South African Journal of Animal Science 2023, 53 (No. 3)

Golden mussel shell (*Limnoperna fortunei*) flour contaminated with cadmium as a calcium source for broiler chickens

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(Submitted 12 September 2022; Accepted 29 March 2023; Published 24 July 2023)

Abstract

The objective of this study was to evaluate the effects of golden mussel flour (GMSF) contaminated with increasing rates of cadmium (Cd) as a replacement for limestone as a Ca source, in broiler chicken feed from 14 to 42 days of age. A total of 60 animals were assigned to four treatments (inclusion rates of Cd: 6.94, 14.55, 22.40, and 30.00 mg Cd kg^{-1}) with five replications in a completely randomized design. At 42 d, blood samples were collected to evaluate serum concentrations of aspartate amino transferase (AST), alanine amino transferase (ALT), gamma glutamyl transferase (GGT), total bilirubin, Ca, P, and Cd. After slaughter, tissues were collected to evaluate Cd concentration in bone parameters. Growth performance of broiler chickens and Cd content in the breast meat were not affected by the inclusion rates of Cd in the GMSF. However, there was an effect of Cd in GMSF on the concentration of Cd in the skin, liver, bones, feathers, and serum; ALT; and total bilirubin. Bone flexibility had a quadratic response to increasing inclusion rates of GMSF; serum Ca concentration increased linearly and there was no effect on serum P concentration. Concentrations of Cd in GMSF above 20 mg kg^{-1} caused high Cd contamination in broiler tissues. Therefore, it was concluded that Cd concentrations above 6.94 mg kg^{-1} in broiler diets caused high Cd concentrations in meat and organs that are above those permitted for human consumption.

Keywords: bone quality, Cd digestibility, serum
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Introduction

The demand for animal protein by the population places constant pressure on the meat production sectors, such as the poultry sector (Xu *et al*., 2015; Bayerle *et al*., 2017). However, this increase in the production of broilers results in an increased need for raw materials to feed the animals. This requirement has driven the research for new food sources that replace or can be used as effective ingredients within the nutritional programs. In this context, the by-products of the food industries become the main alternatives and sources for this purpose (Nunes *et al*.,2018).

The use of by-products in animal diets can reduce the incorrect disposal of these products into the environment (Kar & Patra, 2021). When used in broiler diets, these alternative ingredients can reduce production costs, resulting in more economically viable and nutritionally productive feed (Nunes *et al*., 2018; Olgun *et al*., 2020).

Golden mussel flour can be an economic, nutritional, and environmentally acceptable alternative, especially in regions where its occurrence is unwanted and its acquisition is low-cost. The use of the golden mussel (*Limnoperna fortunei*) as a source of Ca in animal feed is based on its low cost and the fact that this bivalve mollusc is an invasive, exotic species that causes economic and environmental damage (Wachholz *et al*., 2017). Thus, its use in the animal industry would reduce environmental problems of obstruction of the passage of water in pipes, channels and collecting systems of water, filters, pumps, and refrigeration systems (Akter *et al*., 2019).

However, the main obstacle to using this mollusc in animal feeding is the filtering habit of this species, which can lead to the absorption of pollutants from the waters where it lives. Among these pollutants are toxic metals, including cadmium (Cd), which is an environmental pollutant (Marengoni *et*

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v53i3.13

al., 2013), hazardous to humans and animals (Olgun *et al*., 2015; Zwolak, 2020) with potential carcinogenic effects. Cadmium exposure results in bioaccumulation of this element in different tissues, particularly in the liver, kidney, brain, pancreas, intestine, and reproductive organs, which increases oxidative stress at cellular levels due to the overproduction of reactive oxygen species (Zhang *et al*., 2018). These phenomena induce dysfunctions of biologically important cellular molecules, resulting in various gross and histopathological changes in these organs and haemato-biochemical alterations. Consequently, reductions in growth performance can occur in poultry (Kar *et al*., 2018; Akter *et al*., 2019; Olgun et al., 2020). Exposure of livestock including poultry to Cd not only affects health, but also hampers animal production by reducing growth performance and feed efficiency (Olgun *et al*., 2020). Studies have shown that Cd has negative effects on growth performance (Al-Waeli *et al*., 2013; Olgun & Bahtiyarca 2015), while others reported positive effects using low dietary levels (Cigankova *et al*., 2009; Olgun, 2015). The blood profile can also be negatively affected by Cd. Some research has reported liver lesions (Cinar *et al*., 2010; Ali *et al*., 2016; Tahir *et al*., 2017; Gutyj *et al*., 2019) and changes in plasma concentrations of alanine aminotransferase (ALT), triglycerides, and glucose (Ali & Abdulla 2013; Kar & Patra, 2021). Age and species of bird are also factors that can cause variations in the level of ingested Cd, which is toxic when absorbed by the organism. In addition, Cd uptake toxicity can vary according to cell type in the kidneys and liver (Li *et al*., 2018; Olgun *et al*., 2020). Nad *et al*. (2012) evaluated Cd absorption in turkeys and observed an intense accumulation of Cd in bones, muscles, liver, and kidneys. Similar results have been reported by other studies (Ali *et al*., 2016; Jin *et al*., 2018; Kar *et al*., 2018; Vasiljeva *et al*., 2018), suggesting that liver and kidneys are more sensitive to Cd accumulation.

Thus, it is necessary to conduct studies to verify the amount of Cd in golden mussel shells in broiler chickens can utilize without affecting performance or causing tissue contamination (Wachholz *et al*., 2017). In addition, these studies may be useful to determine the potential risks of this heavy metal, as well as its accumulation in tissues, to provide information to local regulatory authorities for future recommendations of maximum allowable values (Khan *et al*., 2016, Huang *et al*., 2019, Jawad *et al*., 2021).

The objective of this study was to evaluate the use of golden mussel shell flour contaminated with different inclusions of Cd in the diet of broiler chickens from 14 to 42 days of age and determine its effects on growth performance, tissue contamination, Cd digestibility, bone quality, and blood metabolites.

