Xylanase and β-glucanase in diets for Japanese laying quails[1]

Xilanase e β-glucanase em dietas para codornas japonesas em postura

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ABSTRACT - The rations of monogastric animals in Brazil are composed of corn and soybean meal. Among the obstacles to the use of cereal grains in diets is the presence of non-starch polysaccharides, constituents of low digestibility, undigested by poultry. Thus, it is important to study non-conventional ingredients and the use of additives, such as enzymes in animal feed. The objective of this study was to evaluate the productivity and metabolism of Japanese laying quails fed with corn or sorghum feeds containing or not xylanase and β-glucanase. The experimental design was completely randomized in a 2x2 factorial scheme, with four treatments, with five replicates and seven birds each, to 140 animals. The treatments were: 1) ration based on soybean meal and maize (RSMM); 2) RSMM with enzymes; 3) ration based on soybean meal and sorghum (RSMS) and 4) RSMS with enzymes. The evaluated variables were: zootechnical performance; metabolizable rations and excreta; quality and oxidative process of eggs; biometrics of organs and serum biochemistry. There was no significant interaction between the factors (ingredients x enzymes) for all evaluated characteristics. The rations did not cause changes in the productive variables, metabolizable, egg quality, biometry of the gastrointestinal tract organs and femur of Japanese laying quails. The total egg mass was higher in the different rations with enzymatic use. Serum calcium and phosphate concentrations were higher in the RSMM and RSMM with enzymes, however, the Ca:P ratios remained, indicating a metabolic normality condition in the Japanese quails from 26 to 35 weeks of age.

Key words: Coturnix coturnix japonica. Egg quality. Enzymes. Performance. Seedor Index.

RESUMO - As rações dos animais monogástricos no Brasil constituem-se de milho e farelo de soja. Dentre os entraves do uso de grãos de cereais nas dietas destaca-se a presença de polissacarídeos não amiláceos, constituintes de baixa digestibilidade, não digeridos pelas aves. Desse modo, faz-se importância estudar ingredientes não convencionais e uso de aditivos, tais como as enzimas nas rações. Objetivou-se avaliar a produtividade e o metabolismo de codornas japonesas em postura, alimentadas com rações à base de milho ou sorgo contendo ou não xilanase e β-glucanase. O delineamento experimental foi inteiramente casualizado, em esquema fatorial 2x2, sendo quatro tratamentos, com cinco repetições e sete aves em cada, perfazendo 140 animais. Os tratamentos foram: 1) ração à base de farelo de soja e milho (RFSM); 2) RFSM + enzimas; 3) ração à base de farelo de soja e sorgo (RFSS); e 4) RFSS + enzimas. As variáveis avaliadas foram: desempenho zootécnico; metabolizabilidade das rações e das excretas; qualidade e processo oxidativo dos ovos; biometria de órgãos e bioquímica sérica. Não houve interação significativa entre os fatores (ingredientes x enzimas) para todas as características avaliadas. As rações não provocaram alterações nas variáveis produtivas, de metabolizabilidade, qualidade dos ovos, biometria dos órgãos do trato gastrintestinal e do fêmur de codornas japonesas em postura. A massa total de ovos apresentou-se maior nas diferentes rações com uso enzimático. As concentrações de cálcio e fosfato séricos foram mais elevadas nas RFSM e RFSM + enzimas, entretanto, as relações Ca:P mantiveram-se, indicando condição de normalidade metabólica nas codornas de 26 a 35 semanas de idade.

Palavras-chave: *Coturnix coturnix japonica*. Desempenho. Enzimas. Índice Seedor. Qualidade do ovo.

INTRODUCTION

The traditional rations of the monogastric animals are corn and soybean meal, and approximately 70% of the corn produced in the world is destined for animal feed, and only 15% for human consumption (OLIVEIRA; SANTOS; CUNHA, 2014).

Corn and soybeans are internationally traded products, their price of agricultural commodities, according to supply in response to the rhythm of demand, limited production in certain years, as well as high prices of inputs, causes an increase in the price of the costs of poultry farming. Such facts have led researchers to test unconventional ingredients in diets (NUNES; MAIER, 2012).

Among the numerous alternatives to substitute maize for poultry feeds, sorghum has a nutritional composition of 1.8 to 7.5% lower in metabolizable energy when compared to maize, however, similar crude protein content (ROSTAGNO *et al.*, 2017).

However, when this food is included in the egg production chain, it is recommended to include pigmentants in the diets of laying hens, since the sorghum contains low levels of carotenoids and xanthophylls, responsible for the pigmentation of the yolk (FREITAS *et al.*, 2014; MOURA; MELO; MIRANDA, 2016).

