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Essential oils and prebiotic on broiler diets as feed additives

Óleos essenciais e prebióticos como aditivos em dietas para frangos de corte

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Highlights:
Broilers fed diets with reduced nutritional levels exhibited a reduction in performance;
Essential oils were similar to antibiotics in terms of improving efficiency of broiler performance;
Broilers fed essential oils and fructooligosaccharides had higher villus heights in the jejunum.

Abstract

The objective of this study was to evaluate the use of a combination of essential oils, prebiotics and antibiotics on broiler production. The treatments consisted of a normal diet meeting the nutritional requirements, and diets with a 5% reduction of nutritional requirements, supplemented or not with antibiotics (En+Sal) (10 g ton^{-1} enramycin and 125 g ton^{-1} salinomycin) or a blend of essential oils (EOFOS) (oregano, anise and lemon) plus fructooligosaccharides (125 g ton^{-1}). A total of 1,152 male one-day-old broilers were randomized by weight and distributed into a 2*3 factorial design, consisting of six treatments with each treatment containing eight replicates of 24 birds per experimental unit. The Student-Newman-Keuls test was performed at 5% probability. Broiler performance was evaluated at 10, 21 and 42 days, intestinal morphometry was evaluated at 21 days, microbiological cecum counts at 33 days, blood parameters at 35 days and carcass and cuts yield at 42 days of age. Broilers fed reduced diets had a reduction in performance ($P < 0.05$). There was an interaction ($P < 0.05$) between diet type and prebiotics for jejunum villus height and villus height:crypt depth ratios for all intestinal segments. There was no effect ($P > 0.05$) of growth promoters and diet type on the *Lactobacillus* and *Bifidobacterium* populations within the gastrointestinal tract. There were no differences for duodenum or ileum villus heights ($P > 0.05$). Broilers fed EOFOS showed higher villus height in the jejunum at 21 days. There were no effects on blood parameters of different diets and growth promoters ($P > 0.05$), nor on carcass and cuts yield, abdominal fat and relative liver weight ($P > 0.05$). Broilers receiving the normal diet with EOFOS showed higher villus height in the jejunum at 21 days when compared to the normal diet with antibiotics and the normal diet without growth promoters. However, there were no effects of growth promoters and diet type on broiler performance at 42 days.

Key words: Diets. Growth promoter. Intestinal morphology. Poultry production.

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Resumo

O objetivo com este estudo foi avaliar o uso de uma combinação de óleos essenciais, prebióticos e antibióticos na produção de frangos de corte. Os tratamentos consistiram em uma dieta normal que atende as exigências nutricionais e uma dieta com redução de 5% das exigências nutricionais suplementada ou não com antibióticos (En+Sal) (10 g ton^{-1} enramicina e 125 g ton^{-1} salinomicina) ou um blend de óleos essenciais (EOFOS) (orégano, anis e limão) + frutooligossacarídeos (125 g ton^{-1}). Um total de 1.152 frangos machos de um dia de idade foram casualizados no peso e distribuídos em um arranjo fatorial 2*3, consistindo em seis tratamentos com cada tratamento contendo oito repetições de 24 aves por unidade experimental. Os dados foram analisados pelo SAS - Versão 9.1, o teste Student Newman Keuls foi realizado a 5% de probabilidade. Aos 10, 21 e 42 dias, avaliou-se o desempenho dos frangos, a morfometria intestinal aos 21 dias, contagem microbiológica de ceco aos 33 dias, parâmetros sanguíneos aos 35 dias e rendimento de carcaça e cortes aos 42 dias de idade. Os frangos alimentados com dietas com redução nas exigências apresentaram uma redução no desempenho ($P < 0.05$). Ocorreu interação ($P < 0.05$) entre tipo de dieta e promotores de crescimento para a altura de vilo no jejuno e para a relação vilo:cripta em todos os segmentos intestinais. Não ocorreu efeito dos promotores de crescimento e tipo de dieta na população de *Lactobacillus* e *Bifidobactérias* do trato gastrointestinal. Não ocorreu diferença na altura de vilos no duodeno ($P > 0.05$). Aves alimentadas com EOFOS demonstraram maior altura de vilos no jejuno aos 21 dias. Não houve efeitos nos parâmetros sanguíneos de diferentes dietas e promotores de crescimento ($P > 0.05$), nem no rendimento de carcaça e cortes, gordura abdominal e peso relativo do fígado ($P > 0.05$). Frangos de corte que receberam dieta normal com EOFOS apresentaram maior altura das vilosidades no jejuno aos 21 dias quando comparados à dieta normal com antibióticos e à dieta normal sem promotores de crescimento. Entretanto, não houve efeito dos promotores de crescimento e tipo de dieta no desempenho dos frangos aos 42 dias.

