Revista Brasileira de Zootecnia
© 2016 Sociedade Brasileira de Zootecnia
ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., 45(5):208-218, 2016

Cottonseed oil in diets for growing broilers

Vânia de Sousa Lima Aguiar[1], Leilane Rocha Barros Dourado[2], João Batista Lopes[3], Luciana Pereira Machado[4], Daphinne Cardoso Nagib do Nascimento[1], Danilo Rodrigo Silva e Silva[4], Edna Teles dos Santos[1], Leonardo Atta Farias[2]

[1] *Universidade Federal do Piauí, Programa de Pós-graduação em Ciência Animal, Teresina, PI, Brazil.*
[2] *Universidade Federal do Piauí, Departamento de Zootecnia, Bom Jesus, PI, Brazil.*
[3] *Universidade Federal do Piauí, Departamento de Zootecnia, Teresina, PI, Brazil.*
[4] *Universidade Federal do Piauí, Departamento de Medicina Veterinária, Bom Jesus, PI, Brazil.*

ABSTRACT - The objective of this study was to evaluate the effect of three levels of crude cottonseed oil on performance, organ weights, and blood parameters of growing broilers. Carcass and cut yields after 33 and 42 days of age and the economic viability of the diets were also evaluated. Male broilers of the Ross line were distributed in a completely randomized design, in a 4 × 2 factorial arrangement (0, 2, 4, and 6% inclusion of cottonseed oil, with and without ferrous sulfate) with five replicates. In the period from 22 to 33 days, quadratic and increasing linear effects were observed on feed intake and weight gain, respectively. Feed conversion during the same period was better with the addition of ferrous sulfate. The addition of ferrous sulfate caused a reduction in heart weight. From 22 to 42 days, carcass and cuts yield, organ weight, and intestine length were not influenced by the levels of oil or by the addition of ferrous sulfate. Supplementation with iron salts provided a lower red blood cell count and increased mean cell volume. Balanced diets formulated with up to 6% of crude cottonseed oil for broilers from 22-33 and 22-42 days of age do not affect their performance or the weight of their organs. Supplementation with ferrous sulfate improved feed conversion up to 33 days. Diets formulated with 4% cottonseed oil supplemented with ferrous sulfate are economically viable in the period from 22 to 42 days.

Key Words: gossypol, poultry, vegetable oil

Introduction

In cotton growing, the material obtained at harvest consists of a product named cotton boll, which is composed of the fiber and the husks. When the lint is open, it reveals the seed, which is rich in oil and thus pointed out as one of the main feedstocks for the edible-oil industry (Freire, 2003). This oil has a great importance for human or animal consumption (Lopes, 2010).

Although cotton by-products can be good alternatives in broiler diets because of their composition, their use in diets for monogastric animals is still restricted due to the presence of a yellow polyphenolic compound called gossypol ($C_{30}H_{30}O_8$), produced by glands present in all parts of the cotton tree (roots, leaves, trunk, and mainly in the seeds) (Nagalakshmi et al., 2007). Gossypol has anti-nutritive properties, as it is responsible for toxic effects in the animal (Santos et al., 2013).

Received November 25, 2015 and accepted February 27, 2016.
Corresponding author: vania_vet06@hotmail.com

http://dx.doi.org/10.1590/S1806-92902016000500002

Gossypol is associated with reduced use of proteins, because in its free form, it binds to proteins or to an amine group free of essential amino acids, especially lysine, reducing the protein biological value (Nagalakshmi et al., 2007). It also has an inhibitory action on the proteolytic enzymes present in the gastrointestinal tract of birds, like pepsin and trypsin, which negatively interferes with the protein digestion, reducing its digestibility (Ryan et al., 1986). There are also reports that gossypol can trigger alterations in the hematological and biochemical parameters of birds (Aletor et al., 1989).

According to Waldroup and Kersey (2002), the adverse effects of gossypol can be minimized by limiting the amount utilized or by neutralizing it with soluble iron salts in a proportion of 2:1 for broilers. Thus, when ferrous sulfate is added, animals tolerate higher levels of free gossypol, reaching 100 ppm and even 200 ppm for the initial and fattening phases (Barbosa and Gattás, 2004).

Given the lack of information about the use of cottonseed oil in broiler feeding, this study aimed to evaluate the effects of including crude cottonseed oil in isoenergetic diets supplemented with or without ferrous sulfate between 22 to 42 days of age, including an economic analysis of the viability of these diets.

Material and Methods

This study was conducted in strict accordance with ethic recommendations and was approved by the Committee of Ethics on Animal Experimentation of Universidade Federal do Piauí (Piauí, Brazil) (#030/12).

A total of 560 male broilers of the Ross line were used in a completely randomized design with a 4 × 2 factorial arrangement (four levels of oil, with or without ferrous sulfate), five replicates, and 14 birds per experimental unit.

At 22 days of age, birds were weighed and distributed according to their average weight into 2-m^2 boxes containing feeder buckets and automatic drinkers, located in a masonry shed covered with ceramic tiles, with concrete floor. Separators between the boxes consisted of flat wire screen, and curtains were used to control the temperature and drafts. Until the beginning of the experimental phase (22 days of age), birds were housed in a conventional shed and fed corn- and soybean meal-based diets according to the requirements described in Rostagno et al. (2011).

The tested cottonseed oil levels were 0, 2, 4, and 6%, following the energy requirement of each phase as recommended by Rostagno et al. (2011). Ferrous sulfate ($Fe_2(SO_4)_3$) was included in the amount of 0.1%, according to Santos et al. (2009).

Experimental diets were formulated to meet the nutritional requirements of the broilers under study, as recommended by Rostagno et al. (2011) (Tables 1 and 2). The cottonseed oil utilized in the experiment was obtained by mechanical pressing; it contained 1.2% gossypol (LABTRON by UV-VIS spectrophotometry) and 9,120 kcal/kg gross energy (LANA-UFPI by calorimetry). For the formulation, the composition of the ingredients described in Rostagno et al. (2011) was adopted, except for the cottonseed oil, for which we adopted the metabolizable energy determined in a previous study according to the method of Sakomura and Rostagno (2007), of 7,732 kcal/kg for the cottonseed oil without ferrous sulfate and 8,270 kcal/kg for the cottonseed oil with ferrous sulfate, whose values were utilized for adjustments of the energy level of diets.

