2	68.8	66.2	106.7
Corn	500.7	213.6	120.0	–	220.0	120.0	–	220.0	129.2	–
S	–	310.0	400.7	514.0	–	–	–	150.0	200.5	250.2
Soybean meal (48%)	375.0	357.9	354.4	349.5	334.0	318.0	300.0	347.7	338.3	325.9
M	–	–	–	–	310.7	420.1	528.2	150.0	200.5	250.2
Soybean oil	28.0	30.0	30.0	30.0	30.0	30.0	30.0	29.0	30.0	30.8
Limestone	3.7	3.3	3.1	3.5	3.7	5.2	5.4	3.9	4.0	4.2
Dicalcium phosphate	21.3	22.7	22.9	23.2	22.2	20.8	20.5	21.7	22.0	22.0
Salt	3.3	2.7	2.5	2.5	3.0	2.9	2.8	2.8	2.9	2.8
Trace mineral premix[1]	4.6	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1
D,L-Methionine	1.2	4.0	1.1	1.2	1.1	1.2	1.3	1.2	1.2	1.3
L-Lysine HCl	0.4	0.7	0.8	1.0	1.1	1.6	2.1	0.8	1.1	1.6
L-Threonine	–	1.0	–	–	–	0.2	0.4	–	–	0.2
Calculated composition (g/kg unless specified)[2]										
CP	219	219	217	218	218	218	218	218	218	218
Crude fat	63.2	61.3	59.7	57.6	64.1	63.6	62.0	61.5	61.8	59.8
Ca	9.0	9.0	9.0	9.1	9.0	9.2	9.1	9.0	9.1	9.1
Av. P	4.5	4.5	4.5	4.50	4.70	4.5	4.5	4.50	4.50	4.5
Dig Lys	11.5	11.1	11.0	11.0	11.1	11.1	11.0	11.0	11.0	11.1
Dig Meth + Cyst	7.9	10.0	7.3	7.3	7.3	7.1	7.0	7.5	7.4	7.3
Dig Thr	7.9	8.5	7.5	7.4	7.4	7.4	7.4	7.5	7.4	7.4
Dig Trp	2.4	2.3	2.4	2.4	2.5	2.6	2.6	2.4	2.5	2.5
Dig Arg	14.2	13.1	12.6	12.2	13.5	13.4	13.2	13.1	13.3	12.7
ME (MJ/kg)[3]	12.5	12.6	12.5	12.6	12.8	12.8	12.8	13.0	12.5	12.8
Analyzed composition (g/kg)										
CP	224	226	219	224	226	221	220	224	223	218
Crude fat	57.7	60.7	60.1	61.2	66.4	70.4	72.7	72.8	62.9	74.5
Condensed tannins	–	0.60	0.54	0.78	–	–	–	0.31	0.40	0.43
NDF	76.8	90.0	81.7	81.4	91.3	101.0	111.8	91.9	95.3	95.2
ADF	30.2	32.1	32.1	33.7	38.3	44.6	50.0	36.7	39.5	39.8
NDL	4.7	5.1	5.6	6.1	4.8	5.8	7.7	5.4	5.1	3.4

C2 = control diet; S = sorghum; M = millet; ME = metabolizable energy.
The number represents the level of substitution of corn.
[1]Premix composition (per kg): vitamin A, 1 100 000 UI; vitamin D_3, 300 000 UI; vitamin E, 4000 UI; vitamin B_1, 250 mg; vitamin B_2, 800 mg; vitamin B_6, 500 mg; vitamin B_{12}, 2.5 mg; vitamin PP, 5000 mg; vitamin C, 10 000 mg; vitamin K_3, 300 mg; Zn, 5400 mg; Cu, 3000 mg; Fe, 6000 mg; I, 124 mg; Se, 29.7 mg; Mn, 8000 mg; Ca, 0.17 mg; Co, 60 mg; Mg, 0.08 g; folic acid, 150 mg; choline chloride, 50 004 mg; biotin, 15 mcg. Premix also supplied titanium 1.0 g/kg of diet and Avatec, 0.6 g/kg of diet.
[2]Nutritional values were calculated, based on Sauvant *et al.* (2004).
[3]ME content estimated using European energy equation (Fisher and McNab, 1987).

