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Black wattle (*Acacia mearnsii*) tannins in broiler diets: intestinal health, bone quality, nutrient digestibility and performance

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ABSTRACT - This study evaluated increasing dietary levels of Black wattle bark extract (BWE) on broiler performance, nutrient digestibility, intestinal histopathological score, and bone quality. A total of 240 male Cobb 500 broilers, eight days old, were distributed into five dietary treatments (0, 300, 500, 700, or 900 mg kg^{-1} BWE) in a randomized block design, housed in metabolic cages, with eight replicates of six birds each. Performance was evaluated from 8 to 28 days of age, nutrient digestibility from 18 to 20 days, and intestinal and bone parameters were assessed at 21 days of age. Supplementation with BWE up to 500 mg kg^{-1} increased crude protein digestibility (5.36%) and ash retention (16.70%) without negatively affecting growth performance. However, the 900 mg kg^{-1} dose reduced feed intake (6.14%) and weight gain (6.4%). In addition, birds fed 300 mg kg^{-1} BWE showed reduced jejunal inflammatory cell infiltration compared with those fed 900 mg kg^{-1}. These findings indicate that moderate supplementation (300–500 mg kg^{-1}) enhance intestinal morphology, and nutrient utilization, while excessive inclusion compromises productive performance. In conclusion, BWE represents a promising natural feed additive for broilers. However, its use should be limited to moderate levels to avoid detrimental effects on growth performance.

Keywords: condensed tannins, gut morphology, mineral retention, protein digestibility

1. Introduction

In poultry production, antibiotics used as growth promoters (AGPs) improve feed intake, weight gain, and feed efficiency. A meta-analysis including 121,643 broilers found that AGP-supplemented diets increased weight gain and improved feed conversion ratio in the initial (1–21 d) and total (1–42 d) phases, resulting in an estimated economic benefit of US$ 0.03 per bird, equivalent to US$ 183.56 million annually (Cardinal et al., 2019). However, their overuse raises public health concerns due to the emergence of antibiotic-resistant bacteria (Iwu et al., 2020). Consequently, alternative nutritional strategies have been developed for antibiotic-free systems (Hernandez-Platan et al., 2023). Phytogenic additives have gained attention for their antimicrobial (Mnisi et al., 2023), antioxidant (Akosile et al., 2023), immunomodulatory (Albarrak et al., 2021), and gut-health-promoting effects (Yang et al., 2021).

The black wattle (*Acacia mearnsii*) extract (BWE) is rich in condensed tannins, consisting of flavan-3-ols or flavan-3,4-diols units (Godoy et al., 2024). These tannins are mainly proanthocyanidins composed of robinetinidol and fisetinidol extension units, with catechin and gallocatechin as terminal units, and contain minor amounts of phenolic acids such as gallic acid (Ogawa et al., 2018). Detailed chemical profiling has shown that approximately 77% of BWE consists of oligomeric proanthocyanidins, with varying degrees of polymerization and structural subtypes identified by high-performance liquid chromatography (HPLC) and mass spectrometry (Chen et al., 2018).

In vitro assays already have demonstrated that BWE exhibits antimicrobial activity against *Campylobacter jejuni* (Kurekci et al., 2012). However, this extract did not reduce *C. jejuni* counts in cecal contents or feces when assessed an *in vivo* assay in broilers (Kurekci et al., 2014). Recent findings indicate that up to 500 mg kg^{-1} of acacia tannins may mitigate the effects of *Salmonella* Heidelberg and necrotic enteritis on broiler performance, improving digestibility and intestinal integrity and reducing *Salmonella* in the cecal content of broilers (Godoy et al., 2024; Maysonnave et al., 2024). Other tannin-rich extracts have been investigated, yielding promising results for broilers, particularly in alleviating oxidative stress associated with *Eimeria* spp. infections. (Wang et al., 2008), and resulting in decreased oocyst counts (Perin et al., 2019).

However, the antinutritional effects of tannins in broiler diets are well-documented, often resulting in decreased performance due to reduced feed intake and impaired digestibility of minerals and proteins, caused by the formation of nutrient-binding complexes (Garcia et al., 2004; Hassan et al., 2020). A level of 900 mg kg^{-1} leads to a reduction in performance and digestibility (Godoy et al., 2024), while the inclusion of 500 mg kg^{-1} of tannins in poultry diets resulted in reduced bone mineralization (Bayerle et al., 2019).

The impact of tannins on health and performance depends on their chemical structure and dosage, with evidence suggesting that moderate levels may enhance nutrient utilization and overall bird health (Redondo et al., 2014; Godoy, 2024). Thus, this study aimed to evaluate increasing levels of black wattle bark extract (BWE) in broilers' diets and their effects on performance, nutrient digestibility, intestinal health, and bone quality. We hypothesized that moderate levels of BWE would improve performance and health parameters without eliciting antinutritional effects.

2. Material and methods

The trial was conducted in accordance with the National Council for Animal Control and Experimentation (CONCEA) rules and approved by the Ethics Committee on Animal Use of the Instituto de Zootecnia of the Universidade Federal Rural do Rio de Janeiro, under protocol number 0118-05-2021.

