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Effect of phytase and chelated minerals on performance and indicators of bone mineralization in broilers

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ADITIONAL KEYWORDS

Bone mineralization.
Chelated minerals.
Enzymes.
Organs weight.
Phytases.

SUMMARY

The objective of the study was to evaluate the effect of phytase and chelated minerals on performance, organs weight and bone mineralization indicators in broiler chickens. 240 male broiler chicken (Cobb 500) were evaluated in three experimental treatments (Basal diet, Basal diet + phytases and with Basal diet + chelated minerals) with 4 replicates (20 birds per replicate). The study was divided into three phases: start, growth and finisher. At the end of each phase, autopsies were performed to weigh the gastrointestinal organs and to evaluate indicators of bone mineralization of the left tibias. Significant differences ($p < 0.05$) were found in the productive performance of chickens in favor of phytase treatment, except in feed intake ($p > 0.05$). Compared to control animals, the birds with phytase showed a significantly lower relative weight of the pancreas ($p < 0.05$) and a statistically ($p < 0.05$) greater intestine weight. Finally, at 42 days of age, statistical differences were observed in bone density ($p < 0.05$) and the Seedor index ($p < 0.05$) in favor of birds supplemented with phytase. Phytase supplementation improved the productive performance, reduced the weight of the pancreas and increased the weight of the intestine, and also increased bone mineralization, being more efficient than chelated minerals with respect to these variables.

Efecto de la fitasas y minerales quelatados en el desempeño e indicadores de mineralización ósea en pollos de engorde

RESUMEN

El objetivo del estudio fue evaluar el efecto de la fitasa y los minerales quelados sobre el rendimiento, el peso de los órganos y los indicadores de mineralización ósea en pollos de engorde. Se evaluaron 240 pollos de engorde machos (Cobb 500) en tres tratamientos experimentales (dieta basal, dieta basal + fitasas y con dieta basal + minerales quelados) con 4 repeticiones (20 aves por réplica). El estudio se dividió en tres fases: inicio, crecimiento y finalización. Al final de cada fase, se realizaron autopsias para pesar los órganos gastrointestinales y evaluar los indicadores de mineralización ósea de las tibias izquierdas. Se encontraron diferencias significativas ($p < 0.05$) en el desempeño productivo de los pollos a favor del tratamiento con fitasa, excepto en el consumo de alimento ($p > 0.05$). En comparación con los animales de control, las aves con fitasa mostraron un peso relativo significativamente menor del páncreas ($p < 0.05$) y un peso intestinal mayor estadísticamente ($p < 0.05$). Finalmente, a los 42 días de edad, se observaron diferencias estadísticas en la densidad ósea ($p < 0.05$) y el índice de Seedor ($p < 0.05$) a favor de las aves suplementadas con fitasa. La suplementación con fitasa mejoró el rendimiento productivo, redujo el peso del páncreas y aumentó el peso del intestino, y también aumentó la mineralización ósea, siendo más eficiente que los minerales quelados con respecto a estas variables.

PALABRAS CLAVE
Mineralización ósea.
Minerales quelatados.
Enzimas.
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INTRODUCTION

The diets of broiler chicken include some cereals such as corn, sorghum or soybeans, that have phytic acid, which is an insoluble molecule that contains six phosphate groups and acts as a chelating agent for minerals such as calcium, iron, zinc and magnesium (Acosta and Cardenas, 2006). It has been suggested that the mechanism of action of phytates is related to their effect on the actual absorption of minerals, due to the inability of chickens to hydrolyse them and the formation of complexes in the neutral pH of the small intestine; together with a decrease in the reabsorption of endogenous minerals due to the reduction of the absorption of nutrients such as sugars and amino acids in the gastrointestinal tract (Woyengo and Nyachoti, 2012). On the other hand, minerals act mainly in protein associations, improving their catalytic activity within the organism (Pirgozliev *et al.*, 2009, Gallardo *et al.*, 2018).

As a result, there is a reduction of performance, food efficiency and nutrient utilization in broiler chickens (Walk and Olukosi, 2019). In addition, due to the low absorption of minerals, mainly calcium and phosphorus, the skeletal development of the bird is affected, generating problems related to a failed mineralization of growing bones such as lameness, fractures, skeletal weakness, valgus or varus. These problems generate economic losses for the producer (Ferket *et al.,* 2009).

Considering all of the above, current formulation and feeding practices seek to reduce the excretion of minerals into the environment, reduce costs and improve the nutritional quality all of which will be reflected by better development of broiler chickens (Campos *et al*., 2014). Therefore, additives such as phytases are now used. This enzyme hydrolyzes phytates to inositol and inorganic phosphate and improves the absorption of minerals such as calcium and phosphorus, proteins, amino acids and nitrogen (Camiruaga *et al.,* 2001).

Another additive that is gaining importance in the formulation of rations of broiler chickens is chelated minerals. These are ions bind to an organic compound, such as proteins, amino acids or carbohydrates, which act as a vehicle for nutrients to cross the intestinal wall, and provide stability and solubility (Pessôa *et al.,* 2012).

Thus, the objective of the present study was to evaluate the effect of phytase and chelated minerals supplementation on productive performance, organs weight and bone mineralization indicators in broiler chickens.

MATERIAL Y MÉTODOS/MATERIAL AND METHODS

The study was conducted at the Unidad Avícola of Unidad Zootecnica y Tecnológica (UZyT), Universidad Cientifica del Sur, Lima, Peru. All the procedures were approved by the Institutional Committee of Ethics in Animal Research and Biodiversity of this institution (CIEI-AB-CIENTÍFICA) on October 1, 2019, with the registration code 143-2019-PRE16.

