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NONRUMINANT NUTRITION

Brazil nut oil in diets for breeder cocks

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ABSTRACT. This study aimed to evaluate the inclusion of Brazil nut oil in diets for breeder cocks on performance, semen analyses, biochemical serum profile and testicular morphology. Forty-two Rhode Island Red breeder cocks with 40 weeks of age were used. The experimental design was completely randomized consisting of seven levels of Brazil nut oil (0; 0.30; 0.60; 0.90; 1.20; 1.50 and 1.80%) in the diets. Data collected were evaluated by polynomial regression. Differences ($p < 0.05$) were detected in feed conversion, semen volume, motility, vigor, pH, swirling and concentration, in which the inclusion of Brazil nut oil in the diets improved the reproductive performance. Differences ($p < 0.05$) were also detected in the biochemical serum profile (triglycerides, cholesterol and blood pH); the inclusion of Brazil nut oil caused a large increase in blood triglycerides and cholesterol levels, consequently, changing the blood pH. The Brazil nut oil can be used as an energetic additive in diets given to breeder cocks, promoting better reproductive performance, without changing semen and testicular morphology, but altering the biochemical serum profile of the birds.

Keywords: additive, fatty acids, metabolism, reproductive performance.

Óleo de castanha do Brasil em rações de galos reprodutores

RESUMO. Objetivou-se avaliar a inclusão de níveis crescentes de óleo de castanha do Brasil (0; 0,30; 0,60; 0,90; 1,20; 1,50 e 1,80%) em rações de galos reprodutores sobre o desempenho, análise andrológica, perfil bioquímico sérico e biometria testicular. Foram utilizados 42 galos reprodutores Rhode Island Red com 40 semanas, distribuídos em um delineamento experimental inteiramente casualizado constituído de sete tratamentos com seis galos (repetições) cada. As estimativas dos níveis de óleo de castanha foram determinadas através de regressão polinomial à 5% de significância. Foram observadas diferenças ($p < 0,05$) nos resultados de conversão alimentar, volume seminal, motilidade, vigor, pH turbilhonamento e concentração seminal a partir da a inclusão de óleo de castanha do Brasil nas rações. Os resultados de perfil bioquímico sérico (triglicerídeos, colesterol e pH sanguíneo) também apresentaram diferenças ($p < 0,05$) onde a inclusão de óleo de castanha do Brasil nas rações ocasionou maior acúmulo de triglicerídeos e colesterol sanguíneo, consequentemente modificando o pH do meio. Concluiu-se que o óleo de castanha do Brasil pode ser utilizado como aditivo energético em rações para galos reprodutores promovendo melhora na conversão alimentar e no desempenho reprodutivo, sem modificar a morfologia espermática e testicular, porém, alterando o perfil bioquímico sérico das aves.

Palavras-chave: ácidos graxos, aditivo, desempenho reprodutivo, metabolismo.

Introduction

Breeders are considered the starting point of the poultry industry, in which cocks, through their genetic material, are responsible for several important reproductive variables (Rufino et al., 2015), which of course confers the need for studies related to productive and reproductive management (Maciel et al., 2011).

In this context, associating nutritional management with reproductive performance, several nutrients are important for sperm production, with lipids presenting remarkable functions integrated to energy metabolism and essential cellular components of biological membranes (Araújo, 2008; Makker, Agarwal, & Sharma, 2009).

Nogueira, Cruz, Tanaka, Rufino and Santana (2014) state that lipid requirements in diets are related to the need of the birds to obtain fatty acids that cannot be synthesized in the body, emphasizing that the high energy value and the high digestibility influence positively their performance. Based on this finding, the use of vegetable oils as a source of fatty acids has some advantages in relation to animal fat,

rich in saturated fatty acids. Oils rich in unsaturated fatty acids are more easily absorbed and, therefore, have higher values of metabolizable energy, promoting better performance of birds (Gaiotto, Menten, Racanicci, & Iafigliola, 2000).

