

Yellowness of egg yolks influences consumer preference for eggs in Ghana

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<u>Abstract</u>

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Introduction

Global experts have consistently advocated for the consumption of nutritious food as a viable approach to address protein and other micronutrient deficiencies prevalent in most developing nations (Thompson and Amoroso, 2011; Pretorius et al., 2021). Eggs are nutrient-dense food sources that contain a variety of essential macroand micronutrients such as proteins, minerals, vitamins, and antioxidants that play crucial roles in promoting human health (Miranda et al., 2015). According to Song and Kerver (2000), egg consumption provides approximately 10% of the recommended daily intake of energy and vitamin B_6 ; 10 - 20% of folate, total, saturated, and polyunsaturated fat; and 20 - 30% of vitamins A, E, and B₁₂. Eggs are also significant sources of omega-3 and omega-6 polyunsaturated

The present work evaluated β -carotene content, colour (L*, a*, b*), and consumer preference for egg yolks from chicken, guinea fowl, and quail, sampled from intensive or semi-intensive rearing systems in Ghana. The β -carotene content of guinea fowl yolk was almost seven times greater (p < 0.001) than that of chicken and quail yolks. The yellowness of guinea fowl yolk (82.18; p < 0.01) was approximately 1.5 and 1.3 times greater than that of chicken and quail yolks, respectively. A consumer preference test showed a significantly greater score (5; p < 0.001) for guinea fowl than for the other egg types. The yellowness of egg yolks had strong positive relationship with β -carotene content (r =0.943; p = 0.216) and consumer preference (r = 0.995; p = 0.064). Therefore, enhancing the yellowness of egg yolks on the Ghanaian market, especially those from chicken, could lead to increased egg consumption in Ghana.

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fatty acids, as well as selenium, ranking third after seafood and meat (McNaughton and Marks, 2002; Meyer *et al.*, 2003).

Egg yolks are reportedly rich in carotenoids and vitamins essential for human health and wellbeing (Ruxton *et al.*, 2010). However, the concentration of carotenoids and vitamins in egg yolks is influenced mainly by the diet of the birds from which the eggs are obtained (Hammond and Renzi, 2013; Zaheer, 2017). Hence, it is crucial to assess the nutritive value of egg yolks to understand the expected benefits of egg consumption, especially in cases where poultry farmers do not provide laying birds with sufficient nutrients. Based on anecdotal evidence, a gradual shift in the colour of cooked eggs has been observed, particularly in eggs obtained from poultry farms in Ghana, where the yolk colour has transitioned from yellow to a grey hue.

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In the Ghanaian market, poultry eggs, including those from chicken and guinea fowl are available (Ayim-Akonor and Akonor, 2014; Naggujja et al., 2020), with chicken eggs being the most common variety. Previous studies have highlighted the nutritional superiority of quail eggs over chicken eggs (Tunsaringkarn et al., 2012; Ali and El-Aziz, generating increased 2019), interest among Ghanaians (Omane et al., 2020). The worldwide average egg consumption per capita is 180 eggs per person per year (IEC, 2018). However, it is important to note that egg consumption in Ghana (approximately 12 eggs per year), is significantly lower than the average per capita consumption worldwide (Ayim-Akonor and Akonor, 2014). The egg consumption might be low in Ghana because the volk colour expectations of consumers are not met (Ali and El-Aziz, 2019; Aygün and Olgun, 2019). While it has been suggested that rearing methods might be a better predictor of egg yolk nutritional quality (Karadas et al., 2006), we hypothesise that if pigmentation is directly related to β-carotene levels in fruits and vegetables, then bird yolks with darker pigmentation are likely to contain higher β-carotene levels.

Researchers worldwide have conducted numerous nutritional experiments to better understand the impact of feed on laying birds, with a specific focus on the effects of feed on yolks. This is because food colour has an undeniable influence on human food consumption patterns (Schlintl and Schienle, 2020). However, achieving this goal becomes challenging when the yolk colour deviates from the normal or expected range (Beardsworth and Hernandez, 2004). Therefore, maintaining the desired yellow colour in yolks would enhance the profitability of poultry farmers within their market niches.

In the present work, we sought to assess consumer preference for yolk colour, and explore the potential association between yolk colour and β carotene content. Additionally, a colour chart was developed to predict egg yolk quality.

Materials and methods

Sampling

Eggs were collected from different agroecological zones in Ghana, including the coastal savannah, semideciduous, transitional, guinea savannah, Sudan savannah, and rainforest zones. The sampled eggs were from quail (*Coturnix coturnix*), guinea fowl (*Numida meleagris*), and chicken (*Gallus gallus domesticus*). Guinea fowl and chicken eggs were collected from both intensive and semiintensive rearing systems, while quail eggs were exclusively obtained from intensive rearing systems.

