The use of Enzymes in Meat Quail Diets Containing Sunflower Seed Cake

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Abstract: We examined the effects of including sunflower cake (SC) associated with an enzyme complex (EC) in the diet of meat quails on nutrient metabolism, performance, carcass characteristics, bone parameters and economic viability. In total, 432 meat quails (7 to 42 days old) were assigned to six treatments in a completely randomised design with 6 replicates with 12 birds each. A 2 × 3 factorial arrangement was adopted (two levels of SC: 10 and 20%; three diet formulation strategies: normal, reduction in the nutrient and energy matrix considering the enzymatic contribution and reduced diet with EC). No interaction effect was observed. The increased levels of SC from 10% to 20% promoted in decrease in the metabolisability coefficients of dry matter and gross energy and in the value of AMEn and increased relative weight of gizzard. The addition of EC in diets containing SC allows a better metabolization of nitrogen and energy, equalize to the normal diet. The best breast yield was obtained in the birds fed the reduced diet with EC, in relation to normal diet. The reduced diet and the reduced diet with EC provided the lowest cost of food and the best economic efficiency index. Up to 20% of the sunflower cake can be included in the diet containing sunflower cake.

Key words: alternative feedstuff, by-products, Coturnix coturnix coturnix, enzyme complex, performance.

INTRODUCTION

In recent years, concerns about environmental and global warming have prompted the introduction of biofuel into the energy mix in several countries, which have their own legislation and demand a larger production of vegetable oils, which are extracted from oilseeds.

Sunflower (Helianthus annuus L.) seeds are used for oil extraction to produce biofuels and foodstuffs. The oil is extracted through mechanical pressing and chemical systems (solvents), resulting in the two by-products the sunflower cake and sunflower meal, respectively (Berwanger et al. 2014, Goiri et al. 2019).

One of the ways to add value to by-products is considering their use in animal feeding as an alternative to replace conventional feedstuffs, given their nutritional value, and to prevent the disposal of waste in the environment (Correia et al. 2012).

Sunflower cake is a by-product obtained from the mechanical pressing of the sunflower seed. It is considered a protein feedstuff because of its high protein content (24.37%). Additionally, it contains 23.80% ether extract,
0.72% calcium and 0.23% phosphorus (Berwanger et al. 2014). However, its use in the feeding of poultry is limited due to the presence of fibre, phytate and chlorogenic acid, which are anti-nutritional factors that may compromise animal performance (Berwanger et al. 2017a).

The high content of fibre in sunflower cake results in a low metabolisable energy for birds, which may limit its inclusion in diets. Phytate can bind to proteins using bivalent mineral elements as bridges, which reduces their availability and, consequently, the availability of the amino acids and minerals involved in the bonds. It also inhibits the activity of various endogenous digestive enzymes (Angel et al. 2002). Chlorogenic acid, in turn, reacts with protein, altering its functionality and reducing the quantity of essential amino acids, the nutritional quality and the digestibility of these nutrients (Zardo et al. 2019).

In this scenario, the use of enzyme complexes composed of carbohydrates, phytase and proteases can improve the digestibility of dietary nutrients, mitigating the negative effects of anti-nutritional factors. Furthermore, they optimise the use efficiency of sunflower cake in poultry feeding as a result of the greater digestion and absorption of nutrients, consequently increasing their metabolisable energy value. This practice also results in a decreased need for dietary supplementation with other sources to meet the nutritional requirements of the birds, ultimately reducing the feeding and total production costs.

The present study thus evaluates the effects of including sunflower cake associated with an enzyme complex in the diet of meat quails (*Coturnix coturnix coturnix*).

**MATERIALS AND METHODS**

The experiment was carried out in Fortaleza - CE, Brazil, located in the coastal zone at an elevation of 15.49 m above sea level, 30º43'02” south latitude and 38º32’35” west longitude. All experimental procedures were approved by the Ethics Committee on Animal Use (case nº 125/2017) of the Federal University of Ceará (UFC).

In total, 432 meat quails were evaluated during 7 to 42 days of age. The birds were allocated to 0.60 × 0.60-m cages containing trough feeders and pressure-cup drinkers.

In the first week of age, from 1 to 7 days, the quails were housed in a protection circle containing a trough feeder, a pressure-cup drinker and poultry heaters. All birds received feed and water *ad libitum*. At 7 days of age, the birds were selected based on the average body weight of the lot and distributed into the plots as recommended by Sakomura & Rostagno (2007). The average weight per plot was 429 ± 4.17 g.

The quails were assigned in a completely randomised design in a 2 × 3 factorial arrangement (sunflower cake levels × diet formulations) composed of six treatments and six replicates, with 12 birds each. The sunflower cake inclusion levels were 10 and 20%, and the following diet formulations were tested: normal (calculated to meet the nutritional requirements of the birds), reduced (reduction of the nutritional and energy matrix considering enzymatic contribution) and reduced with addition of an enzyme complex.