Palavras-chave: Avicultura. Dietas. Morfologia intestinal. Promotor de crescimento.

Introduction

Antibiotics stabilize the gastrointestinal tract microbiota by limiting the growth of pathogenic microorganisms and their toxins, in addition to promoting the growth of beneficial microorganisms. Also, they can inhibit the effects caused by the recruitment of inflammatory intestinal cells, then redirect to the animal growth (Broom, 2018). However, with recent concerns for antibiotic resistance in human health, alternatives to antibiotics are becoming necessary (Brito et al., 2013). These concerns are based on the evidence that indiscriminate use of antibiotics can promote the emergence of bacteria that are resistant to the active ingredients of these additives, which can then be transferred to other organisms (Oguttu, Veary, & Picard, 2008). In response, several countries, mainly those of the European Union, have started a process of withdrawing antibiotics in animal production.

Alternative growth promoters should favor gastrointestinal tract integrity in poultry, which is critical for high performance and overall health of birds. The intestinal microflora of birds is also important for obtaining high productivity through improved intestinal health, performance and control of enteric pathogens (Leite, Mendes, Pereira, Lima, & Lacerda, 2012). Prebiotics show potential as an alternative to antibiotics in feed. Prebiotic is a term used to designate one or more feed ingredients that are not digested by normal digestive enzymes synthesized by the bird, but rather stimulate the growth and activity of beneficial intestinal bacteria. Some of the substances that have been studied as prebiotic feed additives include oligosaccharides, fructooligosaccharides (FOS), glucooligosaccharides (GOS), and mannanoligosaccharides (MOS) (Ricke, Lee, Kim, Park, & Shi, 2020).

Essential oils have been shown to have antimicrobial potential (Costa, Tse, & Miyada, 2007; Santurio et al., 2007), in addition to stimulating pancreatic and digestive enzymes (Jang, Ko,

Kang, & Le, 2007), as well as an animal's immune response (Mellor, 2000). These feed additives may alter the enteric microbiota, making it more stable and beneficial. Essential oils have the potential to modulate the intestinal microbiota, mainly when used in combination with other products (Leite et al., 2012).

The objective of this study was thus to evaluate the use of a combination of essential oils and prebiotics on performance, intestinal morphology, cecal microbiota, blood parameters, and carcass and cut yields in broilers.

Material and Methods

This study was conducted at the Poultry Sector of the Experimental Station of Western Paraná State University (UNIOESTE), Campus Marechal Cândido Rondon - Paraná, Brazil. Birds were handled with care to avoid unnecessary discomfort, and all experimental procedures were approved by the university's ethical review committee (n.º 63/10).

A total of 1,152 male, one day old (Cobb x Cobb 500) broilers were obtained from a commercial hatchery on the day of hatch. Birds were reared in a floor pen (experimental unit [EU]) consisting of a tubular feeder, a nipple drinker, a heat source (250-watt infrared lamps), first-use pine wood shavings. Chicks were randomized by weight and distributed into a 2*3 factorial design (two diets and three dietary treatments), consisting of six treatments with each treatment containing eight replicates of 24 birds per EU.