2.1. Broilers management and challenge

A total of 240 male Cobb 500 broilers, eight days of age, were used. In the pre-experimental period, from day 1 to 7, birds were floor-reared on litter reused for three times to previous flocks to promote early gut microbiota colonization, simulating commercial exposure.

On day 8 the trial began: all birds were weighed (192.67 ± 9 g) and allocated to experimental cages of 0.36 m^2. Each cage housed six birds, resulting in a density of 25.5 kg m^{-2}. Cages were equipped with a trough feeder and a cup drinker, and feed and water were provided *ad libitum*. The experimental period lasted from day 8 to day 28.

On day 8, all birds were orally challenged with a dose corresponding to ten times the manufacturer's recommended dose of a live coccidiosis vaccine (Coccivet®; Vaxxinova, SP, Brazil). The vaccine contained viable oocysts of *Eimeria acervulina*, *E. brunetti*, *E. maxima*, *E. necatrix*, *E. praecox*, *E. tenella*, and *E. mitis*. This model was employed to induce a controlled, mild-to-moderate enteric challenge under experimental conditions, as previously described by Belote et al. (2019) and Lima et al. (2025).

Birds were fed corn–soybean meal diets according to age: starter (days 8–21) and grower (days 22–28). Diets were formulated to meet the recommendations of the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017) and contained no antibiotic growth promoters or anticoccidial agents. The lighting program was 23L:1D from days 8–14 (heat lamps provided both light and supplemental heat) and 14L:10D from days 15–28.

2.2. Diets and experimental design

The black wattle (*Acacia mearnsii*) extract (BWE) was obtained through a steam-pressure process, supplied by SETA TA Company (Nutreset, Estância Velha, RS, Brazil), and standardized to contain a minimum total tannin concentration of 735 g kg^{-1} of dry matter.

Diets were formulated according to the two-phase program previously described (Table 1). Five inclusion levels of BWE were evaluated: (I) reference diet (RD) without the addition of BWE (0 mg kg^{-1}); (II) RD + 300 mg kg^{-1} of BWE (BWE300); (III) RD + 500 mg kg^{-1} BWE (BWE500); (IV) RD + 700 mg kg^{-1} of BWE (BWE700); and (V) RD + 900 mg kg^{-1} of BWE (BWE900). The BWE replaced the inert ingredient (washed sand).

Table 1 - Experimental diets formulated for broilers in the initial (8 to 21 days old) and growth (22 to 28 days old) phases

Item	Composition (g kg^{-1})	
	8 to 21 days	22 to 28 days
Ingredient		
Ground corn	575	594
Soybean meal	357	330
Soybean oil	27.4	39.8
Inert[1]	2.00	2.00
Dicalcium phosphate	17.6	15.7
Limestone	7.74	7.13
Common salt	4.90	4.88
DL-methionine	3.36	2.86
Vitamin supplement[2]	1.00	1.00
Mineral supplement[3]	1.00	1.00
Choline chloride	0.65	0.65
L-Lysine HCL	1.69	0.77
L-Threonine	0.52	-
Antioxidant (BHT)	0.10	0.10
Total	1000	1000
Nutrients	Calculated composition	
Calcium (g kg^{-1})	8.70	7.90
Metabolizable energy (kcal kg^{-1})	3150	3200
Available phosphorous (g kg^{-1})	4.35	3.95
Digestible lysine (g kg^{-1})	11.6	10.3
Digestible methionine + Cystine (g kg^{-1})	9.1	8.33
Digestible methionine (g kg^{-1})	6.18	5.52
Crude protein (g kg^{-1})	216	203
Sodium (g kg^{-1})	2.00	2.00

[1] Inert - washed sand.
[2] Composition per kg of diet: vitamin A, 7500 IU; vitamin D3, 2500 IU; vitamin E, 18 IU; vitamin K3, 1.2 mg; thiamine, 1.5 mg; riboflavin, 5.5 mg; pyridoxine, 2 mg; vitamin B12, 12 mcg; niacin, 35 mg; calcium pantothenate, 10 mg; biotin 0.067 mg.
[3] Composition per kg of diet: iron, 60 mg; copper, 13 mg; manganese, 120 mg; zinc, 100 mg; iodine, 2.5 mg; selenium, 0.5 mg.

The trial was arranged as a randomized complete block design with five treatments and eight replicates of six birds each, totaling 40 experimental units. Blocks were defined by the average body weight at day 8, which was used as the criterion to form homogeneous groups before the random allocation of treatments within each block.

2.3. Data collection and sample processing

The following performance parameters were evaluated weekly: feed intake (FI), weight gain (WG) and feed conversion ratio (FCR). Feed intake was recorded throughout the trial period. Birds were weighed at 8, 15, 21, and 28 days of age. Feed intake was calculated by subtracting the orts from the amount of feed supplied to the feeders. In case of mortality, the amount of feed remaining in the feeder was immediately weighed to calculate the corrected feed intake. Weight gain was determined by subtracting the initial weight from the final weight. Moreover, feed conversion ratio was calculated as the ratio between the average feed intake and weight gain per bird.

