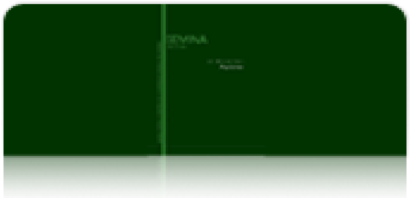

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Golden mussel (*Limnoperna fortunei*) in feed for broiler chicks using tannin as a sequestrant of toxic metals

Mexilhão dourado (*Limnoperna fortunei*) na alimentação de pintos de corte utilizando tanino como sequestrante de metais tóxicos

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Abstract

This study aimed to evaluate the use of wattle tannin as an adsorbent of toxic metals in broilers fed on diets with different levels of replacement of limestone with golden mussel meal. First, we conducted a trial to evaluate the performance of broiler chicks from 1 to 21 days old when fed on wattle tannin. For this we used 720 male broiler chicks that were one day old, in a completely randomized design with six treatments and six replications. The treatments were composed of basal diets with increasing levels of wattle tannin (0, 250, 500, 750, 1000 and 1250 g tonne-1), where the tannin replaced the inert material of the feed. All of the diets were isonutritive and isocaloric. The results showed that 250 g tonne-1 of tannin was detrimental to weight gain, final weight and feed conversion, and the use of 1000 g tonne-1 of wattle tannin, in addition to these effects, caused a drop in consumption of the ration. There was no effect of treatment on morphometric measurements of villi, crypt or the villus:crypt ratio. Next, we conducted a second test to evaluate the performance of broiler chicks from 1 to 21 days of age when fed with increasing levels of replacement (0, 25, 50, 75, 100%) of limestone with golden mussel and even supplementation or not wattle tannin. Five repetitions were used and the birds were housed in a completely randomized design. In diets with the inclusion of tannin, 250 g tonne-1 of wattle tannin was used, which replaced the inert material in the feed. In this test, 1200 male broiler chicks that were one day old were used. The results showed that the golden mussel contains large quantities of calcium, with low concentrations of toxic metals and microbiological contamination within the allowed range. Performance data show that mussel can be used to replace up to 100% of limestone in feed without affecting the variables, but the use of 250 g tonne-1 of wattle tannin affects the performance of chicks. Bone quality was not changed by the treatments. Phosphorus and calcium in the bones remained stable during treatment and only the gray variation showed interaction effects from the use of mussel and wattle tannin.

Key words: Alternative food. Bone structure. Calcium. Intestinal morphology. Limestone. Performance.

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Resumo

Este estudo teve como objetivo avaliar a utilização de tanino de acácia como adsorvente de metais tóxicos em rações para pintos de corte alimentados com diferentes níveis de inclusão da farinha de mexilhão dourado em substituição ao calcário calcítico. Primeiramente foi realizado um ensaio para avaliar o desempenho de pintos de corte de 1 a 21 dias, alimentados com tanino de acácia, utilizando-se 720 pintos de corte, machos, com um dia de idade, distribuídos em delineamento inteiramente casualizado, com seis tratamentos e seis repetições. Os tratamentos foram formados por rações basais com níveis crescentes de tanino de acácia (0, 250, 500, 750, 1000, 1250 g ton^{-1} de tanino de acácia), onde o mesmo substituiu o material inerte da ração, sendo todas as dietas isonutritivas e isoenergéticas. Os resultados apontaram que 250 g ton^{-1} de tanino foi prejudicial para ganho de peso, peso final e conversão alimentar, ao passo que, a utilização de 1000 g ton^{-1} de tanino de acácia, além destes efeitos citados causou queda no consumo de ração. Para as medidas morfometricas de vilo, cripta e relação vilo:cripta, não houve efeitos dos tratamentos. Na sequência foi realizado um segundo ensaio para avaliar o desempenho de pintos de corte de 1 a 21 dias de idade com níveis crescentes de substituição (0; 25; 50; 75; 100%) do calcário calcítico pelo mexilhão dourado e ainda a suplementação ou não de tanino de acácia, com 5 repetições, as aves foram alojadas em delineamento inteiramente casualizado. Nas dietas com a inclusão de tanino utilizou-se 250 g ton^{-1} de tanino de acácia, o qual foi substituto do material inerte da ração. Foram utilizados neste ensaio 1200 pintos de corte, machos, com um dia de idade. Os resultados mostraram que o mexilhão dourado possui grande quantidade de cálcio, com baixas concentrações de metais tóxicos e contaminação microbiológica dentro da permitida. Os dados de desempenho demonstram que a utilização do mexilhão dourado pode ser realizada em até 100% de substituição ao calcário calcítico nas rações sem afetar as variáveis estudadas, porém a utilização de 250 g ton^{-1} de tanino de acácia afeta o desempenho dos pintos de corte. A qualidade óssea não foi modificada pelos tratamentos. Os teores de fósforo e cálcio nos ossos permaneceram estáveis nos tratamentos, sendo que apenas a cinza apresentou variação interação nos efeitos pelo uso de mexilhão dourado e tanino de acácia.