Experimental design, animals and diets

A total of 240 male broiler chickens (Cobb 500) were randomized to receive 3 treatments, with 4 replicates per treatment and 20 birds per replicate. The experimental treatments were: Treatment 1 (control) - basal diet without enzymes or nutritional additives, Treatment 2 - basal diet + phytase, and Treatment 3 - basal diet + chelated minerals. The birds production system was divided into three productive phases: Start: 0 to 14 days of age, growth: 15 to 28 days of age and finish: 29 to 42 days of age.

Water and feed were provided *ad libitum* in starter feeders and baby drinkers in the initial phase, and at day 15 these were exchanged for hopper feeders and automatic drinkers, which were regulated to the height of the crop following the growth of the chickens.

The diets were formulated considering conventional ingredients such as ground corn and soy flour and sources of minerals and vitamins, as well as non-nutritional additives according to the nutritional requirements of Rostagno *et al.* (2017) and the Cobb 500 Management Guide (2018) (**Table I**).

The chickens were raised in floor system, with a wood chip bed approximately 8 cm thick. The pens had an area of 2.4 m2 with a density of 8 birds/m2. The temperature was controlled by 250 watt halogen lamps at 32°C the first week, decreasing to 27°C in the growth phase. Temperature was controlled using Boeco Germany digital thermohygrometers (Boeckel & Co, Hamburg, Germany) with a precision of 1° for temperature and 3% for humidity. Likewise, humidity was controlled at 70 and 75% with this instrument and using curtains and extractors.

Phytases and chelated minerals

Phytases and chelated minerals were added to the diet considering in accordance to the total formulated diet. The phytase used in the present study was a commercial product (Alquerzim FT, Biovet SA, Spain) with an expected activity of 1500 FTU per kg of ration, at a dose of 25 g/t. The chelated minerals were composed of a complex of manganese protein, zinc, iron, potassium and copper iodate, which were included at a dose of 500 g/t of ration. The activity of enzymes in the diets was guaranteed by analysis by the Laboratorio Biovet S.A (Spain).

Productive performance

Birds were weighed weekly, from day 0 to day 42, for which the animals remained without feed for 2 hours, achieved with the removal of feeders. From day 0 to day 42 the birds were weighed weekly, after remaining without food for 2 hours by removal of the feeders. The birds were individually weighed with an electronic platform scale BC30N model with 1 g precision (Henkel, China). In addition, all feeding was recorded in kg and the weekly surplus was weighed. According to this value, the waste calculated in bed was added to determine the amount of feed consumed. With these data, the food conversion index was obtained. Finally, the viability percentage of the treatments was calculated.

Organs weight

At the end of each productive phase, one bird per replicate was randomly selected, weighed with an electronic platform scale BC30N model with precision of 1 g (Henkel, China) and subsequently slaughtered by cervical dislocation, following the procedures of the World Organisation for Animal Health (OIE) (2011). The absolute weight of the liver, spleen, gizzard, proventriculus, pancreas and intestine was determined with a Topscale digital scale model SF-810 with an accuracy of 0.1 g (China). Finally, the relative weight was calculated by determining the relationship between the absolute weight of each organ and the body weight of the bird.

Indicators of bone mineralization

The left tibia of the birds sacrificed for organs weight, was removed and the muscle was dissected. These were labeled and frozen. The bones identified were immersed in boiling water for 15 minutes to remove the adhered fat and remaining tissue (Applegate and Lilburn, 2002) and were then dried at room temperature for 24 hours (Kocabagli, 2001).

Table I. Basal diets given to broiler chickens at the different stages of evaluation[1]. (*Dietas basales para pollos de engorde en las diferentes etapas de evaluación*).

Ingredients	Start 0 to 14 days	Growth 15 to 28 days	Finish 29 a 42 days
Corn	58.951	61.050	62.320
Soy flour, 48%	29.691	21.961	16.225
Whole soy	5.030	10.171	11.939
Wheat by.product	0.670	1.220	2.997
Soy oil	1.000	1.202	1.800
Dicalcium phosphate	1.990	1.875	1.616
Calcium carbonate	1.035	0.970	0.918
Salt	0.282	0.282	0.281
Sodium bicarbonate	0.200	0.200	0.200
DL-Methionine, 99%	0.350	0.323	0.306
L-Lysine HCL, 78%	0.295	0.279	0.265
L-Threonine	0.129	0.074	0.053
Valine	0.036	0.057	0.029
Mycotoxin sequestrant	0.100	0.100	0.100
Premix vit. + min.[2]	0.100	0.100	0.100
Choline Chloride, 60%	0.092	0.087	0.802
Nutritional composition analyzed			
Metabolizable Energy (kcal/kg)	3000	3100	3150
Crude protein (%)	21.091	19.622	18.201
Crude fiber (%)	2.612	2.552	2.692
Ethereal Extract (%)	5.053	6.081	7.010
Total Phosphorus (%)	0.710	0.673	0.631
Phosphorus available (%)	0.451	0.421	0.382
Calcium (%)	0.900	0.840	0.760
Chlorine (%)	0.310	0.300	0.300
Sodium (%)	0.180	0.180	0.180
Potassium (%)	0.831	0.780	0.750

[1]Treatments: Treatment 1: basal-control diet; Treatment 2: basal diet + phytase with activity of 1500 FTU, at a dose of 25 g/t; Treatment 3: basal diet + chelated minerals at doses of 500 g/t.
[2]Premix vitamin and minerals: Folic acid 100 000 mg/kg, Ac. Pantothenic 1620 mg/kg, Biotin 6100 mg/kg, Copper 1220 0000 mg/kg, Hill 60 g/kg, Iron 10.2 g/kg, Iodine 243 mg/kg, mn 12.6 g/kg, Niacin 5000 mg/kg, Selenium 70 mg/kg, Vit. At 1,290,000 IU/kg, Vit. B1 410000 mg/kg, Vit. B12 1,730.00 ug/kg, Vit. B2 800 mg/kg, Vit. B6 400,000 mg/kg, Vit. D3 350,000.00 IU / kg, Vit E 2,500 mg/kg, Vit. K 300,000 mg/kg, Zinc 12630 g/kg.

