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Predictive modelling of skin color in broilers based on sex and dietary xanthophylls

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ABSTRACT

A factorial design was used to predict skin color in broilers depending on the sex and the xanthophylls added to the diet using multiple regression models with interactions. A total of 2160 1-day- old ROSS 308 chicks were distributed across 36 pens, half containing males and half containing females. After a common starter diet, at 14 days of age each pen was assigned to one of three treatments based on the pigment added to a basal diet until 41 days of age: 68 ppm of natural yellow xanthophylls; 34 ppm of synthetic apo-ester or 68 ppm of stabilized natural yellow xanthophylls. Color was measured in the apterial latero-pectoral area and in the hock by means of a MINOLTA 600d spectrophotometer in the CIEL*a*b* color space after 0 and 13 days of pigment intake on the live animal, and after 26 days of pigment intake on the carcass. The reliability of lightness and redness predictions were poor for practical implications. Yellowness predictions were more accurate but a moderate predictive ability was found using cross validation. The models described significant different interactions between sex and days of pigment intake, or natural and synthetic pigments and days of pigment intake. The dose of 34 ppm of synthetic apo-ester was not sufficient to obtain skin color results comparable to those with 68 ppm of natural pigments. Predicting retail color at farm level could serve as a tool for producers to offer the desired color to consumers at the lowest possible cost.

1. Introduction

Xanthophylls are carotenoid derivatives formed in the later stages of the biosynthesis pathway in plants [1]. Their role in plants varies depending on the tissue's photosynthetic ability; in photosynthetic tissues, they help scavenge free radicals, while in non-photosynthetic tissues, they contribute to the yellow, orange and red pigmentation of many plants parts, which serves to attract pollinating insects or herbivores [1]. Birds and mammals are unable to synthesize these compounds, but they can absorb them to a certain extent throughout the digestive system and incorporate them into fatty tissues due to their lipophilic properties, thereby altering the color of those tissues. Among yellow xanthophylls, lutein plays a role in preventing specific degenerative human diseases, such as age-related macular degeneration [2]. However, excessive levels of lutein in the diet may negatively affect the liver [3]. In poultry, marigold flowers (*Tagetes erecta*) has been commonly used as the primary source of lutein in many trials [4–7] because lutein is the main pigment in the skin in broilers [8], sometimes combine with other ingredients to supply additional xanthophylls [3,9], with a focus on enhancing meat color, regardless of potential physiological effects.

Color plays a crucial role in influencing consumer purchasing decisions [10]. Differences in color preferences across regions and countries have been documented by several studies [11–13], as certain consumers demand intensive yellow skin chicken at retail. Historically, the ingredients in the feed were sufficient to provide the necessary pigments to achieve the desired color [5], especially when yellow corn was incorporated due to its carotenoid content. However, the color intensity (Chroma and Hue) achieved from using only corn as the main feed ingredient is often inadequate and may be distributed unevenly in the carcass of modern broilers [13]. Consequently, the poultry industry adds xanthophylls to the feed to ensure good skin pigmentation that meets market demands. These xanthophylls are obtained either through chemical synthesis or by extraction from naturally occurring plants or

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algae [2], but their addition increases feed costs by as much as 10% [5]. Despite the higher cost of synthetic pigments, they offer greater stability and availability [14], which is why the poultry industry widely uses synthetic forms of apo-ester and canthaxanthin.

The addition of adequate dietary supplements can positively affect productivity and improve chicken meat quality [15]. The increasing costs of additives make it necessary for producers to adjust the doses during animal rearing in order to achieve the desired outcomes at the lowest possible cost. Ideally, this adjustment should occur during the growing phase, by either increasing or reducing the concentration of pigments in the feed if the target cannot be reached. To facilitate this, it is crucial to predict the expected color at the processing plant based on the coloration of the live animal on the farm. Regression models are valuable tools for forecasting color intensity based on current values [5], though their accuracy depends on several factors, such as genetics, sex, dietary energy content or the pigment itself. Therefore, this study aimed to evaluate the effects of various natural and synthetic yellow pigments currently available in the industry on skin color development in male and female broilers at commercial inclusion levels, with the goal of predicting the final skin color at the processing plant based on sex and type of natural or synthetic pigment used. Pigment inclusion levels were selected to reproduce standard commercial feeding programs designed to achieve a target skin color accepted by the Spanish market, rather than to explore maximal pigmentation responses.

2. Materials and methods

2.1. Animals

The experiment was conducted with approval from the Ethics Committee for Animal Experiments of the University of Zaragoza (PD19/20NE). The animals were raised in the Experimental Unit of UVESA in Tudela (Navarra) divided into 36 pens (2 × 2.5m), with 60 ROSS 308 1-day-old chicks per pen. Half of the pens housed males, and the other half housed females. The animals were randomly assigned to the pens upon arrival with wood shavings as bedding. Following commercial practices, after a common starter diet for all animals during the first 14 days in the farm, the pens were alternately assigned to one of three diets, each characterized by a specific pigment in the grower phase added to a basal diet: Treatment 1 (NAXa): 68 ppm natural yellow xanthophylls from Capsantal EBS ®; Treatment 2 (SYXa): 34 ppm apo-ester from Carophyll ® Yellow 10%; Treatment 3 (STAXa): 68 ppm stabilized natural yellow xanthophylls from Colortek®. The inclusion rate for each compound (Table 1) was calculated based on commercial dosages and practices in broiler husbandry in Spain, as well as the expected deposition, with the aim of achieving similar color outcomes in the meat (Table 2).

Pigments were sourced from commercial companies specializing in such products. Capsantal EBS® (ITPSA, Barcelona, Spain) is used as a source of yellow xanthophylls, particularly natural lutein, which is its main component. It is derived from marigold flowers (*Tagetes erecta*) and contains at least 40 g/kg of total carotenoids. Carophyll® Yellow 10% (DSM, Madrid, Spain) is a source of ethyl ester of β-apo-8′-carotenoic acid (apo-ester), a synthetic yellow carotenoid known for its higher absorption rate compared to natural pigments. Colortek® (NOVUS, Tarragona, Spain) provides natural yellow carotenoids extracted from marigold flowers, which are processed to enhance stability.

The starter diet was identical for all treatments, containing 34 ppm of stabilized yellow xanthophylls from Colortek® to achieve a commercially acceptable color by the end of the trial, reducing the level of further pigment addition in latter phases when feed intake is higher, favoring sustainability in economic and environmental terms. This is a current commercial practice al farm level. Animals received the grower feed between 14 and 40 days of age. All grower phase diets included 4 ppm of canthaxanthin as a basal red pigment sourced from Carophyll® Red 10%. Considering that the corn used in the feed contained 12 ppm of total xanthophylls, predominantly trans-lutein and zeaxanthin, the inclusion levels of xanthophylls were formulated to remain within the 80 ppm limit [16]. The trial design included 6 replications per sex and diet. Breeding conditions followed industry standards. From the first to the fourth day, the animals were fed on papers placed on the floor, which were set up simultaneously with the feeders. Throughout the study, broilers had access to feed and water ad libitum. Diets were formulated to be isoenergetic and isonitrogenous on digestible lysine basis. The ingredient and nutrient composition of the diets are presented in Table 1.

