Blood Parameters, Digestive Organ Size and Intestinal Microflora of Broiler Chicks Fed Sorghum as Partial Substitute of Corn

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Bulletin UASVM Animal Science and Biotechnologies 74(2)/ 2017

Print ISSN 1843-5262; Electronic ISSN 1843-536X

DOI:10.15835/buasvmcn-asb: 0026

ABSTRACT

A study was conducted to evaluate the effects of dietary white sorghum (WS), as partial substitute of corn on blood parameters, digestive organ size and intestinal microflora of broilers at 35 d. Cobb 500 broilers (n=400), assigned to 2 groups, were fed with isocaloric and isonitrogenous corn-soybean meal control diets (C) or corn-WS-soybean meal diets (WS), where corn was partially replaced (50%) with WS. At 35 d, sixteen broilers per group were selected for blood sampling, gastrointestinal (GIT) measurements, digesta pH and ileum microflora analysis. There was no effect ($P>0.05$) of the dietary WS inclusion on blood biochemistry parameters, GIT development and digesta pH of broilers. In our study, dietary WS significantly reduced the ileal population of *Enterobacteriaceae* (-1.38%; $P<0.0001$) and *E. coli* (-1.16%; $P=0.020$), and increased the *Lactobacillus* spp. (+1.07%; $P=0.014$) compared with the C diet. In conclusion, feeding white sorghum, as partial substitute of corn in broiler diets, did not affect blood parameters, digestive organ size and digesta pH, that support the obtained performance. In addition, sorghum had a positive effect of the ileal microflora increasing the beneficial bacterial *Lactobacillus* spp.

Keywords: broiler, sorghum, blood parameters, gastrointestinal development, ileum microflora

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Monch) is the fifth most important crop in the world (Bryden *et al.*, 2009), with a production of 67.8 million tons in 2014 (FAOSTAT, 2016). In the current context of climate change, it is expected that in the near future heat resistant crops such as sorghum are routinely utilized as food and feed resources for humans and animals (Selle *et al.*, 2010; García *et al.*, 2013). Sorghum is an interesting energy ingredient in poultry diets due to its similar nutritional composition to corn (Leeson and Summers, 2005). Previous study reported that the total or partial replacement of corn with sorghum is a viable option from the first week of life (Torres *et al.*, 2013). Some studies showed that sorghum-based diets impair broiler performance (Jacobs and Parsons, 2013), or had a negative effect

on the intestinal mucosa (Torres *et al.*, 2013), whereas others indicated similar performance as the control diet (Campos *et al.*, 2007; Bozutti, 2009; Fernandes *et al.*, 2013; Tandiang *et al.*, 2014). However, there is some resistance to this replacement, especially in the starter phase, due to the possible presence of antinutritional factors in sorghum grain, such as condensed tannin, which affect broiler performance (García *et al.*, 2013; Tandiang *et al.*, 2014). After hatching the digestive tract of broilers is still not fully developed, but it quickly matures, considering the short life span of these birds (Uni *et al.*, 1998). Understanding broiler digestive morphology and function is essential to obtain maximum growth performance. Dietary manipulation has been shown to be one of the major factors in influencing microbial activity in the gastrointestinal tract of birds,

although little information is available about the main mechanisms regulating the equilibrium of microbial ecology in the gut. Plasma biochemistry, gastrointestinal parameters and intestinal microflora are important markers of health status and nutrients metabolism in the organism (Lokesh *et al.*, 2012). The aim of this study was to evaluate the effects of dietary white sorghum as partial substitute of corn on the blood biochemical parameters, digestive organ size, digesta pH and ileum microflora of broiler chickens.

MATERIALS AND METHODS

Broilers and dietary treatments. The study protocol was approved by the Animal Care Committee of the National Research-Development Institute for Animal Biology and Nutrition (Balotesti, Romania). Birds were treated in accordance with Romanian law no. 305/2006 and EU Directive 2010/63/EU regarding handling and protection of animals used for scientific purposes (OJEU, 2010).