Bone morphometric evaluation: The length of the tibia was determined with a digital micrometer (Truper, Mexico) (Applegate and Lilburn, 2002). Bone width was calculated by the average the lateral-lateral diameter (DLL) and the diameter cranio-caudal (DCC) at the center of the tibia shaft (Kocabagli, 2001). The volume of displaced water was measured by immersing the bone in a graduated glass cylinder. A hole was made in the bone prior to submersion in water to allows water to penetrate the porous interior (Onyango *et al*., 2003).

Bone mineralization indexes: The weight of the left tibia was calculated using a Topscale digital scale model SF-810 with 0.1 g precision (China). The density was calculated by determining the quotient between the weight of the fresh bone and the volume of the bone. The Seedor modifying index was calculated by dividing the weight of the tibia by its length, the value of which is directly proportional to bone density (Seedor *et al*., 1991; Mutus *et al*., 2006). The index of Quetelet was calculated by dividing the weight of the tibia by its length squared, which indicates that the higher the value, the heavier, albeit shorter, the bone (Resenfield, 1972; Mutus *et al*., 2006). The Robustity index was found by dividing the length of the bone by the weight of the bone raised to the power 1/3, showing that the higher the value the lesser the strength of the bone (Rutten *et al*., 2002).

Statistical analysis

The data obtained were analyzed using analysis of variance homogeneity by means of the Barlett test, and normality was determined by the Shapiro-Wilk test. Data with a normal distribution were analyzed by one-way analysis of variance (ANOVA). For the comparison of treatments, the DLS means test was performed, using the generalized linear model (GLM) procedure of

Table II. **Average of weight gain, feed intake, feed conversion rate and viability percentage in broiler chickens supplemented with phytase and chelated minerals in different growth stages.** (*Promedio de aumento de peso, consumo de alimento, tasa de conversión alimenticia y porcentaje de viabilidad en pollos de engorde suplementados con fitasa y minerales quelados en diferentes etapas de crecimiento*).

	Treatments[1]			SEM[2]	P-Value
	Control	Phytase	Chelated minerals		
Start (0 - 14 days)					
Initial weight (g)	43.71	43.25	43.38	0.19	0.241
Final weight (g)	497.58	514.21	499.24	2.82	0.121
Weight gain (g)	453.87	470.96	455.86	2.63	0.134
Feed intake (g)	577.19	565.80	587.25	5.08	0.110
FCR (g/g)	1.27	1.20	1.29	0.13	0.067
Viability (%)	98.59[b]	100[a]	98.59[b]	0.26	0.031
Growth (15 - 28 days)					
Final weight (g)	1642.60[b]	1748.98[a]	1694.32[b]	25.51	0.013
Weight gain (g)	1145.02[b]	1234.77[a]	1195.08[b]	19.69	0.041
Feed intake (g)	1766.58	1826.36	1797.51	12.95	0.097
FCR (g/g)	1.54[a]	1.48[b]	1.50[ab]	0.09	0.017
Viability (%)	98.59	98.61	98.59	0.67	0.104
Finish (29 - 42 days)					
Final weight (g)	3293.49[c]	3467.02[a]	3356.32[b]	34.81	0.015
Weight gain (g)	1650.89[b]	1718.04[a]	1662.00[b]	9.30	0.009
Feed intake (g)	2988.27	2997.20	2994.30	12.95	0.241
FCR (g/g)	1.81[a]	1.74[b]	1.80[a]	0.07	0.001
Viability (%)	97.32	98.61	98.59	0.67	0.154
Total (0 - 42 days)					
Initial weight (g)	43.71	43.25	43.38	0.19	0.241
Final weight (g)	3293.49[c]	3467.02[a]	3356.32[b]	28.56	0.016
Weight gain (g)	3249.78[c]	3423.77[a]	3312.94[b]	28.99	0.017
Feed intake (g)	5322.04	5389.36	5379.06	17.86	0.122
FCR (g/g)	1.64[a]	1.57[b]	1.62[a]	0.23	0.043
Viability (%)	98.82	99.77	99.06	0.40	0.109

[1]Treatments: Treatment 1: basal-control diet; Treatment 2: basal diet + phytase with activity of 1500 FTU, at a dose of 25 g/t; Treatment 3: basal diet + chelated minerals at doses of 500 g/t.
[2]SEM: Standard error of the mean
FCR: Feed conversion ratio
[a,b,c] Values with different letters in the same row indicate that there are significant differences (p <0.05) in the DLS test.

the Statistical Analysis System (SAS) software version 9.3. The data on the percentage of viability and relative weight of organs were transformed to Arcoseno values for analysis of variance.

RESULTS

There were significant differences (p <0.05) among the treatments in relation to the final weight, weight gain, feed conversion rate and viability in the chickens supplemented with phytase and chelated minerals.