Treatments consisted of; 1) 'Normal En + Sal' - meeting the nutritional requirements of each phase, with antibiotics addition (10g ton^{-1} enramycin and 125 g ton^{-1} salinomycin); 2) 'Normal EOFOS' - meeting the nutritional requirements of each phase, with addition of essential oils (EO) (blend of oregano, anise, lemon) + fructooligosaccharides (FOS) (inulin) (125 g ton^{-1} of feed); 3) 'Normal PF' - meeting the nutritional requirements of each phase, without feed additives (Promoter Free - PF); 4) 'Reduced En + Sal' - formulated with 5% reduction of the nutritional requirements and antibiotics addition (10 g t^{-1} enramycin and 125 g t^{-1} salinomycin); 5) 'Reduced EOFOS' - formulated with 5% reduction of nutrient requirements with addition of essential oils (blend of oregano, anise, lemon) + FOS (inulin) (125 g^{-1} ton of feed); 6) 'Reduced PF' - formulated with 5% reduction of nutritional requirements without feed additives. The blend of essential oils and prebiotic was provided by the company Biomin® and was supplied according to the recommendations, replacing the inert feed material.

All diets were fed in mash form and provided *ad libitum*. The experimental diets were based on corn and soybean meal and formulated according to Brazilian Tables for Poultry and Swine (Rostagno et al., 2011), for high-performance male broilers. A pre-starter diet was fed from 1–7 d, a starter from 8–14 d, a grower from 15–21 and finisher from 22–42 d (Table 1).

Weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) were determined on days 10, 21 and 42. Mean individual bird weights and feed intake was calculated and corrected for mortality, which was recorded daily, according to Sakomura and Rostagno (2016).

At 21 d of age, one broiler per EU, with a body weight of ± 5% of the average pen weight, was euthanized by electronarcosis followed by exsanguination according to CONCEA Normative Resolution No. 37 of February 15, 2018. Duodenum, jejunum, and ileum sections were collected for intestinal morphology analysis by histology. Intestinal segments about 5 cm in length were carefully collected, washed with saline solution and fixed in 10% formalin for seven days. The formalin was then replaced by 70% alcohol. For sample dehydration, they were passed through successive baths of 80 and 90% alcohol, and three baths in 100% alcohol. Subsequently, they were subjected to a xylol bath and then placed in histological paraffin (Luna, 1968). The sections were made with microtomes of 7 μm thickness and stained with

Hematoxylin-Eosin. Ten villi and ten crypts were measured per replicate, with 10x magnification for villi and 20x magnification for crypts. The mean intestinal segment villus height, crypt depth, and villus:crypt ratio were calculated for each animal.

Table 1
Composition and calculated nutrient levels of diets used for broilers in each phase

Ingredients g kg^{-1}	Pre-starter		Starter		Grower		Finisher	
	Normal	Reduced	Normal	Reduced	Normal	Reduced	Normal	Reduced
Corn (7.8%)	538.0	599.1	567.3	633.2	595.6	668.7	637.1	709.2
Soybean meal (45%)	388.6	350.0	354.4	320.0	318.5	284.0	278.6	245.0
Soybean oil	26.2	5.0	35.2	5.0	45.1	7.9	44.8	7.5
Dicalcium phosphate	19.3	18.2	18.4	17.3	16.9	15.8	15.4	14.4
Limestone	9.3	9.1	9.0	8.8	8.5	8.3	8.1	7.9
Salt	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Inert[1]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
DL-Methionine (98%)	3.7	3.4	2.7	2.4	2.5	2.2	2.4	2.1
L - Lysine HCL	3.3	3.6	2.1	2.4	2.1	2.3	2.6	2.9
L – Threonine (99%)	1.3	1.3	0.6	0.6	0.5	0.5	0.7	0.7
Vit. and Min. premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Choline chloride (60%)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Antioxidant	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Nutrients g kg^{-1}								
Met. En. (kcal kg^{-1})	2.960	2.812	3.050	2.897	3.150	2.992	3.200	3.040
Crude protein	228.0	216.6	211.4	200.8	193.7	187.4	183.1	173.9
Calcium	9.4	8.9	9.0	8.5	8.4	7.9	7.7	7.6
Av. P	4.7	4.5	4.5	4.3	4.2	4.0	3.9	3.7
Sodium	2.2	2.1	2.2	2.1	2.1	2.0	2.0	1.9
Potassium	5.9	5.6	6.0	5.7	6.0	5.7	6.0	5.6
Dig. Arginine	14.3	13.6	12.5	11.9	11.5	11.0	11.0	10.4
Dig. Lysine	13.6	12.9	11.9	11.3	11.0	10.4	10.5	10
Dig. Met+Cis	9.7	9.2	8.4	8.0	7.9	7.5	7.5	7.2
Dig.Threonine	8.9	8.4	7.7	7.3	7.1	6.8	6.8	6.5
Dig. Tryptophan	2.2	2.1	1.9	1.8	1.9	1.8	1.8	1.7
Dig. Valine	10.2	9.7	8.9	8.5	8.5	8.0	8.1	7.7