The total excreta collection method was used to evaluate nutrient digestibility. Excreta collections were carried out in metal trays covered with plastic material twice daily to avoid fecal fermentation for three consecutive days (18 to 20 days of age). Excreta were weighed, labelled, and immediately frozen. Feed consumption during the collection period was recorded. Subsequently, the samples were thawed and homogenized, and approximately 250 g per experimental unit were weighed for pre-drying.

Samples were dried in a forced-air oven (model TE480, Tecnal, SP, Brazil) at 55 °C for 72 h. Subsequently, they were ground in a Willey-type knife mill to pass a 1-mm screen. The ground material was stored in airtight containers for further analysis.

Dry matter (DM) was determined by drying in a sterilization oven for 24 h at 105 °C (method 930.15; AOAC, 2005), followed by weighing after one hour in a desiccator. Mineral matter (MM) was determined gravimetrically, through the AOAC standard procedure, by burning in a muffle furnace at 600 °C (method 942.05; AOAC, 2005). Crude protein (CP) was determined using the Kjeldahl method (method 954.01; AOAC, 2005) with a 6.25 conversion factor. Gross energy (GE) of feeds and excreta was determined using a Parr calorimeter (model 1341, Parr Instruments, IL, USA), with benzoic acid as the standard.

Using data on feed intake, total excreta, DM, CP, MM, and GE of feeds and excreta, apparent total tract digestibility coefficients were determined for dry matter (ATTDDM), crude protein (ATTDCP), as well as the ash retention coefficient (ARC), in addition to the values of apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen balance (AMEn). Digestibility and retention coefficients, AME, and AMEn were calculated according to equations previously described by Dilelis et al. (2019).

One average-weight bird from each experimental unit, aged 21 days of age, was euthanized by cervical dislocation followed by bleeding to determine bone quality and health parameters. An abdominal incision was made to remove the digestive organs. After visual identification of the ileum (from Meckel's diverticulum to the ileocolic junction), approximately 5 cm of the central region was collected for histological analysis and determination of the intestinal health score "I See Inside". The left leg of each bird was collected for subsequent removal of the tibia to assess bone quality. Thus, 40 samples of ileum and tibia were obtained, eight for each treatment.

For the "I See Inside" methodology (ISI), ileum samples were processed using standard histological procedures and stained with hematoxylin and eosin and Alcian Blue for goblet cell visualization. Twenty intestinal villi were evaluated for each bird using an optical microscope (Nikon Eclipse E200, São Paulo, SP, Brazil). Slides were evaluated without treatment identification to avoid bias. The ISI is based on a numerical score of tissue alterations. According to Belote et al. (2019), ISI scores for the intestine range from 0 to 45, with higher values indicating reduced functional capacity of the organ. The parameters evaluated in the ileal ISI methodology included lamina propria thickness, epithelial thickness, enterocyte proliferation, inflammatory cell infiltration in the epithelium, inflammatory cell infiltration in the lamina propria, goblet cell hyperplasia, vascular congestion, and the presence of *Eimeria* oocysts. Analyses were conducted by the ISI Institute (Curitiba, PR, Brazil).

Left tibias were subjected to the Seedor index analysis (Seedor et al., 1991), bone breaking-strength, and ash concentration. Tibia preparation began by thawing the previously identified legs, followed by immersion in boiling water for ten minutes and manual deboning with scissors to remove all adhered material.

The Seedor index was obtained using a digital caliper (Mitutoyo, Japan) to measure the greatest epiphyseal length of the bone, while bone weight was measured on an analytical scale (model M214AI, BEL, Brazil). The Seedor index was calculated by dividing tibia weight (mg) by length (mm), indicating bone density.

Breaking strength was determined using a texturometer (Texture Analyzer TA.XT Plus) and the Blade Set HDP/BS probe at a speed of 4.0 mm s^{-1}. Each bone was placed on a wooden support with a 2-cm free span attached to the equipment, where the software calculated the force required to cause fracture.

Determination of bone ash was performed after assessing bone strength. The broken bones were weighed and then dried in an oven at 105 °C for 12 h to remove all moisture and determine dry matter. Subsequently, they were burned in a muffle furnace at 580 °C for 6 h to obtain the ash concentration.

2.4. Statistical analysis

Residual normality was assessed with the Shapiro–Wilk test. Data on performance, digestibility, and bone traits were analyzed in PROC MIXED (SAS) under the randomized block design, with treatment as a fixed effect and block as a random effect. The experimental unit was the cage (n = 40, 8 replicate cage per treatment). When the treatment effect was significant (P<0.05), means separation was done using Tukey's HSD. Estimations of optimal responses of supplemented tannin (0, 300, 500, 700, or 900 mg kg^{-1}) were done using linear (L) and quadratic polynomial (QP) regression models.

The general statistical model used for ANOVA was:

$$Y_{ijk} = \mu + T_i + B_j + \varepsilon_{ijk}, \quad (1)$$

in which Y_{ijk} is the observed value of the response variable, μ is the overall mean, T_i is the fixed effect of the *i*-th treatment (BWE inclusion level), B_j is the random effect of the *j*-th block (based on initial bird weight), and ε_{ijk} is the residual error.

For the regression analysis, the following model was applied:

$$Y = \beta 0 + \beta 1X + \beta 2X^2 + \varepsilon, \quad (2)$$

in which Y is the response variable, X is the BWE inclusion level (mg kg^{-1}), $\beta 0$, $\beta 1$ and $\beta 2$ are the regression coefficients, and ε is the random error.

