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Original Article

Lipid Sources in Diets for Hy-Line White Laying: Performance, Biometrics of Digestive Organs, and Bone Characteristics

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ABSTRACT

Dietary inclusion of lipid sources has been one of the methods adopted by nutritionists aiming at better energy balancing. However, alternative energy feedstuffs have been sought in an attempt to reduce production costs. In this regard, lipid sources that can replace conventional ones, such as beef tallow, cottonseed oil, and sunflower oil have been investigated. The objective of this study was to examine the effects of different lipid sources on the performance, egg quality, biometrics of digestive organs, and bone quality of white-egg commercial layers in the first production cycle. A total of 216 Hy-Line White layers at 70 weeks of age, weighing 1.701 ± 0.103 kg, were used in a completely randomized design with four treatments and six replicates. The diets provided to them contained equal amounts of protein, calories, and amino acids, with only the lipid source varying – soybean oil (control), cottonseed oil, beef tallow, and sunflower oil. No significant effect of lipid sources was observed on any of the performance or egg-quality variables assessed ($p>0.05$). Biometric variables were not influenced by lipid sources, except for abdominal fat, which was highest in the birds receiving the beef-tallow treatments and lowest in birds fed the sunflower-oil diets ($p<0.05$). Except for mineral matter, bone traits were not influenced by the treatments; this variable was highest in the control treatments and lowest in the beef-tallow treatments. In conclusion, cottonseed and sunflower oils can be used to fully replace soybean oil in diets for white-egg commercial layers.

INTRODUCTION

In the agribusiness sector, poultry farming has had a marked growth in the last few years. Within this segment, the egg-laying branch has stood out, as verified by the increasing production of eggs at figures never-before seen in Brazil.

For decades, the most relevant components of poultry diets have been corn and soybean meal, the main energy and protein ingredients, respectively. However, as early-maturing animals, today's laying birds are more nutritionally demanding and have low feed-intake rates in their early production life. Thus, the dietary inclusion of lipid sources is a necessary measure to ensure their adequate energy intake (Silva *et al.,* 2014).

Soybean oil has been the lipid source of choice in most poultry diets when the use thereof is a necessity. However, research shows that soybean oil addition to poultry diets should be made judiciously, since elevated levels of this ingredient may worsen eggshell quality (Jiang *et al*., 2014). According to Bavaresco *et al.* (2019), this is due to a likely interference with the mineral metabolism, mainly the calcium retention, through the formation of insoluble soaps during the digestion process. In this context, there has been an ever-growing interest in lipid ingredients that can satisfactory replace those commonly used in diets.

Plant-derived feedstuffs may present compounds called anti-nutritional factors. These are defined as compounds present in plant-based feedstuffs which reduce the nutritional value of these feedstuffs upon being consumed (Benevides *et al.,* 2011). Gossypol is one of these factors; it is present in the seeds and by-products of cotton. Alexander *et al.* (2009) stated that cotton by-products may have some toxic effects, causing losses to poultry farming as a result of weight loss, decreased egg production, and reduced feed intake. Additionally, deleterious effects of it have been reported on organs like the liver and reproductive and immune systems (Gadelha *et al.,* 2014). Because of the concern with the deleterious effects of anti-nutritional factors on animals, their biometrics is used as an instrument of investigation.

Therefore, possible substitutes such as beef tallow, cottonseed oil and sunflower oil, among others, may be viable options. In this scenario, the present study was conducted to evaluate the performance, egg-quality, biometrics of digestive organs, and bone quality of commercial white-egg layers in the first production cycle fed diets containing different lipid sources.

MATERIAL AND METHODS

The project was approved by the local ethics committee (approval no. 001.05.016.UVA.504.03). The experiment was carried out in the egg-laying unit of the Experimental Farm of the State University of Vale do Acaraú - UVA, located in Sobral - CE, for 84 days. A total of 216 Hy-Line White layers at 70 weeks of age, weighing 1.701 ± 0.103 kg, with an average production of 70.97% ± 5.41, were distributed into four treatments with six replicates of nine birds each, in a completely randomized experimental design.