The weight of the birds at the end of the growth and finishing phases (at 28 and 42 days of age, respectively) was significantly affected (p <0.05) by the experimental diets, with the group of animals supplemented with phytases presenting a greater final weight during the periods mentioned. The weight gain and the feed conversion ratio were significantly affected (p <0.05) at 28 and 42 days of age, and when making the evaluation from 0 to 42 days. Compared to the control group, the animals supplemented with phytase presented a greater weight gain followed by the broiler chickens supplemented with chelated minerals. On the other hand, the animals of the control group presented the highest rate of feed conversion compared to the other treatments.

Regarding feed intake, no significant differences (p >0.05) were observed in the diets in the different weeks of evaluation. However, there were numerical differences between the treatments, with the birds supplemented with phytase presenting greater food consumption. The viability of the birds was significantly affected (p <0.05) in the initial phase. However, in the total evaluation from 0 to 42 days of age, no significant differences (p >0.005) were observed among the different treatments.

The live weight of the group of broiler chickens that consumed the phytase diet showed significantly (p <0.05) higher weights at 28 and 42 days of age. Regarding the relative organ weight, the majority maintained statistically similar relative weights (p >0.05). However, the relative weight of the pancreas was significantly lower (p <0.05) in the phytase group compared to the control group at 14 and 28 days of age. On the other hand, intestine weight was significantly (p <0.05) higher in the group of birds supplemented with phytase at 14 and 28 days (**Table III**).

Table IV shows the results of the bone mineralization indicators for the different evaluation phases. In the initial and growth phases, statistical differences (p <0.05) were observed in bone weight, bone density, Seedor index, and the Quetelet and robusticity index. Thus, from 0 to 14 days of age, birds supplemented with phytase had a higher tibia weight, and a higher Seedor and Quetelet index compared to the control treatment, and bone density was significantly (p <0.05) higher in birds supplemented with chelated minerals compared to the other treatments.

At 28 days of age, there were significant differences in the weight of the tibia in birds fed phytase and chelated minerals compared to the control group. Birds supplemented with phytase had (p <0.05) a higher bone density, Seedor and Quetelet indexes, followed by the birds supplemented with chelated minerals. No significant differences (p >0.05) were observed in the robusticity index between birds supplemented with phytase versus chelated minerals; however, the control treatment birds had a higher robusticity index (p <0.05).

At 42 days of age, statistical differences were observed in bone density (p <0.05) and the Seedor index (p <0.05) in favor of birds supplemented with phytase. Bone diameters were not significantly affected (p

Table III. Average of the relative weight (%) of organs in broiler chickens supplemented with phytase and chelated minerals in different growth phases. (*Promedio del peso relativo (%) de órganos en pollos de engorde suplementados con fitasa y minerales quelados en diferentes fases de crecimiento*).

	Treatments[1]				
	Control	Phytase	Chelated minerals	SEM[2]	P-Value
Start (0 - 14 days)					
Broiler weight (g)	425.00	488.50	429.00	24.04	0.144
Proventriculus (%)	0.88	0.87	0.89	0.04	0.128
Gizzard (%)	2.89	2.79	2.79	0.07	0.096
Spleen (%)	0.09	0.09	0.09	0.01	0.245
Liver (%)	3.82	3.75	4.03	0.22	0.087
Pancreas (%)	0.55[a]	0.45[b]	0.50[b]	0.09	0.032
Intestine (%)	8.22[b]	9.56[a]	8.87[ab]	0.15	0.008
Growth (15 - 28 days)					
Broiler weight (g)	1616.67[b]	1835[a]	1601.67[b]	86.63	0.023
Proventriculus (%)	0.49	0.39	0.45	0.05	0.034
Gizzard (%)	1.84	1.87	1.86	0.14	0.131
Spleen (%)	0.09	0.09	0.10	0.01	0.176
Liver (%)	2.52	2.18	2.68	0.63	0.134
Pancreas (%)	0.28[a]	0.21[b]	0.30[a]	0.04	0.024
Intestine (%)	5.86[b]	6.74[a]	6.37[a]	0.00	0.045
Finish (29 - 42 days)					
Broiler weight (g)	3102.00[c]	3467.50[a]	3301.25[b]	119.14	0.012
Proventriculus (%)	0.38	0.33	0.36	0.02	0.144
Gizzard (%)	1.52	1.55	1.62	0.18	0.108
Spleen (%)	0.11	0.10	0.10	0.01	0.230
Liver (%)	2.56	2.18	2.24	0.17	0.097
Pancreas (%)	0.23	0.21	0.21	0.02	0.242
Intestine (%)	3.18	4.12	3.67	0.20	0.095

[1]Treatments: Treatment 1: basal-control diet; Treatment 2: basal diet + phytase with activity of 1500 FTU, at a dose of 25 g/t; Treatment 3: basal diet + chelated minerals at doses of 500 g/t.
[2]SEM: Standard error of the mean
[a,b,c] Values with different letters in the same row indicate that there are significant differences (p <0.05) in the DLS test.