Vegetable oils, depending on the lipid profile presented, can provide numerous benefits to the reproductive metabolism of cocks. In this sense, there is potentiality in the use of Brazil nut oil due to its rich lipid profile, which presents about 13.8% of palmitic acid; 8.7% stearic acid; 31.4% oleic acid and 45.2% linoleic acid, besides small amounts of palmitoleic and myristic acids (Gutierrez, Regitano-D'Arce, & Rauenmiguel, 1997; Freitas, Freitas-Silva, Miranda, & Coelho, 2007), all these fatty acids are considered as essential for the metabolic pathways of birds.

In view of the above, this study was performed with the objective of evaluating the inclusion of Brazil nut oil in breeder cock diets on performance, andrological analysis, serum biochemical profile and testicular morphology.

Material and methods

The experiment was conducted at the Poultry Sector of the Department of Animal and Plant Production (DPAV) of the Faculty of Agricultural Sciences (FCA) of the Federal University of Amazonas (UFAM), located in the Southern Sector of the University Campus, Manaus, State of Amazonas, Brazil.

Forty-two (42) 40-week old Rhode Island Red cocks (mean of 2.251 ± 0.056 kg) identified and housed according to pre-established treatments in poultry houses with water and feed ad libitum.

The experimental period was 49 days with seven days for pre-adaptation of animals to the diets and facilities, 42 days for data collection, and semen data collection and analysis performed at the end. The experiment was a completely randomized design with seven treatments corresponding to the inclusion levels of Brazil nut oil in the diets (0, 0.30, 0.60, 0.90, 1.20, 1.50 and 1.80%) and six cocks (repetitions) each.

The experimental diets (Table 1) were formulated according to the nutritional requirement of cocks using the software SUPERCRAC and reference values provided by Rostagno et al. (2011), except the composition of Brazil nut oil that used the values provided by Gutierrez et al. (1997).

In the performance, feed intake (g bird^{-1} day^{-1}), weight gain (g bird^{-1}) and feed conversion (g feed per 1 mL semen produced) were evaluated. After the experimental period, semen analyses were performed in four cocks of each treatment, with the semen collected by abdominal massage according to the Burrows and Quinn (1937) methodology and immediately analyzed according to the methodology of andrological analysis described by Rufino et al. (2015) and Bezerra et al. (2016).

The semen volume was determined in a graduated Eppendorf tube used at the moment of collection. Then, a drop of semen was mounted between a slide and coverslip for evaluation under light microscopy at 400 x magnification.

Table 1. Composition of the diets containing Brazil nut oil.

Ingredients	Levels of nut oil (%)						
	0.00	0.30	0.60	0.90	1.20	1.50	1.80
Corn (7.88%)	68.84	69.44	69.12	68.75	68.39	68.03	67.67
Soybean meal (46%)	20.82	19.90	19.92	19.99	20.05	20.11	20.18
Brazil nut oil	0.00	0.30	0.60	0.90	1.20	1.50	1.80
Limestone	7.73	7.73	7.73	7.73	7.73	7.73	7.72
Dicalcium phosphate	1.69	1.70	1.70	1.70	1.70	1.70	1.70
Premix Vit. Min.[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-methionine (99%)	0.06	0.07	0.07	0.07	0.07	0.07	0.07
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35
BHT[2]	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrients	Nutritional levels						
Met energy, kcal^{-1} kg^{-1}	2,800	2,800	2,800	2,800	2,800	2,800	2,800
Crude protein, %	14.50	14.50	14.50	14.50	14.50	14.50	14.50
Met. + Cystine, %	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Methionine, %	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calcium, %	3.40	3.40	3.40	3.40	3.40	3.40	3.40
Avilable phosphorus, %	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sodium, %	0.15	0.15	0.15	0.15	0.15	0.15	0.15

