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Addition of a blend based on an emulsifier, monolaurin, and glycerides of butyric acid in broiler chicken feed to replace conventional antibiotics, improves performance and reduces fecal *Escherichia coli* counts

Adição de uma mistura à base de emulsificante, monolaurina e glicerídeos de ácido butírico na alimentação de frangos de corte para substituir os antibióticos convencionais, melhora o desempenho e reduz a contagem de *Escherichia coli* fezes

La adición de una mezcla basada en un emulsionante, monolaurina y glicéridos de ácido butírico en el alimento para pollos de engorde para reemplazar los antibióticos convencionales, mejora el rendimiento y reduce los recuentos fecales de *Escherichia coli*

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Abstract
Brazil is the largest exporter and the third largest producer of chicken meat. However, this activity presents some challenges, the most recent being the banned usage of antimicrobials as growth promoters. The aim of this study was to evaluate whether the addition of a blend (lysolecithins, butyric acid glycerides and lauric acid glycerides) in broiler feed could improve performance, health, meat quality, as well as antimicrobial and coccidiostatic capacity in order to replace conventional growth promoters. Broiler chickens (n=180) were divided into three groups with four replicates per group as follows: positive control - PC (enramycin: 10 mg/kg; coccidiostatic/salinomycin: 64 mg/kg, 1 to 35

days); negative control - NC (basal diet, without antimicrobials); blend - FDH (1 kg/ton, 1 to 42 days). The zootechnical performance was measured on days 1, 14, 21, 35 and 42. Fecal samples were collected for parasitological and bacterial analysis on days 21 and 42. At 42 days, four birds per treatment were euthanized for analysis of meat quality and intestinal morphology. At 42 days, a higher body weight and weight gain, in addition to better feed conversion were observed in the FDH and PC groups compared to the NC group (P = 0.001). The productive efficiency index was higher in the PC and FDH treatments compared to NC. *Escherichia coli* count and total coliform count in the PC and FDH groups were lower compared to NC (P < 0.05) on day 42 of the experiment. Eimeria spp. counts in the feces of FDH birds were lower compared to NC, in contrast to PC (P = 0.047). The villous:crypt ratio was higher in NC followed by FDH as compared to PC (P = 0.001). It is concluded that the use of the blend improved weight gain, body weight and feed conversion, in addition to providing a greater villous:crypt ratio and enhancing coccidiostatic and antimicrobial action. Therefore, the blend demonstrated high potential for replacement of growth promoters in the diet of broilers chickens.
Keywords: Antimicrobial; Butyric acid; Lysolecithins.

Resumo
O Brasil é o maior exportador e o terceiro maior produtor de carne de frango. No entanto, esta atividade apresenta alguns desafios, sendo o mais recente a proibição do uso de antimicrobianos como promotores de crescimento. O objetivo deste estudo foi avaliar se a adição de um blend (lisolecitinas, glicerídeos de ácido butírico e glicerídeos de ácido láurico) na alimentação de frangos de corte poderia melhorar o desempenho, a saúde, a qualidade da carne, bem como a capacidade antimicrobiana e coccidiostática, a fim de substituir o crescimento convencional promotores. Frangos de corte (n=180) foram divididos em três grupos com quatro repetições por grupo da seguinte forma: controle positivo - PC (enramicina: 10 mg/kg; coccidiostático/salinomicina: 64 mg/kg, 1 a 35 dias); controle negativo - NC (dieta basal, sem antimicrobianos); mistura - FDH (1 kg/ton, 1 a 42 dias). O desempenho zootécnico foi medido nos dias 1, 14, 21, 35 e 42. Amostras fecais foram coletadas para análise parasitológica e bacteriana nos dias 21 e 42. Aos 42 dias, quatro aves por tratamento foram eutanasiadas para análise da qualidade da carne e morfologia intestinal. Aos 42 dias, maior peso corporal e ganho de peso, além de melhor conversão alimentar foram observados nos grupos FDH e PC em relação ao grupo NC (P = 0,001). O índice de eficiência produtiva foi maior nos tratamentos PC e FDH em relação ao NC. A contagem de *Escherichia coli* e a contagem de coliformes totais nos grupos PC e FDH foram menores em comparação com NC (P < 0,05) no dia 42 do experimento. A contagens *Eimeria* spp. nas fezes das aves FDH foram menores em comparação com NC, em contraste com PC (P = 0,047). A relação vilo:cripta foi maior no NC seguido pelo FDH em relação ao PC (P = 0,001). Conclui-se que o uso do blend melhorou o ganho de peso, peso corporal e conversão alimentar, além de proporcionar maior relação vilosidade:cripta e potencializar a ação coccidiostática e antimicrobiana. Portanto, o *blend* demonstrou alto potencial para substituição de promotores de crescimento na dieta de frangos de corte.
Palavras-chave: Antimicrobiano; Ácido butírico; Lisolecitinas.