Materials and Methods

This study was conducted at the Poultry Research Centre of Western Paraná State University (Marechal Cândido Rondon, PR, BR). All the procedures were performed according to the National Council for Control and Animal Experimentation and approved by the Animal Use Ethics Committee of the university under the protocol number 04/2017.

Golden mussel shells were collected on the banks of the reservoir from ITAIPU Hydroelectric Power Plant, (Marechal Cândido Rondon - PR, BR), dried by sun exposure, and milled in a hammer mill (4-mm sieve). Golden mussel shell flour (GMSF) was sampled and analysed for the contents of Cd, P, and Ca using atomic absorption spectrometry and its composition was 6.94 mg of Cd kg^{-1}, 3.98 mg P kg^{-1}, and 30.64 mg Ca kg^{-1}.

From days 1 to 14, broiler chickens were raised in an aviary with concrete floor covered with pine wood shavings and received a common initial ration formulated according to the recommendations of Rostagno *et al*. (2011). A total of 60 one-day old male broiler chickens (Cobb 500, Cobb-Vantress Ltd., Cascavel, PR, BR) vaccinated for Marek, Gumboro, fowl pox, and infectious bronchitis, with an initial mean weight of 393.95 ± 14.12 g, were housed in cages of 50 cm^2, with three broiler chickens per experimental unit (cage). The experimental design was completely randomized, with four treatments (inclusion rates of Cd in the flour of mussel shells) and five replications of three broiler chickens per treatment. Mussel shells were used as a Ca source and the concentrations of Cd above 6.94 mg kg^{-1} were obtained by adding Cd nitrate [$Cd(NO_3)^2.4(H_2O)$] to the GMSF. The four treatments were increasing inclusions of Cd in GMSF: 6.94 mg Cd kg^{-1} of GMSF (without induced contamination), 14.55 mg Cd kg^{-1} of GMSF, 22.40 mg Cd kg^{-1} of GMSF, and 30 mg Cd kg^{-1} of GMSF in the starter (14 to 28 d) and grower phases (29 to 42 d). Two experimental diets were used according to the rearing phase of broiler chickens (grower and finisher phases), and the GMSF was the main Ca source in the diets. Broiler chickens received diets to meet the nutritional requirements proposed by Rostagno *et al*. (2011) (Table 1) for males during the grower (14 to 28 d) and finisher (29 to 42 d) phases, with feed and water provided *ad libitum* throughout the experiment.

Table 1 Composition and nutritional values of experimental diets

Item	Composition (% as-fed)	
	Grower phase (d 14 to 28)	Finisher phase (d 29 to 42)
Corn grain	55.608	57.152
Soybean meal	36.498	33.028
Soy oil	3.8	4.854
Monodicalcium Phosphate	1.528	1.653
Golden mussel shells flour	1.185	1.096
NaCl	0.483	0.478
DL-Met (99%)	0.307	0.252
L-Lys (54.6%)	0.338	0.266
L-Thr (98%)	0.068	0.036
Vitamin premix[1]	0.01	0.01
Mineral premix[2]	0.05	0.05
Choline chloride (60%)	0.06	0.06
Antioxidant[3]	0.01	0.01
Avilamycin	0.005	0.005
Salinomycin[4]	0.05	0.05
Celite	0	1.000
Calculated composition		
Metabolizable energy (kcal/kg)	3.050	3.150
Crude protein (%)	21.182	19.78
Digestible Lys (%)	1.217	1.099
Digestible met + cys (%)	0.876	0.791
Thr	0.897	0.714
Trp	0.273	0.222
Ca (%)	0.841	0.837
Available P (%)	0.401	0.418
Na (%)	0.21	0.208
K (%)	0.83	0.776

[1]Vitamin supplement (g/kg of diet): Vit. A 3.240 mg, Vit. D3 120 mg, vit. E 24.1 mg, vit. K3 3.6 mg, vit. B1 2.4 mg, vit. B2 8.4 mg, vit. B6 4.8 mg, vit. B12 18 mg, pantothenic acid 0.014 mg, niacin 0.025 g, folic acid 0.8 mg, biotin 0.06 mg, selenium 0.25 mg
[2]Mineral supplement (g/kg of diet): copper 0.09 g, iron 0.06 g, manganese 0.04 g, cobalt 1 mg, iodine 1 mg, zinc 0.17 g; [3] butylated hydroxytoluene (BHT); [5]salinomycin 4%

Weight gain (WG), feed intake (FI), and feed conversion ratio (FCR) were recorded from day 1 to day 42. Mean individual bird weight and FI were calculated, taking mortalities into consideration (Sakomura & Rostagno, 2016). At 42 days, two birds per pen were randomly selected, fasted for six hours, and blood samples were collected via brachial puncture. Blood was coagulated and centrifuged at 1008 × *g* for 10 min to obtain serum, which was stored at -20 °C. To perform the analyses, serum was thawed at room temperature, centrifuged at 1008 × *g* for 5 min, and then aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), total bilirubin, Ca, and P analyses were performed with a high-performance automatic spectrophotometer (Flexor EL 200, Elitech, Paris, France) with specific kits, calibrated with the appropriate standards (Elical, Elitech, Paris, France). After blood collection, three birds were weighed and euthanized by electrocution followed by exsanguination (Normative Resolution No. 37 of February 15, 2018, Concea) for skin collection (around the chest), muscle (pectoralis major), breast, feathers, bones (femur), and liver to determine the Cd content.

Total excreta collection (Sibbald & Slinger, 1963) was performed from 38–42 d twice a day with an interval of 12 h to minimize fermentation. During this collection period, trays were covered with plastic and placed under each cage to avoid losses and contamination. The material collected was packed in plastic bags according to the experimental unit, duly weighed, and stored in a freezer (-20 °C) until analysis.

For bone quality evaluations, meat from the tibia was removed and thereafter, the stiffness and bone-breaking strength were measured using a CT3 texture analyser (CT3 Texture Analyzer, Brookfield Engineering Laboratories, Inc., Middleboro, MA, US) by applying 200 kgf at a speed of 5 mm/s in the central bone region (diaphysis); the values were expressed as mm and kgf. Bone flexibility is expressed in kgf/cm and calculated from breaking strength and stiffness.

