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Ground Annatto Seeds (*Bixa orellana* L.) in Sorghum-Based Commercial Layer Diets and Their Effects on Performance, Egg Quality, and Yolk Pigmentation

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ABSTRACT

Consumer demands for healthy foods have stimulated the research on the use of natural products in animal nutrition. Annatto can be used a pigmentation source to improve yolk color of commercial eggs. This study aimed at evaluating the effects of the inclusion of ground annatto seeds on the performance and egg quality of layers fed sorghum-based diets. A total of 336 40-w-old hens were distributed according to randomized blocks into seven treatments with six replicates of eight birds each. The following treatments were applied: T1- egg production feed based on corn and soybean meal; T2- egg production feed based on sorghum and soybean meal; T3, T4, T5, T6, and T7- egg production feed based on sorghum supplemented with 0.5, 1.0, 1.5, 2.0, and 2.5% ground annatto seeds, respectively. As to performance parameters, only egg production was influenced, with hens fed the corn-based diet producing more eggs than those fed the sorghum-based diet with inclusion of 1.5 and 2.5% de annatto. The only egg quality parameter affected by treatments was the color of yolk, which pigmentation increased with increasing levels of ground annatto seeds. It is concluded that ground annatto seeds can be supplemented in sorghum-based production feeds for layers, and that the inclusion level of 0.89% is sufficient to promote the same yolk pigmentation as that obtained with corn-based diets.

INTRODUCTION

The current consumer demands for healthy and natural foods have stimulated research in animal nutrition aiming at finding alternatives to replace synthetic ingredients used in animal feeds and that are capable of maintaining or enhancing productivity and reducing production costs.

The Food and Agriculture Organization (FAO) of the United Nations forbids the use of most artificial pigments in animal diets due to their toxic effects to humans (Constant et al., 2002). These guidelines affected several animal production industries, particularly egg production, where pigments are extremely important to provide egg yolk color.

Egg yolk color derives from the deposition of xanthophylls (group of carotenoid pigments). These pigments are not synthesized by the birds, and therefore must be obtained from the diet, and can derive from natural or synthetic sources (Garcia et al., 2002).

In Brazil, poultry feeds are mostly based on yellow corn, which is the main source of energy and natural pigments. When other energy sources that have low pigment content, such as sorghum, wheat, or millet, pigments have to be added to the feeds in order to ensure the pigmentation of broiler skin or egg yolk required by the market.

Sorghum presents excellent nutritional value, similar to corn, which allows it to partially or totally replace corn in poultry diets. Moreover, sorghum price is about 80% of corn price, presenting an interesting economic advantage. However, its low carotenoid content as compared to corn results in yolks with very little pigmentation, which is often below market requirements. This problem may be solved by the inclusion of pigments in layer diets (Assuena et al., 2008).

Annatto seed is a natural pigment derived from a non-carcinogenic and non-toxic native plant (Bixa orellana L.) found in Central and South America, including the Caribbean (Constant et al., 2002). The annatto plant is a shrub that produces fruits which bear approximately 50 orange-reddish seeds.

Annatto seeds present high levels of the carotenoids bixin and norbixin, and their ratio varies according to cultivar, with a general predominance of bixin (Preston & Rickard, 1980). Bixin is non-toxic and can be extracted from the seed pulp (Braz et al., 2007), having several applications in human foods and animal feeds (Franco et al., 2000). Norbixin, on the other hand, is usually present in small amounts in the seeds (Mercadante et al., 1998).

Carotenoids have pigmentation capacity (Ambrosio et al., 2006), are vitamin A precursors, enhance the immune response, and promote anti-oxidant activity (Melendez-Martinez et al., 2004).

