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Symbiotics and *Aloe vera* and *Symphytum officinale* extracts in broiler feed

Simbióticos e Extratos de *Aloe vera* e *Symphytum officinale* na Alimentação de Frangos de Corte

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Abstract

This study aimed to test the effects of dietary *Aloe vera* and *Symphytum officinale* extracts added separately or in combination with symbiotics on the performance, nutrient utilization, serum biochemical parameters, biometrics, and intestinal histomorfometry of broilers. The experiment had a randomized block design with five treatments and six replicates of ten broilers each. Treatments were as follows: negative control and positive control (diet without and with antibiotic, respectively); 0.2% *Aloe vera* (AV); 0.2% *Symphytum officinale* (S); 0.2% functional supplement, composed of symbiotics fermented in *Aloe vera* and comfrey plant extracts (S+PE). At seven days of age, FI of birds fed the *Aloe vera* extracts diets were lower than that observed for birds consuming the diet with *Symphytum officinale* extract and S+PE. Broiler performance remained unaffected by treatments at others ages evaluated. At 10 to 14 days of age the lowest ADCDM ADCCP was shown in group feed NC. The highest ADCCP was observed in PC control group and in diets supplemented with *Aloe vera* and S+PE. Serum levels of cholesterol, triglycerides, and phosphorus were affected by addition of extracts at seven, 21, and 35 days of age. The longest duodenal villi were observed in broilers fed S+PE diets at seven days of age. *Aloe vera* and *Symphytum officinale* extracts and symbiotics can be used in broiler diets as an alternative to growth-promoting antibiotics.

Key words: Antibiotics. Digestibility. Histomorphometry. Performance. Serum parameters.

Resumo

Com este projeto objetivou-se verificar o efeito da inclusão individual dos extratos vegetais de Aloe vera e *Symphytum officinale* e da associação de um simbiótico com estes extratos sobre o desempenho, aproveitamento nutricional, parâmetros séricos bioquímicos, biometria e histomorfometria intestinal de frangos de corte. O delineamento experimental utilizado foi em blocos casualizados com cinco tratamentos e seis repetições de 10 aves cada. Os tratamentos foram: Controle Negativo, Controle Positivo (rações sem e com antibiótico, respectivamente), inclusão 0,2% *Aloe vera*; 0,2% *Symphytum officinale* e 0,2% suplemento funcional (simbióticos+extratos vegetais, S+EV). Aos sete dias de idade, foi observado menor consumo de ração nas aves alimentadas com ração contendo extrato de *Aloe vera* do que nas aves alimentadas com extrato de *Symphytum officinale* e S+EV. O desempenho

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das aves não foi afetado pelos tratamentos em nenhuma das idades avaliadas. Aos 10 a 14 dias de idade, o menor CMAMS e CMAPB foram observados nas dietas do grupo controle negativo. A maior CMAPB foi observada no grupo controle positivo e em dietas suplementadas com *Aloe vera* e S+EV. As concentrações séricas de colesterol, triglicérideos e fósforo foram afetadas pela adição dos extratos aos sete, 21 e 35 dias de idade. A maior altura de vilosidades do duodeno foi observada aos sete dias de idade para o S+EV. A utilização dos extratos de *Aloe vera*, *Symphytum officinale* e do simbiótico na dieta de frangos de corte representa uma alternativa ao uso de antibiótico promotor de crescimento.
Palavras-chave: Antibióticos. Desempenho. Digestibilidade. Histomorfometria. Parâmetros sanguíneos.

Introduction

Consumers are increasingly demanding in their desire for broiler meat grown without antimicrobials. Consequently, nutritionists have been seeking alternative methods of promoting growth that have no effect on animals and humans and do not affect broiler performance.

In this context, the addition of *Aloe vera* and *Symphytum officinale* extracts alone or in combination with symbiotics can improve broiler performance and replace chemical antimicrobials in broiler chicken diets.