Four samplings each were performed from quail and guinea fowl, and ten samplings were performed from chicken. Within each sample, there were 24 whole eggs from quail, 28 from guinea fowl, and 30 from chicken, for a total of 96 quail yolks, 112 guinea fowl yolks, and 120 chicken yolks. The feed provided to the birds mainly contained abundant proteins, carbohydrates, vitamins, dietary minerals, and an adequate water supply. The feed primarily consisted of maize (70% starch, 85 - 90% TDN, 4% oil, and 8 - 12% protein), sorghum (65% starch, 80 -85% TDN, 2 - 3% oil, and 8 - 12% protein), wheat (70% starch, 75 - 80% TDN, and 8 - 14% crude protein, with traces of lysine, threonine, and methionine), rice (80 - 90% starch, 78 - 82% TDN, 8 - 10% crude protein, 9% crude fibre, 1.9% ether extract, and 6.5% ash), and soybean (19.1% starch, 82 - 85% TDN, 18.2% oil, and 33.9% protein) (Belkhanchi et al., 2023). The feed formulations also included by-products of milling, such as wheat bran (15 - 25% starch, 65 - 70% TDN, and 13 - 16% crude protein) and rice bran (72 - 98% starch, 70 - 90% TDN, and 13 - 16% crude protein). All birds were additionally fed spinach, carrot, marigold petals, red pepper, sweet potato peels, forage crops, or other green plants regularly.

Colour determination

The eggs were cracked open, and yolks were separated from the albumen using a spatula. Then, the yolks were transferred to clean and transparent glass Petri dishes (150 mm \times 15 mm), ensuring even distribution across the internal surface area of the Petri dishes until complete coverage was achieved (Figure 1). CIELab measurements of each sample were performed in triplicate for each bird type. These measurements included brightness (L*: 0 = black/dark, 100 = light/white), intensity of red and green hues (a*: -ve = green, +ve = red), and intensity of blue and yellow hues (b*: -ve = blue, +ve = yellow). A handheld Minolta Chromameter (CR-410, Japan) was used for measurements after calibration with a D65 white plate (Y = 80.1; X = 0.3219; y =

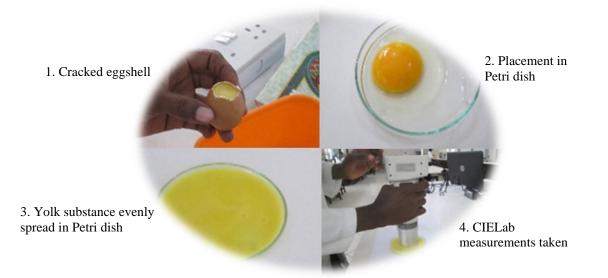


Figure 1. Colour determination protocol.

0.3394). Subsequently, the colour intensity (chroma, C^*) and hue of the yolks were calculated using the values of a^* and b^* , as shown in Eqs. 1a and 1b:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$
 (Eq. 1a)

 $Hue = tan^{-1} \left(\frac{b^*}{a^*}\right)$ (Eq. 1b)

β -carotene determination

β-carotene content was determined using a UV/VIS Excellence UV7 spectrophotometer (Mettler Toledo, Switzerland) following the protocol described by Kpodo (2018). Briefly, 0.1 g of each yolk sample was placed in a test tube with a micropipette. To extract the pigments, 2 mL of acetone was added to each sample, and the mixture was left undisturbed for 30 min. Next, 5 mL of petroleum spirit (40 - 60°C) was added, and the mixture was stirred for 10 min with a stirring rod. Subsequently, 2 mL of distilled water was added to the solution, and the mixture was stirred again. Approximately, 3 mL of each sample was transferred into a cuvette, and absorbance was measured at 663, 505, and 453 nm with a spectrophotometer. The β carotene content was then determined using Eq. 2.

 $\beta - \text{carotene} (mg/g) = \frac{(0.216 \text{ (0.D at 663 nm)} - 0.304 \text{ (0.D at 505 nm)} + (0.D at 453 nm) x V)}{(d x 1000 x W)}$ (Eq. 2)

where, O.D = optical density at a given wavelength, V = final volume of the extract, d = length of the light path, and W = weight of the sample.