The sunflower cake was obtained from the mechanical pressing of sunflower seeds with hulls for the removal of oil, using a Scott Tech (ERT 40-V1) mechanical press with a motor power of 0.75 KW and a seed processing capacity of 6 kg/h.

We used the following enzymes: carbohydrases: α-galactosidase (9,000 U/g), xylanase (100,000 U/g) and β-glucanase (200,000
U/g); proteases (1,10 U/g): elastase, trypsin and chymotrypsin and phytase (400 FTU/g). Enzymes were included in the diet formulations at the ratio of 100 g/t, as recommended in the enzyme matrix of the enzymes. The nutritive and energy contributions of each product are described in Table I.

The experimental diets were based on corn and soybean meal (Table II). The diets were formulated to meet the nutritional requirements of meat quails as proposed by Silva & Costa (2009), considering the chemical composition values of the feedstuffs indicated by Rostagno et al. (2011), except for sunflower cake, for which the nitrogen-corrected apparent metabolisable energy (AMEn), obtained in a metabolic trial with meat quails, was considered. The chemical composition of the diet was determined via laboratory analyses (crude protein, ether extract, neutral detergent fibre, acid detergent fibre, ash), and calcium, available phosphorus, sodium, chlorine, potassium, digestible lysine, digestible methionine, digestible methionine + cystine, digestible threonine and digestible tryptophan were estimated based on FEDNA (2010) tables; the values are shown in Table III.

[The lighting program adopted was 24 h of light (natural + artificial) daily during the entire experimental period (Muniz et al. 2018). Artificial illumination was provided by fluorescent 40-W light bulbs that were distributed at a height of 2.4 m above the floor, so that all birds would receive light uniformly.

Temperature and air relative humidity were measured twice daily (0800 h and 1600 h) throughout the experimental period, using three thermo-hygrometers that were distributed at the main points in the shed. The average minimum and maximum temperatures and the air relative humidity recorded inside the shed during the experimental period were 28.5ºC, 33.3ºC and 58%, respectively.

To evaluate performance, birds and diets were weighed at the start and end of the experiment (7 and 42 days of age) to determine feed intake (g/bird), weight gain (g/bird) and gain-to-feed ratio. The variables were corrected for mortality considering the number of birds

| Nutrient matrix                          | Carbohydrates¹ | Proteases² | Phytase³ | Total contribution |
|-----------------------------------------|----------------|------------|----------|-------------------|
| Metabolizable energy (kcal/kg)          | 30.00          | 25.00      | 49.00    | 104.00            |
| Crude protein (g.kg⁻¹)                  | 0.00           | 5.00       | 4.01     | 9.01              |
| Calcium (g.kg⁻¹)                        | 0.00           | 0.00       | 1.57     | 1.57              |
| Available phosphorus (g/kg)             | 0.00           | 0.00       | 1.43     | 1.43              |
| Sodium (g.kg⁻¹)                         | 0.00           | 0.00       | 0.33     | 0.33              |
| Digestible methionine (g.kg⁻¹)          | 0.00           | 0.14       | 0.16     | 0.50              |
| Digestible met + cys (g.kg⁻¹)           | 0.00           | 0.24       | 0.36     | 0.60              |
| Digestible lysine (g.kg⁻¹)              | 0.00           | 0.32       | 0.16     | 0.48              |
| Digestible threonine (g.kg⁻¹)           | 0.00           | 0.21       | 0.32     | 0.56              |
| Digestible tryptophan (g.kg⁻¹)          | 0.00           | 0.05       | 0.24     | 0.29              |
| Digestible valine (g.kg⁻¹)              | 0.00           | 0.26       | 0.00     | 0.26              |
| Digestible arginine (g.kg⁻¹)            | 0.00           | 0.33       | 0.00     | 0.33              |
| Digestible leucine (g.kg⁻¹)             | 0.00           | 0.46       | 0.00     | 0.46              |

¹Poultrygrow 25; ²ProFar EZ 309; ³Finase.
and the number of days in the period, according to the recommendations by Sakomura & Rostagno (2016).

To evaluate the effect of sunflower cake inclusion with the addition of the enzyme complex on the digestibility of dietary nutrients and on energy use efficiency, the traditional total excreta collection method was performed from 21 to 28 days of age. To this end, at 21 days of age, six birds from each experimental plot were randomly selected and housed in batteries with metabolic cages, following the same experimental design. Each experimental plot had a plastic-coated aluminium tray. At the start and end of the excreta collection period, birds were fed a diet with 1% ferric oxide to mark the start and end of the collection.

