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NONRUMINANT NUTRITION

Natural and synthetic pigments in sorghum-based diets for laying hens

Claudiane Aparecida Rocha Chaves[1], Diogo Alvarenga Miranda[2], Adriano Geraldo[1], Luiz Carlos Machado[1], Jean Kaique Valentim[3*] and Rodrigo Garófallo Garcia[3]

[1]Departamento de Zootecnia, Instituto Federal de Minas Gerais, Faz, Varginha, Minas Gerais, Brasil. [2]Departamento de Zootecnia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Alto da Jacuba, Diamantina, Minas Gerais, Brasil. [3]Departamento de Zootecnia, Universidade Federal da Grande Dourados, Rua João Rosa Góes, 1761, 79825-070, Vila Progresso, Dourados, Mato Grosso do Sul, Brasil. *Author for correspondence. E-mail: kaique.tim@hotmail.com

ABSTRACT. The objective of this work was to evaluate the supplementation of yellow natural pigment levels based on Marigold Flower extract (2%) and yellow synthetic pigment (Carophyll Yellow 10%) in sorghum-based rations for commercial laying hens and their influence on bird performance and egg quality. A completely randomized design was adopted with 5 treatments, 6 replicates, and 5 laying hens in each repetition. The treatments evaluated were: Sorghum based diet without supplementation with pigmentant - Negative Control; 150 g t^{-1} of Yellow Natural Pigment feed; 300 g t^{-1} of yellow natural pigment feed; 450 g t^{-1} of yellow natural pigment feed; 25 g t^{-1} of yellow industrial pigment feed. The Tukey test was applied at 5% for the analysis of the variables of performance and quality of the eggs. The variables related to external and internal egg quality and poultry yield performance did not present significant results ($p > 0.05$). Only the variable color of the yolk obtained significance ($p < 0.01$), with an increase according to the number of pigments included in the diet. Sorghum can be used together supplementation of natural and synthetic pigments in the diet to improve yolk pigmentation. It is recommended to include 450g t^{-1} of natural marigold flower pigment feed (2%) in sorghum-based diets for better pigmentation of the yolk in place of 25 g t^{-1} of yellow synthetic pigmented, by improving the color of the yolk and not interfering in the productive performance of the laying hens and the quality of the eggs.

Keywords: Poultry; carophyll yellow; vegetable extract; marigold.

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Introduction

The supplementation of pigments in diets for commercial laying hens formulated based on sorghum is made to improve the pigmentation of the egg yolk, which is an important tool to adjust this color to be similar and/or superior when using corn-based rations (Mendonça, Correa, Benevides, Mota, & Franca, 2018). This use occurs mainly when using alternative raw materials (sorghum, wheat, millet, oats, etc.) seeking to reconstruct the carotenoids of xanthophylls normally found in basal maize diets (Fassani, Abreu, & Silveira, 2019).

The coloring of egg yolk is extremely important, especially for commercialization, as it is also used as a form of quality evaluation by consumers (Valentim et al., 2019) which is not ideal, because of the color of the yolk does not concern the nutritional value of the egg. This perception of food is used as a selection criterion by consumers at the time of purchase and many of them have a preference for the yolk with higher pigmentation carotenoids (Moura, Melo, & Miranda, 2016). Egg yolk staining is given by the deposition of xanthophylls, carotenoid pigments derived from bird feed (Marounek & Pebriansyah, 2018). There are several foods with high concentrations of carotenoids, such as corn and millet, among others, but there are also foods with low concentrations of carotenoids, such as sorghum (Fayeye, Ojo, Alli, & Adebayo, 2013).

Pigments can be of a natural or synthetic nature, natural pigments are extracted from plant substances, and require a higher concentration to obtain the desired color (Moraleco et al., 2019). Synthetic pigments are extracted from a substance obtained through synthesis with defined chemical composition, small amounts are needed in the concentration to obtain the desired color (Botelho et al., 2017), but their cost is more costly. Marigold flower extract(*Tagetes erecta*)contains 12 g kg^{-1} of xanthophylls, 80 to 90%, and lutein, a yellow carotenoid. The paprika(*Capsicum annuum*)has 4 to 8 g kg^{-1} of xanthophylls, being 50 to 70% capsanthin, a red-orange pigment (Gumus, Oguz, Bugdayci, & Oguz, 2018). The Food and Agriculture Organization [FAO]

(2004) prohibits the use of most artificial pigments in the diet of animals and humans due to their toxic effect, limiting the use of others such as canthaxanthin, the only carotenoid pigment for which an Acceptable Daily Intake (ADI) has been established, is the value of 0.03mg kg^{-1}. With this, seeking natural pigmenting sources is one of the needs of poultry laying.