Grain-based diets have non-starch polysaccharides (PNAs) present in the cell wall of the seeds that forming the viscosity of the digesta (RESENDE *et al.*, 2017). Birds do not secrete the endogenous enzymes necessary for the breakdown of β-glucans, arabinoxylans and other soluble or insoluble fibers present in cereals. Thus, the increase in viscosity results in impaired digestion and absorption of nutrients in the digestive tract of birds (VIANA *et al.*, 2011).

Campos *et al.* (2017), showed that the use of enzymes in bird feeding promotes a positive effect on cell membrane PNA degradation due to the reduction of the viscosity of the digesta, with the release of encapsulated nutrients in the cell wall structures (FERNANDES *et al.*, 2017). The contact between these nutrients and the endogenous enzymes becomes favored, and the indigestible fraction can reach 27% with glucanases and 10% with xylanases. Thus, the study of conventional and/or alternative ingredients combined with different biotechnological inputs (enzymes) is a strategy to be evaluated.

The objective of this study was to evaluate the productive and physiological variables of Japanese laying quails fed with maize or sorghum-based diets containing or not xylanase and β-glucanase.

MATERIAL AND METHODS

The experiment was conducted in an aviary located in Rio Verde-Goiás, Brazil, with a duration of 63 days (three periods of 21 days). The Project was registered with the Committee of Ethics in Research with Animals n°: 3845300616.

A total of 140 quail (*Coturnix coturnix japonica*), 26 to 35 weeks of age, were used. The animal density was 117.85 cm^2 per bird. The experimental design was completely randomized (DCR), in a 2x2 factorial scheme (two rations, with and without enzymes and energy sources), in a total of four treatments, with five replicates of seven birds per experimental unit. The treatments corresponded to the following rations: 1) Ration based on soybean meal and maize (RSMM); 2) RSMM + enzymes; 3) Soybean meal and sorghum meal (RSMS); and 4) RSMS + enzymes.

The rations were formulated following the nutritional recommendations of Rostagno *et al.* (2011), Table 1. The enzymes used were produced by *Trichoderma reesei*: endo-1,4-beta-xylanase (EC 3.2.1.8), and endo-1,3(4)-beta-glucanase (EC 3.2.1.6), with activities of 610 U xylanase and 76 U beta-glucase, respectively.

The light supply was 16 hours a day and was controlled by an automatic timer which enabled the lights to be switched on and off during the night, according to the procedure adopted on commercial farms. Water and feed were provided at will during the trial period. Leftovers and feed wastes were weighed and discounted from the amount of heavy feed initially, and provided at different times. At the end of each 21-day period, the amount of feed consumed by the number of birds of each treatment was divided by the number of days, expressed in grams of feed consumed bird day^{-1}. In the case of poultry mortality, during the experimental period, mean consumption was corrected.

The eggs produced in each replicate were weighed to obtain the mean weight, which in turn was multiplied by the total number of eggs produced in the experimental period, thus obtaining the total egg mass. The feed conversion per dozen eggs was determined by the total feed consumption in grams divided by the dozen eggs produced (g dozen^{-1}) and the conversion by egg mass by egg mass in kilograms (kg kg^{-1}).

On the 19 th, 20 th and 21st days of each period, all the whole eggs collected were subjected to specific gravity analysis, immersed and evaluated in NaCl salt solutions, with a density ranging from 1,055 to 1,100 g cm^{-3}, with intervals of 0.005 g cm^{-3} between them. The density was measured by means of a densimeter (Incoterm-OM-5565®).

Table 1 - Composition of experimental rations, in natural matter

	Treatments			
	T1	T2	T3	T4
Ingredients (g kg⁻¹)	RSMM	RSMM + Enzymes	RSMS	RSMS + Enzymes
Sorghum	-	-	606.703	606.603
Maize	567.891	567.891	-	-
Soybean meal 45%	290.605	290.605	266.419	266.419
Calcium carbonate	73.775	73.775	74.200	74.200
Soybean oil	23.094	23.094	26.956	26.956
Bicalcium phosphate	11.444	11.444	10.936	10.936
L-Lysine	2.458	2.458	3.323	3.323
DL-Methionine	2.296	2.296	2.496	2.496
L-Threonine	0.192	0.192	0.630	0.630
Salt	3.245	3.245	3.238	3.238
Mineral Premix[1]	2.500	2.500	2.500	2.500
Vitamin Premix[2]	2.500	2.500	2.500	2.500
Inert	20.000	19.900	-	-
Butil-hidróxi-tolueno (BHT)	-	-	0.100	0.100
Enzymes (Xilanase e β-glucanase)	-	0.100	-	0.010
Total (kg)	100.000	100.000	100.000	100.000
Calculated levels				
Crude protein, %	18.00	18.00	18.00	18.00
Metabolizable energy, kcal kg⁻¹	2800	2800	2800	2800
Calcium, %	3.00	3.00	3.00	3.00
Phosphorus available, %	0.3040	0.3040	0.3040	0.3040
Sodium, %	0.1460	0.1460	0.1460	0.1460
Lysine dig. birds, %	1.0450	1.0450	1.0450	1.0450
Met + cist dig. birds, %	0.7181	0.7181	0.7034	0.7034
Methionine dig. birds, %	0.4700	0.4700	0.4700	0.4700
Threonine dig. birds, %	0.6270	0.6270	0.6270	0.6270

