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Fiber source and xylanase on performance, egg quality, and gastrointestinal tract of laying hens

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ABSTRACT - The objective of this study was to ascertain the influence of different dietary fiber sources and the usage of xylanase on diet of commercial layers and their influence on productive performance, egg quality, and digestive organ biometry. A total of 864 Lohmann® White hens was fed diets with three different fiber sources (wheat bran, soybean hull, or coffee husk) with or without xylanase inclusion (concentration of 160,000 BXU/g) in a 3×2 factorial arrangement, with six replicates of 24 birds each, from 25 to 44 weeks of age. There were no interactions between dietary fiber and xylanase inclusion. The enzyme supplementation did not influence any parameters evaluated. There were dietary fiber effects on body weight gain, viability, egg weight, eggshell quality, yolk pigmentation, and liver and gizzard relative weights. Wheat bran, soybean hull, and coffee husk can be used in laying hen diets without detrimental effect on productive performance. The enzyme used had no effect on the performance and eggshell quality of laying hens.

Keywords: alternative fiber, enzyme, laying hen, non-starch polysaccharides, viscosity

Introduction

Wheat bran is the main raw material used in laying hen nutrition to decrease energy levels and increase dietary neutral detergent fiber (NDF) levels. However, such an ingredient has been often found at high cost; thus, the search for alternative dietary ingredients to be used to reduce egg production costs with no damage to the performance of birds is needed.

Two points have been basically observed in the use of alternative feed – its chemical composition variation and the presence of non-starch polysaccharides (NSP). The NSP are not only poorly digested by birds but also negatively affect bird physiology, and those adverse effects include altered intestinal transit time, change in intestinal mucosal structure, and hormonal deregulation (Vahouny, 1982). Moreover, the greatest effects are associated with viscosity of NSP and their interaction with the intestinal microbiota (Choct, 1997).

The interest in the effect of high-fiber content in diets on the digestive physiology of animals has increased, especially among the monogastric animals, in which the knowledge of microorganisms involved in fiber breakage is still limited when compared with polygastric species (Castro Júnior et al., 2005).

Studies with exogenous enzymes have been increasingly disseminated, mainly due to their characteristics. According to Lima et al. (2007), the addition of exogenous enzymes to the rations of animals has four distinct objectives: the removal or hydrolysis of anti-nutritional factors, increased digestibility of nutrients, breakdown of NSP, and supplementation of endogenous enzymes. Thus, the exogenous enzymes, in addition to facilitating the feed efficiency use, can improve the use of low-cost ingredients for animal feed, since the viscosity of the digesta decreases with use, potentiating the action of endogenous enzymes on the specific substrates (Ribeiro et al., 2011).

Such hindrances related to wheat bran usage in animal feed have motivated the search for other feedstuffs capable of increasing the fiber levels of poultry diets and decreasing feed energy levels. The efficient usage of alternative feed ingredient could lead to a reduction in egg production costs and a production performance enhancement.

This study aimed at evaluating the effects of different dietary fiber sources with or without enzyme inclusion on performance, egg quality, and biometrics of gastrointestinal tract of laying hens.

Material and Methods

All procedures and animal care followed the ethical principles of animal experimentation. This study was approved by the Ethics Committee on Animal Use (case no. 384/2015). The experiment was conducted in Igarapé, Minas Gerais, Brazil (20°04'13" S, 44°18'06" W).