Most studies on the inclusion of annatto in animal feeds are based on the utilization of its byproduct and oil extracts that are obtained from seeds. In poultry, research essentially focuses on the determination of the optimal levels of annatto seed byproduct inclusion in feeds to maintain productivity and to improve egg yolk and broiler skin and meat color. Silva et al. (2000) studied the effect annatto oil extract in layer feeds on egg yolk pigmentation, and found the addition of 0.1% annatto extract to layer diets containing 40% sorghum produced egg yolk pigmentation similar to that obtained with diets using corn as main energy source. However, in a study using an annatto seed extract in sorghum-based commercial layer diets, Braz et al. (2007) found that 2% extract inclusion did not influence layer performance, but was not sufficient to produce the same egg yolk color as that obtained with corn. Similarly, Silva et al. (2006) concluded that the addition of 4, 8, or 12% of that extract to feeds with up to 40% sorghum did not promote the same yolk color as a corn- and soybean meal-based diet. However, it must be noted that the yolk pigmentation results depend on the variety of annatto used and on pigment extraction method.

One of the first studies on the pigmentation effects of annatto was carried out by Campos (1955), who replaced 30% corn by wheat, and supplemented the feed with 1 or 2% annatto meal, and found that 1% of this product was sufficient to obtain a yolk color similar to that produced by corn-based diets. At 2% inclusion level, annatto meal produced similar yolk color to that of free-range chicken eggs, which are preferred by Brazilian consumers. Arraya (1977) worked with 1.06% inclusion of annatto seed meal in sorghum-based diets and obtained similar yolk color to that produced by corn, with a 9-10 score in the colorimetric fan.

The present study aimed at evaluating the inclusion of ground annatto seed on the performance and egg quality of commercial layers fed sorghum-based diets.

MATERIAL AND METHODS

The experiment was carried out at the School of Veterinary Medicine and Animal Science of the State University of São Paulo (UNESP), Botucatu, São Paulo, Brazil.

A total number of 336 40-week-old commercial layers were distributed according to a randomized block experimental design into seven treatments with six replicates of eight birds each.

Birds were housed in a layer house in 42 metal cages (1.00m long x 45cm deep x 40cm high) placed in two double layers along an aisle. Cages were divided in two internal compartments, with four layers housed per compartment, totaling eight birds per cage. Cages were equipped with individual feeders placed in front of the cages and cup drinkers.

The following treatments were applied: T1 - egg production feed based on corn and soybean meal (SBM); T2 - egg production feed based on sorghum and SBM; T3 - egg production feed based on sorghum and SBM with 0.5% annatto; T4 - egg production feed based on sorghum and SBM with 1% annatto; T5 - egg production feed based on sorghum and SBM with 1.5% annatto; T6 - egg production feed based on sorghum and SBM with 2% annatto; and T7 - egg production feed based on sorghum and SBM with 2.5% annatto. The composition of the experimental diets is shown in Table 1.

A total experimental period of 112 days was used, divided in four periods of 28 days. Feed and water were offered ad libitum. Feed intake and egg weight were weekly recorded, and egg production and mortality were daily controlled. At the end of each 28d cycle, two eggs per replicate were collected for three consecutive days to evaluate egg internal and external quality.

The following parameters were evaluated: egg production; feed intake; average egg weight; egg mass;

Table 1 - Ingredient and calculated compositions of the experimental diets.

Ingredients (%)	Treatments						
	T1	T2	T3	T4	T5	T6	T7
Corn	52.93	-	-	-	-	-	-
Sorghum	-	55.57	55.04	54.50	53.98	53.46	52.93
soybean meal	26.30	20.66	20.60	20.55	20.49	20.43	20.38
Wheat middlings	10.72	13.51	13.59	13.68	13.76	13.84	13.92
Annatto	-	-	0.50	1.00	1.50	2.00	2.50
Calcitic limestone	7.93	7.97	7.97	7.97	7.96	7.96	7.96
Dicalcium phosphate	1.38	1.35	1.36	1.36	1.36	1.36	1.36
Salt	0.35	0.37	0.37	0.37	0.37	0.37	0.37
Vitamin supplement	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral supplement	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-Methionine	0.19	0.23	0.23	0.23	0.23	0.23	0.23
L-Lysine	-	0.14	0.14	0.14	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition							
Metabolizable energy (kcal/kg)	2750	2750	2750	2750	2750	2750	2750
Crude protein (%)	17.50	17.50	17.50	17.50	17.50	17.50	17.50
Methionine (%)	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Lysine (%)	0.89	0.89	0.89	0.89	0.89	0.89	0.89
Methionine + Cystine (%)	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Calcium (%)	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Available phosphorus (%)	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Carotenoids (mg)	0.67	-	0.26	0.52	0.78	1.05	1.31