A total of 400 broilers (Cobb 500; 40.05±1.15

Tab. 1. Ingredient and nutrient composition of broiler diets for grower phases

Ingredients (%)	Starter (1-10 d)		Grower (11-22 d)		Finisher (23-35 d)	
	C ¹	WS ²	C	WS	C	WS
Corn	56.00	27.54	60.00	30.70	65.76	33.27
White sorghum	-	27.54	-	30.70	-	33.27
Soybean meal	31.00	30.00	26.90	25.00	22.00	21.00
Corn gluten meal	5.00	5.93	4.60	5.00	3.60	3.50
Sunflower oil	2.80	3.78	3.50	3.60	4.00	4.30
Monocalcium phosphate	1.82	1.79	1.70	1.62	1.50	1.46
Calcium carbonate	1.47	1.48	1.39	1.40	1.27	1.28
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin-mineral premix ³ with coccidiostatic	1.00	1.00	1.00	1.00	-	-
Vitamin-mineral premix ⁴ , without coccidiostatic	-	-	-	-	1.00	1.00
DL-methionine	0.15	0.15	0.20	0.23	0.19	0.21
L-lysine HCl	0.40	0.43	0.35	0.39	0.32	0.35
Choline HCl	0.06	0.06	0.06	0.06	0.06	0.06
Calculated composition						
ME (MJ/kg) ⁵	12.61	12.62	12.97	12.98	13.31	13.34
CP	22.01	22.06	20.08	20.03	18.02	18.04
Lysine, total	1.32	1.32	1.19	1.19	1.05	1.05
Lysine, digestible	1.18	1.18	1.05	1.05	0.95	0.95
Met + cys, total	0.98	0.98	0.89	0.89	0.82	0.82
Met + cys, digestible	0.88	0.88	0.80	0.80	0.74	0.74
Calcium	0.90	0.90	0.84	0.84	0.76	0.76
Available phosphorus	0.45	0.45	0.42	0.42	0.39	0.39
Crude fiber	3.54	3.43	3.31	3.37	3.04	3.16
Ether extract	5.67	6.08	6.38	6.26	7.01	7.06

¹Control; ²White Sorghum

³Supplied per kg diet: retinyl acetate, 4.47 mg; cholecalciferol, 0.12 mg; DL- α -tocopheryl acetate, 80 mg; menadione sodium bisulphite, 4 mg; thiamine mononitrate, 4 mg; riboflavin, 9 mg; pyridoxine-HCl, 4 mg; cyanocobalamin, 0.020 mg; Ca-panthotenate, 15 mg; niacin, 60 mg; folic acid, 2 mg; Mn, 100 mg; Zn, 100 mg; Fe, 40 mg; Cu, 15 mg; I, 1.0 mg; Se, 0.30 mg; Co, 0.25 mg, lasalocid sodium, 60 mg (Avatec, Alpharma Inc., USA).

⁴Supplied per kg diet: retinyl acetate, 2.90 mg; cholecalciferol, 0.12 mg; DL- α -tocopheryl acetate, 50 mg; menadione sodium bisulphite, 3 mg; thiamine mononitrate, 2 mg; riboflavin, 8 mg; pyridoxine-HCl, 3 mg; cyanocobalamin, 0.015 mg; Ca-panthotenate, 12 mg; niacin, 50 mg; folic acid, 1.5 mg; Mn, 100 mg; Zn, 100 mg; Fe, 40 mg; Cu, 15 mg; I, 1.0 mg; Se, 0.30 mg; Co, 0.25 mg.

⁵calculated using regression equations (NRC, 1994).

g), obtained from a commercial hatchery, were individually weighed, distributed in 8 floor pens (2.5 m x 0.8 m) on litter (wood shavings) and raised in an environmentally controlled house. The pens were assigned to 2 dietary treatments, with 4 replicates per treatment (50 broilers/replicate). Chickens vaccination was carried out according to the usual schedule. Lighting schedule was 23h light:1h darkness.