A total of 864 Lohmann LSL-Lite® birds at 25 weeks of age were distributed into 36 plots, and each plot consisted of four cages with six birds per cage (375 cm^2/bird), totaling 24 birds per plot, for 20 weeks. The experimental design was in a 3×2 factorial arrangement, i.e., three fiber sources (wheat bran, soybean hulls, and coffee husks) and the inclusion or not of a xylanase (at 160,000 BXU/g), totaling six treatments and six replicates/treatment (Table 1). Laying hens were fed diets based on corn and soybean meal formulated to supply their nutritional requirements as described by Rostagno et al. (2011), except for coffee husks and soybean hull, to which a value of 1,035 kcal/kg apparent metabolizable energy was attributed due to the scarcity of studies on energy determination of alternative feedstuffs for layers. A sample of coffee husks was taken to the laboratory for analysis of its chemical composition: 881.9 g kg^{-1} dry matter (DM), 107.5 g kg^{-1} crude protein (CP), 422.4 g kg^{-1} acid detergent fiber (ADF), 2.1 g kg^{-1} total lysine, 1.6 g kg^{-1} total threonine, and 1.1 g kg^{-1} total cysteine. A 14 h light/10 h dark photoperiod was set, with 12 h natural light and 2 h artificial light, half of which was provided at dawn, while the other half was provided at night.

The following traits were evaluated: initial and final laying hen weights, feed intake, egg production, feed conversion (kg of feed/dozen eggs produced and kg of feed/kg of eggs produced), number of eggs per housed bird, egg weight, and viability. Fiber sources used in feeds were sampled and analyzed for NSP concentration according to Englyst et al. (1994).

On the 44th week of age, 24 eggs of each treatment were analyzed for yolk, eggshell, and albumen percentages, eggshell thickness, Haugh unit (HU), eggshell resistance, and yolk color. Yolk, eggshell, and albumen percentages were calculated according to Wu et al. (2005). Eggshell thickness was evaluated through a digital Digimess® micrometer, with 0.001 mm readability. Measurements were performed at three distinct eggshell regions (apical, equatorial, and basal). Results were obtained by the average of the three regions, expressed in mm.

Haugh unit was calculated through a HU-measuring device (model S-8400, Ames, Massachussets, USA) according to Brant et al. (1951). For eggshell resistance evaluation, other 24 eggs of each treatment were randomly sampled and subjected to the compression eggshell fracture force test. A TA-XT2 texture analyzer (Stable Micro Systems, Surrey, England) was used with a stainless steel P4 DIA Cylinder probe (4 mm diameter; 3.0, 0.5, and 5.0 mm/s pre-, during, and post-testing speed, respectively; and 6 mm long); trigger strength was 3.0 g. The whole egg was longitudinally placed onto a ring-shaped metallic support (5 cm diameter) inside a porcelain crucible. Eggshell was pressed until fracture. Yolk color was measured through a color fan (DSM YOLK COLOR FAN, 2005 – HMB 51548).

At the end of the experimental period, one laying hen per replicate was selected within a range of ±10% average weight of the replicate and was slaughtered by cervical dislocation. The following organs were sampled and weighed: gizzard (from which the residual feed content was completely removed), liver with gallbladder, intestine, and pancreas. The intestinal content detected between Meckel's diverticulum and cecum was sampled and centrifuged (Combate®, 3400 rpm for 10 min). The supernatant was collected and then frozen at −30 °C. After thawing at room temperature, the supernatant was analyzed in a viscometer (Brookfield Cone and Plate LVCDVII).

The economic efficiency of each treatment was evaluated through the estimate of average costs with feed per produced egg carton. The prices of inputs were obtained in the region of Minas Gerais State, Brazil, on November 2016.

Means were subjected to analysis of variance (ANOVA) in a factorial arrangement with fiber sources and presence or absence of xylanase as main effects. All possible interactions within and between main effects were evaluated through the SAS software (Statistical Analysis System, version 9.4). Significant means were compared by the Tukey test. Data not showing a normal distribution were compared by the Kruskal-Wallis test through the same software. Significance level was set at $P \leq 0.05$.

