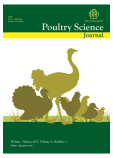

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Bioaccumulation of Heavy Metals in Broilers Fed "Trinidad de Guedes" Phosphorite

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Abstract

In order to use the "Trinidad de Guedes" phosphorite (TGP) as a supplement of calcium and phosphorus in animals, its concentrations of heavy metals were determined and their deposition was analyzed in broilers. Toxic elements were determined using atomic absorption spectrophotometry. The mean levels of heavy metals found in TGP were 82.5 mg/kg lead (Pb), 7.2 mg/kg cadmium (Cd), 1056.9 mg/kg manganese (Mn), 1.3 mg/kg mercury (Hg), 10.4 mg/kg arsenic (As), 210.6 mg/kg zinc (Zn), and 121.3 mg/kg copper (Cu). All concentrations of heavy metals were below harmful levels, with the exception of Pb, which was above its maximum tolerable value. The levels of heavy metals accumulated in bones and feathers of broilers fed TGP were determined in a feeding trial. An amount of 300 day-old broilers from commercial hybrid crossing (HE21) were allocated into three treatments in a completely randomized design, with four replicates of 25 broilers each. The three treatments were a control containing dicalcium phosphate as calcium and phosphorus source, and two experimental treatments with TGP replacing dicalcium phosphate at levels of 50 and 100%. There were no differences between treatments for levels of toxic elements accumulated in feathers, femurs and tarsus of broilers. The amounts of heavy metals accumulated in the analyzed organs showed that TGP can replace dicalcium phosphate (DCP, the traditional source of calcium and phosphate) without causing harm to animal health nor bioaccumulating heavy metals.

Introduction

Phosphorite from "Trinidad de Guedes" (TGP) deposit is a promising source of calcium (Ca) and phosphate (P) for animals. However, as TGP comes from phosphoric rock, it may also contain heavy metals, which could have serious consequences for animal and human health and productivity if bioaccumulated (Martorell, 2009).

In small portions, heavy metals are essentials for maintenance of metabolism in organisms. Higher concentrations, however, can lead to poisoning (Reis *et al.*, 2010). Bioaccumulation is a chemical process of accumulating heavy metals in biological organisms to concentrations greater than its presence in the environment. This process causes severe effects, even fatal, to animal health and productivity. Therefore, it is vital to ensure that the intake of these final products are free of metals, or at least below the established concentrations ranges. In this study, the heavy metal concentrations of TGP were determined. A feeding trial with 300 broilers of commercial hybrid crossing (HE21) was

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performed in order to asses live weight performance as well as the levels of heavy metals accumulated in the bones and feathers.

Materials and Methods

The experiment was performed at the Institute of Animal Science, San José de las Lajas, Mayabeque, Cuba (22º53 and 82º02 LN LO; Herrera, 1976).

Processing of TGP samples for determination of Pb, Cd, Cu, Zn and Mn

The experiment began with a batch of 30 samples of TGP, randomly chosen, from new ores of the deposit of the same name, in the province of Matanzas, Cuba. From each sample, 1 g of phosphorite was dissolved in 10 mL of concentrated HCl (11.66 *M*) in a porcelain crucible for 10 min over a hot iron in a sand bath. Then, the contents were transferred to a 50 mL volumetric flask leveled with deionized water. The supernatant was collected for subsequent determination of Pb, Cd, Cu, Zn and Mn in a Shimadzu AA-6800 Atomic absorption spectrophotometer.

Processing TGP samples for determination of As and Hg

For the determination of As and Hg, 1 g of TGP was dissolved in 10 mL of HNO_3 and 1 mL of H_2O_2 in a porcelain crucible in sand bath on a hot plate. The crucible was covered with a glass lid for 1 hr. Subsequently, the lid was removed, and the contents were dried to remove HNO_3 as much as possible. The sample was allowed to cool at room temperature, and 21 mL of concentrated HCl (11.66 *M*) was added to dissolve the salts. Then, the volume was increased to 50 ml with deionized water, filtered, and the supernatant was collected for determination of As and Hg using cold vapors generation. The samples were processed using HCl (6 M) and 2% $NaBH_4$ to generate volatile species.