Excreta were collected twice daily (0800 h and 1600 h). At the end of each collection, the excreta were weighed, placed in labelled plastic bags and frozen. Prior to analysis, the excreta were thawed at room temperature and homogenised to be pre-dried in a forced-air oven at 55ºC for 72 h. Subsequently, they were ground through a knife mill with a 16-mesh screen with 1-mm pores and sent, together with the samples of experimental diets, to the Laboratory of Animal Nutrition (LANA) at UFC for determinations of dry matter, gross energy and nitrogen contents, in accordance with the methodology described by Silva & Queiroz (2002). Gross energy was determined using an adiabatic bomb calorimeter (PARR model 1241EA). Metabolisability coefficients and AMEn values were calculated using laboratory data, which were entered in equations proposed by Matterson et al. (1965): 

$$EM_A = ME_{RR} + \left(\frac{ME_{RT} - ME_{RR}}{\% \text{ substitution}}\right)$$

where $ME_A$ = metabolisable energy from food, $ME_{RR}$ = metabolisable energy of the reference feed and $ME_{RT}$ = metabolisable energy of the test feed.

For carcass evaluation, at 42 days of age, two birds from each experimental unit (one male and one female) were selected according to the average weight of the plot. After a feed-deprivation period of 6 h, the birds were weighed, euthanised by electronarcosis and subsequently bled, scalded, plucked and eviscerated.

After the removal of the head, neck and feet, the carcass was weighed to determine the carcass yield based on the weight of the fasted animal (prior to slaughtering). Subsequently, the whole breast, drumsticks + thighs and abdominal fat were extracted and weighed to calculate their yield. Drumsticks + thighs were labelled and frozen at −20ºC for later analysis. Liver and gizzard were also weighed to determine their relative weights. The yields of breast, drumsticks + thighs and abdominal fat were expressed relative to the hot carcass weight. The relative weight of liver and gizzard was expressed in relation to the bird’s weight.

To evaluate bone quality, the drumsticks + thighs were thawed in a domestic refrigerator (temperature at 4ºC for a period of 12 h) and placed on bench tops until reaching room temperature. Subsequently, they were immersed in boiling water for 5 min, and a scalpel was used for deboning when all tissues surrounding the bones were removed.

The length and diameter of the femur and tibia were measured with a digital calliper, while their weight was measured on an electronic scale with a precision of 0.01 g. Bone density was assessed based on the Seedor index, which was obtained by dividing the weight (mg) by the length (mm) of the evaluated bone (Seedor et al. 1991).

Bone resistance and deformity were measured in the left tibia and left femur, using a Testop/Ronald triaxial mechanical press (Indústria e Comércio Ronald Top Ltda. Rio de Janeiro, RJ, Brazil) with a capacity of 150 kg.
Table II. Centesimal composition and calculated nutritional levels of experimental diets for meat quail.

| INGREDIENT | Price¹ (R$.kg⁻¹) | Sunflower cake inclusion level | 10 % | 20 % |
|------------|------------------|-------------------------------|------|------|
|            | RN   | RR   | RR+CE | RN   | RR   | RR+CE |
| Corn       | 1.10 | 497.50 | 501.30 | 501.30 | 426.80 | 461.40 | 461.40 |
| Soybean meal | 1.96 | 366.80 | 346.70 | 346.70 | 327.20 | 301.60 | 301.60 |
| Sunflower cake | 0.80 | 100.00 | 100.00 | 100.00 | 200.00 | 200.00 | 200.00 |
| Soybean oil | 2.80 | 7.20  | 0.00  | 0.00  | 17.50  | 0.00  | 0.00  |
| Limestone  | 0.19 | 9.80  | 10.40 | 10.40 | 9.30   | 9.90  | 9.90  |
| Dicalcium phosphate | 2.80 | 9.20  | 1.80  | 1.80  | 9.30   | 1.90  | 1.90  |
| Salt       | 0.60 | 3.50  | 2.80  | 2.80  | 3.50   | 2.70  | 2.70  |
| DL-methionine | 18.83 | 2.60 | 2.20  | 2.20  | 2.40   | 2.00  | 2.00  |
| L-lysine   | 15.18 | 0.40  | 0.40  | 0.40  | 1.00   | 1.10  | 1.10  |
| Vitamin supplement² | 15.29 | 1.50  | 1.50  | 1.50  | 1.50   | 1.50  | 1.50  |
| Mineral supplement³ | 6.84 | 0.50  | 0.50  | 0.50  | 0.50   | 0.50  | 0.50  |
| Anticoccidial | 22.00 | 0.50  | 0.50  | 0.50  | 0.50   | 0.50  | 0.50  |
| Choline chloride | 20.00 | 0.50  | 0.50  | 0.50  | 0.50   | 0.50  | 0.50  |
| Inert      | 0.10 | 0.00  | 31.30 | 31.30 | 0.00   | 16.30 | 16.00 |
| Carbohydrase enzyme | 68.00 | 0.00  | 0.00  | 0.01  | 0.00   | 0.00  | 0.01  |
| Protease enzyme | 38.00 | 0.00  | 0.00  | 0.01  | 0.00   | 0.00  | 0.01  |
| Phytase enzyme | 85.00 | 0.00  | 0.00  | 0.01  | 0.00   | 0.00  | 0.01  |
| Cost (R$.kg⁻¹) | 1.51 | 1.42  | 1.44  | 1.46  | 1.37   | 1.39  | 1.39  |
| TOTAL      | 1000 | 1000  | 1000  | 1000  | 1000   | 1000  | 1000  |