The broilers were subjected to a 3-phase feeding regimen consisting of starter (1 to 10 d), grower (11 to 22 d) and finisher phases (23 to 35 d). Broilers were fed 2 isocaloric and isonitrogenous diets: a control diet (C) based on corn-soybean meal and an experimental diet (WS) that contained white sorghum 27.54% (starter), 30.70% (grower) and 33.27% (finisher), as partial substitute of corn. The diets were formulated to have similar content of sulphur amino acids (met+cys), lysine, calcium and phosphorus (Cobb-Vantress, 2015). The proximate composition of feed ingredients and diets (dry matter, crude protein, crude fiber, ether extract) was analysed using standardized methods (OJEU, 2009). The chemical composition of WS was 87.15% dry matter, 9.91% CP, 3.20% crude fat, 2.56% crude fiber, 3207 kcal/kg ME, 0.266% lysine, 0.344% met+cys., 0.457% threonine, 0.387% arginine. Ingredients and nutrient composition of diets are shown in Table 1. Feed and water were provided *ad libitum*. During 12 h prior slaughter feed was withdrawn.

Sampling and blood analyses. At 35 d of age, sixteen broilers per treatment were randomly selected for blood sampling and gastrointestinal tract (GIT) measurements. Blood samples were collected in heparinized tubes (4 mL) from wing vein (Vacutest®, Italy). The samples were subsequently stored in ice, centrifuged at 2500 x g for 10 min at 4°C (Multifuge 3L-R, Heraeus, Hanau, Germany), and plasma was stored in Eppendorf tubes at -20 °C until analyses. Plasma concentrations of total protein, albumin, total bilirubin, creatinine, urea, glucose, cholesterol, triglycerides, and the activity of alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH) and creatine kinase (CK) were determined on a chemistry analyzer (BS-130, Bio-Medical Electronics Co., China), using commercial kits

Accent-200 MG (Cormay, Wiosenna, Poland).

The broilers were killed by cervical dislocation and immediately bled. Carcasses were eviscerated manually, and the GIT was excised. The absolute weights of the gizzard, liver, pancreas, spleen, duodenum, jejunum, ileum, and cecum and the absolute lengths of the duodenum, jejunum, ileum, and cecum were measured. The jejunum was defined as the portion between the end of the duodenum and the region immediately before Meckel's diverticulum, and the ileum was defined as the portion between Meckel's diverticulum and the region immediately before the ileocecal junction. The results were expressed as relative digestive organ weight (g/kg body weight, BW) and relative length of intestinal tract (cm/kg BW).

pH measurement. The pH of the fresh digesta were determined at the terminal end of each intestinal compartment. Samples of digesta from the ileum were collected aseptically in sterilized plastic tubes and preserved at - 20°C until bacteriological analyses. The intestinal digesta pH measurements were performed with a portable pH meter (WTW ProfiLine pH 3310, WTW GmbH, Weilheim, Germany) by inserting the pH meter electrode (WTW Sentix 41, WTW GmbH, Weilheim, Germany) into the distal sections of the duodenum, jejunum, ileum and caecum.

Microbial population determination. Microbial populations from ileal content samples were determined by counting the colonies on selected media for each microorganism. Briefly, the classical isolation medium G.E.A.M. or Levine were used to determine the *Enterobacteriaceae* and *E. Coli*, *Lactobacillus* was enumerated on De Man-Rogosa-Sharpe (MRS) agar and Rambach-*Salmonella* agar was used to determine the presence or absence of *Salmonella* spp. The results were expressed as a logarithm (base 10) of colony-forming units per gram of sample.

Statistical analysis. The data obtained were analysed using the GLM procedure of the software SPSS (IBM SPSS Statistics version 20.0, 2011). One-way analysis of variance (ANOVA) with the Tukey's comparison test was used to evaluate statistical significance of differences between dietary treatments. The results are given as means and standard error of the mean (SEM). Differences were considered significant at $P \leq 0.05$. For blood biochemistry and GIT parameters each bird was considered as the experimental unit. All

Tab. 2. Effect of dietary sorghum on plasma metabolic profile of broilers at 35 d of age¹

