REVISTA TÓPICOS
ISSN 2965-6672 | Qualis A2

GUAVA BY-PRODUCT MITIGATES THE EFFECTS OF EIMERIA INFECTION IN BROILER CHICKENS CHALLENGED WITH AN ATTENUATED VACCINE

SUBPRODUTO DE GOIABA REDUZ O IMPACTO DA EIMERIA EM FRANGOS DESAFIADOS POR VACINA ATENUADA

Ciências Agrárias • 20/07/2026

REGISTRO DOI: 10.70773/revistatopicos/784430560

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ABSTRACT

This study evaluated the effects of the inclusion of guava by-product flour (GBF) in the diets of broilers under challenged and unchallenged conditions using a live attenuated *Eimeria spp*. vaccine. Performance parameters, intestinal morphometry and fecal oocyst counts were evaluated. Four hundred day-old Cobb-500 chicks were randomly allocated to two rooms with 20 pens each and submitted to four feeding treatments: 0%, 2% and 4% GBF inclusion, and 0% GBF, with the addition of a commercial probiotic based on *Bacillus* spp. On the 15th day, birds in one of the rooms were challenged with an attenuated *Eimeria* vaccine. Performance data were recorded through Day 21, with fecal samples collected on Days 7, 14, and 21 for oocyst counts. Intestinal segments were collected on day 22 for morphometric analysis. In birds without challenge, the inclusion of GBF had no significant effect on performance parameters, although higher villus height in the jejunum and increased villi:crypt ratio were observed in the jejunum (P<0.05) and duodenum (P<0.01), which are indicative of greater intestinal absorptive capacity. Among challenged birds, treatment with 4% GBF resulted in better performance in all variables (P<0.05) and reduced oocyst excretion (P<0.01). It is concluded that the inclusion of 4% GBF may contribute to gut integrity and help mitigate the harmful effects of *Eimeria spp*. infection in broiler chickens.

Keywords: broiler chickens; intestinal morphometry; functional food; guava by-product; oocyst count.

RESUMO

Este estudo avaliou os efeitos da inclusão de farinha de subproduto de goiaba (GBF) nas dietas de frangos de corte com e sem desafio experimental por vacina viva atenuada de *Eimeria spp*. Desempenho produtivo, morfometria intestinal e contagens de

oocistos fecais foram avaliados. Quatrocentos pintos de um dia foram alocados aleatoriamente em duas salas com 20 cercados cada e submetidos a quatro tratamentos alimentares: 0%, 2% e 4% de inclusão de GBF, e 0% de GBF, com a adição de um probiótico comercial baseado em *Bacillus* spp. No 15° dia, aves em uma das salas foram desafiadas com uma vacina atenuada. Os dados de desempenho foram registrados até o dia 21, com amostras fecais coletadas nos dias 7, 14 e 21 para contagem de oocistos. Segmentos intestinais foram coletados no dia 22 para análise morfométrica. Em aves sem desafio, a inclusão do GBF não teve efeito significativo no desempenho, embora tenham sido observados maior altura das vilosidades no jejuno e aumento da relação vilosidade:cripta no jejuno ($P<0,05$) e no duodeno ($P<0,01$), que são indicativos de maior capacidade absortiva. Nas aves com desafio, o tratamento com 4% GBF resultou em melhor desempenho em todas as variáveis ($P<0,05$) e redução da excreção de oocistos ($P<0,01$). Conclui-se que a inclusão de 4% de GBF pode contribuir para a integridade intestinal e ajudar a mitigar os efeitos prejudiciais da infecção por *Eimeria spp.* em frangos de corte.
Palavras-chave: frangos de frango; morfometria intestinal; alimento funcional; subproduto da goiaba; contagem de oocistos.

1. INTRODUCTION

Brazil is the third largest producer of fruits in the world, and a significant part of this production is destined for agro-industrial processing (ABRAFRUTAS, 2023), which generates large volumes of waste material such as peels, seeds, pulp, and bagasse — components that represent a considerable fraction of the raw material used (Maqsood et al., 2025). As the sector has grown, so has the problem of improper disposal of this waste. Guava is a highly nutritious fruit rich in vitamins and minerals, and its by-products have been investigated as alternative ingredients for animal feed, including lamb (Nobre et al., 2024), fish (Furuya et al., 2008), and broiler chickens (Oliveira et al., 2018). Studies have reported benefits in both cost reduction and improved animal health, mainly due to the presence of bioactive compounds.

Further research on guava's agro-industrial by-products could reveal new applications, support the development of value-added products, reduce environmental impacts, and improve the use of important nutrients that would otherwise be wasted.

Brazil is also one of the world's largest producers and the main exporter of chicken meat, known for its competitiveness, sanitary standards, and production efficiency (ABPA, 2025). Bioactive compounds present in guava residue, such as flavonoids, tannins, phenolic acids, and saponins, are potentially useful for the development of intestinal epithelium in chickens (Noleto-Mendonça, 2021), either by trophic action on enterocytes, by promoting microbiota modulation, or by acting against pathogens, such as coccidia, factors that trigger better intestinal absorption and, consequently, better performance parameters (Chen et al. 2024).

Although research indicates desirable effects of the guava by-product on the intestine, there are no studies that have evaluated the effect on animals under challenging conditions. This study aimed to evaluate the effects of guava by-product flour (GBF) in broiler diets on performance parameters, intestinal morphometry and fecal oocyst count in both challenged and unchallenged birds. The hypothesis investigated is that the inclusion of guava by-product in the diet of broilers attenuates intestinal epithelial lesions associated with *Eimeria infection*, preserves the morphometry of the intestinal mucosa, reduces the elimination of oocysts and improves performance parameters in animals subjected to the coccidial challenge.

2. MATERIALS AND METHODS

The experiment was carried out at the Federal Institute of Education, Science and Technology of Rio de Janeiro (IFF) – Bom Jesus do Itabapoana campus, Brazil, in collaboration with the Animal Science Laboratory of the State University of Northern Rio de Janeiro (UENF). The processing of the intestinal segments was carried out at the Laboratory of Animal Morphology and Pathology of the Veterinary Hospital of UENF. The experimental protocol was approved by the Ethics Committee on the Use of Animals (CEUA/REIT/IFFLU, approval No. 37, issued on September 14, 2023).

2.1. Animals and Housing

A total of 400 one-day-old Cobb-500 broiler chickens (males and females) were kept in pens measuring 120X80cm, containing tubular feeders and bowl-type drinkers, on a floor with wood shavings, with feed and water *ad libitum*. Animals with body weight ranging between 35 and 48 g were selected, and then distributed in order to ensure the uniformity of the lots in terms of average weight. Ten birds were allocated to each pen.

The birds were housed in two identical rooms, with 20 pens each, with east-west orientation, masonry side walls and mesh walls on the remaining sides, cement floor and ceramic tile roof with PVC lining. The screened sides were equipped with movable curtains to control ventilation and temperature. Temperature and relative humidity were monitored daily, with curtain management and heating to maintain thermal comfort.