Experimental diets contained equal amounts of protein, calories, and amino acids (Table 1) and were formulated following the nutritional requirements suggested by the manual of the strain. The composition of ingredients used in the formulation followed the recommendation of Rostagno *et al.* (2011). The nutritional composition of cottonseed meal was obtained from Lima *et al.* (2016), and that of sunflower seed from Fedna (2004).

Diets were formulated based on corn, soybean meal, a source of calcium and phosphorus, a vitamin-mineral premix, common salt, and a lipid source, and divided into the following treatments: 1 - soybean oil; 2 - cottonseed oil (no ferrous sulfate supplementation); 3 - beef tallow; and 4 - sunflower oil.

Egg production was recorded daily until the end of each 28-day period, when these data, together with those of feed intake, were used for the calculation of performance variables. The following parameters were evaluated: feed intake (g/bird/day), egg production (%), egg weight (g), egg mass (g/bird/day), feed conversion per egg mass (kg/kg), and feed conversion per dozen eggs (kg/dz).

Table 1 – Centesimal and calculated nutritional composition of experimental diets.

Ingredient (kg)	Lipid Source			
	Soybean	Cottonseed	Beef Tallow	Sunflower
Corn (kg)	61.459	60.723	60.406	61.291
Soybean meal (45%)	21.815	21.947	22.004	21.845
Limestone (kg)	8.147	8.144	8.143	8.146
Meat meal 40% (kg)	5.473	5.479	5.481	5.475
Vitamin-mineral supplement (kg)*	0.400	0.400	0.400	0.400
Common salt (kg)	0.272	0.272	0.272	0.272
Oil	2.311	2.911	3.169	2.448
DL-methionine (kg)	0.121	0.121	0.122	0.121
Metabolizable energy (Kcal/kg)	2900	2900	2900	2900
Crude protein (%)	17.50	17.50	17.50	17.50
Calcium (%)	4.000	4.000	4.000	4.000
Available phosphorus (%)	0.430	0.430	0.430	0.430
Sodium (%)	0.180	0.180	0.180	0.180
Digestible met + cys (%)	0.660	0.660	0.660	0.660
Digestible methionine (%)	0.415	0.415	0.415	0.415
Digestible lysine (%)	0.760	0.760	0.760	0.760

*PX POSTURA 0.4% 500 TEC - Guaranteedlevelsoftheproduct (composition per kg ofproduct): iron (min) - 10.00 g; copper (min) - 2,500.00 mg; zinc (min) - 20.00 g; manganese (min) - 20.00 g; iodine (min) - 208.00 mg; selenium (min) - 75.15 mg; vitamin A (min) - 2,000,000.00 IU; vitamin D3 (min) - 625,000.00 IU; vitamin E (min) - 3,000.00; vitamin K3 (min) - 395.92 mg; folicacid (min) - 74.25 mg; choline (min) - 100.00 g; niacin (min) - 5,025.74 mg; pantothenicacid (min) - 1,805.16 mg; vitamin B1 (min) - 250.09 mg; vitamin B2 (min) - 1,000.00 mg; vitamin B6 (min) - 250.1 mcg; vitamin B12 (min) - 2,400.00; methionine (min) - 125.00 g; colistin (min) - 1,750.00 mg.

At the end of each period, the following egg-quality parameters were also assessed: percentages of albumen, yolk, and eggshell; yolk color; eggshell thickness (mm); and egg specific gravity (g/cm^3). Four eggs were selected per replicate, two of which for the determination of specific gravity and the other two for the other quality analyses.

The eggs were cracked manually, and its components were weighed separately. For the calculation of eggshell thickness, shells were left to dry for 24 h, weighed, and then three measurements were taken from the equatorial region and extremities of the egg using a digital caliper.

Yolk color was determined subjectively by comparing it with the Roche® Yolk Color Fan, whose scores range from 1 to 15, where the first represents the palest yellow and the last the most intense orange (Sandeski *et al.,* 2014).

At the end of the experiment, 20 birds were chosen at random and identified (five per treatment). These were euthanized by the cervical-dislocation method and taken to the laboratory, where they were weighed individually and had their organs removed and emptied during a necropsy for biometric analyses of gizzard, liver, pancreas, and intestines, using a 0.01-g precision scale (adapted from Braz *et al.,* 2011). All weight data were expressed as a percentage of body weight.