[1]Premix mineral of posture, %/kg of feed: crude protein: 2,4347%; ethereal extract: 0.1781%; crude fiber: 0.1495%; calcium: 9.5243%; total phosphorus: 6.5935%; available phosphorus: 11.3059%; sodium 5.9693%; arginine: 0.0262%; lysine: 0.0178%; methionine: 2.8835%; methionine + cystine: 2.9797%; cystine: 0.0136%; tryptophan: 0.0052%; glycine: 0.0234%; histidine: 0.0189%; isoleucine: 0.0200%; leucine: 0.0778%; phenylalanine: 0.0305%; tyrosine: 0.0212%; threonine: 0.1666%; valine: 0.0277%; alanine: 0.0470%; release phosphorus: 0.0101%; phosphorus phytase: 4.7250%; efficiency: 468,7500; serine: 0.0306%; phosphorus dig. birds: 0.0082%; phytic phosphorus: 0.0126%; proline: 0.0833%; ac. glutamic acid: 0.1988%; glycine + serine: 0.0540%; potassium: 2.8675%; chloro: 5.0067%; mineral oil 71.6626%; phenylal + tyrosine: 0.0517%; energy met. matrices: 445 kcal kg⁻¹; energy met. birds: 445 kcal kg⁻¹; linoleic acid: 0.0840%; copper: 666.6666 ppm; iron: 1,666,2500 ppm; manganese: 3,830.6670 ppm; zinc: 3,333,7500 ppm; iodine: 66.7333 ppm; selenium: 13.2917 ppm; Ca-P 0.842%; arg. dig. 0.0234%; lys dig 0.0145%; met. dig. 2.8824%; m+c dig: 2.8945%; cis cysteine: 0.0116%; trp dig: 0.0047%; tre. dig: 0.16660%; val. dig.: 0.0243%; ile. gt; 0.0180%. [2]Premix Vitamin Posture: Vit. A: 406.0000 IU/g; Vit. D₃ 171.0680 IU/g; Vit. E: 2,247,5000 ppm; Vit. K: 94.2388 ppm; Vit B1 (thiamine): 106.5866 ppm; Vit B2 (riboflavin): 417.6000 ppm; Vit. B6 (pyridoxine): 181.2036 ppm; Vit B12 (cyanocobal) 1.5370 ppm; Folic acid: 133.3420 ppm; Nicotinic acid: 1,348.5000 ppm; B.C. Pantothenic: 681,5001 ppm; biotin: 9.7150 ppm; choline: 13,277,8500 ppm; antioxidant: 3,507,2500 ppm; tylosin: 1837.0000 ppm; 1,918,8490 eq.base-meq/kg; humidity: 1.9907%

Weights of yolk, egg albumen and eggshell were evaluated, and four eggs from each replicate were separated on the last three consecutive days of each period. The eggs were weighed individually, broken, having the weight (g), pH, height (mm) and diameter (mm) of the recorded yolk and albumen. The respective shell was washed and air dried, and then weighed. The albumen weight was obtained between the difference of

the weight of the egg and the weight of the yolk plus the weight of the eggshell. The albumen height was determined using a digital caliper, and the pH of albumen and yolk was measured using a portable pH meter with a direct electrode in the content. The percentages of egg components and Haugh Unit (MURAKAMI *et al.*, 2007) were calculated. The measurements of the thickness of the eggshell were performed using digital caliper (Mitutoyo® 0-150 mm, precision 0.001 mm).

To evaluate the oxidative process of the eggs after 40 days of storage, peroxide (IP) and acidity (AI) were determined. The analyzes followed methodologies described in the Compendium of Animal Feeding (SINDICATO NACIONAL DA INDÚSTRIA DE ALIMENTAÇÃO ANIMAL, 2013).