Because of the above, the objective of the present research was to evaluate levels of natural yellow pigment supplementation based on Marigold flower extract and artificial commercial pigment in sorghum-based diets for commercial laying hens and their influence on the productive performance and internal and external quality of eggs.

Methodology

The experiment was conducted at the Experimental Laboratory of Laying Hens of the Federal Institute of Education, Science, and Technology of Minas Gerais (IFMG- Bambuí - *campus)*. The project was submitted to the Ethics Committee on Animal Use (CEUA) of IFMG Bambuí *campus* under protocol 05/2017.

We used 150 laying hens with 67 weeks of age of the Hisex Brown lineage, installed in an experimental shed, consisting of 50 cages with individual dimensions of 0.45m width x 0.50m depth x 0.40m height, with five laying hens in each, with a density of 450 cm^2 ave^{-1}. In each cage contained a nipple and a flower-like feeder.

A completely randomized design was adopted with 5 treatments and 6 replicates with 5 laying hens per plot, totaling 30 experimental plots. The experiment was carried out in two periods of 28 days, totaling 56 experimental days.

The treatments used were: NC - Negative control diet based on Sorghum without supplementation with pigments; 150 g t^{-1} – NC feed supplemented with 150g t^{-1} of Yellow Natural Pigment feed (NP - Wisdom Golden Y-20 - Marigold 2%); 300 g t^{-1} – NC feed supplemented with 300g t^{-1} of yellow natural pigment feed; 450 g t^{-1} – NC feed supplemented with 450g t^{-1} of Yellow Natural Pigment feed; 25 g t^{-1} – NC feed supplemented with 25g t^{-1} of yellow industrial synthetic pigment feed (SP -Carophyll Yellow 10%). Pigments were obtained from commercial companies that offer such products.

The laying hens were fed experimental diets based on sorghum, soybean meal, dicalcium phosphate, supplementation of a mixture of carbohydrates (150 g t^{-1} with a value of 75 kcal), emulsifier based on hydrolyzed lecithin of soybean (250 g t^{-1} with a valuation of 50 kcal), phytase (500 U kg^{-1} and reduction in 0.13 and 0.13 percentage points for available phosphorus and calcium, respectively), following a pattern of the nutritional requirement of the lineage. All diets were isoprotein, isoaminoacid, isocalcic, and isophophoric, according to Table 1.

The light program of 16 hours/day was adopted and daily measured, through a hygrometer term, the temperatures, and relative humidity of the air every 15 minutes. The mean temperature obtained in the experimental period was 21.5°C and a maximum of 29.7°C and relative humidity of 49.6% and a maximum of 76.6%.

Table 1. Composition of experimental diets and nutritional levels applied.

Ingredients	Pigment levels[5]				
	NC	NC + 150g NP	NC + 300g NP	NC + 450g NP	NC +25g SP
Sorghum	68.0700	68.0700	68.0700	68.0700	68.0700
Soybean Meal 45.0	20.8100	20.8100	20.8100	20.8100	20.8100
Dicalcium Phosphate	0.6400	0.6400	0.6400	0.6400	0.6400
Limestone 38.0	9.5800	9.5800	9.5800	9.5800	9.5800
Sodium chloride	0.3900	0.3900	0.3900	0.3900	0.3900
L-Lysine 78.0	0.0800	0.0800	0.0800	0.0800	0.0800
DL-Methionine 99.0	0.1500	0.1500	0.1500	0.1500	0.1500
L-Threonine 98.0	0.0200	0.0200	0.0200	0.0200	0.0200
HCL Betaine 93.0	0.0300	0.0300	0.0300	0.0300	0.0300
Tape 10,000 FTU	0.0100	0.0100	0.0100	0.0100	0.0100
Vitamin Premix[1]	0.1000	0.1000	0.1000	0.1000	0.1000
Mineral Premix [2]	0.1000	0.1000	0.1000	0.1000	0.1000
Pigmentant[3]	0.0000	0.1500	0.3000	0.4500	0.0250
Enzyme[4]	0.0200	0.0200	0.0200	0.0200	0.0200

