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Multi-enzyme supplementation in diets containing sunflower cake for pullets: Mineral digestibility and bone quality

Suplementação multienzimática em dietas contendo torta de girassol para frangas: Digestibilidade de minerais e qualidade óssea

Ednardo Rodrigues Freitas[1], Otoniel Félix de Souza[2], Rafael Carlos Nepomuceno[1], Francislene Silveira Sucupira[4*], Davyd Herik Souza[3], Germano Augusto Jerônimo do Nascimento[4]

ABSTRACT

Sunflower cake is an alternative feed with nutritional potential to be used in poultry feeding. However, the high fiber content and the presence of antinutritional factors can compromise the availability of nutrients in the diet and influence the bone development of pullets, which can be mitigated with the dietary inclusion of exogenous enzymes. In this context, the objective of this study was to evaluate the effect of multi-enzyme supplementation in rations containing sunflower cake to pullets on mineral digestibility and quality and bone mineral composition. A total of 1020 pullets 7-weeks-age from the commercial strain Hy-Line, 540 white pullets and 480 brown pullets, were distributed in a randomized experimental design in a 2×2+1 factorial arrangement, with two sunflower cake levels (10 and 20% for white pullets and 12 and 24 % for brown pullets); two ways of enzymatic supplementation (absence and presence of multi-enzyme complex), and one control diet based on corn and soybean meal, totaling five treatments with six replicates of 18 white pullets and 16 brown pullets. Between week 14 and week 15, a metabolic trial was performed to determine the digestibility coefficient of ration minerals. To assess bone quality of the tibia and femur, one pullet of the 12th and 17th week of age was selected and killed. There was no significant interaction between the tested factors (sunflower cake x multi-enzyme complexes) for any of the variables studied. The inclusion level of sunflower cake did not influence the growth and bone quality parameters of the pullets during the growing phase. However, a presence of the multi-enzyme complex provided significant improvements in Ca, P and Mg digestibility coefficient. It is concluded that the multi-enzyme supplementation in the diets increases the digestibility of the mineral in the rations, contributing to the maintenance of bone quality of pullets fed with up to 24% sunflower cake.

Index terms: Digestibility; exogenous enzymes; bone; bone mineralization; sunflower by-product.

RESUMO

A torta de girassol é um alimento alternativo com potencial nutricional para ser usado na alimentação de aves. Contudo, o elevado teor de fibras e a presença de fatores antinutricionais pode comprometer a disponibilidade dos nutrientes da dieta e influenciar o desenvolvimento ósseo de frangas, o que pode ser mitigado com a inclusão dietética e enzimas exógenas. Nesse contexto, o objetivo da pesquisa foi avaliar o efeito da suplementação multienzimática em rações contendo torta de girassol para frangas sobre a digestibilidade dos minerais da ração e a qualidade e composição mineral óssea. Um total de 1020 frangas com 7 semanas de idade da linhagem comercial Hy-Line, 540 frangas leves e 480 frangas semipesadas, foram distribuídas em delineamento experimental inteiramente casualizado em esquema fatorial 2×2+1, sendo dois níveis de torta de girassol (10 e 20% - para frangas brancas e 12 e 24% para frangas vermelhas), duas formas de suplementação enzimática (com e sem complexo enzimático) e um tratamento controle à base de milho e farelo de soja, totalizando cinco tratamentos com seis repetições de 18 frangas brancas e 16 frangas vermelhas. Entre a 14ª e 15ª semana, foi realizado ensaio de metabolismo para determinar o coeficiente de digestibilidade dos minerais das rações. Para avaliação da qualidade óssea da tíbia foi selecionada e abatida uma ave por parcela na 12ª e na 17ª semana de idade. Nos dois ensaios, não houve interação significativa entre os fatores testados para todas as variáveis estudadas. A inclusão da torta de girassol não influenciou significativamente na digestibilidade dos minerais e os parâmetros de crescimento e qualidade óssea das frangas. Por sua vez, a presença do complexo multienzimático proporcionou melhora significativa na digestibilidade dos minerais, sem influenciar os parâmetros ósseos. Conclui-se que a suplementação multienzimática nas rações aumenta a digestibilidade dos minerais da ração, contribuindo para a manutenção da qualidade óssea de frangas leves e semipesadas alimentadas com até 20% e 24% de torta de girassol, respectivamente.

Termos para indexação: Digestibilidade; enzimas exógenas; osso; mineralização óssea; subproduto do girassol.

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[1]Universidade Federal do Ceará/UFC, Departamento de Zootecnia, Fortaleza, CE, Brasil
[2]Universidade Federal de Minas Gerais/UFMG, Departamento de Zootecnia, Belo Horizonte, MG, Brasil
[3]EMATERCE, Fortaleza, CE, Brasil
[4]Universidade Federal da Paraíba/UFPB, Departamento de Zootecnia, Areia, PB, Brasil

Corresponding author: sucupirafs@ufc.br

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Introduction

The inclusion of fibrous foods in growth rations for commercial layers has been common with the aim of keeping feed density low, increasing feeding time and, therefore, avoiding excessive weight gain and bird losses due to cannibalism (Guzmán et al., 2015; Mens et al., 2020; Patt et al., 2022). Sunflower cake is a fibrous by-product obtained from the mechanical pressing of sunflower seeds (*Helianthus annuus L.*) for oil extraction in the biodiesel, food and cosmetic industries (Cordeiro et al., 2022).

