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Research Article
Evaluation of Tarsus Pigmentation in Chickens Fed with Different Levels of Xanthophyll Pigment: A Practical Application of the CIELab System

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Abstract
Objective: The objective of this study was to evaluate a method to assess dermis pigmentation in broiler chickens in response to xanthophyll pigment inclusion in their feed. **Materials and Methods:** Ninety-six Cobb 500 birds were distributed in a completely randomized design and fed with different diet treatments, including 0.30, 0.50 and 0.90 g kg^{-1} of xanthophyll pigment extract from marigold, or a control diet without pigment inclusion. On 42th day, the pigmentation of the dermis was evaluated. For this study, the DSM® colorimetric score and CIELab system were used for comparative evaluations, the luminosity (L), redness (a*) and yellowness (b*) of the tarsus skin were recorded. The data obtained using both methods were subjected to Pearson's correlation analysis. The CIELab data were subjected to ANOVA, Tukey test was used to compare the treatments mean. The non-parametric data were compared using the Kruskal–Wallis test. The software R was used for statistical analysis. **Results:** The results showed a higher correlation ($p<0.01$) with the yellow spectrum ($b^* = 0.72$) and a greater sensitivity with a significant effect ($p<0.01$) in response to pigment inclusion. **Conclusion:** A practical application of the CIELab spectrum has the capacity to evaluate skin pigmentation "*in vivo*" in broiler chicks with different levels of pigmentation.

Key words: Broiler chicks, natural pigment, marigold, CIELab system, skin pigmentation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Appearance is the most important factor that influences consumer selection through the subjective visual evaluation of the product. Chicken carcass choice in the market is mainly based on this factor and it is considered to indicate its quality[1-4]. Among the principal visual factors, skin pigmentation stands out and this is affected by dietary composition, especially the carotenoid content[5]. For example, the bright yellow color of the skin of chicken is often associated with good health and superior quality[6]. The evaluation of pigmentation can be important to understand the relationship between the level of added pigment in the diet of birds and the final product quality[7]. The first step of quality control is to establish a reliable method to evaluate color. There are simple methods based on visual comparisons, such as the evaluation of coloration that is standardized by scores[8], which was the first method developed for the evaluation of dermis pigmentation in the commercial poultry meat production industry. Due to the dissatisfaction of the visual evaluation, Dalby[9] established a more accurate system to describe the color influence on pigments involving new techniques based on spectrophotometry or reflectance colorimetry (the AOAC method) that is currently used[10]. However, there are limits to its practical application, indicating that it is necessary to implement a method with more practicality and accuracy.

The inclusion of pigment is required mainly because animals cannot synthesize carotenoids, unlike photosynthetic organisms, such as plants and algae, which synthesize carotenoids through a photoprotective mechanism[11,12]. An important industrial pigment extract from marigold flowers (*Tagetes erecta*) is currently used as a natural source of carotenoids and is largely commercialized as a feed additive, proving to be very effective on the carcass's pigmentation[6,8,11,13].

Due to the importance of the use of natural pigments and non-availability of methods to evaluate the pigmentation degree in broiler chicks *in vivo*, this study was conducted to develop a more accurate method to evaluate pigmentation and establish the appropriate dose of these feed additives.

MATERIALS AND METHODS

Animals and management practice: Ninety-six Cobb 500 mixed chickens (21 days of age) obtained from commercial breeding were used in this study. The ambient temperature was maintained between 20-27°C with a light program of 16 h L:8 h D.

One week before the evaluation, an adaptation period (21-28 days) in which a basal corn-soybean meal diet without pigment was provided. Afterward, during the last two weeks of the experiment, each group received the dietary treatments.

All procedures involving animal use were performed according to the Animal Protection and Welfare Law from the Peruvian Congress Legislation (Law No. 30407).

Diet and experimental design: The basal diet was formulated according to the specifications suggested by the Cobb 500-Line Management Guide for the finishing phase (21-42 days). Table 1 describes the composition and nutrient content of the experimental diets. The dietary treatments consisted of supplementation with different amounts (0, 0.3, 0.5, or 0.9 g kg^{-1}) of yellow natural xanthophyll pigment extracted from marigold (*T. erecta*). The birds were placed in a 12-floor pen (4 males and 4 females per pen) using the completely randomized design. Feed and water were offered *ad libitum*.