Sample preparation and visual analysis

The eggs were positioned in cooking pots with the water level 2 cm above the tops of the eggs. Next, the eggs were boiled simultaneously at a temperature between 80 and 90°C for approximately 15 min. Then, the boiled yolks were carefully separated from the albumen, diced into small pieces, and placed on white disposable plates for sensory evaluation. A total of 57 untrained panellists who regularly consume eggs were included as participants in the study to assess consumer preference for boiled egg yolks. Considering the limited availability of quail and guinea fowl yolks from both semi-intensive and extensive rearing systems, all 18 samples were subjected to a combined consumer preference evaluation based on "bird type" without considering the specific rearing systems used. The evaluation was conducted using a five-point hedonic scale, with 1 indicating the "least preferred" option, and 5 indicating the "most preferred" option.

Colour chart

The nutritional analysis and colour determination data were integrated to create a chart that included the different shades of yolks and photographs taken using a handheld camera (Canon PowerShot A810 HD-China).

Statistical analysis

The data collected were analysed with Minitab v16.2.4.4 (Minitab, Inc., State College, PA, USA). β carotene and colour data of the bird types were analysed using One-way analysis of variance. Data for chicken and guinea fowl bird types and rearing systems were analysed using Two-way analysis of variance. The visual data were also analysed using the Kruskal-Wallis non-parametric test. Significance was set at 95% confidence level, and the significance of differences was determined using the Tukey *post hoc* test when needed. Correlational analyses were performed to determine the correlation between yolk colour and β -carotene levels using the Pearson correlation test at 95% confidence level.

Results and discussion

β -carotene content

Figure 2A shows the β -carotene content of the different egg yolks in the present work. Guinea fowl yolks presented significantly greater (p < 0.001) β -carotene concentrations, approximately 6.75 and 6.94

times greater than those of chicken and quail egg yolks, respectively. These findings corroborated previous works by Onyenweaku *et al.* (2018) who also reported higher vitamin A levels in guinea fowl yolks than in chicken, quail, or turkey yolks. Environmental factors such as feed materials and weather conditions likely contributed to this variation in β -carotene content (Minh *et al.*, 2006; Franco *et al.*, 2020; Goto *et al.*, 2022).

As shown in Figure 2B, the β -carotene levels in semi-intensively raised chicken and guinea fowl yolks were approximately three times greater than those in intensively raised chicken yolks. This difference could be attributed to the carotenoid profile of the bird diets. Semi-intensively raised birds have ready access to green leaves and forage materials, which are rich sources of carotenoids, xanthophylls, and vitamin A precursors (Hammond and Renzi, 2013; Zaheer, 2017). In contrast, birds from intensive systems rely solely on feed provided by poultry farmers for their carotenoid supply. These results were aligned with findings from Karadas *et al.* (2006).

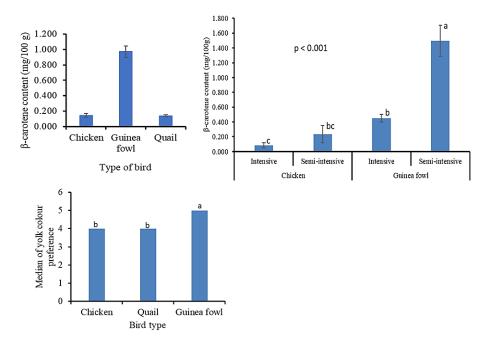


Figure 2. β -carotene contents of egg yolks based on bird type (**A**), rearing system (**B**), and visual preference for egg yolk colour (**C**). Error bars indicate standard deviations. Columns with different lowercase letters are significantly different.

Moreover, guinea fowl yolks from intensive systems had twice the β -carotene content of chicken yolks from semi-intensive systems, possibly due to differences in feed type and production system (Yenice *et al.*, 2016; Bertoncelj *et al.*, 2019; Ortiz *et al.*, 2019; De Souza *et al.*, 2019), as birds are unable to produce carotenoid pigments on their own (Karadas *et al.*, 2006). However, genetic variations in carotenoid accumulation, metabolism, and yolk colour among guinea fowl and chicken could be underlying causes.

Guinea fowl generally require more dietary protein than chicken and quail (Amoah et al., 2018); these proteins play a crucial role in facilitating the transport and absorption of carotenoids from the intestines to the bloodstream (Reboul, 2013). Guinea fowl naturally stand out in their dietary choices as they consume a more varied and carotenoid-rich diet (Prinsloo et al., 2007), especially when they are in free-range systems or have access to additional forage and water, which contributes to an increased intake of carotenoids. By coupling dietary protein with a unique digestive system, these birds can efficiently absorb, extract, and incorporate higher amounts of carotenoids into their tissues and eggs (Karadas et al., 2005). In summary, guinea fowl yolks exhibited higher levels of β -carotene than chicken and quail volks due to their diverse diets, efficient metabolism, and genetic variations among the different bird species, which collectively enhanced β-carotene levels in guinea fowl yolks.