Sunflower cake is a protein source (22.46% to 25.08%), with 17.00 to 24.83% of ether extract, and gross energy values from

4.819 to 5.297 kcal.kg^{-1} (Pinheiro et al., 2013; Berwanger et al., 2014; Kargopoulos et al., 2017; Cordeiro et al., 2022; Souza et al., 2022). However, its use in poultry feed has been limited by low levels of lysine and high crude fibre, high non-starch polysaccharides concentrations, low level of metabolizable energy, the presence of chlorogenic acid and phytic acid (Vasudha, & Sarla, 2021; Mbukwane et al., 2022).

The fiber content of sunflower cake can vary within 12%-32%, which can limit its inclusion level in diets for poultry depending on the degree of hull removal and the method used for the oil extraction (Kargopoulos et al., 2017). According to Jacob et al. (1996), the insoluble fiber content of sunflower cake may be considered the main limiting factor for its use in the feeding of nonruminant animals. There is a possible negative effect of dietary fiber in relation to the absorption of minerals by the gastrointestinal tract, due to the formation of specific bonds (Baye, Guyot, & Mouquet-Rivier, 2015), making the minerals unavailable.

The presence of chlorogenic can promote significant loss in the quality of the protein fraction of the food in response to oxidative processes, resulting in changes in the functional properties of proteins and reduced digestibility of amino acids (Scharlack, Aracava, & Rodrigues, 2017). Phytic acid can make phosphorous unavailable, but also may reduce the bioavailability of bivalent minerals, limiting the use of 05 to 15% (Nout, 2009). Furthermore, it can also compromise the use of other nutrients, including aminoacids, carbohydrates and lipids (Humer, Schwarz, & Schedle, 2015).

Considering the aspects mentioned above, there is a concern about how the inclusion of fibrous foods can influence the use of minerals in the feed and, consequently, influence bone development and quality (Rath et al., 2000).

According to Cruz et al. (2012), the level of development and quality of the bones at the end of the growth phase are important in the formation of a modern layer and, for this, it is essential that food allows adequate bone and muscle development in the rearing phase, as change in nutrition can have direct influences on growth and quality of the bones.

Exogenous enzymes have been added in laying hens diets to minimize the possible harmful effects of the use of sunflower cake (Barbosa et al., 2014). There are several commercial enzyme products that may be added to diets to stimulate fiber digestion, mitigate the adverse effects of antinutritional factors, improve nutrient utilization and increase energy available (Alagawany & Attia, 2015; Berwanger et al., 2017).

There are studies evaluating the effect of enzymes in diets containing sunflower cake for growing layer hens, and their impact on nutrient utilization and growth. However, there is a lack of research on the effect of enzymes on bone development and quality.

This study evaluates the nutrient digestibility, mineral balance (calcium, phosphor and magnesium), mineral composition and bone quality of white layers and brown chickens feeding diets containing different levels of sunflower cake with the addition of a multi-enzyme complex.

Material and Methods

All experimental procedures were approved by the Ethics Committee on Animal Research (Federal University of Ceará, Fortaleza, Brazil) as per the ethical principles of the Brazilian Animal Experimentation Control Council (protocol N° 102/2016). In this study were used data from two experiments which performance results were published by Alencar et al. (2019) and Alencar et al. (2022).

Poultry, housing, design and experimental diets

A total of 1020 pullets from the commercial strain Hy-Line were used, 540 white pullets (Hy Line White W36®) and 480 brown pullets (Hy Line Brown®). The pullets were selected at the 7th week of age based on average initial weight (451 ± 11.95g – white pullets and 505 ± 12.06g – brown pullets) and housed in a conventional shed with galvanized wire cages (50 cm long × 50 cm wide × 45 cm high) equipped with galvanized trough feeders and nipple-type drinkers. The stocking density was 416 cm^2/pullet of the white strain and 468 cm^2/pullet brown strain.

The pullets of each strain were distributed in a completely randomized design in a 2× 2+1 factorial arrangement, including two levels of sunflower cake (SC) (10 and 20% - white layers pullets and 12 and 24% - brown pullets), two ways of enzyme supplementation (with and without multi-enzyme complex - MEC) and a control diet based on corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] meal, totaling five treatments with six replicates of 18 white pullets and 16 brown pullets.

For diets with enzyme supplementation, the contribution of the nutritional and energy matrix of each enzyme used in the formulations was considered (Table 1), to guarantee the same level of nutrients and energy in the diets of the different treatments. Enzyme supplementation was carried out using three commercial products and have been added: carbohydrase (α-galactosidase – 9.000 U per g, xylanase – 100.000 U per g and β-glucanase – 200.000 U per g); protease (1.10 U per g); alkaline serum enzyme that act analogously to trypsin, chymotrypsin and elastase; and phytase (400 FTU per kg) and phytase (400 FTU per kg). The amount indicated for each enzyme matrix was 100g per ton. Therefore, for every 100 kg of diet, 10 g of each enzyme was used, totaling 30g per 100 kg of feed.

The nutritional composition of the sunflower cake was determined in the Laboratory of Animal Nutrition of the Department of Animal Science of UFC, using the method described by Silva and Queiroz (2006), whereas metabolizable energy was obtained from the total collection of excreta in a previous metabolism assay (Sakomura & Rostagno, 2007) and calcium, available phosphorus, sodium, chlorine, potassium, digestible lysine, digestible methionine, digestible methionine + cystine, digestible threonine and digestible tryptophan were estimated based on (Fundação Espanhola para o Desenvolvimento da Nutrição Animal -FEDNA, 2010) tables, whose values are presented in Table 2.