Numerous active compounds are found in *Aloe vera,* including enzymes, mono and polysaccharides, amino acids, vitamins, anthraquinones, saponins, salicylic acid, lignin, and steroids (ARAÚJO et al., 2002; CHRISTAKI; FLOROU-PANERI, 2010). Whole-leaf components proposed to have direct antibacterial properties include antraquinones and saponins (URCH, 1999). However, other substances such as the polysaccharides have been attributed with indirect bactericidal activity through stimulation of phagocytic leukocytes to destroy bacteria (PUGH et al., 2001).

Alemdar and Agaoglu (2009) showed that use of *Aloe vera* juice has antimicrobial activity against gram-positive bacteria (*Micobacteriasmegmatis, Klebsiella pneumoniae, Enterococcus fecaeli, Micrococcus luteus, Candida Albicans and Bacillus sphericus*). The authors concluded that it can be alternative to chemicals used in medication, food and cosmetic sectors.

Symphytum officinale is another plant with medicinal properties that could be used as a growth promoter for broilers. The leaves of this plant contain alkaloids with sedative, anesthetic, analgesic, antimicrobial, spasmolytic, anti-hepatotoxic, diuretic, antimicrobial, and anti-inflammatory properties; the leaves also contain allantoin, saponin glycosides, fixed acids, and tannins (TOLEDO et al., 2003). The antimicrobial activity in these plants against gram-positive bacteria were confirmed for prevention and control of bovine mastitis by Avancini et al. (2008).

In addition to herbal extracts other alternatives to the use of growth promoters have been investigated in the poultry industry. Thus, the utilization of prebiotics, probiotics and symbiotics (FURLAN et al., 2004) are among these alternatives.

In simplest definition, symbiotic is a combination of probiotic and prebiotics. This combination can improve the viability of probiotic microoganisms, since they are able to use prebiotics as a subtract for fermentation (BENGMARK; BENGMARK, 2001; FALAKI et al., 2010). Due containing oligosaccharides and others functional nutrients the herbal extracts can be used as fermentation substrate for symbiotics.

Several studies showed that *Aloe vera (extract or gel)* seems to play an important role in promoting growth in broilers chicks or in their health management (MEHALA; MOORTHY, 2008b; SALARY et al., 2014; FALLAH, 2015). However, few studies have investigated the effects of *Aloe vera* and *Symphytum officinale* plant extracts added alone or in combination with symbiotics to poultry feed.

The objective of this study was to evaluate the effect of dietary *Aloe vera* and *Symphytum officinale* extracts and symbiotics on the histological and morphometric measurements, nutrient utilization, serum biochemical parameters, performance, and carcass and parts yield of broilers.

Materials and Methods

The experimental trial was conducted in the Poultry sector of the Federal Institute of Goiás (Instituto Federal Goiano - IF Goiano) Rio Verde Campus, Goiás State (GO), Brazil. The study was approved by the IF Goiano Animal Research Ethics Committee under protocol number 029/2013.

Three hundred one-day-old male Cobb® broiler chicks were used. The experiment had a randomized block design with five treatments and six replicates of ten broilers each.

The birds were housed in galvanized wire cages measuring 0.90 m x 0.60 m x 0.45 m. Linear feeders and drinking fountains were used, and water and feed were provided *ad libitum* throughout the 35-day experimental period. The feeders were refilled twice a day to avoid waste.

The treatments were as follows:

1. Negative control - corn and soybean meal-based (C+SM) diets without growth-promoting antibiotics; 2. Positive control - basal C+SM diets with 25 ppm of tylosin; 3. Basal diets with 0.2% *Aloe vera* extract; 4. Basal diets with 0.2% *Symphytum officinale* extract; 5. Basal diets with 0.2% symbiotics and plant extracts® (S+PE);

The *Aloe vera* or *Symphytum officinale* extract leaves were purchased commercially as dried herb supplements. S+PE is a commercial product (Estibion®) comprising symbiotics containing prebiotics (mannan oligosaccharide) and polyprobiotics (*Saccharomyces cerevisiae; Lactobacillus acidophilus; Bacillus subtilis; Bifidobacterium bifidum; Enterococcus faecium*) fermented in *Aloe vera* and *Symphytumofficinale*extracts. The *Aloe vera* and *Symphytumofficinale*plant extracts tested are the same as those used to produce S+PE.