Table 1 - Composition (g kg^{-1} as fed) and calculated nutritional values of experimental feeds

	Treatment		
	Wheat bran	Soybean hulls	Coffee husks
Ingredient (g kg^{-1})			
Corn grain	591.67	613.33	610.00
Soybean bran (45% CP)	196.67	196.67	211.67
Wheat bran	78.33	0.000	0.000
Soybean hulls[1]	0.000	50.00	0.000
Coffee husks	0.000	0.000	42.50
Calcitic limestone	85.48	81.54	85.56
Meat-and-bone meal (40% CP)	40.83	51.67	43.33
Salt	3.00	3.22	3.00
Vitamin and mineral supplement[2]	2.00	2.00	2.00
DL methionine	1.50	1.50	1.50
L-lysine HCl	0.11	0.000	0.03
Inert or xylanase[3]	0.075	0.075	0.075
Total	1000.0	1000.0	1000.0
Nutritional values			
Calcium (%)	3.79	3.78	3.82
AMEn (kcal/kg)	2682.0	2680.8	2677.5
Available phosphorus (%)	0.35	0.40	0.35
Digestible lysine (%)	0.75	0.75	0.75
Digestible Met+Cys (%)	0.61	0.61	0.61
Digestible methionine (%)	0.38	0.38	0.38
Crude protein (%)	17.00	17.00	17.00
Sodium (%)	0.18	0.18	0.18
Digestible threonine (%)	0.55	0.55	0.55
Digestible tryptophan (%)	0.16	0.16	0.16

CP - crude protein; AMEn - nitrogen-corrected apparent metabolizable energy.
[1] Soybean hulls with 0.09 pH urease activity.
[2] Composition per kilogram of the product: vitamin A, 5,000,000 IU; vitamin D3, 1,100,000 IU; vitamin E, 4,000 IU; vitamin K3, 1,000 mg; vitamin B1, 520 mg; vitamin B2, 1,500 mg; vitamin B6, 500 mg; vitamin B12, 3,000 mcg; folic acid, 102 mg; biotin, 10 mg; niacin, 10 g; pantothenic acid, 4,600 mg; manganese, 25 g; zinc, 25 g; iron, 25 g; copper, 3,000 mg; cobalt, 50 mg; iodine, 500 mg; selenium, 100 mg; choline 43 g. *Bacillus subtilis* 75×10^{9} cfu.
[3] Xylanase 75 g/ton.

Results

From this work, the main NSP detected in wheat bran were arabinose (5.9%), xylose (9.8%), and glucose (7.5%), which are the main components of hemicelluloses, responsible for changes in intestinal viscosity. On the other hand, soybean hulls and coffee husks had glucose (28 and 16.9%, respectively), xylose (8.0 and 8.1%, respectively), and galacturonic acid (7.7 and 6.6%, respectively) as the main components (Table 2).

In this study, no interaction (P>0.05) was detected between fiber sources and the usage of xylanase for any of the analyzed variables. Initial bird weight was similar among treatments (P>0.05), thus proving their homogeneity at the beginning of the experimental period (Table 3).

Xylanase had no effect on final weight (P>0.05); nevertheless, this trait was influenced by fiber sources (P≤0.05). Birds fed diets with wheat brain had higher final body weight than those fed feed containing soybean hulls and coffee husks; however, both groups did not differ from laying hens fed wheat bran (P≤0.05). Hens fed feed containing wheat bran had higher viability (P<0.05) than

Table 2 - Concentration (%) of non-starch polysaccharides in wheat bran, soybean hulls, and coffee husks