Table 1: Nutritional and energetic contribution and multi-enzyme complex.

Nutritional Matrix	Carbohydrase[1]	Protease[2]	Phytase[3]	Total contribution
Metabolizable energy (kcal/kg)	30.000	25.000	49.000	104.000
Crude protein (%)	0.000	0.500	0.401	0.901
Calcium (%)	0.000	0.000	0.157	0.157
Digestible phosphorus (%)	0.000	0.000	0.143	0.143
Sodium (%)	0.000	0.000	0.033	0.033
Digestible methionine (%)	0.000	0.014	0.016	0.050
Methionine + digestible cystine (%)	0.000	0.024	0.036	0.060
Digestible lysine (%)	0.000	0.032	0.016	0.048
Digestible threonine (%)	0.000	0.021	0.032	0.056
Digestible tryptophan (%)	0.000	0.005	0.024	0.029
Digestible valine (%)	0.000	0.026	0.000	0.026
Digestible arginine (%)	0.000	0.033	0.000	0.033
Digestible leucine (%)	0.000	0.046	0.000	0.046

[1]Poultrygrow 250™; [2]ProFare™ EZ 309; [3]®finase EC.

Table 2: Nutritional and energetic composition of sunflower cake.

Nutrients and energy	Sunflower cake
Analyzed composition[1]	
AMEn[2] (kcal/kg)	2.774
Dry matter (%)	92.81
Crude protein (%)	19.35
Ether extract (%)	15.52
Neutral detergent fiber (%)	43.95
Acid detergent fiber (%)	28.96
Mineral matter (%)	2.66
Calcium (%)	0.28
Available phosphorus (%)	0.09
Sodium (%)	0.03
Clorine (%)	0.08
Potassium (%)	1.16
Digestible lysine (%)	0.59
Digestible methionine (%)	0.59
Digestible methionine + cystine (%)	0.68
Digestible threonine (%)	0.61
Digestible trpyptophan (%)	0.22

[1]Values obtained by the laboratory analysis; [2]Apparent metabolizable energy corrected by nitrogen balance; [3]Estimated based on FEDNA (2010) tables.

The experimental period consisted in two phases: breeding (7 to 12 weeks of age) and rearing (13 to 17 weeks of age). The experimental diets were formulated to be iso-energetic and iso-nutrient (Tables 3 and 4), according to the nutritional requirements of each strain and feedstuffs composition values proposed by Rostagno et al. (2017), except for sunflower cake.

Vaccination program and light program

Vaccination was carried out based on a program elaborated according to the sanitary situation of the region. The birds were immunized with vaccines against: Newcastle disease, developed with the HB1 virus; bronchitis, with the H120 virus; Gumboro, with the intermediate strain; coryza, with aluminum-hydroxide adjuvant; coryza, with mineral-oil adjuvant; avian poxvirus, with the strong strain; and egg drop syndrome.

During the trial period (7th to 17th weeks of age), the pullets received only natural light, approximately 12 hours of light per day.

Digestibility assay to determine mineral balance

The metabolic trial was performed between the 14th and 15th weeks of age, using the total excreta collection method. Pullets were distributed in 5 treatments, 6 replications with 4 birds for experimental unit, with 120 birds. The metabolic trial lasted 8 days, with 4 days of acclimatization and 4 days of sample collection. Aluminum trays lined with plastic were installed under the cages to collect the excreta and avoid content losses. On the first and last day of collection, 1% ferric oxide was added to the diets to identify the excreta. The excreta not marked on the first and last collections were discarded. Daily collection of excreta was performed at 08 00 h and 16 00 h. Next, the total excreta were weighed, packed in labelled plastic bags, weighed and frozen at –20ºC. The feed intake and total excreta produced during the collection period were determined at the end of each experiment.

Table 3: Composition and nutritional and energetic levels of experimental diets provided to white pullets.