In the case of the determination of As, a pre-reduction of As^{V} to As^{III} was performed with KI (5%) 10 minutes before the formation of the corresponding hydride. The metals were determined in a Shimadzu AA-6800 Atomic absorption spectrophotometer using a kit for hydrides that consisted of a three-channel peristaltic pump for $NaBH_4$, HCl, and the sample. Argon carrier gas was used and the measurement was carried out in the Shimadzu Atomic absorption spectrophotometer equipment with quartz T-tube supported on the burner.

Liveweight performance of broilers with different TGP treatments

All experiments approved by the ethical principles of Institute of Animal Science, San José de las Lajas, Mayabeque, Cuba. The development of diseases was avoided through vaccination. All criteria defined by the Farm Animal Welfare Council was taken into account. A total of 300 broilers from the commercial hybrid crossing (HE21) were divided into three treatments in a completely randomized design with four replications of 25 broilers each. The treatments were: control with 100% of DCP, and 50 and 100% of the imported phosphate TGP-sourced DCP (to replace DP). Diets supplemented to the birds at different developmental stages are described in Table 1. Birds had free access to fresh water and feed throughout the experiment.

Birds were weighed at 21, 35 and 42 days to measure live body weight. At the end of rearing period, samples of bone (femur and tarsus) and feathers were taken from 15 birds per treatment to determine accumulated heavy metals.

Determination of heavy metals (Pb, Cu and Zn) in feathers

Feathers were cut into 5 mm fragments with stainless steel scissors and mixed into a homogenous sample. An amount of 1 g of chopped feathers was weighed and dissolved in 10 mL of concentrated HCl in a porcelain crucible in a sand bath on a hot iron. The contents were then transferred to a 50 mL volumetric flask, leveled with deionized water, and filtered. The supernatant was collected for determination of Pb, Cu and Zn with the atomic absorption spectrophotometer.

Determination of heavy metals (Pb, Cd, Cu and Zn) in femur and tarsus

Femurs and tarsus were collected during sampling and dried in an oven at 105° C. Later, samples were ground in a porcelain mortar until completely pulverized. Samples were placed on Whatman filter paper (150 mm) and fat was extracted (to completely clean the ether) with a Soxhlet using petroleum ether as solvent. The dissolution was performed under the same conditions as phosphorite samples.

Table 1. Composition of experimental diets

Raw material	Starter			Grower			Finisher		
	% TGP			% TGP			% TGP		
	0	50	100	0	50	100	0	50	100
Corn	47.82	47.82	47.82	52.65	52.65	52.65	55.02	55.02	55.02
Soybean meal	41.85	41.85	41.85	36.5	36.5	36.5	33.5	33.5	33.5
Vegetable oil	5.2	5.2	5.2	5.65	5.65	5.65	6.7	6.7	6.7
DCP	1.8	0.90	-	1.85	0.925	-	1.63	0.815	-
TGP*	-	0.90	1.8	-	0.925	1.85	-	0.815	1.63
Calcium carbonate	1.6	1.6	1.6	1.55	1.55	1.55	1.4	1.4	1.4
Common salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
DL-Methionine	0.2	0.2	0.2	0.25	0.25	0.25	0.22	0.22	0.22
L-Lysine	0.04	0.04	0.04	0.06	0.06	0.06	0.04	0.04	0.04
Choline chloride	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Butylated hydroxytoluene	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Mineral[1]-vitamin[2] premix	1	1	1	1	1	1	1	1	1
	Nutrient composition (%, unless mentioned) Aportes calc. (%)								
ME (Kcal/kg)	3016	3016	3016	3100	3100	3100	3205	3205	3205
CP	22	22	22	20	20	20	19	19	19
Ca	1.1	1.13	1.22	1.01	1.11	1.20	0.91	0.99	1.08
$P_{available}$	0.50	0.47	0.45	0.50	0.48	0.45	0.45	0.43	0.41
Met + Cys	0.85	0.85	0.85	0.85	0.85	0.85	0.79	0.79	0.79
Lysine	1.26	1.26	1.26	1.15	1.15	1.15	1.05	1.05	1.05

†Di-calcium Phosphate, *TGP: Trinidad de Guedes phosphorite

[1]Vitamin supplement: Vit. A 1000 IU; Vit D_3 2000 IU; Vit E 10 mg; Vit K_3 2 mg; thiamine 1mg; rivoflavin 5 mg; pyridoxine 2 mg; Vit B_{12} 15.4 mg; nicotinic acid 125 mg/Kg

[2]Mineral supplement: selenium 0.1 mg; iron 40 mg, copper 12 mg; zinc 120 mg; magnesium 100 mg; iodine 2.5 mg; cobalt 0.75 mg/Kg.