Table 1

Ingredients and nutrient composition of experimental diets (g/kg as fed basis).

	Starter (1-14d)	Grower (14-41d)		
	Crumble	Pellet		
		NAXa	SYXa	STAXa
Ingredients %				
Corn	15.0	30.0	30.0	30.0
Soybean meal, 47%	30.0	27.0	26.9	26.9
Full fat soybean meal	6.00	0	0	0
Wheat	24.4	25.0	25.0	25.0
Barley	14.6	11.6	11.9	11.8
Animal fat 3/5	1.50	0	0	0
Sunflower meal, 34%	3.00	0	0	0
Soybean oil	0	2.90	2.81	2.83
Mucose, hydrolised	2.00	2.65	0	0
Dicalcium phosphate	0.940	1.060	1.060	1.060
Limestone	0.420	0.720	0.420	0.110
Vitamin-mineral premix[a]	0.500	0.500	0.500	0.500
MHA-Ca[b]	0.450	0.360	0.360	0.360
L-Lysine HCl	0.270	0.240	0.250	0.240
Salt (NaCl)	0.170	0.220	0.220	0.220
Sodium bicarbonate	0.100	0.130	0.140	0.130
L-Threonine	0.130	0.100	0.100	0.100
Colortek ®[c]	0.340	0	0	0.680
Capsantal EBS ®[d]	0	0.170	0	0
Carophyll Yellow ®[e]	0	0	0.340	0
Carophyll Red ®[f]	0	0.080	0.080	0.080
Calculated nutrients %				
Dry matter	88.9	88.6	88.5	88.5
ME poultry, kcal/kg	2980	3100	3100	3100
Crude protein	23.8	18.7	18.7	18.7
Lysine, total	1.47	1.13	1.13	1.13
Lysine, digestible	1.31	1.01	1.01	1.01
Crude fibre	3.79	2.82	2.82	2.83
Crude fat	4.39	5.03	4.97	4.97
Ash	5.56	4.96	4.97	4.97
Calcium	0.950	0.960	0.960	0.960
Phosphorus, total	0.600	0.540	0.540	0.550
Phosphorus, digestible	0.440	0.420	0.420	0.420

[a] Contains per kg premix: vitamin A, 2000000 IU; vitamin D3, 700000 IU; vitamin E, 10000 IU; Vitamin K3, 0.6g; thiamine, 0.5g; vitamin B2, 1.4g; vitamin B6, 0.8g; vitamin B12, 4 mg; biotin, 0.1g; folic acid, 0.3g; niacin, 16g; d-pantothenic acid, 2.8g; choline chloride, 50g; betaine anhydrous, 24g; Fe (iron sulfate monohydrate), 8g; Cu (copper sulfate pentahydrate), 3g; Zn (zinc sulfate monohydratate), 12g; Zn (zinc quelate), 8g; Mn (manganese sulfate monohydrate), 20g; Se (sodium selenite) 0.06g; I, 0.2g. endo-1,4-beta xylanase 390000 EPU; 6-phytase 300000 FTY; narasin, 10g; nicarbazin, 10g

[b] Methionine hydroxy analogue - calcium salts.

[c] Content of 10 % of stabilized natural yellow xanthophylls from Aztec marigold flower (*Tagetes erecta*) (total pigment content: 0.340 g/kg x 0.10 = 34 ppm/0.680 g/kg x 0.10 = 68 ppm).

[d] Content of 40 % of natural yellow xanthophylls from Aztec marigold flower (*Tagetes erecta*) (total pigment content: 0.170 g/kg x 0.40 = 68 ppm).

[e] Content of 10 % of synthetic yellow xanthophylls (apo-ester) (total pigment content: 0.340 g/kg x 0.10 = 34 ppm).

[f] Content of 5 % of synthetic red xanthophylls (total pigment content: 0.080 g/kg x 0.05 = 4 ppm).

Table 2
Color of the surface of the breast 24h after slaughter, influenced by sex and the pigments added to the feed.

	SEX		PIGMENT			SEM	*P*	*P*	*P*
	Female	Male	NAXa	SYXa	STAXa		*Sex*	*Pigment*	*Sex*Pigment*
L*	54.07 x	51.35 y	53.39	52.12	52.54	0.297	<0.001	0.147	0.861
a*	4.99	5.41	4.72 b	5.96 a	4.96 ab	0.152	0.147	0.002	0.159
b*	25.56 y	27.11 x	25.14 b	26.42 ab	27.47 a	0.403	0.041	0.048	0.020
Chroma*	26.07 y	27.68 x	25.61 b	27.12 ab	27.93 a	0.413	0.035	0.048	0.017
Hue^{o}	78.89	78.68	79.31 a	77.21 b	79.77 a	0.311	0.774	0.002	0.867

a, b: mean values with different letters differ significantly between pigments ($P \leq 0.05$); x, y: mean values with different lowercase letters differ significantly between sex ($P \leq 0.05$); NAXa: natural xanthophylls; SYXa: synthetic apo-ester; STAXa: stabilized natural xanthophylls. SEM: standard error of the mean.

2.2. Slaughtering

When animals reached 41 days old, they were transported to the abattoir for less than 30 min after a 6-h fasting period on the farm, with a total feed withdrawal for less than 10 h including lairage. Stunning was performed with CO_2. Carcasses were defeathered, eviscerated, and had their heads, shanks and vents removed prior to chilling. No significant differences in cold carcass weight were found due to pigment intake with an average of 2.07 ± 0.28 kg. However, male carcasses were heavier than female carcasses (2.27 ± 0.23 *vs* 1.87 ± 0.17 kg)

2.3. Color

A reflectance spectrophotometer MINOLTA CM600d (Konika Minolta, Inc, Japan) in the CIEL*a*b* color space [17] with an illuminant D65 and a 10^{o} standard observer was used. The average of three measurements was taken in each location per animal. Lightness (L*), redness (a*) and yellowness (b*) were recorded and Chroma [C*, $\sqrt{(a^2 + b^2)}$] and Hue angle (H^{o}, $\tan^{-1}(b^*/a^*)$ in degrees) were calculated. The color difference (ΔE), which reflects the overall color change between the groups, was also calculated using the equation $\Delta E = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})}$ where ΔL* = L* male—L*female; Δa* = a* male - a* female, and Δb* = b* male—b* female, or between the different pigments. Color measurements were taken from the apterial latero-pectoral (APL) area (which has very sparse feathering, thereby ensuring no interference with skin reflectance readings) and from the hock of the live animal at 14 and 27 days old. On each sampling day, five animals were randomly selected from each pen on the farm, corresponding to 0 and 13 days of pigment intake. Measurements at 14 days of age (0 days of pigment intake) established a baseline pigmentation level prior to the application of dietary pigments during the grower phase. On the afternoon of the slaughter day, which occurred in the morning, color was measured at the processing plant in the same areas of the cold carcass as in the live animals, corresponding to 41-days old animals and 26 days of pigment intake, in randomly selected carcasses. Then, the skin of the breast was lifted, and color was measured on the breast surface at the central area.