Resumen
Brasil es el mayor exportador y el tercer mayor productor de carne de pollo. Sin embargo, esta actividad presenta algunos desafíos, siendo el más reciente la prohibición del uso de antimicrobianos como promotores del crecimiento. El objetivo de este estudio fue evaluar si la adición de una mezcla (lisolecitinas, glicéridos de ácido butírico y glicéridos de ácido láurico) en la alimentación de pollos de engorde podría mejorar el rendimiento, la salud, la calidad de la carne, así como la capacidad antimicrobiana y coccidiostática para reemplazar el crecimiento convencional. promotores Los pollos de engorde (n=180) se dividieron en tres grupos con cuatro repeticiones por grupo de la siguiente manera: control positivo - PC (enramicina: 10 mg/kg; coccidiostático/salinomicina: 64 mg/kg, de 1 a 35 días); control negativo - NC (dieta basal, sin antimicrobianos); mezcla - FDH (1 kg/ton, 1 a 42 días). El desempeño zootécnico se midió los días 1, 14, 21, 35 y 42. Se recolectaron muestras fecales para análisis parasitológico y bacteriano los días 21 y 42. A los 42 días se sacrificaron cuatro aves por tratamiento para análisis de calidad de carne y morfología intestinal. A los 42 días se observó un mayor peso corporal y ganancia de peso, además de una mejor conversión alimenticia en los grupos FDH y PC en comparación con el grupo NC (P = 0.001). El índice de eficiencia productiva fue mayor en los tratamientos PC y FDH en comparación con NC. El recuento de Escherichia coli y el recuento de coliformes totales en los grupos PC y FDH fueron más bajos en comparación con NC (P < 0,05) el día 42 del experimento. Eimeria spp. los recuentos en las heces de las aves FDH fueron menores en comparación con NC, en contraste con PC (P = 0,047). La relación vellosidad:cripta fue mayor en NC seguido de FDH en comparación con PC (P = 0,001). Se concluye que el uso de la mezcla mejoró la ganancia de peso, el peso corporal y la conversión alimenticia, además de proporcionar una mayor relación vellosidades:criptas y potenciar la acción coccidioestática y antimicrobiana. Por lo tanto, la mezcla demostró un alto potencial para reemplazar los promotores del crecimiento en la dieta de los pollos de engorde.
Palabras clave: Antimicrobiano; Ácido butírico; Lisolecitinas.

1. Introduction

In Brazil, meat consumption is estimated at 45 kg per person per year, and 31% of the production is exported, characterizing the country as the largest poultry meat exporter and the third largest producer worldwide (ABPA, 2021). Moreover, poultry production has a great impact on the economy and consumers. Several technologies like antibiotics (ATB) are applied as growth promoters to optimize production and produce safe food. However, it is known that the use of ATB as growth promoters may increase bacterial resistance leading to public health concerns such as the presence of antibiotic residues and multirresistance (Castanon, 2007). In 2006, the European Union banned the use of ATB as growth promoters. In Brazil few ATB are available for this purpose, and there is a tendency of a total ban. In 2018, the Ministry of Agriculture, Livestock and Supply (MAPA) restricted the use of tylosin, lincomycin, virginiamycin, bacitracin and tiamulin in the manufacture of feed as growth-enhancing additives throughout Brazil (Rangel, 2018).

As a result, poultry companies have been searching for alternatives to replace conventional growth promoters but maintain or improve zootechnical performance. As an example, lauric acid is an acid composed of 12 carbon atoms, which exerts metabolic and immunological functions related to a better intestinal development and nutrient absorption which is reflected by a better animal performance (Wu et al., 2021).