Fiber source	Rhamnose			Fucose			Arabinose			Xylose		
	S	I	T	S	I	T	S	I	T	S	I	T
Wheat bran	0	0	0	0	0	0	0.5	5.4	5.9	0.6	9.2	9.8
Soybean hulls	0.1	0.3	0.4	0	0.1	0.1	0.3	3.8	4.1	0	8.0	8.0
Coffee husks	0.2	0.2	0.4	0	0	0	1.2	2.7	3.9	0.5	7.6	8.1
Corn	0	0	0	0	0	0	0.1	1.4	1.5	0	2.2	2.2
Soybean bran	0.1	0.1	0.2	0	0.2	0.2	0.6	1.9	2.5	0.1	1.3	1.4
	Mannose			Galactose			Glucose			Galacturonic acid		
	S	I	T	S	I	T	S	I	T	S	I	T
Wheat bran	0.2	0.2	0.4	0.1	0.6	0.7	0.5	7.0	7.5	0.2	0.2	0.4
Soybean hulls	0	4.1	4.1	0.8	1.7	2.5	0	28.0	28.0	5.0	2.7	7.7
Coffee husks	0.4	0.6	1.0	0.7	1.2	1.9	2.1	14.8	16.9	4.7	1.9	6.6
Corn	0.2	0.3	0.5	0.1	0.5	0.6	0.6	2.5	3.1	0.2	0.2	0.4
Soybean bran	0.5	0.5	1.0	1.1	3.2	4.3	0.1	4.5	4.6	1.00	1.6	2.6

S - soluble; I - insoluble; T - total.

Table 3 - Initial weight (kg), final weight (kg), weight gain (g), and feed intake (g/day) of layers between 25 and 45 weeks of age

Factor	Initial weight[1] (kg)	Feed intake[1] (g/day)	Final weight[1] (kg)
Fiber source (FS)			
Wheat bran	1.43a	101.44a	1.64a
Soybean hulls	1.42a	102.03a	1.60b
Coffee husks	1.43a	102.08a	1.60b
Enzyme (E)			
No	1.42A	101.82A	1.62A
Yes	1.43A	101.88A	1.62A
ANOVA			
FS	0.7666ns	0.6696ns	0.0179*
E	0.3452ns	0.9249ns	0.8674ns
FS×E	0.9179ns	0.7636ns	0.4916ns
CV (%)	1.79	1.91	2.33

CV - coefficient of variation.
[1] ns - non-significant according to the F-test (P≥0.05).
* Significant according to the F-test (P≤0.05).
Means followed by the same letters do not differ by the Tukey test (P≥0.05).

those fed diet with soybean hulls, with no differences in relation to those fed diet with coffee husks (Table 4). Fiber and enzyme use had no effect on egg production, number of eggs per housed bird, and feed conversion (kg/dozen and kg/kg) (P>0.05).

The inclusion of xylanase had no influence on the number of eggs/housed bird (P>0.05). Nevertheless, regardless of the source of fiber feed to the birds, the P-value found (P = 0.056) was rather close to a significant P-value. Therefore, the inclusion of xylanase must be re-evaluated in other experiments.

The use of xylanase had no effect on egg quality (P<0.05). Fiber sources had effect on HU and yolk color score (P≤0.05) of eggs of 44-week-old hens. Eggs from laying hens fed diet with coffee husks had higher HU (P≤0.05) (Table 5), while birds fed feed with soybean hulls had higher yolk color score (P<0.05) (Table 6). However, eggshell quality parameters (%, thickness and resistance) were worse (P≤0.05) when coffee husk was used as a source of fiber (Table 6).

Interactions (P>0.05) were not found for biometrics of organs and intestinal viscosity of birds at 45 weeks of age (Table 7). The inclusion of xylanase (P>0.05) had no effect on none of these traits.

Table 4 - Viability, egg production (%), number of eggs/housed bird, and feed conversion (kg/dozen and kg/kg) of layers between 25 and 45 weeks of age

Factor	Viability[2]	Egg production[1] (%)	Number of eggs/ housed bird[1]	Feed conversion[1] (kg/dozen)	Feed conversion[1] (kg/kg)
Fiber source (FS)					
Wheat bran	99.30a	94.75a	124.54a	1.29a	1.83a
Soybean hulls	96.52b	95.63a	124.37a	1.29a	1.84a
Coffee husks	96.52ab	94.89a	122.63a	1.30a	1.85a
Enzyme (E)					
No	96.75A	94.67A	122.80A	1.30A	1.85A
Yes	98.14A	95.51A	124.89A	1.29A	1.83A
ANOVA					
FS	0.0438*	0.2797ns	0.2747ns	0.6538ns	0.4510ns
E	0.3836ns	0.0856ns	0.0560ns	0.2771ns	0.2142ns
FS×E	-	0.1315ns	0.5037ns	0.6041ns	0.6637ns
CV (%)	-	1.49	2.55	2.21	1.42