2.4. Statistical analyses

For descriptive and inferential analyses of color development during the rearing period, the pen was considered the experimental unit. Pen identity was available for measurements taken on live animals (0 and 13 days of pigment intake) and was accounted for accordingly. However, at slaughter (26 days of pigment intake), individual carcasses could not be traced back to their original pens. Therefore, predictive models for final skin color were fitted at the individual level, assuming independence among observations at this stage. This limitation is acknowledged and should be considered when interpreting the predictive performance of the models.

SPSS 26.0 was used to apply a General Lineal Model (GLM), after assessing normality and homogeneity with Shapiro-Wilk test, to assess the effects of diet, sex and their interactions on color at each measurement time, as well as the effect of days of pigment intake at each sex or diet, with the pen as the experimental unit. Differences between mean values were analyzed using Tukey's multiple range test. For each location (apterial latero-pectoral area and hock) and color value (lightness (L*), redness (a*) and yellowness (b*)) two multiple regression models were fitted. Both models included days of pigment intake (0-26) as a continuous effect and sex (male and female) and pigment in the diet (NAXa, SYXa and STAXa) as categorical effects. The sex and days of pigment intake second order interaction was evaluated in the first model (SEX: y = intercept + pigment + sex + days of intake + sex*days of intake) and pigment in the diet and days of pigment intake was evaluated in the second model (PIGMENT: y = intercept + pigment + sex + days of intake + pigment*days of intake). Continuously, the predictive capacity of the models was evaluated using k-fold cross-validation with five partitions. Because pen identity was unavailable for slaughter measurements, cross-validation was performed at the observation level rather than being blocked by pen. Consequently, predictive performance estimates may be slightly optimistic, particularly for early measurements where within-pen correlation cannot be fully excluded. For each iteration, the same models were fitted to 80% of the data, and predictions were generated for the remaining 20%. Since the partitions did not overlap, all data were predicted. The root mean square error (RMSE) was used as the primary predictive error metric. Additionally, the standard deviation of the RMSE between partitions was calculated as a measure of model performance stability. To facilitate the interpretation of predictive error in relation to data variability, the mean RMSE was normalized by dividing it by the standard deviation of the response variable (NRMSE). A NRMSE close to or below 0.5 was interpreted as moderate predictive capacity, while values close to or above 1 indicated low predictive capacity. Finally, to assess differences between sexes and pigments in the adjusted models, the days of intake-associated marginal means estimated at the time of slaughter were compared in pairs between sex and diet levels, and the slopes of the marginal trends estimated by categories were compared.

3. Results and discussion

The inclusion levels of pigments in the diet were calculated with the aim of achieving similar final breast color. Differences between males and females were not significant for redness or Hue^{o}, but males exhibited darker, more yellow breasts with higher Chroma* values than females (Table 2). This differences were reflected in a ΔE value of 3.16 between males and females. ΔE is a parameter used to assess color differences as perceived by the human eye. Values exceeding 3.0 are generally considered noticeable to the average observer [18]. Sirri et al. [12] previously described females as having higher breast lightness and yellowness, the latter associated with greater subcutaneous fat, a tissue that was not present in the current study at the time of breast measurement. The objective of matching the effects of the natural pigments by including half the dose of synthetic xanthophylls was only partially achieved. No differences in lightness were observed among treatments. The breasts of animals receiving synthetic xanthophylls did not differ from those receiving natural xanthophylls in yellowness or Chroma*, although NAXa group showed lower b* and Chroma* values than STAXa group. Nevertheless, synthetic xanthophylls resulted in higher redness compared with NAXa and lower Hue^{o} compared with both natural pigment treatments. This resulted in differences of ΔE of 2.19 between NAXa and SYXa, 1.50 between SATXa and SYXa and 2.49 between NAXa and STAXa, all of which are below the threshold for color difference perception by the average observer.

Although growth performance was not influenced by the pigment in the diet, as previously found by Karadas et al. [19], all skin color parameters were affected over time by the feed in each sex or pigment group, regardless of the body area ($P < 0.001$). This effect is attributed to the lipophilic nature of the pigments, which accumulate in body fat. However, the differences observed at each measuring time were not consistent across body areas.

No differences between sexes or pigments were observed for lightness in the apterial latero-pectoral (ALP) area at any time. The development has gone towards lighter color with age ($P < 0.001$), likely due to skin thickening and subcutaneous fat accumulation, which would reflect more light. However, females exhibited darker hocks than males at both 14 and 41 days old (Table 3). At 41 days, the intake of stabilized natural yellow xanthophylls also resulted in darker hocks ($P= 0.016$). The highest L* value was observed at 27 days old, although the processing at the abattoir may have influenced the final measurement at 41 days old, making it darker than in the live animal. L* values in the ALP area have been similar to those reported by Karadas et al. [19] in the breast skin, but higher in the hock than those found by the same authors in the leg. Therefore, caution should be exercised when comparing color data from different body areas [13].

Sex differences in redness were found at 14 days of age at the onset of the trial, with females showing higher values in both the ALP area and the hocks (Table 4). These small (0.43 a* units) but significant ($P = 0.002$) differences were likely due to the pigments from the ingredients in the starter diet, which also accumulates in the body fat, as females have greater pigmentation capacity independent of the diet's energy content [5]. However, by the end of the trial differences between sexes disappeared in the ALP area but persisted in the hock at 41 days, with females showing redder hocks than males (10.5 *vs* 9.70; $P < 0.05$). All treatments received 4 ppm of synthetic red xanthophylls during the growing period to achieve a balanced color at display at the trial's conclusion following commercial practices. As a result, redness increased in both sexes and all diets ($P< 0.001$) over time, with increases of 2.3-2.2 a* units in the ALP area and 6.7-7.0 a* units in the hock for males and females, respectively. However, the a* values did not reach those observed by Karadas et al. [19] with 25 ppm of canthaxanthin, which achieved a level of nearly 9 in the breast skin. This value exceeded in more than 2-fold the commercial minimum desired level of 4 [11]. Our trial reached this threshold in the ALP area with a significant lower amount of red xanthophylls in the diet. While the hock was initially 55 to 90% more red than the ALP area, the difference increased to 113-159% after 26 days of pigment intake, likely because canthaxanthin, the main red pigment, is primarily deposited in the shanks [20] and, therefore, the hocks benefit from this higher deposition due to their proximity to the shanks. Unexpected significant differences were found between diets after 26 days of pigment intake in the hocks, with the group receiving stabilized natural yellow xanthophylls showing higher a* values than the other two treatments ($P = 0.005$). At commercial level, synthetic canthaxanthin instead of natural one is included in the diet to enhance redness due to its higher accumulation rate compared to other synthetic pigments [19] and greater stability than natural canthaxanthin. Therefore, we hypothesize that the stabilization process used in Colortek® may have preserved the complex carotenoid profile of botanical extracts from *Tagetes erecta* [21], which could have contributed to the increased redness observed, an effect that was not statistically evident after only 13 days of intake.