Plasma profile	Parameter	C		WS		P-value
		Mean	SEM	Mean	SEM	
Protein	Total protein (g/dL)	2.35	0.14	2.54	0.18	0.274
	Albumin (mg/dL)	1.43	0.17	1.52	0.19	0.515
	Total bilirubin (mg/dL)	0.39	0.05	0.46	0.08	0.107
	Creatinine (mg/dL)	0.65	0.09	0.71	0.05	0.451
	Urea (mg/dL)	6.95	0.84	8.29	0.41	0.168
Energy	Glucose (mg/dL)	196.77	8.00	199.88	6.67	0.770
	Cholesterol (mg/dL)	99.87	4.59	95.89	4.51	0.554
	Triglycerides (mg/dL)	48.33	2.82	50.32	3.72	0.683
Enzymatic	AP (U/L)	41.89	1.94	43.92	4.04	0.659
	AST (U/L)	53.19	3.00	59.55	2.04	0.125
	ALT (U/L)	46.98	2.96	55.85	2.43	0.452
	GGT (U/L)	38.80	2.66	34.77	1.34	0.216
	LDH (U/L)	472.08	26.77	498.16	24.04	0.112
	CK (U/L)	682.94	64.86	570.35	94.43	0.378

C - Control; WS - White Sorghum; SEM - standard error of mean.

¹n=16 samples for each dietary treatment.

microbiological concentrations were subject to base-10 logarithm transformation before analysis.

RESULTS AND DISCUSSIONS

Performance. Dietary sorghum as partial substitute of corn had no significant effect ($P>0.05$) on broilers growth performance (weight gain, feed intake and feed conversion ratio) during overall period (data not shown).

Plasma biochemistry. Effect of dietary sorghum on plasma metabolic profile of broilers at 35 d of age are shown in Table 2. Our results indicate that plasma protein and energy profile was not significantly ($P>0.05$) affected by the dietary inclusion of sorghum as partial replace for corn. It is known that these plasma profile are important indicators of protein and carbohydrate metabolism in organism, also are related with protein synthesis and lipogenesis process (Swennen *et al.*, 2006). Similarly, with our findings, Manwar and Mandal, (2009) studied the effect of sorghum with or without enzymes on production performance and immunocompetence in broiler chicken, and stated that the plasma biochemical parameters such as total cholesterol, triglycerides and uric acid were not affected by the dietary treatments.

The liver is rich in certain enzymes, such as ALT, AST and LDH, and damage often results in abnormally increased concentrations of these enzymes in the

blood (Kaplan *et al.*, 2003). The dietary treatments did not affect ($P>0.05$) plasma enzymes (AP, AST, ALT, GGT, LDH or CK) values (Table 2) and shown that dietary sorghum had no negative effects on biomarkers of liver and kidney function. Kumar *et al.* (2007) suggested that feeding sorghum based diets to broiler chickens did not significantly affect nutrient utilisation, blood biochemicals profile and enzymes, and also, did not cause pathological changes in organs tissues (liver and kidney).

Gastrointestinal development and health. There were no significant dietary treatments effect ($P>0.05$) on relative digestive organ weight and length of the intestinal tract at 35 d of age (Table 3). The results of the present study agree with other research who reported that dietary tannins from sorghum did not affect digestive organ weights (Ahmed *et al.*, 1991; Nyachoti *et al.*, 1996; Nyamambi *et al.*, 2000, 2007) and size or growth of the small intestine in broilers (Nyamambi *et al.*, 2007). Garcia *et al.* (2005) also found no influence of diets based on corn or low and high-tannin sorghum on small intestine or cecum length of broilers. Silva *et al.* (2015) concluded that sorghum grain may replace corn in broiler diets, improving performance and not adversely affect organs weight and intestinal morphometry. Recently, Pasquali *et al.* (2016) stated that partial replacement of corn with sorghum did not affect relative organs weight or small intestine length at 42 d post hatch.

Tab. 3. Effect of dietary sorghum on relative digestive organ weight, relative length of the intestinal tract and intestinal digesta pH of broilers at 35 d of age¹

