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Influence of the natural dyes bixin and curcumin in the shelf life of eggs from laying hens in the second production cycle

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ABSTRACT. This study was developed to evaluate the effect of adding natural pigments on the storage of eggs from laying hens in the second production cycle submitted to diets based on corn and low-tannin sorghum. A total of 160 eggs from Hy-Line Brown laying hens at 90 - weeks old after forced molting were used. The study evaluated the isolated effect and the interaction of two factors: the inclusion of natural pigments (control diet - 50% low-tannin sorghum replacing the corn; RC - 2% powdered dry turmeric rhizome; PU - 2% dry annatto powder; RCPU - 1% turmeric rhizome + 1% dry annatto powder) combined with the storage period (0, 3, 7, 14 and 21 days). The studied variables were: specific gravity, percentages of yolk, albumen and shell in relation to the egg weight, and yolk color. Yolk color was more intense with increasing percentage of annatto. The inclusion of 2% annatto powder promotes and maintains an adequate pigmentation of egg yolk when stored for up to 21 days. Regardless of natural pigment supplementation, the egg quality decreases as storage time increases.

Keywords: coloring, storage, yolk, specific gravity, pigment, sorghum.

Influência dos corantes naturais bixina e curcumina na vida útil de ovos de poedeiras vermelhas no segundo ciclo de produção

RESUMO. A pesquisa foi desenvolvida objetivando-se avaliar o efeito da inclusão de pigmentantes naturais no período de estocagem de ovos de poedeiras em segundo ciclo de produção, submetidas a dietas à base de milho e sorgo baixo tanino. Foram utilizados 160 ovos, provenientes de aves da linhagem Hy-Line Brown de 90 semanas pós-muda forçada. Foi avaliado o efeito isolado e a interação de dois fatores: inclusão de pigmentantes naturais na dieta-controle - 50% sorgo baixo tanino em substituição ao milho; RC - 2% pó de rizoma de cúrcuma seco; PU - 2% pó de urucum seco; RCPU - 1% cúrcuma + 1% urucum associada ao período de estocagem (zero, três, sete, 14 e 21 dias). As variáveis estudadas foram: gravidade específica (GE), percentagens de gema, albúmen e casca em relação ao peso do ovo, e coloração da gema. A coloração da gema apresentou-se mais intensa quanto maior foi a percentagem de urucum utilizada. A inclusão de 2% de pó de urucum promove e mantém adequada a pigmentação das gemas dos ovos quando armazenados por até 21 dias. Independente da suplementação de pigmentantes naturais, a qualidade do ovo decresce com o incremento do tempo de armazenamento.

Palavras-chave: coloração, estocagem, gema, gravidade específica, pigmento, sorgo.

Introduction

The egg is one of the most complete foods of human nutrition and in order to optimize all this nutritious potential, it needs to be preserved during the commercialization period, since some weeks can be spent between the laying moment and the acquisition and preparation. The longer the period, worse will be the internal quality of the eggs, once after the laying the quality is continuously lost. The worsening of quality is associated mainly to the losses of water and carbon dioxide during the storage period, and proportionally to increases of room temperature (LEANDRO et al., 2005). The albumen becomes more liquid when the inappropriate conditions of storage persist. According to Silversides and Villeneuve (1994), this inadequacy occurs, mainly, when they are compared with the fresh eggs produced by different breeds of laying hens, and also when evaluating the albumen quality of stored eggs over different periods.

The egg is properly preserved when the flavor and the nutritional value remains unchanged. Factors such as lineage, egg size, environmental conditions and the poultry age can influence the proportion of the egg constituents. The newly laid eggs have egg-white of quality, depending on the poultry age. The younger laying hens present better quality of egg-white when compared to older

poultries. The egg-white quality and the distance between the blastoderm and the eggshell remain constant during longer periods in young poultries (SANTOS et al., 2009).