Table 1 - Composition of experimental diets for broilers from 22 to 33 days of age

Ingredient (%)	Treatment (cottonseed oil levels)							
	Without ferrous sulfate (%)				With ferrous sulfate (%)			
	0	2	4	6	0	2	4	6
Corn	67.471	66.049	60.896	55.743	67.368	65.690	60.179	54.668
Soybean meal 48%	27.738	27.979	28.854	29.729	27.773	28.040	28.976	29.911
Dicalcium phosphate	1.232	1.233	1.239	1.245	1.232	1.233	1.239	1.245
Limestone	0.880	0.878	0.872	0.865	0.880	0.878	0.872	0.865
Soybean oil	1.274	-	-	-	1.343	-	-	-
Common salt	0.455	0.456	0.458	0.461	0.455	0.456	0.458	0.461
Vitamin-mineral supplement[1]	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400
DL-methionine	0.233	0.234	0.239	0.244	0.233	0.234	0.239	0.244
L-lysine HCL	0.213	0.208	0.190	0.172	0.213	0.208	0.190	0.168
Inert[2]	0.100	0.558	2.849	5.140	-	0.757	3.346	5.936
Cottonseed oil	-	2.000	4.000	6.000	-	2.000	4.000	6.000
Ferrous sulfate	-	-	-	-	0.100	0.100	0.100	0.100
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Nutritional composition								
Linoleic acid (%)	2.190	1.477	1.385	1.293	2.224	1.470	1.372	1.274
Calcium (%)	0.750	0.750	0.750	0.750	0.750	0.750	0.750	0.750
Chlorine (%)	0.326	0.325	0.324	0.323	0.326	0.325	0.324	0.323
AME (Mcal/kg)[3]	3.050	3.050	3.050	3.050	3.050	3.050	3.050	3.050
Available phosphorus (%)	0.335	0.335	0.335	0.335	0.335	0.335	0.335	0.335
Digestible lysine (%)	1.045	1.045	1.045	1.045	1.045	1.045	1.045	1.045
Digestible met. + cys. (%)	0.763	0.763	0.763	0.763	0.763	0.763	0.763	0.763
Crude fiber (%)	2.329	2.315	2.262	2.210	2.327	2.311	2.255	2.199
Potassium (%)	0.780	0.782	0.785	0.789	0.781	0.782	0.785	0.789
Crude protein (%)	19.000	19.000	19.000	19.000	19.000	19.000	19.000	19.000
Sodium (%)	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
Iron (mg/kg)[4]	152	152	152	152	352	352	352	352

[1] Guaranteed levels per kg of product: folic acid - 162.50 mg; chlorohydroxyquinoline - 7,500.00 mg; vitamin A - 1,400,062.50 IU; vitamin B1 - 388.00 mg; vitamin B12 - 2,000.00 mcg; vitamin B2 - 1,000.00 mg; vitamin B6 - 520.00 mg; vitamin D3 - 360,012.00 IU; vitamin E - 2,500.00 mg; vitamin K3 - 300.00 mg; niacin - 7,000.00 mg; salinomycin - 16,500 mg; pantothenic acid - 2,600.00 mg; choline chloride - 71,590 mg; selenium - 75.00 mg; ferrous sulfate 11,250 mg; manganese monoxide - 18,740.00 mg; copper sulfate - 1,996.00 mg; iodine - 187.47 mg; zinc - 17,500.00 mg.
[2] Inert: sand.
[3] Apparent metabolizable energy of cottonseed oil - 7,732 kcal/kg and 8,270 kcal/kg, with and without ferrous sulfate, respectively.
[4] Considering the iron content in corn, soybean meal, dicalcium phosphate, vitamin-mineral supplement, and ferrous sulfate.

Humidity and maximum and minimum temperature in the shed were monitored using a thermo-hygrometer placed in the center of the shed at the height of the back of the birds, with values read daily. Water was supplied *ad libitum*, and changed twice daily to prevent heating and fermentation. A continuous lighting program (natural + artificial light) was adopted, and diets were available *ad libitum*. Mean values for the maximum and minimum temperatures and relative humidity of the air recorded inside the shed in the period from 22 to 33 days were 31.8 ºC, 25.1 ºC, and 70%, respectively. From 34 to 42 days, the observed maximum and minimum temperatures were 31.6 ºC and 24.7 ºC, respectively, and recorded humidity was 70%.

At 33 and 42 days of age, feed intake (FC) was evaluated, calculated as the difference between the amount of feed supplied and the orts from the experimental diets. Weight gain (WG) was determined as the difference in weight of the birds at the beginning and end of the period. Feed conversion (FC) was calculated from feed intake and weight gain data in the period. Feed intake and average WG were calculated as a function of the number of birds in each plot, and in cases of mortality, they were defined as a function of the number of birds corrected according to Sakomura and Rostagno (2007).

At 33 and 42 days of age, one bird with weight close to the average weight of the experimental plot was selected and fasted for six hours for the removal of organs (heart, liver, gizzard + proventriculus, bursa, spleen, pancreas, and intestine) to determine their relative weight, which was calculated considering the live weights of the birds.

Also in this period, three birds with weight close to the average of the pen were slaughtered after six hours of fasting for evaluation of the carcass and cuts yields. After the fasting period, birds were weighed individually to determine their live weight, and then slaughtered, bled, and plucked. The carcass yield was defined as the ratio between the empty carcass weight and the weight of the fasted bird. To determine the empty carcass weight, we considered the weight of the slaughtered fasted birds without feathers, organs, head, neck, and feet. Immediately afterwards, the

Table 2 - Composition of experimental diets for broilers from 34 to 42 days of age

Ingredient (%)	Treatment (cottonseed oil levels)							
	Without ferrous sulfate (%)				With ferrous sulfate (%)			
	0	2	4	6	0	2	4	6
Corn	71.089	69.640	64.487	59.335	71.089	69.281	63.770	58.2594
Soybean meal 48%	24.688	24.933	25.808	26.683	24.688	24.994	25.930	26.865
Dicalcium phosphate	1.016	1.018	1.023	1.029	1.232	1.018	1.023	1.029
Limestone	0.781	0.779	0.772	0.766	0.781	0.779	0.772	0.766
Soybean oil	1.265	-	-	-	1.265	-	-	-
Common salt	0.443	0.444	0.445	0.446	0.443	0.444	0.446	0.448
Vitamin-mineral supplement[1]	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
DL-methionine	0.204	0.205	0.209	0.214	0.204	0.205	0.209	0.214
L-lysine HCL	0.213	0.208	0.190	0.172	0.213	0.208	0.188	0.168
Inert[2]	0.100	0.572	2.862	5.153	-	0.770	3.359	5.948
Cottonseed oil	-	2.000	4.000	6.000	-	2.000	4.000	6.000
Ferrous sulfate	-	-	-	-	0.100	0.100	0.100	0.100
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Nutritional composition								
Linoleic acid (%)	2.231	1.522	1.430	1.338	2.231	1.520	1.418	1.319
Calcium (%)	0.650	0.650	0.650	0.650	0.650	0.650	0.650	0.650
Chlorine (%)	0.319	0.318	0.317	0.316	0.319	0.318	0.317	0.316
AME (Mcal/kg)[3]	3.100	3.100	3.100	3.100	3.100	3.100	3.100	3.100
Available phosphorus (%)	0.290	0.290	0.290	0.290	0.290	0.290	0.290	0.290
Digestible lysine (%)	0.969	0.969	0.969	0.969	0.969	0.969	0.969	0.969
Digestible met. + cys. (%)	0.707	0.707	0.707	0.707	0.707	0.707	0.707	0.707
Crude fiber (%)	2.264	2.249	2.197	2.144	2.264	2.246	2.189	2.134
Potassium (%)	0.727	0.728	0.732	0.735	0.727	0.728	0.732	0.735
Crude protein (%)	17.800	17.800	17.800	17.800	17.800	17.800	17.800	17.800
Sodium (%)	0.195	0.195	0.195	0.195	0.195	0.195	0.195	0.195
Iron (mg/kg)[4]	117	117	117	117	326	326	326	326