Only the tibiae were used in the evaluation of bone quality. The bone length was measured with a digital caliper, and its weight was determined on an electronic scale with 0.01-g precision. Bone density was obtained using the Seedor index, by dividing its weight (mg) by its length (mm) (Seedor *et al.,* 1991).

Analyses of bone resistance and deformity were performed using a mechanical press, where the left tibiae were placed in horizontal position and then received a compression force onto their center. The maximum amount of force applied to the bone to its disruption was considered breaking resistance (kgf/cm²), which was measured using a digital strain gauge. Deformity (mm) was measured using an analogical strain gauge until the bone broke. These analyses took place in the Laboratory of Soil Mechanics at the Department of Transportation Engineering of the Federal University of Ceará.

Mineral matter (MM) was determined at the Laboratory of Animal Nutrition (LANUT) of UVA. After deboning, the right tibiae were weighed and dried in a forced-air oven at 105 ºC for 72 h. Next, they were weighed and ground with a pestle and mortar. Ground samples were then identified for determination of the MM according to the methodology described by Silva & Queiroz (2002).

The data were subjected to analysis of variance and means were compared by the Tukey test at the 5% probability level, using the Statistical Analysis System software (SAS, 2000).

RESULTS

No effect of the evaluated lipid sources was observed on the performance variables (Table 2).

Table 2 – Mean values for feed intake, egg production, egg weight, egg mass, feed conversion per egg massand feed conversion per dozen eggs of Hy-line White commercial layers fed diets containing different lipid sources, in the period of 70 to 82 weeks of age.

Lipid Source	Intake (g/bird/day)	Production (%)	Egg weight (g)	Egg mass (g/bird/day)	FCEM[1] (kg/kg)	FCDZ[2] (kg/dz)
Soybean control	90.00	66.30	65.41	43.35	2.080	1.634
Cottonseed	90.56	62.76	65.25	41.00	2.224	1.737
Beef tallow	90.14	62.70	64.24	40.28	2.253	1.735
Sunflower	92.02	62.10	66.13	41.06	2.256	1.789
Mean	90.69	63.50	65.30	41.47	2.201	1.723
SEM[3]	0.839	0.534	0.227	0.370	0.031	0.023
p-value	0.9450	0.1148	0.1230	0.1141	0.3557	0.2867

[1]Feed conversion per egg mass; [2]Feed conversion per dozen eggs; [3]Standard error of the mean.

None of the egg-quality variables was influenced ($p>0.05$) by the lipid sources tested in this study (Table 3).

Irrespective of the lipid source used, no significant differences were found ($p>0.05$) for the relative weights of gizzard, liver, pancreas, and intestines. However, the abdominal fat was heavier in the birds from the treatments with beef tallow in relation to the diet containing sunflower oil ($p<0.05$). The treatments including cottonseed and soybean oils, in turn, did not differ from the others (Table 4).

There was no effect of lipid sources on the bone-quality variables, except for mineral matter, whose highest value was found in the control treatment

Table 3 – Mean values for the percentages of albumen, yolk, and shell, eggshell thickness and specific gravity of Hy-line White layers fed diets containing lipid sources, in the period of 70 to 82 weeks of age.

Lipid source	Variable					
	% Albumen	% Yolk	% Shell	Yolk color	ET[1] (mm)	SG[2](g/cm^3)
Soybean Control	61.22	26.70	8.48	6.82	0.372	1.084
Cotton	60.20	27.41	8.80	6.93	0.380	1.085
Beef tallow	60.00	27.97	8.47	6.96	0.370	1.083
Sunflower	60.28	27.31	8.51	6.80	0.372	1.083
Mean	60.43	27.38	8.57	6.87	0.373	1.084
SEM[3]	0.158	0.154	0.049	0.053	0.002	0.001
p-value	0.2109	0.1088	0.1705	0.7758	0.5036	0.7685

[1]Eggshell thickness; [2]Specific gravity; [3]Standard error of the mean.

Table 4 – Relative weight of the digestive organs of Hy-line Whitelayers fed diets containing lipid sources, in the period of 70 to 82 weeks of age.

