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Emulsifier and enzymatic complex in diet for free range broiler chicken in the western amazon

Emulsificante e complexo enzimático em dieta para frangos de corte de linhagem caipira na Amazônia ocidental

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Abstract
This study aimed to evaluate the effects of adding levels of emulsifier based on soy lecithin and enzymatic complex (xylanase, β-glucanase, galactosidase, protease, amylase, and β-mannanase) on the basal diet during the raising of free-range broiler chickens. The experimental design was completely randomized, with five treatments and six replications. Treatments were: T1: control diet, without enzymatic complex and emulsifier; T2: 0.2 kg t^{-1} of enzymatic complex; T3: 0.5 kg t^{-1} of emulsifier; T4: 0.2 kg t^{-1} of enzymatic complex + 0.5 kg t^{-1} of emulsifier; and T5: 0.3 kg t^{-1} of enzymatic complex + 1 kg t^{-1} of emulsifier. Performance data were collected every 14 days, and the animals were euthanized at 70 days to obtain carcass, breast, drumstick, and thigh yield and intestine collection for pH analysis and morphometry. The periods from 1 to 56 and 1 to 70 days showed a difference ($P<0.05$) in the parameters of zootechnical performance, the feed intake was lower in T4 and T5, and weight gain was better in T1, T2, T3, and T4. Feed conversion was better in T1, T2, and T4. No difference ($P>0.05$) was observed for poultry livability, carcass yield, viscera, and pH of duodenum and cecum. A difference was found for intestinal morphometry ($P<0.05$), and T5 showed the best villus/crypt ratio. The level with 0.3 kg t^{-1} of enzymatic complex and 1 kg t^{-1} of emulsifier indicated a better relationship between villi and crypts. However, the level with 0.2 kg t^{-1} of enzymatic complex and 0.5 kg t^{-1} of emulsifier added to the commercial diet led to benefits such as decreased feed intake, without affecting weight gain, thus inducing a good feed conversion.
Keywords: Additives; Performance; Intestinal morphometry; Carcass yield.

Resumo
Objetivou-se avaliar os efeitos da adição de níveis de emulsificante à base de lecitina de soja e complexo enzimático (Xilanase, β-Glucanase, Galactosidase, Protease, Amilase, β-Mananase) na ração basal durante a criação de frangos de corte de linhagem caipira. O delineamento experimental foi inteiramente casualizado com 5 tratamentos e 6 repetições. Os tratamentos foram: T1: ração controle, sem complexo enzimático e emulsificante; T2: 0,2 kg t^{-1} de complexo enzimático; T3: 0,5 kg t^{-1} de emulsificante; T4: 0,2 kg t^{-1} de complexo enzimático + 0,5 kg t^{-1} de emulsificante; T5: 0,3 kg t^{-1} de complexo enzimático + 1 kg t^{-1} de emulsificante. A cada 14 dias foram coletados os dados do desempenho zootécnico e após 70 dias as aves foram eutanasiadas para obtenção do rendimento de carcaça, peito, coxa, sobrecoxa e coleta do intestino para análise de pH e morfometria. Nos períodos de 1 a 56 e 1 a 70 dias houve diferença ($P<0,05$) nos parâmetros de desempenho zootécnico, o consumo de ração foi menor no T4 e T5, e o ganho de peso foi melhor no T1, T2, T3 e T4. A conversão foi melhor em T1, T2 e T4. Não houve diferença ($P>0,05$) na viabilidade das aves, no rendimento de carcaça, cortes de vísceras e no pH de duodeno e cecos. Houve diferença na morfometria intestinal ($P<0,05$), sendo o T5 o que obteve melhor relação vilo/cripta. O nível 0,3 kg t^{-1} complexo enzimático com 1 kg t^{-1} emulsificante indicou melhor efeito na relação vilosidade e criptas. O nível com 0,2 kg t^{-1} do complexo enzimático com 0,5 kg t^{-1} do emulsificante adicionado a dieta comercial trouxe benefícios como diminuição do consumo de ração, sem afetar o ganho de peso, tendo assim uma boa conversão alimentar.
Palavras-chave: Aditivos; Desempenho Zootécnico; Morfometria Intestinal; Rendimento de Carcaça.