The intestinal health score data evaluated by the "I See Inside" (ISI) methodology were analyzed using the non-parametric Kruskal-Wallis test, appropriate for data that do not meet the assumptions of normality. When significant differences were detected (P<0.05), the means were compared using Dunn's multiple comparison test.

3. Results

From 8 to 14 days of age, dietary supplementation with BWE produced no significant changes in feed intake, body weight gain, or feed conversion ratio (P>0.05; Table 2). Between days 15 and 21 (Table 2), feed intake decreased linearly with increasing BWE inclusion. Tukey's test confirmed a dose-dependent effect, with birds fed 300 mg kg^{-1} showing the highest intake and those at 700 and 900 mg kg^{-1} showing the lowest values. For broilers from 22 to 28 days, the reduction in feed intake was accompanied by a decrease in weight gain (P<0.05), with lower weight gain in the groups fed 900 mg kg^{-1}, while broilers receiving 0 and 300 mg kg^{-1} of acacia tannins showed greater weight gain.

Considering the entire growth cycle (8 to 28 days), only feed intake was significantly influenced by the treatments (P = 0.02), with a linear reduction as the inclusion level of BWE increased. Broilers fed 300 mg kg^{-1} of BWE exhibited the highest cumulative feed intake, whereas those receiving 900 mg kg^{-1} showed the lowest values throughout the evaluated period. It is important to note that performance was evaluated only up to 28 of age under cage housing conditions, which limits direct extrapolation to commercial grow-out conditions typically extending to 42 days.

The BWE inclusion in broilers' diets resulted in a linear response in dry matter and protein digestibility (Table 3), and a quadratic response was observed for ash retention. The proposed quadratic model estimated 40.77% as the maximum ash retention at an inclusion level of 495 mg kg^{-1} of BWE.

Table 2 - Performance of broilers supplied with different levels of black wattle extract (BWE)

	BWE inclusion level (mg kg^{-1})					SEM	P-value	Regression
	0	300	500	700	900			
Day 8 to 14								
Feed intake (g)	453.7	443.5	443.5	428.9	428.2	8.59	0.563	NS
Weight gain (g)	326.2	324.6	307.6	316.0	310.0	5.19	0.604	NS
Feed:gain ratio	1.390	1.367	1.394	1.405	1.382	0.021	0.595	NS
Day 15 to 21								
Feed intake (g)	643.3ab	669.2a	631.7ab	613.1b	614.9b	10.81	0.013	L[1]
Weight gain (g)	476.2	480.0	461.9	473.2	457.4	7.12	0.461	NS
Feed:gain ratio	1.351	1.394	1.370	1.327	1.346	0.017	0.070	NS
Day 22 to 28								
Feed intake (g)	1007ab	1021a	975.1ab	985.6ab	959.8b	17.21	0.006	L[2]
Weight gain (g)	716.2a	711.3a	684.8ab	696.5ab	669.7b	15.89	0.009	L[3]
Feed:gain ratio	1.407	1.438	1.425	1.415	1.435	0.017	0.666	NS
Day 8 to 28								
Feed intake (g)	2104ab	2134a	2035ab	2042ab	2003b	28.87	0.021	L[4]
Weight gain (g)	1519	1516	1454	1485	1437	23.33	0.072	NS
Feed:gain ratio	1.386	1.408	1.401	1.373	1.394	0.010	0.138	NS

SEM - standard error of the mean; L - linear effect; NS - non-significant.
Means followed by the same letter within a row do not differ according to Tukey's test (P<0.05) (n = 8).
[1] $y = -0.048x + 657.51$; $R^2 = 0.5282$.
[2] $y = -0.0569x + 1017.1$; $R^2 = 0.6506$.
[3] $y = -0.0483x + 718.88$; $R^2 = 0.7828$.
[4] $y = -0.129x + 2125.57$; $R^2 = 0.7019$.

Table 3 - Nutrient digestibility coefficients and metabolizable energy content of broiler diets containing different levels of black wattle extract (BWE)

	BWE inclusion level (mg kg^{-1})					SEM	P-value	Regression
	0	300	500	700	900			
ATTDDM (%)	69.03b	71.94a	71.34ab	70.73ab	71.76ab	0.673	0.034	L[1]
ATTDCP (%)	63.28c	66.88ba	68.64a	64.44cb	68.57a	0.859	<0.01	L[2]
ARC (%)	27.53c	39.67a	44.23a	29.35cb	32.96b	1.310	<0.01	Q[3]
AME (kcal kg^{-1})	3227	3277	3326	3265	3312	32.33	0.242	NS
AMEn (kcal kg^{-1})	2995	3029	3050	3031	3049	30.30	0.706	NS

ATTDDM - apparent total tract digestibility of dry matter; ATTDCP - ATTD of crude protein; ARC - ash retention coefficient; AME - apparent metabolizable energy; AMEn - nitrogen-corrected apparent metabolizable energy; SEM - standard error of the mean; L - linear; Q - quadratic; NS - non-significant.
Means followed by the same letter within a row do not differ according to Tukey's test (P<0.05) (n = 8).
[1] $Y = 0.0021x + 69.934$; $R^2 = 0.4037$.
[2] $Y = 0.0039x + 64.458$; $R^2 = 0.3265$.
[3] $Y = -0.00005x^2 + 0.0495x + 28.519$; $R^2 = 0.5507$.