[1] Guaranteed levels per kg of product: folic acid - 162.50 mg; chlorohydroxyquinoline - 7,500.00 mg; vitamin A - 14,000.00 IU; vitamin B1 - 388.00 mg; vitamin B12 - 2,000.00 mcg; vitamin B2 - 1,000.00 mg; vitamin B6 - 520.00 mg; vitamin D3 - 1,600.00 IU; vitamin E - 2,500.00 mg; vitamin K3 - 300.00 mg; zinc - 70 ppm; niacin - 7,000.00 mg; salinomycin - 16,500 mg; pantothenic acid - 2,600.00 mg; choline chloride - 71,593.49 mg; selenium - 75.00 mg; ferrous sulfate 11,250 mg; manganese monoxide - 18,750.00 mg; copper sulfate - 2,000.00 mg; iodine - 187.50 mg; zinc oxide - 17,500.00 mg; antioxidant additive - 25,000.00 mg; halquinol - 7,500.00 mg.
[2] Inert: sand.
[3] Apparent metabolizable energy of cottonseed oil - 7,732 kcal/kg and 8,270 kcal/kg, with and without ferrous sulfate, respectively.
[4] Considering the iron content in corn, soybean meal, dicalcium phosphate, vitamin-mineral supplement, and ferrous sulfate.

breast, drumstick, thigh, wings, and abdominal fat were cut and weighed. The cuts yields were determined in relation to the empty-carcass weight.

On the 38th, 39th, 40th, 41st, and 42nd days of age, 3 mL of blood samples were collected from the jugular vein of one bird per experimental unit, totaling five birds per treatment. Anticoagulant EDTA (ethylenediaminetetraacetic acid) was used at the rate of 0.1 mL for 1.0 mL of blood for blood collection. The following hematological tests were run: count of red and white blood cells, using the blood sample and a Natt and Herrick's solution at a dilution ratio of 1:200, with the count performed (N/mL) in a Neubauer chamber; hematocrit or globular volume, by the microhematocrit method; measurement of plasma total proteins, by refractometry, utilizing the plasma from the same capillary used for the microhematocrit (Goldenfarb et al., 1971); and hemoglobin concentration, by the cyanmethaemoglobin method (Collier, 1944). With these results, the Wintrobe (1934) hematimetric indices mean corpuscular volume and mean corpuscular hemoglobin concentration were calculated using standard formulae.

For the study of economic viability of the inclusion of crude cottonseed oil with and without addition of ferrous sulfate to the diet, the following primary variables were considered: average feed intake (AFI, kg), feed cost (FC, kg), average weight gain (AWG, kg), average live weight (ALW, kg), and price of the live chicken (PLC, kg). Based on the observed values for these primary variables, the following economic indicators were obtained, according to Togashi (2004): average feed cost (AFC) = AFI × FC; AFC/AWG ratio; average gross income (AGI) = ALW × PLC; and average gross margin (AGM) = AGI – (AFC + AFC in the period from 1 to 21 days). An AFC of R$0.84 was adopted, relative to average values determined in previous studies with chickens of the same line, origins, and at 1 to 21 days of age.

Gross margin (GM) was calculated as GM = (kg of chicken produced × chicken sale price) – (feed price × feed intake), involving the prices of the ingredients in the diets. The Bioeconomic Index (BEI) was used to evaluate the effect on profitability, obtained by the following formula: BEI = weight gain – (Z × FC), in which Z is the ratio between the kilogram of feed and the price of the kilogram of the live chicken.

The average prices of the kilogram of live chicken (R$2.69), corn, and soybean meal were obtained on the poultry production website Avisite (2014), whereas the price of the kilogram of diet was calculated based on the prices of the ingredients in the period of March 2013, as follows: corn - R$0.51; 48% soybean meal - R$0.82; dicalcium phosphate - R$2.80; limestone - R$0.27; soybean oil - R$3.45; common salt - R$1.40; vitamin-mineral supplement - R$11.00; DL-methionine - R$19.00; L-lysine HCL - R$13.00; inert (sand) - R$0.00; crude cottonseed oil - R$1.65; and ferrous sulfate - R$23.60.

Data were subjected to evaluation of homogeneity and normality; any outliers identified were removed. Subsequently, the data were subjected to analysis of variance, using the GLM procedure of SAS (Statistical Analysis System, version 9.0). Estimates based on the level of cottonseed oil were established by linear and polynomial regression models. Means were compared by the SNK test with $\alpha = 0.05$.

Results and Discussion

From 22 to 33 days of age, no interaction ($P>0.05$) was observed between the levels of cottonseed oil (COL) with or without ferrous sulfate on the variables feed intake, weight gain, or feed conversion (Table 3). Feed conversion was not influenced by the level of cottonseed oil in the diet. However, a quadratic effect ($P<0.05$) was detected on this variable, according to the equation $FI = 1.423 + 0.036COL - 0.0049\ COL^2$ ($R^2 = 0.86$), with the highest intake obtained with 3.75% of inclusion of cottonseed oil in this period.