CV - coefficient of variation.
[1] ns - non-significant according to the F-test (P≥0.05). * Significant according to the F-test (P≤0.05). Means followed by the same letters do not differ by the Tukey test (P≤0.05).
[2] ns - non-significant according to the Kruskal-Wallis test (P≥0.05). * Significant according to the Kruskal-Wallis test (P≤0.05). Means followed by the same letters do not differ by the Mann & Whitney test (P≥0.05).

Table 5 - Egg weight, yolk and albumen percentages, and Haugh unit (HU) of 44-week-old layers

Factor	Egg weight[1]	Yolk (%)[1]	Albumen (%)[1]	HU[1]
Fiber source (FS)				
Wheat bran	62.01a	27.20a	63.27a	94.35b
Soybean hulls	61.17a	26.53a	64.00a	93.12b
Coffee husks	61.54a	27.20a	63.73a	96.34a
Enzyme (E)				
No	61.87A	26.96A	63.70A	94.04A
Yes	61.28A	26.99A	63.64A	95.17A
ANOVA				
FS	0.5079ns	0.0689ns	0.1276ns	0.0004*
E	0.3184ns	0.8932ns	0.8361ns	0.0842ns
FS×E	0.1121ns	0.1781ns	0.1331ns	0.4546ns
CV (%)	5.78	6.04	2.75	4.12

CV - coefficient of variation.
[1] ns - non-significant according to the F-test (P≥0.05). * Significant according to the F-test (P≤0.05). Means followed by the same letters do not differ by the Tukey test (P≥0.05).

When it comes to organ weight, fiber sources did not influence intestine + pancreas weight (P>0.05). Laying hens fed diet with coffee husks had higher relative gizzard weight when compared with birds fed wheat bran diets (P<0.05), and both groups did not differ from birds fed diet with soybean hulls. However, liver weight was affected by fiber sources (P<0.05).

No effect (P>0.05) of treatments was detected on pancreas characteristics (length and width), thus showing that there were no alterations in the intestine that could lead to greater pancreas activity and, consequently, a hypertrophy of this organ.

No difference in the feed cost per egg carton with or without the inclusion of xylanase was found (P>0.05). The feed containing coffee husks had a lower cost per egg carton compared with the wheat bran diets (P≤0.05). The soybean hulls treatment did not differ from the other two fiber sources treatments regarding the cost per egg carton (Table 8).

Table 6 - Eggshell quality traits and yolk color of 44-week-old layers

Factor	Eggshell (%)[1]	Eggshell thickness (mm)[2]	Eggshell resistance (kg/cm^2)[1]	Yolk color[2]
Fiber source (FS)				
Wheat bran	9.53a	0.391a	5.56a	7.15c
Soybean hulls	9.48a	0.386a	5.48a	7.81a
Coffee husks	9.07b	0.373b	5.27a	7.23b
Enzyme (E)				
No	9.35A	0.38A	5.56A	7.44A
Yes	9.37A	0.38A	5.31A	7.35A
ANOVA				
FS	0.0003*	<0.0001*	0.1443ns	<0.0001*
E	0.7341ns	0.8228ns	0.0515ns	0.2346ns
FS×E	0.6576ns	0.3456ns	0.5792ns	0.0939ns
CV (%)	7.46	6.41	13.77	5.24

CV - coefficient of variation.
[1] ns - non-significant according to the SNK test (P≥0.05). * Significant according to the SNK test (P≤0.05). Means followed by the same letters do not differ by the SNK test (P≥0.05).
[2] ns - non-significant according to the Kruskal-Wallis test (P≥0.05). * Significant according to the Kruskal-Wallis test (P≤0.05). Means followed by the same letters do not differ by the Mann & Whitney test (P≥0.05).