the levels of substitution used in Experiment 1. Therefore, three other experimental diets were formulated by replacing 60%, 80% or 100% of the corn with sorghum (S60, S80 and S100). Three millet-based diets (M60, M80 and M100) were formulated in the same way. In order to assess the interactions that might exist when these ingredients are used simultaneously, three other diets were formulated by substituting different proportions of the corn with both S and M (S30/M30, S40/M40, S50/M50). Birds were given *ad libitum* access to feed and water throughout the experiment. All diets were formulated to be isocaloric (12.69 ± 0.05 MJ/kg) and isonitrogenous (222.5 ± 0.9 g/kg) and formulated to meet Ross broiler nutrient specifications (Aviagen, 2007) as in Experiment1. TiO_2 was included at 1 g/kg in all diets as digestibility marker.

Measurements

Performance (Experiments 1 and 2). Chicken BW was measured at days 8, 15, 21 and 28 in Experiment 1. In Experiment 2, birds were weighed at days 8, 14 and 27. Feed intake was measured every week by weighing feed refusals in all experiments. Due to practical reasons, feed intake and BW were not measured at day 20 in Experiment 2. Feed conversion ratio (FCR) was calculated as the ratio between total feed intake and BW gain of all birds in each cage. Feed intake, BW gain and FCR were calculated after correcting for mortalities.

Apparent ileal digestibility (AID) (Experiments 1 and 2). At the end of each experimental period, all birds were slaughtered by cervical dislocation. The contents of the terminal part of the ileum, defined as the region between 17 and 2 cm

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proximal to the ileocaecal junction were collected for ileal digestibility determinations. Digesta of birds were pooled per cage, freeze-dried and ground before analysis. All digesta samples were analyzed for dry matter (DM), nitrogen and concentration of titanium as indigestible marker. The AID coefficient of nutrients in the two experiments was calculated according to the following equation:

$$\mathrm{AID}\ (\%) = \left\{1 - \left(\frac{\mathrm{Ti}_{\mathrm{Diet}}}{\mathrm{Ti}_{\mathrm{ileum}}} \times \frac{\mathrm{Nutrient}_{\mathrm{ileum}}}{\mathrm{Nutrient}_{\mathrm{Diet}}}\right)\right\} \times 100$$

where $\mathrm{Ti}_{\mathrm{Diet}}$ and $\mathrm{Ti}_{\mathrm{ileum}}$ represent concentration of titanium in feed and ileal digesta, respectively. $\mathrm{Nutrient}_{\mathrm{Diet}}$ and $\mathrm{Nutrient}_{\mathrm{ileum}}$ are the nutrients (protein and energy) in feed and ileal digesta.

Total tract digestibility (Experiment 2). In the second experiment, plastic trays were placed under cages for total excreta collection. Representative samples of excreta were then collected from six cages per treatment over a period of 3×24 h from day 22 to 24. All excreta samples were freeze-dried and then ground to pass through 0.5 mm sieve. Samples of diets and excreta were analyzed for DM, gross energy (GE), starch, fat, total N and protein N. Apparent metabolizable energy (AME) was calculated as the difference between energy intake and excretion, relative to feed intake. AME was then corrected for the energy of N retention (AMEn) according to Hill and Anderson (1958).

Chemical analysis

Chemical composition of feeds and faeces was determined following standard procedures (AOAC International, 1995). DM was determined with oven drying at 103°C. Crude fat contents were determined after a saponification with determination by gas chromatography (Büchi, B820). GE was determined using a bomb calorimeter (C2000 Basic; IKA, Germany). Total nitrogen analysis was carried out with a Leco N analyzer (LECO, FP528) and CP was then calculated as $N \times 6.25$. Starch was measured using a polarimeter (ADP 410; Bellingham and Stanley, UK). Total sugars were determined according to Luff-Schoorl method. NDF, ADF and ADL in feed ingredients and diets were measured on Fibertech according to Van Soest and Wine (1967). Titanium concentrations were determined by Induction Coupled Plasma after HNO_3/NH_4F mineralization in microwaves. Total tract protein nitrogen (Terpstra and de Hart, 1974) and total tract starch were determined by Near Infrared Spectroscopy (NIRS) according to Bastianelli *et al.* (2010). Condensed tannins were determined by vanillin assay and expressed as catechin equivalent. Gossypol contents were determined by colorimetry following the standard ISO6866.