Measurements: On the 42th day, a subjective evaluation of the degree of tarsus pigmentation of each bird *in vivo* was performed using the DSM® fan colorimetric method to score pigmentation by visual comparison on a scale of one to fifteen, with ascending values proportional to the degree of pigmentation, according to the indications suggested by the DSM Pigmentation Guidelines. By using the CIELab system, images were captured with a simple smartphone camera (16 Mpx.), three replicates of each reference point were

Table 1: Composition and nutritional values of the basal diet for the finishing phase (21-42 days old; % of feed)

Ingredients	Basal diet
Corn	35.00
Soybean meal	25.26
Corn gluten	20.00
Starch	12.00
Oil	4.50
Dicalcium phosphate	1.39
Limestone	1.25
Methionine-DL	0.23
HCl Lysine-L	0.19
Inert	0.10
L-threonine	0.04
Vitamin and trace minerals premix[7]	0.04
Total	100.00
Calculated nutritional content	
ME (kcal kg^{-1})	2961.00
Crude protein	19.00
Calcium	0.92
Available phosphorus	0.38
Dig. lysine	0.95
Dig. Met+Cys	0.74

analyzed by the colorimeter app version 3.5.2 (Research Lab Tools, Sao Paulo, Brazil)[14], which used the pixel color variations on the tarsal surface to determine pigmentation in the real scale. The brightness (L), red (a*) intensity and yellow (b*) intensity were recorded, obtaining positive values from 0-100.

Statistical analyses: The correlation analysis between the pigmentation data obtained from the DSM® fan (ADSM) and CIELab system was analyzed using Pearson's correlation. Subsequently, an assessment of the pigment supplementation was performed, in which quantitative data in the CIELab system were analyzed using a one-way ANOVA and linear effects model. The DSM® fan colorimetric method to score pigmentation was analyzed by the Kruskal-Wallis test, reporting the frequency of the occurrence of each score for each treatment. All data were analyzed by software R version 3.5.3 ® Foundation for Statistical Computing, Vienna, Austria). Differences of $p<0.05$ were considered statistically significant.

RESULTS

Figure 1 shows the distribution of CIELab spectrum values of L, a* and b* on the ADSM scores. The spectrum a* and b* reported a high ($p<0.001$) and medium ($p<0.01$) positive correlation, respectively, whereas the spectrum L presented a moderate negative correlation ($p<0.01$). These results showed that the CIELab system can be an explanatory variable of the ADSM pigmentation score.

In the linear response model, the yellow pigmentation (b*) of the tarsus of each bird was significantly affected ($p<0.01$) by the inclusion of marigold extract, which increased the intensity of yellow skin pigmentation. The inclusion of the xanthophyll pigment had an effect ($p<0.01$) on the appearance of pallor (L) compared to the control group, while the birds supplied with the pigment did not show pigmentation differences between them. The red pigmentation (a*) did not show a significant difference ($p>0.05$) in reference to the inclusion of pigment or to the inclusion levels (Table 2).

Table 2: CIELab and DSM fan score values of tarsus pigmentation of broilers fed with different amounts of pigment at 42 days old

	CIELab system			
Pigment (g kg^{-1})	L	a*	b*	ADSM score
0	72.05±6.6^{a}[1]	5.57±2.9	24.45±6.9^{a}	1.76^{c}[2]
0.3	64.17±6.9^{b}	7.20±3.9	30.33±5.4^{b}	2.92^{b}
0.5	63.84±8.5^{b}	8.23±3.5	37.84±5.7^{c}	4.88^{a}
0.9	59.03±6.5^{b}	8.22±3.9	44.35±5.7^{d}	4.64^{a}
Probability	<0.05	ns	<0.05	<0.01
R^{2aj} linear	0.861	0.671	0.965	
SEM	51.57	12.65	36.26	

[1]μ±SD. [2]μ×frequency of each score. SEM: Standard error of the mean. [a,b,c,d]Different superscripts represent a statistical difference by Tukey test. ns: Non-significant effect. L: Brightness, a*: Red and b*: Yellow