Table 7 - Organ weight in relation to bird weight (%), pancreas characteristics, and intestinal viscosity in of 45-week-old layers

Factor	Liver[2] (%)	Intestine + pancreas[1] (%)	Gizzard[1] (%)	Pancreas length[1]	Pancreas width[3]	Viscosity (cP[4])[2]
Fiber source (FS)						
Wheat bran	2.20b	4.67a	1.16b	9.48a	0.63a	4.01a
Soybean hulls	2.77a	4.78a	1.26ab	9.72a	0.57a	4.58a
Coffee husks	2.82a	5.00a	1.30a	9.76a	0.63a	4.65a
Enzyme (E)						
No	2.54A	4.76A	1.21A	9.61A	0.64A	4.90A
Yes	2.65A	4.88A	1.27A	9.69A	0.58A	4.07A
ANOVA						
FS	0.0054*	0.1213ns	0.0043*	0.7430ns	0.3389ns	0.6160ns
E	0.5092ns	0.3677ns	0.0660ns	0.7893ns	0.0812ns	0.3105ns
FS×E	0.8529ns	0.1188ns	0.3637ns	0.5544ns	-	0.1213ns
CV (%)	18.47	8.09	8.19	10.06	-	46.53

CV - coefficient of variation.
[1] ns - non-significant according to the F-test (P≥0.05). * Significant according to the F-test (P≤0.05). Means followed by the same letters do not differ by the Tukey test (P≥0.05).
[2] ns - non-significant according to the F-test (P≥0.05). * Significant according to the F-test (P≤0.05). Means followed by the same letters do not differ by the Duncan test (P≥0.05).
[3] ns - non-significant according to the Kruskal-Wallis test (P≥0.05). * Significant according to the Kruskal-Wallis test (P≤0.05). Means followed by the same letters do not differ by the Mann & Whitney test (P≥0.05).
[4] Centipoise.

Table 8 - Feed costs per egg carton (R$/carton)

Enzyme	Fiber source			Means
	Wheat bran	Soybean hulls	Coffee husks	
No	30.68	30.10	29.68	30.15A
Yes	30.39	30.27	29.94	30.20A
Means	30.53b	30.19ab	29.81a	

Means followed by the same uppercase letters in the column and lowercase letters in the line do not differ by the Tukey test (P≥0.05). Coefficient of variation = 2.3%.

Discussion

The influence of fiber on performance and physiological traits of non-ruminant animals depends not only on the cell wall content incorporated into the diet but also on the cell wall components, considering their chemical composition and association with other nutrients.

Galacturonic acid, which is found in large proportion in soybean hulls and coffee husks, is the most abundant component of pectin and is considered the principal substance responsible for gel formation in the gastrointestinal tract of the animals (Palenzuela et al., 1998).

Non-starch polysaccharides are divided into three major groups: cellulose (insoluble in water, alcohol, or diluted acids), non-cellulosic polysaccharides (arabinoxylans, mixed-linkage of glucans, mannans, galactans, xyloglucans, and fructans, which are partially soluble in water), and pectinic polysaccharides (polygalacturonic acids, which can be replaced by arabinans, galactans, and arabinogalactans, which are partially soluble in water) (Sakomura et al., 2014). However, the solubility of such groups cannot be defined by itself (as depicted in Table 2). Each component of NSP has a soluble and an insoluble fraction, and the arrangement of those fractions combined with the characteristics of the environment in which they are located will define the final NSP solubility.

Xylose, arabinose, glucose, mannose, and glucuronic acid are the main components of hemicelluloses, which belong to the soluble cell wall fraction. The main characteristic of the soluble fraction is the capacity to increase the viscosity of intestinal contents, resulting in less enzyme and chyme contact, lower nutrient absorption, and reduced gastrointestinal flow rate. Consequently, a reduction in feed intake and a higher substrate availability for proliferation of microorganisms are normally observed (Bedford, 1996).