Next to that, glycerol monolaurate (GML), composed of lauric acid and glycerol, is a medium-chain fatty acid ester that can be found in palm and coconut, with several antimicrobial properties (Lan et al., 2021). Glycerol monolaurate can improve poultry performance and health in addition to having an antiparasitic effect (Fortuoso et al., 2019). For these reasons, the use of lysolecithins and butyric acid glycerides to improve animal performance can be highlighted as an increasingly common practice. Lysolecithins are produced through enzymatic hydrolysis of lecithin and improve apparent metabolizable energy availability, emulsification of fats making them more available for digestion and absorption (Brautigan et al., 2017).

Short-chain fatty acids such as butyric acid are known to improve gastrointestinal development. The usage of short-chain fatty acids may decrease gastric pH, thus enhancing the production of pepsin from pepsinogen and decreasing the formation of insoluble metal-phytate complexes. It is possible to observe an increase in the absorption surface of the small intestine due to the stimulation of production of epithelial cells, and thus, a better use of nutrients after the ingestion of butyric acid (Nari et al., 2020). Also, short-chain acids exert antimicrobial functions. Therefore, the aim of this study was to evaluate whether the addition of a blend of lysolecithins, monolaurin and glycerides of butyric acid in broiler feed could improve animal performance, health, meat quality due to a coccidiostatic and antimicrobial effect capable of replacing conventional growth promoters.

2. Methodology

The ethics committee approved the project on animals' use in research (CEUA nº 7175220620) at the Universidade do Estado de Santa Catarina (UDESC).

2.1 Products

The blend was purchased from FRAmelco and it is based on a combination of lysolecithins, butyric acid glycerides and lauric acid glycerides.

2.2 Animals accommodation and diet

A total of 180 male, 1-day-old chicks (Cobb 500) were purchased from a commercial company located in the city of Chapecó, Santa Catarina State, Brazil. They were weighed upon arrival and randomly distributed into three groups with four

replicates each and 15 birds per replicate. The birds were kept in pens (1m x 1.8m) with wood shavings previously used to raise 5 flocks of broiler chickens. A light regime was used as recommended by the lineage manual.

The basal diet was formulated according to the nutritional requirements described by the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017 – Table 1). The basal diet was based on corn and soybean meal, and different formulations were used for the starter (1 to 21 days), grower (22 to 35 days) and finisher (36 to 42 days) periods. All groups received the same basal diet, except for the group that received the blend and a reformulated diet with minimal nutrients in line with the nutritional matrix of the product (Table 2).

Table 1: Composition of basal diet used for the experimental groups: positive control (salinomycin and enramycin) and negative control.

Ingredients	Feeding phase		
	1 – 21	22–35	36–42
Corn (g/kg)	479.57	509.73	614.49
Soybean meal (g/kg)	433.08	391.84	304.46
Soybean oil (g/kg)	44.95	61.35	49.18
Bicalcium phosphate (g/kg)	18.94	14.53	10.92
Limestone (g/kg)	8.32	8.36	7.14
Salt (g/kg)	4.60	4.25	4.07
DL-methionine (g/kg)	3.34	3.16	2.54
L-lysine HCL (g/kg)	2.21	1.81	2.22
L-threonine (g/kg)	0.82	0.66	0.64
L-valine (g/kg)	0.17	0.31	0.31
Premix of vitamins[1] (g/kg)	2.00	2.00	2.00
Premix of minerals[2] (g/kg)	2.00	2.00	2.00
Calculated chemical composition			
Metabolizable energy (Kcal/kg)	3.050	3.200	3.250
Crude protein (%)	24.3	22.62	19.54
Calcium (%)	0.94	0.83	0.66
Available phosphorus (%)	0.47	0.38	0.31
Digestible lysine (%)	1.36	1.23	1.06
Digestible methionine + cysteine (%)	0.96	0.91	0.79
Digestible threonine (%)	0.88	0.81	0.70
Digestible tryptophan (%)	0.28	0.26	0.21
Digestible valine (%)	1.00	0.95	0.82
Sodium (%)	0.22	0.21	0.20