[1] Levels of guarantee per kilogram of product: Vitamin A 2,000,000 IU, Vitamin D3 400,000 IU, Vitamin E 2,400 mg, Vitamin K3 400 mg, Vitamin B1 100 mg, Vitamin B2 760 mg, Vitamin B6 100 mg, Vitamin B12 2,400 mcg, Niacin 5,000 mg, Calcium Pantothenate 2,000 mg, Folic Acid 50 mg, Coccidiostatic 12,000 mg, Choline 50,000 mg, Copper 1,200 mg, Iron 6,000 mg, Manganese 14,000 mg, Zinc 10,000 mg, Iodine 100 mg. Selenium 40 mg. Vehicle quantum sufficit for 1,000 g. [2] Butyl hydroxy toluene, antioxidant.

The variables of andrological analysis were motility (percentage of motile spermatozoa during analysis from 0 to 100%), vigor (straight and uniform movement of sperm in a scale from zero to five), density (score from zero to five, in which zero is the wide space and five, total absence of space among spermatozoa), texture (between zero and three, in which aqueous texture is represented by zero, and creamy texture is represented by three), color (score of zero to three, with zero representing the aqueous white coloration and three creamy white color), swirling (progressive movement of spermatozoa mass in scoring between zero and five) and seminal pH (determined using pH meter coupled to probe penetration with fine tips directly in the semen samples).

To evaluate the sperm concentration, semen samples were diluted 1: 800 in methylene blue solution, and the spermatozoa were counted in a Neubauer chamber under light microscopy at 640x magnification.

For sperm morphology analysis, semen samples were placed in identified Eppendorf tubes containing sodium citrate with 4% formalin. A drop of this solution was placed on a microscopic slide and emerged in a 3-step Panotic kit. Then, 200 spermatozoa were counted per sample at 640x magnification, observing the abnormal forms. Sperm defects were classified as head defects, tail defects, and normal cells.

For biochemical analysis of the blood, four birds of each treatment were selected for collection of 1 mL blood directly from the ulnar vein. Blood samples were sent to the Laboratory of Poultry Technology of the UFAM Poultry Industry to measure glucose, triglyceride, total cholesterol and pH using a portable biochemical analyzer (Accucheck Trend, ROCHE).

After these analyses, three birds of each treatment were selected to evaluate the morphology of the testis. The cocks were slaughtered by cervical dislocation and the testicles immediately removed, separated (right and left), weighed on a scale accurate to 0.01 g and measured for diameter and height by means of an electronic caliper. A manual micrometer was used to measure the thickness of the protective testicular membrane.

Statistical analysis was performed using the Statistical Analysis System [SAS] (2008) software and the treatments estimates were tested by a polynomial regression analysis at 5% significance.

Results and discussion

Differences ($p < 0.05$) were detected in feed conversion ($y = 8.95x^2 – 7.168x + 331.69$; $r^2 = 0.85$), where the best result was obtained (188.69 g mL^{-1}) from the inclusion of 0.40% Brazil nut oil in the diets (Table 2). These results diverge from Bezerra et al. (2016), who did not observe significant differences with the inclusion of vegetable oils in poultry diets on performance. On the other hand, according to Freitas et al. (2013), excess energy in diets can lead the animal to meet its energy requirements with lower feed intake and, consequently, cause a reduction in feed conversion similar to that observed in this study. However, they also state that the specific lipid profile of each vegetable oil may affect the performance of the birds differently when evaluated individually.

Regarding andrological analysis (Table 3), differences ($p < 0.05$) were observed in the semen volume ($y = - 0.017x^2 + 0.033x + 0.65$; $r^2 = 0.62$) where the best semen volume, 0.66 mL, was obtained from the inclusion of 0.97% of Brazil nut oil. Among the nutrients that can affect sperm biology, stand out lipids because of their energetic metabolic function as cellular components of biological membranes and stimulants for the production of spermatozoa. And depending on the lipid profile of each oil and its level of inclusion in the diets, there may be substantial variations in the semen volume produced by the cock, in addition to the direct influence on other indices for evaluation of reproductive performance.