CALCULATED NUTRITIONAL AND ENERGY COMPOSITION

|                     | 10 % | 20 % |
|---------------------|------|------|
| Metabolizable energy (kcal.kg⁻¹) | 2950 | 2846 |
| Crude protein (g.kg⁻¹) | 230.0 | 221.0 |
| Neutral detergent fiber (g.kg⁻¹) | 157.3 | 155.0 |
| Acid detergent fiber (g.kg⁻¹) | 77.6 | 76.1 |
| Calcium (g.kg⁻¹) | 7.5 | 5.9 |
| Available phosphorus (g.kg⁻¹) | 2.9 | 1.5 |
| Sodium (g.kg⁻¹) | 1.6 | 1.3 |
| Chlorine (g.kg⁻¹) | 2.7 | 2.2 |
| Digestible lysine (g.kg⁻¹) | 11.4 | 10.9 |
| Digestible met + cys (g.kg⁻¹) | 8.9 | 8.3 |
| Digestible methionine (g.kg⁻¹) | 5.8 | 5.3 |
| Digestible threonine (g.kg⁻¹) | 7.7 | 7.6 |
| Digestible tryptophan (g.kg⁻¹) | 2.6 | 2.5 |

RN: Normal diet; RR: Reduced diet; RR+EC: Reduced diet + enzyme complex; ¹Prices of ingredients obtained in July/2017, in Fortaleza-CE, Brazil; ²Composition per kilogram of product: vit. A - 9,000,000.00 IU; Vit. D3 - 2,500,000.00 IU; vit. E - 20,000.00 mg; vit. K3 - 2,500.00 mg; vit. B1 - 2,000.00 mg; vit. B2 - 6,000.00 mg; vit. B12 - 15.00 mg; niacin - 35,000.00 mg; pantothenic acid - 12,000.00 mg; vit. B6 - 8,000.00 mg; folic acid - 1,500.00 mg; selenium - 250.00 mg; biotin - 100.00 mg; ³Composition per kilogram of product: iron - 100,000.00 mg; copper - 20.00 g; manganese - 130,000.00 mg; zinc - 130,000.00 mg; iodine - 2,000.00 mg.
Bones were placed in the horizontal position, supported on their edges by a piece of wood, and a compression force was exerted by a piston (at a descent speed of 0.4064 mm.min⁻¹) on their centre. Breaking strength was determined as the maximum force applied to the bone until it broke (kgf.cm⁻²), which was measured using a digital extensometer. Deformity (mm) was measured using an analogical extensometer until the moment of breaking by the action of the applied force. After the analyses, the bones were placed together with their right counterparts for bone composition analyses.

For bone composition, the bones (tibia and femur) were thawed at room temperature and weighed. Subsequently, they were placed in labelled containers and dried in a forced-air oven at 55°C for 72 h. After drying, they were weighed and ground with a mortar and pestle. Ground samples were then identified to determine their mineral matter content, following the methodology described by Silva & Queiroz (2002).

To determine the economic viability of including sunflower cake in the diets in association with the enzyme complex, the diet cost per kilogram of body weight gain was determined by using the following equation proposed by Bellaver et al. (1985): \[ Yi = \frac{(Qi \times Pi)}{Gi} \]
where \( Yi \) = cost of diet per kilogram of body weight gained in treatment \( i \); \( Pi \) = price of kilogram of diet used in treatment \( i \); \( Qi \) = amount of feed consumed in treatment \( i \); \( Gi \) = weight gain in treatment \( i \).

The economic efficiency index (EEI) and the cost index (CI), proposed by Fialho et al. (1992), were calculated as follows: EEI = \( \frac{(LCeɪ)}{(TCeɪ)} \) × 100 and CI = \( \frac{(CTeɪ)}{(LCeɪ)} \) × 100, where \( LCeɪ \) = lowest cost of the diet per kilogram of gain observed among the treatments; \( TCeɪ \) = cost of treatment \( i \) considered.

To calculate the cost of the diets, we considered the compositions of each diet and the prices of ingredients quoted in July 2017 in Fortaleza - CE, Brazil.

For statistical data analysis, we used the ANOVA procedure of the Statistical Analysis System (SAS 2000), according to a 2 × 3 factorial arrangement (two sunflower cake inclusion levels × three diet formulations). Means were compared by the SNK test at the 5% significance level. The statistical model of the design was as follows:
\[
Xijk = \mu + Ai + Bj + ABij + eijk
\]
in which \( Xijk \) = the record of each observation, \( \mu \) = the mean, \( Ai \) = sunflower cake inclusion effect, \( Bj \) = effect of diet formulations, \( ABij \) = the interaction effect of sunflower cake inclusion and diet formulations, and \( eijk \) = error effect.