The diets for the different production stages (pre-initial from 1 to 7 days of age, initial from 8 to 21days of age and grower from 22 to 35 days of age) were formulated according to the nutritional recommendations of Rostagno et al. (2011) (Table 1).

The plant extracts, symbiotics, and antimicrobials (tylosin at 25 ppm) were added to the diets to replace the inert substance (washed sand).

Performance, morphological and histological measurements

The following parameters were measured to evaluate performance: average weight gain (WG), feed intake (FI), and feed conversion (FC). The broilers and diets were weighed at the beginning and end of each production stage.

At seven, 21 and 35 days two broilers with similar average weights from each pen, were fasted for 12 hours then euthanized by cervical dislocation to evaluate the morphological measurements, the digestive organs were weighed (proventriculus+gizzard (Prov+giz), small and large intestines (SI and LI, respectively), liver and pancreas).The spleen and bursa of Fabricius were weighed to assay the responses of the immune system of the broilers in each treatment using the organ weights.

The carcass yield (%) was measured only at 35 days of age of broilers. The percentages of each organ weight and carcass in relation to broiler body weight were estimated.

The villi and crypts of the duodenum were also evaluated at seven, 21 and 35 days of age.

Clean duodenum samples were fixed in a buffered 10% formol solution for 8 h, washed in water and stored in 70% ethanol solution until use. A 0.5 cm^2 subsample of each duodenum sample was then stained using eosin-hematoxilin reagents. At least 10 villi and 10 crypts from each subsample were dissected under a microscope and then photographed. Villus height and crypt depth were measured using the image analysis software Leica (Image-Pro Plus version 4.5.0.27).

Table 1. Proximate composition and nutritional levels calculated from the experimental diets at the pre-initial, initial, and growth stages.

Ingredients	Natural Matter %		
	Pre-Initial	Initial	Growth
Corn kernel	57.37	59.85	62.82
45% soybean meal	37.05	34.27	30.66
Soybean oil	1.35	2.26	3.19
Dicalcium phosphate	1.50	1.29	1.16
Lime	1.06	1.02	0.91
Common salt	0.44	0.42	0.40
DL-Methionine	0.35	0.18	0.17
L-Lysine	0.34	0.29	0.29
Mineral Supplement[1]	0.01	0.04	0.03
Vitamin Supplement[2]	0.08	0.04	0.03
Antioxidant BHT	0.01	0.01	0.01
Inert substance[3]	0.20	0.20	0.20
Total	100.00	100.00	100.00
Calculated levels			
Metabolizable energy (Kcal/kg)	2,960	3,050	3,150
Crude protein, %	22.40	21.20	19.80
Digestible lysine, %	1.32	1.21	1.13
Digestible methionine+cystine, %	0.95	0.47	0.45
Digestible threonine, %	0.81	0.79	0.73
Calcium, %	0.92	0.84	0.75
Available phosphorus, %	0.39	0.35	0.32
Sodium, %	0.22	0.21	0.20

[1]Composition per kg of product: manganese - 45,0000 g/kg; iron - 30,0000 g/kg; zinc -40.0083 g/g; copper - 75,0000 g/kg; cobalt - 3,0000 mg/kg; iodine - 500,0000 mg/kg; and qsp vehicle -1,000 g.[2]Composition per kg of product: Folic acid - 697,6595 mg/kg; pantothenic acid- 6,997.58 mg/kg; BHT - 508,2839 mg/kg; Biotin - 69,9408 mg/kg; Vitamin A - 196,448.00 UI/kg; Vitamin B1 - 911,5614 mg/kg; Vitamin B12 - 5,245.56 mcg/kg; Vitamin B2 - 2,797.63 mg/kg; Vitamin B6 - 1,395.55 mg/kg; Vitamin D3 - 1,293,904.80 UI/kg; Vitamin E - 14,085,954.36 UI/kg; Vitamin K - 1,747.25 mg/kg; and qsp vehicle -1,000 g. [3]Washed sand.