Only birds from one of the rooms were submitted to the experimental challenge. The two groups were identified as "no challenge" (NC) and "with challenge" (WC)

The light and temperature program followed recommendations from the lineage manual (COBB-VANTRESS, INC., 2021). The temperature and humidity of the house were monitored with thermo-hygrometers and adjusted using electric heaters, fans and curtains. For the lighting, LED lamps and timers were used.

2.2. Guava By-Product Flour (GBF)

Guava by-products were collected from the fruit processing agroindustry at the Federal Institute of Espírito Santo (Ifes) – Itapina Campus, located in Colatina, Espírito Santo, Brazil. The material was dried at 60 °C in a circulating air dehydrator, with the moisture content monitored by the constant weight method, until it reached values below 13%. The dried by-product was then ground and stored in sealed plastic bags under freezing conditions (–7 °C).

The analysis of the physicochemical composition of the GBF used was published by Assis et al. (2025). The centesimal composition of the guava by-product flour presented the following values: neutral detergent fiber (NDF) of 37.42 ± 0.244%; crude protein of 9.37 ± 0.103%; moisture from 11.94 ± 0.58%; ashes from 2.04 ± 0.109%; lipids of 5.76 ± 0.328%; total carbohydrates from 76.65 ± 0.301%; non-fiber carbohydrates from 2.18 ± 0.283%; and total tannins of 0.0051 ± 0.175%.

2.3. Experimental Treatments

Four treatments consisting of balanced diets suitable for each growth phase were evaluated. Three treatments included 0%, 2% and 4% levels of GBF, which was considered in this study as a functional ingredient with additive effects, rather than a nutritional substitute for corn or soybean flour. The fourth treatment used the same formulation with 0% GBF, but included a commercial probiotic for poultry and swine (PAS-TR, Imeve®) at the minimum dose recommended by the manufacturer (100 g/ton), containing *Bacillus cereus* and *Bacillus subtilis* (4.0×10^{12} CFU/kg). The inclusion of this treatment aimed to compare the effect of GBF with the commercial alternative most commonly accessible to poultry farmers in the region, used to promote the modulation of the intestinal microbiota and improve performance. The four treatments were designated as follows:

- 0%: Basal diet without GBF;
- 2%: Diet with 2% GBF;
- 4%: Diet with 4% GBF;
- 0+P: Basal diet with probiotic supplementation.

The experimental diets were formulated to be nutritionally equivalent (isoenergetic and isoprotein) and to meet the nutritional needs of the pre-onset (up to seven days) and early (eight to 21 days) phases, according to the recommendations of Rostagno et al. (2024) and the Cobb strain management guide (COBB-VANTRESS, 2025). The ingredients, nutritional composition and satisfaction of requirements are presented in Table 1. Coccidiostats and antibiotics were not included in the premixed formulation.

Table 1. Ingredient composition and calculated nutritional composition of experimental diets with graded levels of guava agro-industrial by-product and probiotic supplementation for broiler chickens during the pre-starter (1–7 d) and starter (8–21 d) phases.

Ingredients (g/kg)		1–7 days			
		0%	2%	4%	0+P
Corn (7.86%)		55.80	52.91	49.46	55.8
Soybean meal (46%)		34.10	34.20	34.38	34.1
Guava by-product		–	2.00	4.00	–

⚠ Esta tabela possui muitas colunas e foi cortada para impressão. Para visualizá-la completa, acesse o artigo original em: https://revistatopicos.com.br/artigos/guava-by-product-mitigates-the-effects-of-eimeria-infection-in-broiler-chickens-challenged-with-an-attenuated-vaccine?noblockage

2.4. Experimental Challenge

On the 15th day of the experiment, birds housed in one of the two rooms were orally inoculated with a high dose of a live attenuated commercial vaccine against avian coccidiosis (Vaxoon® Coccivet R), containing strains of *Eimeria acervulina*, *E. brunetti*, *E. maxima*, *E. necatrix*, *E. praecox*, *E. tenella* and *E. mitis*. Each bird received a dose 25 times higher (0.75mL) than the immunizing dose (0.03mL), based on the methodology described by Zhang et al. (2016). The objective was to induce a controlled infection in the animals.

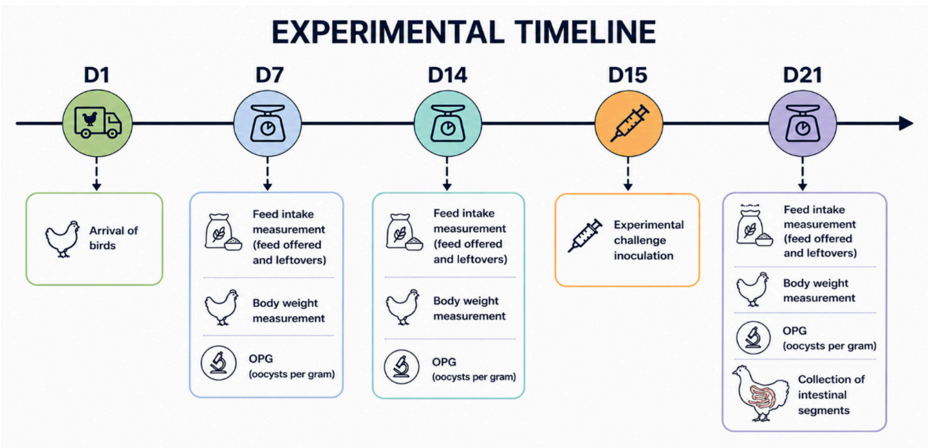
2.5. Data And Sample Collection And Processing

The mean values for performance variables in each experimental unit were recorded on days 7, 14 and 21. Feed intake (FI) was measured as the difference between the amount of feed offered and leftovers. Body weight (BW) was assessed by weighing the birds using an electronic scale. Weight gain (WG) was calculated as the difference between body weight at the beginning and end of each week. The feed conversion ratio (FCR) was calculated as the ratio between FI and WG. Bird mortality was recorded throughout the test and accounted for in the calculations.

For intestinal morphometry, one bird per replication was selected based on the body weight closest to the average of its pen, representing the experimental unit. On the 22nd day, the selected birds were euthanized by exsanguination after electric stunning to collect intestinal segments: duodenum, jejunum and ileum. 3.0 cm sections were collected from the midpoint of each segment. Following the methodology described by Torres (2010), intestinal samples were opened longitudinally to expose the lumen, fixed on labeled cardboard strips, rinsed with 0.9% saline solution to remove luminal content, and immersed in 10% neutral buffer formalin for 72 hours. After fixation, the samples were transferred to 70% ethanol and processed for histology slide preparation. The slides were stained with hematoxylin and eosine. Using an optical microscope with 5× magnification, the images were captured and analyzed using the QuPath® software (version v0.6.0-rc3). Ten villi and ten crypts were measured by replica, selecting those with the best integrity and visualization. The villus height (VH) was measured from the base to the apex, and the crypt depth (CD) was measured from the base to the villi–crypt junction. The villi:crypt (V:C) ratio was calculated as the average villus height divided by the average crypt depth for each sample.

For oocyst counting, fecal samples from each experimental unit were collected on days 7, 14, and 21 and analyzed using the McMaster technique (HAUG et al., 2006) to determine oocysts per gram (OPG). To avoid contamination of the sand, plastic sheets were placed under the feeders. Each sample consisted of feces from at least two birds per experimental unit. Samples were collected on days 7 and 14, and on day 21 in the NC group, to confirm the absence of accidental coccidial infection. Figure 1 shows the schedule of experimental procedures.