Yellowness was the most affected color attribute in this trial (Table 5) since all compounds tested are considered yellow xanthophylls, and skin color is related to the amount of these compounds in the feed [9]. At the start of the grower phase, no differences were found in the hock between sexes, but females exhibited higher yellowness than males in the ALP area ($P < 0.05$). As previously explained in redness, the greater pigmentation deposition in females contributes to the accumulation of fat-soluble pigments in the subcutaneous fat. As all animals received 34 ppm of stabilized yellow xanthophylls in the diet in the starter feed to achieve an acceptable color at the processing plant, sex differences were apparent as early as 14 days of age at the start of the grower phase. These differences between sexes in the ALP area increased over the course of the trial, from 0.62 b* units at the beginning to 1.7 b* units after 13 days of intake and 3.4 b* units after 26 days, although no significant differences were observed in the hock. Despite the higher pigment intake in broiler males compared to females [22] due to greater feed consumption, this difference was insufficient to compensate for the higher fat deposition in females, and thus, the greater pigment accumulation in the skin, as previously found by other authors [5,22]. However, the deposition of yellow pigments did not differ significantly between sexes in the hocks throughout the trial, unlike the findings for redness (a* values), which suggests a preference for the deposition of red pigments (canthaxanthin) rather than yellow pigments (lutein) in this area [9]. Additionally, other yellow pigments, such as zeaxanthin, have shown an affinity for deposition at the shanks along with the red canthaxanthin [20], although this was not the primary xanthophyll used in our study. It is important to note that the dosage used in our trial was designed to align with commercial practices, promoting sustainability by avoiding unnecessarily high levels of pigments, which would increase costs. Since

Table 3
Development of skin lightness (L*) in various body areas of broilers throughout the intake period, influenced by sex and the pigments added to the feed.

	SEX		PIGMENT			SEM	*P*	*P*	*P*
	Female	Male	NAXa	SYXa	STAXa		*Sex*	*Pigment*	*Sex*Pigment*
Apterial latero-pectoral area									
0 days	62.8 Z	62.9 Y	62.9 Y	62.8 Y	62.9 Y	0.197	0.845	0.982	0.103
13 days	64.1 Y	63.1 Y	63.6 Y	63.5 Y	63.8 Y	0.269	0.065	0.910	0.447
26 days	66.5 X	67.3 X	67.9 X	65.7 X	66.7 X	0.301	0.184	0.072	0.093
SEM	0.227	0.231	0.315	0.263	0.262				
P Days	<0.001	<0.001	<0.001	<0.001	<0.001				
Hock									
0 days	67.5 yZ	69.2 xY	67.9 Z	68.9 Y	68.3 Y	0.274	0.002	0.322	0.406
13 days	72.7 X	73.4 X	73.3 X	73.3 X	72.5 X	0.229	0.118	0.281	0.100
26 days	69.2 yY	70.4 xY	69.8 aY	70.2 aY	68.9 bY	0.167	0.001	0.016	0.753
SEM	0.226	0.238	0.311	0.286	0.265				
P Days	<0.001	<0.001	<0.001	<0.001	<0.001				

a, b: mean values with different letters differ significantly between pigments within age ($P \leq 0.05$); x, y: mean values with different lowercase letters differ significantly between sex within age ($P \leq 0.05$); X, Y, Z: mean values with different uppercase letters differ significantly between age within sex or pigment ($P \leq 0.05$); NAXa: natural xanthophylls; SYXa: synthetic apo-ester; STAXa: stabilized natural xanthophylls. SEM: standard error of the mean.

Table 4

Development of skin redness (a*) in various body areas of broilers throughout the intake period, influenced by sex and the pigments added to the feed.

	SEX		PIGMENT			SEM	*P*	*P*	*P*
	Female	Male	NAXa	SYXa	STAXa		*Sex*	*Pigment*	*Sex*Pigment*
Apterial latero-pectoral area									
0 days	2.15 xY	1.72 yZ	1.80 Z	2.01 Y	2.00 Y	0.071	0.002	0.385	0.264
13 days	2.61 Y	2.47 Y	2.50 Y	2.59 Y	2.53 Y	0.108	0.540	0.941	0.728
26 days	4.37 X	4.04 X	3.80 X	4.55 X	4.43 X	0.155	0.305	0.064	0.039
SEM	0.107	0.231	0.118	0.133	0.140				
P Age	<0.001	<0.001	<0.001	<0.001	<0.001				
Hock									
0 days	3.50 xZ	3.02 yZ	3.42 Z	3.26 Z	3.10 Z	0.100	0.016	0.423	0.448
13 days	6.01 Y	5.70 Y	5.75 Y	6.06 Y	5.76 Y	0.175	0.391	0.713	0.488
26 days	10.5 xX	9.70 yX	9.83 bX	9.70 bX	11.1 aX	0.188	0.040	0.005	0.697
SEM	0.211	0.213	0.253	0.253	0.287				
P Age	<0.001	<0.001	<0.001	<0.001	<0.001				

a, b: mean values with different letters differ significantly between pigments within age ($P \leq 0.05$); x, y: mean values with different lowercase letters differ significantly between sex within age ($P \leq 0.05$); X, Y, Z: mean values with different uppercase letters differ significantly between age within sex or pigment ($P \leq 0.05$); NAXa: natural xanthophylls; SYXa: synthetic apo-ester; STAXa: stabilized natural xanthophylls. SEM: standard error of the mean.

Table 5

Development of skin yellowness (b*) in various body areas of broilers throughout the intake period, influenced by sex and the pigments added to the feed.

	SEX		PIGMENT			SEM	*P*	*P*	*P*
	Female	Male	NAXa	SYXa	STAXa		*Sex*	*Pigment*	*Sex*Pigment*
Apterial latero-pectoral area									
0 days	8.18 xZ	7.56yZ	8.07 Z	7.56 Z	7.99 Z	0.156	0.047	0.348	0.164
13 days	13.0 xY	11.3 yY	12.4 aY	10.9 bY	13.3 aY	0.285	0.002	0.002	0.857
26 days	28.7 xX	25.3 yX	29.4 aX	23.4 bX	29.9 aX	0.567	0.001	<0.001	0.155
SEM	0.607	0.496	0.745	0.599	0.758				
P Age	<0.001	<0.001	<0.001	<0.001	<0.001				
Hock									
0 days	21.9 Z	21.9 Z	22.1 Z	22.6 Z	21.0 Z	0.352	0.990	0.176	0.268
13 days	33.0 Y	32.1 Y	33.6 Y	30.9 Y	33.2 Y	0.697	0.521	0.250	0.506
26 days	57.9 X	56.5 X	58.8 bX	51.9 cX	61.6 aX	0.628	0.206	<0.001	0.030
SEM	1.017	0.986	1.278	1.071	1.363				
P Age	<0.001	<0.001	<0.001	<0.001	<0.001				

a, b, c: mean values with different letters differ significantly between pigments within age ($P \leq 0.05$); x, y: mean values with different lowercase letters differ significantly between sex within age ($P \leq 0.05$); X, Y, Z: mean values with different uppercase letters differ significantly between age within sex or pigment ($P \leq 0.05$); NAXa: natural xanthophylls; SYXa: synthetic apo-ester; STAXa: stabilized natural xanthophylls. SEM: standard error of the mean.

there is competition between carotenoids in the uptake and incorporation into micelles [23] and most carotenoids are not absorbed [24], it is possible that at lower doses differences between sexes disappear in the hocks due to a reduced fat deposition in this area and competition for pigment deposition between tissues [25,26].