Statistical analysis

Data analyses were performed using R version 3.0.2 (R Core Team, 2013).

The mortality recorded for all treatments in each experiment was submitted to a one-way ANOVA. In Experiment 1, data collected was analyzed based on three periods: (1) 8 to 15 days; (2) 15 to 21 days (3) 21 to 27 days. In Experiment 2, analyses were performed based on two periods: (1) 8 to 14 days and (2) 14 to 27 days. A one-way ANOVA was performed in each period of age to test the treatment effect (nine levels in Experiment 1; 10 levels in Experiment 2) on feed intake, weight gain and FCR. Data were also submitted to a one-way ANOVA to evaluate the effect of the type of ingredient irrespective of its level: C1 *v.* S *v.* CM *v.* S/CM in Experiment 1 and C2 *v.* S *v.* M *v.* S/M in Experiment 2. To assess the effect of the level of substitution, treatments of each ingredient were submitted to a Kruskal–Wallis test at each period of age. These analyses were also realized on the data collected for AID and total tract digestibility.

Tested factors were considered significant if $P < 0.001$, $P < 0.01$ and $P < 0.05$, discussed as a trend if $P < 0.10$, and not significant if $P > 0.10$. A Bonferroni–Dunnet pairwise comparison was used to compare differences between mean after each ANOVA test being performed. A multiple comparisons *post hoc* test (Siegel and Castellan, 1988) was realized to compare differences between means for the level of substitution effect in each ingredient-based diets.

Results

Experiment 1: simultaneous use of sorghum and cottonseed meal

Performance. The overall mortality from 8 to 28 days was $4.17 \pm 0.91\%$ and was not influenced by the treatments.

Feed intake was not affected by treatments throughout the experimental period (Table 4). However, from 21 to 28 days and 8 to 28 days, it tended to be influenced by the type of ingredient with the lowest values in CM-based diets and the highest in S-based diets ($P = 0.07$). Feed intake of the control diet and S/CM-based diets were intermediate. There was no significant effect of the level of substitution of each ingredient on feed intake.

Weight gain was similar among treatments and was not affected by the inclusion of sorghum or cottonseed meal. A high weight gain value (82.7 g/b per day) was recorded for S30 diet at days 15 to 21. This induced a trend for a level effect in sorghum at this period.

CM-based diets resulted in a similar FCR to control diet (1.49 *v.* 1.50). Higher FCR were obtained with sorghum- (1.55) and S/CM-based diets (1.54). The lowest FCR value was observed with CM15 (1.47) while the highest was obtained with S45/CM25 (1.58). However, only a few significant differences of FCR were obtained in the first two periods regarding the dose–response effect of each ingredient. FCR increased with the level of substitution of S and CM. When the two ingredients were included in the same diet from 8 to 15 days, FCR was affected due to a low value in S60/CM15.

AID of protein and energy. Table 5 shows the effect of treatments on AID of protein and energy. AID of protein of all experimental treatments was lower to control diet ($82.93 \pm 0.70\%$). This difference was not significant for diets

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Table 4 *Effect of diets with sorghum (S), cottonseed meal (CM) or their combination (S/CM) on the performance of broilers fed from 8 to 28 days old (Experiment 1)*