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Introduction

Poultry farming is an expanding sector, and great advances in genetics, management, nutrition, and ambient have allowed an increase in broiler meat quality. In addition to the fast-growing broiler industry, there is also an important slow-growing broiler sector. But known as "capoeira" in the Northeast, "caipira" in the North and Southeast, and colonial in the South. Free-range broiler chickens are an excellent option for raising in the climate of the Western Amazon, as they have better resistance to high temperatures. In addition, the different organoleptic characteristics of the meat, such as more tender and stronger flavor compared to industrial broiler chickens.

The reduced papers published with these slow-growing broiler lines leads to a need for studies, mainly in tropical climate regions. This sector may represent a promising source of income for family producers, and a minimum of technification can add value to their production. However, alternative birds still have physiological limitations in the digestive system, which can be overcome using exogenous strategies to improve their performance[1]. The use of feed additives can be an example of technification accessible to the producer, which will bring advantages for poultry raising.

Among them, the use of enzymes and emulsifiers are alternatives that can improve production, as these additives act by making more nutrients available to the animal, improving the performance of alternative birds since there are no specific feeds for slow-growing chickens on the market. According to Fortes et al.[2] the addition of an enzymatic complex to diets composed of corn and soybean accelerates the degradation of cell wall fibers, promoting better nutrient digestibility and optimizing nutrient digestibility, which can promote weight gain in animals because there are more nutrients available.

The addition of an emulsifier to broiler chicken diets is a less frequent practice compared to other feed supplements. Emulsifiers act by increasing the active surface of fats, allowing the action of lipase, which hydrolyzes triglyceride molecules and improves the formation of micelles, creating a diffusion gradient that increases the absorption of fatty acids, monoglycerides, and fat-soluble nutrients, providing greater use of energy, which may improve bird performance[1].

This study aimed to evaluate the effect of supplementing diets with levels of an enzymatic complex and an emulsifier, alone and associated, on the performance, carcass yield, pH of duodenum and cecum, and duodenum morphometry of free-range broiler chickens.

Material and methods

The experiment was conducted from August to October 2019 in a poultry farm located in the municipality of Rio Branco, Acre, Brazil, at 143 meters of altitude, and geographic coordinates 9°58′26″ S and 67°48′27″ W. According to the Köppen classification, the area is located in the tropical zone characterized by rains and monsoons[3]. The project was approved on 06/14/2018 by the Ethics Committee on the Use of Animals (CEUA-UFAC), process 23107.010003/2018-04, protocol 15/2018.

A total of 300 one-day female slow-growing broiler chickens of the "*Vermelho Pesadão"* lineage were used. The birds were acquired in a certified hatchery, being already vaccinated against Marek, Gumboro, and fowl pox. The vaccination against Newcastle disease was carried out at 14 days. A poultry litter was placed about 48 hours before the arrival of the chicks. The material consisted of wood shavings with superimposed newspaper sheets in all pens. Drinkers and tray feeders were placed around 24 hours before. An incandescent lamp was placed in each pen to maintain the temperature of the chicks.

The birds were observed and weighed upon arrival to achieve homogeneity in the lot and obtain the initial weight. Then, they were taken to the pens. Management was carried out twice a day, early in the morning and late in the afternoon, always at the mildest times to avoid thermal stress. Also, drinkers were cleaned, and water and feed were supplied to the birds, thus ensuring food and water ad libitum. Commercial feed was used, with the manufacturer's composition levels shown in Table 1.