### Table III. Nutritional and energetic composition of sunflower seed cake.

| Constituents                  | Sunflower seed cake |
|-------------------------------|---------------------|
| Metabolizable energy (kcal.kg⁻¹) | 104.00              |
| Crude protein (g.kg⁻¹)         | 230.0               |
| Ether extract (g.kg⁻¹)         | 161.0               |
| Neutral detergent fiber (g.kg⁻¹) | 473.3              |
| Acid detergent fiber (g.kg⁻¹)  | 312.0               |
| Ash (g.kg⁻¹)                   | 28.7                |
| Calcium (g.kg⁻¹)               | 3.0                 |
| Available phosphorus (g.kg⁻¹)  | 1.0                 |
| Sodium (g.kg⁻¹)                | 0.3                 |
| Chlorine (g.kg⁻¹)              | 0.9                 |
| Potassium (g.kg⁻¹)             | 12.8                |
| Digestible lysine (g.kg⁻¹)     | 7.0                 |
| Digestible methionine (g.kg⁻¹) | 4.9                 |
| Digestible methionine + cystine (g.kg⁻¹) | 7.3 |
| Digestible tryptophan (g.kg⁻¹) | 2.7                 |

Values crude protein, ether extract, neutral detergent fiber, acid detergent fiber, ash, obtained from laboratory analyses; Values calcium, available phosphorus, sodium, chlorine, potassium, digestible lysine, digestible methionine, digestible methionine + cystine, digestible threonine, digestible tryptophan, which were estimated by the authors based on FEDNA (2010) tables.
RESULTS

In the metabolism trial with the experimental diets (Table IV), there was no significant interaction effect ($P > 0.05$) between diet type and sunflower cake level on the metabolisability coefficients of dry matter (MCDM), nitrogen (MCN) and gross energy (MCGE) or on the nitrogen-corrected apparent metabolisable energy (AMEn) values of the diets.

Considering the effect of sunflower cake levels, better MCDM MCGE and AMEn values were obtained with the diets containing 10% sunflower cake. However, no significant effect was observed on MCN.

The diet in which the nutrient matrix was reduced provided a lower MCN and a lower AMEn value than the treatment with reduction plus enzyme supplementation and the normal diet without reductions in the nutrient matrix or enzyme addition. When the reduced diet was supplemented with the enzyme complex, the observed MCDM, MCN, MCGE and AMEn values were similar to those of the diet without alterations in its nutrient matrix.

In terms of performance, no significant interaction effect ($P > 0.05$) between the analysed factors was observed on feed intake, weight gain or gain-to-feed ratio. These variables were also not influenced ($P > 0.05$) by the sunflower cake levels or by the different diet formulations tested (Table V).

There was no significant interaction effect ($P > 0.05$) between the studied factors on carcass characteristics (Table VI). However, a significant difference was detected in breast yield when the different diet formulations were considered and in the relative weight of the gizzard between the two sunflower seed levels tested. These factors did not affect the other variables.

In the evaluation of growth and bone quality (Table VII), there was no interaction effect ($P > 0.05$) between the sunflower cake levels and diet formulations on any of the bone parameters measured in the femur and tibia of the meat quail. Similarly, no significant effect ($P > 0.05$) of sunflower cake or diet formulation was observed on the weight, length, diameter, Seedor index, resistance, deformity, dry matter or ash of the femur and tibia of the animals.

When evaluating economic viability (Table VIII), we detected no significant interaction effect between the sunflower cake levels and diet formulations ($P > 0.05$) on feeding cost per live-weight gain, economic efficiency index (EEI) or cost index (CI). The sunflower cake levels also

Table IV. Metabolization coefficients and metabolizable energy values of diets for meat quail containing 10 or 20% sunflower cake (SC) with and without enzyme complex (EC) supplementation.

| Item      | Diet      | SC level | SEM | P-value         |
|-----------|-----------|----------|-----|-----------------|
|           | RN        | RR       | RR + EC | 10%   | 20%   |       |       |       |
| MCDM (g.kg$^{-1}$) | 670.1 | 661.6 | 674.2 | 682.4$^b$ | 654.9$^c$ | 0.544 | 0.556 | 0.008 | 0.152 |
| MCN (g.kg$^{-1}$)   | 445.2$^c$ | 377.0$^c$ | 475.1$^c$ | 419.4 | 445.5 | 1.448 | 0.008 | 0.285 | 0.076 |
| MCGE (g.kg$^{-1}$)  | 726.8 | 728.5 | 743.3 | 753.7$^b$ | 712.0$^b$ | <0.0001 | 0.020 | 0.003 | 0.446 |
| AMEn (kcal.kgDM$^{-1}$) | 3.115$^a$ | 3.035$^b$ | 3.134$^c$ | 3.142$^c$ | 3.047$^c$ | 0.017 | 0.167 | 0.232 |