The concentration of Cd in feed and excreta (mg kg^{-1}) were determined using an indigestibility factor (IF), which was obtained from the acid insoluble ash (AIA). Apparent metabolizable Cd (AMCd) was obtained according to Sakomura & Rostagno (2007) and the apparent digestibility coefficient of Cd (ADCCd) was adapted from Rostagno & Featherston (1977). Data were expressed on a dry matter basis and calculated according to the following formulae:

$$AMCd = (Cd\ in\ feed - Excreted\ Cd) \times IF, \quad (1)$$

where: IF = AIA from diet/AIA from excreta.

$$ADCCd = (\left(Cd\ from\ feed - \frac{fecal\ Cd \times IF}{Cd}\ from\ feed\right) \times 100. \quad (2)$$

Data were subjected to analysis of variance at 5% probability level and subsequent polynomial regression for the inclusion rates of GMSF. Statistical analyses were performed using SAS software (SAS Inst. Inc., Cary, NC, US) in a completely randomized design, considering the cages as the experimental units.

Results and Discussion

The contamination with cadmium nitrate – $Cd(NO_3)^2$ – in the GMSF was not enough to cause toxicity to the animals, nor reduce its growth performance (Table 2). This result was probably related to the low inclusion of Cd in the GMSF.

Table 2 Growth performance of broiler chickens fed with the inclusion of golden mussel shells flour (GMSF) contaminated with increasing inclusions of Cd

Cd (mg kg^{-1})	days 14 to 42			
	LW[1] (kg)	WG[2] (kg)	FI[3] (kg)	FCR[4] (g/g)
6.94	2.677	2.263	3.834	1.695
14.55	2.784	2.319	3.867	1.668
22.4	2.626	2.232	3.796	1.702
30	2.648	2.240	3.814	1.703
SEM[5]	29.060	13.884	24.221	0.012
P-value	0.380	0.173	0.287	0.753

[1]LW (Live weight); [2]WG (Weight gain); [3]FI (Feed intake); [4]FCR (Feed conversion ratio); [5]SEM (Standard error of mean)

In the study by Olgun *et al*. (2020), it was observed that cadmium concentrations of 10–20% provide toxicity and reduce the performance of cutting chickens up to 45 days of age. Lisunova & Tokarev (2016) observed that the inclusion of 0.002 mg Cd kg^{-1} in diets was sufficient to cause a 6.2% reduction in weight gain in cutting chickens up to 49 days of age.

In the study of Al-Waeli *et al*. (2013) with broiler chickens fed diets with 10 mg kg^{-1} Cd, negative effects on growth performance and mortality were not observed. However, when 100 mg kg^{-1} Cd per unit body mass was offered, feed intake decreased and feed conversion increased. These observations suggest that Cd, besides causing metabolic and pathological changes in different organs, can directly impair nutrient digestion and absorption in the gut, resulting in poor feed conversion ratio and production performance of poultry (Kar & Patra, 2021). There were no effects of Cd inclusion on its concentration in the pectoralis major muscle (Table 3). However, the Cd concentration used for all the diets evaluated was above the recommended by FAO (2000) (0.05 mg kg^{-1}).

Studies have shown that Cd accumulates preferentially in the inner organs and in the liver and kidneys (Kurnaz & Filazi, 2011; Karimi *et al*., 2017). However, in the present study, a high Cd concentration was also found in the skin. Cadmium concentration was higher in serum (2.95 mg kg^{-1}) followed by bone, feathers, skin, liver, and muscle. Zhuang *et al*. (2014) evaluated broiler chickens fed with metal-enriched rice (0.24 mg Cd kg^{-1}) and obtained a higher content of Cd in the liver (9.36 mg kg^{-1}) followed by kidney, feather, muscle, and blood (4.64, 0.51, 0.059, and 0.042 mg kg^{-1} of dry weight, respectively). Cinar *et al*. (2010) evaluated lower doses of Cd, and no such remarkable changes were found in chicks. However, severe hydropic degeneration was reported in liver tissues with Cd administration (60 mg kg^{-1}) in a basal diet of broiler chickens for 42 d.

Table 3 Cadmium concentrations in different tissues of broiler chickens fed gold mussel shell flour contaminated with increasing inclusions of Cd

Cd (mg kg^{-1})	Cd concentration (mg kg^{-1}, wet weight basis)					
	Pectoralis major	Skin	Liver	Bone	Feathers	Serum
6.94	0.531	1.301	0.762	1.911	1.404	1.602
14.55	0.742	2.562	1.553	2.312	2.714	3.431
22.4	0.742	2.574	1.552	2.974	2.982	3.442
30	1.032	2.732	1.191	2.984	2.413	3.432
[1]SEM	0.072	0.221	0.142	0.224	0.243	0.332
P-value	0.160	<0.01(Q)	<0.01(Q)	<0.01(Q)	<0.01(Q)	<0.01(Q)
		Regression Equations				R^2
Cd in the skin =		0.297995 + 0.283476Cd – 0.006653Cd^2				0.99
Cd in the liver =		-0.360925 + 0.198602Cd – 0.004934Cd^2				0.98
Cd in the bone =		0.538293 + 0.252988Cd – 0.005837Cd^2				0.81
Cd in the feathers =		-0.539816 + 0.336893Cd – 0.007962Cd^2				0.99
Cd in the serum =		-0.386185 + 0.352766Cd – 0.007656Cd^2				0.93

[1]SEM (Standard error of mean)

Singh *et al*. (2016) showed that daily administration of Cd (50 mg L^{-1} in drinking water) for 45 d to broiler chickens induced degenerative changes in the hepatocytes; increases in sinusoidal spaces; and swollen, fragile, and focal necrotic spots in livers. Gabol *et al*. (2014) investigated the effect of Cd at various doses (low dose of 10 µg kg^{-1} body weight and a higher dose of 20 µg kg^{-1} body weight) and observed that the higher doses of Cd caused increased hepatic cell size, damage and necrosis of the hepatic cells, and infiltrations of numerous macrophages in the liver. In addition, cadmium induces alterations in normal liver function, causing a wide range of alterations in blood profiles (Akter *et al*., 2019; Ali *et al*., 2021; Jawad *et al*., 2021; Kar & Patra, 2021). Gutyj *et al*. (2019) reported that Cd (2 and 4 mg kg^{-1} body weight) for white laying hens (78 weeks of age) for 30 d resulted in deviations from normal values of blood biochemical profile due to pathological changes in the organs. As Cd causes extensive pathological changes in different vital organs, particularly in the liver, kidney, lungs, and reproductive organs, these alterations are reflected in the blood metabolites.