Lipid source	Variable				
	% Gizzard	% Liver	% Pancreas	% Intestine	% Abdominal fat
Soybean Control	1.05	2.32	0.16	3.36	6.35ab
Cottonseed	1.09	2.39	0.18	3.12	6.64ab
Beef tallow	1.18	2.42	0.17	3.08	7.07a
Sunflower	1.17	2.48	0.17	3.13	5.33b
Mean	1.21	2.40	0.17	3.17	6.35
SEM[1]	0.025	0.064	0.003	0.059	0.195
p-value	0.4165	0.9386	0.5983	0.7188	0.0386

[1]Standard error of the mean. Different letters in the same column differ statistically by the Tukey test at the 5% level.

(soybean oil) in comparison with the beef-tallow treatment. The diets containing cottonseed and sunflower oils, in turn, did not differ from the other treatments (Table 5)

Table 5 – Weight, length, Seedor Index (SI), resistance, deformity, and mineral matter of the tibiae of Hy-line White layers fed diets containing lipid sources, in the period of 70 to 82 weeks of age.

Lipid source	Variable					
	Weight (g)	Length (mm)	SI[1](mg/mm)	Resistance (kgf/cm^2)	Deformity (mm)	Mineralmatter (g/kg)
Soybean control	7.06	113.00	62.46	7.36	1.42	54.69a
Cottonseed	6.80	111.59	60.90	6.54	1.29	50.94ab
Beef tallow	7.30	112.17	64.99	6.56	1.09	49.50b
Sunflower	7.12	114.17	62.34	6.87	1.27	51.26ab
Mean	7.07	112.73	62.67	6.83	1.26	51.59
SEM[2]	0.081	0.380	0.564	0.230	0.056	0.552
p-value	0.4900	0.3411	0.3428	0.7541	0.4501	0.0250

[1]Seedor Index; [2]Standard error of the mean. Different letters in the same column differ statistically by the Tukey test at the 5% level.

DISCUSSION

Oil addition to diets is normally indicated as a means to improve their palatability (Silva *et al.,* 2014), consequently stimulating intake. However, in the present study, no alterations in feed intake were seen as a function of the tested lipid sources. Therefore, the lack of effects of such sources on the *intake* variable suggests that, at the levels used in the experimental diets, they may be indicated for use on a commercial scale.

As observed, none of the lipid sources affected egg production. This was likely because the layers used in the experiment were in the post-peak production, when their nutritional requirements are lower, and also because the diets contained equal amounts of energy and nutrients, ensuring adequate nutrient supply across all treatments, which led to the obtained results.

Oliveira *et al.* (2010) conducted two experiments with young layers (20 to 28 weeks), the second with birds in the post-peak phase (54 weeks) using lipid sources (soybean, sunflower, and linseed oils) and reported that, in the second trial, no performance variable was influenced by the tested lipid sources. These results are similar to those found in the present study. Conflicting results were reported by Küçükersan *et al.* (2010), who worked with sunflower, fish, soybean, and hazelnut oils and reported that the lowest egg production was obtained in the treatment with sunflower oil (p<0.05).

Santos *et al.* (2009) used three lipid sources (soybean, linseed, and cottonseed oils) at the inclusion levels of 2 and 4% of the diets of white-egg layers and found that their performance was not influenced by the treatments.

Lima *et al.* (2016) used different levels of cottonseed oil (0, 2, 4, and 6%) with and without addition of ferrous sulfate in pre-starter diets (1 to 7 days) for broilers and reported that no effects of the treatments were observed on the performance variables.

According to Jiang *et al.* (2014), elevated levels of soybean oil (7 or 10%) in the diet of brown-egg layers have detrimental effects on eggshell quality. In this study, no effect of the treatments was observed on the percentage of eggshell, eggshell thickness, or specific gravity, probably because the inclusion levels of the lipid sources tested here were lower than those mentioned by the above author.

In an experiment with commercial brown-egg layers at 36 weeks of age, Küçükersan *et al.* (2010) used 3% of four different lipid sources (fish, sunflower, soybean, and hazelnut oils) and concluded that none of the egg-quality parameters was influenced by the lipid sources.

Santos *et al.* (2009) used soybean, linseed, and cottonseed oils at the inclusion levels of 2 and 4% in experimental diets for white-egg layers and reported that the inclusion of vegetable oils in the diets, irrespective of the source, did not change the egg quality or its cholesterol level.