Palavras-chave: Alimento alternativo. Calcário calcítico. Cálcio. Desempenho. Estrutura óssea. Morfometria intestinal.

Introduction

With the growth and modernization of the Brazilian poultry industry, there has been an increase in the production of poultry meat and consequently in the use of raw materials for the production of feed. To maintain this growth in feed production, nutritionists seek alternative sources that can replace the foods commonly used. The low cost of alternative ingredients is crucial for its use in the feed, as is the case for the meal obtained from the processing of mollusk valves. One of these mollusk species that is used in animal feed is the golden mussel, Limnoperna fortunei. It is composed mainly of calcium carbonate ($CaCO_3$), serving as a source of calcium for animals. As this species is not native to South America, it is considered exotic and invading, and has produced an environmental imbalance due to its high degree of infestation and proliferation, caused by its fast growth rate.

The use of L. fortunei in diets can provide a calcium source for animals. However, because it is a filtering species, the golden mussel can be contaminated by toxic metals (MARENGONI et al., 2013). When ingested, these metals have accumulative effects in organisms and may cause serious health problems in humans. The use of tannin through the sequestering activity of the golden mussel could be an interesting practice to lessen the effects of toxic metals (HARIKISHORE et al., 2010) and enable the use of the golden mussel in poultry feed.

Thus, the aim of this experiment was to evaluate the use of tannin as an adsorbent of toxic metals in broiler chicks fed on diets with different levels of replacement of limestone in diets with golden mussel meal.

Material and Methods

Two experiments were conducted at the experimental aviary of State University of West Parana – Unioeste, Campus of Marechal Cândido Rondon – PR.

To evaluate the amount of wattle tannin to be used in broiler chick diets, 720 male, 1-day old broiler chicks of Cobb 500 lineage were acquired from a commercial hatchery and vaccinated in the hatchery for the diseases Marek, Gumboro, Avian Bouba and Infectious Bronchitis.

The birds were distributed in a completely randomized design across 36 experimental units, which included six treatments and six replicates, with 20 birds in each. The average initial weight of the chicks was 42.5 ± 0.47 g.

The lighting program was 24 hours of light, and food and water were fed ad libitum. All diets were isonutritive and isocaloric, based on corn and soybean meal and formulated to meet the nutritional requirements for chicks of 1-21 days of age (Table 1). The treatments consisted of increasing levels of wattle tannin (tannin acid 72%) at the concentrations: 0, 250, 500, 750, 1000 and 1250 g ton-1 of wattle tannin.

Table 1. Diet composition.

Ingredients (%)	Initial
Corn grain	53.85
Soybean meal	37.00
Degummed soy oil	3.250
Monocalcium phosphate	1.713
Limestone	1.317
Salt	0.503
DL-methionine 99%	0.308
L-lysine.HCl 78%	0.212
L-threonine 99%	0.052
Vitamin suplement[1]	0.100
Mineral suplement[2]	0.050
Choline chloride 60%	0.060
Antioxidant[3]	0.020
Coxistac[4]	0.060
Stafac 500[5]	0.005
Inert/Tannin	1.500
Total	100.00
	Calculated composition
Metabolizable Energy (kcal/kg)	2960
Crude Protein (%)	21.40
Digestible lysine (%)	1.217
Digestible methyonine + cystin (%)	0.876
Digestible threonine (%)	0.791
Calcium (%)	0.920
Available phosphorous (%)	0.470
Sodium (%)	0.218