The metabolizability coefficients of crude protein, ethereal extract and crude fiber were determined. Under the floor of the cages were placed trays of metal plates galvanized, coated with plastics, properly identified. The total excreta collection, twice a day, was carried out during the last five consecutive days of each experimental period. The marker used was ferric oxide (2%). Excreta and feed were analyzed following procedures described in Silva and Queiroz (2006).

At the end of the experimental period (63 rd day), a bird per treatment was weighed, sacrificed by cervical dislocation and had blood (serum), bone (femur) and organs of the digestive tract (liver, pancreas, proventriculus + gizzard and intestine) removed. The viscera were immediately weighed; the length of the intestine was measured using a metric ruler, measured from the beginning of the duodenum to the cloaca.

In the right femur *in natura*, all adherent tissue was removed, using scissors and tweezers, and then weighed in analytical balance, and measurements of the diameter (horizontal) and length (mm) were performed using a digital caliper. The Seedor index was obtained by dividing bone weight (mg) by bone length (mm), and taking into account measures bone density (SEEDOR; QUARRACCIO; THOMPSON, 1991), therefore the higher the index, the higher the density of the bone piece.

The collected blood was conditioned in a properly identified test tube; and centrifuged at 6000 rpm for 15 minutes to obtain serum (RAMOS *et al.*, 2014), and then stored at -20 °C until the time of the evaluations. Analyzes of serum minerals, calcium and phosphorus were performed with commercial kits.

Data were submitted to analysis of variance in DCR, factorial scheme (2x2), considering the level of 5% of probability, and in case of significance the means were compared by means Tukey's test. The analyses were performed using SAEG (2007) software, version 9.5.

RESULT AND DISCUSSION

The use of maize or sorghum, with or without xylanase and β-glucanase in feed, did not influence the productive variables: feed consumption (total egg bird^{-1} day^{-1}), egg weight (g), feed conversion by mass of eggs and per dozen produced and viability of birds (Table 2). The results verified resemble those of Moura *et al.* (2010). The researchers did not find significant effects on the production characteristics of Japanese laying quails, from nine to 25 weeks of age, by replacing corn with sorghum (0; 25; 50; 75 and 100%) in the rations.

The total egg mass was altered by the inclusion of xylanase and beta-glucanase in the rations, presenting a higher amount when compared to the rations without the enzymes. The use of the enzymes was possibly efficient in the degradation of the non-starch polysaccharides (PNAs), improving the energetic utilization of the diets. According to Viana *et al.* (2011), xylanase supplementation in diets containing reduced energy (2,755 kcal ME kg^{-1}) improved the egg production and egg mass of commercial laying hens from 24 to 48 weeks.

Iwahashi *et al.* (2011), stated that the supplement with xylanase and β-glucanase enzyme complex was efficient in providing reduced dietary performance in ME and amino acids for the initial and growth stages of quails.

The sorghum has a similar bromatological profile to corn, with the exception of the amount of metabolizable energy - ME (ROSTAGNO *et al.*, 2017) and carotenoids, which are smaller. Thus, the absence of significant difference between the rations evaluated, indicated the possibility of safe use of this feed in Japanese quail rations. However, the inclusion of synthetic pigments may be necessary to improve egg yolk color.

The accumulated mortality was 15.90% (1.77% week^{-1}), and the viability was not influenced by the ingredients and/or enzymes; this result was due to the advancing age of birds. Togashi, Soares and Murakami (2008) reported that at the end of the batch life, mortality may reach 30%, and despite numerous reports of cloacal prolapse problems, many deaths have indeterminate causes.

There was no significant interaction between ingredients and enzymes (p>0.05) in these variables (Table 3). However, rations with soybean meal and maize (RSMM) showed higher crude protein metabolizability coefficient - CMCF (p=0.0396) when compared to soybean meal and sorghum (RSMS) rations. Also, with the enzymatic addition, the crude fiber metabolizability coefficient - CMCF verified were lower (p=0.0209).

Dissimilar to that found in this study, Iwahashi *et al.* (2011), verified an improvement in five percentage points in the metabolizability coefficient of neutral detergent fiber (CMNFD) due to the enzymatic addition (Xylanase + β-glucanase) in the feeds (negative controls) for quails. The explanation is the greater availability of intracellular nutrients contained in the vegetal wall.

Regarding egg quality, there was no significant interaction between feed and enzymes, as well as differences between treatments (Table 4). The internal characteristics of the egg: weight (g), pH, diameter (mm),

height (mm) of albumen and yolk; were not influenced ($p>0.05$). Similarly, Viana *et al.* (2011), Oba *et al.* (2013), Geraldo *et al.* (2014), and Vargas *et al.* (2017), did not observe effects of the use of enzymatic complexes under the quality of the eggs, indicating that the requirements of the birds were met with the diets used.