[1]Sand. 2Composition per kg of feed: vit. A - 9000 IU. vit. D3 - 2500 IU. vit. E - 20 IU. vit K3 – 2500 mg. vit. B1 – 1500 mg; vit. B2 – 6000 mg; vit. B6 – 3000 mg; vit. B12 - 12000 mcg. Biotin – 60 mg. Folic acid – 800 mg. Nicotinic acid – 25000 mg. Panteonic acid – 12000 mg; Selenium – 250 mg. Manganese - 160 mg. Iron – 100 mg. Zinc - 100 mg. Copper – 20 mg. Cobalt – 2 mg. Iodine – 2 mg; Normal (meeting the nutritional requirements of each phase); Reduced (with nutritional reduction of 5% of the nutritional requirement).

At 33 d of age, two broilers per EU were selected and euthanized by electronarcosis followed by exsanguination, to collect the cecal contents. Analyzes were performed in MRS agar (De Man, Rogosa, & Sharpe) for *Lactobacillus* spp., and *Bifidobacterium* agar for *Bifidobacterium* spp. Plates were incubated and the colonies were counted after 48 hours. Results were expressed as log base 10.

At 35 d of age, two birds per EU were randomly selected and fasted for 6 h. Blood samples were collected via brachial puncture. Blood was rested for coagulation and centrifuged at 3000 rpm for 10 min to obtain serum, which was stored at -20°C (Nunes et al., 2018). The serum was analyzed for calcium, cholesterol and triglycerides using a high-performance automatic spectrophotometer (Flexor EL 200, Elitech, Paris, France) with specific kits, calibrated with standards (Elical, Elitech).

At 42 d of age, two birds per pen were randomly selected and fasted for 6 h. The eviscerated carcass weight was considered (without feet, head, neck and abdominal fat) in relation to the weight of the bird pre-slaughter. Breast, thigh, drumstick and wing yields were considered the weight of the eviscerated carcass. Abdominal fat was obtained from fat removed from the cloaca and around the gizzard. Finally, the relative liver weight (% of live weight) was determined.

Data were submitted to analysis of variance (ANOVA), and means were compared by the Student-Newman-Keuls Test (SNK) using a significance level of 5%. Analyzes were carried out in SAS version 9.1 (Statistical Analysis System Institute [SAS Institute], (2011).

Results and Discussion

There were no significant effects ($P > 0.05$) of the different growth promoters and the nutritional composition of diets on broiler body weight (BW) or WG at 10 d (Table 2). There was an interaction between diet type and additives ($P < 0.05$) on FCR; broilers fed 'Normal EOFOS' showed better FCR than those fed 'Reduced EOFOS', and the same occurred to broilers fed 'Reduced PF' (without additives) and 'Normal PF' diets. Broilers fed the 'Reduced PF' diet showed the worst FCR compared to those fed 'Reduced EOFOS'.

Table 2
Performance of broilers fed diets supplemented with feed additives

	Body weight (g)		Weight gain(g)		Feed intake (g)		Feed Conv. Ratio (g g^{-1})	
				1-10 d				
	Normal	Reduced	Normal	Reduced	Normal	Reduced	Normal	Reduced
En+Sal[1]	250	248	206	204	286	284	1.38^{Aa}	1.39^{Aab}
EOFOS[2]	253	252	209	208	277	288	1.33^{Bb}	1.38^{Ab}
PF[3]	254	250	210	206	282	293	1.35^{Bab}	1.42^{Aa}
CV (%)	2.528		3.067		3.150		2.463	
				1-21 d				
En+Sal	839^{Aa}	805^{Ba}	795^{Aa}	761^{Ba}	1.211	1.243	1.530	1.640
EOFOS	847^{Aa}	819^{Ba}	803^{Aa}	775^{Ba}	1.239	1.281	1.540	1.660
PF	845^{Aa}	786^{Bb}	802^{Aa}	742^{Bb}	1.200	1.279	1.500	1.690
CV (%)	2.014		2.124		3.655		3.345	
				1-42 d				
En+Sal	2.613	2.518	2.569	2.474	4.447	4.559	1.730	1.840
EOFOS	2.679	2.597	2.635	2.553	4.480	4.717	1.700	1.850
PF	2.614	2.533	2.570	2.489	4.475	4.646	1.740	1.870
CV (%)	2.834		2.884		2.544		2.889	