	Feed intake (g/b per day)				Weight gain (g/b per day)				FCR			
Age (days)	8 to –15 days	15 to 21 days	21 to 28 days	8 to 28 days	8 to 15 days	15 to 21 days	21 to 28 days	8 to 28 days	8 to 15 days	15 to 21 days	21 to 28 days	8 to 28 days
C1	64.1	115	151	111	50.3	80.8	89.2	74.2	1.27^{a}	1.43^{a}	1.70	1.50^{ba}
S30	67.0	121	156	115	49.8	82.7	90.0	74.1	1.35^{ab}	1.46^{a}	1.73	1.55^{bc}
S45	65.1	118	161	115	48.7	78.2	95.1	74.0	1.33^{ab}	1.50^{a}	1.70	1.55^{b}
S60	66.8	124	160	117	49.8	79.1	94.3	74.4	1.34^{ab}	1.57^{b}	1.69	1.57^{b}
CM15	65.0	113	148	109	50.2	81.1	90.0	73.8	1.29^{ab}	1.40^{a}	1.65	1.47^{a}
CM40	65.2	119	153	112	49.5	79.6	94.6	74.6	1.32^{ab}	1.49^{ab}	1.62	1.51^{ba}
S30/CM40	67.3	114	157	112	49.2	78.8	92.5	73.5	1.37^{ab}	1.44^{a}	1.72	1.53^{b}
S45/CM25	67.4	115	159	114	48.6	79.6	89.9	72.6	1.38^{b}	1.51^{a}	1.78	1.58^{c}
S60/CM15	65.9	118	154	113	50.1	80.2	92.7	74.3	1.31^{ab}	1.48^{a}	1.67	1.52^{ba}
SEM	1.3	3	4	2	0.7	2.1	2.7	1.2	0.02	0.03	0.03	0.01
Treatment effect[1]	ns	ns	ns	ns	ns	ns	ns	ns	*	**	ns	***
Type of ingredient[2]	ns	ns	†	†	ns	ns	ns	ns	**	*	ns	***
Level of substitution[3]												
S	ns	ns	ns	ns	ns	†	ns	ns	ns	*	ns	ns
CM	ns	ns	ns	ns	ns	ns	ns	ns	*	**	ns	ns
S and CM	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns

C1 = control diet; FCR = feed conversion ratio.
The number represents the level of substitution of corn for S-based diets or soybean meal for CM-based diets.
[1]Effect of treatments: ANOVA on nine diets.
[2]Effect of the type of ingredient: ANOVA on four treatments (control *v.* S-based diets *v.* CM-based diets *v.* S/CM-based diets).
[3]Effect of the level of substitution (dose–response effect) within each type of ingredient.
[a,b,c]Values with the same superscript are not significantly different at $P<0.05$.
$***P<0.001$; $**P<0.01$; $*P<0.05$; $\dagger P\leqslant 0.10$. ns: not significant at $P>0.10$.

Table 5 *Apparent ileal digestibility of protein and energy of broilers fed sorghum- (S) and/or cottonseed meal- (CM) based diets measured at 28 days (Experiment 1)*

	AID of protein (%)	AID of energy (%)
C1	82.9[a]	78.1[a]
S30	81.3[a]	77.1[ac]
S45	80.5[ab]	76.2[ac]
S60	80.3[ab]	75.1[bcd]
CM15	79.6[ab]	75.7[ab]
CM40	78.0[bc]	72.7[de]
S30/CM40	74.7[c]	70.2[e]
S45/CM25	78.0[b]	73.3[bd]
S60/CM15	77.4[bc]	72.7[de]
SEM	0.77	0.60
General treatment effect[1]	***	***
C *v.* S *v.* CM *v.* S/CM[2]	***	***
Level of substitution[3]		
S	ns	†
CM	ns	***
S and CM	*	*

C1 = control diet; AID = apparent ileal digestibility.
The number represents the level of substitution of corn for S-based diets or soybean meal for CM-based diets.
[1]Effect of treatments: ANOVA on nine diets.
[2]Effect of the type of ingredient: ANOVA on four treatments (control *v.* S-based diets *v.* CM-based diets *v.* S/CM-based diets).
[3]Effect of the level of substitution (dose–response effect) within each type of ingredient.
[a,b,c,d,e]Values with the same superscript are not significantly different at $P<0.05$.
***$P<0.001$; **$P<0.01$; *$P<0.05$; †$P\leqslant0.10$. ns: not significant at $P>0.10$.

based on sorghum (80.69 ± 0.34%), but it was significant for CM-based diets (78.81 ± 0.59%). AID of protein of S/CM-based diets was the lowest (76.71 ± 0.55%), with values significantly lower from control and S-based diets. No effect of the level of substitution was found on AID of protein in S- and CM-based diets. However, the combination of these two ingredients affected AID of protein in relation to the low value in S30/CM40.