[1]Minimal vitamin levels per kg of feed: vit. A (5000000 IU); vit. D3 (1.000.000 IU); vit. E (15000 IU); vit. K3 (1.500 mg); vit. B1 (1.500 mg); vit. B2 (3.000 mg); vit. B6 (2.000 mg); vit. B12 (7.000 mcg); folic acid (500 mg); nicotinic acid (15 g); pantothenic acid (7000 mcg); choline (80 g); biotin (100 mg); minimum humidity (40 g); maximum mineral matter (500 g).
[2]Minimal mineral levels per kg of feed: copper (10 g); iron (50 g); iodine (1.000 mcg); manganese (80 g); selenium (300 mg); zinc (70 g); minimum humidity (20 g); maximum mineral matter (980 g). Growth promoter (enramycin, 10 mg/kg of feed); coccidiostatic agent (salinomycin, 64 mg/kg of feed). Source: Authors.

Table 2: Composition of basal diet used for experimental groups: blend.

Ingredients	Feeding phase		
	1-21	22-35	36-42
Corn (g/kg)	508.59	538.75	643.51
Soybean meal (g/kg)	428.27	387.03	299.66
Soybean oil (g/kg)	20.27	36.68	24.52
Bicalcium phosphate (g/kg)	18.80	14.39	10.78
Limestone (g/kg)	8.42	8.46	7.25
Salt (g/kg)	4.59	4.24	4.06
DL-methionine (g/kg)	2.99	2.81	2.19
L-lysine HCL (g/kg)	2.25	1.85	2.26
L-threonine (g/kg)	0.65	0.49	0.46
L-valine (g/kg)	0.16	0.30	0.30
Premix of vitamins[1] (g/kg)	2.00	2.00	2.00
Premix of minerals[2] (g/kg)	2.00	2.00	2.00
Calculated chemical composition			
Metabolizable energy (Kcal/kg)	2.920	3.070	3.120
Crude protein (%)	24.3	22.62	19.54
Calcium (%)	0.94	0.83	0.66
Available phosphorus (%)	0.47	0.38	0.31
Digestible lysine (%)	1.33	1.20	1.03
Digestible methionine + cysteine (%)	0.94	0.89	0.77
Digestible threonine (%)	0.87	0.80	0.69
Digestible tryptophan (%)	0.28	0.26	0.21
Digestible valine (%)	1.00	0.95	0.82
Sodium (%)	0.22	0.21	0.20

[1]Minimal vitamin and mineral levels per kg of product: vit. A (5.000.000 UI); vit. D3 (1.000.000 IU); vit. E (15.000 UI); vit. K3 (1.500 mg); vit. B1 (1.500 mg); vit. B2 (3.000 mg); vit. B6 (2.000 mg); vit. B12 (7.000 mcg); folic acid (500 mg); nicotinic acid (15 g); pantothenic acid (7000 mcg); choline (80 g); biotin (100 mg); copper (10 g); iron (50 g); iodine (1.000 mg); manganese (80 g); selenium (300 mg); zinc (70 g); minimum humidity (20 g); maximum mineral matter (980 g). Source: Authors.

The following three treatment groups were defined: positive control (PC), birds that received the basal diet and enramycin (10 mg/kg) + salinomycin (64 mg/kg) at days 1 to 35; negative control (NC), birds that received the basal diet without antimicrobials; blend (FDH), birds that received reformulated diet and the blend (1 kg/ton, 1 to 42 days). Water and feed were provided ad libitum during the entire experimental period (42 days).

At 14 days of age, the birds all groups were challenged orally with a commercial live vaccine (BIOVET) containing the following species of Eimeria: *E. acervulina, E. brunetti, E. maxima, E. necatrix, E. praecox, E. tenella* and *E. mitis*, using a dosage eight times greater than the manufacturer's recommendation (28,000 oocysts/bird).