The yolk β -carotene content of eggs on the Ghanaian market is lower than those reported in Nigeria (6.00 - 8.00 mg/100 g; Okonkwo, 2009) and European (0.75 - 8.70 mg/100 g; Bovšková *et al.*, 2014) markets. Therefore, our findings suggested that the nutritional content of eggs is closely tied to the composition of feed materials.

Visual evaluation

Yolk colour is a crucial factor determining egg quality for consumers because it is a strong indicator of nutritional content, including vitamins and their precursors (Bertoncelj *et al.*, 2019; Ortiz *et al.*, 2019; De Souza *et al.*, 2019). Eggs with deeply hued yolks are associated with higher levels of these essential nutrients. Accordingly, consumers showed a preference for guinea fowl yolks (Figure 2C) due to their rich, deep yellow colour (Ayim-Akonor and Akonor, 2014; De Souza *et al.*, 2019).

These findings were in line with those of a comparative study by Mizrak *et al.* (2012) in which more than 81% of 2,241 consumers preferred eggs with deep yellow yolks. In the present work, consumer preference for guinea fowl yolks was significantly different (p < 0.001) from that of chicken or quail yolks, but no significant difference was observed between consumer preference for chicken and quail yolks (Figure 2C).

Colour

Table 1 shows that laying bird type did not significantly (p > 0.05) influence the L* (lightness), a* (redness), or hue of the egg yolks. However, there were noticeable differences in the yellowness (b*) and colour intensity (chroma) of the yolks, with guinea fowl yolks exhibiting values approximately 1.27 and 1.46 times greater than quail and chicken yolks, respectively. Both rearing system (state) and bird type (chicken or guinea fowl) had significant (p < 0.05) effects on the colour profile of the yolks (Table 1). As expected, the yellowness indices followed a pattern similar to that described earlier for the β -carotene content: semi-intensively raised birds displayed more intense yellow colour than birds from intensive-care systems. This observation was attributed to the composition of the feed material, as postulated by Kowalska et al. (2021).

Type of bird		L*	a*	b*	Chroma	Hue
Chicken		$76.79\pm5.16^{\rm a}$	$7.96 \pm 4.50^{\rm a}$	56.16 ± 14.96^{a}	$56.87 \pm 15.05^{\text{b}}$	981.59 ± 3.89^{a}
Guinea fowl		74.49 ± 8.48^{a}	$11.73\pm9.59^{\rm a}$	82.18 ± 3.99^{b}	83.57 ± 2.46^a	81.75 ± 3.99^a
Quail		73.79 ± 5.34^{a}	$9.89\pm9.68^{\rm a}$	64.86 ± 5.69^{a}	$66.19\pm6.57^{\rm c}$	81.66 ± 7.71^{a}
p-value		0.248	0.297	<0.001	<0.001	0.997
Bird type	Rearing system	L*	a*	b*	Chroma	Hue
Chicken	Intensive	$80.44 \pm 1.35^{\rm a}$	$6.98 \pm 1.43^{\text{b}}$	$45.21\pm7.53^{\rm c}$	$45.79\pm7.38^{\rm c}$	80.92 ± 2.52^{ab}
	Semi-intensive	71.31 ± 3.57^{b}	9.45 ± 6.79^{b}	72.58 ± 3.40^{b}	73.49 ± 3.31^{b}	82.59 ± 5.32^{ab}
Guinea fowl	Intensive	71.17 ± 10.87^{b}	$17.62\pm10.90^{\rm a}$	79.51 ± 4.49^{ab}	$82.09 \pm 1.58^{\text{a}}$	77.44 ± 7.93^{b}
	Semi-intensive	77.20 ± 4.25^{ab}	5.84 ± 0.42^{b}	84.84 ± 2.39^{a}	85.05 ± 3.37^{a}	86.06 ± 0.38^a
p-value		<0.001	<0.001	<0.001	<0.001	0.027

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Values are mean \pm standard deviation. Means in the same column with different lowercase superscripts are significantly different (p < 0.05).