No significant differences were found between pigment treatments in either of the two body areas at the beginning of the intake trial. However, differences became evident starting from 13 days of intake in the ALP area, and latter also in the hock by the end of the trial. In every case, SYXa exhibited lower b* values than NAXa and STAXa, both of which contained 68 ppm of natural xanthophylls. Despite the lower concentration of synthetic apo-ester, the absorption of the synthetic compound is higher than that of the natural xanthophylls [11], although higher absorption may not necessarily correlate with higher deposition [11]. With half the dose in the feed, b* values for SYXa after 26 days of intake were between 20.4 and 21.7 % lower in the ALP area and 11.7-15.7% lower in the hock compare to treatments with natural xanthophylls. At an equal concentration of 65 ppm for both natural and synthetic xanthophylls, in 49 days old broilers Castañeda et al. [11] found 11.7% less yellowness in the ALP area when using synthetic xanthophylls compared to natural ones, a difference that increased to 30.5 % when the dose was raised to 85 ppm.

The xanthophylls doses used in our study were lower than those employed by other authors [22], who aimed to reach the intensity levels required by consumers in their countries (20 b* units). This minimum yellowness was not achieved with 65 ppm of xanthophylls in the diet after 49 days of age and 28 days on the feed. Tépox-Pérez et al. [22] needed at least 92 ppm to reach the desired level with sorghum as the main ingredient in the feed. In contrast, in the same body area we were able to achieve at least 23.4 b* units with just 34 ppm of synthetic apo-ester at 41 days of age after 26 days of intake, with corn as the primary ingredient. The yellowness in the ALP area in our study with 68 ppm of natural yellow xanthophylls was even higher (nearly 30 b* units). Gut integrity could explain the lack of carotenoid incorporation in adipose tissues due to reduced absorbance in unhealthy animals [9] which was not a factor in our study. We supplemented 34 ppm of stabilized yellow xanthophylls in the starter phase, which allowed us to reduce the concentration in later stages, when feed consumption is higher, thus reducing costs. Also different ingredients in the diet result in varying basal carotenoid concentrations, which should be considered when formulating the addition of xanthophylls.

Throughout the intake period, the increase in color intensity was substantial in both treatments and body regions. After 26 days of intake, b* values in the ALP area were 3 to 3.7 times higher than those measured at 14 days old, and in the hock, they were 2.3 to 2.9 times higher.

Chroma* (Table 6), which reflects color saturation, followed a pattern similar to that observed in the b* values. Significant differences between sexes were found in the ALP area at all times, but not in the hock. Additionally, differences between synthetic pigments became evident from 13 days of intake onward in the ALP area, and only at the end of the trial in the hock.

Differences in Hue° between sexes were only observed at the

Table 6
Development of skin chroma* in various body areas of broilers throughout the intake period, influenced by sex and the pigments added to the feed.

	SEX		PIGMENT			SEM	*P*	*P*	*P*
	Female	Male	NAXa	SYXa	STAXa		*Sex*	*Pigment*	*Sex*Pigment*
Apterial latero-pectoral area									
0 days	8.53 xZ	7.81 yZ	8.32 Z	7.88 Z	8.31 Z	0.153	<0.001	0.662	0.715
13 days	13.3 xY	11.6 yY	12.7 aY	11.2 bY	13.6 aY	0.290	0.002	0.003	0.889
26 days	29.1 xX	25.7 yX	29.6 aX	23.9 bX	30.3 aX	0.576	0.002	<0.001	0.136
SEM	0.610	0.500	0.747	0.606	0.763				
P Age	<0.001	<0.001	<0.001	<0.001	<0.001				
Hock									
0 days	22.2 Z	22.1 Z	22.4 Z	22.9 Z	21.3 Z	0.355	0.204	0.256	0.360
13 days	33.6 Y	32.7 Y	34.1 Y	31.6 Y	33.7 Y	0.710	0.513	0.294	0.496
26 days	58.9 X	57.4 X	59.6 bX	52.8 cX	62.6 aX	0.640	0.185	<0.001	0.041
SEM	1.034	1.004	1.298	1.094	1.388				
P Age	<0.001	<0.001	<0.001	<0.001	<0.001				

a, b, c: mean values with different letters differ significantly between pigments within age ($P \leq 0.05$); x, y: mean values with different lowercase letters differ significantly between sex within age ($P \leq 0.05$); X, Y, Z: mean values with different uppercase letters differ significantly between age within sex or pigment ($P \leq 0.05$); NAXa: natural xanthophylls; SYXa: synthetic apo-ester; STAXa: stabilized natural xanthophylls. SEM: standard error of the mean.

beginning of the feeding trial in the hock (Table 7). Therefore, the significant differences in redness or yellowness found in both areas were not large enough to produce noticeable differences in overall color. This finding supports the practice of selling chickens without distinguishing sexes based on color, unlike what happens in other species such as beef. However, differences in Hue^o appeared between pigments from 13 days of intake onward, with the synthetic apo-ester producing lower values in both body areas, while the natural xanthophyll treatments showed the highest Hue^o in both areas after 26 days of intake. Therefore, even when the same level of inclusion is used, the final color can vary depending on the processing or nature of the additives.

Over time, females experienced a decreased of approximately 1 point in Hue^o, while males showed a decrease of around 2 points in the hock. In contrast, Hue^o increased by 6.6 units in females and 5.1 units in males in the ALP area. This increase in the ALP area was similar in NAXa and STAXa treatments, which contain natural xanthophylls (6.1-6.8 units), but lower in SYXa with the synthetic xanthophylls (4.7). However, the trend in the hock was different: the synthetic apo-ester resulted in a greater decrease (2.3) compared to the stabilized xanthophylls (1.8) and the non-stabilized xanthophylls (0.5). A study should be conducted to determine whether consumers are able to differentiate these color differences at retail, as although statistically significant between treatments, the differences are small in absolute terms.

At the time of formulating the feed composition, the cost per unit of stabilized natural xanthophylls was 42.6% lower than that of synthetic xanthophylls, whereas natural xanthophylls without additional stabilization could be up to 90% cheaper than synthetic xanthophylls. Since synthetic xanthophylls were included at half the dosage of natural xanthophylls, using non-stabilized yellow xanthophylls instead of synthetic xanthophylls would result in a 50% reduction in pigment costs in the feed. In contrast, replacing stabilized xanthophylls with synthetic xanthophylls would reduce costs by 12.8 %. Currently, stabilized xanthophylls have reached a significantly lower market price than synthetic xanthophylls. Therefore, consumer acceptability becomes crucial when offering a retail product that meets quality expectations at the most competitive price.