AID of energy was different among treatments with the highest values in control (78.11 ± 0.66%) and the lowest in S/CM-based diets (72.05 ± 0.52%). AID of energy obtained with S-based diets (76.12 ± 0.31%) was not significantly different from the control whereas in CM-based diets (74.19 ± 0.51%) it was lower than the control. The level of substitution of sorghum tended to affect AID of energy ($P=0.09$) while in CM-based diets, AID of energy decreased with the increasing level of substitution. Combination of sorghum and cottonseed meal affected AID of energy with the lowest values in S30/CM40 diet and the highest in S45/CM25 diet.

Experiment 2: total corn substitution by millet and sorghum in broiler diets

Performance. The overall mortality recorded in this experiment was 2.41 ± 0.62% and was not affected by the treatments.

Simultaneous utilization of sorghum and millet resulted in similar feed intake and growth performance as the control diet (Table 6). As main effects, sorghum and millet did not have a significant impact on feed intake, weight gain and FCR.

Table 6 *Effect of diets with sorghum (S), millet (M) or their combination (S/M) on the performance of broilers fed from 8 to 27 days old (Experiment 2)*

	Feed intake (g/b per day)			Weight gain (g/b per day)			FCR		
	8 to 14 days	15 to 20 days	8 to 27 days	8 to 14 days	15 to 27 days	8 to 27 days	8 to 14 days	15 to 20 days	8 to 27 days
C2	62.7	134	109	47.3	84.6	71.6	1.33	1.58	1.52
S60	61.6	131	107	45.9	85.3	71.5	1.34	1.54	1.50
S80	61.9	132	108	45.8	85.8	71.8	1.35	1.54	1.50
S100	63.9	132	108	47.6	82.8	70.4	1.34	1.60	1.54
M60	63.6	130	107	46.4	85.9	72.1	1.37	1.51	1.48
M80	62.1	134	109	44.8	86.5	71.9	1.38	1.55	1.51
M100	62.4	132	108	46.4	85.0	71.5	1.35	1.55	1.51
S30/M30	59.8	128	104	45.3	81.7	68.9	1.32	1.56	1.51
S40/M40	63.1	132	108	45.0	84.6	71.2	1.42	1.55	1.52
S50/M50	63.3	133	109	48.2	84.2	71.6	1.34	1.58	1.52
SEM	1.5	2.0	1.5	1.3	1.6	1.2	0.02	0.02	0.02
Treatment effect[1]	ns	ns	ns	ns	ns	ns	ns	ns	ns
Type of ingredient[2]	ns	ns	ns	ns	ns	ns	ns	ns	ns
Level of substitution[3]									
S	ns	ns	ns	ns	ns	ns	*	ns	ns
M	ns	ns	ns	ns	ns	ns	ns	ns	ns
S and M	ns	ns	ns	ns	ns	ns	ns	ns	ns

C2 = control diet; FCR = feed conversion ratio.
The number represents the level of substitution of corn.
[1]Effect of treatments: ANOVA on 10 diets.
[2]Effect of the type of ingredient: ANOVA on four treatments (control *v.* S-based diets *v.* M-based diets *v.* S/M-based diets).
[3]Effect of the level of substitution (dose–response effect) within each type of ingredient.
*$P<0.05$; ns: not significant at $P>0.10$.

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Table 7 *Apparent ileal digestibility of protein and energy of broilers fed sorghum- (S) and/or millet- (M) based diets measured at 27 days (Experiment 2)*

	AID of protein (%)	AID of energy (%)
C2	80.4^{ac}	74.6^{a}
S60	82.8^{abc}	75.9^{ab}
S80	81.3^{abc}	74.6^{a}
S100	82.4^{abc}	76.3^{ab}
M60	84.3^{b}	78.4^{b}
M80	84.2^{b}	77.9^{b}
M100	82.6^{abc}	75.9^{ab}
S30/M30	83.2^{abc}	77.0^{ab}
S40/M40	83.7^{ab}	77.7^{b}
S50/M50	79.5^{c}	74.6^{a}
SEM	0.69	0.54
Treatment effect[1]	***	***
Type of ingredient[2]	**	**
Level of substitution[3]		
S	ns	†
M	**	**
S and M	**	*