The mean values for AST activity (184.06 U L^{-1}) and GGT (11.88 U L^{-1}) were within the normal range for broiler chickens at 42 d, as described by Borsa *et al*. (2006) (17–24 U L^{-1} and 251.6 U L^{-1}, respectively) (Table 4).

Table 4 Blood metabolites of broiler chickens fed with golden mussel shell flour contaminated with increasing inclusions of Cd

Cd (mg kg^{-1})	[1]AST (U L^{-1})	[2]ALT (U L^{-1})	[3]GGT (IU L^{-1})	Total Bilirubin (mg dL^{-1})
6.94	176.653	9.223	10.203	0.323
14.55	187.452	5.883	12.452	0.292
22.4	186.321	5.454	12.223	0.542
30	185.832	8.643	12.643	0.692
[4]SEM	1.812	0.854	0.413	0.072
P-value	0.985	<0.01(Q)	0.532	<0.01(L)
		Regression Equations		R^2
ALT =		15.6 – 8.16Cd + 1.60Cd^2		0.99
Total Bilirubin =		0.1030 + 0.4110Cd		0.88

[1]AST: aspartate aminotransferase, [2]ALT: alanine aminotransferase; [3]GGT: gamma glutamyl transferase, SEM: standard error of mean

The minimum estimated value of ALT (5.454 mg Cd kg^{-1}) observed in the present study corroborates the value reported by Adaramoye & Akanni (2016) in a study with rats, where Cd contamination caused changes in the ALT activity. When the circulatory Cd is greater than the binding capacity of metallothionein, the free Cd induces free radicals and lipid peroxidases and can damage kidneys and liver (Gałazyn-Sidorczuk *et al*., 2009; Heshmati & Salaramoli, 2015; Korénekova *et al*.,

2017; Olgun *et al*., 2020). An increased Cd concentration in serum may be related to the higher activity of the enzyme, ALT, at higher inclusions, since Cd can induce injuries in the liver and kidney due to its ability to enhance free radical formation *in vivo* (Abdo & Abdulla, 2013; Gutyj *et al*., 2019).

Ali *et al*. (2016) used 10 mg Cd kg^{-1} of diet for 4 w in the diet of broiler chickens and found an increased activity of ALT and AST. When compared to the control group, the authors attributed this effect to hepatocellular damage or cellular degradation by this heavy metal, possibly in the liver, but in the heart or muscle. Karimi *et al*. (2017) evaluated Cd in the diets of Japanese quail with 100 mg kg^{-1} for 60 d and obtained an increase in ALT activity. Similarly, the same group of researchers in two experiments with male Japanese quail reported that administration of Cd at 25 mg kg^{-1} of feed (in both experiments) increased total serum protein and ALT activity (Karimi *et al*., 2015; 2016). Corroborating our results, it is clear that Cd increases liver enzyme activities. The increase in serum bilirubin is associated with fat digestion and absorption (Andjelkovic *et al*., 2019). Silva *et al*. (2007) obtained total bilirubin values of 0.41 mg dL^{-1}, similar to those obtained in the present study (0.45 mg dl^{-1}).

According to Oliveira *et al*. (2014), resistance and flexibility are the most important factors in cases of bone fractures. Liao *et al*. (2017) evaluated Cd in the diet of ducks and obtained a reduction in bone quality; studies evaluating Cd contamination in humans also found a reduction in bone quality (Chen *et al*., 2014; Birr *et al*., 2015). The serum concentration of P was not influenced by the inclusion of GMSF contaminated with Cd in the diet (Table 5). However, the concentration of Ca increased linearly with Cd inclusion in the diet.

Table 5 Serum concentrations of Ca and P of broiler chickens fed with golden mussel shell flour contaminated with increasing inclusion rates of Cd.

Cd (mg kg^{-1})	Ca (mg dL^{-1})	P (mg dL^{-1})
6.94	4.442	3.650
14.55	4.662	3.263
22.4	5.042	3.863
30	5.962	3.852
SEM	0.245	0.122
P-value	0.040(L)	0.610
	Regression equations	R^2
Ca =	3.8441079 + 0.064091 Cd	0.91

SEM: standard error of mean

Cadmium can affect bone health, reducing the absorption of Ca from the intestines, increasing its excretion from kidneys, and preventing Ca incorporation and collagen production in bone cells (Wachholz *et al*., 2019). In addition, minerals such as P and Mg are affected by Cd in animals (Olgun *et al*., 2015) and can affect the biomechanical properties of bone, such as resistance to breaking (Olgun *et al*., 2020). In the present study, only flexibility was influenced (quadratically), and the values obtained with the increased inclusion rates of Cd were above those obtained for the basal diet. The bone parameters, deformation and breaking strength were not altered by the inclusion of Cd in the GMSF (Table 6). Corroborating that, the study of Olgun *et al*. (2015) evaluated increasing inclusion rates of Cd (5, 15, and 45 mg kg-¹) for laying hens and found no effects on Ca, P, and Zn content in the tibia of laying hens. However, the authors observed that higher doses of cadmium (>10 mg kg^{-1}) lead to toxicity symptoms, worsening productive performance in poultry.