Two linear models, that included sex, pigment and days of intake effects, and days of intake × sex interaction in SEX model, and days of intake × pigment interaction in PIGMENT model, were fit for the three color parameters (L*, a* and b*) measured at both locations, apterial latero-pectoral area and hock. Results are presented in Table 8. In all models, a relationship between the parameter and the included effects was found ($P > 0.001$). However, the variability explained for L* values were low, and ranged from 8% in SEX model in Hock data to 18% in apterial latero-pectoral area using PIGMENT model. Consequently, their prediction ability tested with cross validation showed a low predictive performance. Therefore, the effects evaluated in this trial did not account for the variability observed in broilers, making it difficult to predict lightness throughout the rearing phase and at the processing plant. These results are consistent with the limited biological influence of dietary xanthophylls on skin lightness, which appears to be primarily determined by structural and processing-related factors, as observed in the most extensively studied meats [27].

In the case of redness, the variability explained by the models were

Table 7
Development of skin Hue^o in various body areas of broilers throughout the intake period, influenced by sex and the pigments added to the feed.

	SEX		PIGMENT			SEM	*P*	*P*	*P*
	Female	Male	NAXa	SYXa	STAXa		*Sex*	*Pigment*	*Sex*Pigment*
Apterial latero-pectoral area									
0 days	74.8 Z	75.9 Y	76.7 Y	74.4 Y	75.0 Y	0.555	0.301	0.201	0.036
13 days	78.4 Y	77.7 Y	78.5 aY	76.2 bY	79.4 aX	0.455	0.412	0.011	0.608
26 days	81.4 X	81.0 X	82.8 aX	79.1 cX	81.8 bX	0.249	0.455	<0.001	0.053
SEM	0.384	0.432	0.467	0.491	0.511				
P Age	<0.001	<0.001	<0.001	0.001	<0.001				
Hock									
0 days	80.9 yX	82.1 xX	81.0	81.8 X	81.6 X	0.235	0.012	0.390	0.566
13 days	79.7 Y	80.2 Y	80.5 a	79.1 bY	80.3 aY	0.196	0.196	0.005	0.641
26 days	79.8 Y	80.3 Y	80.5 a	79.5 bY	79.8 bY	0.135	0.067	0.003	0.092
SEM	0.150	0.194	0.208	0.224	0.200				
P Age	0.001	<0.001	0.502	<0.001	<0.001				

a, b: mean values with different letters differ significantly between pigments within age ($P \leq 0.05$); x, y: mean values with different lowercase letters differ significantly between sex within age ($P \leq 0.05$); X, Y, Z: mean values with different uppercase letters differ significantly between age within sex or pigment ($P \leq 0.05$); NAXa: natural xanthophylls; SYXa: synthetic apo-ester; STAXa: stabilized natural xanthophylls. SEM: standard error of the mean.

Table 8
Performance of fitted models for broiler skin color parameters (L*, a*, b*) across different body regions, using SEX model (y = intercept + sex + pigment + age + age* sex) and PIGMENT model (y = intercept + sex + pigment + age + age* pigment), and their predictive ability using 5-fold cross evaluation.

					Model		Cross Validation		
		SE	R^2	R^{2*}	*P*	DF	RMSE	SD	NRMSE
Apterial latero-pectoral area									
L*	SEX	3.298	0.175	0.167	<0.001	488	50.031	0.739	13.848
	PIGMENT	3.283	0.184	0.174	<0.001	487	50.020	0.718	13.847
a*	SEX	1.418	0.299	0.292	<0.001	488	14.598	1.126	8.663
	PIGMENT	1.417	0.301	0.293	<0.001	487	14.597	1.128	8.663
b*	SEX	4.750	0.731	0.728	<0.001	488	4.801	0.509	0.527
	PIGMENT	4.707	0.736	0.733	<0.001	487	4.723	0.592	0.518
Hock									
L*	SEX	3.557	0.083	0.074	<0.001	487	38.268	0.716	10.355
	PIGMENT	3.555	0.086	0.075	<0.001	486	38.272	0.737	10.356
a*	SEX	2.011	0.652	0.648	<0.001	487	32.330	0.683	9.535
	PIGMENT	1.995	0.658	0.654	<0.001	486	32.325	0.642	9.534
b*	SEX	7.981	0.753	0.750	<0.001	487	7.875	0.366	0.499
	PIGMENT	7.801	0.764	0.761	<0.001	486	7.830	0.277	0.490

NAXa: natural xanthophylls; SYXa: synthetic apo-ester; STAXa: stabilized natural xanthophylls.
SE: Standard error; R^2: Coefficient of determination; R^{2*}: Adjusted coefficient of determination; *P*: p-value; DF: Degrees of freedom; RMSE: Root mean square error; SD: Standard deviation; NRMSE: Normalized RMSE.

higher, ranging from 30 % in SEX model in ALP area to 66% in Hock area for PIGMENT model. These rates were approximately two times higher in the hock than in the ALP area. However, in the case of predictivity, both APL and Hock measurements exhibit a reduced predictive ability. This fact may be the consequence of overfitting of the models, so models capture systematic structure in the data and are not stable enough to support prediction and probably evaluated parameters capture sample-specific structure rather than generalizable signal.

Models assessing changes in the yellowness increment demonstrated strong explanatory power, with coefficients of determination, R^2, ranging from 0.73 for the SEX model applied to APL measurements to 0.76 for the PIGMENT model applied to hock measurements. Furthermore, when their predictive ability was evaluated using cross validation, these models achieved a moderate ability to predict. Thus, the results of this manuscript provide a plausible predictive capacity of the b* value at the time of slaughter considering sex, pigment included in the diet and days of administration. Additionally, although further research is required, it is expected that measuring more individuals and over a greater number of days throughout the growth period will allow for a better fit and predictive capacity of the model. This would also allow for the possibility of a quadratic rather than linear fit that might be expected in the case of the yellowness given its cumulative nature.

The average difference in the model predictions at slaughterhouse moment and differences in the slopes between sexes in SEX model and

Table 9
Average and slope differences of interaction effects, predicted for broiler skin color parameters (L*, a*, b*) at abattoir age, using SEX model (y = intercept + sex + pigment + age + age* sex) and PIGMENT model (y = intercept + sex + pigment + age + age* pigment) across different body regions.