In the study by Moura *et al.* (2010), the substitution of maize by sorghum in feed had no effect on yolk weight, eggshell, albumen and eggshell thickness. In the present study, eggshell thickness (mm) was altered as a function of the ingredient used, so corn rations (RSMM and RSMM +

Table 2 - Performance of Japanese laying quails fed with corn or sorghum feeds, with and without enzymes

Rations	Enzymes		MR	P Value			CV (%)	SEM
	With	Without		Rations	Enzymes	ExR		
Total feed consumption (g)								
Maize	2495.40	2551.80	2523.60	>0.05	>0.05	0.0782	2.52	0.026
Sorghum	2525.40	2475.40	2500.40	-	-	-	-	-
ME	2510.40	2513.60	-	-	-	-	-	-
Feed consumption (g bird^{-1} day^{-1})								
Maize	29.71	30.38	30.04	>0.05	>0.05	0.0782	2.52	0.025
Sorghum	30.06	29.47	29.76	-	-	-	-	-
ME	29.88	29.92	-	-	-	-	-	-
Egg weight (g)								
Maize	11.25	10.96	11.10	>0.005	>0.05	0.1129	3.26	0.033
Sorghum	11.13	11.38	11.25	-	-	-	-	-
ME	11.19	11.17	-	-	-	-	-	-
Egg mass (g)								
Maize	1620.53	1501.39	1560.95	>0.05	0.0001	>0.05	3.29	0.102
Sorghum	1624.89	1512.66	1568.77	-	-	-	-	-
ME	1622.71A	1507.02B	-	-	-	-	-	-
Feed conversion per egg mass (kg kg^{-1})								
Maize	2.64	2.77	2.70	>0.05	>0.05	0.2048	2.92	0.044
Sorghum	2.70	2.59	2.64	-	-	-	-	-
ME	2.67	2.68	-	-	-	-	-	-
Feed conversion per dozen of eggs (g duz^{-1})								
Maize	356.48	364.54	360.51	>0.05	>0.05	0.0782	2.52	0.0.25
Sorghum	360.77	353.63	357.20	-	-	-	-	-
ME	358.63	359.08	-	-	-	-	-	-
Viability (%)								
Maize	86.43	82.14	84.28	>0.05	0.1261	>0.05	10.03	0.099
Sorghum	87.86	80.00	83.93	-	-	-	-	-
ME	87.14	81.07	-	-	-	-	-	-

CV = coefficient of variation; ExR = Interaction Enzyme x Ration; ME = media rations with on without enzymes; MR = media rations; SEM = standard error of the mean

enzymes) provided eggs with a thicker eggshell (p<0.05). Murakami *et al.* (2007), also observed variations in eggshell thickness and Haugh Unit of eggs of commercial laying hens fed with the inclusion of multienzymatic complex in the rations.

When evaluating the quality of the egg, some characteristics should be analyzed together, such as the thickness of the shell and the specific gravity. In general, the highest value of specific gravity is considered to be related to a thicker egg shell, a characteristic desirable by

Table 3 - Metabolizability coefficients of crude protein, ethereal extract, and crude fiber of Japanese laying quails fed with corn or sorghum feeds, with and without enzymes

Ratios	Enzymes		MR	P Value			CV (%)	SEM
	With	Without		Rations	Enzymes	ExR		
Metabolizability coefficients of crude protein (%)								
Maize	88.86	89.93	89.39	0.0571	0,0952	0,1515	1.89	0.020
Sorghum	90.98	90.95	90.96	-	-	-	-	-
ME	89.92	90.44	-	-	-	-	-	-
Metabolizability coefficients of ethereal extract (%)								
Maize	89.79	91.76	90.77	0.0668	0.0716	0,2150	1.73	0.0196
Sorghum	91.79	92.56	92.17	-	-	-	-	-
MR	90.79	92.16	-	-	-	-	-	-
Metabolizability coefficients of crude fiber (%)								
Maize	91.22	93.80	92.51A	0.0396	0.0209	0.1411	1.53	0.0191
Sorghum	90.79	91.42	91.10B	-	-	-	-	-
MR	91.00B	92.61A	-	-	-	-	-	-

CV = coefficient of variation; ExR = Interaction Enzyme x Ration; ME = media rations with on without enzymes; MR = media rations; SEM = standard error of the mean

Table 4 - Egg quality of Japanese laying quails fed with maize or sorghum feeds, with and without enzymes