Calculated nutritional levels					
Crude Protein (%)	16.0000	16.0000	16.0000	16.0000	16.0000
Ethereal extract (%)	2.3000	2.3000	2.3000	2.3000	2.3000
Crude fiber (%)	2.8000	2.8000	2.8000	2.8000	2.8000
Calcium (%)	4.0700	4.0700	4.0700	4.0700	4.0700
Total phosphorus (%)	0.2600	0.2600	0.2600	0.2600	0.2600
Phosphorus available (%)	0.4000	0.4000	0.4000	0.4000	0.4000
Sodium (%)	0.1700	0.1700	0.1700	0.1700	0.1700
Chlorine (%)	0.2700	0.2700	0.2700	0.2700	0.2700
EMA available (kcal/kg)	2780.0	2780.0	2780.00	2780.0	2780.0
Total lysine (%)	0.6400	0.6400	0.6400	0.6400	0.6400
Digestible lysine (%)	0.7100	0.7100	0.7100	0.7100	0.7100
Total methionine (%)	0.2900	0.2900	0.2900	0.2900	0.2900
Met+Cis dig. (%)	0.3600	0.3600	0.3600	0.3600	0.3600
Met+Cis total (%)	0.4200	0.4200	0.4200	0.4200	0.4200
Met+Cis dig. (%)	0.5500	0.5500	0.5500	0.5500	0.5500
Total tryptophan (%)	0.1400	0.1400	0.1400	0.1400	0.1400
Tryptophan dig. (%)	0.1700	0.1700	0.1700	0.1700	0.1700
Total threonine (%)	0.3900	0.3900	0.3900	0.3900	0.3900
Threonine dig. (%)	0.5000	0.5000	0.5000	0.5000	0.5000

[1]Vitamin premix guarantee level per kg of product: Calcium(Min) 170g, Calcium(Max) 210 g, Phosphorus(Min) 45 g, Methionine(Min) 10 g, Choline(Min) 5000 mg, Sodium(Min) 30 g, Vitamin A (Min)140000U. I, Vitamin D3(Min)35000U. I, Vitamin E(Min) 140U. I, Vitamin K3(Min) 30 mg, Thiamine B1(Min) 10 mg, Riboflavin B2(Min) 75 mg, Piroxidine B6(Min) 20 mg, Vitamin B12(Min) 120 mcg, Folic Acid(Min) 6 mg, Niacin(Min) 300 mg, Calcium Pantotheate (Min). [2]Mineral premix guarantee level per kg of product: 120 mg, Copper(Min) 160 mg, Iron (Min) 630 mg, Iodine(Min) 20 mg, Manganese(Min) 1600 mg, Selenium(Min) 6 mg, Zinc(Min) 1300 mg, Phytase(Min) 10000FTU, Zinc Bacitracin 500 mg. [3]Natural Pigmentant (NP) guarantee level: Carotenoids (Min.) 20.00 g kg^{-1}. Guarantee level Synthetic Pigmentant (SP): 20-40 ppm carophyll® yellow. [4]Enzyme Composition U g^{-1}: Xylanase >10000. Protease ⩾ 4000. Amylase ⩾ 800. Beta Mananase ⩾ 1000. Betaglucanase ⩾ 700. Galactose Ward ⩾ 100.

Zootechnical performance

Egg production

The average egg production in percentage per bird/day of each plot was obtained by recording daily the number of eggs produced including cracked, broken, and abnormal eggs.

Feed consumption

At the end of each week, the leftovers of the feeder and the bucket of each plot were weighed and the feed intake determined and expressed in grams of feed consumed per bird per day.

Egg mass

The egg mass was obtained by using the product of the number of viable eggs produced in each period by the average weight of the eggs in grams.

Feed conversion by mass

It was calculated by dividing the average feed intake (g) by the average mass of eggs produced (g), which is expressed in grams of feed consumed per gram of egg produced.

Feed conversion per dozen

It was calculated by dividing the average feed intake (g) by the number of dozens produced.

Egg quality

At the end of two final days of each experimental week, two eggs per plot, totaling 120 eggs, were collected, taken to the Laying Hens Laboratory to perform the internal and external quality analyses of the eggs.

Weight of eggs

Egg weight was obtained by individuals weighing them on a digital scale with an accuracy of 0.01g.