The maize is an ingredient that contributes around 65% of the laying hens diet, and represents around 40% of its costs. In this scenario, the decision of the United States of America, to employ this grain for biofuel production, has contributed to increases in maize prices (COSTA et al., 2006). The maize importance on the costs, and its use for ethanol production have led to a greater interest by the use of alternative foods in the animal diets. Among these foods, the sorghum stands out, for presenting good nutritional value, similar to maize, being able to be used in partial or total maize replacement. However, the low presence of carotenoids in this grain, in comparison with maize, results in yolk of very light pigmentation, which sometimes does not meet the market requirements. Such problem can be solved by the insertion of pigments on the poultry diet.

Because of the consumer restrictions and the legislations of countries that prohibit the addition of synthetic dyestuffs to animal rations and to human foods, the choice for the use of natural dyestuffs increases continuously. The natural dyestuffs most used in food industry are: annatto, turmeric, carmine, beetroot red, paprika, anthocyanin (YABIKU, 1992) and chlorophyll.

Among the carotenoids, stands out bixin, obtained from the annatto seeds (*Bixa orellana* L.), representing 80% of the whole carotenoid in the plant, whose dyestuff is largely used in the food industry. The annatto pulp and other parts possess astringent properties, are also used in cases of dysentery and kidney diseases. In India, the leaves are used in the jaundice treatment (CHACO et al., 1969).

The commercial turmeric powder comes from the dry and pulverized rhizome from the *Curcuma longa* L., an herbaceous plant belonging to the family Zingiberaceae from the South and South East Asia. The main dyestuff extracted from the *C. longa* L. rhizome is the curcumin which possesses colors varying from the bright yellow to orange, and must be used with a minimum of 90% purity. The curcumin presents antifungal, anti tumor and antibacterial properties, and the annatto has antioxidant properties.

This study evaluated the levels of dietary inclusion of natural dyestuffs (curcumin and norbixin) for laying hens in the second cycle of production and their effects on the shelf life of these eggs.

Material and methods

A total of 160 brown shelled eggs were used, obtained from poultries (forced post-molting) of the breed Hy-Line Brown, 90 weeks-old on the date of the eggs collection. The experimental rations were all isonutritious (ROSTAGNO et al., 2000) and formulated using maize and sorghum, except for the content of pigments (Table 1).

Table 1. Calculated and Percentage composition of the diets used in the experimental period.

Ingredients (%)	Treatments[1]			
	Control	SU	RC	SURC
Maize (8% PB)	32.24	31.78	31.86	31.67
Sorghum (11% PB)	33.95	32.79	32.72	32.89
Soy Bran (45% PB)	22.88	22.93	22.89	22.91
Annatto (11% PB)	-	2.0	-	1.0
Turmeric (8% PB)	-	-	2.0	1.0
Calcium Limestone	7.99	7.98	7.97	7.97
Dicalcium Phosphate	1.54	1.52	1.52	1.52
Salt	0.35	0.37	0.37	0.37
Vitamin Supplement [2]	0.10	0.10	0.10	0.10
Mineral Supplement [3]	0.10	0.10	0.10	0.10
DL-Methionine 99%	0.16	0.17	0.17	0.17
Washed Sand	0.69	0.26	0.30	0.30
TOTAL	100.00	100.00	100.00	100.00
Calculated Composition				
Metabolisable Energy (kcal kg^{-1})	2800	2800	2800	2800
Crude Protein (%)	16.09	16.09	16.09	16.09
Methionine (%)	0.56	0.56	0.56	0.56
Lysine (%)	0.74	0.74	0.74	0.74
Methionine + Cystine (%)	0.68	0.82	0.82	0.82
Calcium (%)	4.00	4.00	4.00	4.00
Available Phosphorus (%)	0.38	0.38	0.38	0.38

[1]Reference ration + 2% of Turmeric Rhizome and SURC - Reference ration + 1% of Annato Seed + 1% of Turmeric Rhizome.[2]Basic composition of the product: Vitamin A, vitamin D3, vitamin E, vitamin K, vitamin B1, vitamin B2, vitamin B6, vitamin B12, Niacin, Folic Acid, Pantothenic Acid, Sodium Selenite, Antioxidant Additive , Vehicle Q.S.P. Assurance level per kg of product: Vitamin A 10,000,000 U.I, Vitamin D3 2,500,000 U.I, Vitamin E 6,000 U.I, Vitamin K 1,600 mg, Vitamin B12 11,000 mg, Niacin 25,000 mg, Folic Acid 400 mg, Pantothenic Acid 10.000 mg, Selenium 300 mg, Antioxidant 20 g. [3]Basic composition of the product: Manganese monoxide, zinc oxide, iron sulfate, copper sulfate, calcium iodide, vehicle Q.S.P. Assurance level per kg of product: Manganese 150,000 mg, Zinc 100,000 mg, Iron 100,000 mg, Copper 16,000 mg, Iodine 1,500 mg.