Table 6 Deformation, resistance, and flexibility of bones in broilers fed diets containing golden mussel shell flour contaminated with increasing inclusions of Cd

Cd (mg kg^{-1})	Deformation (mm)	Breaking strength (Kgf)	Flexibility (Kgf mm^{-1})
6.94	3.972	8.692	21.013
14.55	3.673	9.332	24.572
22.4	3.923	9.072	23.382
30	4.073	8.333	22.142
SEM	0.062	0.172	0.632
P-value	0.081	0.192	<0.01(Q)
	Regression equations		R^2
Flexibility =	18.806977 + 0.781672 Cd – 0.020397 Cd^2		0.84

SEM (standard error of mean)

The apparent metabolizable Cd increased linearly (*P* <0.05) as well as the coefficient of apparent digestibility (CAD) of Cd (P<0.05) (Table 7).

Table 7 Apparent metabolizable Cd (AM Cd), coefficient of apparent digestible Cd (CAD Cd) in broiler chickens fed with golden mussel shell flour contaminated with Cd

Cd (mg kg^{-1})	Digestibility of Cd	
	AM Cd (mg kg^{-1})	CAD Cd (%)
6.94	1.132	41.884
14.55	3.122	67.914
22.4	3.883	73.824
30	4.584	78.672
SEM	0.534	5.922
P-value	<0.01(L)	<0.01(L)
	Regression equations	R^2
AM Cd =	0.510976 + 0.144216 Cd	0.93
CAD Cd =	31.711855 + 1.50806 Cd	0.84

SEM (Standard Error of Mean).

Apparent metabolizable Cd increased (AMCd) and its metabolizable coefficient increased with Cd inclusion. After absorption, this metal is excreted in urine, faeces, and bile, but has a low excretion rate (Suttle, 2010). According to ATSDR (2007), the estimated absorption rate of Cd by oral exposure for humans may vary from 1.1–10.6%. Therefore, a tolerable monthly Cd intake of 25 µg kg^{-1} body weight (Chen *et al*., 2021) may occur in some industrial areas where human inhabitants may be vulnerable to Cd exposure through foods. In this context, with advances in Brazilian poultry farming, concern about the biosafety of ingredients used in the diets of farm animals has increased in recent years (Bayerle *et al*., 2017). Contamination of poultry feed is a problem and the scientific community strives to find solutions to increase food quality and safety (Wachholz *et al*., 2019). Heavy metals such as Cd are toxic agents involved in several disorders of animals and humans and limits in rations must be constantly updated (Wolf & Cappai, 2021). Distribution of Cd driven from feed in target organs is a key for estimating health risk from this exposure and determines the safety of animal products. In general, Cd can influence the absorption of Ca, as Cd uses the same transporters as Ca in animals (Reeves & Chaney, 2008). However, it is not possible to completely eliminate the damage in the liver and kidney and the injuries caused by this exposure (Akter *et al*., 2019; Gutyj *et al*., 2019; Ali *et al*., 2021; Kar & Patra, 2021). This occurs even on a small scale, as this contaminant is highly absorbed by animals and can cause economic losses (Chen *et al*., 2021).

Therefore, it is concluded that the use of golden mussels as a source of Ca contaminated with up to 30 mg kg^{-1} of Cd does not affect broiler chicken growth performance. However, Cd concentrations above 6.94 mg kg^{-1} in broiler chicken diets cause high Cd concentrations in meat and organs, which are above the levels permitted for human consumption.

Acknowledgements
The authors acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) for financial support for the PhD. scholarship for the first author - Finance Code 001.

Author contributions
Cristine Kaufmann, Edinan Hagdon Cirilo, Jomara Broch, Lucas Wachholz, Gabrieli Toniazzo, and Paulo Levi de Oliveira Carvalho: investigation, methodology, data curation, formal analysis, software, and project administration; Ricardo Vianna Nunes: conceptualization, methodology, and project administration, supervision, validation, and visualization; Cinthia Eyng and Cleison de Souza: roles/writing - original draft; André Sanches de Avila and Thiago dos Santos Andrade: writing - review and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of interest
The authors declare no competing interests.

Ethics approval
The protocol of this research was in accordance with the Brazilian Normative Act No. 37, from April 07, 2017, by the National Animal Experimentation Control Board.

References

Abdo, K.S.A. & Abdulla H., 2013. Effect of cadmium in drinking water on growth, some hematological and biochemical parameters of chicken. Eur. J. Exp. Biol., 3, 287–291. https://www.researchgate.net/publication/263393784_Effect_of_cadmium_in_drinking_water_on_growth_some_haematological_and_biochemical_parameters_of_chicken; accessed 10 March 2022

Adaramoye, O.A. & Akanni, O.O., 2016. Modulatory effects of methanol extract of *Artocarpus altilis* (Moraceae) on cadmium-induced hepatic and renal toxicity in male Wistar rats. Pathophysiology, 846, 1-8. https://doi.org/10.1016/j.pathophys.2015.07.003

Akter, M.T., Ferdous, K. A., Rahaman, T, Hassan, M.A. & Monjur, T., 2019. Exposure to environmental heavy metal (cadmium) through feed and its effect on bio-histomorphological changes in commercial quail. J. Entomol. Zool. Stud., 7,965–971. https://www.researchgate.net/publication/337339555_Exposure_to_environmental_heavy_metal_cadmium_through_feed_and_its_effect_on_bio-histomorphological_changes_in_commercial_quail; accessed 10 March 2022.

Al-Waeli, A., Zoidis, E., Pappas, A.C., Demiris, N., Zervas, G. & Fegeros, F., 2013. The role of organic selenium in cadmium toxicity: Eeffects on broiler performance and health status. Animal, 7, 386-393. https://doi.org/10.1017/S1751731112001590.