[1]ROVIMIX – Vitamin suplement for poultry. Content: Vit A (min) 9000000 UI; Vit D_3 (min) 2500000 UI; Vit E (min) 20000 UI; Vit K_3 (min) 2500 mg; Vit B_1 (min) 1500 mg; Vit B_2 (min) 6000 mg; Vit B_6 (min) 3000 mg; Pantotenic acid (min) 12 g; Niacin (min) 25 g; Folic acid (min) 800 mg; Se (min) 250 mg; [2]ROLIGOMIX – Mineral suplement for poultry. Content: Cu (min) 20g; Fe (min) 100g; Mn (min) 2000 mg; Zn (min) 100 g; [3]BHT; [4]Salinomycin 12%; [5]Virginamycin.

Environmental variables were measured twice a day, once in the morning and once in the afternoon, and the maximum and minimum values were recorded using a digital thermo-hygrometer that was installed near the birds. During the experimental period, the average temperature ranged between 24.25°C and 29.68°C and the relative humidity ranged from 42.48% to 63.76%.

During the experimental period, dead birds were removed and the feed intake was recorded for corrections in intake and feed conversion ratio according to Sakomura and Rostagno (2007). At 21 days old, all birds and feed were weighed to determine weight gain (WG), feed intake (FI) and feed conversion rate (FCR).

At the end of the experiment, two birds per experimental unit (± 5% average weight and starved for 8 h) were slaughtered. A length of 4 cm from the distal duodenum was collected for morphometric analysis by microscopy, and histological sections were prepared according to Luna (1968). The image capture from the slides was carried out using an optical microscope with an image capture system. The villi and crypts were measured with an objective lens of 4x magnification. The height of villi was measured from the basal region to the top and the depth of crypts was the base to the transition region. The relationship of crypt:villi was calculated using the villus heights and crypt depths.

To evaluate the effect of wattle tannin on all variables studied, an analysis of variance followed by a Dunnett's test was performed, using the basal diet as a control. Subsequently, polynomial regression analysis was used to assess the effect of increasing levels of wattle tannin on all variables. Significance of results was judged using the 5% level of probability and analyses were performed using the Statistical and Genetics Analysis System – SAEG (UNIVERSIDADE FEDERAL VIÇOSA, UFV, 1999).

In the second experiment, 1200 male, 1-day old broiler chicks of the Cobb 500 lineage were used. The birds were individually weighed and were found to have an average weight of 46.02 ± 0.17 g. They were evenly distributed across 50 experimental units, with 24 birds each, housed in a completely randomized design. A 2 x 5 factorial scheme was used, where the first factor was the supplementation or not of wattle tannin (tannic acid) and the second factor was the increasing replacement levels (0, 25, 50, 75, 100%) of the limestone in the feed by golden mussel meal, with five replicates per treatment.

The golden mussel was collected at the Itaipu Binacional reservoir, in the municipality of Santa Helena – PR, by using floating net cages. The mussels were exposed to the sun for drying, then bagged and stored.

The golden mussels were milled using a hammer mill type and the following components of the samples of meal were determined: dry matter, crude protein, mineral matter (SILVA; QUEIROZ, 2006), chromium (Cr), cadmium (Cd), lead (Pb), calcium (Ca) and phosphorus (P). To quantify Cr, Cd, Pb and Ca, nitropercloric digestion (AOAC, 2005) and atomic absorption spectrometry modality flame (AAS-flame) were used. ultraviolet-visible spectroscopy (UV-Vis) was used to quantify P. Microbiological analysis for coliforms and Salmonella sp. in the golden mussel was performed in the Microbiology Laboratory, according to the methodology described by Silva et al. (1997). The diets were formulated to meet the nutritional requirements of the birds and 250 g ton-1 of wattle tannin was used; this quantity was determined in the first trial (Table 2).

Environmental variables were measured twice a day, in the morning and afternoon, and the points of maximum and minimum values were recorded using a digital thermo-hygrometer that was installed near the birds. During this experimental period, the average temperature ranged between 22.60°C and 25.00°C and the relative humidity ranged from 46.33% to 55.04%.