RN: Normal diet; RR: Reduced diet; RR+EC: Reduced diet + enzyme complex; MCDM: metabolizability coefficients of dry matter; MCN: metabolizability coefficients of nitrogen; MCGE: metabolizability coefficients of gross energy; AMEn: nitrogen-corrected apparent metabolizable energy; SEM: standard error of the mean; Means followed by superscript uppercase letters within a row differ from each other according to the SNK test ($P < 0.05$).
did not affect (P > 0.05) these variables. However, a significant difference was observed between the different diet formulations. In relation to the diets formulated with the enhancement of the nutritional matrix of the enzymes (reduced diets), the diets formulated with normal levels showed a higher cost of feeding per kilogram of weight gain and, consequently, worse EEI and CI.

DISCUSSION

The reduction of MCDM, MCGE and AMEn in the diet in which the sunflower cake inclusion level was increased from 10 to 20% may be associated with the increase in its fibre content, provided by the sunflower cake. According to Rostagno et al. (2017), the fibre in sunflower meal is mainly insoluble. The consumption of feedstuffs with a high level of insoluble fibre induces increased peristaltic movements of the gastrointestinal tract and increased motility of the feed bolus. As a consequence, the residence time of the feed in the gastrointestinal tract, the time for enzymatic action on the substrate, and the availability of nutrients for absorption all decrease (Mateos et al. 2002).

According to the hypothesis tested in this study, the exogenous enzymes added should act on the cell wall present in the fibrous fraction of the diet, making the nutrients within the cell available. Thereby, they would ensure the hydrolysis of the protein fraction and of phytate, reducing chelate formation and improving the use of amino acids, energy and minerals from the diet. In this way, the results obtained for the MCN and AMEn of the diets demonstrate the positive effects of enzyme action, since the birds fed the diet with reduction in the nutrient and energy matrix associated with EC and those fed the diet calculated to meet their nutritional requirements showed similar results.

The results obtained in the metabolism trial with the experimental diets revealed a decrease in the AMEn of the diets with a reduced nutrient matrix. With the inclusion of 20% sunflower cake, feed intake was expected to increase, since it is regulated by the amount of metabolisable energy in birds (Leeson & Summers 2001). However, this was not observed, which suggests that the magnitude of reduction of the nutrient matrix and the negative effects of increasing the fibrous fraction of the diet through the addition of 20% sunflower cake were not sufficient to alter the feed intake and, consequently, the performance of the birds.

Because the nutritional composition of these feedstuffs varies (e.g. type of seed and oil extraction process), experiments with sunflower seed by-products in poultry feeding have yielded varying results. For example, Alagawany et al. (2015) observed increased feed intake, decreased weight gain and lower feed conversion with increasing sunflower seed levels to substitute soybean meal in the diet. The worst results were

| Item           | Diet         | SC level | SEM | P-valor |
|----------------|--------------|----------|-----|---------|
|                | RN           | RR       | RR+EC | 10% | 20%     | Diet | Level | Diet×Level |
| FG (g/bird⁻¹)  | 1103.25      | 1055.73  | 1033.42 | 1048.62 | 1079.66 | 15125 | 0.150 | 0.292 | 0.242 |
| WG (g/bird⁻¹)  | 236.79       | 239.06   | 229.09 | 232.74 | 233.89 | 1.414 | 0.093 | 0.684 | 0.924 |
| G:F            | 0.21         | 0.22     | 0.22  | 0.22  | 0.22  | 0.003 | 0.593 | 0.378 | 0.203 |

RN: Normal diet; RR: Reduced diet; RR+EC: Reduced diet + enzyme complex; FG: feed intake; WG: weight gain; G:F: gain-to-feed ratio; SEM: standard error of the mean; Statistical effect insignificant according to the SNK test (P > 0.05).
obtained when 75% of the soybean meal were replaced. Ozturk (2017) reported that feed intake did not vary significantly and that weight gain declined with the inclusion of sunflower meal replacing soybean meal in broiler diets; enzyme addition was not effective at lessening the negative effects on weight gain. Similarly, Bilal et al. (2017) described a decreased feed conversion in chickens fed diets with 25% sunflower meal. However, the negative effects at that inclusion level were not reversed by NSP-ase addition, and these enzymes were most effective in the diets containing 15 and 20% inclusion of sunflower meal.

Nevertheless, according to the researchers, the addition of an enzyme complex can mitigate the negative effects of the larger proportion of sunflower meal in the diet. Berwanger et al. (2017a, b), on the hand, did not detect significant effects of the use of sunflower cake with or without the association of an enzyme complex on the performance of broilers at different ages.