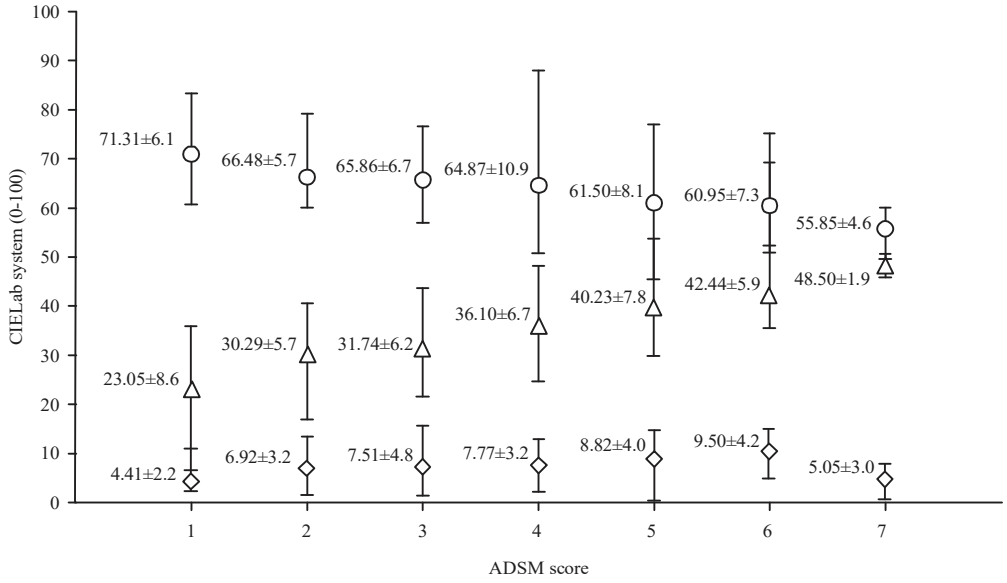

Fig. 1: Distribution of CIELab spectrum values on the ADSM scores. [1]μ±SD

The visual comparison of the pigmentation score showed an effect ($p<0.01$) compared to the control group, which showed a lower frequency of the ADSM score, different from the other treatments; however, the inclusion of 0.5 and 0.9 g kg^{-1} were not significantly different (Table 2).

DISCUSSION

Several studies have been conducted using Minolta photocolorimetry to evaluate the pigmentation of chicken meat, as reported by Martínez *et al.*[15] and Karaoğlu *et al.*[16], who obtained similar L spectrum values (70.50 and 63.48, respectively). However, Melluzi[2], Rajput *et al.*[16] and An[5] reported different values of 79.00, 47.81 and 45.89, respectively. That difference is attributed to the type of evaluation since *in vivo* compared with post-mortem evaluations, the method of slaughter and storage after the slaughter influence the intensity and brightness[16]. The a* spectrum values described by Martínez *et al.*[15], Karaoğlu *et al.*[16] and Rajput *et al.*[17] (3.34, 4.56 and 6.6, respectively) were similar to our results, even though it is considered that this spectrum describes a color (red) that is expressed at low levels in the tarsal epithelial pigment. Rajput *et al.*[17] and Barbosa *et al.*[18] reported no difference in the values of a* and mentioned that the color of red pigment can be differentiated with greater intensity in animals exposed to environments of high temperatures or conditions that stimulate an increase in peripheral circulation or dehydration. The spectrum of yellow color (b*) has been more widely evaluated because it predominates in the expression of a commercially desired coloration. For example, Wang *et al.*[4] reported values of 12.38; Rajput *et al.*[17] reported values of 10.92 for chest pigmentation and 11.19 for the yellow pigment in legs; and Rajput *et al.*[17] reported lower values of 8.47 with natural carotenoids. Furthermore, Meluzzi *et al.*[2] and Morales *et al.*[19] obtained values similar to those found in this experiment (31.66 and 34.78, respectively). The variability in these values could be explained by the nature of the pigment used[5] or carcass process because the yellow pigment is the most affected by this type of process[20]. Rajput *et al.*[17] reported that at higher inclusion level of the xanthophyll pigment, a progressive increase in pigmentation (b*) occurs. Similarly, Sirri *et al.*[1] reported that yellow pigment is expressed with greater intensity in the tarsus of the bird, unlike other sections of the carcass.

Understanding the pigmentation of the final product has a commercial objective, which encompasses the concept of quality. It is also a manifestation of the biological and physiological variations, which occur in response to the health condition of the animal[21], being that this is typically observed in live birds, the tarsal dermis, the beak and crests[22].

Even though the objective evaluation using the CIELab system better represents the quality of meat in color appearance, it does not provide a strong foundation to determine the value of the final product. Therefore, future studies are necessary to evaluate other parameters and strengthen the description of the quality of chicken meat.

CONCLUSION

CIELab system is a viable procedure for the evaluation of the degree of pigmentation. The inclusion of marigold extract (*T. erecta*) increased the intensity of yellow skin pigmentation in the tarsus of birds.

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