Mineral supplementation per kg feed: copper: 8mg, iron: 50mg, manganese: 70mg, zinc: 50mg, iodine: 1.2mg, selenium: 0.2mg, vehicle QSP: 1g. Vitamin supplementation per kg feed: vitamin A: 7,000IU, vitamin D3: 2,000IU, vitamin E: 5mg, vitamin K3: 1.6mg, vitamin B2: 3mg, vitamin B12: 8mcg, niacin: 20mg, pantothenic acid: 5mg, antioxidant: 15mg, vehicle QSP: 1g. T1 -feed based on corn and soybean meal (SBM); T2 - feed based on sorghum; T3 -feed based on sorghum with 0.5% annatto; T4 - feed based on sorghum with 1% annatto; T5 - feed based on sorghum with 1.5% annatto; T6 - feed based on sorghum with 2% annatto; and T7 -feed based on sorghum with 2.5% annatto.

feed conversion ratio per dozen eggs and per kg eggs; specific gravity; eggshell thickness; eggshell, albumen, and yolk percentages; Haugh units; and yolk color evaluated according to the colorimetric fan.

Yolk color was measured on days 3, 7, 10, 14, 20, and 28 in order to evaluate the evolution of yolk color up to the plateau color, i.e., the value where yolk color stabilized, for each annatto inclusion level and the time required for color stabilization.

The ground annatto seeds were chemically analyzed at the Food Chemistry Lab of the aforementioned school, and the results are presented in Table 2.

Using the chemical analysis results, annatto seed metabolizable energy was determined using the equation of Jansen (1989) for the prediction of metabolizable energy in low-tannin sorghum (ME = 31.02 x CP + 77.03 x EE + 37.67 x NNE), where: ME: metabolizable energy; CP: crude protein; EE: ether extract; NNE: non-nitrogen extract. The results was ME= 3597.152 kcal/kg.

Carotenoid content in corn and in ground annatto seeds was determined by spectrophotometry in the Food Technology Lab of UNICAMP. Results showed 1.27 mg/100g total carotenoids in corn, and 2.81 mg/100g bixin and 2.42 mg/100g norbixin in annatto seeds.

The obtained data were submitted to analysis of variance, and means were compared by the test of Tukey ($p<0.05$) using Sisvar 4.6 statistical package (Ferreira, 1998).

RESULTS AND DISCUSSION

Table 3 shows the performance results of commercial layers fed diets based on corn or sorghum and supplemented with increasing levels of annatto seeds.

The dietary inclusion of ground annatto seeds did not influence ($p>0.05$) feed intake, egg weight, feed conversion ratio per egg mass or dozen eggs, or egg mass. These results are consistent with the findings of Braz et al. (2007), Sanchez (1965), and Arraya et al. (1977), who did not observe any significant effect ($p>0.05$) of the dietary inclusion of annatto residual seeds or annatto extract on the performance of commercial layers.

Table 2 - Chemical analysis of ground annatto seeds.

Annatto	DM (%)	CP (%)	EE (%)	MM (%)	CF (%)	NNE (%)
	91.32	12.99	5.0	5.1	2.34	74.57

DM: dry matter; CP: crude protein; EE: ether extract; MM: mineral matter; CF: crude fiber; NNE: non-nitrogen extract.

Table 3 - Performance of commercial layers fed diets containing different levels of ground annatto seeds.