		Variable 1	Variable 2	Average difference	SE	*P*	Slope Difference	SE	*P*
Apterial latero-pectoral area									
L*	SEX	Females	Males	0.152	0.524	0.772	−0.006	0.030	0.841
	PIGMENT	NAXa	STAXa	0.926	0.615	0.289	0.048	0.035	0.360
		NAXa	SYXa	**1.818**	**0.618**	**0.010**	0.082	0.035	0.052
		STAXa	SYXa	0.892	0.618	0.319	0.034	0.035	0.595
a*	SEX	Females	Males	0.342	0.225	0.126	0.001	0.013	0.955
	PIGMENT	NAXa	STAXa	−0.470	0.265	0.180	−0.015	0.015	0.583
		NAXa	SYXa	−0.591	0.267	0.070	−0.019	0.015	0.408
		STAXa	SYXa	−0.120	0.267	0.894	−0.004	0.015	0.955
b*	SEX	Females	Males	**4.530**	**0.754**	**<0.001**	**0.163**	**0.043**	**<0.001**
	PIGMENT	NAXa	STAXa	−0.828	0.882	0.613	−0.027	0.050	0.851
		NAXa	SYXa	**5.264**	**0.886**	**<0.001**	**0.204**	**0.050**	**<0.001**
		STAXa	SYXa	**6.092**	**0.886**	**<0.001**	**0.231**	**0.050**	**<0.001**
Hock									
L*	SEX	Females	Males	**−1.780**	**0.566**	**0.002**	−0.019	0.032	0.545
	PIGMENT	NAXa	STAXa	1.110	0.669	0.222	0.053	0.038	0.340
		NAXa	SYXa	−0.110	0.672	0.985	0.025	0.038	0.786
		STAXa	SYXa	−1.220	0.669	0.163	−0.028	0.038	0.740
a*	SEX	Females	Males	**0.832**	**0.320**	**0.010**	0.019	0.018	0.293
	PIGMENT	NAXa	STAXa	**−1.101**	**0.375**	**0.010**	**−0.061**	**0.021**	**0.014**
		NAXa	SYXa	−0.065	0.377	0.984	−0.003	0.021	0.988
		STAXa	SYXa	**1.035**	**0.375**	**0.017**	**0.057**	**0.021**	**0.021**
b*	SEX	Females	Males	**3.100**	**1.27**	**0.015**	0.130	0.072	0.070
	PIGMENT	NAXa	STAXa	−2.330	1.470	0.251	−0.146	0.084	0.190
		NAXa	SYXa	**6.680**	**1.470**	**<0.001**	**0.284**	**0.084**	**0.002**
		STAXa	SYXa	**9.020**	**1.470**	**<0.001**	**0.430**	**0.084**	**<0.001**

NAXa: natural xanthophylls; SYXa: synthetic apo-ester; STAXa: stabilized natural xanthophylls. SE: Standard error; *P*: p-value. In bold, significant average and slope differences.

between pigments in PIGMENT model were calculated. These differences indicate whether the interactions of these parameters with the days of intake generates a significant change between them. The results are present in Table 9. As can be observed, there is a significant average difference between NAXa and SYXa pigments, probably a consequence of the difference in slope between these parameters which is close to be significant. However, this difference is not observed when L* parameter is measured in the Hock.

In the case of redness, there are not significant differences when it is measured in the APL area, but are found when measured in the hock. This discrepancy is not accompanied by a difference in slope, which indicates that this difference may be due to systematic change between sexes, captured into the sex effect of the model. Dissimilarities between NAXa and STAXa slopes and STAXa and SYXa slopes were significant, which also generated significant differences between averages at slaughterhouse.

Finally, average significant differences between sexes were found in yellowness when it was measured both in APL area and hock, and slope significant differences were found in the APL area. In the case of pigments interaction differences, significant differences were found between natural and artificial pigments (NAXa vs. SYXa and STAXa vs. SYXa) in both measurement locations. Hormone-dependent regulation of avian coloration across the spectrum of androgens and estrogens has been described [28] and may partially explain these differences between sexes. However, the precise regulatory mechanisms governing skin coloration remain unknown, despite the identification of some genes associated with color regulation in chickens [8].

Figs. 1, 2 and 3, (as supplementary Figures) are a graphical representation of the linear regression for each diet at each sex for lightness, redness and yellowness parameters, respectively. Different regressions were fit for each measurement location.

4. Conclusions

The regression models presented should be interpreted as within-study predictive tools aimed at comparing pigment strategies and biological factors, rather than as externally validated predictive models. Skin color prediction for animals at the processing plant can be done on the farm for yellowness in both the apterial latero-pectoral area and the hock. Predictive models of yellowness reflect significant differences between pigment deposition evolution between sexes and, consequently, the final color as seen in a human eye perceptible ΔE. Similarly, predictive models show differences between the deposition of natural (NAXa and STAXa) and synthetic (SYXa) xanthophylls, although no differences can be predicted between both studied natural ones at the dosage studied. The synthetic xanthophylls dosage used in this study, which was half that of natural xanthophylls, was insufficient to achieve the skin results obtained with natural xanthophylls, although it was sufficient to attain similar breast yellowness and Chroma*. Nevertheless, Hue^{o} in the breast was lower than the one achieved by the studied natural pigments. Therefore, more than 34 ppm of synthetic xanthophylls should be used to achieve similar results in the skin as those with 68 ppm of natural xanthophylls. This would potentially increase the cost of the feed, as synthetic xanthophylls are more expensive than natural ones, even after the stabilization process. The applicability of these results is limited to production systems using fast-growing genotypes and corn-based diets targeting yellow skin pigmentation, and extrapolation to other systems should be undertaken with caution. Market conditions fluctuate according to seasonal variations and demand dynamics, which can significantly alter the economic scenario of products. Nevertheless, producers should be aware of consumer color preferences to provide the desired color at retail. Farm-level color prediction can serve as a useful tool for producers, allowing them to lower feed pigment costs while promoting greater uniformity of products at the abattoir.

CRediT authorship contribution statement

Maria del Mar Campo: Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Leticia Mur:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **David López-Carbonell:** Methodology, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maria del Mar Campo reports a relationship with University of Zaragoza that includes: employment. Leticia Mur reports a relationship with University of Zaragoza that includes: non-financial support. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2026.102969.

Data availability

Data will be made available on request.

References

[1] G.E. Bartley, P.A. Scolnik, Plant carotenoids: pigments for photoprotection, visual attraction and human health, Plant Cell 7 (1995) 127–1038, https://doi.org/10.1105/tpc.7.7.1027.

[2] S. Gayathri, S.R. Radhika Rajasree, R. Kirubagaran, L. Aranganathan, T.Y. Suman, Spectral characterization of b, ε-carotene-3, 30-diol (lutein) from marine microalgae *Chlorella salina*, Renew. Energy 98 (2016) 78–83, https://doi.org/10.1016/j.renene.2016.04.065.

[3] W. Yu, Q. Kun, X. Qin, S. Fulong, X. Xiao, Effects of different types of xanthophyll extracted from marigold on pigmentation of yellow-feathered chickens, Anim Biosci 36 (2023) 1853–1859, https://doi.org/10.5713/ab.23.0097.

[4] M. Martínez Peña, A. Cortés Cuevas, E. Avila González, Evaluation of three pigment levels of marigold petals (Tagetes erecta) on skin pigmentation of broiler chicken, Tec. Pecu. Mex. 42 (2004) 105–111.

[5] J.I. Muñoz-Díaz, B. Fuente-Martínez, X. Hernández-Velasco, E. Ávila-González, Skin pigmentation in broiler chickens fed various levels of metabolizable energy and xanthophylls from Tagetes erecta, J. Appl. Poult. Res. 21 (2012) 788–796, https://doi.org/10.3382/japr.2011-00507.