2.3 Animal performance

On days 1, 14, 21, 35 and 42 of the experiment, the animals were weighed using a digital scale and feed intake was recorded. Weight gain was obtained considering the final weight and the initial weight of each repetition (pen). Daily weight gain (g/bird/day) and daily feed intake (g/bird/day) were calculated from the weight gain and feed intake data per period, respectively. Feed conversion ratio (FCR) was calculated using the measured weight gain and feed intake per period. The productive efficiency index (PEI) was calculated as follows (Galli et al., 2021):

PEI = ((Daily weight gain x viability (%))/FCR) x100

2.4 Sampling

On day 42 of the experiment, four birds were slaughtered per group in a commercial slaughterhouse by cervical dislocation according to the animal welfare and euthanasia rules described by the CONCEA Euthanasia Practice Guidelines (BRASIL/MCTI, 2013). During slaughter, fragments of the intestine (jejunum) were collected for histology examination and the Pectoralis muscle was used for meat quality analysis.

2.5 Histopathology

The intestinal samples were stored in 10% formalin and H&E stained for histological examination. As described by Caruso and Demonte (2005), the villous length and depth of the crypts were determined with the aid of histological images captured on a digital microcamera in the Electronic Eyepiece Camera Video model, which was coupled to a trinocular microscope of the TNB-41T-PL, OPTON through a specific program (J Images) for capturing histological images. To calculate the length of the villous blades, a straight line from the tip of the villi to the upper part of the crypt was used. The depth of the crypt was also determined by using a straight line from the base of the crypt to the top.

2.6 Fecal analysis

2.6.1 Oocyst count

Fecal samples were collected on days 21 and 42 of the experiment and processed by the fluctuation centrifuge technique (Monteiro, 2010), where 1 g of feces was diluted into 15 ml of sucrose solution, centrifuged for 5 minutes and then examined under an optical microscope (100x) to count the oocysts.

2.6.2 Total coliform count and *Escherichia coli*

On days 21 and 42, fecal samples were aseptically collected for total and fecal coliform counts. After serial dilution, 1 mL of the last dilution (106) of each sample was inoculated onto a Petrifilm™ 6404 (3™) plate to obtain total coliforms (CT) counts and Escherichia coli counts. After incubation at 37 ºC for 24 hours, colony count was performed and results were expressed in colony-forming units per g of feces (CFU/g).

2.7 Meat quality analysis

The breast meat samples were kept refrigerated for five hours after which the pH was measured using an electrode, and L * (brightness), a * (red intensity) and b * (yellow intensity) were evaluated using a Minolta Chrome meter. The water holding capacity and cooking weight loss (CWL) were measured using the methods as described by Hamm (1960) and Honikel (1987), respectively. The same meat samples used for determining the cooking weight loss were used to analyze the shear force (kgf/cm) during which 1.5 cm cuts were used and positioned with the muscle fibers oriented perpendicularly to the blade of the device (Texture Analyzer TA-XT2i), coupled to another device (Warner-Bratzler) that generated the force necessary to cut the sample (Lyon et al., 1998).

2.8 Statistical analysis

All variables were submitted to the normality test (Shapiro-Wilk); variables that did not have a normal distribution were transformed into logarithms. Subsequently, the data were submitted to analysis of variance (ANOVA) and Tukey's test, in which differences between groups were considered significant at $P<0.05$.

3. Results

3.1 Animal performance

Birds of the PC group showed a better FCR as compared to the other groups (P = 0.001) on days 1 to 14 (Table 3); however, there was no difference (P > 0.05) in body weight and weight gain among groups. From days 1 to 21, a better FCR was observed in the PC group followed by the FDH group as compared to NC (P = 0.001). From days 1 to 35, the highest body weights and weight gain were demonstrated in PC followed by FDH compared to the NC (P = 0.001). From day 1 to 42, it was observed that birds in the PC and FDH groups showed a higher body weight and weight gain, and lower FCRs compared to NC (P = 0.001). There was no difference in feed intake in all periods analyzed (P > 0.05; Table 3). For the results of the productive efficiency index, the groups PC and FDH showed better results compared to the group NC (P = 0.001; Table 4).

Table 3: Performance (mean and standard deviation) of broilers.