Treat.[1]	Feed intake (g)	Egg production (%)	Egg weight (g)	Egg mass (g)	FCR/dz	FCR/kg
T1	120.24	95.53a	59.91	56.98	1.51	2.13
T2	114.41	91.97ab	60.60	54.98	1.51	2.09
T3	115.41	92.00ab	59.57	55.10	1.51	2.15
T4	116.43	91.44ab	61.89	57.06	1.52	2.05
T5	113.94	90.21b	59.03	53.52	1.58	2.25
T6	118.58	91.28ab	61.02	54.18	1.58	2.17
T7	115.19	91.17b	62.39	56.13	1.57	2.12
Mean	116.31	91.94	60.63	55.42	1.54	2.14
C.V.(%)	3.66	2.62	3.70	4.76	4.26	6.10

Means followed by different letters in the same column are different by the test of Tukey (p<0.05). 1 - T1 -feed based on corn and soybean meal (SBM); T2 - feed based on sorghum; T3 -feed based on sorghum with 0.5% annatto; T4 - feed based on sorghum with 1% annatto; T5 - feed based on sorghum with 1.5% annatto; T6 - feed based on sorghum with 2% annatto; and T7 -feed based on sorghum with 2.5% annatto.

Birds fed the corn-based diet produced more eggs ($p<0.05$) than those fed the treatment diets with 1.5 and 2.5% annatto. The other treatments presented intermediate values and were not significantly different.

These results are different from those obtained by Silva et al. (2006), who worked with annatto residual seeds in diets with 40% sorghum and observed higher egg production as annatto inclusion increased.

Table 4 presents egg quality results. There were no differences among treatments ($p>0.05$) as to none of the evaluated egg parameters, except for egg yolk. Similar results were found by Silva et al. (2000), Pereira et al., (2000), Braz et al., (2007), Harder (2005), and Kishibe et al., (2000), who worked with different annatto products and byproducts at different dietary inclusion levels and did not find any significant differences in egg specific gravity, eggshell thickness, or yolk, albumen, and eggshell percentages.

Treatments significantly influenced yolk color. The analysis of regression showed a significant linear effect of the dietary inclusion level of ground annatto seeds on yolk color as measured by the colorimetric fan. This effect is expressed by the equation $Y=3.15 + 4.79X$, $R^2 = 90.61\%$, where X is the inclusion level of annatto seeds, and Y is egg yolk color. According to this equation, 0.89% of ground annatto seed inclusion in the sorghum-based feed would be sufficient to achieve the same color (7.44) as that obtained with the diet based on corn. These results are consistent with those of Garcia et al. (2002), who fed different canthaxanthin levels to commercial layers and found a quadratic effect of pigment levels on yolk color.

Table 5 and Figure 1 show the regression equations of annatto levels for yolk color, the time required to achieve the plateau color, as well as the plateau color.

Yolk color quadratically improved with time. The time required to reach the plateau color ranged between 3.25 a 7.74 days within annatto levels. The treatment with sorghum and no inclusion of ground annatto seeds took 8.55 days to loose the yolk color obtained with the corn-based diet supplied before the experimental period, i.e., to reach value 1 in the colorimetric fan. The yolk of birds fed the diet with 0.5% inclusion of ground annatto seeds took 7.74 days to achieve the plateau color of 5.16 in the colorimetric fan, color 7.45 was achieved in 3.25 days with 1% inclusion, color 11.16 in 7.54 days for the treatment with 1.5% inclusion, color 12.37 in 7.29 days for the treatment with 2%, and

Table 4 - Egg quality of commercial layers fed diets based on corn or sorghum and containing different levels of ground annatto seeds.

