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Use of Phytase and Chelated Mineral Supplementation on Productive Performance, Organs Weight and Indicators of Bone Mineralization in Broiler Chickens

(Uso de fitasas y minerales quelatados en el desempeño productivo, peso de órganos e indicadores de mineralización ósea en pollos de engorde)

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Abstract

The broiler chickens diets are formulated based on corn and soybeans, which have phytic acid; that inhibits the absorption of minerals and cannot be degraded by birds. Phytases can degrade phytic acid and improve mineral absorption, while chelated minerals are bound to organic compounds. The aim of this study was to evaluate the use of phytase and chelated minerals supplementation on the productive performance, organs weight, and bone mineralization indicators in chickens from 0 to 42 days of age. Three treatments (Control diet, diet plus phytase and diet plus chelated minerals) were performed with 4 replicates per treatment and 20 chickens per replicate. The study was divided into three phases: start, growth, and finisher. Productive performance was evaluated by weight gain, feed intake, feed conversion ratio, and viability. At the end of each phase, the gastrointestinal organs and to evaluate indicators of bone mineralization of the left tibias were evaluate. Variance analysis of the data was performed, considering the significant differences of the DLS, test. Significant differences ($p < 0.05$) were found in the productive performance of chickens in favor of phytase, except in feed intake ($p > 0.05$). The birds with phytase supplementation showed a significantly ($p < 0.05$) lower relative pancreas weight and higher intestine weight. At 42 days of age, differences were observed in bone density ($p < 0.05$) and the Seedor index ($p < 0.05$) in birds supplemented with phytase. Phytase supplementation in broiler chickens 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 concerning these variables.

Key word: broiler chickens ǀ chelated minerals ǀ enzymes ǀ mineralization ǀ organs ǀ phytase

Resumen

Las dietas de los pollos de engorde están formuladas principalmente a base de ingredientes vegetales, como el maíz y la soya, los cuales poseen ácido fítico, que impide el aprovechamiento de minerales y no puede ser degrado por los pollos de engorde. Las fitasas son capaces de degradarlo y mejorar la absorción de minerales, mientras que los minerales quelatados son minerales unidos a un compuesto orgánico. El objetivo de este studio fue evaluar la suplementación de fitasas y minerales quelatados en el desempeño productivo, peso de órganos y los indicadores de mineralización ósea en pollos de engorde de 0 a 42 días de edad. Se realizaron 3 tratamientos (Dieta control, dieta más fitasa y dieta más minerales quelatados) con 4 repeticiones por tratamiento y 20 pollos por repetición, El estudio se dividió en tres fases: inicio, crecimiento y acabado. Se evaluó el desempeño productivo mediante la ganancia de peso, consumo de alimento, conversión alimenticia y viabilidad. Al concluir cada fase se realizaron necropsias para pesar órganos gastrointestinales y se evaluaron indicadores de mineralización ósea de las tibias izquierdas. Se realizó el Análisis de Varianza de los datos, considerando las diferencias significativas mediante la prueba de de DLS. Se encontraron diferencias significativas ($p < 0.05$) en el desempeño productivo de pollos suplementados con fitasa, excepto en el consumo de alimento ($p > 0.05$). En los tratamientos con fitasa el peso relativo del páncreas fue significativamente más bajo ($P < 0.05$), mientras que el peso del intestino fue estadísticamente mayor ($p < 0.05$). Finalmente, a los 42 días de edad, se observó diferencias estadísticas en la densidad ósea ($p < 0.05$) y el índice de Seedor ($p < 0.05$) favor de las aves al suplementadas con fitasa. La suplementación de fitasas en pollos de engorde mejoró el desempeño productivo, redujo el peso de páncreas e incrementó el peso del intestino y mejoró la mineralización ósea; siendo más eficiente que los minerales quelatados con respecto a estas variables.

Palabras claves: pollos de engrode l minerales quelatados l enzimas l mineralización l órganos l fitasa

Introduction

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 chickens unable to hydrolyze due to insufficient enzyme activity. So, the complexes formation in neutral pH in the small intestine, together with a decrease in the reabsorption of endogenous minerals, due to the absorption reduction 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, feedd efficiency, and nutrient utilization in chickens (Walk and Olukosi, 2019). Also, 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).

Additionally, 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 birds (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 birds 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.

Materials and methods

All the procedures were approved by the Institutional Committee of Ethics in Animal Research and Biodiversity of the Universidad Cientifica del Sur (CIEI-AB-CIENTÍFICA) with the registration code 143-2019-PRE16.

Experimental design, animals and diets

For the study, 240 male broiler chickens (Cobb 500) were distributed in 3 treatments (control diet, phytase and chelated minerals), with 4 replicates and 20 birds per replicate. Water and feeding were provided ad libitum. The diets were formulated according to the nutritional requirements of Rostagno *et al*. (2017) and the Cobb 500 Management Guide (2018) (Table 1).

Table 1. Basal diets given to broiler chickens at the different stages of evaluation[1]

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
Common 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
Calculated nutritional composition			
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 25g/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 mcg / 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.*

Phytases and chelated minerals

Phytases and chelated minerals were added to the diet considering in accordance with the total formulated diet. The phytase used in the present study was a commercial product derived from modified *Escherichia coli* expressed in Trichoderma reesei, 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 25 g/t of ration. The activity of enzymes in the diets was guaranteed by analysis by the Laboratorio Biovet S.A (Tarragona, Spain).