Rations	Enzymes		MR	P Value			CV (%)	SEM
	With	Without		Rations	Enzymes	ExR		
Albumin weight (g)								
Maize	6.78	6.32	6.55	>0.05	>0.05	0.0958	5.67	0.0644
Sorghum	6.53	6.95	6.74	-	-	-	-	-
ME	6.65	6.63	-	-	-	-	-	-
pH of the albumin								
Maize	7.92	7.30	7.61	>0.05	0.3185	>0.05	9.43	0.0919
Sorghum	7.76	7.72	7.74	-	-	-	-	-
MR	7.84	7.51	-	-	-	-	-	-
Height of the albumin (mm)								
Maize	5.08	4.45	4.76	>0.05	>0.05	>0.05	6.82	0.0910
Sorghum	4.92	5.07	4.99	-	-	-	-	-
MR	5.00	4.76	-	-	-	-	-	-
Diameter of the albumin (mm)								
Maize	31.33	29.95	30.64	>0.05	>0.05	>0.05	7.88	0.0744
Sorghum	30.83	31.17	31.00	-	-	-	-	-
MR	31.08	30.56	-	-	-	-	-	-

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Continued Table 4

Weight yolk (g)								
Maize	3.71	3.70	3.70	>0.05	>0.05	>0.05	10.60	0.1014
Sorghum	3.91	3.62	3.76	-	-	-	-	-
ME	3.81	3.66	-	-	-	-	-	-
pH yolk								
Maize	7.04	6.17	6.60	>0.05	>0.05	>0.05	7.57	0.0909
Sorghum	6.78	7.19	6.98	-	-	-	-	-
ME	6.91	6.68	-	-	-	-	-	-
Height of the yolk (mm)								
Maize	11.39	9.61	10.50	0.2549	>0.05	>0.05	5.28	0.1039
Sorghum	11.00	11.67	11.33	-	-	-	-	-
ME	11.19	10.64	-	-	-	-	-	-
Diameter of yolk (mm)								
Maize	24.94	22.93	23.94	0.0534	0.3310	>0.05	4.46	0.0649
Sorghum	25.60	25.48	25.54	-	-	-	-	-
ME	25,27	24.20	-	-	-	-	-	-
Weight shell (g)								
Maize	0.92	0.94	0.93	>0.05	>0.05	>0.05	7.87	0.0729
Sorghum	0.94	0.92	0.93	-	-	-	-	-
ME	0.93	0.93	-	-	-	-	-	-
Albumin (%)								
Maize	59.46	57.28	58.37	0.2273	>0.05	0.2683	4.75	0.0477
Sorghum	59.59	60.30	59.94	-	-	-	-	-
ME	59.52	58.79	-	-	-	-	-	-
Yolk (%)								
Maize	32.47	34.06	33.26	>0.05	0.2786	0.07	5.45	0.108
Sorghum	39.00	31.93	35.46	-	-	-	-	-
ME	35.73	32.99	-	-	-	-	-	-
Eggshell (%)								
Maize	8.15	8.21	8.18	>0.05	>0.05	>0.05	6.56	0.0609
Sorghum	8.36	8.24	8.30	-	-	-	-	-
ME	8.25	8.22	-	-	-	-	-	-
Eggshell thickness (mm)								
Maize	0.30	0.33	0.32A	0.00	>0.1370	>0.05	9.62	0.181
Sorghum	0.23	0.24	0.23B	-	-	-	-	-
ME	0.27	0.29	-	-	-	-	-	-

CV = coefficient of variation; ExR = Interaction Enzyme x Ration; ME = media rations with on without enzymes; MR = media rations; SEM = standard error of the mean

the processing industries. Despite the modification of the thickness of the eggshell found, no change was observed in the specific gravity of the eggs, thus indicating the maintenance of quality.

In Table 5, the values of specific gravity (g cm^{-3}), Haugh Unit and the indicators of egg oxidative process, peroxide index and acidity, are presented after 40 days of storage. There was no influence of the treatments in isolation, or even interaction between the ingredients and the enzymes.

Oba *et al.* (2013), have pointed out that the addition of enzymes is able to reduce some antinutritional properties present in foods, such as PNAs, which have the capacity to increase the viscosity of the digesta, as it has high capacity to bind to water, forming a viscous gel, thus hindering the action of enzymes and the absorption of the digested nutrients. Despite the improvement in viscosity of the digesta, in general, the productive and egg quality variables were not influenced independently of the enzymatic use.

The biometry of the organs of the gastrointestinal tract (GIT) of quails was in accordance with those verified in the zootechnical performance, indicating that there was no physiological involvement of the birds (Table 6). It is important to consider the alterations of the digestive system, since these interrelate deeply with productivity, as a function of the influence on the utilization of all nutrients (ARTONI *et al.*, 2014).