Ingredients (kg)	7 to 12 weeks					13 to 17 weeks				
	0% SC[1]	10% SC	20% SC	10% SC+MEC[2]	20% SC+MEC	0% SC	10% SC	20% SC	10% SC+MEC	20% SC+MEC
Corn, grain	65.41	61.16	53.33	63.70	58.33	71.95	66.59	59.51	64.63	59.27
Soybean meal	26.97	23.32	20.32	20.93	17.50	22.59	19.15	16.03	17.57	14.14
Sunflower cake	0.00	10.00	20.00	10.00	20.00	0.00	10.00	20.00	10.00	20.00
Soybean oil	1.90	1.53	2.36	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Multi-enzyme complex	0.00	0.00	0.00	0.03	0.03	0.00	0.00	0.00	0.03	0.03
Calcitic limestone	1.12	1.07	1.02	1.14	1.09	1.20	1.14	1.09	1.21	1.15
Dicalcium phosphate	2.01	2.01	2.03	1.28	1.29	1.93	1.94	1.95	1.21	1.22
Common salt	0.38	0.38	0.38	0.30	0.30	0.41	0.40	0.40	0.33	0.33
Min. and vit. supplement[3]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
DL-methionine	0.19	0.17	0.16	0.13	0.12	0.13	0.12	0.10	0.08	0.06
L-lysine HCL (78.5%)	0.09	0.15	0.19	0.16	0.21	0.04	0.09	0.14	0.00	0.14
Washed sand (inert)	1.72	0.00	0.00	2.12	0.93	1.56	0.37	0.00	4.66	3.47
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated nutritional and energy composition										
Metabolizable energy (kcal/kg)	3000	3000	3000	2896	2896	2950	2950	2950	2846	2846
Crude protein (%)	17.55	17.55	17.55	16.65	16.65	16.00	16.00	16.00	15.10	15.10
Dry matter (%)	88.73	88.99	89.58	88.96	89.39	88.41	88.78	89.30	89.11	89.48
Digestible lysine (%)	0.89	0.89	0.89	0.84	0.84	0.75	0.75	0.75	0.70	0.70
Digestible methionine + cystine (%)	0.68	0.68	0.68	0.62	0.62	0.59	0.59	0.59	0.52	0.52
Digestible methionine (%)	0.43	0.43	0.43	0.38	0.38	0.36	0.36	0.36	0.31	0.31
Digestible threonine (%)	0.60	0.60	0.60	0.56	0.55	0.54	0.54	0.53	0.51	0.50
Digestible tryptophan (%)	0.18	0.18	0.18	0.17	0.17	0.16	0.16	0.16	0.15	0.15
Acid detergent fiber[4] (%)	4.38	7.91	11.36	7.80	11.31	4.25	7.75	11.23	7.56	11.07
Neutral detergent fiber[5] (%)	11.52	16.80	21.75	16.78	21.96	11.69	16.88	21.90	16.42	21.61
Calcium (%)	1.00	1.00	1.00	0.84	0.84	1.00	1.00	1.00	0.84	0.84
Available phosphorus (%)	0.47	0.47	0.47	0.33	0.33	0.45	0.45	0.45	0.31	0.31
Sodium (%)	0.17	0.17	0.17	0.14	0.14	0.18	0.18	0.18	0.15	0.15

[1]Sunflower cake; [2]Multi-enzyme complex; [3]Provided per kilogram of diet: vitamin A - 5,500,000 IU, vitamin B1 - 500 mg, vitamin B12 - 7,500 mcg; vitamin B2 – 2.502 mg; vitamin B6 - 750 mg; vitamin D3 - 1,000,000 IU, vitamin E - 6,500 IU; vitamin K3 - 1,250 mg; biotin - 25 mg; niacin - 17,5 g; folic acid - 251 mg; pantothenic acid – 6.030 mg; cobalt - 50 mg; copper - 3,000 mg; iron - 25 g; iodine - 500 mg; manganese – 32.5 g; selenium – 100.05 mg; zinc – 22.49 g; [4]Acid detergent fiber expressed including residual ash; [5]Neutral detergent fiber determined in a heat-stable amylase test and expressed including residual ash.

Table 4: Composition and nutritional and energetic levels of experimental diets provided to brown pullets.

Ingredients	7 to 12 weeks					13 to 17 weeks				
	0% SC[1]	12% SC	24% SC	12% SC+MEC[2]	24% SC+MEC	0% SC	12% SC	24% SC	12% SC+MEC	24% SC+MEC
Corn, grain	67.74	61.30	52.63	59.35	52.91	68.62	62.16	55.72	60.21	53.76
Soybean meal (45%)	26.66	22.54	18.83	20.96	16.84	23.30	19.31	15.24	17.71	13.65
Sunflower cake	0.00	12.00	24.00	12.00	24.00	0.00	12.00	24.00	12.00	24.00
Soybean oil	0.00	0.00	0.76	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Multi-enzyme complex	0.00	0.00	0.00	0.03	0.03	0.00	0.00	0.00	0.03	0.03
Calcitic limestone	1.27	1.20	1.14	1.27	1.20	1.19	1.13	1.07	1.19	1.13
Dicalcium phosphate	1.79	1.80	1.81	1.07	1.08	1.93	1.94	1.95	1.21	1.22
Common salt	0.38	0.38	0.38	0.30	0.30	0.41	0.40	0.40	0.33	0.33
Mineral and vitamin premix[3]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
DL-Methionine	0.17	0.15	0.13	0.11	0.10	0.10	0.08	0.06	0.04	0.03
L-Lysine HCL	0.01	0.07	0.12	0.06	0.12	0.00	0.00	0.04	0.00	0.03
Washed land (Inert)	1.78	0.36	0.00	4.65	3.23	4.25	2.77	1.32	7.08	5.62
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated nutritional and energy composition										
Metabolizable energy (kcal/kg)	2900	2900	2900	2796	2796	2850	2850	2850	2746	2746
Crude protein (%)	17.50	17.50	17.50	16.60	16.60	16.00	16.00	16.00	15.10	15.10
Dry matter (%)	88.48	88.99	89.50	89.33	89.33	88.70	89.20	89.70	89.54	90.04
Digest. lysine (%)	0.82	0.82	0.82	0.77	0.77	0.72	0.68	0.67	0.64	0.62
Digestible methionine + cystine (%)	0.66	0.66	0.66	0.60	0.60	0.56	0.56	0.56	0.50	0.50
Digestible methionine (%)	0.41	0.41	0.41	0.36	0.36	0.32	0.32	0.32	0.28	0.28
Digestible threonine (%)	0.60	0.59	0.58	0.56	0.55	0.55	0.54	0.53	0.51	0.50
Digestible tryptophan (%)	0.18	0.18	0.18	0.17	0.17	0.16	0.16	0.16	0.15	0.15
Acid detergent fiber[4] (%)	4.44	8.63	12.82	8.44	12.63	4.20	8.40	12.59	8.20	12.40
Neutral detergent fiber[5] (%)	11.76	17.90	24.04	17.45	23.59	11.40	17.56	23.70	17.10	23.25
Calcium (%)	1.00	1.00	1.00	0.84	0.84	1.00	1.00	1.00	0.84	0.84
Available phosphorus (%)	0.43	0.43	0.43	0.29	0.29	0.45	0.45	0.45	0.31	0.31
Sodium (%)	0.17	0.17	0.17	0.14	0.14	0.18	0.18	0.18	0.15	0.15