C2 = control diet; AID = apparent ileal digestibility.
The number represents the level of substitution of corn.
[1]Effect of treatments: ANOVA on 10 diets.
[2]Effect of the type of ingredient: ANOVA on four treatments (control *v.* S-based diets *v.* M-based diets *v.* S/M-based diets).
[3]Effect of the level of substitution (dose–response effect) within each type of ingredient.
[a,b,c]Values with the same superscript are not significantly different at $P<0.05$.
$***P<0.001$; $**P<0.01$; $*P<0.05$; $†P\leqslant0.10$. ns: not significant at $P>0.10$.

No difference of level of substitution of the feed ingredient was observed for M-based diets and S/M-based diets. However, there was a significant difference between the three S-based diets from 8 to 14 days of age with no significant *post hoc* effect.

AID of protein and energy. The effects of the type of cereal on AID of protein and energy are shown in Table 7. AID of protein of all experimental treatments was higher than the control diet (80.40 ± 1.44%). This difference was not significant for S- (82.17 ± 0.44%) and S/M-based diets (82.15 ± 0.60%). However, it was significant for millet-based diets (83.73 ± 0.23%). AID of protein was affected with the increasing level of millet when it was used separately or combined with sorghum. However, no significant difference was found between these diets with the *post hoc* test. No dose–response effect of sorghum was found on AID of protein.

Similar results were obtained for the AID of energy with the highest values in M-, followed by S/M-, S-based and control diets in descending order. The level of substitution tended to affect AID of energy in S-based diets ($P = 0.09$) while it significantly affected AID of energy in M-based diets and S/M-based diets.

Total tract digestibility of DM, protein, fat and starch and AMEn values. Total tract digestibility of nutrients is shown in Table 8. There were significant differences in DM digestibility, although none of the experimental treatments was significantly different from the control diet (68.81 ± 0.46%). Lower DM digestibility was observed with S-based diets (67.52 ± 0.17%) compared with M-based diets (69.15 ± 0.30%). S/M-based diets were intermediate (68.57 ± 0.25%). In S and S/M, there was a significant reduction of DM digestibility when the level of substitution increased.

Table 8 *Total tract nutrient digestibility and nitrogen-corrected apparent metabolizable energy (AMEn; MJ/kg DM) of S- and/or M-based diets of broilers measured on faeces collected from 22 to 24 days (Experiment 2)*

	Nutrient digestibility (%)				
	DM	Protein	Fat	Starch	AMEn (MJ/kg DM)
C2	68.8^{abc}	81.5^{a}	82.2^{abc}	97.2^{ab}	13.3^{ab}
S60	68.1^{abc}	80.5^{ab}	79.2^{a}	96.4^{abc}	13.2^{ab}
S80	67.5^{bc}	78.9^{ab}	80.1^{ab}	95.7^{cd}	13.1^{ab}
S100	66.9^{c}	78.7^{abc}	80.2^{ab}	94.9^{d}	13.0^{b}
M60	69.3^{ab}	78.7^{ab}	82.7^{abc}	97.6^{a}	13.4^{a}
M80	69.3^{ab}	77.6^{abc}	83.2^{abc}	96.9^{ae}	13.4^{a}
M100	68.8^{abc}	75.0^{c}	84.0^{bc}	97.0^{abe}	13.3^{ab}
S30/M30	69.5^{a}	79.4^{ab}	86.1^{c}	96.8^{ae}	13.4^{a}
S40/M40	68.4^{abc}	77.3^{bc}	82.1^{abc}	96.1^{bce}	13.3^{ab}
S50/M50	67.8^{abc}	77.0^{bc}	83.4^{abc}	95.8^{de}	13.2^{ab}
SEM	0.38	0.75	0.87	0.24	0.07
Treatment effect[1]	***	***	***	***	***
Type of ingredient[2]	***	***	***	***	**
Level of substitution[3]					
S	*	†	ns	*	†
M	ns	†	ns	†	ns
S and M	*	ns	**	*	ns

C2 = control diet; S = sorghum; M = millet; DM = dry matter.
The number represents the level of substitution of corn.
[1]Effect of treatments: ANOVA on 10 diets.
[2]Effect of the type of ingredient: ANOVA on four treatments (control *v.* S-based diets *v.* M-based diets *v.* S/M-based diets).
[3]Effect of the level of substitution (dose–response effect) within each type of ingredient.
[a,b,c,d,e]Values with the same superscript are not significantly different at $P<0.05$.
$***P<0.001$; $**P<0.01$; $*P<0.05$; $†P\leqslant0.10$. ns: not significant at $P>0.10$.