The increase in feed intake up to this level of 3.75% can be explained by the beneficial effect of the fats, by the lower heat increment projected onto the diet, and by the property of improving the palatability of the feed, stimulating consumption, according to Lipstein and Bornstein (1975). However, after this level, the gossypol present in the oil might have caused a reduction in feed intake, although it did not impair the weight gain of these animals, because a linear increase ($P<0.05$) was observed in this variable, according to the equation $WG = 0.848 + 0.03COL$ ($R^2 = 0.63$), in which weight gain was increased as the levels of inclusion of cottonseed oil in the diets was elevated. Similar results were obtained by Andreotti et al. (2004), who added soybean oil at the levels of 0.0, 3.3, 6.6, and 9.9% in diets for broilers in the period from 21 to 42 days and observed a quadratic effect on weight gain, revealing a tolerance to soybean oil up to the level of 9.63%.

The linear increase in weight gain may also be related to the increase in feed intake up to the level of 3.75% of inclusion of the oil, demonstrated in this same period, and to the better utilization of these nutrients resulting from the reduction of the digesta transit time, thereby subjecting the nutrients to the enzymatic action for a longer time. This was observed by Andreotti et al. (2004), who found that increasing levels of inclusion of soybean oil caused a linear decrease in the feed transit time at 22 and 42 days of age.

According to Santos et al. (2009), the addition of lipid sources improves the palatability and provides greater digestive and absorptive efficiency of non-lipid components of ingredients, in the diets. Thus, the increase in weight gain and the improvement in feed conversion observed with the inclusion of fat in isoenergetic diets for broilers have been attributed to the extra-caloric effect of fats, as they provide better energetic efficiency, resulting from the lower heat increment from the fat, which increases the available dietary net energy for animal production (Sakomura et al., 2004).

Still addressing the period from 22 to 33 days of age, feed intake and weight gain were not influenced by the addition of ferrous sulfate. On the other hand, feed conversion was improved (Table 3), corroborating the results found by Heidarinia and Malakian (2011), who, evaluating the use of cottonseed meal in broiler diets with and without addition of ferrous sulfate to chelate gossypol, observed that the feed conversion of the birds fed the meal plus 0.04% ferrous sulfate was significantly better than that of the birds that consumed the meal without ferrous sulfate. Likewise, Karakaş Oğuz et al. (2006) obtained positive effects of supplementation with ferrous sulfate in diets containing cottonseed meal for broilers and concluded that supplementation with iron was more effective than the control diets, improving weight gain.

Buitrago et al. (1970) evaluated the effect of dietary iron (400, 800, and 1,200 mg/kg) on the accumulation of gossypol and its elimination in the liver and pigs and found that feed intake was significantly greater in the animals that received diets with higher levels of iron, although the daily weight gain and feed conversion were not influenced. Additionally, the authors observed a significant linear effect of the dietary iron level on the iron content in the liver, indicating its accumulation in the tissues.

As regards the performance of the birds in the entire phase (22 to 42 days of age), no interaction ($P<0.05$) was observed between the levels of cottonseed and the addition or lack of ferrous sulfate in the diets, without influence of the oil levels (Table 3). In general, research conducted with cotton by-products in diets for monogastrics has demonstrated a decline in performance of animals caused by increments in the levels of inclusion of these ingredients; however, this was not demonstrated in the current study, which implies that the addition of up to 6% of cottonseed oil

Table 3 - Effect of cottonseed oil levels (COL), with or without addition of ferrous sulfate, on feed intake, weight gain, and feed conversion of broilers from 22 to 33 and from 22 to 42 days of age

Variable (kg)	FS	Cottonseed oil level (%)				Mean	CV (%)	P>F		
		0	2	4	6			FS	COL	FS × COL
				22 to 33 days						
Feed intake	Without	1.419	1.505	1.479	1.448	1.463	3.43	0.874	0.020	0.441
	With	1.417	1.475	1.476	1.492	1.465				
	Mean[1]	1.418	1.490	1.477	1.470					
Weight gain	Without	0.855	0.851	0.892	0.840	0.859	6.83	0.060	0.054	0.075
	With	0.812	0.909	0.925	0.941	0.897				
	Mean[2]	0.834	0.880	0.909	0.890					
Feed conversion	Without	1.661	1.781	1.660	1.739	1.710A	6.22	0.04	0.320	0.043
	With	1.751	1.623	1.598	1.587	1.640B				
	Mean	1.706	1.702	1.629	1.663					
				22 to 42 days						
Feed intake	Without	2.983	3.058	3.084	2.953	3.020	3.23	0.955	0.201	0.345
	With	2.966	3.055	3.019	3.045	3.018				
	Mean	2.970	3.060	3.050	2.990					
Weight gain	Without	1.668	1.653	1.672	1.613	1.645	4.22	0.132	0.307	0.068
	With	1.603	1.690	1.730	1.730	1.681				
	Mean	1.634	1.672	1.701	1.670					
Feed conversion	Without	1.826	1.852	1.847	1.863	1.847	3.04	0.059	0.527	0.005
	With	1.880	1.810	1.767	1.775	1.800				
	Mean	1.836	1.831	1.807	1.819					

FS - ferrous sulfate; CV - coefficient of variation.
Means followed by a common letter in the column do not differ statistically according to the SNK test ($P<0.05$).
[1] Quadratic effect (Feed intake = $1.423 + 0.036COL - 0.0049COL^2$. $R^2 = 0.86$).
[2] Linear effect (Weight gain = $0.848 + 0.03COL$. $R^2 = 0.63$).

in diets for growing broilers does not have a negative impact on the performance variables. This fact may be related to the level of free gossypol present in the oil utilized, the period of consumption, and the stress conditions to which the animals were subjected, which were not sufficient to compromise their feed intake, weight gain, or the feed conversion (Gamboa et al., 2001).

No effects (P>0.05) of supplementation with ferrous sulfate were observed on the feed conversion of the birds or on the other performance variables (Table 3). Because of the lack of positive responses from animal performance to the iron salts in the total period, this supplementation may be unnecessary depending on the level of free gossypol present in the cotton by-products. Evaluating the effect of layer diets containing cottonseed meal treated with and without ferrous sulfate, Panigrahi et al. (1989) obtained lower feed intake and fewer spots in the egg yolk, attributed to the treatment of cottonseed meal with the ferrous sulfate heptahydrate solution.

No interaction (P>0.05) was observed between the levels of cottonseed oil and the inclusion of ferrous sulfate (Table 4) for any of the carcass and cuts yield variables at 33 days. The levels of cottonseed oil had no effects on the carcass and cuts yield, except for the drumstick yield, on which the oil levels had a quadratic effect, according to the equation $DSY = 14.077 + 0.208COL – 0.004COL^2$ ($R^2 = 0.59$), with the lowest drumstick yield obtained at the level of 2.6%. However, the increase in the amount of cottonseed oil in the diet might have provided a better use of the nutrients, benefiting the protein synthesis and consequently leading to a better drumstick yield after this level of inclusion of the oil. On the other hand, Andreotti et al. (2004) did not observe influences of the increasing levels of soybean oil on the yields of drumstick and other cuts from broilers.