Day 14				
Treatment	Body weight (g)	Weight gain (g)	Feed intake (g)	FCR
Negative control	442 ± 50	395 ± 49	592 ± 80	1.49 ± 0.03[a]
Positive control	461 ± 16	413 ± 17	594 ± 32	1.43 ± 0.04[b]
Blend	442 ± 46	395 ± 47	588 ± 28	1.49 ± 0.11[ab]
P-value	0.461	0.458	0.849	**0.001**
Day 21				
Treatment	Body weight (g)	Weight gain (g)	Feed intake (g)	FCR
Negative control	853 ± 90	805 ± 89	1281 ± 168	1.58 ± 0.07[a]
Positive control	918 ± 48	870 ± 48	1261 ± 61	1.44 ± 0.01[c]
Blend	871 ± 54	823 ± 54	1256 ± 53	1.52 ± 0.05[b]
P-value	0.485	0.524	0.847	**0.001**
Day 35				
Treatment	Body weight (g)	Weight gain (g)	Feed intake (g)	FCR
Negative control	2227 ± 83[b]	2154 ± 72[b]	3143 ± 163	1.45 ± 0.03
Positive control	2320 ± 56[a]	2272 ± 55[a]	3304 ± 71	1.44 ± 0.05
Blend	2295 ± 92[ab]	2247 ± 92[ab]	3200 ± 140	1.42 ± 0.01
P-value	**0.039**	**0.011**	0.695	0.107
Day 42				
Treatment	Body weight (g)	Weight gain (g)	Feed intake (g)	FCR
Negative control	2444 ± 177[b]	2392 ± 170[b]	3989 ± 260	1.67 ± 0.03[a]
Positive control	2678 ± 219[a]	2630 ± 218[a]	4121 ± 163	1.57 ± 0.06[b]
Blend	2654 ± 179[a]	2607 ± 178[a]	4122 ± 166	1.58 ± 0.05[b]
P-value	**0.001**	**0.001**	0.580	**0.001**

Different letters on the same column indicate significant differences between groups using the Tukey test. Source: Authors.

Table 4: Production efficiency index values of broilers.

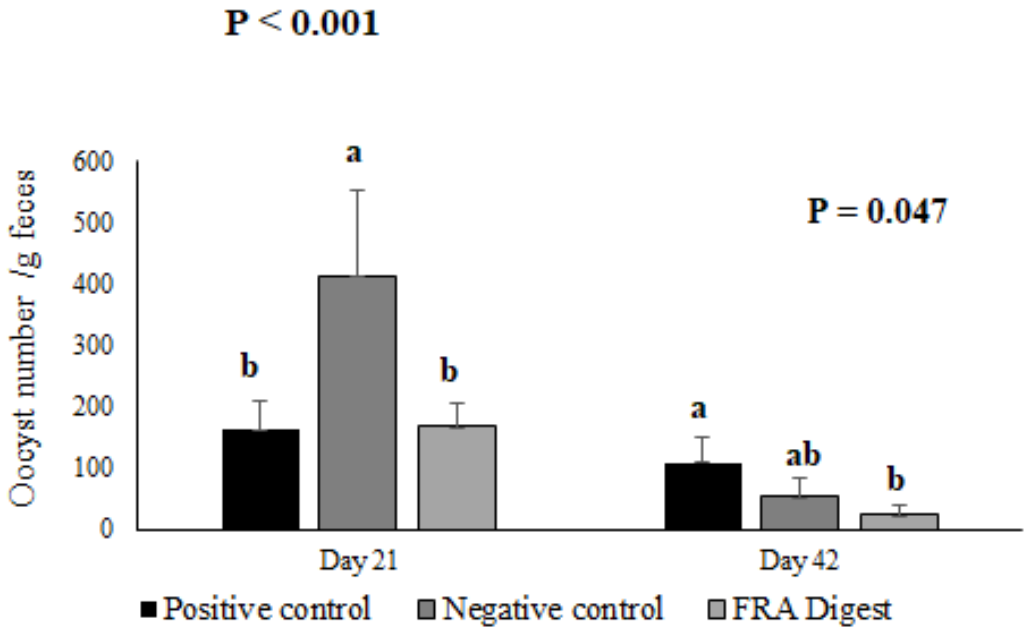
Treatment	Productive Efficiency Index (PEI)
Negative control	317.0 ± 28.2[b]
Positive control	398.0 ± 19.7[a]
Blend	387.9 ± 20.4[a]
P-value	**0.001**

Different letters on the same line indicate significant differences between groups using the Tukey test. Source: Authors.