Table IV. Average of indicators of bone mineralization in the left tibia of broiler chickens supplemented with phytase and chelated minerals in different growth phases. (*Promedio de indicadores de mineralización ósea en la tibia izquierda de pollos de engorde suplementados con fitasa y minerales quelados en diferentes fases de crecimiento).*

	Treatments[1]			SEM[2]	P-Value
	Control	Ahitase	Chelated minerals		
Start (0 - 14 days)					
Weight (g)	0.803[c]	1.067[a]	0.967[b]	0.11	0.014
Density (mg/cm^3)	533.33[c]	893.33[b]	933.33[a]	98.87	<0.001
LL diameter[3] (mm)	4.40	4.44	4.47	0.03	0.203
CC diameter[4] (mm)	4.31	4.280	4.30	0.04	0.142
Total diameter (mm)	4.36	4.360	4.39	0.01	0.221
Length (mm)	49.93	50.96	49.21	0.71	0.078
Seedor index (mg/mm)	16.09[b]	20.93[a]	19.64[a]	1.04	<0.001
Quetelet index (mg/mm^2)	0.322[b]	0.41[a]	0.40[a]	0.03	0.006
Robusticity index (mm/g$^{(1/3)}$)	53.72[a]	49.88[b]	49.77[b]	1.83	0.001
Growth (15 - 28 days)					
Weight (g)	3.45[b]	4.30[a]	4.00[ab]	0.35	0.008
Density (mg/cm^3)	756.25[c]	913.33[a]	840.00[b]	34.17	<0.001
LL diameter[3] (mm)	7.76	7.79	7.35	0.20	0.142
CC diameter[4] (mm)	6.37	6.89	6.70	0.21	0.087
Total diameter (mm)	7.07	7.34	7.03	0.14	0.140
Length (mm)	63.94	58.27	58.69	6.17	0.092
Seedor index (mg/mm)	53.96[c]	73.79[a]	68.15[b]	5.53	<0.001
Quetelet index (mg/mm^2)	0.84[b]	1.26[a]	1.16[ab]	0.30	<0.001
Robusticity index (mm/g$^{(1/3)}$)	42.316[a]	35.836	36.974	3.85	0.001
Finish (29 - 42 days)					
Weight (g)	9.37	9.93	9.27	0.10	0.231
Density (mg/cm^3)	965.45[b]	1035.30[a]	999.81[ab]	28.51	0.032
LL diameter[3] (mm)	9.99	10.51	9.93	0.26	0.053
CC diameter[4] (mm)	8.93	9.64	8.31	0.53	0.144
Total diameter (mm)	9.46	10.07	9.12	0.39	0.055
Length (mm)	99.95	101.58	98.90	2.00	0.076
Seedor index (mg/mm)	93.72[b]	97.79[a]	93.73[b]	0.79	0.035
Quetelet index (mg/mm^2)	0.94	0.96	0.95	0.02	0.061
Robusticity index (mm/g$^{(1/3)}$)	47.41	47.25	47.08	0.76	0.207

[1]Treatments: Treatment 1: basal-control diet; Treatment 2: basal diet + phytase with activity of 1500 FTU, at a dose of 25 g/t; Treatment 3: basal diet + chelated minerals at doses of 25g/t.
[2]SEM: Standard error of the mean
[3]LL: Latero-lateral
[4]CC: Craneo-caudal
[a,b,c] Values with different letters in the same row indicate that there are significant differences (p <0.05) in the DLS test.

>0.05) by experimental treatments in any of the phases evaluated.

DISCUSSION

To start with, the greater live weight of the chickens observed in the phytase treatment compared to the control group, can be partially explained by a better feed conversion rate in favor of the phytase group. This higher rate could be due to the enzymatic activity that degrades phytates of the diet and releases phosphorus. However by degrading these compounds, it also releases other energy nutrients and amino acids, which are assimilated in greater amounts at the intestinal level. According to Ravindran et al. (1995), the enzymatic activity of phytases increases ileal digestibility of crude protein by 2.4% and energy by 3.9%. In addition, in the study by Sebastian *et al*. (1997) improvements were observed in the digestibility of 1.8% amino acids and 4.3% protein in broilers supplemented with microbial phytases.

When minerals and other nutrients bind to the phytic acid molecule, they are not totally or partially available, that is, they are not digested (Gallardo *et al*., 2018). Phytic acid can also be integrated with positive ions of proteins, amino acids, carbohydrates, lipids and digestive enzymes (Kornegay, 2001), which can affect the productive performance of broilers. Thus, the use of phytases could improve weight gain and the feed conversion ratio, possibly in association with the breakdown of phytic acid-nutrient complexes, favoring their absorption. Ptak *et al*. (2015) verified the effect of phytase at 5000 FTU/kg in chickens, which showed an increased in weight gain, feed intake and a reduction in the feed conversion ratio in the start phase (1 to 14 days). This trend was maintained in the growth phase (15 to 21 days), except that phytase supplementation had no impact on feed intake. Finally, in the finishing phase (22 to 42 days), only the feed conversion ratio improved. In general terms of production (0 to 42 days), weight gain increased and the feed conversion ratio decreased due to the addition of phytase in the diet. This information coincides, in part, with the results showing no significant differences in weight gain, food consumption or feed conversion ratio in the start phase.

The study by Momeneh *et al*. (2018), reported no effect of phytase at different levels (500 and 2500 FTU/kg) on weight gain, feed intake or the feed conversion ratio. These authors associated these results with a diet with a higher phosphorus level which meets the requirements of the broiler but does not achieve a correct calcium: phosphorus balance, because phytases release more phosphorus, which is then excreted and cannot be used by birds.