The highest breast yield was observed in the quails fed the diet with reduced nutrient and energy matrix levels and supplemented with enzymes. The worst result for this variable, in turn, was found in the birds fed the normal diet, whose formulation took into consideration the application of the levels to meet the nutritional requirements of the birds. The better breast yield may be related to the action of the added enzymes, mainly proteases and phytase, on the protein fraction and phytates of the diet. This increased the availability of amino acids as a consequence of their higher digestibility and lower chelation with phytate, as shown by the positive effect observed on the metabolisability coefficient of nitrogen (Table IV). Accordingly, the higher availability of dietary amino acids improved the energy: amino acid ratio, which contributed to protein synthesis and to muscle deposition in the breast.

The present results differ from those mentioned by Alagawany et al. (2015), who did not observe significant effects of enzyme supplementation on the carcass characteristics of broilers fed sunflower meal. Berwanger et al. (2017a) observed an increase in the yields of carcass, breast and drumsticks + thighs of broilers from 1 to 21 days of age that were fed enzyme-supplemented diets.

The increasing gizzard weight seen in the birds fed the diet containing 20% sunflower cake may be related to the greater development of the organ, which required greater activity for the mechanical process of grinding the fibrous

| Item             | Diet         | SC level | SEM       | P-valor          |
|------------------|--------------|----------|-----------|------------------|
|                  | RN           | RR       | RR + EC   | 10%              | 20%   | Diet | Level | Diet x Level |
| Carcass (%)      | 67.44        | 67.95    | 67.25     | 67.88            | 67.22 | 0.40 | 0.776     | 0.431 | 0.606 |
| Breast (%)       | 42.71<sup>B</sup> | 43.50<sup>AB</sup> | 44.32<sup>A</sup> | 43.31            | 43.71 | 0.22 | 0.012     | 0.329 | 0.845 |
| Drumstick+ thigh (%) | 24.93    | 23.95    | 24.63     | 24.63            | 24.38 | 0.35 | 0.543     | 0.741 | 0.604 |
| Abdominal Fat (%)| 2.56         | 2.40     | 2.48      | 2.37             | 2.59 | 0.07 | 0.596     | 0.031 | 0.582 |
| Gizzard (%)      | 2.57         | 2.58     | 2.44      | 2.34<sup>B</sup> | 2.72<sup>AB</sup> | 0.05 | 0.051     | 0.001 | 0.688 |
| Liver (%)        | 1.95         | 1.90     | 1.90      | 1.92             | 1.91 | 0.03 | 0.851     | 0.065 | 0.267 |

RN: Normal diet; RR: Reduced diet; RR+EC: Reduced diet + enzyme complex; SEM: standard error of the mean; Means followed by superscript uppercase letters within a row differ from each other according to the SNK test (P < 0.05).
fraction of the diet. As a result, the feed was reduced to smaller particles to facilitate nutrient digestion and absorption in the intestine (Ribeiro et al. 2002).

These results corroborate those reported by Bilal et al. (2017), who obtained a higher relative weight of the gizzard as the fibre content in the diet of broilers was elevated. However, this effect was not observed by Ozturk (2017) when substituting soybean meal protein with 50 and 100% sunflower cake in broiler diets.

In the evaluation of bone quality, considering that this study was conducted in the age range in which the bone structure of the birds is still under formation and that the minerals derived from the diet are mostly used for the mineralisation of bone tissue, our hypothesis was that a higher inclusion of sunflower cake would result in diets with increased concentrations of fibre and phytate. As a consequence, the availability of minerals would decrease and bone development would be compromised, especially in the diets in which the level of available phosphorus in the nutrient matrix was reduced. The negative effects would then be minimised by the addition of enzymes - mainly phytase -, since dietary supplementation is aimed at making available the phosphorus complexed to the phytate molecule (Pereira et al. 2010) as well as reducing the predisposition of the phytic acid molecule to chelate with other bivalent minerals, improving their bioavailability.

Table VII. Bone parameters of meat quail fed diets containing 10% or 20% sunflower cake with and without enzyme complex (EC) supplementation.

| Item     | Diet      | SC level     | SEM       | P-value      |
|----------|-----------|--------------|-----------|--------------|
|          | RN     | RR     | RR+EC | 10% | 20% | Diet | Level | Diet x Level |
| BW (g)   | 0.721  | 0.699  | 0.717 | 0.706 | 0.720 | 9.798 | 0.631 | 0.483 | 0.352 |
| BL (mm)  | 45.58   | 45.16  | 45.79 | 45.71 | 45.48 | 0.183 | 0.246 | 0.539 | 0.383 |
| BD (mm)  | 3.29    | 3.34   | 3.28  | 3.34  | 3.28  | 0.026 | 0.578 | 0.229 | 0.069 |
| SI (mg/mm²) | 15.72 | 15.47  | 15.65 | 15.62 | 15.80 | 0.187 | 0.859 | 0.330 | 0.305 |
| BR (kgf/cm²) | 3.24 | 3.99   | 3.92  | 3.49  | 3.93  | 0.155 | 0.085 | 0.146 | 0.403 |
| DFO (mm) | 1.26    | 1.17   | 1.29  | 1.20  | 1.27  | 0.033 | 0.309 | 0.325 | 0.739 |
| BDM (%)  | 73.40   | 73.26  | 71.58 | 72.47 | 73.01 | 0.456 | 0.633 | 0.577 | 0.846 |
| BAS (%)  | 50.55   | 49.48  | 48.26 | 48.85 | 50.00 | 0.431 | 0.071 | 0.152 | 0.010 |