3.2 Bacterial and coccidiostatic effect

Figure 1 shows counts of *Eimeria* spp. oocysts found in fecal samples. On day 21, PC and FDH birds had the lowest oocyst counts compared to NC ($P < 0.001$). Additionally, on day 42 the FDH group had the lowest oocyst count followed by NC compared to PC ($P = 0.047$). Microbiological analysis revealed that the total coliform count on days 21 and 42 in the FDH group was lower compared to the NC group ($P = 0.002$, $P = 0.024$). The *E. coli* counts on day 21 were lower in the PC and FDH groups compared to the NC ($P = 0.0035$). On day 42, the birds of the FDH group had lower *E. coli* counts compared to PC ($P = 0.043$). For total coliforms there was no difference between groups ($P > 0.05$; Table 5).

Figure 1. Counts of *Eimeria* sp oocyst in fecal samples of broilers at 21 and 42 days.



Source: Authors.

Table 5. Microbial count in feces of broilers on day 21 and 42.

Treatment	Day 21	Day 42
Coliforms total (CFU x10^6 / g)		
Negative control	17.9 ± 5.78	21.8 ± 14.2
Positive control	14.8 ± 10.8	21.5 ± 19.3
Blend	10.8 ± 4.74	14.3 ± 8.96
P-value	0.597	0.569
***Escherichia coli* (CFU x10^6 / g)**		
Negative control	6.74 ± 1.96^a	3.71 ± 2.19^a
Positive control	3.28 ± 2.04^b	4.69 ± 3.04^{ab}
Blend	2.73 ± 1.85^b	0.38 ± 0.64^b
P-value	**0.035**	**0.043**

Different letters on the same line indicate significant differences between groups using the Tukey test. Source: Authors.

3.3 Meat physicochemical parameters

There was no difference for the variables shear force, pH, luminosity (L), red intensity (a*), yellow intensity (b*), water retention capacity and cooking loss between groups (P>0.05; Table 6).

Table 6: Physical-chemical parameters in the meat of broilers.

Treatment	Negative control	Positive control	Blend	P-value
pH	5.69 ± 0.2	5.72 ± 0.14	5.73 ± 0.15	0.942
Color L	58.4 ± 2.4	55.2 ± 4.1	58.0 ± 2.19	0.128
Color a	-0.55 ± 1.2	-1.28 ± 0.4	-0.58 ± 1.56	0.539
Color b	9.41 ± 3.7	7.90 ± 1.76	8.92 ± 0.42	0.401
Water holding capacity	67.4 ± 3.9	71.6 ± 2.78	69.2 ± 1.25	0.084
Loss of water by cooking	26.1 ± 3.2	24.0 ± 3.4	23.3 ± 1.6	0.254
Shear force (x10^3)	2.42 ± 0.7	2.42 ± 0.6	2.54 ± 0.7	0.384

There was no difference between groups regarding meat quality. Source: Authors.

3.4 Histopathology

It was observed that the FDH and NC groups had the smallest crypt depths compared to the PC group (P=0.001). The villous:crypt ratio was higher in the NC group followed by FDH when compared to the PC group (P=0.001; Table 7).

Table 7: Intestinal histopathology of broilers.

	Villi height (µm)	Crypt depth (µm)	Villi/crypt ratio
Negative control	1281 ± 59	157 ± 32 ^{b}	8.15 ± 1.25 ^{a}
Positive control	1248 ± 74	235 ± 10 ^{a}	5.31 ± 0.75 ^{c}
Blend	1280 ± 106	193 ± 19 ^{b}	6.63 ± 0.57 ^{b}
P-value	0.681	**0.001**	**0.001**

Different letters on the same line indicate significant differences between groups using the Tukey test. Source: Authors.

4. Discussion

Brazilian poultry production continuously uses new technologies in order to optimize resources and to improve animal performance, resulting in the production of safer and healthier products such as meat and eggs. These technologies involve the use of acids in animal feed, such as the use of blends in order to enhance animal performance by thesynergistic effects of some molecules (Adewole et al., 2021). This was verified in our study where the blend, a combination of lysolecithins, lauric and

butyric acid glycerides, proved to be an excellent choice of performance enhancer in place of enramycin, one of the most commonly used antibiotics for broiler chickens.