The intestinal length (cm), well the weights of each intestine (thin and thick) of the birds were not altered by receiving the different rations in an interactive and/ or isolated way. Saar *et al.* (2015), studied the intestinal morphometry of Japanese quails fed diets based on sorghum grain. These authors also did not find significant differences for the weight of the liver, gizzard and intestines, as well as intestinal size (cm). Also, because they did not compromise the gastrointestinal tract of the birds, they recommended the use of sorghum in the total or partial substitution of corn in the feed of quails.

The Table 7 shows the bone metric variables as well as the serum minerals (Ca and P) values. The femur weight, length (mm) and diameter (mm) were not

Table 5 - Specific gravity, Haugh unit, peroxide index and acidity of laying Japanese quail eggs fed with maize or sorghum feeds, with and without enzymes

Rations	Enzymes		MR	P Value			CV (%)	SEM
	With	Without		Rations	Enzymes	ExR		
Specific gravity (g cm^{-3})								
Maize	1.06	1.06	1.06	>0.05	>0.05	>0.05	0.27	0.0021
Sorghum	1.06	1.06	1.06	-	-	-	-	-
ME	1.06	1.06	-	-	-	-	-	-
Haugh unit								
Maize	92.52	87.08	89.80	0.2630	>0.05	0.0632	4.87	0.0522
Sorghum	90.86	93.34	92.10	-	-	-	-	-
MR	91.69	90.21	-	-	-	-	-	-
Peroxide index								
Maize	0.4543	0.4340	0.4441	0.0608	>0.05	>0.05	9.77	0.2010
Sorghum	0.4860	0.4834	0.4847	-	-	-	-	-
MR	0.4701	0.4587	-	-	-	-	-	-
Acidity								
Maize	9.2732	9.2468	9.2600	>0.05	>0.05	>0.05	3.22	0.101
Sorghum	9.3800	9.2860	9.3330	-	-	-	-	-
MR	9.3266	9.2664	-	-	-	-	-	-

CV = coefficient of variation; ExR = Interaction Enzyme x Ration; ME = media rations with on without enzymes; MR = media rations; SEM = standard error of the mean

Table 6 - Biometry of digestive tract organs of Japanese laying quails fed on corn or sorghum feeds, with and without enzymes

Rations	Enzymes		MR	P Value			CV (%)	SEM
	With	Without		Rations	Enzymes	ExR		
Intestine length (cm)								
Maize	47.81	49.43	48.62	>0.05	>0.05	>0.05	6.74	0.0648
Sorghum	46.92	48.03	47.47	-	-	-	-	-
ME	47.36	48.73	-	-	-	-	-	-
Gastrointestinal tract weight (g)								
Maize	10.27	10.51	10.39	0.1919	0.2596	>0.05	7.97	0.0807
Sorghum	9.59	10.20	9.89	-	-	-	-	-
ME	9.93	10.35	-	-	-	-	-	-
Slender intestine (g)								
Maize	2.78	2.71	2.74	>0.05	>0.05	>0.05	10.99	0.102
Sorghum	2.78	2.65	2.72	-	-	-	-	-
MR	2.78	2.68	-	-	-	-	-	-
Thick Intestine (g)								
Maize	1.08	1.04	1.06	>0.05	>0.05	>0.05	8.97	0.103
Sorghum	0.93	1.15	1.04	-	-	-	-	-
MR	1.00	1.09	-	-	-	-	-	-
Liver (g)								
Maize	2.78	3.05	2.91	0.2326	0.1291	>0.05	7.81	0.099
Sorghum	2.31	2.68	2.49	-	-	-	-	-
MR	2.54	2.86	-	-	-	-	-	-
Pancreas (g)								
Maize	0.25	0.24	0.24	>0.05	0.2992	0.2143	9.90	0.097
Sorghum	0.24	0.27	0.25	-	-	-	-	-
ME	0.24	0.26	-	-	-	-	-	-
Proventric + gizzard (g)								
Maize	2.62	2.60	2.61	>0.05	>0.05	0.2609	7.15	0.069
Sorghum	2.52	2.70	2.61	-	-	-	-	-
ME	2.57	2.65	-	-	-	-	-	-

MR = media rations; ME = media rations with on without enzymes; ExR = Interaction Enzyme x Ration; CV = coefficient of variation; SEM = standard error of the mean

Table 7 - Bone morphometry and serum minerals of Japanese laying quails fed with maize or sorghum feeds, with and without enzymes