No interaction was detected (P>0.05) between the oil levels and the addition or non-addition of ferrous sulfate on the weight of the organs or on the intestinal length of the broilers at 33 days of age. The relative weight of organs and intestinal length of birds in this phase was not influenced (P>0.05) by the cottonseed oil levels added to the diet. Addition of ferrous sulfate interfered (P<0.05) with the weight of the heart of these animals, which decreased (Table 5). Liver cells are the main sites where iron is stored, as a protein named ferritin. Therefore, it is one of the main organs affected by high iron levels in the body, and so its excess intake leads to an accumulation of liver iron (Siqueira et al., 2006), but no difference was observed in the liver of animals fed diets with and without iron supplementation. However, in addition to the liver, other organs can be compromised, since there are reports that high iron stores in tissues may lead to the formation of free radicals, which may be associated with progressive tissue lesions in several organs (Siah et al., 2006). These lesions, in turn, may be related to the lower weight of this organ found in the animals of the present study, which had their diets supplemented with ferrous sulfate.

No interaction (P>0.05) was observed between the levels of cottonseed oil and the supplementation or lack of supplementation of ferrous sulfate for any the carcass or

Table 4 - Effect of cottonseed oil levels (COL), with or without addition of ferrous sulfate, on yields of carcass and cuts from broilers at 33 days of age

Variable (%)	FS	Cottonseed oil level (%)				Mean	CV (%)	P>F		
		0	2	4	6			FS	COL	FS × COL
Carcass yield	Without	71.181	71.510	70.951	70.114	70.94	2.53	0.470	0.851	0.445
	With	70.819	70.261	69.717	71.265	70.52				
	Mean	71.000	70.885	70.334	70.690					
Breast yield	Without	34.473	35.965	33.739	35.012	34.80	3.64	0.572	0.100	0.614
	With	34.656	35.033	34.179	34.367	34.56				
	Mean	34.564	35.499	33.959	34.690					
Drumstick yield	Without	14.464	14.042	14.406	14.026	14.17	3.70	0.343	0.043	0.120
	With	13.828	14.163	14.536	13.521	14.01				
	Mean[1]	14.146	14.103	14.471	13.77					
Thigh yield	Without	15.626	15.021	15.719	15.443	15.45	4.88	0.330	0.436	0.870
	With	15.560	15.532	15.970	15.716	15.69				
	Mean	15.593	15.277	15.844	15.579					
Wing yield	Without	12.158	11.705	11.902	12.093	11.96	6.21	0.820	0.583	0.264
	With	11.775	12.002	12.642	11.662	12.02				
	Mean	11.966	11.854	12.272	11.877					

CV - coefficient of variation; FS - ferrous sulfate.
[1] Quadratic effect (Drumstick yield = $14.077 + 0.208COL – 0.004COL^2$. $R^2 = 0.59$).

cuts yield variables at 42 days, and nor was any influence of the levels of oil or addition or non-addition of ferrous sulfate (P>0.05) (Table 6).

It is known that the carcass yield and the abdominal fat content can be affected by the levels of oil added to the diet (Roll et al., 2011). However, when animals are subjected to high-energy diets, there is an increase in the deposition of fat underlying the skin, around the organs, and especially in the abdominal region (Summers and Leeson, 1979). This was not observed in the present study, since the amount of fat increased as the levels of cottonseed oil were increased, indicating that this oil can be utilized at up to 6% of inclusion for broilers in the phase from 22 to 42 days of age without the need for addition of iron salts, because it does not incur losses in the yields of carcass, breast, drumstick, thigh, or wings, and neither does it elevate the abdominal fat contents.

Regarding the weight of the animals at 42 days, no interaction was observed (P>0.05) between the levels of oil and the addition or non-addition of ferrous sulfate. The relative weights of organs and the intestinal length of birds in this phase were not influenced (P>0.05) by the levels of cottonseed oil or by the ferrous sulfate added to the diet (Table 7).

No interaction (P>0.05) was found between the levels of cottonseed and the addition or non-addition of ferrous sulfate to the diets on the blood parameters of the animals. Also, no alterations (P>0.05) were observed in these

Table 5 - Effect of cottonseed oil levels (COL), with or without addition of ferrous sulfate, on weight of organs from broilers at 33 days of age

Variable	FS	Cottonseed oil level (%)				Mean	CV (%)	P>F		
		0	2	4	6			FS	COL	FS × COL
				Relative weight (%)						
Heart	Without	0.441	0.479	0.414	0.413	0.437A	15.90	0.005	0.431	0.190
	With	0.558	0.468	0.472	0.537	0.509B				
	Mean	0.499	0.474	0.443	0.475					
Liver	Without	2.175	2.183	2.225	2.123	2.176	12.80	0.820	0.970	0.976
	With	2.178	2.179	2.139	2.126	2.155				
	Mean	2.176	2.181	2.182	2.124					
Gizzard	Without	2.656	2.762	3.041	2.920	2.845	14.66	0.682	0.363	0.734
	With	2.595	2.948	2.914	2.701	2.790				
	Mean	2.625	2.855	2.978	2.811					
Bursa	Without	0.175	0.177	0.190	0.175	0.179	27.30	0.165	0.956	0.896
	With	0.171	0.147	0.156	0.156	0.157				
	Mean	0.173	0.162	0.173	0.165					
Pancreas	Without	0.253	0.266	0.267	0.233	0.255	15.20	0.160	0.140	0.980
	With	0.236	0.254	0.249	0.210	0.237				
	Mean	0.244	0.260	0.258	0.221					
Spleen	Without	0.094	0.096	0.087	0.085	0.091	25.01	0.870	0.916	0.851
	With	0.086	0.091	0.087	0.094	0.090				
	Mean	0.090	0.094	0.087	0.090					
Intestine	Without	3.993	4.005	4.716	4.638	4.338	12.65	0.788	0.083	0.750
	With	4.184	4.306	4.691	4.376	4.389				
	Mean	4.088	4.156	4.703	4.507					
				Intestinal length (cm)						
Intestinal length	Without	165.95	175.20	201.60	177.75	180.12	8.59	0.610	0.060	0.122
	With	184.80	177.00	185.00	184.50	182.82				
	Mean	175.38	176.10	193.30	181.13					
Duodenum length	Without	28.58	28.80	32.20	32.50	30.52	11.84	0.893	0.160	0.655
	With	31.40	28.40	31.30	31.63	30.68				
	Mean	29.99	28.60	31.75	32.06					
Jejunum length	Without	65.38	66.60	78.40	77.00	71.84	12.22	0.662	0.368	0.149
	With	72.20	76.20	73.04	71.13	73.14				
	Mean	68.79	71.40	75.72	74.06					
Ileum length	Without	65.50	73.86	77.60	77.55	73.63	13.94	0.353	0.580	0.514
	With	77.20	77.58	78.56	74.25	76.89				
	Mean	71.35	75.72	78.08	75.90					