[1]Sunflower cake; [2]Multi-enzyme complex; [3]Provided per kilogram of diet: vitamin A - 5,500,000 IU, vitamin B1 - 500 mg, vitamin B12 - 7,500 mcg; vitamin B2 – 2.502 mg; vitamin B6 - 750 mg; vitamin D3 - 1,000,000 IU, vitamin E - 6,500 IU; vitamin K3 - 1,250 mg; biotin - 25 mg; niacin - 17,5 g; folic acid - 251 mg; pantothenic acid – 6.030 mg; cobalt - 50 mg; copper - 3,000 mg; iron - 25 g; iodine - 500 mg; manganese – 32.5 g; selenium – 100.05 mg; zinc – 22.49 g; [4]Acid detergent fiber expressed including residual ash; [5]Neutral detergent fiber determined in a heat-stable amylase test and expressed including residual ash.

For the analysis, the excreta were thawed at room temperature and homogenized. A sample was then taken and pre-dried in a forced-air oven at 55 ºC for 72 h. Subsequently, the samples were ground through a knife mill with a 16-mesh sieve with 1-mm pores. Samples of excreta were dried and analyzed for dry matter (DM) according to the methodology described by (Association of official analytical chemists - AOAC, 2005).

For mineral analysis of excreta and feed samples, a mineral solution was prepared using a mixture of nitric acid (HNO_3 65%) and perchloric acid ($HClO_4$ 72%), in a 3:1 ratio, according to the methodology described by Silva et al. (2009). The reading of the minerals was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES), using Perkin Elmer simultaneous spectrometer (Otima 4300DV).

The mineral content of the feed and excreta was expressed in g/kg. The amount of calcium, phosphorous and magnesium was obtained through total feed intake multiplied by the content of the respective mineral in the feed. The amount of calcium, phosphorus and magnesium in excreta was obtained from the amount of total excreta produced multiplied by the content of each mineral in the excreta.

After obtaining the results, the individual balance of calcium, phosphorus and magnesium was calculated based on the difference between the amount excreted.

Bone quality assessment

The bone chosen for analysis was the tibia due to its representative appearance, size and ease of removal. At the end of the 17th week of age, one pullet per experimental unit were selected, based on the mean weight, and after fasting for 6 h, they were stunned by electronarcosis, slaughtered, plucked, and eviscerated. Subsequently, the thigh and drumstick were removed, identified, and frozen at -20 °C, thus remaining until the moment of boning.

To carry out boning, pieces were initially defrosted in a domestic refrigerator (temperature of 4 °C for 12 hours) and left on benches until the material reached room temperature. Subsequently, the pieces were immersed in boiling water for 10 minutes and then deboned with a scalpel.

After boning, the length and diameter of the right femur were measured by a digital caliper, and the weight was determined on a precision scale (0.01 g). Bone density (mg/mm) was calculated using the Seedor index, obtained by dividing the weight (mg) by the length (mm) of the evaluated bone (Seedor, Quartuccio, & Thompson, 1991).

The parameters of bone resistance and bone deformity was assessed for the left bone, where the resistance and deformity were determined in the bone in natura with the aid of a Testo/Ronald Triaxial mechanical press (Ronald Top Ltda., Brazil) with a 150-kg capacity. The bones were placed horizontally on a wooden support and a force applied to the center of each bone. The maximum amount of force applied to the bone before breaking was the breaking resistance (kgf/cm^2), and the amount of force at the time the bone broke was the deformity (mm).

The bones used in bone quality tests were used in the analysis to determine dry matter and mineral matter and mineral composition. The chemical composition of the bones was determined at the Animal Nutrition Laboratory (LANA) of the Department of Animal Science of the Federal University of Ceará. The bones were weighed and dried in a forced ventilation oven at 55 °C for 72 h. Subsequently, they were removed from the oven and degreased in a Soxhlet extractor for eight hours, returning to a forced ventilation oven at 55 °C for another 72 hours, being weighed again to obtain the degreased pre-dried matter. After weighing, the bones were crushed in a ball mill, and the samples ground and placed in duly labelled plastic bags for the dry matter (DM), mineral matter (MM) and calcium, phosphorus and magnesium content using the same analysis procedures adopted for feed and excreta samples.

Statistical Analysis

The data were analyzed by the ANOVA procedure of the SAS software (SAS, 2000), according to a factorial model 2× 2+1. Means comparison was performed using the F test at 5% probability. Dunnet's test (5%) was also applied to compare the other treatments to the control treatment.