Treat.	SG[1] (g/cm^3)	BS[2] (gf)	Yolk[3] (%)	Albumen[4] (%)	EST.[5] (mm)	HU[7]	Yolk color*
T1	1.085	3059.23	28.92	61.84	0.396	87.29	7.44
T2	1.087	3100.20	28.51	62.12	0.395	90.64	1.16
T3	1.087	3116.29	28.89	62.04	0.396	87.84	6.33
T4	1.088	2977.11	29.78	61.07	0.395	87.86	9.38
T5	1.087	3232.87	28.95	61.39	0.394	88.02	11.72
T6	1.084	2848.99	29.03	61.70	0.397	90.24	12.44
T7	1.087	2978.28	28.65	61.75	0.397	87.07	13.80
Mean	1.087	3044.71	28.96	61.70	0.396	88.42	8.90
C.V. (%)	0.35	11.51	3.58	3.78	0.77	6.36	10.89
Signif.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	<0.01

1 - Specific gravity; 2 - Breaking strength; 3 - Yolk percentage; 4 - Albumen percentage; 5 - Eggshell thickness; 6 - Haugh units. T1 -feed based on corn and soybean meal (SBM); T2 - feed based on sorghum; T3 -feed based on sorghum with 0.5% annatto; T4 - feed based on sorghum with 1% annatto; T5 - feed based on sorghum with 1.5% annatto; T6 - feed based on sorghum with 2% annatto; and T7 -feed based on sorghum with 2.5% annatto. * Significant quadratic effect.

Table 5 - Regression equations of egg yolk color, time required to achieve the plateau color, and the plateau color estimated by Linear Response Plateau.

Annatto levels (%)	LRP			
	Regression equation	R^2	Time (days)	Plateau color
0.0	$6.8 - 1.2x + 0.067x^2$	0.99	8.55	1.0
0.5	$5.8 - 0.2x + 0.035x^2$	0.99	7.74	5.16
1.0	$1.36 + 2.1x - 0.12x^2$	0.99	3.25	7.45
1.5	$3.05 + 1.7x - 0.08x^2$	0.99	7.54	11.16
2.0	$3.62 + 1.8x - 0.08x^2$	0.99	7.29	12.37
2.5	$2.10 + 2.7x - 0.16x^2$	0.99	5.77	12.69

color 12.69 in 5.77 days for the treatment with 2.5% inclusion of ground annatto seeds in the diet.

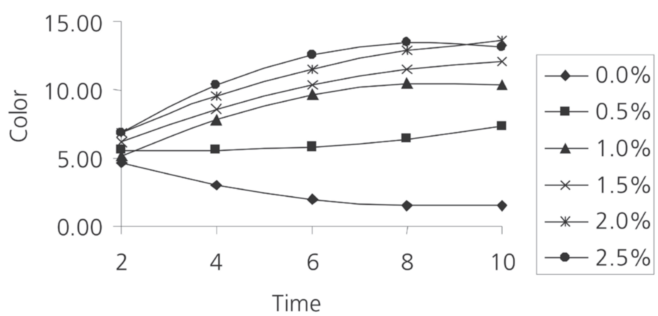

Figure 1 - Effect of ground annatto seed dietary level and time of product utilization on yolk color.

The time required for plateau color stabilization in the present study was shorter than that obtained by Garcia et al. (2002), who included different canthaxanthin level in commercial layer diets based on corn and soybean meal and achieved the plateau color between 5.43 and 9.01 days.

Saldanha et al. (2009) observed a significant linear effect of the use of 1 and 2% annatto in layer feeds with 50% replacement of corn by sorghum for 28 days on yolk color. Estimated yolk color values were 7.68 and 8.52 for the levels of 1 and 2% of annatto, respectively, after seven days of annatto inclusion. These results are different from those obtained in the present study, where yolk color values of 7.45 after 3 days for the treatment with 1% inclusion of annatto seed and 12.37 after 7 days for the treatment with 2% of annatto. This difference may be explained by the fact that the former replaced only 50% of corn by sorghum, whereas in the present experiment, corn was completely replaced by sorghum.

CONCLUSIONS

Ground annatto seeds can be added to commercial layer feeds based on sorghum, and a dietary level of 0.89% of these seeds is sufficient to promote similar yolk color as to that obtained with corn-based diets.

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