Also, differences ($p < 0.05$) were observed in motility ($y = 0.095x^2 – 0.196x + 4.55$; $r^2 = 0.85$), vigor ($y = 0.104x^2 – 0.22x + 4.41$; $r^2 = 0.78$) and swirling ($y = 0.067x^2 – 0.144x + 4.35$; $r^2 = 0.76$), where from the inclusion of 1.03% of Brazil nut oil in the diets, the results obtained were 4.52; 4.29 and 4.35; respectively.

According to Bongalhardo, Leeson and Buhr (2009), the lipid profile of the sperm membrane is one of the most determinant factors for sperm morphology, presenting a high content of polyunsaturated fatty acids (PUFA). In this context, from a lipid supplementation by the inclusion of vegetable oils rich in these fatty acids, such as Brazil nut oil, there can be a substantial improvement in the production and quality of spermatozoa, considering the fundamental role of lipids in their formation.

In the results of pH ($p < 0.05$) ($y = 0.0063x^2 – 0.0205x + 7.05$; $r^2 = 0.73$), the best semen pH (7.03) was obtained from the inclusion of 1.62% of Brazil nut oil in feed. These results corroborate with

those observed by Rybnick, Horbanczuk, Naranowicz, Lukaszewicz, and Malecki (2007), Modupe, Livinus, & Ifeanyi (2013) and Bezerra et al. (2016), who verified semen pH values between 6.4 and 8.0; where they also affirm that the use of lipid sources in diets for cocks directly influences semen pH and, consequently, sperm performance and viability, in which a basic pH is considered ideal for the maintenance of spermatozoa activity.

For sperm concentration, differences ($p < 0.05$) were verified ($y = -0.0030x^2 + 0.0085x + 1.77$; $r^2 = 0.71$) where from the inclusion of 1.41% Brazil nut oil, the ideal sperm concentration was achieved (1.77). According to Bongalhardo (2013), sperm concentration and semen volume are considered important tools for determining the number of females that can be inseminated by a male. Nevertheless, evaluation of the functional gamete is also necessary when selecting a cock, and the concentration should act in conjunction with other variables to determine an accurate reproductive diagnosis.

There were no significant differences ($p > 0.05$) in the results of semen morphology (Table 4). Bongalhardo (2013) further states that good-quality semen must have a number of live cells with normal morphology greater than or equal to 90%. It was verified in this study that from the inclusion of Brazil nut oil in diets for cocks, the results of normality are within the technical recommendations.

In the concentration of blood triglycerides (Table 5), differences ($p < 0.05$) ($y = 5.51x^2 – 16.69x + 316.64$; $r^2 = 0.85$) were found, obtaining the best concentration (184.00 mg dL^{-1}) from the inclusion of 1.51% Brazil nut oil. Minafra et al. (2008; 2010) argue that because of the faster metabolism, birds require a greater mobilization of fatty acids and steroid hormones for various metabolic functions in relation to other domestic animals. And, depending on the composition of the diets, significant changes in the concentration of triglycerides in the blood can occur, especially when using energy additives.

In this context, the blood cholesterol level also presented differences ($p < 0.05$) ($y = 0.47x^2 – 1.69x + 167.14$; $r^2 = 0.76$) with the best blood cholesterol level (165.62 mg dL^{-1}) from the inclusion of 1.79% Brazil nut oil in the diets. It should be noted that in cocks, as the commercial product is the production of semen, there should be attention with fatty acid metabolism and this relationship with sperm production. In this way, the level of triglycerides and cholesterol in blood may be directly related to the fatty acid profile observed in the sperm, and may be mobilized from the bloodstream to produce spermatozoa under occasional metabolic conditions.