The birds and feed were weighed at the beginning and at the end of the experiment to assess WG, FI and FCR. At the end of the trial period, two birds per experimental unit were slaughtered to collect the breast, thigh, liver and kidneys. The breast and the thigh were deboned to obtain the breast meat and the tibia bone. The left tibia was weighed and the length measured using a digital caliper, and these measurements were used to calculate the Seedor Index. Bone strength was determined using CT3 texture analyzer equipment from Brookfield. The organs (liver and kidneys), the breast meat and the right tibia were dried in a forced ventilation oven at 55°C, milled and then chemically analyzed in order to quantify Ca, P, Cr, Cd and Pb using same methodology previously described for the analysis of these minerals in the mussel. The analysis of Ca and P were performed only for the tibia. The methodology described by Silva and Queiroz (2006) was used to determine dry matter and mineral matter.

Table 2. Experimental diets composition.

	Replacement level (%) of the limestone by golden mussel				
Ingredients (%)	0	25	50	75	100
Corn grain	53.51	53.46	53.36	53.24	53.13
Soybean meal	39.33	39.32	39.33	39.36	39.38
Degummed soy oil	2.500	2.506	2.540	2.582	2.620
Monocalcium phosphate	1.900	1.900	1.900	1.900	1.900
Limestone	0.912	0.684	0.456	0.228	0.000
Golden mussel	0.000	0.282	0.563	0.844	1.126
Salt	0.508	0.508	0.508	0.508	0.508
DL-methionine 99%	0.360	0.360	0.360	0.360	0.360
L-lysine.HCl 78%	0.272	0.273	0.272	0.272	0.272
L-threonine 99%	0.097	0.097	0.097	0.097	0.097
L – valine 99%	0.068	0.068	0.068	0.068	0.068
Vitamin suplement[1]	0.100	0.100	0.100	0.100	0.100
Mineral suplement[2]	0.050	0.050	0.050	0.050	0.050
Choline chloride 60%	0.060	0.060	0.060	0.060	0.060
Coxistac[3]	0.060	0.060	0.060	0.060	0.060
Antioxidant[4]	0.020	0.020	0.020	0.020	0.020
Stafac 500[5]	0.005	0.005	0.005	0.005	0.005
Inert (washed sand)	0.250	0.250	0.250	0.250	0.250
Total	100.00	100.00	100.00	100.00	100.00
	Calculated composition				
Metabolizable Energy (kcal/kg)	2961	2960	2960	2960	2960
Crude Protein (%)	22.62	22.62	22.62	22.62	22.62
Digestible lysine (%)	1.324	1.324	1.324	1.324	1.325
Digestible methyonine + cystin (%)	0.953	0.953	0.953	0.953	0.953
Digestible threonine (%)	0.861	0.861	0.861	0.861	0.861
Digestible valine (%)	1.020	1.020	1.020	1.020	1.020
Calcium (%)	0.920	0.920	0.920	0.920	0.920
Available phosphorous (%)	0.470	0.470	0.470	0.470	0.470
Sodium (%)	0.220	0.220	0.220	0.220	0.220

[1]ROVIMIX – Vitamin suplement for poultry. Content: Vit A (min) 9000000 UI; Vit D_3 (min) 2500000 UI; Vit E (min) 20000 UI; Vit K_3 (min) 2500 mg; Vit B_1 (min) 1500 mg; Vit B_2 (min) 6000 mg; Vit B_6 (min) 3000 mg; Pantotenic acid (min) 12 g; Niacin (min) 25 g; Folic acid (min) 800 mg; Se (min) 250 mg; [2]ROLIGOMIX – Mineral suplement for poultry. Content: Cu (min) 20g; Fe (min) 100g; Mn (min) 2000 mg; Zn (min) 100 g; [3] Salinomycin 12%; [4]BHT; [55]Virginamycin.

Performance results, presence of toxic metals and bone characteristics (bone strength and Seedor index) were statistically evaluated using analysis of variance to verify the effects of limestone replacement levels with golden mussel meal using polynomial regression at a 5% significance level, and to evaluate the effect of wattle tannin the F test was applied at a 5% significance level. Analyses were conducted using the Statistical and Genetics Analysis System – SAEG (UNIVERSIDADE FEDERAL VIÇOSA, UFV, 1999).