Ali, K.A., Lakal, M.A. & Obad, K., 2016. Changes in some biochemical parameters and body weight of chicken exposed to cadmium. World J. Pharm. Res., 5, 227–234. https://www.researchgate.net/publication/296443573_CHANGES_IN_SOME_BIOCHEMICAL_PARAMETERS_AND_BODY_WEIGHT_OF_CHICKEN_EXPOSED_TO_CADMIUM; accessed 10 March 2022

Ali, S., Bashir, S., Mumtaz, S., Shakir, H.A., Ara, C., Ahmad, F., Tahir, H.M., Faheem, M, Irfan, M., Masih, A, Ulhaq, M. & Andleeb, S., 2021. Evaluation of cadmium chloride-induced toxicity in chicks via hematological, biochemical parameters, and cadmium level in tissues. Biol. Trace Elem. Res, 9, 3457-3469. https://doi.org/10.1007/s12011-020-02453-9

Ali, K. S. & Abdulla, Huda., 2013. Effect of cadmium in drinking water on growth, some haematological and biochemical parameters of chicken. Eur. J. Exp. Biol., 3, 287-291. https://www.researchgate.net/publication/263393784_Effect_of_cadmium_in_drinking_water_on_growth_some_haematological_and_biochemical_parameters_of_chicken; accessed 10 March 2022

Andjelkovic, M., Buha-Djordjevic, A., Antonijevic, E., Antonijevic, B., Stanic, M., Kotur-Stevuljevic, J., Spasojevic-Kalimanovska, V., Jovanovic, M., Boricic, N., Wallace, D.& Bulat, Z., 2019. Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney. Int. J. Environ. Res. Public Health, 2, 273-274. https://doi.org/10.3390/ijerph16020274

Bayerle, D.F., Nunes, R.V., Gonçalves Junior, A.C., Wachholz, L., Scherer, C., Silva, I. M., Bruxel, T. M.O. & Vargas Jr, J.G., 2017. Golden mussel (*Limnoperna fortunei*) in feed for broiler chicks using tannin as a sequestrant of toxic metals. Semin. Ciencias Agrarias, 38, 843-854. http://dx.doi.org/10.5433/1679-0359.2017v38n2p843

Birr, E., Ho, M. & Kwon, H., 2015. Association between blood cadmium level and bone mineral density reduction modified by renal function in young and middle-aged men. J. Trace Elem. Med. Biol., 32, 60-65. https://doi.org/10.1016/j.jtemb.2015.06.002

Borsa, A., Kohayagawa, A., Boretti, L.P., Saito, M.E. & Kuibida, K., 2006. Serum levels of hepatic enzyme function in clinically healthy broiler chickens. Braz. J. Vet. Res. Anim. Sci., 58, 675-677. https://www.researchgate.net/publication/262704595_Serum_levels_of_hepatic_enzyme_function_in_clinically_healthy_broiler_chickens; accessed 10 March 2022

Chen, Z., Shi, K., Kuang, W. & Huang, L., 2021. Exploration of the optimal strategy for dietary calcium intervention against the toxicity of liver and kidney induced by cadmium in mice: An *in vivo* diet intervention study. PLoS ONE, 5, e0250885. https://doi.org/10.1371%2Fjournal.pone.0250885

Chen, X., Wang, K., Wang, Z., Gan, C., Ele, P., Liang, Y., Jin, T. & Zhu, G., 2014. Effects of lead and cadmium co-exposure on bone mineral density in a Chinese population. Bone, 63: 76-80. https://doi.org/10.1016/j.bone.2014.02.017

Cigankova V, Almasiova, V. & Holovska, K., 2009. Morphological changes in duodenal epithelium of Japanese quail after chronic cadmium exposure. Pol. J. Environ. Stud., 19, 275–282. http://www.pjoes.com/pdf-88381-22239?filename=Morphological%20Changes%20in.pdf; accessed 10 March 2022.

Cinar, M., Yigit, A.A. & Eraslan, G. 2010. Effects of vitamin C or vitamin E supplementation on cadmium induced oxidative stress and anaemia in broilers. Revue de Medecine Veterinaire, 161, 449–454. https://www.cabdirect.org/cabdirect/abstract/20103370669; accessed 10 March 2022.

European Commission. Directive n 2005/87/Ce of Commission of December. Official Journal of European Union, l318/19–l318/24. http://data.europa.eu/eli/dir/2005/87/oj

FAO Agricultural Data (2000) Food and Agriculture Organization of the United Nations (http://apps.fao.org/default.htm)

Gabol, K., Khan, Z.M., Umair, A.K.M., Khan, P., Fatima, F. & Siddiqui, S., 2014. Induced effects of lead, chromium, and cadmium on morphological changes in duodenal epithelium of Japanese quail after chronic cadmium exposure. *Gallus domesticus*. Can J. of Pure & Appl. Sci., 8, 3035–3042. http://www.pjoes.com/Morphological-Changes-in-Duodenal-Epithelium-of-Japanese-Quail-after-Chronic-Cadmium,88381,0,2.html; accessed 12 September 2022.

Gałazyn-Sidorczuk, M., Brzóska, M.M., Jurczuk, M. & Moniuszko-Jakoniuk, J. 2009. Oxidative damage to proteins and DNA in rats exposed to cadmium and/or ethanol. Chem. Biol. Interact., 180, 8-30. https://doi.org/10.1016/j.cbi.2009.01.014

Gutyj, B.V., Ostapyuk, A.Y., Sobolev, O.I., Vishchur, V.J., Gubash, O.P., Kurtyak, B.M., Kovalskyi, Y.V., Darmohray, L.M., Hunchak, A.V., Tsisaryk, O.Y., Shcherbatyy, A.R., Farionik, T.V., Savchuk, L.B., Palyadichuk, O.R. & Hrymak, K., 2019. Cadmium burden impact on morphological and biochemical blood indicators of poultry. Ukr. J. Ecol., 9, 236–239. https://www.ujecology.com/abstract/cadmium-burden-impact-on-morphological-and-biochemical-blood-indicators-of-poultry-19001.html; accessed 12 September 2022.

Heshmati, A. & Salaramoli, J., 2015. Distribution pattern of cadmium in liver and kidney of broiler chicken: An experimental study. J. Food Qual. Hazard. Cont., 2, 15–19. https://www.researchgate.net/publication/323970630_Distribution_pattern_of_Cadmium_in_liver_and_kidney_of_broiler_chicken_An_experimental_study; accessed 12 September 2022.