Total tract digestibility of protein was higher in the control diet (81.50 ± 0.85%) compared with all other treatments. M- (77.09 ± 0.62%) and S/M-based diets (77.89 ± 0.54%) were significantly lower than the control diet whereas S-based diets (79.35 ± 0.32%) were not significantly different. Total tract digestibility of protein in S- and M-based diets tended to decrease linearly with the increasing level of substitution of corn. However, there was no effect of the level of substitution on total tract digestibility of protein when both sorghum and millet are simultaneously used in the diet.

No significant differences were observed between the control diet (82.23 ± 1.64%) and the experimental treatments. However, S-based diets (79.82 ± 0.46%) showed lower total tract digestibility of fat compared with M- (83.30 ± 0.42%) and

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S/M-based diets (83.83 ± 0.62%). The level of substitution of sorghum or millet did not impact the total tract digestibility of fat. However, the combination of these two ingredients in broiler diet significantly affected fat digestibility with the highest level in the S30/M30 diet and the lowest level with the S50/M50 diet.

Higher total tract digestibility of starch was obtained with the control diet (97.21 ± 0.17%) and M-based diets (97.17 ± 0.14%) compared with the S-based diets (95.66 ± 0.22%). Total tract digestibility of starch in S/M-based diets was intermediate (96.28 ± 0.16%) and not different from the control diet. The level of substitution of millet tended to affect total tract digestibility of starch whereas a significant reduction was observed when the level of substitution increased in S and S/M-based diets.

The treatment significantly affected AMEn with the highest values obtained with M-based diets (13.38 ± 0.05 MJ/kg DM). Results observed with the control diet (13.26 ± 0.07 MJ/kg DM) were similar to S- (13.11 ± 0.03 MJ/kg DM) and S/M-based diets (13.29 ± 0.05 MJ/kg DM). AMEn tended to decrease ($P = 0.06$) with the level of substitution in sorghum-based diets. No dose–response effect was found in millet or when this latter was mixed with sorghum.

Discussion

Partial or total substitution of corn by sorghum and millet, and soybean meal replacement by cottonseed meal had no significant effect on broiler's feed intake and growth performance. This is in accordance with reports of Jacob *et al.* (1996), Hidalgo *et al.* (2004) and Azman and Yilmaz (2005). However, these results are not in agreement with those reported by Jacobs and Parsons (2013) who observed a reduction in feed efficiency with sorghum-based diets, and related it to the feed particle size, which induces a higher feed intake. However, the present findings confirm the results obtained with the meta-analysis (Batonon-Alavo *et al.*, 2015) in which no significant difference in feed intake was observed between sorghum- or millet-based diets and corn-based diets, and between cottonseed meal and control diets. However, sorghum-based diets increased FCR in Experiment 1 while no difference was found between the control- and sorghum-based diets in Experiment 2. In both experiments, there were differences between formulated and calculated ME and CP contents in all treatments (Tables 2 and 3). This is because estimated ME and CP contents of major feed ingredients were used in formulating diets and these differ from determined CP contents and recalculated ME contents based on the laboratory determination of starch, sugar and fat. The trend of higher feed consumption in sorghum-based diets in Experiment 1 could then be related to their lower recalculated ME content (11.9 MJ/kg) compared with the control diet (12.3 MJ/kg). In addition, sorghum-based diets were lower in analyzed fat content (39.3 ± 1.65 g/kg) than the control diet (53.4 g/kg) in Experiment 1. Since bird tend to decrease its consumption with the level of energy in the diet (Pérez-Bonilla *et al.*, 2012) it is possible that ME and fat contents contributed to explain the increased FCR observed in sorghum-based diets in Experiment 1 instead of the sorghum effect *per se*. In addition, there was no difference in lysine intake and Met + Cyst intake. Therefore, no deficiency in these amino acids seems to exist with young birds.