Results and Discussion

In the first experiment, 1- to 21-day old birds fed with 1000 and 1250 g ton-1 of wattle tannin showed significantly lower FI compared to the control diet (Dunett's test at 5% probability; Table 3). The effect of tannin level on FI was quadratic, with higher FI (1191.71 g) at the level of 367.21 g ton-1 of wattle tannin. These values were obtained through the derivation of adjusted regression equations for the variable.

Table 3. Performance of broilers from 1 to 21 days old fed different levels of tannin.

Tannin (g ton^{-1})	Feed intake (g)	Final weight (g)	Weight gain (g)	Feed conversion rate (g/g)
0 (control)	1160.34^{a}	802.07^{a}	759.89^{a}	1.527^{a}
250	1183.87^{a}	776.17^{b}	733.19^{b}	1.615^{b}
500	1195.70^{a}	747.67^{b}	705.33^{b}	1.695^{b}
750	1152.49^{a}	709.86^{b}	667.47^{b}	1.727^{b}
1000	1078.33^{b}	650.78^{b}	607.83^{b}	1.775^{b}
1250	990.30^{b}	620.40^{b}	578.08^{b}	1.713^{b}
LSD	37.05	20.79	20.61	0.039
CV (%)	2.444	2.153	2.269	1.732
			Probability	
Linear	<0.001	<0.001	<0.001	<0.001
Quadratic	<0.001	0.327	0.312	<0.001
	Regression equations		R^2	
Feed intake	$1156.17 + 0.193600X – 0.000263607X^2$		0.89	
Final weight	$823.500 – 0.163367X$		0.92	
Weight gain	$780.698 – 0.163091X$		0.92	
Feed conversion rate	$1.48774 + 0.000570719X – 0.000000307X^2$		0.71	

[a,b,c] Values followed by different letters in the same column differ from the control treatment at the 5% level of significance; LSD = Least significant difference; CV= Coefficient of variation (%).

At 21 days old, there was a linear relationship between final weight (FW) and WG. The inclusion of tannin had a significantly negative effect on both of these characteristics (Dunnett's test at 5% probability), resulting in decreased performance. Similarly, the FCR exhibited a quadratic response with maximum conversion (2.134) at the level of 929.51 g of tannin ton^{-1} of feed, also featuring a reduction in performance.

Torres et al. (2013) evaluated diets containing sorghum tannin and noted that replacing up to 100% of the low-tannin sorghum with corn did not influence the WG, FI or FCR in broiler chicks up to 21 days old. They also reported that a concentration of up to 2.6 g kg^{-1} of tannins in the diet did not significantly affect the performance of broiler chickens, but in this experiment, it was possible to note a reduction in broiler performance with the maximum tannin concentration of 0.9 g kg^{-1}.

Moyle et al. (2012) observed that when fed on increasing levels of Sericea lespedeza, a grass rich in tannin, poultry decreased in weight in the second and third week of life, and showed an increase in FI and consequently worsening FCR. This decline in performance is related to ingredients with low nutrient levels in the diet, such as tannins. They cause reductions in growth due to their toxicity and their negative impact on nitrogen retention and use of amino acids by the body, due to a decrease in protein digestibility.

The decline in performance may also be related to hypertrophy of the pancreas, which can change the production and levels of trypsinogen and alpha-amylase. This is turn influences the action of trypsin and alpha-amylase in the duodenum and jejunum and can cause liver hyperplasia, which impairs regulatory functions, such as detoxification of the body, bile production, synthesis, storage and breakdown of glycogen, cholesterol synthesis, and conversion of ammonia to urea (NELSON; COX, 2008).

The morphometric analysis of histological sections of the duodenum at 21 days old revealed no significant effect (P > 0.05) of tannin on villus height, crypt depth, or the relationship of villus:crypt (Table 4).

Table 4. Duodenum intestinal morphometry of broilers at 21 d-old fed diets with different inclusion levels of tannin.

Tannin (g ton^{-1})	Villus height (μm)	Crypt depth (μm)	Relation Villus height:Crypt depth
0	1364.00	115.93	12.19
250	1554.43	121.03	13.11
500	1577.13	125.14	13.02
750	1397.97	137.60	10.34
1000	1617.42	134.34	12.60
1250	1528.66	142.13	11.42
CV (%)	13.721	21.476	23.854
	Probability		
Linear	0.965	0.164	0.315
Quadratic	0.564	0.845	0.560

CV= Coefficient of variation (%).