The following variables were studied: intestinal villi height, crypt depth (20 readings per slide), and villi height/crypt depth ratio. The villi measurements were taken from the basal region coincident with the upper portion of the crypts to the villi apex. The CD measurements were taken from the basal region of the villi to their boundary with the basal mucosa.

Digestibility of nutrients

The total fecal collection method was used at two different ages to determine nutrient utilization. The fecal samples were collected from the broilers at 10 to 14 days of age (initial stage) and at 28 to 32 days of age (growth stage), resulting in four days of collection.

The dry matter and crude protein analyses were performed according to the method described by Silva and Queiroz (2002).

After the chemical analyses were performed, the apparent digestibility coefficients of dry matter and crude protein (ADCDM and ADCCP) were calculated using the equations:

Apparent digestibility (%) = (Nutrient intake - Nutrient excretion x 100)/Nutrient intake

Serum biochemical parameters

The serum biochemical parameters and morphological and histological measurements were performed in the broilers at seven, 21, and 35 days of age.

Before slaughter, 3 mL of blood was collected from each broiler via venipuncture of the left brachial vein according to the method described by Gonçalves et al. (2010).

The serum biochemical analyses were performed in duplicate to determine the concentration of calcium (Ca), phosphorus (P), triglycerides (Tri), and cholesterol (Chol) in the serum. All of the analyses were performed using commercial kits according to the manufacturer's instructions (LABORCLIN®, 2013).

Statistical analyses

The data were evaluated using the SISVAR program (system for analysis of variance, version 5.0) and subjected to analysis of variance; the means were compared by Student Newman Keuls (SNK) test at 5% significance.

Results and Discussion

At seven days of age, FI of birds fed the *Aloe vera* extracts diets were lower than that observed for birds consuming the diet with *Symphytum officinale* extract and S+PE. FI of birds fed NC and PC diets elicited intermediate intakes (Table 2).

In contrast, greatest FI were observed with the addition of *Aloe vera* extracts and gel in drinking water of broilers chickens (SALARY et al., 2014; FALLAH, 2015).

WINDISCH et al. (2009) analyzed literature data for proven effects of phytobiotic feed additives in different poultry species. In average phytobiotics as plant extracts reduced feed intake by 2.1% (-8 to +3). These effects of phytogenic extracts on the FI can be attributed to changes in feed palatability and its influences in appetite stimulation of broilers (SALARY et al., 2014).

None of the variables of performance analyzed at 21, and 35 days of age differed significantly among the treatments (Table 2).

These results are similar to those reported by Rizzo et al. (2010) who found that the final weight, weight gain, and feed intake of broilers fed plant extracts (clove, thyme, cinnamon, and pepper) did not differ among treatments at 21 days of age.

Mehala and Moorthy (2008b) also found that the weight gain, average weight, and feed intake of broilers fed *Aloe vera* and *turmeric* extracts at 0.1 and 0.2% supplement levels did not differ.

Table 2. Weight gain (WG), feed intake (FI), and feed conversion (FC) in broilers at seven, 21, 35 days of age andcarcass yield (%) at 35 days of age.