The commercial feed was supplemented with incresing dosis of an enzyme complex and an emulsifier additive. The enzymatic complex is called Tecnase® and is composed of the following enzymes: xylanase (11,500 U/g), β-glucanase (855 U/g), galactosidase (110 U/g), protease (4,230 U/g), amylase (850 U/g), and β-mannanase (1,210 U/g). The emulsifier is called Lipidol Ultra® and is composed of soy lecithin. Thus, the five treatments were distributed as follows:

T1: Control feed: without enzymatic complex and emulsifier

T2: Control feed: with 0.2 kg t^{-1} of enzymatic complex

T3: Control feed: with 0.5 kg t^{-1} of emulsifier

T4: Control feed: with 0.2 kg t^{-1} of enzymatic complex + 0.5 kg t^{-1} of emulsifier

T5: Control feed: with 0.3 kg t^{-1} of enzymatic complex + 1.0 kg t^{-1} of emulsifier

Table 1. Composition of the commercial feed (initial and growth) used for free-range chicken broilers

Component	Initial	Growth	Component	Initial	Growth
	%			%	
Moisture (max.)	12	12	Choline (min)	0.000035	0.025
Crude protein (min)	19	17	Iron (min)	0.00525	0.0006
Ether extract (min)	30	30	Iodine (min)	0.000126	0.025
Crude fiber (max.)	50	50	Manganese (min)	0.007	0.005
Ashes (max.)	110	110	Methionine (min)	0.000216	0.00012
Calcium (min)	0.7	7	Niacin (min)	0.0032	0.006
Calcium (max.)	1.5	15	Selenium (min)	0.00003	0.00017
Phosphorus (min)	0.6	0.6	Zinc (min)	0.0063	0.0024
Sodium (min)	0.014	0.014	Nicarbazin (min)	0.005	-
Folic acid (min)	0.00006	0.00005	Narasin (min)	0.005	-
Pantothenic acid (min)	0.0008	0.0008	Salinomycin (min)	-	0.0066
Biotin (min)	0.000006	3,00E-06	Halquinol (min)	-	0.003
Copper (min)	0.00063	0.0006			

Source: Nutrak.

Birds and the feed were weighed every 14 days to determine the mean weight gain (kg), feed intake (kg), and feed conversion. Mortality was considered to calculate livability (%). Thus, the experimental periods were 1 to 14, 1 to 28, 1 to 42, 1 to 56, and 1 to 70 days of age. Regarding performance, intake was determined by the difference between the feed offered and feed leftovers in the feeder at the end of each experimental period. Birds were weighed at the beginning and end of each period to determine mean weight gain and feed intake. Feed conversion was calculated by the ratio between intake and weight gain. Livability was calculated by the difference in the percentage of mortality.

The carcass yield was determined using two birds representing the average of each experimental unit, which were fasted for eight hours until they were sacrificed in the poultry sector of the Universidade Federal do Acre. The defeathered and eviscerated carcass weight (with feet and head) was considered relative to the live weight after fasting. The yield of cuts (breast, drumstick, and thigh), abdominal fat, and viscera (liver, gizzard, and heart) was calculated relative to the weight of the eviscerated carcass (with feet and head).

The pH was determined on the day of slaughter using the duodenum and cecum, whose digesta contents were placed in a beaker containing 20 mL of distilled water and stabilized for 10 minutes to perform the reading with a portable pH meter. Duodenum samples were also collected along the length of the first intestinal loop (4 cm), washed in a 10% formalin solution, and submitted to histomorphometry analysis. Duodenum segments were washed in saline solution, opened by their mesenteric border, extended by the serous tunic, and fixed in 10% formalin for 24 hours, after which the solution was changed. Subsequently, the samples were reduced and placed in alcohol and diaphonized in xylene for inclusion in histological paraffin. After embedding in paraffin, 6-µm thickness sections were made using a microtome for making slides, which were later stained with Hematoxylin-Eosin.

The villus height and crypt depth of duodenum samples were measured after the staining procedure using an optical microscope coupled to a Leica image analyzer system (Image-Pro Plus version 1.0.0.1), using a 40x objective lens. Villus height measurements were taken from the basal region, coinciding with the upper portion of the crypts up to the apex. Height measurement was performed from one end of the villus to the other, while the crypts were measured from the base to the transition region between crypt and villus[4].

The design was completely randomized, with five treatments and six replications. The experimental unit or plot consisted of 10 birds per box (initially), and the means of the quantitative variables of these birds were

considered for determining the avian performance. The mean was corrected in case of mortality to avoid overestimated values. An exploratory data analysis was performed to indicate possible violations of the model assumption for analysis of variance (ANOVA). Subsequently, ANOVA was performed to verify possible differences between the effect of treatments for each analyzed variable. The Scott-Knott test was performed when the F-test showed a significant difference between the effects of treatments to verify which treatments differed from each other for each of the analyzed variables. All analyses were performed at a 5% significance level using the statistical software SISVAR version 5.6[5].