Figure 1. Experimental timeline of the broiler trial, indicating the main procedures performed throughout the experimental period: bird arrival (D1: day 1; D2: day 2; etc.).



2.6. Experimental Design and Statistical Analysis

From day 1 to 14, a randomized block design was used, with rooms (blocks) considered a source of random variation.

On the 15th day, the experimental challenge was introduced. Therefore, for data analysis covering the period from day 1 to day 21, a completely random design (CRD) was adopted in a 2×4 factorial arrangement. The factors were the experimental challenge (with or without challenge) and the food treatment (0%, 2%, 4% and 0+P), considering the main effects of each factor and their interaction. In addition, a simple CRD was also applied in each room (with or without challenge) to identify possible differences between treatments that could be masked by the strong effect of the challenge.

Data were initially subjected to outlier detection and removal using the interquartile range (IQR) and Z-score methods. Subsequently, analysis of variance (ANOVA) was performed and, when significant differences were detected, means were compared using Tukey's test. Statistical analyses were performed using the RStudio® software (version 2024.12).

3. RESULTS

3.1. Performance Before the Experimental Challenge

The mean performance values for the periods from day 1 to 7 and from day 1 to 14 are presented in Table 2.

Table 2. Effects of dietary guava by-product inclusion on growth performance of broiler chickens during the pre-challenge period (1–7 and 1–14 days of age).

Treatments	FI (g/bird)	WG (g/bird)	

			1 a 7 d		
0%	177		131	ab	1,34
2%	179		126	bc	1,42
4%	177		136	a	1,30

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FI was not affected during the period from day 1 to 7. However, there was a significant effect during the period from day 1 to 14, with broilers fed the 0+P diet showing lower FI compared to treatment with 0%.

From day 1 to 7, WG was significantly higher in birds fed the 4% GBF diet compared to those fed 2% and 0+P. From day 1 to 14, no statistical differences were observed between the treatments.

The FCR from day 1 to 7 was improved in broilers fed the GBF diet of 4%, compared to 2% and 0+P. From day 1 to 14, FCR was also better in the 4% and 0+P treatments compared to the 0% treatment.

The inclusion of 4% GBF in the diet from day 1 to 7 increased the average BW compared to the 2% and 0+P treatments. From day 1 to 14, numerically the highest BW was observed in the treatment of 4%, followed by 2%, 0% and 0+P; however, the result was at the threshold of significance (P = 0.06).

3.2. Performance after the Experimental Challenge

Table 3 presents the performance results of broilers from day 1 to 21 days of age, considering the effect of the experimental challenge introduced on day 15.

A highly significant challenge effect was observed for all performance parameters, as expected, as infection with *Eimeria spp.* compromises the intestinal integrity of broilers. Therefore, the differences observed between WC and NC birds can be attributed to intestinal damage caused by experimental challenge.

Table 3. Effects of dietary guava by-product inclusion and experimental challenge on growth performance of broilers from 1 to 21 d of age.

Treatments	FI (g/bird)	WG (g/bird)	FCR (g/g)	BW (g/bird)
Treatments				
0%	1266 a	778 a	1.635 a	819 a
2%	1269 a	819 a	1.557 a	860 a
4%	1198 a	843 a	1.429 a	885 a
0% + probiotic	1159 a	778 a	1.490 a	819 a
SEM	42	29	0.037	29
Challenge effect				
No challenge	1308	883 a	1.486	924 a
With challenge	1138	726 b	1.569	767 b
SEM	22	15	0.022	15

P-value				
Challenge	****	****	**	****
Treatment	*	**	***	**
C × T	*	NS	*	NS
CV (%)	10.7	11.4	7.5	10.8

0%, 2%, and 4% = diets containing 0, 20, and 40 g/kg of guava by-product, respectively; 0% + probiotic = basal diet supplemented with probiotic. FI = feed intake; WG = weight gain; FCR = feed conversion ratio; BW = body weight; SEM = standard error of the mean; CV = coefficient of variation; C × T = interaction between challenge and treatment. Statistical significance: NS = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Means followed by different lowercase letters within a column differ according to Tukey's test ($P < 0.05$).

The treatment effect was significant for all performance variables according to the analysis of variance. However, Tukey's test did not detect significant differences. The highly significant effect of the challenge factor may have increased the residual error, thereby reducing the sensitivity of the Tukey test.

The inclusion of 4% of GBF resulted, numerically, in the highest WG and BW, as well as in the lowest FI and FCR. The downward trend in WG and BW continued with the 2%, 0%, and 0+P treatments. However, the multiple comparison test did not detect significant differences between the treatments.

The challenge × treatment interaction (C × T) was significant only for FI and FCR. The interaction was further explored in Table 4.

Table 4. Interaction effects between experimental challenge and dietary guava by-product inclusion on feed intake and feed

conversion ratio of broilers from 1 to 21 d of age.

Treatment		FI (g/bird)		FCR (g/g)	
No challenge					
0%		1297	ab	1.533	abc
2%		1345	a	1.490	bc
4%		1275	ab	1.402	c
0% + probiotic		1314	a	1.517	bc

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The 0+P treatment of the WC group resulted in the lowest FI, except in comparison with the treatment 4% of the WC group, which also presented lower values than those observed in the 0+P and 2% treatments of the NC group. FCR was better in NC broilers fed the 4% diet compared to WC birds that received the 0% and 2% treatments.

Additional analyses (ANOVA and Tukey), performed separately per room, allowed the investigation of the effects of the treatment under different sanitary conditions. The results, presented in Table 5, refer exclusively to the effect of the treatments under the specific conditions of the WC group.

Table 5. Growth performance of broilers from 1 to 21 d of age fed diets containing different levels of guava agro-industrial by-product

and subjected to an *Eimeria spp.* vaccine challenge at 15 d of age.

	FI (g/bird)		WG (g/bird)		
With challenge					
0%	1234	a	711	ab	1,74
2%	1192	a	735	ab	1,62
4%	1121	ab	771	a	1,46
0% + probiotic	1004	b	687	b	1,46

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Significant differences between treatments were observed only in the WC group, for all performance variables. The 4% treatment resulted in higher WG and BW compared to broilers that received the 0+P treatment. The FI in the 0% and 2% treatments was higher than in the 0+P group. Regarding FCR, both the 4% and 0+P treatments showed better results compared to the 0% group.

Although the values observed in the NC birds indicated a trend towards higher WG and BW with the inclusion of GBF in the diet, no statistically significant differences were detected.

3.3. Intestinal Morphometry

The results of the intestinal morphometric evaluation of the duodenum, jejunum, and ileum segments at 21 days of age, seven days after the experimental challenge, are presented in Table 6.

Table 6. Intestinal morphometric parameters of the duodenum, jejunum, and ileum of broilers at 21 d of age fed diets containing different levels of guava agro-industrial by-product and subjected to an experimental challenge at 15 d of age.