Variables	TREATMENTS						
	Negative control[1]	Positive control[2]	*Aloe vera*	*S. officinale*	S + PE[3]	Pr>F[4]	CV (%)[5]
1 to 7 days of age							
MW (g)	146.80	152.20	133.00	157.80	152.20	0.2014	6.90
WG (g)	103.05	107.06	89.88	113.60	107.10	0.5628	9.75
FI (g)	151.60[b]	154.70[b]	133.40[c]	172.98[a]	174.13[a]	0.0001	6.33
FC (g:g)	1.49	1.45	1.52	1.53	1.62	0.1085	7.66
1 to 21 days of age							
MW (g)	763.60	799.40	723.60	810.40	758.00	0.0987	6.58
WG (g)	719.85	755.23	679.66	766.44	713.70	0.1012	7.01
FI (g)	1068.59	1037.17	1011.75	1116.73	1042.46	0.0700	5.16
FC (g:g)	1.48	1.37	1.50	1.46	1.46	0.4786	7.88
1 to 35 days of age							
MW (g)	1852.60	1879.00	1765.20	1841.40	1717.40	0.2974	7.18
WG (g)	1808.85	1834.83	1721.26	1797.44	1673.10	0.2971	7.36
FI (g)	2664.27	2722.36	2593.70	2825.22	2669.63	0.5952	8.45
FC (g:g)	1.48	1.49	1.51	1.57	1.61	0.8119	13.12
Carcass yield (%)	71.83	71.78	73.25	72.46	72.74	0.7252	2.68

*Means followed by different letters in the columns significantly differ by SNK test ($p<0.05$).
[1]Diets without antibiotics; [2]Diets with 25 ppm of tylosin; [3]Diets with symbiotics+ plant extracts; [4]Probability; [5]Coefficient of variation.

At the initial stage (10 to 14 days of age), lower ADCDM were found with the broilers fed diets without antibiotics. The groups fed diets supplemented with 25 ppm of tylosin (CP) showed ADCDM 14.8% higher in comparison with the negative control group. Intermediate results were observed for this variable in broilers from the other treatment groups (Table 3).

According to Barbosa et al. (2008), ADCDM reflects nutrient digestibility, i.e., higher coefficients indicate a higher rate of nutrient absorption from the diet. Thus, the lower ADCDM obtained in the diets without antibiotics may be explained by the fact that the broilers were not able to metabolize the nutrients present in the feed and, therefore, continued to ingest feed to meet their needs.

At 10 to 14 days of age the lowest ADCCP was shown in group feed NC and with 0.2 of *Symphytum officinale* extract. The highest ADCCP was observed in PC control group and in diets supplemented with *Aloe vera* and S+PE.

Table 3. Apparent digestibility coefficients of dry matter (ADCDM) and crude protein (ADCCP) in broilers at the initial and growth stages.

Treatments	Variables (%)	
	ADCDM	ADCCP
10 to 14 days of age		
Negative control[1]	68.85[b]	60.06[b]
Positive control[2]	79.06[a]	69.67[a]
Aloe vera	77.29[ab]	65.82[a]
S. officinale	70.88[ab]	54.67[b]
S+PE[3]	77.65[ab]	64.93[a]
Pr>F[4]	0.0200	0.0109
CV (%)[5]	6.68	9.51
28 to 32 days of age		
Negative control[1]	83.15	71.98
Positive control[2]	82.84	71.52
Aloe vera	81.04	67.02
S. officinale	83.79	73.43
S+PE[3]	83.40	66.44
Pr>F[4]	0.5766	0.1836
CV (%)[5]	3.34	7.53

*Means followed by different letters in the columns significantly differ by SNK test (p<0.05).
[1]Diets without antibiotics; [2]Diets with 25 ppm of tylosin; [3]Diets with symbiotics+plant extracts; [4]Probability; [5]Coefficient of variation.

These results tested the hypothesis that plant extracts such as *Aloe vera*but no *Symphytum officinale* extract acted as a digestibility enhancer, balancing the gut microflora and stimulating the secretion of endogenous digestive enzymes thus improving ADCCP (SALARY et al., 2014). Pharmacologically active substances are supposed to enhance feed digestion and absorption by stimulating secretion of digestive enzymes leading to better feed utilization and assimilation (LANGHOUT et al., 2000).

At the growth stage (28 to 32 days of age), none of the analyzed variables differed significantly among treatments (Table 3). Similarly for the results at growth stage, Rizzo et al. (2010) replaced antibiotics with a variety of plant extracts in broiler diets and found no effect on the apparent digestibility coefficient for crude protein (ADCCP).