Lecithin is ingested with feed. It generally consists of a mixture of phospholipids, glycolipids, carbohydrates and glycerides. It is an excellent source of phospholipids which supports bile salts in the emulsification of fats, the formation of micelles and the preparation of the environment for lipid hydrolysis. The pancreatic enzyme phospholipase A2 hydrolizes phospholipids, for instance PC, into the lysolecithins (i.e. lysophosphatidylcholine (LPC)) that is an even more potent emulsifier and enhances micelle formation and provides a more optimal environment for lipid hydrolysis, thereby improving fat absorption by enterocytes (Brautigan et al., 2017). Lysolecithins are composed of phospholipids containing one fatty acid, and have been used to increase feed efficiency of laying hens and enhance the performance of broilers by improving the availability of fat (Han et al., 2010; Zhang et al., 2011). Boontiam et al. (2019) mentioned that the use of lysophospholipid for broilers increases the digestibility of nutrients, ether extract and protein due to the improvement of the emulsification of fat. Thus, with the results obtained, it is believed that the use of lysolecithin positively affects animal performane by increasing and improving the fat emulsification, digestion and absorption.

Butyric acid acts directly in the provision of energy to the intestinal mucosa. As the consumption of this acid leads to the availability and production of pepsin generated from pepsinogen is also affected. Pepsin has the function of unfolding proteins into simpler peptides and, in this way, butyric acid promotes an increase in the amount of peptides available for absorption (Nari & Ghasemi 2020; Abdelqader & Al-Fataftah, 2016). With the supplementation of butyric acid, an improvement in the animal performance was observed, which might be related to the anti-inflammatory and antibacterial effect which reduces the release of macrophages and pro-inflammatory cytokines. This leads to a lower energy expenditure and tissue damage resulting from the induction of acute phase proteins to hepatocytes, which interferes with the synthesis of adenosine triphosphate (ATP) which is essential in energy production and negatively affects the loss of growth proteins (Fortuoso et al., 2019).

The addition of the blend reduced the excretion of intestinal *E. coli*, an effect that may be related to the consumption of lauric and butyric acid glycerides, which have antibacterial action. In addition, butyric acid is used by the animal as an energy source when it undergoes a beta-oxidation process forming acetyl-CoA which is used in the Krebs cycle to generate ATP. In this process, oxygen is used, which makes the medium anaerobic, disfavoring the reproduction and maintenance of opportunistic bacteria such as *E. coli* (Han et al., 2020). In addition, there is the release of H+ ions that acidify the medium, and, in this way, the bacterial cytoplasm is harmed (Han et al. 2020). Another point that deserves attention is the fact that butyric acid influences the intestinal morphology by stimulating epithelial cell turnover as reflected by the increased villous to crypt ratio and, consequently, the nutrient absorption area (Jazi, 2018). This is because these acids are used by colon cells in the mitochondrial oxidation process forming NADH, H^+ and acetyl-CoA which are used to form ATP (Han et al., 2020). In addition, what participates in the mitochondrial citric acid cycle, which allows the intestinal cells to obtain energy quickly, reducing intestinal permeability and enterocyte hypertrophy (Liu et al., 2017; Vieira, 2012). Wu et al. (2021) supplemented 1000 mg of glycerides/kg for chickens and noted an improved growth performance, increased intestinal villi length, better villus/crypt ratio, enhanced absorption capacity and immune functions, modulation of lipid metabolism and beneficial change in cecal microbial composition (Wu et al. 2021).

The use of glycerides and lysolecithins in their separate forms demonstrates their potential to replace growth promoters; however, there are up to now, few studies using the combination of these components. Adewole et al. (2021) found that the combined use of organic acids (fumaric, sorbic, malic and citric acids) increases poultry productivity. Therefore, the association of these acids can be used as a strategy to reduce the use of antibiotics in broiler chickens production. It is possible

to conclude, that the blend composed of butyric and lauric acid glycerides, in addition to lysolecithins can replace conventional growth promoters, and even be superior to them in some aspects.

4. Conclusion

The blend of lysolecithins, butyric acid glycerides and lauric acid glycerides in broiler feed can replace conventional growth promoters, as it has coccidiostatic and antimicrobial action. In addition, this blend improved animal performance and production efficiency.

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