Despite the significant linear effect, a low coefficient of determination was observed. The Tukey's HSD test (Table 3) showed a higher dry matter digestibility value when 300 mg kg^{-1} of BWE was supplied compared with the treatment without BWE inclusion. For protein digestibility, the best value was observed with the inclusion of 500 and 900 mg kg^{-1} of BWE. Generally, the highest digestibility values were observed at inclusion levels up to 500 mg kg^{-1} concentrations. Dietary values of AME and AMEn were not affected (P>0.05) by the BWE inclusion in broiler diets.

No differences (P>0.05) were found in the total "I See Inside" index (Table 4) in the ileum of broilers slaughtered at 21 of age. In the analyzed indices of intestinal health, only inflammatory cell infiltration in the epithelium, goblet cells, and oocyst presence showed significant alterations (P<0.05). The broiler group fed 900 mg kg^{-1} of BWE showed the worst results (P<0.05) for inflammatory cell infiltration in the epithelium compared with animals receiving only 300 mg kg^{-1} of the additive. In addition, the animals fed with the diet with the lowest BWE concentration had higher goblet cell counts than those supplied with 500 mg kg^{-1} of BWE. Finally, the group fed 900 mg kg^{-1} of BWE showed a higher presence of oocysts than all others.

Keeping in view the bone parameters (Table 5), adding BWE in broiler feed did not influence (P>0.05) any of the bone quality characteristics in tibia.

Table 4 - Ileum intestinal health indexes ("I See Inside") of 21-day-old broilers supplied with different levels of black wattle extract (BWE)

Variable	BWE inclusion level (mg kg^{-1})					SEM	P-value
	0	300	500	700	900		
Own lamina thickness	1.01	0.88	1.11	0.79	0.96	0.11	0.562
Epithelium thickness	0.53	0.46	0.60	0.64	0.60	0.06	0.174
Enterocyte proliferation	0.48	0.43	0.59	0.57	0.60	0.05	0.143
Inflammatory cell infiltration into epithelium	0.38ab	0.31b	0.47ab	0.45ab	0.56a	0.05	0.011
Inflammatory cell infiltration in the lamina propria	0.83	0.70	1.04	0.69	0.99	0.11	0.227
Goblet cells	0.72ab	0.50ab	0.47b	0.91a	0.91a	0.10	<0.01
Congestion	0.04	0.08	0.01	0.08	0.07	0.03	0.578
Presence of oocysts	0.00b	0.00b	0.02b	0.00b	0.17a	0.02	<0.01
Total score	3.99	3.36	4.34	4.13	4.86	0.40	0.247

SEM - standard error of the mean.
Means followed by the same letter within a row do not differ according to Dunn test (P<0.05) (n = 8).

Table 5 - Bone parameters of tibia of 21-day-old broilers supplied with different levels of black wattle extract (BWE)

Variable	BWE inclusion level (mg kg^{-1})					SEM	P-value	Regression
	0	300	500	700	900			
Seedor index	77.47	77.96	73.95	75.21	77.09	2.243	0.686	NS
Breaking strength (kgf)	21.32	18.27	19.53	18.00	20.68	1.531	0.466	NS
Ash (g)	1.11	1.10	1.10	1.12	1.14	0.046	0.967	NS
Dry matter content (%)	44.54	43.35	44.98	45.38	45.72	0.567	0.091	NS
Ash content (%, in dry matter)	47.93	48.74	49.65	50.17	50.33	0.695	0.102	NS

NS - non-significant (P>0.05) (n = 8); SEM - standard error of the mean.

4. Discussion

The inclusion of black wattle extract (BWE) suppressed feed intake in a dose-dependent manner, with birds fed the highest level (900 mg kg^{-1}) consuming less than those fed 300 mg kg^{-1}. This finding is consistent with the well-known astringent properties of tannins, which can reduce palatability and feed acceptance (Butler et al., 1984). Meta-analyses by Soares et al. (2020) and Hidayat et al. (2021) have consistently associated tannin supplementation to reduced feed intake, weight gain, and feed conversion ratio.

A key finding of this study was the improvement in crude protein and dry matter digestibility, as well as ash retention at BWE inclusion levels up to 500 mg kg^{-1}. This result is particularly interesting given that tannins are known for their protein and metal binding capacity, which is typically associated with reduced amino acid digestibility (Hidayat et al., 2021), inhibition of digestive enzymes (Medugu et al., 2012) and reduced mineral retention by birds (Hassan et al., 2020). Godoy et al. (2024) reported similar improvements in protein digestibility in broilers receiving 500 mg kg^{-1} of acacia tannins under a necrotic enteritis challenge. Antioxidant and anti-inflammatory effects of tannins may counteract their chelating properties, allowing reduced epithelial damage and better utilization of nutrients in the diet (Choi and Kim, 2020).

Despite improved dry matter and protein digestibility, these changes did not translate into better dietary energy utilization. This discrepancy may be explained by the fact that the improvements in digestibility were more pronounced in nitrogenous and mineral fractions rather than in primary energy-yielding components, such as fats or carbohydrates.