We used an completely randomized design, in subdivided plots scheme, in which the plots were represented by natural pigments (control-50% low-tannin sorghum replacing maize); RC - 2% dry turmeric rhizome powder; PU – 2% dry annatto powder; RCPU – 1% dry turmeric rhizome powder + 1% dry annatto powder) and the subplots characterized by the storage period (0, 3, 7, 14 and 21 days), with six repetitions, being the experimental unit represented by eight eggs.

The analyses of external and internal quality of the eggs were carried out at the Laboratório do Polo Regional do Leste Paulista.

The analyses of shelf life have begun after the poultries had consumed experimental diets for 56 days, by the collection of 160 eggs. Of this amount, a fraction with 32 eggs was used as control and evaluated 2h after the collect. The 128 remaining eggs were identified,

weighed, placed in cardboard trays and stored in laboratory at room temperature.

After the 3, 7, 14 and 21-day period, the following variables were evaluated: the specific gravity parameters, yolk percentage, albumen and shell in relation to the egg weight, and yolk coloring (colorimetric range). To determine the weight of yolk, albumen and eggshell, the eggs were weighed in balance with 0.01 g precision, broken and the yolks were manually separated and weighed. The eggshells were dried in forced ventilation oven for 24 hours at 105ºC and weighed again. The albumen weight was obtained by the difference between the egg weight and the weight of the shell and yolk. The specific gravity was obtained by immersing the eggs into different saline solutions of densities varying between 1.050 and 1.100, from the lowest to the highest density, and the solution in which the egg floated the gravity was determined.

The analysis of the yolk color was carried out using the colorimetric score DSM® (color chart), in which the yolk color, exposed in a Petri dish, on a white surface was compared using a color chart, and according to the visual similarity, obtained by a single and trained evaluator, it was attributed an average value between 1 and 15, as described by Galobart et al. (2004).

An analysis of variance was performed, and for the means comparison of the natural pigments, it was applied the Tukey's test and for the storage time effect was applied the polynomial regression analysis.

Results and discussion

The results for the yolk color of eggs stored during 21 days at room temperature are presented in Tables 2 and 3. The analysis of variance detected significant differences for the pigments used ($p < 0.05$) and storage time ($p < 0.05$) for the factor 'yolk color'.

There was no significant interaction between the pigment and the storage time for the yolk color parameter, showing the similar trend for all natural pigments used, including that of the control.

Table 2. Mean scores of yolk pigmentation from eggs stored during 21 days in ambient temperature.

	Storage time (Days)					
Treatments	0	3	7	14	21	Mean
Testimony	3.5d	3.5d	3.75c	3.75c	3.75c	3.65d
2% Annatto	11.5[a]	11.25[a]	11.25[a]	11.25[a]	11.25[a]	11.3[a]
2% Curcumine	4.5c	4.5c	4c	4c	4.5c	4.3c
1% Annatto + 1% Curcumine	9.25b	9.75b	9b	9b	9b	9.2b
General Mean: 7.11						
CV 1 (%): 7.86%						
CV 2 (%): 6.09%						

Mean values with different letters in the same column are significant different ($p < 0.05$) by the Tukey's test.

Table 3. Regression equations for specific gravity (g cm^{-3}), yolk %, albumen % and yolk color, from eggs of poultries which received pigments in the ration and were stored at room temperature for 21 days.