On the other hand, Camiruaga *et al*. (2001) evaluated the effect of phytase on two different substrates: corn and triticale. The corn-based phytase diet achieved a 9.78% increase in total weight gain, but there was no effect on the feed conversion ratio. However, the triticale-based diet did show a significant difference in this parameter ($p < 0.01$), and the authors described that enzymes act differently according to the substrate used. This is complemented by the results obtained by Aguilar *et al.* (2018), who found that a diet with a normal phosphorus level without enzymes, had a better effect on weight gain and final live weight compared to a phosphorus deficient diet with phytase. They suggested that this may occur because the matrix of commercial phytases considers the phosphorus available, but not other nutrients such as crude protein, amino acid, calcium and metabolizable energy.

In relation to the results obtained with the supplementation of chelated minerals, Térreas *et al*. (2000) found statistical differences between chickens supplemented with organic minerals (1.36 mg/kg/d) in water versus control treatment, with an increase in live weight of 79.33 g and a decrease in the feed conversion ratio of 0.09 ($p < 0.05$). These results coincide with those of the present study in which the best accumulated weight gain (0 to 42 days) was observed in chickens supplemented with chelated minerals. However, Nollet *et al*. (2007) found no statistical difference when supplementing organic minerals to broilers in any of the productive phases. These results are similar to those of Manangi *et al*. (2012), who concluded that although there is no improvement in the productive performance of chickens supplemented with organic minerals at low dose, the same can be observed in chickens supplemented with inorganic minerals, thereby being an optimal option to decrease the excretion of minerals to the environment.

As for the relative weight of the organs, the lower weight of the pancreas might indicate that phytases likely improve digestion. Thus, the bolus of food could be simpler with a lower need for secretion of endogenous enzymes produced at that level, thereby leading to a lower weight by a reduction of activity. The opposite occurs with the weight of the intestine, which was greater. This could be related to the greater absorption of nutrients by successful absorption at the level of the intestinal villi. However, in this study histological evaluation at the intestinal level was not performed.

The relative weight of the organs is a great indicator of the digestive capacity of the animals, highlighting the weight of the pancreas, liver and intestine (Brito *et al*., 2004). Phytin is the main storage form of phosphates, myoinositol and cations during seed germination (Selle *et al*., 2007). In the study by Maenz (1999), they determined that phytin is a protein that can form complexes through electrostatic bonds between phosphate groups and the amino terminal group of proteins. These protein-phytin complexes can form an acidic pH from dietary proteins, which can affect the rate of passage of food (Selle *et al*., 2003).

According to Pirgozliev *et al*. (2009), phytase supplementation does not affect the intestine weight of broilers, since they did not observe increased growth of the villi in the ileum. Wang *et al*. (2013) observed there was no effect on the weight of the duodenum and ileum in birds fed with phytase. However, Akyurek *et al*. (2011) observed that the weight of the intestine of broilers can increase by adding phytases to the diets.

Finally, in regard to indicators of mineralization, it is important to know the changes in bone development in chickens occurres up to four weeks of age. These changes are mainly due to the proportion of calcium and phosphorus in the diet as well as their absorption rate in the digestive tract. A deficit in these components results in late calcification, affecting bone weight and density, but not the normal bone fibrocartilage synthesis (Santos *et al*., 2013).

Bone mineralization indicators were better in broiler chickens with diets supplemented with phytase followed by animals with diets supplemented with chelated minerals. This could be related to the greater availability of phosphorus and calcium as well as the availability of other minerals in the digestive tract of birds. There was an increase in the substrate content for bone development, verified by the increase in the weight and density of the tibia at 14 and 28 days of age. The phosphorus and calcium consumed by the broilers and used for bone mineralization were improved by the addition of phytase in the diet possibly due to the hydrolysis of minerals linked to phytic acid. In addition, the response in terms of bone indicators at the level of the tibia was greater in broilers receiving phytase than birds supplemented with chelated minerals.

Since the minerals found in the greatest amounts in the bone matrix are calcium and phosphorus, greater availability of these minerals through the addition of phytases leds to improvements in weight, density and resistance to bone breakage (Ahmad *et al*., 2000; Lan *et al*., 2002). On the other hand, the effects of the addition of chelated minerals to the diet of broilers were not as marked compared to phytase, possibly because micro-minerals are distributed to a lesser extent in the bone matrix.

Chung *et al*. (2013) evaluated the productive response and bone density in broilers 1 to 42 days of age supplemented with two different types of phytases from a bacteria and a yeast respectively. They found that regardless of the origin, both phytases improved bone density, with a mean of 9% for tibia and 13% for femur compared to the control group.

Bone mineralization indexes have been studied in experimental diets with phytases, but not with chelated minerals. Thus, Kocabagli (2001) indicated that with phytase supplementation at a dose of 300 FTU/kg, the tibias of broilers have a robusticity index of 4.8 mm/g (1/3), compare to controls with values of 5.1 and 5.2 mm/g (1/3). This result, together with a higher tibiotarsal index, indicated that the bone density of chickens supplemented with phytase is higher, since this enzyme promotes the bioavailability of phosphorus and calcium, and therefore, better bone development. These results are similar to those obtained in the study by Somkuwar *et al.* (2010), in which the broilers of two phytase treatments had a lower robustness index than the controls (3.91,4.19 and 4.27 mm/g (1/3) respectively), regardless of the total phosphorus of the formulated diet. In addition, Aguilar *et al.* (2018) found a lower rate of robustness in broilers supplemented with phytases, regardless of the type and dose used. Finally, they found that the Quetelet index showed no statistical differences between the control and phytase treatments, but the Seedor index was higher (56.23 mg/mm) in the treatment with microbial phytase.

CONCLUSIONS

In conclusion, supplementation with phytase and chelated minerals in the diets of broiler chickens improved productive performance and bone mineralization, with the phytase treatment achieving better results. In addition, broilers receiving supplementation with this enzyme presented a lower pancreas weight and a greater intestine weight.