According to Sandeski *et al.* (2014), yolk color values higher than 8, as measured using the Roche yolk color fan, are overall preferred among consumers. In this experiment, despite the lack of differences (p>0.05) between the treatments for this variable, the value was below that recommended by the afore-mentioned authors. The corn used in the experimental diets likely showed a lower xanthophyll content that led to the yolk color values found.

The diets used in this experiment were formulated so as to meet the nutritional requirements of the birds in the production stage in which they were. Therefore, there were no alterations in the nutritional levels of the diets across the treatments, and these factors might influence the weight and quality of the eggs, which did not occur. Because the only difference between the treatments was the lipid source, one may suggest that they did not interfere with the digestibility of the dietary nutrients, which promoted their use in the nutrition of commercial layers.

Although the data obtained in the present experiment showed that the different lipid sources were not able to improve the performance or egg quality of the commercial layers, these results are relevant, considering that they were similar to those obtained in the control group, containing soybean oil, an ingredient commonly used in the formulation of poultry diets but found at high prices in the market. In this way, the cottonseed and sunflower oils and beef tallow are viable alternative lipid sources in the feeding of commercial layers.

Gossypol is the anti-nutritional factor present at the largest concentration in the seeds of cotton (He *et al.,* 2015), released upon their processing. These compounds have a cumulative effects and cause liver degeneration (Gadelha *et al.,* 2014), which is evidenced by an increase in the size of these organs, these effects being more pronounced in young animals. In the current experiment, the biometrics of digestive organs was not influenced by the treatments containing cottonseed oil, probably because the birds were at 70 weeks of age at the onset of the experiment and thus more resistant to the deleterious effects of the above-mentioned anti-nutritional factors, coupled with the inclusion value of cottonseed oil in the diet, which did not reach 3%.

Despite not being a commonly evaluated parameter in laying birds, the increase in abdominal fat found in these birds should be taken into account, mainly because laying birds deposit more abdominal fat as they age, and this excess fat may negatively affect the performance (Xing *et al.,* 2009; Gewehr *et al.,* 2011) and productive lifespan of those animals. Thus, factors favoring an increase in abdominal fat deposition should be avoided.

The type of lipid source used in the bird diets affects total body fat deposition (Fouad and El-Senousey, 2014), and the fatty acid profile of a feedstuff directly influences the digestibility of the lipid source. The increasing percentage of abdominal fat following the use of beef tallow may be related to the saturation level of the lipid source, since it is high in saturated fatty acids. Furthermore, as stated by Murakami *et al.* (2010), saturated fatty acids are less digestible when compared to unsaturated fatty acids.

Conflicting results were reported by Duarte *et al.* (2010), who worked with beef tallow (BT), degummed soybean oil (DSO), poultry viscera oil (PVO), BT + DSO, and PVO + DSO in broiler diets and found no effect of beef tallow alone or associated on the percentage of abdominal fat in the carcasses.

According to Mazzuco (2006), a decrease in the omega-6/omega-3 polyunsaturated fatty acids ratio may protect the body from loss of bone mass due to a reduction in the production of prostaglandin (PG), an eicosanoid involved in the stimulus to the bone-resorption function. The lipid composition of soybean oil includes high concentrations of linoleic acid (56%), besides oleic acid (23%) and linolenic acid (5.21%) (Faitarone *et al.,* 2012). Thus, the above-mentioned composition of soybean oil led to the mineral matter results found in the tibiae.

Contrary results were reported by Potença *et al.* (2008), who worked with cottonseed oil, chicken viscera oil, beef tallow and their combinations with soybean oil in the feeding of broilers. Those authors evaluated bone-quality variables (Seedor Index and bone resistance) and concluded that the lipid sources did not influence the bone characteristics of the birds, regardless of being plant- or animal-derived.

Faitarone *et al.* (2012) evaluated the bone quality of commercial layers fed diets containing different lipid sources (linseed, canola, and soybean oils at the rates of 2.5%, 5%, and their association at the rate of 2.5% of each lipid source). The researchers concluded that dietary supplementation of vegetable oils high in omega-3 and omega-6 polyunsaturated fatty acids did not influence the bone resistance of the layers or their bone formation/remodeling.

In conclusion, cottonseed and sunflower oils can be used to fully replace soybean oil in diets for commercial white-egg layers without affecting their performance, egg-quality, digestive organs, or bone quality.

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