Results

Avian performance

Table 2 shows the results of performance for feed intake (FI, kg), weight gain (WG, kg), feed conversion (FC), and raising livability (RV, %) of free-range broiler chicken according to treatment and raising period.

Table 2. Mean feed intake (kg), mean weight gain (kg), and feed conversion of free-range broiler chickens fed diet with added levels of enzyme complex and emulsifier

Treatment (kg t^{-1})	Period (days)				
	1 to 14^{ns}	1 to 28^{ns}	1 to 42^{ns}	1 to 56*	1 to 70*
	Mean feed intake				
Basal feed	0.248	0.937	1.90	3.09 b	4.29 b
0.2 enzyme complex	0.257	0.900	1.92	3.11 b	4.31 b
0.5 emulsifier	0.259	0.902	1.91	3.11 b	4.31 b
0.2 enzyme complex + 0.5 emulsifier	0.247	0.983	1.87	3.03 a	4.20 a
0.3 enzyme complex + 1.0 emulsifier	0.253	0.911	1.91	3.05 a	4.25 a
CV (%)	3.72	5.85	3.49	1.84	1.22
P-value	0.1390	0.1607	0.8850	0.673	0.0059
Standard error	0.00384	0.0243	0.0271	0.0231	0.0212
	Mean weight gain				
Basal feed	0.107	0.417	0.864	1.33 a	1.86 a
0.2 enzyme complex	0.111	0.397	0.858	1.34 a	1.87 a
0.5 emulsifier	0.106	0.391	0.837	1.31 a	1.83 a
0.2 enzyme complex + 0.5 emulsifier	0.111	0.403	0.844	1.33 a	1.86 a
0.3 enzyme complex + 1.0 emulsifier	0.113	0.405	0.836	1.25 b	1.77 b
CV (%)	5.98	5.02	3.03	1.89	2.31
P-value	0.3020	0.3997	0.2462	0.0000	0.0042
Standard error	0.0027	0.4017	0.0104	0.0101	0.0173
	Feed conversion				
Basal feed	2.32	2.24	2.18	2.31 a	2.31 a
0.2 enzyme complex	2.31	2.28	2.24	2.32 a	2.30 a
0.5 emulsifier	2.45	2.30	2.28	2.37 b	2.36 b
0.2 enzyme complex + 0.5 emulsifier	2.23	2.44	2.24	2.27 a	2.26 a
0.3 enzyme complex + 1.0 emulsifier	2.25	2.25	2.28	2.44 b	2.40 b
CV (%)	5.84	8.21	4.06	2.41	2.47
P-value	0.0837	0.4623	0.3608	0.0003	0.0037
Standard error	0.0551	0.0852	0.0372	0.0229	0.0234

ns: non-significant difference between means in the column. *Means followed by the same letter in the column do not differ (p>0.05) from each other by the Scott-Knott test at the 5% probability level. CV: coefficient of variation.

The periods 1–14, 1–28, and 1–42 showed no difference (P>0.05) between the means of treatments for feed intake, weight gain, and feed conversion. However, periods 1–56 and 1–70 showed a difference (P<0.05) for these variables. Table 3 shows the livability of all raising periods.

The raising livability at 70 days ranged from 96.66 to 100%, being considered satisfactory, as 10% mortality is acceptable for free-range broiler chicken farming, that is, the ideal is that the livability is above 90%.

Carcass yield

Table 4 shows the results regarding carcass yield and prime cuts (breast, drumstick, and thigh) of free-range broiler chickens at 70 days. Carcass, breast, drumstick, and thigh yield showed no differences (P>0.05). The carcass yield values ranged from 69.85 to

76.92%. Breast yield varied between 21.21 and 22.25%. Drumstick yield ranged between 14.43 and 16.01%. Thigh yield ranged from 16.39 to 16.95%. Table 5 shows the results regarding the yield of edible viscera (gizzard, liver, and heart), intestine, and abdominal fat of free-range broiler chickens at 70 days.