[6] N. Rajput, M. Naeem, S. Ali, Y. Rui, W. Tian, Effect of dietary supplementation of marigold pigment on immunity, skin and meat color, and growth performance of broiler chickens, Braz. J. Poult. Sci. 14 (2012) 233–304, https://doi.org/10.1590/S1516-635X2012000400009.

[7] R. Riveros Lizana, O. Zea Mendoza, C. Vilchez Perales, Evaluation of tarsus pigmentation in chickens fed with different levels of xanthophyll pigment: a practical application of the CIELab system, Int. J. Poult. Sci. 19 (2020) 265–269, https://doi.org/10.3923/ijps.2020.265.269.

[8] R. Huang, X. Deng, J. Wu, W. Luo, Genetic and metabolic factors influencing skin yellowness in yellow-feathered broilers, Poult. Sci. 104 (2025) 104534, https://doi.org/10.1016/j.psj.2024.104534.

[9] A.M. Pérez-Vendrell, J.M. Hernández, L. Llauradó, J. Schierle, J. Brufau, Influence of source and ratio of xanthophyll pigments on broiler chicken pigmentation and performance, Poult. Sci. 80 (2001) 320–326, https://doi.org/10.1093/ps/80.3.320.

[10] T. Singh, V.K. Pandey, K.K. Dash, S. Zanwar, R. Singh, Natural bio-colorant and pigments: sources and applications in food processing, J. Agric. Food Res. 12 (2023) 100628, https://doi.org/10.1016/j.jafr.2023.100628.

[11] M.P. Castañeda, E.M. Hirschler, A.M. Sams, Skin pigmentation evaluation in broilers fed natural and synthetic pigments, Poult. Sci. 84 (2005) 143–147, https://doi.org/10.1093/ps/84.1.143.

[12] F. Sirri, M. Petracci, M. Bianchi, A. Meluzzi, Survey of skin pigmentation of yellow-skinned broiler chickens, Poult. Sci. 89 (2010) 1556–1561, https://doi.org/10.3382/ps.2009-00623.

[13] M.M. Campo, L. Mur, Carcass color in broilers when replacing wheat with corn in the diet, Foods 14 (15) (2025) 2558, https://doi.org/10.3390/foods14152558.

[14] B.S. Gebregziabher, H. Gebremeskel, B. Debesa, D. Ayalneh, T. Mitiku, T. Wendwessen, E. Habtemariam, S. Nur, T. Getachew, Carotenoids: dietary sources, health functions, biofortification, marketing trend and affecting factors - a review, J. Agric. Food Res. 14 (2023) 100834, https://doi.org/10.1016/j.jafr.2023.100834.

[15] H. Kumar, I. Bhardwaj, E. Nepovimova D.S. Dhanjal, S.S. Shaikh, R. Knop, D. Atuahene, A.M. Shaikh, K. Béla, Revolutionising broiler nutrition: the role of probiotics, fermented products, and paraprobiotics in functional feeds, J. Agric. Food Res. 21 (2025) 101859, https://doi.org/10.1016/j.jafr.2025.101859.

[16] European Commission, Commission Implementing Regulation (EU) 2021/420 of 9 March 2021 correcting Implementing Regulation (EU) 2020/1097 concerning the authorisation of lutein-rich and lutein/zeaxanthin extracts from Tagetes erecta as feed additives for poultry (except turkeys) for fattening and laying and for minor poultry species for fattening and laying, OJEU 83 (2021) 17–21.

[17] C.I.E. (Commission Internationale de L'Eclairage). Colorimetry; C.I.E.: Vienna, Austria (1986).

[18] A. Desai, M.A. Brennan, C.S. Brennan, The effect of semolina replacement with protein powder from fish (*Pseudophycis bachus*) on the physicochemical characteristics of pasta, Food Sci. Technol. 89 (2018) 52–57, https://doi.org/10.1016/j.lwt.2017.10.023.

[19] F. Karadas, S. Erdoğan, D. Kor, G. Oto, M. Uluman, The effects of different types of antioxidants (Se, vitamin E and carotenoids) in broiler diets on the growth performance, skin pigmentation and liver and plasma antioxidant concentrations, Braz. J. Poult. Sci. 18 (2016) 101–116, https://doi.org/10.1590/18069061-2015-0155.

[20] J.C. Branellec, La pigmentation du poulet de chair, Aliscope 85 (1985) 1–13.

[21] Y. Qiu, R. Wang, E. Zhang, Y. Shang, G. Feng, W. Wang, Y. Ma, W. Bai, W. Zhang, Z. Xu, W. Shi, X. Niu, Carotenoid biosynthesis profiling unveils the variance of flower coloration in *Tagetes erecta* and enhances fruit pigmentation in tomato, Plant Sci. 347 (2024) 112207, https://doi.org/10.1016/j.plantsci.2024.112207.

[22] M.A. Tepox-Pérez, B. Fuente-Martínez, X. Hernández-Velasco, M. Quiroz-Pesina, E. Ávila-González, G. Téllez, Absorption and cutaneous deposition of yellow pigment in male and female broilers in response to different levels of xanthophylls from *Tagetes erecta*, Austral. J. Vet. Sci. 49 (2017) 167–173, https://doi.org/10.4067/s0719-81322017000300167.

[23] D.M. Deming, J.W. Erdman Jr., Mammalian carotenoid absorption and metabolism, Pure Appl. Chem. 71 (1999) 2213–2223, https://doi.org/10.1351/pac199971122213.

[24] H. Hencken, Chemical and physiological behavior of feed carotenoids and their effects on pigmentation, Poult. Sci. 71 (1992) 711–717, https://doi.org/10.3382/ps.0710711.

[25] D. Phelan, A. Prado-Cabrero, J.M. Nolan, Analysis of lutein, zeaxanthin, and meso-zeaxanthin in the organs of carotenoid-supplemented chickens, Foods 7 (2018) 20, https://doi.org/10.3390/foods7020020.

[26] Y. Wang, D.R. Illingworth, S.L. Connor, P.B. Duell, W.E. Connor, Competitive inhibition of carotenoid transport and tissue concentrations by high dose supplements of lutein, zeaxanthin and beta-carotene, Eur. J. Nutr. 49 (2010) 327–336, https://doi.org/10.1007/s00394-009-0089-8.

[27] M.M. Campo, L. Mur, A. Guerrero, M. Barahona, V.C. Resconi, D.R. Magalhaes, E. Lisbinski, B. Boito, I.M.D. Oliveira, J.L. Olleta, Differentiating breast myopathies through color and texture analyses in broiler, Foods 9 (2020) 824, https://doi.org/10.3390/foods9060824.

[28] R.T. Kimball, Hormonal control of coloration, in: G.E. Hill, K. McGraw (Eds.), Bird Coloration I: Measurements and Mechanisms, Harvard Univ. Press, Cambridge (US), 2006, pp. 431–468.