Results and Discussion

Digestibility coefficient and mineral balance

According to the metabolic trial, the results for digestibility coefficient and calcium, phosphorus and magnesium balance (Table 5 and 6) indicate that there were no significant differences among the control treatment and the treatments that included sunflower cake with or without multi-enzyme complex. There was also no significant interaction between sunflower cake levels and the enzyme supplementation tested. When comparing the levels of sunflower cake, there was no significant difference in the variables studied, indicating that the inclusion of up to 20% in the diet of white birds and 24% in the diet of brown birds did not compromise the use of calcium, phosphorus and magnesium. It was observed that the presence of the multi-enzyme complex in diets with sunflower cake resulted in increases in the digestibility coefficients of calcium, phosphorus and magnesium, without affecting the balance of these minerals.

The highest apparent digestibility coefficients of minerals determined for the treatments that had the multi-enzyme complex supplementation can be associated to the combined effect of the enzymes carbohydrase, protease and phytase. The hydrolytic action of the multi-enzyme complex present in the feed allows the breakdown of the PNA structure of the vegetable cell wall and the phytate molecule, making it possible to increase the efficiency in the use of phytic phosphorus and other complexed minerals (Choct, 2006; Olukosi, Cowieson, & Adeola, 2007).

Table 5: Digestibility coefficient and mineral balance of the experimental diets for white replacement pullets containing sunflower cake and multi-enzyme complex.

Diets	CADCa[1] (%)	CaB[2] (g)	PADC[3] (%)	PB[4] (g)	MgADC[5] (%)	MgB[6] (g)
0% SC[7]	46.92	4.99	47.25	2.61	24.73	0.31
10% SC	44.44	4.61	44.53	2.53	25.48	0.35
20% SC	44.04	4.99	44.65	2.78	27.26	0.42
10% SC+MEC[8]	50.97	4.94	49.73	2.60	30.10	0.41
20% SC+MEC	50.49	5.25	48.36	2.53	29.33	0.41
Mean	47.37	4.95	46.91	2.61	27.38	0.38
SEM[9]	0.766	0.087	0.598	0.041	0.859	0.015
Sunflower cake levels						
10%	47.71	4.77	47.13	2.57	27.79	0.38
20%	47.26	5.12	46.51	2.66	28.30	0.42
Multi-enzyme complex						
Absence	44.24b	4.80	44.59b	2.66	26.37b	0.39
Presence	50.73a	5.09	49.05a	2.57	29.72a	0.41
ANOVA[10]	*p-value*					
Diets	0.3457	0.2476	0.2570	0.3030	0.2035	0.0531
SC	0.6729	0.0917	0.5851	0.3098	0.8033	0.2629
MEC	0.0001	0.1398	0.0011	0.3098	0.1167	0.4096
SC × MEC	0.9681	0.8409	0.5161	0.0760	0.5370	0.3160

[1]Calcium apparent digestibility coefficient; [2]Calcium balance; [3]Phosphorous apparent digestibility coefficient; [4]Phosphorous balance; [5]Magnesium apparent digestibility coefficient; [6]Magnesium balance; [7]Sunflower Cake; [8]Multi-enzyme complex; [9]Standard error of the mean; [10]Analysis of variance (p<0.05); *Different from the 0% SC treatment by Dunnett test; Means followed by different letters in the in the column differ by the F test at 5% probability.

Table 6: Digestibility coefficient and mineral balance of the experimental diets for replacement brown pullets containing sunflower cake and multi-enzyme complex.

Diets	CADCa[1](%)	CaB[2] (g)	PADC[3] (%)	PB[4] (g)	MgADC[5] (%)	MgB[6] (g)
0% SC[7]	45.93	5.04	42.55	3.07	35.37	0.642
12% SC	44.43	4.78	43.60	3.09	35.62	0.662
24% SC	43.73	5.22	41.13	3.26	34.59	0.722
12% SC+MEC[8]	48.88	5.32	48.25	3.08	41.56	0.680
24% SC+MEC	47.38	5.19	48.71	3.27	41.24	0.720
Mean	46.07	5.11	44.85	3.15	37.68	0.69
SEM[9]	0.492	0.071	0.693	0.038	0.801	0.013
Sunflower cake levels						
10%	46.66	5.05	45.92	3.09	38.59	0.67
20%	45.56	5.20	44.92	3.26	37.91	0.72
Multi-enzyme complex						
Absence	44.08b	5.00	42.36b	3.17	35.11b	0.69
Presence	48.13a	5.25	48.48a	3.18	41.40a	0.70
ANOVA[10]	*p-value*					
Diets	0.3985	0.1404	0.1751	0.2519	0.6596	0.2037
SC	0.1307	0.3346	0.2124	0.1462	0.6034	0.1046
MEC	0.0001	0.1231	0.0001	0.9712	0.0002	0.7866
SC × MEC	0.5690	0.0852	0.0766	0.0852	0.7844	0.7352

[1]Calcium apparent digestibility coefficient; [2]Calcium balance; [3]Phosphorous apparent digestibility coefficient; [4]Phosphorous balance; [5]Magnesium apparent digestibility coefficient; [6]Magnesium balance; [7]Sunflower Cake; [8]Multi-enzyme complex; [9]Standard error of the mean; [10]Analysis of variance (p<0,05); *Different from the 0% SC treatment by Dunnett test; Means followed by different letters in the in the column differ by the F test at 5% probability.