With the exception of the relative weights of the LI, none of the digestive tract morphometric variables or the weights of the spleen and bursa were affected by the treatments at seven, 21, or 35 days of age. At 21 days of age, the broilers fed diets containing the *Aloe vera* extract exhibited higher LI % than the broilers fed the other treatments. The lowest LI % was observed in the birds of S+PE treatment (Table 4).

Oliveira and Moraes (2007) observed reduced intestinal weights in poultry fed growth-promoting antibiotics and prebiotics; they attributed this reduction to a decreasing in the bacterial count of the gastrointestinal tract.

At 35 days of age, the following carcass yield did not differ among the treatments (Table 4).

Similar results were found by Mehala and Moorthy (2008b) added *Aloe vera* and *turmeric* to broiler diets and found no significant effects on the carcass yield or abdominal fat. Though, Darabighane et al. (2011) observed better performance and heavier dressing percentage in broilers fed 15 ppm of antibiotic virginiamycin or 2% of *Aloe vera* gel.

The serum levels of Chol, Tri, and P differed among the treatments at different ages evaluated (Table 5).

At seven and 21 days of age, the broilers fed the diets with 0.2% of *Aloe vera*, *Symphytum officinale* and S+PE had higher serum levels of Chol. The lower serum levels of Tri was observed in diets group NC and with 0.2% of *Aloe vera*at seven days and highest level of Tri was found in S+PE diets group at 21 days of age.

Increases in serum Chol levels may be attributed to the inhibition of the active enzyme hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-COA) (SHARIFI et al., 2013). It is proposed that medicinal plants results in a reduction of HMG-COA in the liver (key enzyme in cholesterol synthesis (MIDDLETON; HUI, 1982; YU et al., 1998). Salary et al. (2014) also found that the level of plasma HDL was significantly increased by addition of 0.4% *Aloe vera* and *Glycyrrhiza glabra.*

Table 4. Morphological measurements of *proventriculus + gizzard (Prov+giz)*, pancreas, small intestine (SI), large intestine (LI), liver, spleen, and bursa at seven, 21 and 35 days and Carcass yield.

	TREATMENTS						
Variables (%)	Negative control[1]	Positive control[2]	*Aloe vera*	*S. officinale*	S + PE[3]	Pr>F[4]	CV (%)[5]
7 days of age							
Prov+giz	7.44	6.86	6.67	7.24	7.45	0.1939	8.40
Pancreas	0.59	0.52	0.67	0.56	0.54	0.1029	14.55
SI	8.39	7.65	8.40	7.93	8.53	0.3668	9.43
LI	1.66	1.37	1.10	1.49	1.52	0.2715	27.50
Liver	2.82	2.72	2.75	2.99	3.10	0.4726	13.23
Spleen	0.09	0.09	0.09	0.08	0.09	0.3684	12.19
Bursa	0.18	0.18	0.12	0.17	0.18	0.0709	19.37
21 days of age							
Prov+giz	3.54	3.34	3.56	3.21	3.47	0.5876	11.25
Pancreas	0.37	0.36	0.41	0.36	0.36	0.7815	18.44
SI	3.75	3.70	3.82	3.73	3.72	0.9552	6.76
LI	1.07[ab]	1.04[ab]	1.15[a]	0.99[ab]	0.86[b]	0.0315	12.63
Liver	2.55	2.50	2.43	2.74	2.56	0.5913	11.78
Spleen	0.13	0.15	0.09	0.10	0.12	0.3553	40.21
Bursa	0.33	0.30	0.32	0.29	0.43	0.2480	28.66
35 days of age							
Carcass yield	71.83	71.78	73.25	72.46	72.74	0.7252	2.68
Prov+giz	2.36	2.19	2.32	2.36	2.25	0.6127	8.41
Pancreas	0.23	0.23	0.25	0.22	0.25	0.2889	11.53
SI	2.36	2.26	2.39	2.53	2.40	0.4253	8.87
LI	0.72	0.64	0.84	0.64	0.68	0.4628	27.21
Liver	1.64	1.59	1.57	1.62	1.64	0.8528	7.52
Spleen	0.12	0.13	0.13	0.12	0.13	0.8613	18.02
Bursa	0.26	0.28	0.25	0.27	0.24	0.6921	18.16

*Means followed by different letters in the rows significantly differ by SNK test ($p<0.05$).
[1]Diets without antibiotics; [2]Diets with 25 ppm of tylosin; [3]Diets with symbiotics+plant extracts; [4]Probability; [5]Coefficient of variation.