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BIBLIOGRAPHY

Acosta, A & Cárdenas M 2006, `Enzimas en la alimentación de las aves. Fitasas', *Revista Cubana de Ciencia Agrícola*, vol. 40, no. 4, pp. 377-87.

Aguilar, J, Zea, O, & Vílchez, C 2018, `Rendimiento productivo e integridad ósea de pollos de carne en respuesta a suplementación dietaria con cuatro fuentes de fitasa comercial', *Revista de Investigaciones Veterinarias del Perú*, vol. 29, no.1, pp. 169-79.

Ahmad, T, Rassol, S, Sarwar, M, Haq, A, & Hasan, Z 2000, `Effect of microbial phytase produced from a fungus Aspergillus niger on biovailability of phosphorus and calcium in broiler chickens', *Animal Feed Science and Technology*, vol. 83, no. 2, pp. 103-14.

Akyurek, H, Ozduven, M.L, Okur, A.A, Koc, F, & Samli, H.E 2011, `The effect of supplementing an organic acid blend and/or microbial phytase to a corn-soybean based diet fed to broiler chickens', *African Journal of Agricultural Research*, vol. 6, no.3, pp. 642-49.

Applegate, T.J & Lilburn, M.S, 2002, `Growth of the Femur and Tibia of a Commercial Broiler Line', *Poultry Science*, vol. 81, no.9, pp. 1289-94.

Brito, A.B, Stringhini, M.B, Café, M.B, Xavier, S.A.G, Muramatsu, K, & Andrade, M.A 2004, `Níveis de metionina + cistina em rações de frangos de corte na fase pré-inicial (1-7 dias)', *Ars Veterinaria*, vol. 20, no. 1, pp. 009-015

Camiruaga, M, Garcia, F, Elera, R, & Simonetti, C 2001, `Respuesta productiva de pollos broilers a la adición de enzimas exógenas a dietas basadas en maíz o triticale', *Ciencia e investigación agraria,* vol. 28, no. 1, pp. 23-36.

Chung, T.K, Ruhterfurd, S.M, Thomas, D.V, & Moughan, P.J 2013, `Effect of two microbial on mineral availability and retention and bone mineral availability and retention and bone mineral density in low-phosphorus diets for broilers', *British Poultry Science*, vol.54, no. 3, pp. 362-73.

Cobb Vantress, Cobb 500 2018 `Suplemento informativo sobre rendimiento y nutrición de pollos de engorde'.

Ferket, P.R, Oviedo-Rondón, E.O, Mente, P.L, Bohórquez, D.V, Santos, A.A, Grimes, J.L, & Felts, V 2009, `Organic trace minerals and 25-hydroxycholecalciferol affect performance characteristics, leg abnormalities, and biomechanical properties of leg bones of turkeys', *Poultry Science*, vol. 88, no.1, pp. 118–31.

Gallardo, C, Dadalt, J.C, Trindade, M.A 2018, `Nitrogen retention, energy, and amino acid digestibility of wheat bran, without or with multicarbohydrase and phytase supplementation, fed to broiler chickens', *Journal of Animal Science*, vol. 96, no. 6, pp. 2371-79.

Kocabagli, N 2001, `The effect of dietary phytase supplementation at different levels on tibial bone characteristics and strength in broilers' *Turkish Journal of Veterinary and Animal Sciences*, vol. 25, no.5, pp. 797-902.

Kornegay, E.T 2001 `Digestion of phosphorus and other nutrients: the role of phytases and factors influencing their activity' En: Bedford MR, Partridge GG. Enzymes in farm animal nutrition. Wallingford, Cab Publishing, pp. 432.

Lan, G.Q, Abdullah, N, Jalaudin, S, Ho, Y.W 2002, `Efficacy of supplementation of a phytase-producing bacterial culture on the performance and nutrient use of broiler chickens fed corn-soybean meal diets', *Poultry Science*, vol. 81, no. 10, pp. 1522-32.

Maenz, D.D, Irish, G.G, & Classen, H.L 1999 `Carbohydrate-binding and agglutinating lectins in raw and processed soybean meals', *Animal Feed Science and Technology*, vol. 76, no.3-4, pp. 335-43.

Manangi, M.K, Vazquez-Añon, M, Richards, J.D, Carter, S, Buresh, R.E, & Christensen, K.D 2012, `Impact of feeding lower levels of chelated trace minerals versus industry levels of inorganic trace minerals on broiler performance, yield, footpad health, and litter mineral concentration', *The Journal of Applied Poultry Research*, vol. 21, no. 4, pp. 881-90.

Momeneh, T, Karimi, A, Sadeghi, G, Vaziry, A, & Bedford, M.R 2018, `Evaluation of dietary calcium level and source and phytase on growth performance, serum metabolites, and ileum mineral contents in broiler chicks fed adequate phosphorus diets from one to 28 days of age', *Poultry Science*, vol. 97, no. 4, pp.1238-89.

Mutus, R, Kocabagli, N, Alp, M, Acar, N, Eren, M, & Gezen, S, 2006, `The Effect of Dietary Probiotic Supplementation on Tibial Bone Characteristics and Strength in Broilers', *Poultry Science*, vol. 85, no. 9, pp. 1621-25.

Nollet L, Van der Klis JD, Lensing M, & Spring, P 2007, `The effect of replacing inorganic with organic trace minerals in broiler diets on productive performance and mineral excretion', *The Journal of Applied Poultry Research*, vol. 16, no. 4, pp.592-97.