Statistical Analysis

For analysis of data, InfoStat computerized statistical package (Di Rienzo *et al.* 2012) was used. The mean values were compared by the test of Duncan (1955) when necessary.

Results and Discussion

Table 2 shows the concentrations of heavy metals in TGP and their maximum permissible values reported in the literature for food for poultry. Heavy metal concentrations determined in the new ores of TGP are below the values recorded as reference in the literature, with the exception of Pb, which is above its maximum tolerable value. The concentrations determined in this study are in the range reported by Suttle (2010) who reported that Pb doses between 20-200 mg kg^{-1} in the diet are high for birds. However, when metal concentrations in TGP are compared to that supplied daily in broiler diet, the amounts received by the birds are lower than the maximum limits in literature. In the case of Pb, the metal with the highest concentration in TGP, birds received about 0.2 mg Pb/kg of consumed dry matter (DM) when 100% of the DCP was replaced for TGP in the diet. This amount is lower than the "normal" dietary intake of 1-10 mg Pb/kg of consumed DM in these birds (Suttle, 2010).

Table 2. Mean concentrations of certain heavy metals in TGP* and reference values in poultry feeds

Element	Mean (mg kg^{-1})	Coefficient of variation	Standard deviation	Referenced Value (mg kg^{-1})
Pb	82.5	15.44	0.0013	10
Cd	7.2	5.83	0.0000	10
Mn	1056.9	11.86	0.0100	2600
Hg	1.3	22.89	0.3000	2
As	10.4	1.92	0.2000	30
Zn	210.6	6.02	0.0013	1500
Cu	121.3	9.77	0.0012	900

*TGP: Trinidad de Guedes phosphorite

Referenced values were taken from NRC (2005).

Similar trends were observed with Cd. Experimental birds received 0.014 mg Cd /kg of consumed DM. Values established as normal, according to Suttle (2010), ranged from 0.1-0.8 mg Cd/kg of consumed DM, so the quantities supplied are markedly lower. Similarly, when birds were supplemented with 100% TGP, they received 0.24 and 0.42 mg of Cu and Zn per kg of consumed DM, respectively. The amounts considered as normal in the diet are 200 mg Cu/kg and 1-2 g Zn/kg of consumed DM (Suttle, 2010). Likewise, the amounts provided by the diet are lower than those reported as harmful in the literature.

Results regarding live weight of the birds are reported in Table 3. There were no significant difference in live body weight between treatments over the experimental period. These results differ from those obtained by Acosta *et al.* (2009) who found greater weight gain and final body weight in laying hens fed 100% TGP. These differences may be mainly caused by the fact that diet formulations for broilers and laying hens are different. Further, the duration of the productive cycle for broilers is shorter than laying hens.

Table 3. Liveweight performance of broilers with different treatments

Days	Live body weight (g)			Standard error
	†DCP (100%)	*TGP (50%)	*TGP (100%)	
21	632	601	534	50.1
35	1442	1366	1307	67.7
42	1793	1782	1745	25.1

†DCP: Di-calcium Phosphate, *TGP: Trinidad de Guedes phosphorite

As seen in the Table 4, there were no significant differences in metal concentrations in feathers between treatments. Feathers are considered general indicators of exposure to heavy metals, as they are irrigated by blood vessels: metals are included into the structure of keratin and become immobilized upon deposition (Lewis and Furness, 1991; Monteiro and Furness, 1996).Therefore, this may be considered a mechanism for metal removal. However, growing feathers are physiologically connected to the rest of the body, and blood is exchanged between feathers and soft tissues (Nam *et al.*, 2005). Therefore, metal concentrations in feathers should reflect their concentration in blood, at least during the first weeks of feather formation (Motas *et al.*, 2011). Therefore, this suggests that levels of heavy metals in birds fed the two treatments of TGP are within normal ranges.