| Item     | Diet      | SC level     | SEM       | p-value      |
|----------|-----------|--------------|-----------|--------------|
|          | RN     | RR     | RR+EC | 10% | 20% | Diet | Level | Diet x Level |
| BW (g)   | 0.844  | 0.823  | 0.815 | 0.824 | 0.832 | 13.029 | 0.672 | 0.758 | 0.413 |
| BL (mm)  | 56.76   | 55.88  | 56.90 | 55.58 | 56.44 | 0.273 | 0.279 | 0.803 | 0.049 |
| BD (mm)  | 3.21    | 3.21   | 3.10  | 3.20  | 2.14  | 0.027 | 0.027 | 0.295 | 0.038 |
| SI (mg/mm) | 14.86 | 14.71  | 14.33 | 14.56 | 14.71 | 0.206 | 0.571 | 0.709 | 0.181 |
| BR (kgf/cm²) | 1.76 | 1.76   | 1.72  | 1.65  | 1.84  | 0.073 | 0.971 | 0.028 | 0.047 |
| DFO (mm) | 1.46    | 1.42   | 1.38  | 1.44  | 1.39  | 0.055 | 0.872 | 0.690 | 0.732 |
| BDM (%)  | 76.10   | 75.88  | 74.90 | 75.48 | 75.57 | 0.586 | 0.733 | 0.944 | 0.779 |
| BAS (%)  | 51.97   | 50.46  | 49.70 | 50.03 | 51.39 | 0.456 | 0.016 | 0.022 | 0.346 |

RN: Normal diet; RR: Reduced diet; RR+EC: Reduced diet + enzyme complex; SEM: standard error of the mean; BW: bone weight; BL: bone length; BD: bone diameter; SI = Sedor index; BR = bone resistance; DFO: bone deformity; BDM: bone dry matter; BAS: bone ash (dry matter basis); Statistical effect insignificant according to the SNK test (P > 0.05).
Bone quality was not changed, regardless of mineral deficiency or enzyme supplementation. In this regard, Conte et al. (2003) suggested that this effect may be due to the homeostasis mechanism that adjusts the absorption of minerals, reducing or increasing their excretion.

Regarding economic viability, irrespective of enzyme supplementation, reducing the nutrient and energy matrix allowed for a reduction in feeding costs, consequently improving the economic efficiency of quail feeding. This may be associated with the fact that animal performance data, which were used in the calculation of economic variables, were not influenced by the treatments. Moreover, the cost of enzyme supplementation did not exceed the cost reduction obtained with the diets whose nutrient and energy matrix had been reduced.

Considering the results obtained for the performance and economic viability of food costs, up to 20% of sunflower cake can be included in quail diets with or without enzyme supplementation. However, the use of the enzyme complex composed of carbohydrases, proteases and phytase can favor the metabolization of nitrogen and the energy of the diet containing sunflower cake.

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Table VIII. Economic assessment of 10% or 20% sunflower cake (SC) with and without enzyme complex (EC) supplementation for meat quail from 7 to 42 days of age.

| Item               | Diet                      | SC level | SEM | P-valor |
|--------------------|---------------------------|----------|-----|---------|
| CD (R$\cdot$kg\(^{-1}\) of gain) | | | | |
| RN                 | 6.91\(^a\)               | 6.29\(^b\) | 6.37\(^b\) | 6.55 | 6.50 | 0.010 | 0.020 | 0.761 | 0.225 |
| RR+EC              |                          |          |     |         |
| RR                 |                          |          |     |         |
| CD (R$\cdot$kg\(^{-1}\) of gain) | | | | |
| EEI (%)            | 89\(^b\)                | 98\(^a\) | 97\(^a\) | 94 | 95 | 1.457 | 0.017 | 0.836 | 0.297 |
| CI (%)             | 113\(^a\)              | 103\(^b\) | 104\(^b\) | 107 | 106 | 1.650 | 0.019 | 0.812 | 0.233 |

RN: Normal diet; RR: Reduced diet; RR+EC: Reduced diet + enzyme complex; CD: Cost of diet; EEI: economic efficiency index; CI: Cost index; SEM: standard error of the mean; Means followed by different letters in the same lean differ from each other according to the SNK test (P < 0.05). Means followed by superscript uppercase letters within a row differ from each other according to the SNK test (P < 0.05).
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