The increase observed in digestibility coefficients was also observed by Tavernari et al. (2008) who evaluated the inclusion of sunflower cake (0 and 20%) with and without enzyme supplementation (cellulase, β-glucanase, xylanase and phytase) in diets for broiler chicken with no changes in the nutritional levels and verified a significant increase in the digestibility coefficient of calcium and phosphorus.

The improvement in the digestibility coefficients of calcium, phosphorus and magnesium in the diet through the supplementation of exogenous enzymes contributes to the reduction of the inclusion of mineral sources in the formulations. According to Matos (2008) the animal organism can benefit from the greater quantity of these minerals available for various organic functions in which they are required, whether in the homeostasis of bone tissue, participation in the stimulation of the nervous system, muscle contraction, blood coagulation, eggshell calcification and regulation of hormonal secretions.

The results of bone mineralization observed for both experiments (Table 7 and 8) indicate that there was no significant difference between the control treatment in relation to the treatments that included sunflower cake with or without enzyme supplementation for the percentage of dry matter, ash, calcium, phosphorus and magnesium in the bone. There was no significant interaction between the levels of sunflower cake and enzyme supplementation. When comparing the levels of sunflower cake or enzyme supplementation, there was no significant difference for the variables analyzed.

According to Bromfield et al. (2021), bone mineral concentration responded to enzyme supplementation mainly due to the presence of phytase in the composition. This enzyme was also present in the multi-enzyme complex evaluated in this research and the positive effect of enzyme supplementation on mineral digestibility and bone mineralization in diets containing sunflower cake are similar to those reported by Kim et al. (2018), that the addition of the enzyme phytase to broiler chicken feed containing sunflower meal provided a higher ash content in the tibia.

Table 7: Tibia bone mineral composition of white replacement pullets fed diets containing sunflower cake and supplemented with multi-enzyme complex.

Diets	Dry matter (%)	Mineral matter (%)	Calcium (g/kg)	Phosphorous (g/kg)	Magnesium (g/kg)
0% SC[1]	60.51	57.89	240.15	102.16	3.55
10% SC	63.11	57.61	238.29	101.82	3.56
20% SC	61.99	57.77	234.92	100.98	3.69
10% SC+MEC[2]	61.82	56.85	247.82	105.73	3.70
20% SC+MEC	59.90	56.52	243.55	104.76	3.68
Mean	61.47	57.33	240.95	103.09	3.63
SEM[3]	0.666	0.319	2.838	1.209	0.045
			Sunflower cake levels		
10%	62.47	57.23	243.06	103.78	3.63
20%	60.95	57.14	239.24	102.87	3.68
			Multi-enzyme complex		
Absence	62.55	57.69	236.61	101.40	3.69
Presence	60.86	56.68	245.70	105.25	3.62
ANOVA[4]			p-value		
Diets	0.6065	0.6118	0.6909	0.7171	0.7313
SC	0.3690	0.9040	0.6057	0.7761	0.6728
MEC	0.3188	0.1667	0.2290	0.2343	0.5780
SC × MEC	0.8122	0.7640	0.9518	0.9826	0.5117

[1]Sunflower Cake; [2]Multi-enzyme complex; [3]Standard error of the mean; [4]Analysis of variance (p<0,05).

Table 8: Tibia bone mineral composition of brown replacement pullets fed diets containing sunflower cake and supplemented with multi-enzyme complex.

Diets	Dry matter (%)	Mineral matter (%)	Calcium (g/kg)	Phosphorous (g/kg)	Magnesium (g/kg)
0% SC[1]	48.15	56.96	240.82	102.63	3.44
12% SC	47.26	56.79	249.53	103.91	3.35
24% SC	46.23	56.50	256.68	108.50	3.59
12% SC+MEC[2]	46.93	57.41	247.82	99.37	3.70
24% SC+MEC	44.62	57.95	235.99	98.88	3.52
Mean	46.64	57.12	246.17	102.66	3.52
SEM[3]	0.8877	0.2813	5.6052	2.402	0.0742
			Sunflower cake levels		
10%	47.10	57.10	248.68	101.64	3.52
20%	45.43	57.22	246.33	103.69	3.55
			Multi-enzyme complex		
Absence	46.75	56.68	253.11	106.21	3.47
Presence	45.77	57.68	241.91	99.12	3.61
ANOVA[4]			*p-value*		
Diets	0.8065	0.5487	0.8305	0.7489	0.6466
SC	0.4463	0.8296	0.8587	0.7181	0.8805
MEC	0.6550	0.0733	0.4003	0.2217	0.4230
SC × MEC	0.7674	0.4539	0.4747	0.6549	0.2268

[1]Sunflower Cake; [2]Multi-enzyme complex; [3]Standard error of the mean; [4]Analysis of variance (p<0,05).

When evaluating bone quality of chickens when they reached 17 weeks of age, the results obtained in both experiments indicate that there was no significant difference between the control treatment and the treatments that included sunflower cake with or without enzyme supplementation to weight, length, diameter, Seedor index and deformity and bone strength (Table 9 and 10). There was also no significant interaction between sunflower cake levels and the enzyme supplementation tested. When comparing the levels of sunflower cake or multi-enzyme complex supplementation, there was no significant difference for the variables studied.