CV - coefficient of variation; FS - ferrous sulfate.
Means followed by common letters in the column do not differ statistically according to the SNK test (P<0.05).

parameters according to the levels of cottonseed oil added. However, the inclusion of ferrous sulfate resulted in a decrease ($P<0.05$) in the red blood cell count and an increase in mean corpuscular volume (Table 8). Possibly, the lower red blood cell count observed in the animals that received diets with ferrous sulfate is related to the decomposition of H_2O_2 (hydrogen peroxide), an iron-dependent oxygen metabolite that has its toxicity increased by 10,000 times in the presence of this metal. This reaction results in the formation of hydroxyl radicals (OH^+), which are potentially capable of reacting with the lipids from the red cell membrane, causing hemolysis of these cells (Eaton, 1991). However, the lower red blood cell counts observed were not sufficient to compromise the performance of the animals, perhaps because even with this reduction, the red cells are within normal parameters for the Ross line (Furlan et al., 1999), since none of the performance variables was influenced by supplementation with ferrous sulfate.

Gaber et al. (2012) observed that the counts of red blood cells, hematocrit, and hemoglobin in Nile tilapia were elevated with increasing levels of inclusion of iron in the diets containing cottonseed meal, indicating that they can be affected by the dietary iron, contrasting the results found in the present study.

The average feed cost varied between 2.25 and 2.38 (Table 9). The feed cost was increased with the inclusion of crude cottonseed oil in the diets; however, the diets with the highest level of the oil, 6%, showed a similar cost to the diet without the oil (0%). When ferrous sulfate was added to the diet, it was observed that these costs became higher compared with the diet without supplementation of ferrous sulfate, generating an increase of 3.0, 2.0, 0.4, and 5.0% in the diets containing 0, 2, 4, and 6% of cottonseed oil with ferrous sulfate, respectively.

The AFC/AWG ratio increased with the inclusion of the crude cottonseed oil in the diets, with the diets formulated with the highest level of the oil, 6%, without ferrous sulfate, providing a 4% increase compared with the diet containing 0% cottonseed oil. When the ferrous sulfate was added to the diets containing 0 and 2% of the cottonseed oil, an increase of 7 and 0.7% was observed in the AFC/AWG ratio compared with the diet with the same levels without ferrous sulfate. On the other hand, addition of ferrous sulfate to the diets with 4 and 6% of inclusion of the cottonseed oil provided a reduction of 3 and 2% in the AFC/AWG ratio. No differences were observed in this ratio with the addition of ferrous sulfate at the level of 2% of cottonseed oil. The largest difference in the average feed cost/average weight gain ratio between the treatments was observed in the diet without cottonseed oil, with iron supplementation, which provided a 7% higher AFC/AWG ratio than the diets with 0% cottonseed oil and no ferrous sulfate.

As the level of inclusion of the crude cottonseed oil was increased in the diets with and without supplementation of ferrous sulfate, an increase was observed in the average

Table 6 - Effect of cottonseed oil levels (COL), with or without addition of ferrous sulfate, on yields of carcass and cuts from broilers at 42 days of age

Variable (%)	FS	Cottonseed oil level (%)				Mean	CV (%)	P>F		
		0	2	4	6			FS	COL	FS × COL
Carcass yield	Without	72.571	71.996	71.969	71.676	72.053	1.80	0.689	0.241	0.704
	With	71.493	71.420	72.324	72.166	71.851				
	Mean	72.032	71.708	72.147	71.921					
Breast yield	Without	35.155	34.944	35.992	36.436	35.632	3.98	0.755	0.425	0.311
	With	36.377	36.375	36.812	36.204	36.442				
	Mean	35.766	35.660	36.402	36.320					
Drumstick yield	Without	14.046	14.046	13.837	13.966	13.974	7.08	0.766	0.694	0.373
	With	13.833	14.462	14.482	14.112	14.222				
	Mean	13.939	14.254	14.160	14.039					
Thigh yield	Without	15.427	15.178	14.920	14.597	15.030	6.16	0.124	0.328	0.675
	With	14.659	15.179	15.279	15.866	15.246				
	Mean	15.043	15.179	15.099	15.231					
Wing yield	Without	11.275	11.393	11.243	11.413	11.331	5.20	0.970	0.540	0.514
	With	11.362	11.760	11.567	11.561	11.562				
	Mean	11.318	11.576	11.405	11.487					
Abdominal fat	Without	2.798	2.996	3.022	3.102	2.980	21.73	0.379	0.516	0.420
	With	3.172	3.232	2.941	3.033	3.095				
	Mean	2.985	3.114	2.982	3.068					

CV - coefficient of variation; FS - ferrous sulfate.

Table 7 - Effect of cottonseed oil levels (COL), with or without addition of ferrous sulfate, on weight of organs from broilers at 42 days of age

Variable	FS	Cottonseed oil level (%)				Mean	CV (%)	P>F		
		0	2	4	6			FS	COL	FS × COL
		Relative weight (%)								
Heart	Without	0.42	0.39	0.40	0.46	0.42				
	With	0.43	0.39	0.44	0.40	0.41	12.01	0.593	0.293	0.143
	Mean	0.43	0.39	0.42	0.43					
Liver	Without	1.88	1.99	1.96	1.94	1.94				
	With	1.96	1.95	1.93	1.93	1.94	11.70	0.491	0.898	0.791
	Mean	1.92	1.97	1.95	1.94					
Gizzard	Without	2.46	2.58	2.34	2.44	2.45				
	With	2.04	2.23	2.69	2.57	2.38	13.41	0.514	0.272	0.039
	Mean	2.25	2.40	2.52	2.51					
Bursa	Without	0.13	0.12	0.12	0.13	0.12				
	With	0.12	0.12	0.12	0.13	0.12	29.25	1.000	0.901	1.000
	Mean	0.12	0.12	0.12	0.13					
Pancreas	Without	0.20	0.20	0.19	0.19	0.19				
	With	0.20	0.20	0.19	0.19	0.19	16.10	0.721	0.663	0.726
	Mean	0.20	0.20	0.19	0.19					
Spleen	Without	0.07	0.09	0.08	0.10	0.09				
	With	0.08	0.09	0.08	0.08	0.08	20.31	0.487	0.040	0.298
	Mean	0.07	0.09	0.08	0.09					
Intestine	Without	3.86	3.89	3.44	4.01	3.80				
	With	3.67	3.83	3.77	3.73	3.75	12.87	0.742	0.580	0.507
	Mean	3.76	3.86	3.60	3.87					
		Intestinal length (cm)								
	Without	127.60	125.50	121.40	136.00	127.63				
Intestinal length	With	121.00	126.50	139.75	122.50	127.44	9.87	0.92	0.644	0.084
	Mean	124.30	126.00	130.58	129.25					
	Without	35.80	34.20	33.20	35.00	34.55				
Duodenum length	With	34.60	34.60	33.20	35.20	34.40	10.42	0.949	0.622	0.981
	Mean	35.20	34.40	33.20	35.10					
	Without	70.80	63.40	66.60	70.70	67.87				
Jejunum length	With	67.00	70.30	69.20	63.60	67.51	13.57	0.929	0.972	0.423
	Mean	68.90	66.83	67.90	67.13					
	Without	64.60	72.40	70.20	72.80	70.00				
Ileum length	With	70.40	67.60	69.00	70.20	69.30	11.26	0.996	0.505	0.284
	Mean	67.50	70.00	69.60	71.50					