Table 3. Viability of free-range broiler chickens fed different levels of enzyme complex and emulsifier

Treatment (kg t^{-1})	Period (days)				
	1–14	1–28	1–42	1–56	1–70
Basal feed	100	100	100	96.66	96.66
0.2 enzyme complex	100	100	100	98.33	98.33
0.5 emulsifier	100	100	98.33	98.33	98.33
0.2 enzyme complex + 0.5 emulsifier	100	100	100	100	100
0.3 enzyme complex + 1.0 emulsifier	100	100	98.33	98.33	98.33

Table 4. Carcass yield and prime cuts (%) relative to clean carcass weight of free-range broiler chickens fed basal diets containing levels of enzyme complex and emulsifier

Treatment (kg t^{-1})	YIELD (%)			
	Carcass ^{ns}	Breast ^{ns}	Drumstick ^{ns}	Thigh ^{ns}
Basal feed	73.58	22.25	15.99	16.85
0.2 enzyme complex	73.36	21.21	15.62	16.82
0.5 emulsifier	69.85	21.29	14.43	16.95
0.2 enzyme complex + 0.5 emulsifier	76.92	21.76	16.01	16.94
0.3 enzyme complex + 1.0 emulsifier	72.75	21.29	15.71	16.39
CV (%)	10.79	10.62	10.72	9.33
P-value	0.3146	0.7712	0.1372	0.9050
Standard error	2.283	0.6609	0.4811	0.4522

ns: non-significant difference between means in the column. CV: coefficient of variation.

Table 5. Viscera yields (%) relative to clean carcass weight of free-range broiler chickens fed basal diets containing levels of enzyme complex and emulsifier

Treatment (kg t^{-1})	YIELD (%)				
	GY ^{ns}	LY ^{ns}	HY ^{ns}	IY ^{ns}	AFI ^{ns}
Basal feed	3.03	2.43	0.66	6.11	5.07
0.2 enzyme complex	2.77	2.22	0.58	5.55	5.38
0.5 emulsifier	2.71	2.17	0.58	5.73	5.45
0.2 enzyme complex + 0.5 emulsifier	2.89	2.29	0.56	5.98	5.57
0.3 enzyme complex + 1.0 emulsifier	2.67	2.44	0.56	5.16	5.51
CV (%)	11.35	10.47	17.39	19.32	25.56
P-value	0.5999	0.7277	0.1057	0.2563	0.2343
Standard error	0.1735	0.1706	0.0295	0.3183	0.1631

ns: non-significant difference between means in the column. CV: coefficient of variation. GY: gizzard yield; LY: liver yield; HY: heart yield; IY: intestine yield; AFI: abdominal fat yield.

The yield of the edible viscera gizzard, liver, and heart showed no difference (P>0.05). The gizzard results ranged from 2.67 to 3.03%. Liver yield ranged from 2.17 to 2.44%. Heart yield varied between 0.56 and 0.66%. The yield of the non-edible viscera intestine and abdominal fat varied between 5.16 and 6.11% and 5.07 to 5.57%, respectively.

Intestinal morphometry and pH

Table 6 shows the results regarding villus height, crypt depth, and the villus/crypt ratio of free-range broiler chickens at 70 days.

Table 6. Villus height (µm), crypt depth (µm), and villus/crypt ratio of the duodenum of free-range broiler chickens fed basal diets containing levels of enzyme complex and emulsifier at 70 days of age

Treatment (kg t^{-1})	Villus height (µm)*	Crypt depth (µm)*	V/C*
Basal feed	81.68 b	13.94 c	5.88 c
0.2 enzyme complex	76.37 c	11.77 a	6.49 b
0.5 emulsifier	82.91 b	13.03 b	6.37 b
0.2 enzyme complex + 0.5 emulsifier	85.13 b	12.99 b	6.60 b
0.3 enzyme complex + 1.0 emulsifier	98.80 a	13.96 c	7.09 a
CV (%)	4.10	5.54	7.07
P-value	0.000	0.0001	0.0028
Standard error	1.421	0.2970	0.1873

*Means followed by the same letter in the column do not differ (p>0.05) from each other by the Scott-Knott test at the 5% probability level. CV: coefficient of variation.