Another component of the soluble fraction of the cell wall is pectin, which is mainly constituted of galacturonic acid. Pectins have great affinity for water leading to a high capacity of gel formation and, consequently, altering the viscosity of gastrointestinal contents.

The effects of NSP and their changes in the gastrointestinal tract of birds are only related to ingredients used as fiber sources. However, the main ingredients used in animal feed including corn and soybean meal have also considerable levels of NSP. The NSP in corn and soybean meal when added to the NSP of the other ingredients can boost the negative effects of NSP on the performance and on the gastrointestinal tract of birds.

The non-effect of treatments on the feed intake of laying hens may indicate that the used fiber sources and the inclusion or not of xylanase have no influence upon palatability of birds. The lack of effect on feed intake of birds fed diet with soybean hulls and coffee husks in comparison with those fed diet containing wheat bran indicates that the changes caused by the composition of such fiber sources in the viscosity of gastrointestinal contents probably were not significant enough to compromise feed intake. Those results agree with Araujo et al. (2008), who observed that the inclusion of up to 9% wheat bran in the feed did not interfere with its intake. According to the authors, the physiological maturity of the gastrointestinal tract of animals is an important factor to be observed in adjusting the levels of alternative feeds in diets to digestive capacity of layers, especially the low digestibility of the nutrients in these feeds.

Insoluble NSP are the main components of fiber sources used in experimental feeds. Due to their physicochemical properties, NSP reduce the retention time of gastrointestinal contents and nutrient uptake, besides increasing water retention capacity, thus leading to a decrease in the use of dietary nutrients (Montagne et al., 2003). In the present study, the NSP contents of wheat bran did not hinder the uptake and digestion of other feed nutrients, thus assuring a higher final weight of the birds when compared with layers fed feed with coffee husks and soybean hulls.

With regard to viability (Table 4), no justification was found for the above-mentioned effects.

There was no effect of the fiber sources for egg weight, percentage of yolk, and percentage of albumen (Table 5). According to Leeson and Summers (2005), protein, amino acid, and fat contents are the most important nutritional factors that affect egg weight and, consequently, the proportion of its components, especially in younger birds. As in this study the laying hens received diets formulated to contain similar nutrient contents and are older birds, probably the nutrients ingested by the birds were sufficient for the egg components to remain stable.

The eggs from birds that received coffee husks as a source of fiber presented higher Haught unit ($P \leq 0.05$), but no explanation was found for this fact.

Egg quality parameters such as eggshell percentage and eggshell thickness were lower in eggs from birds fed diet with coffee husks ($P \leq 0.05$). Coffee husks are the source of fiber that presented higher concentration of pectin, which has high capacity of gel formation. According to Conte et al. (2003), the increased viscosity of chymos, promoted by the NSP, can lead to decreased digestion of proteins, fats, carbohydrates, and also micronutrients, since they are less available to the enzymatic action in the small intestine. Moreover, they have a strong ionic bonding ability with mineral elements, causing the fiber-rich diets to interfere negatively in the absorption of minerals (Arruda et al., 2003). Another possibility would be a possible interaction between caffeine and calcium absorption. This may reduce the availability of calcium for egg shell formation, but this reduction is not detrimental to the marketing of eggs.