In contrast to the results of this study, Mehala and Moorthy (2008a) added 0.1 and 0.2% of *Aloe vera* and *turmeric*, respectively, to broiler diets and found that the serum levels of glucose, total cholesterol, and triglycerides were similar among all treatments.

According to Silva et al. (2001), the addition of flavonoids to rat diets results in reduced Tri serum levels, suggesting that these compounds increase lipoprotein lipase activity and lead to higher mobilization (hydrolysis) of Tri for the liver, muscle tissue, and adipose tissue.

Although *Symphytum officinale* and *Aloe vera* crude extracts contain flavonoid glycosides (TOLEDO et al., 2003), it is possible that the concentrations used in this study were not sufficiently high to affect serum The P serum content differed among the broilers in the different treatments at 21 and 35 days of age. At 21 days of age, the broilers fed S+PE exhibited the lowest P serum values. At 35 days of age, the lowest serum values were observed in the broilers fed diets containing *Aloe vera* and *Symphytum officinale*. lipid levels in the broilers.

Table 5. Serum levels of cholesterol (Chol), triglycerides (Tri), calcium (Ca), and phosphorus (P) in broilers.

Treatments	VARIABLES (mg/dL)			
	Chol	Tri	Ca	P
		7 days of age		
Negative control[1]	202.58[b]	198.11[b]	10.09	5.36
Positive control[2]	213.56[ab]	209.34[a]	10.79	5.32
Aloe vera	229.05[a]	188.25[b]	10.55	5.27
S. officinale	220.08[a]	207.77[a]	10.72	5.19
S + PE[3]	198.95[b]	214.16[a]	10.43	5.38
Pr>F[4]	0.0044	0.0004	0.1310	0.6106
CV (%)[5]	3.33	2.01	2.91	2.88
		21 days of age		
Negative control[1]	200.00[bc]	188.60[b]	10.77	5.14[ab]
Positive control[2]	180.19[c]	193.68[b]	9.97	4.87[ab]
Aloe vera	190.64[ab]	192.43[b]	10.64	4.81[ab]
S. officinale	192.36[ab]	190.90[b]	9.52	5.40[a]
S + PE[3]	209.00[a]	205.26[a]	9.89	4.70[c]
Pr>F[4]	0.0040	0.0270	0.3906	0.0008
CV (%)[5]	3.18	2.61	8.36	2.54
		35 days of age		
Negative control[1]	218.00	225.04	10.33	5.43[a]
Positive control[2]	205.92	226.35	10.12	5.03[ab]
Aloe vera	205.75	217.79	10.32	4.85[b]
S. officinale	219.58	210.96	10.06	4.85[b]
S + PE[3]	213.33	213.73	10.19	5.05[ab]
Pr>F[4]	0.0304	0.2166	0.6849	0.0318
CV (%)[5]	2.45	3.97	2.62	3.80

*Means followed by different letters in the columns significantly differ by SNK test ($p<0.05$).
[1]Diets without antibiotics; [2]Diets with 25 ppm of tylosin; [3]Diets with symbiotics+plant extracts; [4]Probability; [5]Coefficient of variation.

Although the broilers exhibited different P serum levels at 21 and 35 days of age, the calcium:phosphorus ratio remained close to 2:1 (1.76 from 2.21: 1) among all the treatments, which is a value considered ideal in living organisms (MINAFRA et al., 2010).

At seven days of age, the broilers fed S+PE had longer duodenal villi. The smaller villus height was observed in the broilers fed diets without antibiotics. However, no significant difference was observed between the herbal extracts and the antibiotic group (Table 6).