The results regarding villus height, crypt depth, and villus/crypt ratio showed a difference (P<0.05). The treatment with 0.3 kg t^{-1} of enzymatic complex and 1 kg t^{-1} of emulsifier expressed the highest villus height, while the treatment with 0.2 kg t^{-1} of enzymatic complex presented the lowest villus height. Figure 1 shows the image obtained using an optical microscope coupled to an image analyzer system for measurements of villus height and crypt depth of duodenum samples. Table 9 shows the results regarding the pH of the duodenum and ceca of free-range broiler chickens at 70 days.

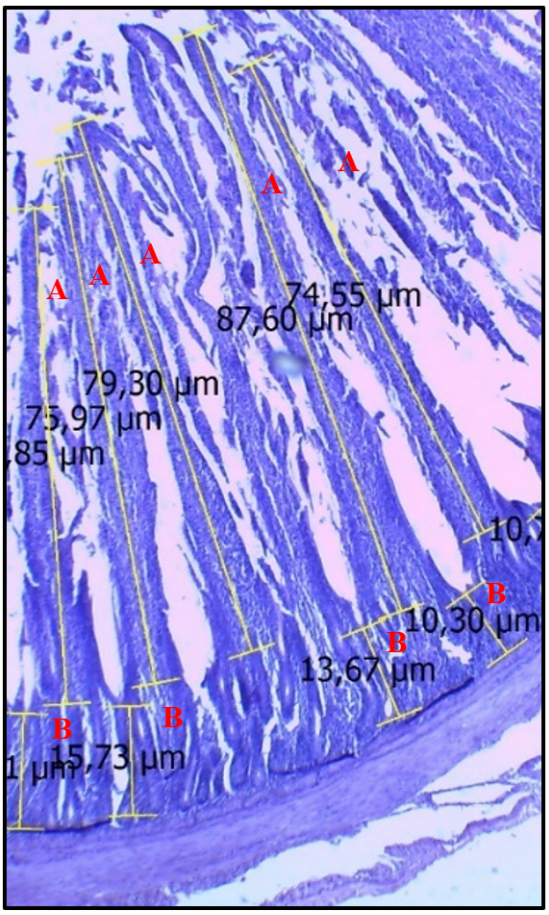

Figure 1. Photomicrograph showing duodenum of free-range broiler chicken: **A** - Height of villi and **B** - Depth of duodenum crypts of free-range broiler chicken. 40X. Stained by the eosin-hematoxylin method.

Table 7 – pH values in the duodenum and ceca at 70 days of age of free-range broiler chickens fed basal feed containing levels of enzyme complex and emulsifier

Treatment (kg t^{-1})	pH	
	DUODENUM ^{ns}	CECA ^{ns}
Basal feed	6.48	6.94
0.2 enzyme complex	6.37	6.77
0.5 emulsifier	6.31	6.83
0.2 enzyme complex + 0.5 emulsifier	6.13	6.99
0.3 enzyme complex + 1.0 emulsifier	6.20	6.96
CV (%)	2.10	2.54
P-value	0.4824	0.6001
Standard error	0.2051	0.2309

ns: non-significant difference between means in the column. CV: coefficient of variation.

No differences (P>0.05) were observed in the pH of the duodenum and ceca of free-range broiler chickens at 70 days. The pH of the duodenum ranged from 6.13 to 6.48 and that of the ceca ranged from 6.77 to 6.99.

Discussion

Avian performance

Feed intake in the periods 1–56 and 1–70 was lower in treatments with the addition of enzymatic complex and emulsifier, that is, the addition of these additives associated influenced the results most likely due to their joint action. Enzymes act to increase digestion reactions and the emulsifier has action in the use of lipids. These reactions provide more nutrients and energy to the animal, providing a higher feeling of satiety in birds.