Normal: meeting the nutritional requirements of each phase; Reduced: with nutritional reduction of 5% of the nutritional requirement. [1]En+Sal: 10 g ton^{-1} enramycin and 125 g ton^{-1} salinomycin; [2]EOFOS – Essential oil (blend of oregano, anise, lemon + FOS (inulin) 125 g ton^{-1} of feed; [3]PF: (promoter free), without the addition of growth promoters. Means followed by different uppercase letters in the row differ by the F test at 5% probability; Means followed by different lowercase letters in column differ by the SNK test at 5% probability.

From 1–21 d of age, there was a significant interaction between diets and growth promoters on broiler BW and WG ($P < 0.05$) (Table 2). Broilers fed normal diets did not differ in BW and WG when comparing the incorporation or not of growth promoters. On the other hand, broilers fed diets with reduced nutrient levels had a lower BW and WG than those receiving normal diets ($P < 0.05$).

In this study, even when the diet was deficient in nutrients, EOFOS was significantly similar to antibiotics (En + Sal), proving the efficiency of essential oils on broiler performance. The same was observed by Hong, Steiner, Aufy and Lien (2012), who evaluated the supplementation effect of essential oils from oregano, anise and citrus peel on broiler performance and found that dietary supplementation with essential oils improved FCR. However, the supplementation with antibiotics increased broiler WG. The WG of broilers fed EOFOS was intermediate and did not differ significantly when compared to broilers fed the reduced nutrient diets with antibiotics. There were no differences in broiler performance from 1–42 d.

After 21 d, duodenum and ileum villus height did not show significant differences between treatments ($P > 0.05$) (Table 3). In the jejunum, villi height showed a significant interaction ($P < 0.05$), with broilers that were fed 'Normal EOFOS' showing better results compared to 'Normal En + Sal' and 'Normal PF' treatments. Broilers fed treatment with 'Reduced En + Sal' and 'Reduced PF' diets showed a higher villus height than those fed normal diets.

Table 3
Intestinal morphometry of broilers fed diets supplemented with feed additives at 21 days

	Duodenum		Jejunum		Ileum	
	Villus height (µm)					
	Normal	Reduced	Normal	Reduced	Normal	Reduced
En+Sal[1]	1127.02	1236.36	785.91^{Bb}	1043.25^{Aa}	663.75	693.03
EOFOS[2]	1255.09	1179.75	1070.97^{Aa}	947.00^{Aa}	704.22	687.85
PF[3]	1213.77	1138.23	780.52^{Bb}	944.63^{Aa}	677.51	728.33
CV (%)	12.61		16.70		14.08	
	Crypts Depth					
En+Sal[1]	258.36^{Ab}	272.27^{Ab}	241.35^{Ab}	273.79^{Ab}	241.14^{Aa}	246.45^{Ab}
EOFOS[2]	287.93^{Aab}	322.43^{Aa}	289.02^{Ba}	338.44^{Aa}	260.61^{Aa}	287.86^{Aa}
PF[3]	306.42^{Aa}	247.45^{Bb}	286.28^{Aa}	268.49^{Ab}	277.31^{Aa}	223.72^{Bb}
CV (%)	13.54		12.52		11.80	
	Villus:crypt					
En+Sal[1]	4.36^{Aa}	4.57^{Aa}	3.29^{Aab}	3.83^{Aa}	2.76^{Aa}	2.86^{Aab}
EOFOS[2]	4.53^{Aa}	3.67^{Bb}	3.76^{Aa}	2.82^{Bb}	2.73^{Aa}	2.40^{Ab}
PF[3]	3.97^{Ba}	4.63^{Aa}	2.73^{Bb}	3.56^{Aa}	2.47^{Ba}	3.33^{Aab}
CV (%)	14.55		17.76		18.77	