	DUODENUM				
	VH	CD	V:C		VH
0%	1345 a	291	5.88 a		864
2%	1474 a	314	6.18 a		973
4%	1569 a	313	6.81 a		1084
0% +	1342 a	285	5.52 a		947

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A significant effect of the experimental challenge was observed, with increased CD, reduced VH and lower V:C ratio in all three intestinal segments of WC birds. A significant effect of the treatment was found for VH and V:C ratio in the duodenum and ileum, and for V:C ratio in the jejunum. However, the overall comparison of the treatment means revealed no statistically significant differences.

The VH in the duodenum, as well as the VH and the V:C ratio in the ileum, showed a significant treatment effect, but no interaction effect. The 4% GBF treatment resulted in the highest numerical values, followed by the 2% GBF treatment, for all variables. However, despite the statistically significant P values ($P < 0.05$), the multiple comparison test did not detect significant differences between the means, possibly due to the strong challenge effect that reduced the sensitivity of the test.

The challenge×treatment interaction (C × T) showed significant effects on CD and V:C in the duodenum, and on V:C in the jejunum. The breakdown of these interactions is shown in Table 7.

The CD in the duodenum was significantly affected by the interaction × treatment (C × T) ($P < 0.05$), but not by the treatment itself. Analysis of the interaction revealed the same pattern observed under the main effect of the challenge: all four challenged treatments had higher CD values than their NC counterparts. In other words, all treatments were affected by the challenge, with no significant differences between treatments within the same health condition.

For the V:C in the duodenum, the treatments containing GBF showed the highest values. The 4% GBF treatment was significantly higher than 0% and 0+P, while 2% was higher only than 0+P when considering NC birds. No significant differences were found between the WC group treatments, and all of them had lower means than their NC equivalents.

In the jejunum, only the V:C was significantly affected by the interaction. All WC treatments had lower V:C values than NC. Among NC birds, the treatment with GBF of 4% had the highest average, significantly higher than all other treatments.

Table 7. Interaction effects between challenge and dietary treatment on selected intestinal morphometric parameters of broilers at 21 d of age fed diets containing different levels of guava agro-industrial by-product.

	DUODENUM				
Treatment	CD		V:C		
No challenge					
0%	170	b	8.81	bc	
2%	168	b	10.1	ab	
4%	156	b	11.2	a	

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In the analyses performed separately for each sanitary condition, significant differences were detected only in the NC group (table 8), for duodenal V:C and for VH and V:C in the jejunum. In the duodenum, the V:C of the treatment with 4% was significantly higher than that of 0% and 0+P. In the jejunum, 4% GBF resulted in the highest VH, significantly higher than 0%. For the V:C, the 4% GBF treatment was also higher than 0+P and 0%.

Table 8. Intestinal morphometric parameters of broilers at 21 d of age fed diets containing different levels of guava by-product flour under no-challenge conditions.

	DUODENUM				
Treatment	VH	CD	V:C		VH
No challenge					
0%	1490	170	8.81	b	923
2%	1680	168	10.08	ab	1154
4%	1732	156	11.24	a	1314
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

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3.4. Faecal oocyst Count

The OPG (oocysts per gram) analyses performed on days 7 and 14 confirmed the absence of *Eimeria* infection in the birds. On day 21 of the experiment, the NC birds maintained a result of zero oocysts per gram of feces in all replications. The WC birds showed the results presented in Table 9.

Treatments with 4% and 2% GBF had the lowest oocyst counts, followed by the 0% group and the 0+P group. Broilers that received GBF (2% and 4%) had significantly lower oocyst counts than those that received 0% plus the commercial probiotic.

Table 9. Oocyst shedding (oocysts per gram of feces, OPG) at 7 d after experimental infection in broilers fed diets containing different levels of guava by-product flour.

Treatment	OPG		SE	CV (%)
0%	30470	ab	7844	57.6
2%	19160	b	2547	29.7
4%	17680	b	2153	27.2
0% + probiotic	64530	a	20694	71.7
SEM	11190			
P-value	**0.03**			
CV (%)	75.9			

0%, 2%, and 4% = diets containing 0, 20, and 40 g/kg of guava by-product, respectively; 0% + probiotic = basal diet supplemented with probiotic. OPG = oocysts per gram of feces; SE = standard error within each treatment; SEM = pooled standard error of the mean; CV = coefficient of variation. CV (%) values in the column represent within-treatment variation, whereas the final CV (%) represents the overall coefficient of variation of the experiment. Means followed by different lowercase letters within a column differ by Tukey's test ($P < 0.05$).

4. DISCUSSION

Chickens fed 4% GBF in the diet showed better performance indices during the 1–7 and 1–14 day phases. In the period from 1 to 21 days, although no statistically significant differences were detected in the absence of challenge, the results suggest a beneficial effect of GBF under sanitary challenge conditions. These findings reinforce the potential of GBF as a functional ingredient.

Bioactive compounds present in the by-product have antioxidant and microbiota-modulating activity, which can increase nutrient utilization even under enteric stress (El-Ghareeb et al., 2023;

Galamatis et al., 2025). This hypothesis is consistent with the best performance indices observed in WC birds receiving 4% GBF, indicating possible mitigation of the negative effects of *Eimeria spp. infection.*

The literature reports variable responses regarding the use of guava by-products or their derivatives during the initial phases of breeding. Oliveira et al. (2018) observed a linear increase in BWG with the inclusion of up to 1.5% of GBF, although without significant effects on FCR and FI. Similarly, Noleto-Mendonça et al. (2021), using a standardized guava by-product extract, reported a linear reduction in FCR and improvement in BWG at the intermediate inclusion levels of the evaluated substance.

In the present study, the positive effects associated with GBF on performance parameters were evident only in WC birds, which can be attributed to the stronger expression of the benefits of bioactive compounds in conditions of intestinal injury. The experimental challenge amplified the differences between treatments, making the effects of GBF more noticeable.

In NC birds, the effects of GBF on performance were more discrete, which is consistent with previous studies. Ogega et al. (2022), Oliveira et al. (2018), and Lira et al. (2009) reported no performance losses, including up to 5%, 1.5%, and 12% GBF, respectively, in broiler chickens, while Vieira et al. (2023) confirmed this safety in slow-growing broilers. On the other hand, Radaman et al. (2023), working with Japanese quails, and Barakat & El-Garhy (2019), with ducks, found performance improvement with the inclusion of GBF, demonstrating a positive effect of the by-product on different poultry species.

The experimental challenge with the attenuated vaccine against *Eimeria* spp. compromised the intestinal morphology, with a reduction in VH, an increase in CD, and a decrease in the villi:crypt ratio, as expected in cases of intestinal damage (Cloft et al., 2023). The relationship between villus height and crypt depth is widely recognized as a functional marker of mucosal integrity, with higher values indicating greater absorptive efficiency and adequate epithelial turnover (Rysman et al., 2023).

Among NC birds, the 4% GBF treatment showed statistical superiority in the V:C in the duodenum ($P<0.01$), in the VH in the jejunum ($P<0.05$) and also in the V:C in the jejunum ($P<0.05$). The means for VH and V:C in the ileum were statistically identical, but numerically, the 4% treatment showed better results, with a P value (0.07) at the threshold of significance.