Specific egg gravity

According to Freitas, Sakomura, Gonzalez, and Barbosa (2004) methodology the eggs were weighed in air and distilled water, with water temperature control as described in the methodology. The specific gravity was calculated by dividing the weight of eggs in the air by the weight of eggs in water.

Yolk coloring

After that, the yolk stain was obtained through the use of the Yolk Color Fan DSM colorimetric disc, where on top of a white surface, the egg was broken and the color of its yolk evaluated, considering the same environment, room and closed curtains and lights lit in all periods so that there was no subjectivity in the evaluation of the color, were all performed by the same person.

Egg components

The eggs were broken and the shells, yolk, and albumen were separated on a surface, then the egg constituents were weighed on a digital scale with a precision of 0.01g for the evaluation of the percentage of the shell, egg, and albumen, taking into account the total weight of the egg. The shells were washed and dried in a forced ventilation oven at 65°C for 72 hours, then weighed on a digital precision scale and the percentage of the shell was calculated about the total weight of the eggs.

Shell thickness

The shell thickness (EC) was measured in the washed and dried shells, with the aid of a 0.001 mm precision micrometer at three different points in the median region of the eggshell.

Haugh Unit

The *Haugh* unit was calculated using the mathematical model, according to Alleoni & Antunes methodology (2001):

$$UH = 100\,log\,(H + 7.57 - 1.7W0,37)$$

Where: H = height of dense albumen (mm); W = egg weight (g).

Statistical analysis

The variables studied were submitted to the statistical premises of normality of residues and the homogeneity of variances and after the analysis of variance was performed through the Sisvar program (Ferreira, 2011) with the application of the Tukey test at the level of 5% probability.

Results and discussion

There were no significant differences ($p > 0.05$) of the variables of productive performance, feed conversion by mass and per dozen, egg production and feed intake, according to the treatments with pigments used in the study, according to Table 2.

As reported by Botelho et al. (2017) it is feasible to replace corn with sorghum with low tannin in the feeding of commercial laying hens without interfering in the productive characteristics of the zootechnical performance. The use of alternative ingredients to maize in diets, in addition to a need to reduce costs in egg production, is an opportunity to find other energy sources that replace corn, without harming animal performance (Bittencourt et al., 2019).

Table 2. Performance of commercial laying hens receiving diets with supplementation levels of natural and synthetic pigments in the diet.

	Pigment levels[5]						
Variables	NC	NC + 150g NP	NC + 300g NP	NC + 450g NP	NC +25g SP	CV (%)[5]	P-value
EP (%)[1]	86.14	88.85	75.20	91.85	84.00	9.7	0.213
FI (kg)[2]	0.117	0.112	0.108	0.118	0.110	7.5	0.654
FC Mass[-3]	2.08	1.92	2.22	1.97	2.00	7.3	0.423
FC Dozen[-4]	1.65	1.52	1.76	1.56	1.58	7.3	0.345

[1st]EP (%): Egg Bird^{-1} Day^{-1} Production. [2th]FI (kg): Feed Ave^{-1} Day^{-1} Intake (kg). [3th]FC/Mass: Feed conversion by mass (kg of feed kg^{-1} of eggs). [4th]FC/Dozen: Feed conversion per dozen (kg of Feed Dozen^{-1} Eggs). [5th]NC – Negative control ration based on Sorghum without supplementation with pigments; 150 g t^{-1} - NC diet supplemented with 150 g t^{-1} of yellow natural pigment feed (Wisdem Golden Y-20 - Marigold 2%); 300 g t^{-1} – NC feed supplemented with 300 g t^{-1} of yellow natural pigment feed; 450 g t^{-1} – NC feed supplemented with 450 g t^{-1} of Yellow Natural Pigment feed; 25 g t^{-1} – NC feed supplemented with 25g t^{-1} of yellow industrial pigment feed (Carophyll Yellow 10%). [6th]CV: coefficient of variation (%).

Sorghum in diets for laying hens may have advantages, as it is marketed at a price around 80% of the price of corn, despite the nutritional differences between both (Fassani et al., 2019). Corn has lower protein content, more oil and energy, and more lysine and methionine than sorghum, having similar tryptophan quantity between both, the digestibility of some essential amino acids of corn and sorghum is, respectively, 93% and 83% for methionine, 90% and 78% for lysine, 87% and 78% for threonine and 78.2% and 74.5% for tryptophan, which demonstrates lower availability of sorghum amino acids about corn (Maciel et al., 2019).