From a pathophysiological standpoint, the *Eimeria* challenge induces oxidative stress and intestinal inflammation (Masood et al., 2013). Tannins are potent antioxidants, with a reduction potential exceeding that of vitamins E and C (Shi et al., 2003). In this context, we observed a lower infiltration of inflammatory cells in the epithelium of birds supplemented with BWE300 compared with those receiving BWE900, indicating a beneficial, dose-dependent anti-inflammatory effect. This aligns with Tonda et al. (2018), who reported lower intestinal lesion scores in birds fed tannin extracts, and with Godoy et al. (2024), who reported improved villus height.

However, BWE supplementation did not alter the total intestinal lesion score (ISI) in our study. While the ISI methodology is highly correlated with performance (Belote et al., 2019), the lack of effect on both ISI and performance suggests that the tested BWE levels were safe but insufficient to promote significant gut integrity recovery or growth promotion under our specific challenge conditions. The overall ISI scores were relatively low compared with those reported by Belote et al. (2019), possibly because housing birds in metabolic cages limited oocyst reinfection, thereby diminishing the severity of the challenge compared with floor-pen systems.

Age may be another factor contributing to the different outcomes observed. In our study, we challenged birds at 8 days of age, whereas Belote et al. (2019) challenged birds that were only at 1 day of age. The developing immune system and gut barrier of neonatal chicks may respond more severely to an *Eimeria* challenge, potentially explaining the milder lesion scores recorded in our trial. Lima et al. (2025), for instance, reported that this same 10x challenge model, performed in metabolic cages, induces age-dependent changes in the expression of genes related to intestinal integrity. Therefore, analyzing these genes should be considered in future studies to confirm the gut health-promoting effects of tannins at a molecular level, especially when gross lesions are not pronounced.

Although an increase in ash retention was observed, this did not translate into improvements in tibia bone quality parameters. This apparent contradiction highlights the complex mechanisms involved in bone and mineral homeostasis. Bone deposition is not a passive outcome of mineral availability but is tightly controlled by parathyroid hormone (PTH), vitamin D, calcitonin, and fibroblast growth factor-23, as well as by the activity of osteoblasts and osteoclasts (Shao et al., 2019). Future studies should explore these variables to better understand the role of tannins in mineral metabolism after absorption.

While improved gut health can enhance mineral absorption, tannins are also known to chelate metals, which can reduce their bioavailability and impair bone mineralization (Hassan et al., 2020). Deleterious effects of tannins on bone quality have been previously reported (Keshavarzi et al., 2017; Ghaedi et al., 2018; Bayerle et al., 2019). In our study, the inclusion of acacia tannins up to 900 mg kg^{-1} did not impair tibia mineralization or breaking strength. These findings reinforce that the impact of tannins on skeletal development is strongly dose- and source-dependent, with adverse effects occurring only when mineral binding is sufficient to alter mineral deposition.

5. Conclusions

The dietary inclusion of black wattle extract at levels up to 500 mg kg^{-1} effectively improved nutrient digestibility and intestinal health in broilers under an *Eimeria* challenge, positioning it as a promising functional feed additive, particularly for antibiotic-free production systems. However, the intake suppression observed at 900 mg kg^{-1} underscores that the optimal dose represents a critical balance between the beneficial effects and the inherent antinutritional properties of tannins.

Data availability

The data that support this study will be shared upon reasonable request to the corresponding author.

Author contributions

Conceptualization: Dilelis, F. and Maysonnave, G. S. **Formal analysis:** Dilelis, F. **Funding acquisition:** Maysonnave, G. S. **Investigation:** Reis, T. L.; Miranda, C. C. M.; Bravo, A. M. and Guariento, E. F. **Methodology:** Reis, T. L. and Machado, N. J. B. **Project administration:** Dilelis, F. **Resources:** Guariento, E. F. and Lima, C. A. R. **Supervision:** Lima, C. A. R. **Writing – original draft:** Dilelis, F. **Writing – review & editing:** Miranda, C. C. M.; Bravo, A. M. and Machado, N. J. B.

Conflict of interest

The authors declare no conflict of interest.

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References

Akosile, O. A.; Kehinde, F. O.; Oni, A. I. and Oke, O. E. 2023. Potential implication of *in ovo* feeding of phytogenics in poultry production. Translational Animal Science 7:txad094. https://doi.org/10.1093/tas/txad094

Albarrak, S. M. 2021. Supplementation with the methanolic extract of *Lycium shawii* or *Rhanterium epapposum* enhances immune responses of broilers. Advances in Animal and Veterinary Sciences 9:1816-1828.

AOAC - Association of Official Analytical Chemistry. 2005. Official method of analysis. 18th ed. AOAC International, Washington, DC.

Bayerle, D. F.; Nunes, R. V.; Wachholz, L.; Bruxel, T. M. O.; Vargas Junior, J. G.; Sangalli, G.; Giron, T. V. and Schone, R. A. 2019. Use of golden mussel and wattle tannin in the supply of cut chickens. Semina: Ciências Agrárias 40:1951-1964.