Differences ($p < 0.05$) were also found in blood pH ($y = -0.0092x^2 + 0.0228x + 7.21$; $r^2 = 0.81$), obtaining the best pH (7.22) in the inclusion level of 1.23% Brazil nut oil. These results show a direct relationship between pH and level of other blood components, especially lipids, where there was an increase in blood pH from the inclusion of Brazil nut oil, evidencing sensitivity in the blood metabolism of the birds from the inclusion of lipids in the diets and the immediate metabolic changes in the acid-base balance of the blood (Minafra et al., 2010).

In turn, for the results of testicular morphology (Table 6), no significant differences were detected ($p > 0.05$), but, higher values were verified for weight, diameter and height in the left testicle, regardless of the oil level of Brazil nut oil included. In agreement with Ecthes (1996), while in chickens only the left ovary is functional and much larger than the right, in cocks, both testes are functional. However, the left testicle is usually larger than right.

The results of testicular morphological are show in Table 6. Differences ($p > 0.05$) weren't observed in testicular morphology variables, but better results were observed for weight, diameter and height in the left testicle, independent of chestnut of Brazil oil level used. According Ecthes (1996), while in chickens only the left ovary is functional and much biggest than the right, in the cocks both testicles are functional. But, the left testicle usually is largest than the right.

Table 2. Feed intake (FI), weight gain (WG) and feed conversion (FC, g mL^{-1}) of cocks fed diets containing increasing levels of Brazil nut oil.

Variables	Levels of inclusion of Brazil nut oil (%)							p-value	Effect	CV, %
	0	0.3	0.6	0.9	1.2	1.5	1.8			
FI, g $bird^{-1}$ day^{-1}	84.16	78.87	77.14	76.23	86.44	92.32	83.99	0.93	ns	17.70
WG, g $bird^{-1}$	15.83	17.08	25.41	11.66	20.41	17.91	19.16	0.91	ns	17.10
FC, g mL^{-1}	326.12	160.40	125.31	224.26	299.84	173.06	258.75	0.04	Q	4.42

CV – coefficient of variation. p-value – coefficient of probability. Q - quadratic. ns – non-significant.

Table 3. Andrological analysis of cocks fed diets containing increasing levels of Brazil nut oil.

Variables	Levels of inclusion of Brazil nut oil (%)							p-Value	Effect	CV, %
	0	0.3	0.6	0.9	1.2	1.5	1.8			
Volume, mL	0.26	0.57	0.63	0.36	0.35	0.55	0.33	0.01	Q	18.36
Motility	5.00	5.00	4.00	3.75	5.00	4.50	5.00	0.01	Q	9.17
Vigor	5.00	4.87	3.75	3.75	5.00	4.62	5.00	0.01	Q	10.54
Density	4.87	4.62	4.00	4.75	5.00	4.50	5.00	0.30	ns	13.60
Texture	2.50	2.75	2.50	3.00	2.37	2.50	2.62	0.63	ns	18.95
Color	2.50	3.00	2.62	3.00	2.50	2.62	2.75	0.49	ns	16.33
Swirling	5.00	4.62	3.50	3.75	4.87	3.83	4.16	0.01	Q	14.02
pH	7.08	7.20	7.38	7.46	7.56	7.41	7.96	0.01	Q	2.79
Concentration, $x10^9$	1.92	1.71	1.90	1.72	2.16	2.01	1.83	0.02	Q	9.47

CV - Coefficient of variation. p-value- Coefficient of Probability. Q - Quadratic. ns – non-significant.

Table 4. Sperm morphology of cocks fed diets containing increasing levels of Brazil nut oil.