Normal: meeting the nutritional requirements of each phase; Reduced: with nutritional reduction of 5% of the nutritional requirement. [1]En+Sal: 10 g ton^{-1} enramycin and 125 g ton^{-1} salinomycin; [2]EOFOS – Essential oil (blend of oregano, anise, lemon + FOS (inulin) 125 g ton^{-1} of feed; [3]PF: (promoter free), without the addition of growth promoters. Means followed by different uppercase letters in the row differ by the F test at 5% probability; Means followed by different lowercase letters in column differ by the SNK test at 5% probability.

For crypt depth, a significant interaction ($P < 0.05$) was observed between diet type and growth promoters for all intestinal segments evaluated (duodenum, jejunum and ileum). Broilers fed the 'Normal PF' diet showed higher duodenum crypt depth than those fed 'Normal En + Sal'. Duodenum crypt depth for 'Reduced EOFOS' diets showed higher values than those fed 'Reduced En + Sal' and 'Reduced PF'. Broilers fed 'Normal PF' diets had higher duodenum crypt depth than the 'Reduced PF' diet, and the 'Normal PF' treatment promoted a higher crypt depth than the 'Reduced PF' diet.

Broilers fed 'Normal En + Sal' diets showed a lesser jejunum crypt depth than those fed 'Normal EOFOS' and 'Normal PF'. Broilers fed 'Reduced EOFOS' had longer jejunum compared to those fed 'Reduced En + Sal' and 'Reduced PF' diets; these birds might have a longer jejunum to increase surface for more nutrient absorption. The 'Reduced EOFOS' diet promoted a higher crypt depth than the 'Normal EOFOS' diet.

For ileum crypt depth, broilers receiving the 'Normal PF' diet showed a higher ileum crypt depth than those fed 'Reduced PF'. The 'Reduced EOFOS' diet promoted a higher ileum crypt depth than the 'Reduced En + Sal' and 'Reduced PF' diets. There was no significant difference ($P > 0.05$) between the promoters and the normal diet for the ileum.

There was an interaction ($P < 0.05$) between diet type (normal and reduced) and different growth promoters used for villus:crypt ratio of the duodenum, jejunum and ileum. The 'Reduced EOFOS' diet had a lesser value for villus:crypt ratio of duodenum compared to the 'Reduced En+Sal' and 'Reduced PF' diets. The 'Normal EOFOS' treatment promoted a higher value for villus:crypt ratio for duodenum and jejunum compared to reduced diets. Broilers fed the 'Reduced PF' diet had a higher value for ileum villus:crypt ratio than those fed 'Normal PF'.

Villus height is indicative of the absorptive capacity of the intestinal mucosa; the higher the villus height, the greater the capacity to absorb nutrients. According to the results obtained in this study, when the normal diet was supplemented with EOFOS, there was increased villus height in the jejunum, as well as larger crypt depths.

Maiorka, Santin, Sugeta, Almeida and Macari, (2001) mention that the lower the contamination of the intestinal tract with undesirable microorganisms, the higher the growth of the villi. Since essential oils are considered antimicrobials, this may explain the increase in villus height seen in the jejunum of broilers fed diets containing EOFOS. These feed additives can also stimulate the growth of beneficial bacteria as well as inhibiting or inactivating pathogenic microorganisms.

The different feed additives used in the diets did not show significant effects ($P > 0.05$) on cecum microorganism counts (Table 4). Jung, Houde, Baurhoo, Zhao and Lee. (2008) showed that antibiotics act on pathogenic and non-pathogenic bacteria, thus reducing the overall bacterial population. According to Ricke et al. (2020), prebiotics represent feed additives that potentially select gastrointestinal tract bacteria and potentially benefit the host in a variety of ways, including bird health, prevention of pathogens establishing and even improving performance. However, these effects were not observed in the present study.