Belote, B. L.; Soares, I.; Tujimoto-Silva, A.; Sanches, A. W. D.; Kraieski, A. L. and Santin, E. 2019. Applying I see inside histological methodology to evaluate gut health in broilers challenged with *Eimeria*. Veterinary Parasitology 276:100004. https://doi.org/10.1016/j.vpoa.2019.100004

Butler, L. G.; Riedl, D. J.; Lebryk, D. G. and Blytt, H. J. 1984. Interaction of proteins with sorghum tannin: Mechanism, specificity and significance. Journal of the American Oil Chemists' Society 61:916-920. https://doi.org/10.1007/BF02542166

Cardinal, K. M.; Kipper, M.; Andretta, I. and Ribeiro, A. M. L. 2019. Withdrawal of antibiotic growth promoters from broiler diets: Performance indexes and economic impact. Poultry Science 98:6659-6667. https://doi.org/10.3382/ps/pez536

Chen, X.; Xiong, J.; Huang, S.; Li, X.; Zhang, Y.; Zhang, L. and Wang, F. 2018. Analytical profiling of Proanthocyanidins from *Acacia mearnsii* bark and *in vitro* assessment of antioxidant and antidiabetic potential. Molecules 23:2891. https://doi.org/10.3390/molecules23112891

Choi, J. and Kim, W. K. 2020. Dietary application of tannins as a potential mitigation strategy for current challenges in poultry production: A review. Animals 10:2389. https://doi.org/10.3390/ani10122389

Dilelis, F.; Gomes, A. V. C.; Lima, C. A. R.; Corrêa, D. C. B. and Reis, T. L. 2019. Metabolizable energy of rice bran, cottonseed meal and wheat bran for slow-growing broilers at two ages. Ciência Animal Brasileira 20:1-10. https://doi.org/10.1590/1089-6891v20e-46357

Garcia, R. G.; Mendes, A. A.; Sartori, J. R.; Paz, I. C. L. A.; Takahashi, S. E.; Pelícia, K.; Komiyama, C. M. and Quinteiro, R. R. 2004. Digestibility of feeds containing sorghum, with and without tannin, for broiler chickens submitted to three room temperatures. Brazilian Journal of Poultry Science 6:55-60. https://doi.org/10.1590/S1516-635X2004000100007

Ghaedi, L.; Houshmand, M. and Parsaei, S. 2018. Performance and tibia characteristics of broilers fed diets containing raw or treated oak acorn (*Quercus brantii lindl*). The Journal of Animal and Plant Sciences 28:38-45.

Godoy, G. L.; Rodrigues, B. N.; Agilar, J. C.; Biselo, V.; Brutti, D. D.; Maysonnave, G. S. and Stefanello, C. 2024. Effects of *Acacia mearnsii* tannins on growth performance, footpad dermatitis, nutrient digestibility, intestinal permeability, and meat quality of broiler chickens. Animal Feed Science and Technology 308:115875. https://doi.org/10.1016/j.anifeedsci.2024.115875

Hassan, Z. M.; Manyelo, T. G.; Selaledi, L. and Mabelebele, M. 2020. The effects of tannins in monogastric animals with special reference to alternative feed ingredients. Molecules 25:4680. https://doi.org/10.3390/molecules25204680

Hernandez-Patlan, D.; Tellez-Isaias, G.; Hernandez-Velasco, X. and Solis-Cruz, B. 2023. Editorial: Technological strategies to improve animal health and production. Frontiers in Veterinary Science 10:1206170. https://doi.org/10.3389/fvets.2023.1206170

Hidayat, C.; Irawan, A.; Jayanegara, A.; Sholikin, M. M.; Prihambodo, T. R.; Yanza, Y. R.; Wina, E.; Sadarman, S.; Krisnan, R. and Isbandi, I. 2021. Effect of dietary tannins on the performance, lymphoid organ weight, and amino acid ileal digestibility of broiler chickens: A meta-analysis. Veterinary World 14:1405-1411. https://doi.org/10.14202/vetworld.2021.1405-1411

Iwu, C. D.; Korsten, L. and Okoh, A. I. 2020. The incidence of antibiotic resistance within and beyond the agricultural ecosystem: A concern for public health. MicrobiologyOpen 9:e1035. https://doi.org/10.1002/mbo3.1035

Keshavarzi, S.; Houshmand, M. and Bahreini Behzadi, M. R. 2017. Age-specific response of broilers to dietary inclusion of a high-tannin feedstuff. Poultry Science Journal 5:83-90.

Kurekci, C.; Al Jassim, R.; Hassan, E.; Bishop-Hurley, S. L.; Padmanabha, J. and McSweeney, C. S. 2014. Effects of feeding plant-derived agents on the colonization of *Campylobacter jejuni* in broiler chickens. Poultry Science 93:2337-2346. https://doi.org/10.3382/ps.2014-03950

Kurekci, C.; Bishop-Hurley, S. L.; Vercoe, P. E.; Durmic, Z.; Al Jassim, R. A. M. and McSweeney, C. S. 2012. Screening of australian plants for antimicrobial activity against *Campylobacter jejuni*. Phytotherapy Research 26:186-190. https://doi.org/10.1002/ptr.3526

Lima, P. P. A. B. M.; Paulino, P. G.; Silva, N. M.; Galdino, K. C. P.; Rabello, C. A.; Souza, F. G.; Reis, T. L.; Machado, L. S.; Sousa, F. D. R. and Santos, H. A. 2025. Dose and age-dependent effects of *Eimeria* spp. infection on cytokine and intestinal integrity gene expression in broiler chickens. Veterinary Parasitology 338:110550. https://doi.org/10.1016/j.vetpar.2025.110550

Masood, S.; Abbas, R. Z.; Iqbal, Z; Mansoor, M. K.; Sindhu, Z. U. D.; Zia, M. A. and Khan, J. A. 2013. Role of natural antioxidants for the control of coccidiosis in poultry. Pakistan Veterinary Journal 33:401-407.