These observations agree with previous studies that have reported beneficial changes in gut morphology with the use of guava byproducts. Oliveira et al. (2018) found that inclusion levels of up to 1.5% reduced crypt depth and increased villi:crypt ratio in the ileum. Noleto-Mendonça et al. (2021) observed that a guava by-product extract increased villus height and reduced crypt depth in the duodenum and jejunum.

In addition, there is ample evidence on the role of plant-derived bioactive compounds in maintaining gut integrity, even under coccidia infection (Khan et al. 2025). Flavonoids, tannins, phenolic acids, and saponins exert antioxidant effects, promote beneficial microbiota, and stimulate enterocyte regeneration (El-Ghareeb et al., 2023; Galamatis et al., 2025). These mechanisms act synergistically to help preserve mucosal structure and maintain absorptive function

(Jelveh et al., 2023). It is also important to consider the potential contribution of metabolizable energy and amino acids, especially in the 4% group, although the level of inclusion is low and the impact on nutritional value is minimal, according to physicochemical data on the composition of guava by-product flour reported by other authors.

In a study conducted by Rysman et al. (2023) on 50 commercial farms, a positive correlation was observed between the villi:crypt ratio in the duodenum and performance parameters such as weight gain and body weight, confirming that changes in gut architecture directly affect the performance of birds, even under field conditions. In the present study, although the best performance of the WC group was recorded in the treatment of 4% — suggesting greater nutrient absorption and, consequently, higher VH and V:C — the morphometric data did not statistically confirm this effect of GBF under challenge. The shortening of the villi and the deepening of the crypt caused by the infection reduced the differences between the treatments, which were significant only in the NC group. However, in the WC group, despite the absence of statistical significance, the mean VH and V:C in the three intestinal segments were numerically higher in GBF-fed birds, especially at the 4% inclusion level, suggesting a possible attenuating effect of the by-product against infection-induced lesions. This observed trend reinforces the functional potential of the ingredient in mucosal integrity and intestinal recovery.

This study was limited to morphometric analysis; however, complementary histological evaluations could provide valuable information to better characterize the mechanisms underlying the observed morphometric trends.

In addition to the beneficial effects on the intestinal mucosa, bioactive compounds present in plant-based ingredients demonstrate direct activity against *Eimeria sp.*, interfering with essential stages of its life cycle, such as oocyst sporulation, sporozoite integrity, and its ability to invade the intestinal epithelium (Madlala et al., 2021; El-Ghareeb et al., 2023). Substances such as artemisinin, curcumin, tannins, flavonoids, and terpenoids have documented anticoccidial activity, with effects comparable to conventional chemotherapeutic agents (Chen et al., 2024; Ghafouri et al., 2023).

Studies using different plant sources reinforce this activity, showing significant reductions in oocyst excretion with the use of natural compounds (Jelveh et al., 2023; Shahininejad et al., 2024; Zhang et al., 2024; Galamatis et al., 2025). In the present study, the inclusion of 4% GBF in the diet was associated with lower fecal oocyst excretion in WC birds. Despite the high coefficient of variation (CV), the statistical power of the test was extremely high (99.7%), which provides confidence in the results.

To date, no studies have been found that directly evaluated the effect of GBF on oocyst counts in broiler chickens, suggesting that this research may be a pioneer in this regard. However, research involving other fruit by-products, such as cranberry pomace (Pereira, 2023), pomegranate and lemon peels (Hafeez et al., 2023a, 2023b), and mixed berry residues (Das, 2020), indicates that bioactive-rich plant compounds may play a functional role in controlling coccidiosis.

It is important to note, however, that fecal oocyst count is a variable naturally subject to high variability, with reported CVs between 37% and 251% (Mohamed et al., 2021; Hauck & Pacheco, 2021; Liu et al., 2022). Therefore, the interpretation of the data requires methodological caution, including standardized sampling procedures, adequate sample size, and presentation of standard deviations or standard errors by group (Hauck & Pacheco, 2021).

Finally, an alternative hypothesis for the reduction in oocyst count observed with the use of GBF is mechanical in nature. Oikeh et al. (2019) suggest that ingredients rich in indigestible fiber can increase fecal volume and dilute the concentration of oocysts per gram of feces. Although this effect cannot be ruled out, the association with bioactive compounds and the available literature point to a direct effect as the most likely explanation. Additional studies should be carried out to clarify the mechanisms involved.

In the present study, the inclusion of probiotics did not promote consistent improvement in performance parameters, intestinal morphometry or oocyst excretion at 21 days of age, except for feed conversion in the WC group ($P < 0.001$). These results are similar to those reported by other researchers who also did not observe significant effects of *Bacillus subtilis* in broilers raised under low sanitary challenge (Domingues et al., 2014) and in birds challenged with *C. perfringens* (Frazier et al., 2015).

On the other hand, a recent meta-analysis by Ghimire et al. (2024) showed that research demonstrates benefits of *B. subtilis* in broilers subjected to the challenge of necrotic enteritis (*Eimeria* spp. and *C. perfringens vaccines*), with improvements in performance and lesion scores at levels comparable to those achieved with conventional growth promoters.

The absence of a favorable response to the probiotic may be related to the strain used. Unlike *B. subtilis*, the use of *B. cereus* as a probiotic is controversial. Although some specific strains, such as var. *toyoi* (Vilà et al., 2009) and the BC13 strain (Liu et al., 2025) have shown positive effects; most of the literature describes the species as pathogenic, associated with food poisoning and severe hospital infections (Hirao et al., 2025). The presence of enterotoxin resistance and antimicrobial genes contributes to *B. cereus* being considered unsafe for use of probiotics in humans (Duc et al., 2004). In fact, commercial preparations containing *B. cereus* have been banned from the European market due to safety concerns (Sánchez et al., 2009). Identifying safe strains is complex due to genetic proximity within the *B. cereus group*, whose pathogenic potential is broad and diverse (Glasset et al., 2021). The microorganism is also identified as a feed contaminant, capable of causing immunosuppression and aggravating other diseases in poultry and pigs (Zuo et al., 2020; Li et al., 2020).

Zho et al. (2016) reported serious problems in commercial probiotics containing *B. cereus* in China, including contamination, mislabeling, transferable antimicrobial resistance genes, and the presence of enterotoxin genes. The authors warned about the global risk posed by the commercialization of these products.

To date, no studies have reported the use of *B. cereus* under experimental challenge with *Eimeria* species . However, data from the present study indicate some inferior results in the probiotic-supplemented WC group, raising the hypothesis of a possible negative interaction between *B. cereus* and *Eimeria*, with worsening of intestinal lesions and greater release of oocysts. Another possibility is that increased oocyst count is associated with reduced feed intake, resulting in lower fecal volume and higher relative concentration of oocysts. Specific studies on its interaction with enteropathogens, such as *Eimeria* spp., are needed to clarify its potential effects and establish safety criteria.

The findings of this study reinforce the potential of GBF as a functional ingredient and indicate that its inclusion in 4% in broiler diets may be a viable strategy to mitigate the effects of enteropathogens, while highlighting the need for greater rigor in the evaluation of probiotics used in poultry production.