Similar data were observed by Garcia et al. (2005), whereby there was no significant effect of replacing corn with high or low tannin sorghum on the length of the duodenum, jejunum or ileum. Torres et al. (2013) noted the same, also finding no significant effect on the cell growth of duodenal crypt, villus height or crypt depth in broiler chicks fed diets containing 0, 50 or 100% low-tannin sorghum in replacement of corn. On the other hand, these authors observed significant changes in the intestinal mucosa of broilers fed diets containing sorghum; namely increased mitotic index and loss of epithelium.

The integrity of the membrane and epithelial enzymes is essential to ensure the digestion and absorption of nutrients from the intestinal lumen. According to Gonçalves et al. (2014), the condensed tannins may decrease the absorption of nutrients through the intestinal wall due to metabolic phenomena that inhibit the action of enzymes present in the digestive system. Nyamambi et al. (2007) observed that villus height and crypt depth of the duodenum decreased with increasing tannin levels in the diet. Moreover, Torres et al. (2013) observed reduced aminopeptidase activity in the jejunum. This enzyme is responsible for almost all peptidase activity on the jejunum and ileum brush border. However, this reduction did not reduce the birds' performance.

The calcium level in the golden mussel (Table 5) was 30.55%, which was higher than that found by Almeida et al. (2006), who observed 15.91% Ca in mussel meal, but similar to that found by Canzi (2011), who observed mean values of 27.27% Ca and 28.70% Ca in whole mussels and mussel valves meal, respectively. The chemical composition of golden mussel is similar to other calcium sources, such as oyster meal. According Çath et al. (2012) oyster meal contained 38.90% calcium, and a study by Silva Fernandes and Peixoto (2000) reported values of 36.60% calcium.

Canzi (2011) assessed the use of golden mussel meal to fish and found that *Limnoperna fortunei* contained 12.95% crude protein and Almeida et al. (2006) reported values of 7.38% crude protein. These two values are higher than that found in this experiment, which was 0.50%. Even as the first author, the quantity of mineral matter was 80.53%, which was similar to the value of 87.60% found in this study, showing that this mollusk contains a high concentration of minerals. The value of potassium found by Canzi (2011) was 1.73%, which was close to the value of 1.63% observed in this study. For other minerals, there were discrepancies between the values found by Canzi (2011) and those in this study: phosphorus and zinc were found to have values of 0.11 and 15 mg g^{-1} respectively (CANZI, 2011), while in this experiment, observed values were 0.38 and 79 mg g^{-1}, respectively. This difference found in the mineral values was probably due to the environment from which these mollusks were collected.

Table 5. Chemical composition and levels of toxic metals of the golden mussel.

Dry matter (%)	Ash (%)	Crude protein (%)	Calcium (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)
98.11	87.60	0.50	30.55	0.38	1.63	0,37

Zinc (mg g^{-1})	Manganese (mg g^{-1})	Iron (mg g^{-1})	Cadmium (mg g^{-1})	Chromium (mg g^{-1})	Lead (mg g^{-1})
79.00	311.00	1520.00	<0.005	<0.001	0.46

The concentration of toxic metals such as cadmium and chromium in the golden mussel meal was within the limits allowed by Brazilian law. The lead concentration was the highest (0.46 mg g^{-1}), but still remained within the maximum permitted (2.00 mg g^{-1}). Although the concentrations were found to be within the limits allowed by law, the cumulative effect of toxic metals can be a problem when consumed in large quantities. The low concentrations of toxic metals in the golden mussel can be explained by the fact that the mussels were collected at the surface of the water column. According to Ferreira et al. (2010) and Marengoni et al. (2013), the concentrations of metals in sediments are greater than those found in the water column. However, these toxic metal particles are available to organisms such as the golden mussel, and some characteristics may facilitate this process such as particle size, type of metal ion, organic content and metal concentrations present in the water.