Similar results on the use of enzymes in broiler's diet were found by Dalólio et al.[6], who used an enzymatic complex based on phytase, protease, xylanase, β-glucanase, cellulase, amylase, and pectinase in corn- and soybean-based diets and did not observe differences in feed intake of broiler chickens compared to the control treatment. Regarding the use of emulsifiers, Guerreiro Neto et al.[1] used the fat sources soybean oil, viscera oil, and their association and did not observe differences in feed intake when adding an emulsifier, a result similar to that observed in this study when using the emulsifier alone in the commercial feed.

According to Guerreiro Neto et al.[1], the lack of results of weight gain in the first days may be related to the emulsifier action not being very efficient in the first days of life, as there is low activity of the lipase enzyme in this period. This fact results in lower lipid digestion and absorption, which, consequently, reduces the energy destined for production, thus not positively interfering with the animal growth in the initial period. Weight gain at the final raising stages was lower in the treatment with 0.3 kg t^{-1} enzime complex and 1 kg t^{-1} emulsifier, showing that the treatment with 0.2 kg t^{-1} enzime complex and 0.5 kg t^{-1} emulsifier is enough to guarantee the weight of the birds. The increased level would be a waste, as it will not be used by the bird, negatively affecting weight gain.

This study corroborates with Cho et al.[7], who used an emulsifier and an enzymatic complex composed of α-galactosidase, galactomannanase, xylanase, and β-glucanase and did not find differences in weight gain for broilers fed basal feed. On the other hand, the use of these additives in low-energy density diets improved weight gain compared to the low energy diet without the enzymatic complex and emulsifier.

Unlike this study, Guerreiro Neto et al.[1] used soybean oil as a source of lipids in the diet of broilers and observed higher weight gain with the inclusion of an

emulsifier. Barbosa et al.[8] also found different results regarding the use of an enzymatic complex. These authors used xylanase, protease, amylase, and phytase in corn-and soybean-based diets with negative energy control and found higher weight gain in chickens compared to treatments without the inclusion of the enzymatic complex. In turn, Dalólio et al.[6] found no influence of phytase, protease, xylanase, β-glucanase, cellulase, amylase, and pectinase on the weight gain of birds.

The best treatments for feed conversion in the periods 1–56 and 1–70 feed basal without additives, 0.2 kg t^{-1} enzime complex, 0.5 kg t^{-1} emulsifier, 0.2 kg t^{-1} enzime complex, and 0.5 kg t^{-1} emulsifier. The treatment with the addition of 0.2 kg t^{-1} enzime complex and 0.5 kg t^{-1} emulsifier stood out positively, showing a lower feed intake, without affecting weight gain and resulting in better feed conversion.

The results of this study contrast with those found by Cho et al.[7], who used both emulsifier and enzymatic complex and did not observe differences in feed conversion of broilers fed basal diet. Guerreiro Neto et al.[1] used lipid sources and found higher weight gain with the inclusion of emulsifiers. Barbosa et al.[8] used enzymatic complex in corn-and soybean-based diets and energy imbalance and detected better feed conversion in broilers relative to treatments without the inclusion of the enzymatic complex.

Carcass yield

Carcass yield and prime cuts represent the percentage to be marketed, which shows the importance of this knowledge. In this study, the use of enzymatic complex and emulsifier alone and associated did not interfere with the percentages of carcass yield of free-range broiler chickens. The carcass yield result corroborates with Dalólio et al.[6], who observed that phytase, protease, xylanase, β-glucanase, cellulase, amylase, and pectinase did not influence the carcass yield of birds. On the other hand, these authors observed higher breast yield, which was not observed in the present study. Controversies in the results can be explained by variations in the type and level of enzymatic complex supplementation, as well as in the formulation and quality of the diet ingredients, the interactions between birds and the raising environment, and management[9].

In contrast to this study, Fonseca et al.[10] used a soy lecithin emulsifier and obtained a better yield of prime cuts compared to the control treatment. However, similar to the present research, Kamran et al.[11] studied emulsifier levels in different lipid sources in the diet of broilers and observed no influence on carcass yield despite the obtained differences in productive performance.