Yolk color was influenced by the treatments (Table 6). Eggs from birds that consumed diet with soybean hulls had higher rates of yolk color compared with those fed the other treatments ($P \leq 0.05$). As the xanthophyll contents of the diets were similar, these results indicate that fiber may interfere with the absorption of pigment substances. Laudadio et al. (2014) also observed an increase in the yolk color score when they included 15% of low-fiber alfalfa in the diet in relation to the group that received the control diet with soybean meal

A higher liver weight was observed for laying hens fed feed with soybean hulls and coffee husks in comparison with those fed diet with wheat bran. The higher liver weight may be related to the harmful effect of some types of fibers contained in the soybean hulls and coffee husks on the digestion process of nutrients, requiring a higher hepatic metabolic activity. Researchers have reported that viscosity caused by NSP enhances the secretion of bile acids, and, consequently, larger amounts of bile acids will be lost into the excreta. Therefore, the great affinity of NSP for bile acids will lead to higher excretion rates in the manure, and, consequently, larger hepatic synthesis of bile acids will be needed aiming at reestablishing the normal concentrations of bile acids in the gastrointestinal tract (Ikegami et al., 1990).

When fed diets containing high fiber levels, birds are capable of changing the metabolism and characteristics of the gastrointestinal tract to improve the use of nutrients. According to Braz et al. (2011), using increasing NDF levels (14.5, 16.5, and 18.5%) in rations for growing birds (7 to 17 weeks of age), observed an increase in intestinal weight in birds fed ration with higher NDF content. The authors associated the greater weight of the intestines with the negative effects of the greater amount of fiber on the digestion and absorption of nutrients; these effects would induce greater activity of this organ attempting to improve the digestion and absorption with diets of high viscosity, causing greater development of the organs. Since no significant difference in intestinal weight was observed in this study, the NDF content may not have been enough to lead to increased organ development.

Hetland et al. (2003), including whole wheat and wood chips in the diet of laying hens from 15 to 29 weeks of age, observed that the insoluble fiber fraction stimulated gizzard development and led to an increase in fiber and bile acid levels of its content, thus improving starch digestibility, similar to what was found in this study. The insoluble fiber has the characteristic to increase the time of permanence of the feed content in the gizzard, increasing the mechanical reduction, which increases the area of contact for later enzymatic action and, therefore, a better utilization of the nutrients, mainly starch. This explained the greater relative weight of this organ.

No effect of treatments was detected on pancreas characteristics (length and width), showing that there were no alterations in the intestine that could lead to greater pancreas activity and to a consequent hypertrophy of this organ. Similar results were reported by Mourão and Pinheiro (2009), who evaluated the inclusion of rye and wheat combined with the inclusion of xylanase and did not observe differences in pancreas weight of broiler chickens. Some authors detected that birds fed diets with high NSP levels can present physiological adaptations including an increase in pancreas weight, secretion of pancreatic enzymes, and length and uptake area of the gastrointestinal tract (Brenes et al., 1993; Jorgensen et al., 1996). All those changes in the physiology of the gastrointestinal tract are aimed to compensate the anti-nutritional effects of some compounds of the fiber; however, it might modify nutrient digestion and uptake.

Intestinal viscosity showed no difference between treatments, which can be explained by the fact that older birds probably have a more developed gastrointestinal tract. Therefore, they are better able to adapt to possible changes caused by NSP.

In the present study, the inclusion of xylanase had no effect on any evaluated trait, probably due to the fact that the enzymes have not found a sufficient amount of xylans for their action, not leading to an improvement in performance and other evaluated trait. Enzymes depend on the substrate concentration correspondent for their performance. Wheat bran, which contains higher xylan levels, may be used at higher amounts and might be benefited with the use of xylanase. Enzymatic activity is also influenced by pH. Xylanases have optimal activity at a lower pH and can lose their efficiency at non-optimal pH values if the enzyme is not protected. Therefore, further studies must be performed aiming at better evaluating the enzymatic action on animal performance, thus identifying the most suitable conditions for its better activity

Conclusions

Wheat bran, soybean hulls, and coffee husks can be used in the feed up to the studied inclusions with no damage to the performance of layers.

The evaluated enzyme is not efficient to improve the performance and quality of eggshells.

Fiber sources change traits related to egg quality and biometrics of organs, therefore leading to a higher gizzard development and relative liver weight.

Coffee husks reduce feed costs per egg carton when compared with the use of wheat bran.

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