Table 4. Mean levels of heavy metals in the feathers of birds

Element	Metal concentration (mg/kg)			Standard error
	†DCP (100)	*TGP (50)	*TGP (100)	
Pb	0.86	1.14	1.10	0.18
Cu	0.80	0.97	0.87	0.09
Zn	16.50	17.19	17.24	1.18

†DCP: Di-calcium Phosphate, *TGP: Trinidad de Guedes phosphorite

Table 5 shows the content of Pb, Cd, Zn and Cu in the femurs of birds fed TGP. There were no significant differences between treatments. The high Pb content in TGP ores was not directly reflected in the bioaccumulated amount in the bones of birds. According to Bergqvist *et al.* (1991), birds are on the highest level of sensitivity to heavy metals of commercial species for humans. However, adverse effects typical of Pb poisoning were not observed in the birds used in the experiment. Henry and Miles (2001) stated that the Pb is highly accumulated in bones (with little removal), liver and pancreas. Therefore, it is likely that the amounts of Pb found in the femur indicated the cumulative concentrations of this element in birds. In addition, as stated by Sipos *et al.* (2003), after prolonged exposure to Pb, Pb accumulates in the skeleton and reaches equilibrium very slowly with blood and organs. Therefore, this reinforces that Pb concentrations in bone after six weeks of the productive cycle are similar to the Pb concentrations in animal organs.

There were no differences in cadmium concentrations in bones of birds fed TGP. Cd and Pb share similarities in chemical reactivity, and both have similar metabolic pathways. Induction of metallothionein synthesis in the intestinal tract is the primary protective mechanism in chickens against absorption of Cd. Therefore, the element is sequestered by the protein, and together detach from the epithelial tissue and are removed through feces (Henry and Miles, 2001). A test to explore heavy metals from the beds of animals would provide a more complete understanding of these mechanisms used to remove heavy metals.

Table 5. Mean levels of heavy metals in the femurs of birds

Element	Metal concentration (mg/kg)			Standard error
	†DCP (100)	*TGP (50)	*TGP (100)	
Pb	2.08	1.71	2.08	0.13
Cd	0.25	0.26	0.21	0.19
Cu	0.62	0.72	0.74	0.07
Zn	14.83	16.88	15.39	1.06

†DCP: Di-calcium Phosphate, *TGP: Trinidad de Guedes phosphorite

Table 6 shows the results of heavy metals accumulated in the tarsus. No significant differences were found between treatments. According to Ross (2000), Ca supplements are sometimes rich in Pb. In this case, concentrations of Pb in the TGP are above the maximum value established in the literature (NRC, 2005). However, the accumulated levels of this metal in the tarsus are not high due to the small amount of metal that reaches the animal through diet. Sullivan *et al.* (1994) argued that the most important exogenous sources of Cd are superphosphates. Nevertheless, TGP does not contain high levels of this metal, so their bio-accumulative levels in tarsus (and in birds as a whole) is insignificant. However, it is necessary to sample other target organs to better understand the fate of dietary exposure to heavy metals in birds.

Table 6. Mean levels of heavy metals in the tarsus of birds

Element	Metal concentration (mg/kg)			Standard error
	†DCP (100)	*TGP (50)	*TGP (100)	
Pb	1.16	1.37	1.35	0.11
Cd	0.17	0.16	0.14	0.01
Cu	0.61	0.63	0.61	0.04
Zn	9.08	9.67	9.48	0.38

†DCP: Di-calcium Phosphate, *TGP: Trinidad de Guedes phosphorite

These results broaden our understanding of the chemical composition of new TGP ores, particularly of the amounts of toxic elements present. The low amounts of most heavy metals in TGP used in this study did not exceed reference values for bird feed – with the exception of Pb -- and therefore permitted acceptable use in poultry diet. Nonetheless, the real amount of Pb received by the animal through diet is minimal. TGP did not affect final weight of the birds nor did it bioaccumulate in bones and feathers. Therefore, these results support the use of phosphorite from TGP ores as a source of Ca and P in broilers. Future studies can evaluate the deposition of heavy metals in other target organs and assess heavy metals deposition in other types of chickens.

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