Item	C		WS		<i>P-value</i>
	Mean	SEM	Mean	SEM	
<i>Weight (g/kg BW)</i>					
Gizzard	15.19	0.73	15.49	0.56	<i>0.751</i>
Liver	21.17	0.81	21.02	0.83	<i>0.896</i>
Pancreas	2.21	0.08	2.40	0.17	<i>0.353</i>
Spleen	1.01	0.04	0.84	0.06	<i>0.143</i>
Duodenum	6.65	0.08	6.60	0.11	<i>0.780</i>
Jejunum	15.35	0.51	15.22	0.14	<i>0.811</i>
Ileum	10.99	0.38	10.09	0.73	<i>0.310</i>
Caecum	6.68	0.17	5.86	0.19	<i>0.111</i>
<i>Length (cm/kg BW)</i>					
Duodenum	14.41	0.66	14.39	0.39	<i>0.978</i>
Jejunum	38.68	0.59	36.30	1.53	<i>0.186</i>
Ileum	37.47	0.97	36.83	0.40	<i>0.560</i>
Caecum	8.79	0.15	8.40	0.24	<i>0.243</i>
pH					
Duodenum	5.70	0.057	5.59	0.058	<i>0.125</i>
Jejunum	6.02	0.058	5.91	0.064	<i>0.216</i>
Ileum	6.25	0.087	6.17	0.061	<i>0.366</i>
Caecum	6.70	0.061	6.56	0.034	<i>0.542</i>

C - Control; WS - White Sorghum; SEM - standard error of mean.

¹n=16 samples for each dietary treatment.

Contrary, Fernandes *et al.* (2013) observed that the inclusion of different sorghum particle size (partially ground or whole grain) in the broiler diets significantly increase gizzard and small intestine weight, which could be explained due to a greater development of the intestinal mucosa (Tarachai and Yamauchi, 2000).

The pH in the digestive tract segments is very important in protein digestion as it affects the processing of endogenous proteases into their mature form, but also the solubility of the dietary proteins (Recoules *et al.*, 2017). Our results shown that the value of intestinal digesta pH (Table 3) was similar in broilers fed the WS compared with C diet ($P>0.05$) and as expected, the pH value increased in the different segments of the digestive tract from 5.59 (duodenum) to 6.56 (caecum). According to Ao *et al.* (2008) the dietary pH for monogastric animals vary between 5.5 and 6.5, and changes as digesta transit different segments of the GIT.

The efficient feed utilisation of broiler chickens could be associated with changes in the intestinal functions, such as microbial population

(Zulkifli *et al.*, 2009). In our study, the inclusion of sorghum in chicken diets significantly reduced the ileal population of *Enterobacteriaceae* (-1.38%; $P<0.0001$) and *E. coli* (-1.16%; $P=0.020$), and increased *Lactobacillus spp.* (+1.07%; $P=0.014$) compared with control diet (Table 4). The samples of ileal digesta was negative for *Salmonella spp.* It is known that the microbes inhabiting the GIT play pivotal role in digestion, metabolism, growth and overall health of the birds (Torok *et al.*, 2011). Despite their beneficial effects, these microbes also compete for energy and nutrients utilization with the host-animal. However, from our knowledge little scientific literature are available about the effect of dietary sorghum on microbial population of ileal content of broilers for comparison. Recently, Fagundes *et al.* (2017) concluded that replacing corn with sorghum in broiler diets alters the intestinal microbiota, by lowering the counts of *Clostridium* and increasing the *Lactobacillus* population in the small intestine and caeca, without adverse effect of broilers

Tab. 4. Effect of dietary sorghum on colony-forming units of bacterial species (Log10 cfu) per gram of ileal content of broilers at 35 d of age¹

Item	C		WS		P-value
	Mean	SEM	Mean	SEM	
Enterobacteriaceae	7.212 ^A	0.008	7.112 ^B	0.003	<0.0001
E. coli	5.608 ^a	0.011	5.543 ^b	0.017	0.020
<i>Lactobacillus</i> spp.	6.383 ^b	0.009	6.452 ^a	0.018	0.014
Salmonella	absent	-	absent	-	-

C - Control; WS - White Sorghum; SEM - standard error of mean.

^{a,b}Means within a row with no common superscript differ significantly (P< 0.05).^{AB}Means within rows with different superscripts are significantly different (P< 0.01).¹n=16 samples for each dietary treatment.

performance.

CONCLUSION

In conclusion, feeding white sorghum, as partial substitute of corn in broiler diets, did not affect blood parameters, digestive organ size and digesta pH, that support the obtained performance. In addition, sorghum had a positive effect of the ileal microflora increasing the beneficial bacterial such as *Lactobacillus* spp.

Acknowledgements. This work was supported by funds from the Romanian Ministry of Research and Innovation, National Project PN 16 41 01 05.

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