Table 4
Microbiological count cecum of broilers fed diets supplemented with feed additives at 33 days

	Bifidubacterium (Log_{10})		*Lactobacillus* (Log_{10})	
	Normal	Reduced	Normal	Reduced
En+Sal[1]	7.96	7.91	7.63	7.90
EOFOS[2]	8.04	8.29	7.79	8.21
PF[3]	8.42	8.60	8.46	8.41
CV (%)	6.258		7.188	

Normal: meeting the nutritional requirements of each phase; Reduced: with nutritional reduction of 5% of the nutritional requirement. [1]En+Sal: 10g ton^{-1} enramycin and 125 g ton^{-1} salinomycin; [2]EOFOS – Essential oil (blend of oregano, anise, lemon + FOS (inulin) 125 g ton^{-1} of feed; [3]PF: (promoter free), without the addition of growth promoters.

There was no interaction ($P > 0.05$) between diet type and additives for blood parameters (Table 5). Blood parameter analysis is an important tool to assess health status and to detect abnormalities in poultry metabolic changes that may have occurred (Nunes et al., 2018; Eler et al., 2019). In general, the blood values of broilers fed different treatments were within normal ranges. The blood test is a sensitive indicator of the bird's metabolic status so it must be combined with other parameters of the production (performance, processing) in order to be a useful tool (Hosseintabar et al., 2015).

Table 5
Blood parameters of broilers fed diets supplemented with feed additives at 35 days

	Triglycerides (mg dl^{-1})		Cholesterol (mg dl^{-1})		Calcium (mg dl^{-1})	
	Normal	Reduced	Normal	Reduced	Normal	Reduced
En+Sal[1]	83.71	68.81	111.24	104.44	9.85	8.88
EOFOS[2]	87.73	78.95	108.62	112.96	10.57	11.15
PF[3]	101.54	89.99	106.41	120.97	13.16	10.38
CV (%)	19.753		13.927		22.453	

Normal: meeting the nutritional requirements of each phase; Reduced: with nutritional reduction of 5% of the nutritional requirement. [1]En+Sal: 10g ton^{-1} enramycin and 125 g ton^{-1} salinomycin; [2]EOFOS – Essential oil (blend of oregano, anise, lemon + FOS (inulin) 125 g ton^{-1} of feed; [3]PF: (promoter free), without the addition of growth promoters.

There was no interaction between diet type and growth promoters ($P > 0.05$) on carcass yield, breast, wings, drumstick, thigh, liver and abdominal fat (Table 6). Similarly, other researchers (Yalcinkaya et al., 2012; Méndez Zamora, Durán Meléndez, Hume, & Silva Vázquez, 2017) reported no significant improvement in carcass yield of broilers when fed with additives.

Table 6
Carcass and cuts yield (%) of broilers fed diets supplemented with feed additives at 42 days

	En+Sal[1]		EOFOS[2]		PF[3]		Means		CV (%)
	Normal	Reduced	Normal	Reduced	Normal	Reduced	Normal	Reduced	
Carcass	71.18	70.64	70.15	70.80	71.44	71.17	70.92	70.87	2.24
Breast	34.63	34.61	33.77	33.79	34.17	34.23	34.19	34.21	4.40
Wings	11.48	11.66	11.81	11.51	11.68	11.50	11.66	11.56	5.40
Drumstick	16.45	16.84	17.00	17.11	16.45	16.98	16.63	16.98	3.11
Thigh	14.75	14.74	14.62	15.10	15.07	14.84	14.81	14.89	3.78
Liver	2.28	2.35	2.37	2. 51	2.16	2.38	2.27	2.41	14.03
Abd. Fat	1.62	1.95	1.70	1.93	1.65	2.10	1.66	1.99	24.96

Normal: meeting the nutritional requirements of each phase; Reduced: with nutritional reduction of 5% of the nutritional requirement. [1]En+Sal: 10g ton^{-1} enramycin and 125 g ton^{-1} salinomycin; [2]EOFOS – Essential oil (blend of oregano, anise, lemon + FOS (inulin) 125 g ton^{-1} of feed. [3]PF: (promoter free), without the addition of growth promoters.

Conclusion

Broilers receiving the diet meeting requirements (Normal) with EOFOS showed higher villus height in the jejunum at 21 days when compared to the Normal diet with antibiotics and the Normal diet without growth promoters. However, there were no effects of diet type and growth promoters on broiler performance at 42 days.

Conflict of interest declaration

The authors declared no conflict of interest.

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