According to the observed in this study, Costa et al. (2007), demonstrated that the combination of extracts of clove and oregano provided growth performance close to that of pigs fed with antimicrobial.

Maintenance of normal microarchitecture in the small intestine is very important for proper growth and development. Changes in intestinal morphology, suchas reduced villus height or increased crypt depth, can indicate the presence of toxins (YASON et al., 1987).

Sohail et al. (2012) found that the application of probiotic and symbiotic improved the ileal villus width, surface area, and crypt depth. Increase in villus height and width provide a greater surface area for nutrient digestion and absorption pursuant to increased mucosal enzymes, absorption, and nutrients transport system (FARTHING, 2004).

Table 6. Villi height (villi), crypt depth (CD), and villi/crypt ratio (VI/CD)of the duodenum in broilers at seven, 21 and 35 days of age.

Variables	Treatments						
	Negative control[1]	Positive control[2]	*Aloe vera*	*S. officinale*	S+PE[3]	Pr>F[4]	CV (%)[5]
		Duodenum		7 days of age			
Villi (µm)	496.93[b]	545.68[ab]	549.48[ab]	595.09[ab]	692.98[a]	0.0416	17.18
CD(µm)	146.80	147.47	170.32	146.84	187.30	0.0600	15.33
V/C	3.41	3.77	3.33	4.09	3.72	0.3170	16.37
			21 days of age				
Villi (µm)	659.20	766.15	604.98	614.75	540.68	0.0577	18.11
CD (µm)	133.00	154.82	136.48	131.89	148.17	0.7878	24.62
VI/CD	5.28	4.99	4.62	4.86	3.80	0.1044	17.57
			35 days of age				
Villi (µm)	701.90	822.68	700.13	711.89	691.25	0.1989	14.87
CD (µm)	168.32	167.41	145.82	147.59	144.00	0.2905	15.06
V/C	4.42	4.96	4.82	4.86	4.87	0.6889	13.05

*Means followed by different letters in the rows significantly differ by SNK test ($p<0.05$).
[1]Diets without antibiotics; [2]Diets with 25 ppm of tylosin; [3]Diets with symbiotics+plant extracts; [4]Probability; [5]Coefficient of variation.

Darabighane et al. (2011) coment that short-chain fatty acids, as the final product of fermentation by lactobacillus, may reduce intestinal pH and make the environment unfavorable for pathogens and increase in useful bacteria count can improve intestinal health by reduction in the level of damage to intestine mucosa and in level of repairs. These results at seven days of age, suggest that S+PE inhibited microbial colonization, which may have benefited intestinal mucosa and favored the structure of the villi.

At 21 and 35 days of age, the treatments had no effects on broiler duodenal intestinal histomorphometry (Table 6). These results are similar to those obtained by Fukayama et al. (2005) who added oregano extract to broiler diets and found no effect on the broiler villi at 21 days of age. However, Darabighane et al. (2011) observed that longer villi in the groups treated with 2% and 2.5% *Aloe vera* gel as the result of larger lactobacillus in small intestine.

According to Viola and Vieira (2007), crypt depth is a measurement of cellular proliferation in the mucosa, and shallower crypts indicate a healthy intestinal state.

Montagne et al. (2003) claim that the villi:crypt ratio is an indicator of the digestive ability of the small intestine. An increase in this ratio indicates better digestion and absorptive ability.

Thus, the lack of difference in villi:crypt ratios among the treatments observed in this study suggests that a combination of the evaluated additives will not necessarily result in an improved ability to gain nutrients in broilers at seven, 21, and 35 days of age.

Studies examining the effects of *Aloe vera*, *Symphytum officinale* and S+PE on broiler performance are rare; however, it appears that these additives can be used to replace growth-promoting antibiotics, as they produce the same performance of diets containing antibiotics. However, for more conclusive results and to verify that these additives can functionally replace antibiotics, future studies must subject animals to health-related challenges.

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