Onyango, E.M, Hester, P.Y, Stroshine, R, & Adeola, O 2003, `Bone Densitometry as an Indicator of Percentage Tibia Ash in Broiler Chicks', *Poultry Science*, vol. 82, no. 11, pp. 1787-91.

Pessôa, G.B, Tavernari, F.C, Alves, R, & Albino, L.F 2012, `Novos conceitos em nutrição de aves', *The Revista Brasileira de Saúde e Produção Animal*, vol. 13, no. 3, pp. 755–74.

Pirgozliev, V, Oduguwa, O, Acamovic, T, & Bedford, M.T 2009 `Effects of dietary phytase on performance and nutrient metabolism in chickens', *Brish Poultry Science*, vol. 49, no. 2, pp. 144-54.

Ptak, A, Bedford, M.R, Świątkiewicz, S, Żyła, K, & Józefiak, D 2015, `Phytase Modulates Ileal Microbiota and Enhances Growth Performance of the Broiler Chickens', *PloS One*, vol. 10, no. 3, pp. e0119770.

Ravindran, V, Kornegay, E.T, Potter, L.M, Ogunabameru, O.B, Welton, M.K, Wilson, J.H, & Potchanakorn, M 1995, `An evaluation of various response criteria in assessing biological availability of phosphorus for broilers', *Poultry Science*, vol. 74, no.11, pp. 1820-30.

Reisenfield, A 1972, `Metatarsal Robusticity in Bipedal Rats', *The American Journal of Physical Anthropology*, vol. 36, no.2, pp. 229-34.

Rostagno, H.S, Teixeira, L.F, Hannas, M.I, Donzele, J.L, Sakomura, N.K, Perazzo, F.G, Saraiva, A, Teixeira, M.L, Borges, P, De Oliveira, R.F, De Toledo, S.L, & De Oliveira, C 2017, `Tablas brasileñas para aves y cerdos: Composición de alimentos y requerimientos nutricionales. 4a ed., Viçosa, Brasil: Universidad Federal de Viçosa.

Rutten, M, Leterrier, C, Constantin, P, Reiter, K, & Bessei, W 2002, `Bone development and activity in chickens in response to reduced weight-load on legs', *Animal Research*, vol. 51, vol. 4, pp. 327-36.

Santos, T.T, Srinongkote, S, Bedford, M.R, & Walk, C.L 2013 `Effect of high phytase inclusion rates on performance of broilers fed diets not severely limited in available phosphorus', *Asian Australasian Journal of Animal Sciences*, vol. 26, no.2, 227-32.

Statistical Analisys System 2012, `SAS/STAT User's Guide. Proceeding Version 9.3. SAS Institute Incorporation. Cary, NC, USA.

Sebastian, S, Touchburn, S.P, Chavez, E.R, & Lague, P. C 1997, `Apparent digestibility of protein and amino acids in broiler chickens fed a corn-soybean diet supplemented with microbial phytase', *Poultry Science*, vol. 76, no.12, pp. 1760-69.

Seedor, J.G, Quartuccio, H.A, & Thompson, D.D 1991, `The Bisphosphonate Alendronate (MK.-217) Inhibits Bone Loss Due to Ovariectomy in Rats', *Journal of Bone and Mineral Research*, vol. 6, no.4, pp. 339-46.

Selle, P.H & Ravindran, G 2007, `Microbial phytase in poultry nutrition', *Animal Feed Science and Technology*, vol. 135, no. 1-2, pp. 1-41.

Selle, P.H, Walker, A.R, & Bryden, W.L 2003, `Total and phytate-phosphorus contents and phytase activity of Australian sourced feed ingredients for pigs and poultry', *Australian Journal of Experimental Agriculture*, vol. 43, no. 5, pp. 475–479.

Somkuwar, A.P, Ravikanth, K, Maini, S, Rekhe, D.S, Patil, M.K, & Ballurkar, B 2010, `Phytase with Synergistic Herbs: An Option to Reduce Environmental Pollution by Partial Replacement of Inorganic Phosphorus in Broiler Ration', *International Journal of Poultry Science*, vol. 9, no. 4, pp. 390-394.

Térreas, J.C, Sandoval, G.L, Fernández, R.J, Revidatti, F.A, Barcht, A, Campos, M.V, & Dellamea, S 2000, `Efectos de la suplementación con quelatos orgánicos sobre el rendimiento productivo de pollos parrilleros en una zona subtropical', *Revista Veterinaria de Mexico,* vol. 31, no, 2, pp. 95-99.

Walk, C.L & Olukosi, O.A 2019, `Influence of graded concentrations of phytase in high-phytate diets on growth performance, apparent ileal amino acid digestibility, and phytate concentration in broilers from hatch to 28 D post-hatch', *Poultry Science*, vol. 98, no. 9, pp. 3884-3893.

Wang, W, Wang, Z, Yang, H, Cao, Y, Zhu, X, & Zhao, Y 2013, `Effects of phytase supplementation on growth performance, slaughter performance, growth of internal organs and small intestine, and serum biochemical parameters of broilers', *Journal of Animal Science*, vol. 3, no. 3, pp. 236-241.

World Organisation for Animal Health (OIE) 2011, `Matanza de animales con fines profilácticos. pp. 23.

Woyengo, T.A & Nyachoti M 2013, `Review: Anti-nutritional effects of phytic acid in diets for pigs and poultry current knowledge and directions for future research', *Canadian Journal of Animal Science*, vol. 93, no. 1, pp. 9-21.