Maysonnave, G. S.; Brutti, D. D.; Silva, V. M. and Stefanello, C. 2024. Evaluation of increasing levels of *Acacia mearnsii* tannins on growth performance and intestinal morphometrics of broiler chickens undergoing a *Salmonella* Heidelberg challenge. Poultry 3:284-297. https://doi.org/10.3390/poultry3030021

Medugu, C. I.; Saleh, B.; Igwebuike, J. U. and Ndirmbita, R. L. 2012. Strategies to improve the utilization of tannin-rich feed materials by poultry. International Journal of Poultry Science 11:417-423.

Mnisi, C. M.; Mlambo, V.; Gila, A.; Matabane, A. N.; Mthiyane, D. M. N.; Kumanda, C.; Manyeula, F. and Gajana, C. S. 2023. Antioxidant and antimicrobial properties of selected phytogenics for sustainable poultry production. Applied Sciences 13:99. https://doi.org/10.3390/app13010099

Ogawa, S. and Yoshikazu Y. 2018. Tannins from *Acacia mearnsii* De Wild. Bark: Tannin determination and biological activities. Molecules 23:837. https://doi.org/10.3390/molecules23040837

Perin, G.; Baldissera, M. D.; Fernandes, M.; Barreta, M.; Casagrande, R. A.; Griss, L. G.; Fortuoso, B. F.; Volpato, A.; Stefani, L. M.; Boiago, M. M.; Cristo, T. G.; Santiani, F. and Silva, A. S. 2019. Effects of tannin-containing diets on performance, gut disease control and health in broiler chicks. Animal Production Science 59:1847-1857. https://doi.org/10.1071/AN18393

Redondo, L. M.; Chacana, P. A.; Dominguez, J. E. and Fernandez Miyakawa, M. E. 2014. Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry. Frontiers Microbiology 5:118. https://doi.org/10.3389/fmicb.2014.00118

Rostagno, H. S.; Albino, L. F. T.; Hannas, M. I.; Donzele, J. L.; Sakomura, N. K.; Perazzo, F. G.; Saraiva, A.; Abreu, M. L. T.; Rodrigues, P. B.; Oliveira, R. F.; Barreto, S. L. T. and Brito, C. O. 2017. Brazilian tables for poultry and swine. 4th ed. UFV, Viçosa, MG.

Seedor, J. G.; Quartuccio, H. A. and Thompson, D. D. 1991. The bisphosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. Journal of Bone and Mineral Research 6:339-346. https://doi.org/10.1002/jbmr.5650060405

Shao, Y; Sun, G.; Cao, S.; Lu, L.; Zhang, L.; Liao, X. and Luo, X. 2019. Bone phosphorus retention and bone development of broilers at different ages. Poultry Science 98:2114-2121. https://doi.org/10.3382/ps/pey565

Shi, J.; Yu, J.; Pohorly, J. E. and Kakuda, Y. 2003. Polyphenolics in grape seeds-biochemistry and functionality. Journal of Medicinal Food 6:291-299. https://doi.org/10.1089/109662003772519831

Soares, S.; Brandão, E.; Guerreiro, C.; Soares, S.; Mateus, N. and Freitas, V. 2020. Tannins in food: Insights into the molecular perception of astringency and bitter taste. Molecules 25:e2590. https://doi.org/10.3390/molecules25112590

Tonda, R. M.; Rubach, J. K.; Lumpkins, B. S.; Mathis, G. F. and Poss, M. J. 2018. Effects of tannic acid extract on performance and intestinal health of broiler chickens following coccidiosis vaccination and/or a mixed-species *Eimeria* challenge. Poultry Science 97:3031-3042. https://doi.org/10.3382/ps/pey158

Wang, M. L.; Suo, X.; Gu, J. H.; Zhang, W. W.; Fang, Q. and Wang, X. 2008. Influence of grape seed proanthocyanidin extract in broiler chickens: Effect on chicken coccidiosis and antioxidant status. Poultry Science 87:2273-2280. https://doi.org/10.3382/ps.2008-00077

Yang, C.; Diarra, M. S.; Choi, J.; Rodas-Gonzalez, A.; Lepp, D.; Liu, S.; Lu, P.; Mogire, M.; Gong, J.; Wang, Q. and Yang, C. 2021. Effects of encapsulated cinnamaldehyde on growth performance, intestinal digestive and absorptive functions, meat quality and gut microbiota in broiler chickens. Translational Animal Science 5:txab099. https://doi.org/10.1093/tas/txab099