This study evaluated the effects of the inclusion of guava agro-industrial by-products in the diet of chickens challenged with *Eimeria spp*. only during the initial phase. Further research is needed to confirm these effects at different stages of production and to elucidate the mechanisms underlying the observed responses.

5. CONCLUSION

Dietary inclusion of 4% GBF was associated with increased villus height and villus-to-crypt ratio, reduced oocyst shedding, and attenuation of the performance losses associated with Eimeria spp. infection. These findings suggest that guava by-product may serve as a functional ingredient in broiler nutrition.

REFERENCES

ASSIS, H. F.; OLIVEIRA, D. B.; MORAES, L. P.; FIGUEIREDO, F. C.; PAIXÃO, M. V. S Composição centesimal e principais características químicas da farinha de resíduo do processamento agroindustrial de *Psidium guajava L. Revista Aracê*, São José dos Pinhais, v. 7, n. 2, p. 7197–7207, 2025. DOI: https://doi.org/10.56238/arev7n2-156.

ASSOCIAÇÃO BRASILEIRA DE PROTEÍNA ANIMAL – ABPA. *Relatório Anual 2025.* São Paulo: ABPA, 2025. Disponível em: https://abpa-br.org/. Acesso em: 14 jun. 2025.

ASSOCIAÇÃO BRASILEIRA DOS PRODUTORES EXPORTADORES DE FRUTAS E DERIVADOS – ABRAFRUTAS. *Brasil bate recorde de exportação de frutas em 2023.* 2024. Disponível em: https://abrafrutas.org/2024/03/brasil-bate-recorde-de-exportacao-de-frutas-em-2023. Acesso em: 5 maio 2025.

BARAKAT, H.; EL-GARHY, O. H. Incorporating guava seed powder into a commercial diet improves the growth performance, nutritional and quality characteristics of Muscovy duck meat. Journal of Food Chemistry & Nanotechnology, vol. 5, no. 2, p. 20–29, 2019. https://doi.org/10.17756/jfcn.2019-067.

CHEN, P.; LIU, K.; YUE, T.; LU, Y.; LI, S.; JIAN, F.; HUANG, S. Plants, plant-derived compounds, probiotics and postbiotics as green agents to combat poultry coccidiosis: a review. *Animal Research and One Health*, v. 1, p. 1–21 (2024). DOI: https://doi.org/10.1002/aro2.96.

CLOFT, S. E.; MISKA, K. B.; JENKINS, M. C.; PROSZKOWIEC-WEGLARZ, M.; KAHL, S.; WONG, E. A. Temporal gene changes associated with intestinal homeostasis in broilers after a single infection by Eimeria acervulina. *Poultry Science*, v. 102, n. 4, p. 102537, 2023. DOI: https://doi.org/10.1016/j.psj.2023.102537.

COBB-VANTRESS, INC. *Supplement to the Cobb500 Broiler Management Guide.* Arkansas: Cobb-Vantress Inc., 2021. 60 p. Available at: https://www.cobb-vantress.com/assets/Cobb-Files/productguides/broiler/Cobb500BroilerPerformanceandNutritionSupplement-PT.pdf. Accessed on: May 5, 2025.

DAS, Quail. *Organic Berry Pomaces to Improve the Health of Broiler Chickens*. 2020. 229 f. Thesis (PhD in Food Science) – University of Guelph, Ontario, Canada, 2020. Available at: atrium.lib.uoguelph.ca/server/api/core/bitstreams/44dfeddf-1fe5-4533-8a64-01668c661fe9/content. Accessed on: 25 May 2025.

DOMINGUES, C. H.; SILVA, K. M. da; SANTOS, M. A. dos; OLIVEIRA, J. F. de; LIMA, R. M. de. Evaluation of performance and carcass yield of broilers fed diets containing probiotics in different phases of rearing. *Revista Agrocientífica*, v. 1, n. 1, p. 7–16, 2014. Available at: https://periodicos.unoesc.edu.br/agrocientifica/article/view/4859.

DUC, Le H.; HONG, Huynh A.; BARBOSA, Teresa M.; HENRIQUES, Adriano O.; CORTES, Simon M. Characterization of Bacillus probiotics available for human use. *Applied and Environmental Microbiology*, Washington, v. 70, n. 4, p. 2161–2171, Apr. 2004. DOI: https://doi.org/10.1128/AEM.70.4.2161-2171.2004.

EL-GHAREEB, W. R.; GAD, W. A.; ALI, M. M.; SOLIMAN, M. M.; EL-TAWIL, O. S.; AHMED, ALSO KNOWN AS ALJUFAIMI, M. A.; ABDEL-DIAM, R. M.; AMIN, H. K.; ZUBAIDAH, A. M. New antioxidant insights of myricetin on broiler performance and relief of experimental infection with *Eimeria* spp.: Conversation between oxidative stress and inflammation. *Antioxidants*, v. 12, n. 5, p. 1026, 2023. DOI: https://doi.org/10.3390/antiox12051026.

FRAZIER, Anthony Nathan. *The use of Bacillus subtilis as a direct-fed microbial and its effects on the production and colonization of Salmonella Enteritidis and Clostridium perfringens in production* *broilers.* 2015. 94 f. Dissertation (Master's Degree in Poultry Science) – Auburn University, Auburn, 2015. Advisors: Kenneth Macklin, Joseph Giambrone, Mark Liles. Available at: https://www.proquest.com/openview/53c579b9464097f9cc91a3cc084d5913/1?pq-origsite=gscholar&cbl=18750&diss=y, accessed on 11/06/2025.

FURUYA, W. M.; SALES, J. P.; SANTOS, D. L. dos; SANTOS, L. D. dos; FURUYA, V. R. B. Composição química e coeficientes de digestibilidade aparente dos subprodutos desidratados das polpas de tomate e goiaba para tilápia do Nilo (*Oreochromis niloticus*). *Boletim do Instituto de Pesca*, São Paulo, v. 34, n. 4, p. 505–510, 2008. Disponível em: https://institutodepesca.org/index.php/bip/article/view/820. Acesso em: 25 maio 2025.

GALAMATIS, D.; KOUTSOUSTOGLOU, M.; TSAPRAILI, E.; GAZOULIS, G. N.; KOROZIS, D.; SKOUFOS, I.; CRONIS, E. N.; TSIPLARIDIS, I.; TSIOURIS, V. Evaluation of a natural phytobiotic mixture as a feed additive for broilers: studies on animal performance, intestinal health and antioxidant status after experimental infection with Eimeria spp. *Aves*, v. 4, n. 1, p. 4, 2025. doi: https://doi.org/10.3390/poultry4010004.

GHAFOURI, S. A.; GHANIEI, A.; TAMANNAEI, A. E. T.; SADR, S.; CHARBGOO, A.; GHIASSI, S.; ABUALI, M. Anticoccidial effects of tannin-based herbal formulation (Artemisia annua, Quercus infectoria and Allium sativum) against coccidiosis in broilers. *Journal of Parasitic Diseases*, v. 47, n. 4, p. 820–828, 2023. DOI: https://doi.org/10.1007/s12639-023-01627-1.