Considering that the research was conducted in the age range of birds in which the bone structure is being formed; that minerals from food are mostly directed towards the mineralization of bone tissue; and that the results obtained by Scheideler, Jaroni and Puthpongsiripron (1998) indicated lower digestibility of calcium and phosphorus with the addition of fibrous foods in chicken feed, it was hypothesized that the negative action of increasing the fibrous fraction and the amount of phytate in the feed with the addition of sunflower cake reduced the availability of minerals and, thus, influenced the development or quality of bones, which was not confirmed by the results obtained. However, the results obtained in the present research are similar to those reported by Cruz et al. (2012), who did not observe a significant effect of increasing the level of neutral detergent fiber in the feed with the inclusion of wheat bran on the growth and quality of chicken bones.

When applying the nutritional and energetic contribution values of the multi-enzyme complex in the formulation of rations containing sunflower cake, there was a reduction in the amounts of corn, soybean bran and, mainly, dicalcium phosphate, without compromising the resistance parameters and bone deformity at the end of the growth phase. These results demonstrate the efficiency of the multi-enzyme mixture added to the diets containing sunflower cake, which increased the digestibility of minerals, guaranteeing the growth and quality of chicken bones.

Table 9: Bone quality parameters of the tibia of white replacement pullets fed diets containing sunflower cake and supplemented with multi-enzyme complex.

Diets	Weight (g)	Length (mm)	Circumference (mm)	Seedor index (mg/mm)	Deformity (mm)	Resistance (kgf/cm^2)
0% SC[1]	5.67	116.07	7.23	48.85	3.37	12.37
10% SC	5.42	114.90	7.05	47.18	3.25	12.86
20% SC	5.54	114.94	7.04	48.20	3.35	11.96
10% SC+MCE[2]	5.16	111.42	7.19	46.19	3.56	12.83
20% SC + MCE	5.29	112.11	7.17	47.14	3.27	12.89
Mean	5.42	113.89	7.14	47.51	3.36	12.57
SEM[3]	0.090	0.796	0.067	0.645	0.102	0.259
Sunflower cake levels						
10%	5.29	113.16	7.12	46.67	3.40	12.84
20%	5.41	113.52	7.11	47.67	3.31	12.40
Multi-enzyme complex						
Absence	5.48	114.92	7.05	47.69	3.30	12.39
Presence	5.22	111.76	7.18	46.66	3.41	12.86
ANOVA[4]	*p-value*					
Diets	0.4262	0.2849	0.8807	0.7525	0.9061	0.7370
SC	0.5727	0.8509	0.9385	0.5493	0.7250	0.4544
MEC	0.2544	0.1118	0.3771	0.5307	0.6528	0.4231
SC × MEC	0.9671	0.8652	0.9473	0.9829	0.4464	0.3948

[1]Sunflower Cake; [2]Multi-enzyme complex; [3]Standard error of the mean; [4]Analysis of variance ($p<0,05$).

Table 10: Bone quality parameters of the tibia of brown replacement pullets fed diets containing sunflower cake and supplemented with multi-enzyme complex.

Diets	Weight (g)	Length (mm)	Circumference (mm)	Seedor index (mg/mm)	Deformity (mm)	Resistance (kgf/cm²)
0% SC[1]	7.07	120.22	8.38	58.74	2.77	14.34
12% SC	6.99	118.91	8.53	58.85	2.80	13.82
24% SC	7.14	119.07	8.37	59.88	3.35	15.23
12% SC+MEC[2]	6.68	117.18	8.24	56.97	3.05	14.31
24% SC + MEC	7.39	117.62	8.51	62.77	2.94	14.40
Mean	7.05	118.60	8.41	59.44	2.98	14.42
SEM[3]	0.134	0.584	0.070	1.061	0.096	0.495
Sunflower cake levels						
10%	6.84	118.04	8.38	57.91	2.92	14.06
20%	7.26	118.34	8.44	61.32	3.15	14.82
Multi-enzyme complex						
Absence	7.06	118.99	8.45	59.37	3.08	14.52
Presence	7.04	117.40	8.37	59.87	3.00	14.36
ANOVA[4]	p-value					
Diets	0.4194	0.6170	0.2051	0.3572	0.7033	0.5650
SC	0.1873	0.388	0.7087	0.1867	0.3340	0.5120
MEC	0.9328	0.2840	0.6417	0.8421	0.7394	0.8848
SC × MEC	0.3808	0.9260	0.1971	0.3509	0.1600	0.5680

[1]Sunflower Cake; [2]Multi-enzyme complex[3]; Standard error of the mean; [4]Analysis of variance (p<0,05).

Conclusions

The inclusion of sunflower cake in rations during the growth phase (7 to 17 weeks of age) at levels of up to 20% for white birds and 24% for brown birds does not compromise the use of the minerals Ca, P and Mg in the ration, the mineralization and bone quality of pullets. The multi-enzyme complex supplementation evaluated provides an increase in the digestibility coefficients of minerals in the feed, guaranteeing the composition and bone quality of chickens fed sunflower cake.

Authors Contribution

Conceptual idea: Freitas, E. R.; Nepomuceno, R. C.; Methodology design: Freitas, E. R.; Nepomuceno, R. C.; Data collection: Souza, O. F.; Souza, D. H.; Data analysis and Interpretation: Freitas, E. R.; Nepomuceno, R. C.; Writing and editing: Souza, O. F.; Sucupira, F. S.; Nascimento, G. A. J.

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