The quantity of coliforms was found to be lower than 0.4 MPN g^{-1}, which is within acceptable levels. No contamination by *Salmonella* sp. was found, indicating that the golden mussel meal was not microbiologically contaminated beyond the permitted limits. Some authors evaluated the presence of other possible microbiological contaminants in the golden mussel, finding values less than 1.0 CFU g^{-1} for enterobacteria and 2.4 x 10^{-6} CFU g^{-1} for mesophilic bacteria. Almeida et al. (2006) observed values less than 10 CFU g^{-1} for coliforms at 45°C and less than 10^2 CFU g^{-1} for *Salmonella* sp., however, the presence of *Staphylococcus aureus* was not found.

The performance results for chicks from 1 to 21 days old showed no interaction ($P < 0.05$) between the use of tannin and the level of replacement with golden mussel (Table 6). Golden mussel can be used as a source of calcium for broilers from 1 to 21 days old by replacing limestone in feed by up to 100% without affecting FI, WG, FCR and viability. For FW, WG, FI and FCR, there was no interaction between the calcium source substitution level and the diet inclusion of tannin ($P > 0.05$). However, analysis of variance indicated that in isolation, the tannin addition significantly decreased FW, WG, FI ($P < 0.05$), and consequently worsened the FCR ($P < 0.05$).

According to Melo and Moura (2009), higher solubility of Ca sources improves bioavailability and intestinal absorption of Ca. They also emphasize that organic sources of this mineral (such as seaweed, shells, bones and eggshell) present higher solubility compared to inorganic sources (MELO; MOURA, 2009). Despite this, inorganic sources are the most commonly used in animal feed because they are abundant and inexpensive, such as limestone and dicalcium phosphate. In this context, the performance of Ca from golden mussels in this experiment was satisfactory, indicating that the tested absorption rates were sufficient to meet the metabolic demands of birds at this stage. This hypothesis is supported by the fact that the birds possess the ability to regulate the use of Ca to meet their physiological needs (PINTO et al., 2010). Other factors studied here, such as FI, also suggest that the amount of calcium in the golden mussel was inline with the requirements of the animals, because according to Mello et al. (2012), a diet that is deficient in this mineral causes increased FI, whereas excess Ca could result in a reduction in FI, due to the low palatability of Ca.

Table 6. Performance of broilers from 1 to 21 days old fed diets with diferentes levels of replacement of limestone by golden mussel, with or without tannin addition.

Inclusion level	Final weight (g)		Weight gain (g)		Intake (g)		Feed conversion rate (g/g)	
	Tannin							
	No	Yes	No	Yes	No	Yes	No	Yes
0	840.56	814.58	794.51	768.73	1149.12	1141.29	1.446	1.485
25	881.59	814.22	835.61	768.12	1205.30	1140.11	1.443	1.484
50	833.33	809.90	787.38	764.02	1131.71	1123.54	1.437	1.471
75	869.58	815.42	823.58	769.28	1180.50	1124.00	1.433	1.461
100	830.80	820.42	784.71	774.27	1135.82	1133.54	1.448	1.464
Mean	851.17[a]	814.91[b]	805.16[a]	768.87[b]	1160.49[a]	1132.50[b]	1.442[a]	1.473[b]
	Probability							
Tannin	<0.01		<0.01		0.012		<0.01	
Golden mussel	0.140		0.141		0.091		0.211	
Interaction	0.121		0.118		0.188		0.617	
CV (%)	3.210		3.392		3.276		1.304	

[a,b] Values followed by different letters in the same line differs on F test at the 5% level of significance; CV= Coefficient of variation (%).

The use of wattle tannin at a concentration of 250 g ton^{-1} of ration caused a reduction in FI ($P < 0.05$), and hence a decrease in weight ($P < 0.05$). This reduction was probably due to the effect of tannin on digestibility, which then reduces the WG and negatively affects the FCR ($P < 0.05$). The causes of alterations in the performance of birds due to tannin in their diets include: decreased food palatability and voluntary intake, digestibility of proteins, carbohydrates, starch and lipids, and the inhibition of certain enzymes in the gastrointestinal tract, hindering the absorption of nutrients.

The values of the Seedor index and bone strength (kgf) of broiler chickens at 1 to 21 days old were not affected (P > 0.05) by the studied factors (Table 7). These results demonstrate that 100% inclusion of golden mussel can be used as a source of Ca for broilers from 1 to 21 days old without affecting their bone development. Differences in Seedor index and bone strength were not observed. This result allows us to affirm that the use of golden mussel and tannin in diets of broilers did not cause the bones to suffer calcification problems.