Rations	Enzymes		MR	P Value			CV (%)	SEM
	With	Without		Rations	Enzymes	ExR		
Femur weight (g)								
Maize	0.50	0.51	0.50	0.0762	>0.05	>0.05	7.15	0.080
Sorghum	0.56	0.54	0.55	-	-	-	-	-
ME	0.53	0.52	-	-	-	-	-	-

Continuation Table 7

Femur length (mm)								
Maize	38.58	37.39	37.98	>0.05	>0.05	0.0875	2.81	0.056
Sorghum	37.96	38.52	38.24	-	-	-	-	-
ME	38.27	37.95	-	-	-	-	-	-
Femur diameter (mm)								
Maize	2.91	2.67	2.79	>0.05	>0.05	0.2005	3.67	0.028
Sorghum	2.82	2.90	2.86	-	-	-	-	-
MR	2.87	2.78	-	-	-	-	-	-
Seedor Index								
Maize	12.96	13.64	13.30	0.0692	>0.05	0.0775	6.08	0.177
Sorghum	14.75	14.01	14.38	-	-	-	-	-
MR	13.85	-	-	-	-	-	-	-
Serum calcium (mg dL^{-1})								
Maize	12.92	12.72	12.82A	0.0022	>0.05	>0.05	14.30	0.215
Sorghum	10.30	10.00	10.15B	-	-	-	-	-
MR	11.61	11.36	-	-	-	-	-	-
Serum phosphorus (mmol L^{-1})								
Maize	4.98	5.28	5.13A	0.0001	>0.05	0.0853	14.16	0.074
Sorghum	3.39	4.12	3.75B	-	-	-	-	-
ME	4.19	4.70	-	-	-	-	-	-

MR = media rations; ME = media rations with on without enzymes; ExR = Interaction Enzyme x Ration; CV = coefficient of variation; SEM = standard error of the mean

influenced by diets (p>0.05). The Seedor Index (SI) is used as an indication of bone density, so that the higher the index, the greater the density of the piece and vice versa. No changes were observed in the SI values of the studied bones, such results indicate the integrity in the filling of the organic bone matrix.

Serum calcium and phosphate levels were altered as a function of the feed ingredient. There was no significant interaction between the factors studied. The highest blood concentrations of Ca and P were observed in birds fed corn and soybean meal (RSMM and RSMS + enzymes), independent of the receipt of xylanase and β-glucanase.

The results of serum minerals found were within the normal range, similar to the values described by Silva *et al.* (2017), who evaluated turmeric in sorghum-based diets for Japanese laying quails. The investigators mentioned did not verify differences due to treatments for serum Ca and P, as well as performance, protein metabolism, ethereal extract, internal and external egg quality, biometry of the gastrointestinal tract, tibial and femoral morphometry.

Normal concentrations of Ca in birds may reach values much higher than those tolerated in mammalian species, reaching 30 g L^{-1}; while the bands for P are 7 to 9 g L^{-1} (THRALL; BAKER; CAMPBELL, 2004). It should be noted that the changes in the mineral amounts of blood occur as a function of the physiological phase of laying hens, and in the reproductive stage the transport of Ca to the ovary, induced by estrogens, by the increase of Ca binding proteins such as vitellogenin and albumin (CAPITELLI; CROSTA, 2013; SILVA *et al.*, 2017).

The Ca: P ratios were: 2.59:1; 2.40:1; 3.03:1 and 2.42:1 for RSMM, RSMM + enzymes; RSMS and RSMS + enzymes, respectively. The maintenance of these relationships results from the fact that laying hens maintain plasma Ca and phosphate proportional levels (CHOI; MILES; HARMS, 1979). In this study, the Ca and P values in diets provided were in accordance with the physiological needs of the laying quails under the conditions evaluated.

Lecznieski (2006) explained that in diets with adequate or slightly deficient levels, the addition of enzymes can release nutrients that the animal does not

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need or even cannot convert into higher productive indexes. Such justification became plausible in the absence of additive effects of enzymatic use on the productive and physiological results found. Still, it is sometimes desired not to impair the productive and biochemical-physiological characteristics, and not only the improvement of these, especially in the case of the use of alternative foods.

CONCLUSIONS

1. In general, there were no significant differences in the main productive variables, metabolizability coefficient, egg quality, biometry of the gastrointestinal tract and organs of Japanese laying quails fed with rations containing maize or sorghum supplemented or not with xylanase + β-glucanase;

2. Based on the physiological responses obtained, an alternative is the optional use of the enzymes evaluated with maize or sorghum in Japanese quail rations, from 26 to 35 weeks of age.

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