CV - coefficient of variation; FS - ferrous sulfate.

gross income when compared with the diets containing 0% cottonseed oil with and without ferrous sulfate, especially for the diets with 4% of cottonseed oil. Diets with 2, 4, and 6% of inclusion of this oil supplemented with ferrous sulfate also provided a higher average gross income compared with those without ferrous sulfate.

The worst bioeconomic index among the diets with different levels of cottonseed oil was obtained with the diet containing 0% crude cottonseed oil with ferrous sulfate (0.74), compared with the other diets. In contrast, the diet formulated with 4% inclusion of the oil with ferrous sulfate provided the best BEI (0.86).

The results of the economic analysis, especially the average gross margin, indicated that in the period from 22 to 42 days of age, the inclusion of 4% crude cottonseed oil with ferrous sulfate provided the best economic result (R$3.27) among the diets. Therefore, this diet provided the best economic viability for broilers in this phase from 22 to 42 days of age.

The decision of the best level of incorporation of cottonseed oil to broiler diets should be made after a specific evaluation for each case, considering not only the price of this ingredient, but also the performance of animals using it compared with the traditional lipid source.

Table 8 - Effect of cottonseed oil levels (COL), with and without ferrous sulfate, on blood parameters of broilers at 42 days of age

Variable	FS	Cottonseed oil level (%)				Mean	CV (%)	P>F		
		0	2	4	6			FS	COL	FS × COL
GV (%)	Without	29.00	27.80	29.60	30.00	29.10	9.13	0.330	0.591	0.834
	With	30.80	29.20	30.00	29.80	29.95				
	Mean	29.90	28.50	29.80	29.90					
PTP (%)	Without	3.32	3.52	3.64	3.60	3.52	10.09	0.182	0.564	0.705
	With	3.32	3.40	3.28	3.48	3.37				
	Mean	3.32	3.46	3.46	3.54					
RBC (10^3/µL)	Without	2587	2520	2424	2494	2506A	5.01	0.004	0.861	0.179
	With	2331	2406	2415	2398	2388B				
	Mean	2459	2463	2419	2446					
HB (g/dL)	Without	8.34	9.02	8.92	8.86	8.79	13.10	0.124	0.542	0.845
	With	9.04	9.64	8.98	9.86	9.38				
	Mean	8.69	9.33	8.95	9.36					
MCV (µm^3)	Without	112.16	114.85	122.38	120.30	117.42B	5.75	0.025	0.117	0.738
	With	120.70	121.60	124.26	124.42	122.75A				
	Mean	116.43	118.23	123.32	122.36					
MCHC (%)	Without	28.82	31.73	30.21	29.58	30.08	13.30	0.413	0.630	0.732
	With	30.08	31.53	30.07	33.06	31.18				
	Mean	29.45	31.63	30.14	31.32					
WBC (10^3/µL)	Without	25	23	26	29	25.75	24.19	0.110	0.391	0.379
	With	23	24	19	25	22.75				
	Mean	24	24	23	27					

CV - coefficient of variation; FS - ferrous sulfate; GV - globular volume; PTP - plasma total proteins; RBC - red blood cells; HB - hemoglobin; MCV - mean corpuscular volume; MCHC - mean corpuscular hemoglobin concentration; WBC - white blood cells.
Means followed by common letters in the column do not differ statistically according to the SNK test (P<0.05).

Table 9 - Economic indicators of experimental diets containing crude cottonseed oil for broilers from 22 to 42 days of age

Vaariable	Treatment (cottonseed oil levels)							
	Without ferrous sulfate (%)				With ferrous sulfate (%)			
	0	2	4	6	0	2	4	6
Average feed cost (R$)	2.25	2.27	2.31	2.25	2.32	2.32	2.32	2.38
AFC/AWG ratio (R$/kg)	1.35	1.37	1.38	1.40	1.45	1.38	1.34	1.37
Average gross income (R$)	6.26	6.30	6.31	6.28	6.02	6.31	6.45	6.45
Average gross margin (R$)	3.17	3.18	3.12	3.16	2.86	3.15	3.27	3.20
Bioeconomic index	0.83	0.81	0.81	0.78	0.74	0.82	0.86	0.85

AFC - average feed cost; AWG - average weight gain.

Conclusions

Inclusion of up to 6% of crude cottonseed oil in diets balanced for broilers in the period from 22 to 42 days of age does not impair the performance of these animals. Supplementation with ferrous sulfate to chelate the gossypol provides a lower count of red blood cells and an increase in mean corpuscular volume in these animals, in addition to improving the feed conversion of birds in the period from 22 to 33 days of age. Diets containing 4% cottonseed oil with addition of ferrous sulfate are more profitable for broilers in this rearing phase.

References

Aletor, V. A. 1989. Effect of varying levels of oyster shell on serum constituents and erythrocyte indices in growing chicken fed gossypol-containing Nigerian cottonseed cake. Die Nahrung 33:905.

Andreotti, M. O.; Junqueira, O. M.; Barbosa, M. J. B.; Cancherini, L. C.; Araújo, L. F. and Rodrigues, E. A. 2004. Tempo de trânsito intestinal, desempenho, característica de carcaça e composição corporal de frangos de corte alimentados com rações isoenergéticas formuladas com diferentes níveis de óleo de soja. Revista Brasileira de Zootecnia 33:870-879.