GHIMIRE, A.; ALFARO, D.; SCHMIDT, C. J.; WATKINS, S. E.; BEHRENS, L. M.; BAXTER, M. F. A.; BUMP, J. A.; LOAR, R. E.; ADELEYE, A. A.; HUBBARD, R. D. Efficacy of the probiotic *Bacillus subtilis* in the prevention of necrotic enteritis in chicken engo chickens: a systematic review and meta-analysis. **Poultry Science,** v. 103, n. 3, p. 103451, 2024. https://doi.org/10.1080/03079457.2024.2359596

GLASSET, Benjamin; SPERRY, Mylène; DERVYN, Rozenn; HERBIN, Sabine; BRISABOIS, Anne; RAMARAO, Nalini. *The cytotoxic potential of Bacillus cereus strains of various origins*. *Microbiologia* Alimentar, [S. l.], v. 98, p. 103759, 2021. Available at: https://doi.org/10.1016/j.fm.2021.103759. Accessed on: June 13, 2025.

HAFEEZ, A.; AHMAD, I.; NAZ, S.; ALONAIZAN, R.; AL-AKEEL, R. K.; KHAN, R. U.; TUFARELLI, V. Effect of Lemon (citrus lemon, L.) Peeling powder on oocyst drop, gut health, and performance of broilers exposed to E. tenela challenge. *Animals*, v. 13, p. 3533, 2023. DOI: https://doi.org/10.3390/ani13223533.

HAFEEZ, A.; PIRAL, P.; NAZ, S.; ALMUTAIRI, M. H.; ALREFAEI, A. F.; AYASAN, T.; KHAN, R. U.; LOSACCO, C. Ameliorative Effect of Pomegranate Peel Powder on Growth Indexes, Oocyst Drop, and Intestinal Health of Chicken Under Experimentally Induced Coccidiosis Condition. *Animals*, v. 13, p. 3790, 2023. DOI: https://doi.org/10.3390/ani13243790.

HAUCK, R.; PACHECO, W. J. Detection of *Coccidia oocysts* in sand and feces of broilers in a trial in a floor enclosure. *Journal of Parasitology*, v. 107, n. 6, p. 878–881, 2021. DOI: 10.1645/21-8.

HAUG, A.; WILLIAMS, R. B.; LARSEN, S. Coccidial oocyst count in chicken feces: A comparative study of a standard McMaster technique and a new rapid method. *Veterinary Parasitology*, v. 136, n. 3–4, p. 233–242, 2006. DOI: https://doi.org/10.1016/j.vetpar.2005.11.024.

HIRAO, Yuki; MORIOKA, Hiroshi; AGATA, Tomomasa; IIMURA, Masaki; TAKI, Shunichi; YAGI, Tetsuya. Prolonged Bacillus cereus bacteremia: case report and literature review. Journal of Infection and Chemotherapy, [s. l.], v. 31, n. 7, p. 102736, 23 maio 2025. https://doi.org/10.1016/j.jiac.2025.102736

JELVEH, K.; MOTTAGHITALAB, M.; MOHAMMADI, M. Effect of green tea phytosome on ooism per gram and growth performance in broilers challenged with mixed species *of Eimeria* . *Veterinary Medicine and Science*, v. 9, n. 6, p. 2227–2235, 2023. doi: https://doi.org/10.1016/j.psj.2023.102627.

KHAN et al., 2025. Molecular prevalence and genetic characterization of *Eimeria tenella* in backyard chickens. Pak Vet J, 45(3): 1190-1198. http://dx.doi.org/10.29261/pakvetj/2025.225

LI, Xiaohui; LI, Qiang; WANG, Yihui; HAN, Zhenhai; QU, Guanggang; SHEN, Zhiqiang; HUANG, Shujian; HIM, Cheng. Gastric ulcer and immune suppression in weaned piglets associated with feed-borne *Bacillus cereus* and *Aspergillus fumigatus*. *Toxins*, Basel, v. 12, n. 11, p. 703, 2020. DOI: https://doi.org/10.3390/toxins12110703.

LIRA, R. C.; RABELLO, C. B. V.; FERREIRA, P. V.; LANA, G. R. Q.; LUDKE, J. V. and DUTRA JUNIOR, W. M. Inclusion of guava residues in broiler feed. *Revista Brasileira de Zootecnia* 38: 2401-2407. 2009. https://doi.org/10.1590/S1516-35982009001200016.

LIU, Fei; YU, Jiang; CHEN, Zhi; ZHANG, Shuzhi; ZHANG, Yue; ZHANG, Lin; ZHANG, Yuyu; LI, Jianda; DING, Luogang; WU, Jiaqiang. *Bacillus* *cereus isolation and its probiotic effect on growth performance, antioxidant capacity, and intestinal barrier protection of broilers* . *Ciência Avícola*, [S. l.], v. 104, p. 104944, 2025. DOI: https://doi.org/10.1016/j.psj.2025.104944.

LIU, H.; CHEN, P.; LV, X.; ZHOU, Y.; LI, X.; MA, S.; ZHAO, J. Effects of chlorogenic acid on performance, anticoccidial indicators, immunity, antioxidant status, and intestinal barrier function in coccidia-infected broilers. *Animals* 2022, 12,963. https://doi.org/10.3390/ani12080963.

MADLALA, T.; OKPEKU, M.; ADELEKE, M. A. Understanding the interactions between Eimeria infection and intestinal microbiota, aiming at the control of chicken coccidiosis: a review. *Parasite*, v. 28, p. 48, 2021. EDP Sciences. DOI: https://doi.org/10.1051/parasite/2021047.

MAQSOOD, S.; KHALID, W.; KUMAR, P.; BENMEBAREK, THAT IS; UL-ISLAM, M.; AL-DUAIJ, O. R.; CHAUDHARY, R.; ABDULRAHMAN, A. A.; MANIKANDAN, S.; ADELOYE, A. O.; BASHEER, A. A. Valorization of agro-industrial residues of plant origin and by-products for the production of polysaccharides: Towards a more circular economy. *Applied Food Research*, v. 5, n. 7, p. 100954, May 2025. DOI: https://doi.org/10.1016/j.afres.2025.100954.

MOHAMED, E. R. A.; ELAZAB, M. F.; EL-HABASHI, N.; ELHAWARY, N.; MOKHBATLY, A. A. Anticoccidial effect of Origanum majoranum aqueous extract on *Eimeria tenella*-infected chicken. *Tropical Biomedicine*, v. 38, n. 1, p. 62–72, 2021. DOI: https://doi.org/10.47665/tb.38.1.011.

NOBRE, P. T.; COSTA, R. G.; DIAS-SILVA, T. P.; RIBEIRO, N. L.; GURGEL, A. L. C.; BELTRÃO, L. G. C.; CRUZ, G. R. B.; BORGES, I.; LORENZO, J. M. Effect of feeding with agro-industrial residues of guavas on nutrient utilization, lamb performance and economic analysis. *Animal Husbandry Archives*, v. 67, p. 541-549, 2024. DOI: https://doi.org/10.5194/aab-67-541-2024.

NOLETO-MENDONÇA, R. A.; MARTINS, J. M. da S.; CARVALHO, D. P.; ARAÚJO, I. C. S.; STRINGHINI, J. H.; CONCEIÇÃO, E. C.; CAFÉ, M. B.; LEANDRO, N. S. M. R. Performance, nutrient digestibility and intestinal histomorphometry of broilers fed a diet supplemented with guava extract standardized in phenolic compounds. *Revista Brasileira de Zootecnia,* Viçosa, MG, v. 50, p. e20210026, 2021. DOI: https://doi.org/10.37496/rbz5020210026.