Sorghum is an alternative food to corn, but its grains have a deficient amount of carotene and pigments such as shamans, and therefore its supply to the bird induces the depigmentation of the egg yolk and the skin of the chicken (Moura et al., 2011). The content of tannins present in sorghum is an important factor to be considered, as they can form complexes with carbohydrates and proteins, thus reducing their digestibility and palatability, besides promoting astringent flavor to sorghum (Almeida et al., 2016). Being inferred that sorghum can be a substitute for corn without altering animal performance.

As reported by Valentim et al. (2020) natural or phytogenic additives have antioxidant or nutraceutical properties that influence the physiological metabolism of animals and consequently their productive performance, however, the form of administration, dose and physiological status of the animal may interfere in the absorption of these compounds. This may explain the non-difference ($p > 0.05$) between treatments for performance variables.

The variables of yolk, shell and albumen percentage, specific gravity, average egg weight, shell thickness, and Haugh unit did not present significant differences ($p > 0.05$) as a function of the carotenoids of inclusion of pigments according to Table 3. Only the variable yolk color showed significant difference ($p < 0.01$), with higher yolk color according to the increase in the inclusion of the natural pigment.

The addition of pigmenting plant extracts was not able to alter the internal and external quality of the eggs, but there was an improvement in the color of the yolk due to the higher deposition of pigmenting carotenoids. The addition of synthetic pigmentant ensured better pigmentation when compared to natural pigmenting levels, this fact can be explained by the chemical pigmentation capacity of this product.

The color of the yolk is due to the deposition of xanthophylls, carotenoid pigments derived from the feeding of laying hens. After ingestion and digestion of food, pigments are transferred to the bloodstream and quickly the yolk (Oliveira et al., 2017), this deposition of pigments in specific tissues depends on the amount in the diet, the rate of deposition in the tissue and the ability of the bird to digest, absorb and metabolize them.

Table 3. Internal and external quality of eggs of commercial laying hens fed diets with different levels of supplementation of natural and synthetic pigments.

	Pigment levels[5]						
Variables	NC	NC + 150g NP	NC + 300g NP	NC + 450g NP	NC +25g SP	CV (%)[6]	P-value
Yolk (%)	25.99	25.48	24.96	25.23	25.06	3.82	0.124
Shell (%)	9.90	9.71	9.62	9.74	9.75	2.85	0.543
Albumen (%)	64.20	64.92	65.51	65.22	65.34	1.56	0.221
SG (g cm^{-3})[1]	1.15	1.11	1.12	1.13	1.13	0.27	0.524
EW (g)[2]	64.92	66.13	66.72	67.74	64.66	3.28	0.198
ST (mm)[3]	0.41	0.42	0.42	0.42	0.43	2.89	0.265
HU[4]	76.11	77.25	75.58	76.22	76.39	2.53	0.302
YC[5]**	2.96e	4.14d	4.82c	5.33b	6.56a	5.37	0.001

**Average followed by different letters in the row differ statistically by the Tukey test ($p < 0.01$). NP - natural pigmentant. SP - synthetic pigmentant. [1st] SG (g cm^{-3}): Specific egg gravity (g cm^{-3}). [2nd] EW (g): Average Egg Weight (g). [3th] ST (mm): Shell Thickness (mm). [4th] HU: Haugh Unit. [5th] NC - Negative control ration based on Sorghum without supplementation with pigments; 150 g t^{-1} – NC feed supplemented with 150g t^{-1} of Yellow Natural Pigment feed (Wisdem Golden Y-20 - Marigold 2%); 300 g t^{-1} – NC feed supplemented with 300 g t^{-1} of yellow natural pigment feed; 450 g t^{-1} – NC feed supplemented with 450 g t^{-1} of Yellow Natural Pigment feed; 25 g t^{-1} – NC feed supplemented with 25 g t^{-1} of yellow industrial pigment feed (Carophyll Yellow 10%). [5th]YC: Yolk color. [6th]CV: coefficient of variation (%).

Free carotenoids, after being absorbed with fatty acids, are transported by lipoproteins into the blood (Klasing, 1998). Pigments are absorbed in the ileum together with fatty acids in the form of micelles, are esterified, and stored mainly in adipose tissue and skin as hydroxyxinoids (Pérez-Vendrell, Hernandez, Llauradó, Schierle, & Brufau, 2001). These results corroborate those of Galobart et al. (2004) and Santos-Bocanegra, Ospina-Osorio, and Oviedo-Rondón (2004) that natural pigments do not influence the productivity and quality of laying hens' eggs, only in the color of yolks. The coloring of the yolk is widely used by consumers as a quality analysis tool, the desired color varies between markets, even not indicating its nutritional value, as stated Hernandez, Blanch, and Roche (2000).