In this study, the use of enzymatic complex and emulsifier alone and associated did not affect viscera yield, as observed by Cho et al.[7], who used an emulsifier and an enzymatic complex and did not find differences in viscera yield from broiler chickens fed basal diet. Fonseca et al.[10] studied three types of emulsifiers, including soy lecithin, used in the present study, and found no differences in viscera yield. According to Zhao et al.[12], the inclusion of an emulsifier reduces abdominal fat yield, but this result was not observed in the present study. In fact, Oliveira et al.[13] found no influence on the use of emulsifiers on fat deposition in the abdominal region of birds.

Intestinal morphometry and pH

The small intestine wall is made up of four tunics: mucosa, submucosa, muscularis, and serosa. The small intestine mucosa has many microscopic evaginations called villi, which provide an increase in the internal surface of the organ, which is the area of intestinal digestion and absorption[14]. The mucous tunic also has invaginations called crypts, which are simple tubular glands formed between the base of the adjacent villi and the epithelium towards the lamina propria[14].

The treatment with 0.2 kg t^{-1} of enzymatic complex showed the smallest crypt depth, while the treatments basal diet and with 0.3 kg t^{-1} of enzymatic complex and 1.0 kg t^{-1} of emulsifier had the highest crypt depth. The best villus/crypt ratio was observed in the treatment with 0.3 kg t^{-1} of enzymatic complex and 1.0 kg t^{-1} of emulsifier. This high ratio indicates ideal circumstances for better nutrient absorption with less loss of energy used for cell renewal, meaning a good intestinal health[15].

Similarly, Kubis et al. (2020) used xylanase enzyme and an emulsifier in wheat-based diets and observed higher crypt depth in treatments that had both the enzyme and the emulsifier. However, these authors observed no differences in villus height, unlike our results. In turn, Wickramasuriya et al.[17] used lipase enzyme and an emulsifier in broilers and noticed a reduction in crypt depth in the treatment that used only the emulsifier with negative energy control, thus increasing the villus/crypt ratio.

Nutrient absorption depends on the functional integrity of villus cells, villus height depends on the number of cells that compose it. Thus, the more the number of cells, the larger the villus size, maximizing the area for nutrient absorption[14]. The variation in the effectiveness of exogenous emulsifiers can be attributed to many factors, such as type of fat, bird age, lipase activity, and hydrophilic-lipophilic balance, in addition to differences in the interactions between the type of dietary fat and emulsifiers or enzymes used in the experiments[17].

According to Verdal et al.[18], birds with inefficient gastric compartments and digestive enzymes have higher

villus height and higher villus/crypt ratio just to compensate for this low functionality, improving the absorptive processes. The intestine with higher development conditions better absorption and use of nutrients, which reflects in weight gain, but it did not occur in the present study.

Lipid emulsification and hydrolysis by pancreatic lipase occur in the duodenum. The addition of enzymatic complex and emulsifier in this study could not change the homeostasis capacity of birds, which allows digesta lipids to reach the intestine to find the ideal pH to enable the action of emulsifiers and lipases,[19] without the effect of additives alone or synergistically to modify intestinal pH. This study corroborates with Vaz et al.[9], who used an enzymatic complex composed of the enzymes phytase, protease, xylanase, ß-glucanase, cellulase, amylase, and pectinase and also obtained no differences in the pH of duodenum and ceca.

Conclusion

Supplementation of diets with levels of enzymatic complex and emulsifier separately had no positive effect on the productive characteristics of the birds. Already combined at the level 0.2 kg t-1 enzymatic complex and 0.5 kg t-1 emulsifier demonstrated positive effects on the characteristics of avian performance. The level of 0.3 kg t-1 enzymatic complex with 1 kg t-1 emulsifier indicated a better effect on the villus and crypts ratio. The addition of enzymatic complex and emulsifier had no effect on carcass yield, cuts, viscera, duodenum and cecum pH.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: A. M. do Nascimento and F. A. Gomes; *Formal analysis:* A. M. do Nascimento, F. A. Gomes; S. F. da C. Rodrigues, G. A. Pires and C. A. Guato; *Supervision:* E. M. de Souza and H. J. de Freitas.

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