Huang, S., Kuang, J., Zhou, F., Jia, Q., Lu, Q., Feng, C., Yang, W. & Fan, G., 2019. The association between prenatal cadmium exposure and birth weight: A systematic review and meta-analysis of available evidence. Environ. Pollut., 251, 699-707. https://doi.org/10.1016/j.envpol.2019.05.039

Li, H., Li, X., Xiang, L., Zhao, H.M., Li, Y.W., Cai, Q.Y., Zhu, L., Mo, CH. & Wong, M.H., 2018. Phytoremediation of soil co-contaminated with Cd and BDE-209 using hyperaccumulator enhanced by AM fungi and surfactant. Sci. Total Environ., 1, 613-614. https://doi.org/10.1016/j.scitotenv.2017.09.066

Jawad, S.T. Abedali, S.T. & Khalef, W.F., 2021. Determination of heavy metals concentration in liver, meat, and blood chicken of Thi-Qar province, Iraq. Nat. Volatiles Essent. Oils, 6, 2562-2570. https://www.nveo.org/index.php/journal/article/view/3928; accessed 22 March 2022.

Jin, X., Jia, T., Liu, R., Xu, S., 2018. The antagonistic effect of selenium on cadmium-induced apoptosis via PPAR-γ/PI3K/Akt pathway in chicken pancreas. J. Hazard. Mater, 357, 355–362. https://doi.org/10.1016/j.jhazmat.2018.06.003

Kar, I., Mukhopadhayay, S.K., Patra, M.A.K & Pradhan, S., 2018. Bioaccumulation of selected heavy metals and histopathological and hematobiochemical alterations in backyard chickens reared in an industrial area, India. Environ. Sci. Pollut. Res., 25, 3905–391. https://doi.org/10.1007/s11356-017-0799-z

Kar, I. & Patra, A.K., 2021. Tissue bioaccumulation and toxicopathological effects of cadmium and its dietary amelioration in poultry: A review. Biol. Trace Elem. Res., 199, 3846–3868. https://doi.org/10.1007/s12011-020-02503-2

Karimi, O., Hesaraki, S. & Mortazavi, S.P., 2015. Morphological and functional changes of Japanese quail (*Coturnix japonica*) liver exposed to cadmium. J Pharm Bioallied Sci., 4, 491-497. https://dx.doi.org/10.3923/ajpsaj.2016.165.174

Karimi, O., Hesaraki, S. & Mortazavi, S.P., 2016. Histological and functional changes of Japanese quail (*Coturnix japonica*) kidneys exposed to cadmium. The Caspian Sea Journal, 10, 61-64. http://ijt.arakmu.ac.ir/browse.php?a_code=A-10-412-1&sid=1&slc_lang=en; accessed 12 March 2022.

Karimi, O., Hesaraki, S. & Mortazavi, S.P., 2017. Histological and functional alterations in the liver and kidney and the response of antioxidants in Japanese quail exposed to dietary cadmium. Iran J. Toxicol. 11, 19–26. https://ijt.arakmu.ac.ir/article-1-551-en.html; accessed 12 March 2022.

Khan, A., Khan, S., Alam, M., Khan, M.A., Aamir, M., Qamar, Z., Rehman, Z.U. & Perveen, S., 2016. Toxic metal interactions affect the bioaccumulation and dietary intake of macro- and micro-nutrients Chemosphere. 146, 121–128. https://doi.org/10.1016/j.chemosphere.2015.12.014

Korénekova, B., Skalická, M., Nad, P. & Korének, M. 2017. Effects of cadmium and zinc on the quality of quail's eggs. Biol. Trace Elem. Res., 116, 103-109. https://doi.org/10.1007/BF02685923

Kurnaz, E. & Filazi, A., 2011. Determination of metal levels in the muscle tissue and livers of chickens. Fresen Environ Bull. 20, 2896–2901. https://www.academia.edu/49270164/Determination_of_Metal_Levels_in_the_Muscle_Tissue_and_Livers_of_Chickens accessed 10 March 2022.

Liao, Y., Cao, H., Xia, B., Xiao, Q., Liu, P., Hu, G. & Zhang, C., 2017. Changes in trace element contents and morphology in bones of duck exposed to molybdenum or/and cadmium. Biol. Trace Elem. Res., 175, 449-457. https://doi.org/10.1007/s12011-016-0778-0

Lisunova, L.I. & Tokarev, V.S. 2016. Meat quality of broiler chickens affected by cadmium toxicity. Russ. Agric. Sci., 42, 268–270. https://doi.org/10.3103/S1068367416030149

Marengoni, N.G., Klosowski, E.S., Oliveira, K.P., Chambo, A.P.S. & Gonçalves Junior, A.C., 2013. Bioaccumulation of heavy metals and nutrients in golden mussel from the Itaipu Hydroelectric Dam. Quím. Nova. 36, 359-363. https://doi.org/10.1590/S0100-40422013000300002

Nad, P., Skalicka, M. & Korenekova, B., 2012. The effects of long-term cadmium exposure in turkeys: Accumulation and zinc prevention. J. Environ. Sci. Health - Toxic/Hazard., 47, 1239-43. https://doi.org/10.1080/10934529.2012.672074

Nunes, R.V., Broch, J., Wachholz, L., De Souza, C., Damasceno, J.L., Oxford, J.H., Bloxham, D.J., Billard, L. & Pesti, G.M., 2018. Choosing sample sizes for various blood parameters of broiler chickens with normal and non-normal observations. Poult. Sci., 97, 3746-3754. https://doi.org/10.3382/ps/pey217

Oliveira, A.F.G.; Bruno, L.D.G.; Martins, E.N. De Moraes, G.E.R., Monteiro, A.C., Paula, L.M.C., Pozza, P.C. & Sangali, C.P., 2014. Effect of stocking density and genetic group on mineral composition and

development of long bones of broilers. Semina: Ciências Agrárias, 35, 1023- 1034. http://dx.doi.org/10.5433/1679-0359.2014v35n2p1023