Another aim of this study was to evaluate the effects of simultaneous inclusion of sorghum and cottonseed meal or millet on broiler performance and nutrient digestibility. Most substitution studies involved only one ingredient and interactions are therefore not studied (Hidalgo *et al.*, 2004; Azman and Yilmaz, 2005). The present results showed that a combination of cottonseed meal and sorghum in the same diet did not decrease bird performance. Simultaneous replacement of corn by sorghum and millet also resulted in equivalent performance as the control diet. However, significant differences were observed in AID of protein and energy between feed ingredients, with the cottonseed meal- and sorghum/cottonseed meal-based diets having lower protein and energy digestibility than corn-based diets (Experiment 1). These results are consistent with those reported by Li *et al.* (2012) and Gonzalez-Vega and Stein (2012) who observed a decline in nitrogen and energy digestibility of growing and finishing pigs fed with various sources of cottonseed meal. Free gossypol in cottonseed meal is known to have an inhibitory action on certain enzymes in bird gastrointestinal tract like pepsinogen, pepsin and trypsin by binding with their free epsilon amino groups of lysine, thus reducing protein digestibility (Sharma *et al.*, 1978). However, in this study free gossypol levels were too low (5 to 13 ppm) to induce any detrimental effect on growth performance (Nagalakshmi *et al.*, 2007); but the effect of free gossypol could not be excluded from the reduction of digestibility with cottonseed meal-based diets. Similarly, lower total tract digestibility of fat and starch and lower AMEn values were obtained with sorghum-based diets consistently with previous reports (Selle *et al.*, 2010; Mahmood *et al.,* 2014) where digestibility coefficients were found inferior in sorghum than in other cereals due to the presence of phytate and phenolic compounds. Despite the lower fat and starch digestibility, our results suggest that birds fed low tannin sorghum-based diets were able to demonstrate equivalent performance as the control group since protein was equivalently available for both groups. Birds in millet-based diets have the same ability as those in corn-based diets to metabolize the energy and have a good feed efficiency. Higher coefficients of digestibility were obtained in millet-based diets consistently with Yin *et al.* (2002) and Baurhoo *et al.* (2011) and might be the factors leading to equivalent performance to the control diets.

Slaughtering method can have an effect on the results when digestibility is estimated by collection in the digestive tract. It should be noted that in the current study, birds were killed by cervical dislocation. Despite a debate on the best method of slaughtering the birds (i.e. cervical dislocation *v.* CO_2 asphyxiation) in ileal collection, Poureslami *et al.* (2012) suggest that cervical dislocation, by the means of cutting off

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the nervous stimulation from the brain to the gut, might actually slow or stop peristalsis sooner and therefore is more advantageous than asphyxiation slaughtering method.

The results obtained in the two experiments also suggest that the level of substitution of the experimental feed ingredients does not affect broiler performance, thus confirming the meta-analytic findings (Batonon-Alavo *et al.*, 2015). Corn can be partially replaced by sorghum (Experiment 1) or totally substituted by sorghum or millet without any damages on bird performance consistently with several authors (Davis *et al.*, 2003; Manwar and Mandal, 2009). Likewise, soybean meal substitution at various levels by cottonseed meal did not impact bird's response. However, the present study demonstrated that nutrient digestion can be compromised with these feed ingredients. The inclusion of exogenous enzymes in poultry diets is an established practice to improve performance and proteases are of particular relevance since they can be used to reduce the levels of trypsin inhibitors and lectins, thus improving protein digestibility (Bedford and Partridge, 2010). Other feed processing technologies like hydrothermal processes combined with reducing agents may enhance the solubility and digestibility of sorghum protein by either cleaving disulphide linkages or preventing their formation (Liu *et al.*, 2013). Consequently, further investigations are required on the best strategies to enhance the nutritional value of these feed ingredients and lead to an increasing utilization in poultry.

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