The main Ca reserve of chickens is in their bones, accounting for 98-99% of this mineral in the body (SAKOMURA et al., 2014). When Ca concentration in the blood decreases, Ca from bones is mobilized to raise the levels in the blood. This loss of Ca results in decalcification and weakening of bones. Other characteristics may demonstrate symptoms of calcium deficiency in birds, such as rickets, which produces characteristic swollen joints, enlargement of bone ends and rubberized beaks, due to poor deposition of Ca in these structures (SAKOMURA et al., 2014). According to these authors, osteomalacia is another indication of Ca deficiency that can compromise the development of older birds. However, in this study, none of these symptoms were observed.

Table 7. Seedor index and bone strength of broilers from 1 to 21 days old fed diets with differents levels of replacement of limestone by golden mussel, with or without tannin adition.

Golden mussel levels	Seedor index		Bone strength (kgf)	
	Tannin			
	Yes	No	Yes	No
0	54.60	50.67	12.70	12.99
25	53.27	49.77	12.63	12.60
50	51.75	54.55	13.16	13.74
75	49.98	48.08	13.49	12.61
100	52.02	49.97	14.72	14.32
Mean	52.32	50.61	13.34	13.25
CV (%)	10.005		19.352	
	Probability			
Tannin	0.100		0.863	
Golden mussel	0.109		0.159	
Interaction	0.258		0.909	

CV= Coefficient of variation (%).

The results of P, Ca and mineral matter in bones (Table 8) demonstrate that there was an interaction between factors (P < 0.05) for mineral matter. By breaking down the data and evaluating the effect of limestone replacement by golden mussel into isolated factors, it was observed that, regardless of the studied factors, there was no significant adjustment of the models. These results demonstrate that the availability of Ca and P in the diets did not affect the deposition of Ca, P and mineral matter in the bones of the birds in the initial phase.

Calcium homeostasis is influenced by the production of parathormone, calcitonin and renal hydrolase for the activation of cholecalciferol, which acts on intestinal absorption and metabolic utilization (NELSON; COX, 2008). According to Macari et al. (2002), this homeostatic mechanism has an important function in preventing excessive absorption or toxicity of Ca in birds. The calcium concentration of bones undergoes a dynamic process of mineralization due to remodeling and renewal processes in the plasma. The balancing of Ca levels in the plasma is fundamental because it can be absorbed and used for the growth of chickens, i.e. it is needed for satisfactory bone mineralization for structural development of the animals.

Incidence of toxic metals (Cd, Cr and Pb) was not detected in breast meat, tibia bones or organs (liver and kidneys), showing that the contamination by toxic metals in feed (derived from the golden mussel) was not high enough to transfer to the poultry meat. This is likely due to the low concentrations of these heavy metals found in the golden mussel, providing a satisfactory result for the consumption of these birds.

Table 8. Calcium and phosphorus levels in bones of broilers from 1 to 21 days old fed diets with differents levels of replacement of limestone by golden mussel, with or without tannin adition.

Golden mussel levels	Phosphorus (%)		Calcium (%)		Ash (%)	
	Tannin					
	Yes	No	Yes	No	Yes	No
0	2.27	2.30	17.79	17.90	44.94	46.35
25	2.28	2.28	18.00	18.26	47.08	45.94
50	2.28	2.29	18.13	18.27	46.81	46.30
75	2.29	2.26	19.14	18.38	46.81	47.79
100	2.24	2.27	17.67	18.16	46.29	48.00
Mean	2.27	2.28	18.15	18.19	46.39	46.88
CV (%)	2.843		13.397		3.092	
	Probability					
Tannin	0.504		0.921		0.092	
Golden mussel	0.434		0.779		0.004	
Interaction	0.621		0.943		0.007	

CV= Coefficient of variation (%).

Conclusions

The use of tannin to sequester heavy metals in the golden mussel adversely affects the performance of chicks. The golden mussel can be used as a source of Ca for broiler chicks from 1 to 21 days old. It does not alter the development, intestinal morphology or the bones of the birds, and the use of this ingredient does not contaminate broiler chickens with toxic heavy metals.

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