Avisite. 2014. Estatísticas e preços. Available at: <http://www.avisite.com.br/economia/index.php?acao=frangovivo>. Accessed on: Aug. 26, 2015.

Barbosa, F. F. and Gattás, G. 2004. Farelo de algodão na alimentação de suínos e aves. Revista Eletrônica Nutritime 1:147-156.

Buitrago, J. A.; Clawson, A. J. and Smith, F. H. 1970. Effects of dietary iron on gossypol accumulation in and elimination from porcine liver. Journal Animal Science 31:554-558.

Collier, H. B. 1944. Standardization of blood haemoglobin determinations. Canadian Medical Association Journal 50:550.

Eaton, J. W. 1991. Catalases and peroxidases and glutathione and hydrogen peroxide: mysteries of the bestiary. Journal of Laboratory and Clinical Medicine 118:3-4.

Freire, R. M. 2003. Cultura do algodão herbáceo na agricultura familiar: Subprodutos do algodão. Revista Embrapa Algodão 2.ed., versão eletrônica. Available at: <http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Algodao/AlgodaoAgriculturaFamiliar/subprodutos.htm>. Accessed on: Aug. 26, 2015.

Furlan, R. L.; Morais, V. M. B.; Malheiros, R. D.; Secato, E. R. and Macari, M. 1999. Alterações hematológicas e gasométricas em diferentes linhagens de frangos de corte submetidos ao estresse calórico agudo. Revista Brasileira de Ciência Avícola 1:77-84.

Gaber, M. M.; Elhalfawy, M. M. and Ramadan, A. M. 2012. Utilization of cottonseed meal supplemented with iron for detoxification of gossypol in nile tilapia, broodstock and their impact on the hatchability of their progenies. Journal Aquaculture Research & Development 3:2-5.

Gamboa, D. A.; Calhoun, M. C.; Kuhlmann, S. W.; Haq, A. U. and Bailey, C. A. 2001. Use of expander cottonseed meal in broiler diets formulated on a digestible amino acid basis. Poultry Science 80:789-794.

Goldenfarb, P. B.; Bowyer, F. P.; Hall, E. and Brosius, E. 1971. Reproducibility in the hematology laboratory: the microhematocrit determinations. American Journal of Clinical Pathology 56:35-39.

Heidarinia, A. and Malakian, M. 2011. Nutritional evaluation of cottonseed meal with and without ferrous sulphate for broiler chickens. Research Journal of Poultry Sciences 4:14-17.

Karakaş Oğuz, F.; Mustafa, N. O.; Şefika, H. and Mehmet, S. G. 2006. The effects of iron sulphate supplementation to diets containing cottonseed meal on performance and haematological parameters of broilers. Journal of Faculty of Veterinary Medicine 3:9-14.

Lipstein, B. and Bornstein, S. 1975. "Extra-caloric" properties of acidulated soybean-oil soapstock for broilers during hot weather. Poultry Science 54:396-404.

Lopes, A. M. 2010. Uso de subprodutos do algodão na alimentação de ruminantes. Ciências Veterinárias nos Trópicos 13:24-37.

Nagalakshmi, D.; Rao, S. V. R.; Panda, A. K. and Sastry, V. R. B. 2007. Cottonseed meal in poultry diets: a review. The Journal of Poultry Science 44:119-134.

Panigrahi, S.; Plumb, V. E. and Machin, D. H. 1989. Effects of dietary cottonseed meal with and without iron treatment on laying hens. British Poultry Science 30:641-651.

Roll, A. P.; Lopes, D. C. N.; Azambuja, S.; Pires, P. G. S.; Xavier, E. G.; Roll, V. F. B. and Rutz, F. 2011. Efeito de diferentes níveis de energia da dieta no desempenho de frangos de corte entre os 43 e 48 dias de idade. Revista Portuguesa de Ciências Veterinárias 110:69-74.

Rostagno, H. S.; Albino, L. F. T.; Donzele, J. L.; Gomes, P. C.; Oliveira, R. F.; Lopes, D. C.; Ferreira, A. S.; Barreto, S. L. T. and Euclides, R. F. 2011. Tabelas brasileiras para aves e suínos. Composição de alimentos e exigências nutricionais. 3.ed. Universidade Federal de Viçosa, Viçosa, MG.

Ryan, J. R.; Kratzer F. A.; Grats G. R. and Vohrra, P. 1986. Gbudless cottonseed cake for laying and breeding hens and broiler chicks. Poultry Science 65:549-955.

Sakomura, N. K.; Longo, F. A.; Rabello, C. B.; Watanabe, K.; Pelícia, K. and Freitas, E. R. 2004. Efeito do nível de energia metabolizável da dieta no desempenho e metabolismo energético de frangos de corte. Revista Brasileira de Zootecnia 33:1758-1767.

Sakomura, N. K. and Rostagno, H. S. 2007. Métodos de pesquisa em nutrição de monogástricos. Funep, Jaboticabal.

Santos, M. D.; Rodrigues, R. S.; Freitas, S. H.; Costa, D. S.; Ruas, J. R. M.; Miranda, E. J. and Simões, M. J. 2013. Qualidade seminal, morfologia dos testículos e epidídimos de touros submetidos à dieta contendo gossipol. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 65:975-980.

Santos, M. S. V.; Espíndola, G. B.; Lôbo, R. N. B.; Fuentes, M. F. F.; Carvalho, L. E. and Santos, A. B. E. 2009. Desempenho e qualidade dos ovos de poedeiras comerciais submetidas às dietas com diferentes óleos vegetais. Revista Brasileira de Saúde Produção Animal 10:654-667.

Siah, C. W.; Ombiga, J.; Adams, L. A.; Trinder, D. and Olynyk, J. K. 2006. Normal iron metabolism and the pathophysiology of iron overload disorders. Clinical Biochemical Reviews 27:5-16.

Siqueira, E. M. A.; Almeida, S. G. and Arruda, S. 2006. Papel adverso do ferro no organismo. Comunicação em Ciências da Saúde 17:229-236.

Summers, J. D. and Leeson, S. 1979. Composition of poultry meat as affected by nutritional factors. Poultry Science 58:536-542.

Togashi, C. K. 2004. Teores de colesterol e ácidos graxos em tecidos e soro de frangos de corte submetidos a diferentes programas nutricionais. Tese (D.Sc.). Universidade Estadual do Norte Fluminense, Rio de Janeiro.

Waldroup, P. W. and Kersey, J. H. 2002. Nutrient composition of cottonseed meal surveyed. Feedstuffs 4:11-22.

Wintrobe, M. M. 1934. Variations in the size and hemoglobin content of erythrocytes in the blood of various vertebrates. Folia Haematololiga 51:32-49.