Moura et al. (2011) working with the inclusion of natural pigments in sorghum feed in the diet of Japanese quails, observed higher pigmentation efficiency of paprika extract (*Capsicum annuone*) about marigold flower (*Tagetes erectus*), however, the association of the two sources of pigments was more efficient than its use alone, since it enhanced the increase in the colorimetric score of egg yolks about laying hens of the other experimental groups.

The preference for the degree of pigmentation of the yolk varies between consumers from different countries, or even between regions of the same country. In the United States and Brazil, consumers prefer stains between 7 and 10 on the DSM colorimetric scale (DYCF), on the other hand, in Europe and Asia, consumers prefer more pigmented gems, between 10 and 14 in dycf (Galobart et al., 2004).

Due to market requirements, natural pigments have been increasingly used to enhance the color of egg yolks (Seibel, Schoffen, Queiroz, & Soares, 2010). The color of the yolk is due to the deposition of xanthophylls, carotenoid pigments derived from the feeding of laying hens. After ingestion and digestion of food, the pigments are transferred to the bloodstream and the yolk is quickly transferred (Oliveira, Fonseca, Soares, Ferreira, & Thiébaut, 2007) Most of the components of the egg are metabolized in the liver, and the accumulation of nutrients in the blood circulation causes them to be transported to the ovary, where the deposition of the liposoluble compounds, lipids, phospholipids, cholesterol and the carotenoids that give the yellow-orange color of the yolk occurs (Lopes et al., 2011).

In laying hens the metabolism of pigments such as carotenes present in food is due to the absorption of light from the intestinal lumen, where carotenoids are transported together with lipids to the liver where lipogenesis occurs and enter cells by lipoproteins present in the cell membrane. From the liver, these pigments are transported for accumulation in the cells of several lipid-rich tissues, such as egg yolk (Faehnrich, Lukas, Humer, & Zebeli, 2016).

Marigold extract contains 12 g kg^{-1} of xanthophylls, 80 to 90% of lutein, a yellow carotenoid (Galobart et al., 2004). According to Moura et al. (2011) the corn-based and soybean meal feed meets the need for pigmentation in the yolk satisfactorily, the sorghum-based diet without the addition of pigments provides a depigmentation of the yolk and the sorghum-based diets with the inclusion of pigments can give color to the yolk with more intensity.

From a technical point of view, there are several benefits obtained with the use of synthetic dyes in the food industries, considering that they have low cost, better tinctorial power, good stability (Silva et al., 2019).

Although the current trend is the replacement of synthetic dyes by natural ones, the former is still widely used in the Western world, despite the high cost. The use of synthetic carotenoids for pigmentation has the advantage of using minimum quantities necessary to achieve the desired effect (Moraleco et al., 2019). As reported by Garcia et al. (2009) pigments can be obtained through natural and synthetic sources and although less costly, natural sources have lower pigmentation efficiency when compared to synthetic sources.

The Food and Agriculture Organization (FAO) of the United Nations prohibits the use of most artificial pigments in the diet of animals and humans, due to its toxic effect (Constant, Stringheta, & Sandi, 2002), limiting the use of others such as canthaxanthin, the only carotenoid pigment for which an Acceptable Daily Intake (IDA) has been established, is the value of 0.03mg kg^{-1}. According to Stringheta and Silva (2008), the toxicity of many artificial dyes has led the responsible organs of several countries to restrict or even prohibit the use of a variety of them.

However, these compounds have a high cost. With this the use of natural pigments becomes an important aspect in the current market, generating more options of additives and inputs such as sorghum aiming at reducing production costs and ensuring animal productivity.

This is a natural supplementation option to meet consumer requirements, even obtaining about 1 point below the color scale obtained with the use of the synthetic pigmentation commonly used in the field in sorghum-based diets.

Conclusion

It is recommended to include 450g t^{-1} of feed of the natural pigment of Marigold flower (2%) in diets based on sorghum for better pigmentation of the yolk and for not interfering in the productive performance of laying hens and in the eggs quality.

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