OGEGA, E. B.; OJWANG, B. O.; OKOTH, J. K.; NYANGWESO, J. M. Effects of the inclusion of guava fruit processing by-products in the diets of broiler chickens on performance. Livestock Research for Rural Development, v. 34, n. 10, 2022. Available at: https://www.lrrd.org/lrrd34/10/3492ogeg.html.

OIKEH, I.; SAKKAS, P.; TAYLOR, J.; GIANNENAS, I.; BLAKE, D. P.; KYRIAZAKIS, I. Effects of reduction of growth rate by diet dilution on bone mineralization, performance and carcass yield of broiler chickens infected with coccidia. *Scientific Aviculture* , v. 98, p. 5477–5487, 2019. DOI: https://doi.org/10.3382/ps/pez400.ResearchGate.

OLIVEIRA, M. D.; MELLO, S. S. C.; STRINGHINI, J. H.; MASCARENHAS, A. G.; ARNHOLD, E.; CONCEIÇÃO, E. C.; MARTINS, J. M. S.; SILVA JÚNIOR, A. J. Antioxidant effect of guava by-product on the diet of chickenians in the initial phase. *Brazilian Journal of Animal Science*, Viçosa, MG, v. 47, p. e20160290, 2018. DOI: https://doi.org/10.1590/rbz4720160290.

PEREIRA, A. *Cranberry pomace as a functional feed ingredient in broiler chickens: gut health and immune modulation in a coccidiosis challenge model*. 2023. Dissertation (Master's Degree in Animal Science) – University of Guelph, Guelph, ON, Canada. Available at: https://atrium.lib.uoguelph.ca/server/api/core/bitstreams/21af8455-5f44-4123-9d87-ae795422f9df/content. Accessed on: 5 Apr. 2025.

RADAMAN, M. S. E.; ABUZIED, A. M. F.; ABDEL WAHED, S. M.; RAGAB, M. S.. Effect of the use of guava and olive residues with or without the addition of enzymes in the diet on the performance of Japanese quail cultivation. *Fayoum Journal of Agricultural Research and Development*, Fayoum, v. 37, n. 2, p. 310–334, 2023. https://fjard.journals.ekb.eg/article_297297.html.

ROSTAGNO, H. S.; ALBINO, L. F. T.; DONZELE, J. L.; GOMES, P. C.; OLIVEIRA, R. F.; LOPES, D. C.; FERREIRA, A. S.; BARRETO, S. L. T.; EUCLIDES, R. F. Food composition and nutritional requirements. In: ROSTAGNO, H. S.; ALBINO, L. F. T.; DONZELE, J. L.; GOMES, P. C.; OLIVEIRA, R. F.; LOPES, D. C.; FERREIRA, A. S.; BARRETO, S. L. T.; EUCLIDES, R. F. (eds.). *Brazilian tables for poultry and swine: feed composition and nutritional requirements*. 4. ed. Viçosa, MG: UFV, 2017. p. 1–252.

RYSMAN, K.; EECKHAUT, V.; DUCATELLE, R.; GOOSSENS, E.; VAN IMMERSEEL, F. *The performance of chicken-fattening correlates with intestinal morphology and intestinal inflammation under field conditions*. *Avian Pathology* , v. 52, n. 4, p. 232–241, 2023. DOI: https://doi.org/10.1080/03079457.2023.2201169.

Sánchez, B., Arias, S., Chaignepain, S., Denayrolles, M., Schmitter, J.M., Bressollier, P. & Urdaci, M.C. (2009). Identification of surface proteins involved in the adhesion of a probiotic strain of Bacillus cereus to mucin and fibronectin. AVIAN PATHOLOGY 259 Microbiology, 155, 1708–1716. https://doi.org/10.1099/mic.0.025288-0.

SHAHININEJAD, H.; RAHIMI, S.; KARIMI TORSHIZI, M. A.; ARABKHAZAELI, F.; AYYARI, M.; BEHNAMIFAR, A.; ABUALI, M.; GRIMES, J. Comparing the effect of phytobiotics, coccidiostats, toltrazuril and vaccine on the prevention and treatment of coccidiosis in broilers. *Poultry Science*, v. 103, p. 103596, 2024. DOI: https://doi.org/10.1016/j.psj.2024.103596.

TORRES, Karoll Andrea Alfonso. *Avaliação do desempenho zootécnico, da função da mucosa intestinal e da microbiota ileal quando da substituição do milho pelo sorgo na ração de frangos de corte.* xiii, 75 f. Thesis (Doctorate in Animal Science) – São Paulo State University, Faculty of Agrarian and Veterinary Sciences, Jaboticabal, 2010. Available at: Microsoft Word - TESE KAROLL.doc, accessed on 05/05/25.

VIEIRA, S. S. et al. By product of dehydrated guava in the diet for slow-growing broiler chickens. *Frontiers in Animal Science*, Vol. 4, 2023. DOI: https://doi.org/10.3389/fanim.2023.1189291.

VILÀ, B.; FONTGIBELL, A.; BADIOLA, I.; ESTEVE-GARCIA, E.; JIMÉNEZ, G.; CASTILLO, M.; BRUFAU, J. *Reduction of colonization and invasion of Salmonella enterica var. Enteritidis by Bacillus cereus var. toyoi in poultry feed. Poultry Science*, Savoy, v. 88, p. 975–979, 2009. DOI: https://doi.org/10.3382/ps.2008-00483.

ZHANG et al., 2024. Coccidiostat activity of *Mahonia bealei (Fort.)* leaves extract against *Eimeria tenella* in chickens. Pak Vet J, 44(2): 292-297. http://dx.doi.org/10.29261/pakvetj/2024.176.

ZHANG, L.; ZHANG, H. J.; WU, S. G.; YU, S. H.; QIN, L.; LIU, Y.; QIAO, X.; YANG, C. B.; YANG, Z. B. Feed clove essential oil relieves intestinal oxidative stress and alters the gut microbiota in broilers exposed to heat stress. Journal of Animal Science and Biotechnology, v. 7, p. 57, 2016. DOI: https://doi.org/10.1186/s40104-016-0114-3.

ZHO, K., Hölzel, C.S., Cui, Y., Mayer, R., Wang, Y., Dietrich, R., Didier, A., Bassitta, R., Märtlbauer, E. & Ding, S. (2016). Probiotic strains of Bacillus cereus, a potential public health risk in China. Frontiers in Microbiology, 7, 718.

ZUO, Zhiheng; LI, Qinqin; GUO, Yuxi; LI, Xiaohan; HUANG, Shijie; HEGEMANN, Johannes H.; HIM, Chen. *Feed-borne Bacillus cereus aggravates respiratory distress in broilers infected with Chlamydia psittaci by inducing hemorrhagic pneumonia*. **Avian Pathology,** Abingdon, vol. 49, no. 3, p. 251–260, 2020. DOI: https://doi.org/10.1080/03079457.2020.1716940.

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