Olgun, O., Yildiz, A.O. & Şahin A., (2020). Evaluation of dietary presence or use of cadmium in poultry. Worlds Poult Sci J., 76, 64-73. https://doi.org/10.1080/00439339.2020.1729669

Olgun, O. & Bahtiyarca, Y., 2015. Effects of dietary cadmium and boron supplementation on performance, eggshells quality and mineral concentrations of bone in laying hens. Biol. Trace Elem. Res., 167, 56–62. https://doi.org/10.1007/s12011-015-0291-x

Reeves, P.G. & Chaney, R.L., 2008. Bioavailability as an issue in the risk assessment and management of dietary cadmium: A review. Sci Total Environ., 398, 1-3. https://doi.org/10.1016/j.scitotenv.2008.03.009

Rostagno, H.S. & Featherston, W.R., 1977. Studies of methods for determining amino acid availability. Brazilian Journal of Animal Science, 6, 64-75.

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., 2011. Brazilian tables for poultry and swine: Food composition and nutritional requirements. 3rd edition. Viçosa, BR (in Portuguese).

Rostagno, H.S., Albino, L.F.T., Hannas, M.I., Donzele, J.L., Sakomura, N.K., Perazzo, F.G., Saraiva, A., Teixeira, M.L., Rodrigues, P.B., Oliveira, R.F., Barreto, S.L.T. & Brito, C.O., 2017: Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4 ed. Universidade Federal de Viçosa, Viçosa, MG.

Sakomura, N.K., Rostagno, H.S., 2007. Métodos de pesquisa em nutrição de monogástricos. Jaboticabal: FUNEP, 283p.

Sakomura, N.K. & Rostagno, H.S., 2016. Métodos de pesquisa em nutrição de monogástricos. 2.ed. Jaboticabal: FUNEP, 262p.

Silva, P.R.L., Freitas Neto O.C., Laurentiz A.C., Junqueira O.M. & Fagliari J.J. 2007. Blood serum components and serum protein test of Hybro-PG broilers of different ages. Braz. J. Poult. Sci., 9, 229-232. https://doi.org/10.1590/S1516-635X2007000400004

Sibbald, I.R. & Slinger, S.J., 1963. A biological assay for metabolizable energy in poultry feed ingredients together with findings which demonstrate some of the problems associated with the evaluation of fats. Poult. Sci., 42, 313-325. https://doi.org/10.3382/ps.0420313

Singh, R., Srivastava, A.K., Gangwar, N.K., Giri, D.K., Singh, R. & Kumar, R., 2016. Pathology of subchronic cadmium and chlorpyrifos toxicity in broilers. Indian J. Vet. Pathol., 40, 331–336. https://doi.org/10.5958/0973-970X.2016.00076.6

Suttle N.F. 2010. Copper, p.255-305. In: Ibid (Ed.), Mineral Nutrition of Livestock. 4th ed. London, CABI.

Tahir, M.W., Saleemi, M.K., Khan, A., Yousaf, M., Butt, S.L., Siriwong, W., Muhammad, F., Bh atti, S.A., Qureshi, A.S., 2017. Hematobiochemical effects of cadmium intoxication in male Japanese quail (*Coturnix japonica*) and its amelioration with silymarin and milk thistle. Toxin Rev. 36, 187–193. https://doi.org/10.1080/15569543.2017.1287088

Vasiljeva, S., Basova, N. & Smirnova, G., 2018. Disturbance of the functionality in immune competent organs of chickens due to accumulation of cadmium. Food Sci., 1, 222–226. https://doi.org/10.22616/rrd.24.2018.035

Wachholz, L., Nunes, R.V., Broch, J. & Souza, C., 2017. Possibility of using golden mussel contaminated with toxic metals in diets for broilers. Colombian Journal of Animal Science, 9, 227-235. https://doi.org/10.24188/recia.v9.n2.2017.562

Wachholz, L., Nunes, R.V., Gonçalves Junior, A.C., Eyng, C., Castilha, L.D., Cirilo, E.H., Broch, J., & Souza, C. de. 2019. Effect of the use of golden mussel flour contaminated with lead as a source of calcium on the performance of broilers. Semina: Ciências Agrárias, 40, 2783-2794. https://doi.org/10.5433/1679-0359.2019v40n6p2783

Wolf, P. & Cappai, M.G., 2021. Levels of Pb and Cd in single feeding and compound feeds for poultry. Biol. Trace Elem. Res., 199, 1074-1079. https://doi.org/10.1007/s12011-020-02197-6

Xu, F., Liu. S. & Li. S., 2015. Effects of selenium and cadmium on changes in the gene expression of immune cytokines in chicken splenic lymphocytes. Biol. Trace Elem. Res., 165, 214-221. https://doi.org/10.1007/s12011-015-0254-2

Zhang, D., Li, Y., Zhang, T., Liu J., Jahejo, A.R., Yang, L., Chen, P., Ning, G. & Huo, N.H., 2018. Protective effects of zinc and N-acetyl-L-cysteine supplementation against cadmium induced erythrocyte cytotoxicity in Arbor Acres broiler chickens (*Gallus Gallus domesticus*). Ecotox Environ Safe. 163, 331-333. https://doi.org/10.1016/j.ecoenv.2018.07.069

Zwolak, I., 2020. The role of selenium in arsenic and cadmium toxicity: an updated review of scientific literature. Biol. Trace Elem. Res., 193, 44-63. https://doi.org/10.1007/s12011-019-01691-w

Zhuang, P., Zou, B., Lu, H., Zhian, L., 2014. Heavy metal concentrations in five tissues of chickens from a mining area. Pol. J. Environ. Stud., 23, 2375-2379. http://www.pjoes.com/Heavy-Metal-Concentrations-in-Five-Tissues-r-nof-Chickens-from-a-Mining-Area,89423,0,2.html; accessed 14 September 2022.