Performance, organs weight and bone mineralization indicators

The birds and feed were weighed weekly with an electronic platform scale BC30N model with 1 g precision (Henkel, China). The viability was calculated daily.

At the end of each productive stage, one broiler per repetition 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 Organization 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. Finally, the relative weight was calculated by determining the relationship between the absolute weight of each organ and the live weight of the animal.

The left tibia of the animals sacrificed for organ weighing 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).

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 craniocaudal (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 before submersion in water to allows water to penetrate the porous interior (Onyango *et al.*, 2003)

Bone mineralization index: 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, determining the quotient between the fresh bone weight 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

Data were analyzed by one-way analysis of variance (ANOVA), using the generalized linear model (GLM) procedure of the Statistical Analysis System (SAS, version 9.3). For the comparison of treatments, the LSD means comparison test was performed. The data on the percentage of viability and relative weight of organs were transformed to Arcoseno values for analysis of variance.

Results

Productive performance

In table 2 are show the results of performance. There were significant differences ($p < 0.05$) among the treatments to the final weight, weight gain, feed conversion rate, and viability in the chickens supplemented with phytase and chelated minerals. The weight gain and the feed conversion ratio were significantly affected ($p < 0.05$) at 28 and 42 days of age, and when evaluating 0 to 42 days. Compared to the control group, the animals supplemented with phytase presented a more significant weight gain, followed by the birds supplemented with chelated minerals. The control birds presented the highest feed conversion rate compared to the other treatments. The viability of the birds was significantly affected ($p < 0.05$) in the initial phase.

Table 2. Average weight gain, feed intake, feed conversion rate and percentage of viability in broiler chickens supplemented with phytase and chelated minerals in different growth stages

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

Organs weight

The relative organs 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 3).

Table 3. Average of relative organs weight (%) in broiler chickens supplemented with phytase and chelated minerals in different growth phases.

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

Indicators of bone mineralization

In Table 4 are shown the results of the bone mineralization indicators. 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 tibia weight in birds fed phytase and chelated minerals. Birds supplemented with phytase had ($p < 0.05$) a higher bone density, Seedor, and Quetelet indexes, followed by the birds supplemented with chelated minerals. 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.

Table 4. Indicators of bone mineralization in the left tibia of broiler chickens supplemented with phytase and chelated minerals in different growth phases.

	Treatments[1]			SEM[2]	P-Value
	Control	Phytase	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	179.87	<0.001
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
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
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: Control diet; Treatment 2: diet + phytase with activity of 1500 FTU, at a dose of 25 g/t; Treatment 3: diet + chelated minerals at doses of 25 g/t.*
[2]***SEM:*** *Standard error of the mean*
[a,b,c] *Values with different letters in the same row indicate significant differences (p <0.05) in the DLS test.*

Discussion

The higher body weight observed in the phytase treatment compared to the control group can be partially explained by a better feed conversion rate in favor of this 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 more considerable amounts at the intestinal level. 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 birds. 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. According to Ravindran *et al.* (1995), the enzymatic activity of phytases increases digestibility of crude protein by 2.4% and energy by 3.9%.

Ptak *et al.* (2015) verified the effect of phytase at 5000 FTU/kg in broiler chickens, which showed an increase in weight gain, feed intake and a reduction in the feed conversion ratio in the start stage (1 to 14 days). This trend was maintained in the growth stage (15 to 21 days), except that phytase supplementation had no impact on feed intake. Finally, in the finishing stage (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 stage.

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 animals.

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 broiler chickens in any of the production stages. These results are similar to those of Manangi *et al.* (2012), who concluded that although there is no improvement in the 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.

The lower weight of the pancreas might indicate that phytases likely improve digestion. Thus, the bolus of food could be simple 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 higher. This could be related to the more excellent 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 an excellent 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.*, 2007).

According to Pirgozliev *et al.* (2009), phytase supplementation does not affect the intestine weight of broiler chickens, 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 chickens could increase by adding phytases to the diets.

Bone mineralization indicators were better in birds with phytase suplementation followed by 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 broiler chickens 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 broiler chickens 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 leads to improvements in weight, density, and resistance to bone breakage (Ahmad *et al.*, 2000). The effects of the addition of chelated minerals to the diet of broiler chickens were not as marked compared to phytase, possibly because microminerals are distributed to a lesser extent in the bone matrix.

Chung *et al.* (2013) evaluated the productive response and bone density in broiler chickens 1 to 42 days of age supplemented with two different types of phytases from bacteria and 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 birds 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 broiler chickens 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.

Besides, Aguilar *et al.* (2018) found a lower rate of robustness in broiler chickens 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.

In conclusion, supplementation with phytase and chelated minerals in the diets of broiler chickens improved productive performance and bone mineralization, concluding than the phytase treatment achieved better results than chelated minerals. Also, the chickens receiving supplementation with this enzyme presented a lower pancreas weight and a higher intestine weight.

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