Evaluated parameters	Equations	R^2 (%)
Specific gravity (g cm^{-3})	$\hat{Y} = 1.092 - 0.005\,X$	96.91
Yolk percentage (%)	$\hat{Y} = 23.133 + 0.820\,X$	94.09
Albumen percentage (%)	$\hat{Y} = 69.224 - 1.096\,X$	95.51
Yolk color	$\hat{Y} = 8.78 + 1.05\,X + 0.14\,X^2$	94.07

The eggs from poultries fed with control ration, containing sorghum without the pigment (control treatment), have had yolks with little pigmentation (3.65 points), an expected result once the sorghum is poor in xanthophylls. By including 2% of annatto rhizome powder (RC), the coloring increased significantly (mean pigmentation score of 4.3), when compared to the control ration, however, not enough to pigment the egg yolks to reach acceptable value to meet the requirements of Brazilian consumers, which is around 7.0 points.

According to Klassing (1998), the pigment deposition in specific tissues depends on the appropriate quantity in the diet, the deposition rate in the growing tissue and the capacity of the bird to digest, absorb and metabolize. The limiting step of the pigment use is the hydrolytic action by specific intestinal esterase, of low digestion, when the pigment is esterified with long chain fatty acids. The free carotenoids are absorbed together with the fatty acids dissolved in the micelles and transported by lipoprotein in the blood.

Better results of yolk pigmentation were obtained with annatto supplemented diets. The addition of annatto 1% resulted in a similar pigmentation to that usually is obtained with the ration containing maize as the main source of energy (9.2 points), compared to the inclusion of annatto 2% (with mean pigmentation score of 11.3). Similar results were obtained by Campos (1995), mentioned by Silva et al. (2000), using annatto meal in rations in which the maize was replaced in 30% by adlai (*Coix lacrima* Jobi, Lin). The addition of 2% of the product has produced yolks of stronger orange color than the obtained with 1% of addition, since this is the preferred color by the Brazilian consumer. In other study, Silva et al. (2006) evaluated the supplementation of annatto seed residues (4.8 and 12%) for hens under sorghum based diets (40%) and observed that the higher inclusion level, improved the yolk color. Sanchez (1965) also recommended the use of 3% of annatto meal in rations in which maize was replaced at 30 and 50%, by the food poor in xanthophylls.

The analysis of variance did not detect significant differences among the used pigments ($p > 0.05$) over parameters of specific gravity, yolk percentage, albumen and eggshell. Also, there was no significant interaction among studied factors ($p > 0.05$).

Significant differences were observed for the storage period ($p < 0.05$), with a decreasing linear effect of the regression equations for the specific gravity and albumen percentage, and an increasing linear effect for the yolk percentage. The yolk coloring parameter presented quadratic effect (regression equations are presented in Table 3).

The values registered for GE, albumen % and yolk %, were significantly superior at the start of the experiment when compared to other storage periods. The albumen and yolk percentage, characteristics related to the egg internal quality, linearly worsened during the storage period. The yolk percentage was inversely proportional to the albumen percentage. This occurs because, at the time of egg laying there is an osmotic pressure gradient between the albumen and the yolk, which gradually becomes more pronounced as the water moves from the albumen to the yolk. During the storage period, the moisture of the yolk can vary from 46 to 59%, depending on the time and the storage conditions (SOUZA-SOARES; SIEWERDT, 2005).

According to Sauver (1993), the reduction of the egg specific gravity is linear, estimated around 0.0016 unities per day, at room temperature (15 to 22ºC). Abdallah et al. (1993) reported that the egg's specific gravity has direct relationship with the eggshell percentage. Thus, in the present study, the worsening of the specific gravity occurred without significant effect ($p > 0.05$) on the eggshell percentage.

Other studies using the sorghum at different levels in the laying hen diet also did not influence the eggs quality (MORENO et al., 2007).

Conclusion

The evaluated characteristics have allowed concluding that regardless the supplementation of natural pigments, the egg quality decreases with the storage time increase, affecting negatively the physical characteristics of the eggs, such as specific gravity and percentages of albumen and yolk. The inclusion of annatto was more effective for the maintenance of the yolk pigmentation during the storage period for up to 21 days, being the yolk color stronger as higher the percentage of annatto used. The turmeric pigment did not show efficacy in yolk coloring during the storage period.

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