Variables	Levels of inclusion of Brazil nut oil (%)							p-Value	Effect	CV, %
	0	0.3	0.6	0.9	1.2	1.5	1.8			
Rolled head, %	0.250	0.280	0.125	0.130	0.200	0.100	0.150	0.10	ns	1.20
Defective head, %	0.125	0.125	0.125	0.250	0.075	0.100	0.125	0.80	ns	17.78
Bent head, %	0.500	0.250	0.125	0.125	0.100	0.125	0.125	0.63	ns	15.10
Isolated head, %	0.450	0.675	0.300	0.550	0.150	0.100	0.250	0.32	ns	18.73
Defective tail, %	0.180	0.250	0.200	0.075	0.100	0.125	0.100	0.12	ns	10.01
Broken tail, %	0.375	0.500	0.125	0.500	0.250	0.100	0.125	0.32	ns	14.51
Bent tail, %	0.125	0.250	0.750	0.125	0.000	0.000	0.125	0.06	ns	14.02
Presence of drop, %	0.375	0.300	0.500	0.625	0.125	0.100	0.250	0.28	ns	7.58
Normal, %	97.62	97.37	97.75	97.62	99.00	99.25	98.75	0.10	ns	1.11

CV - coefficient of variation. p-value - coefficient of probability. ns – non-significant.

Table 5. Blood biochemical analysis of cocks fed diets containing increasing levels of Brazil nut oil.

Variables	Levels of inclusion of Brazil nut oil (%)							p-value	Effect	CV, %
	0	0.3	0.6	0.9	1.2	1.5	1.8			
Glucose, mg dL^{-1}	194.5	185.5	221.0	209.0	197.5	191.5	178.0	0.61	ns	11.79
Triglycerides, mg dL^{-1}	292.0	192.5	193.5	199.5	193.5	194.0	192.5	0.01	Q	1.65
Total cholesterol, mg dL^{-1}	163.5	169.5	158.0	160.0	163.0	178.0	165.0	0.04	Q	2.88
pH	6.86	6.94	7.04	7.08	6.99	7.01	7.06	0.01	Q	0.44

CV - coefficient of variation. p-value - coefficient of probability. Q - quadratic. ns – non-significant.

Table 6. Testicular morphology of cocks fed diets containing increasing levels of Brazil nut oil.

Variables	Levels of inclusion of Brazil nut oil (%)							p-value	Effect	CV, %
	0	0.3	0.6	0.9	1.2	1.5	1.8			
Weight of the left testicle, g	16.60	11.66	14.00	15.66	13.66	17.00	18.33	0.82	ns	18.44
Diameter of the left testicle, cm	4.83	4.50	4.66	5.00	4.50	4.66	4.83	0.95	ns	14.08
Height of the left testicle, cm	10.33	10.00	9.66	11.00	9.33	9.00	10.00	0.31	ns	10.10
Thickness of the left testicle membrane, μm	56.00	46.00	46.00	36.00	36.00	40.00	46.00	0.06	ns	17.07
Weight of the right testicle, g	10.33	10.33	10.33	10.00	11.66	10.66	13.00	0.91	ns	20.15
Diameter of the right testicle, cm	4.00	3.83	4.00	4.16	4.16	4.00	4.16	0.89	ns	16.40
Height of the right testicle, cm	8.00	8.66	7.66	9.00	7.66	8.66	7.66	0.73	ns	15.79
Thickness of the right testicle membrane, μm	33.00	50.00	46.00	56.00	40.00	36.00	36.00	0.36	ns	21.39

CV - coefficient of variation. p-value - coefficient of probability. ns – non-significant.

The same author also states that the weight variation between the testicles is usually between 0.5 to 3.0g. However, our results showed variations of approximately 7.0 g, with the left testicle heavier, with higher height and total diameter, in relation to the right, but with no difference in the thickness of the membrane.

It is noteworthy that the testicular membrane can be defined as a tissue film that covers the testicles and provides an initial protective barrier. According to Rutz, Anciuti, Xavier, Roll, & Rossi (2007), a deficient diet or modifications in the content of the feed can cause anatomical and physiological modifications in testicles, and consequently, this membrane can present changes in its structure.

Conclusion

Brazil nut oil can be used as an energy additive in diets for breeding cocks, presenting better feed conversion and semen yields, without negatively affecting sperm morphology, testicular morphology and blood biochemical metabolism.

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