earlier In studies (Seemann, 2000; Hammershøj et al., 2010), researchers showed a strong association between egg pigmentation and the type of feed consumed rather than genetic makeup and breed. Therefore, ensuring a well-balanced diet that contains appropriate amounts of fats, proteins, and other essential nutrients is crucial because it enhances the absorption and utilisation of carotenoids (Westphal and Böhm, 2015). All birds in the present work were fed supplementary items (i.e., spinach, marigold petals, red paprika, forage crops, and other green plants) in addition to their primary feed (i.e., maize, sorghum, wheat, rice, soybean, wheat bran, and rice bran). These supplements were excellent sources of xanthophylls known to yield deeper hues of yolk yellowness than cereal diets (Karadas et al., 2006; Hammershøj et al., 2010; Kljak et al., 2021). However, yolks from semi-intensively raised chicken and guinea fowl had deeper coloured yolks because the birds were free-roaming, and complemented their diets with insects and worms. The ingested insects and worms provided the complete protein profiles essential for optimal bird growth and development (Rumpold and Schlüter, 2015), arguably surpassing nutrition provided by conventional meat sources (Payne et al., 2016). A wide array of essential amino acids is necessary for protein synthesis, and due to the increased carotenoid intake from the consumption of insects, worms, and additional forage materials, the birds efficiently absorbed carotenoids through the intestine, and transported them directly to the bloodstream. Consequently, the yolks of these birds took on a deeper yellow colour, signifying greater nutritional value and greater visual appeal to Ghanaian consumers.

The colour values recorded in the present work were consistent with the ranges reported for chicken yolks by Kljak *et al.* (2012) and the a* values reported by Dvořák *et al.* (2010), but notably higher than the results of Aygün and Olgun (2019) for chicken and quail yolks. In the study conducted by Aygün and Olgun (2019), birds were strictly housed in cages, and fed daily rations of 17 - 20% HP with no additional carotenoid supplementation noted. However, poultry production is important in Ghana, as it serves as a major source of income for individuals with limited formal education. It is widely practiced on different scales, depending on capital and expansion opportunities. Therefore, the demand for vibrant volks in Ghanaian poultry markets likely prompted local producers prioritise carotenoid to supplementation to enhance yolk colour. Owing to sustainable integrated crop-livestock operations, farmers had access to abundant waste forage materials from crop farming, which served as a costeffective source for supplementing carotenoids in poultry feed. The presence of essential macronutrients such as nitrogen, phosphorus, and potassium, as well as micronutrients such as magnesium, zinc, and iron in the soil due to farming practices, soil composition, and varying climatic and geographical factors, may have contributed to increased carotenoid production in the forage materials fed to Ghanaian poultry. In essence, the need to meet consumer expectations for visually appealing yolks led to a focus on consistent carotenoid supplementation to maintain market relevance in the poultry industry.

The inclusion of carotenoids in avian poultry diets has a significant impact on overall bird health and egg quality (Langi et al., 2018). Modification of yolk colour through carotenoid supplementation primarily depends on the concentration, source, and ratio of supplemental feed material (Kljak et al., 2021). Adequate sunlight exposure further facilitates the transformation of provitamin A carotenoids (such as β -carotene) into more potent forms (Toomey and McGraw, 2016), such as retinol (vitamin A), in birds. Therefore, the β -carotene levels in quail yolks may be lower because farmers do not consistently provide them with sufficient supplemental materials with carotenoids, and actively deprive the birds of sunlight. These factors likely contributed to the less intense yellow colour observed in quail yolks compared to guinea fowl yolks in general. Other significant factors that influence carotenoid transfer from feed to yolks could also determine carotenoid bioavailability (Sarmiento-Garcia et al., 2023) and the presence of lipid vesicles throughout egg layers (Yunitasari et al., 2022).

Correlation

Correlation analysis (Table 2) revealed strong positive associations between β -carotene content and redness (a*) (r = 0.857; p = 0.345) and yellowness (b*) (r = 0.943; p = 0.216). Approximately 73 and 89% of the variability in β -carotene was explained by its association with redness and yellowness,

Parameter	L*	a*	b*	β-carotene	Preference
L*	1	-0.807	-0.671	-0.386	-0.594
a*	-0.807	1	0.979	0.857	0.955
b*	-0.671	0.979	1	0.943	0.995
β-carotene	-0.386	0.857	0.943	1	0.972
Preference	-0.594	0.955	0.995	0.972	1

Table 2. Correlation matrix between β -carotene content and colour.

respectively. This indicated that yolk redness (a*) and yellowness (b*) were directly related. However, as expected, the association between β -carotene and lightness (L*) was negative and weak (r = -0.386; p =0.747), with only 15% of the variation explained. Kljak et al. (2012) also reported similar associations between carotenoid content and these colour parameters. Regarding yolk colour preference, strong positive associations were observed with redness (r =0.955; p = 0.193, yellowness (r = 0.995; p = 0.064), and yolk β -carotene content (r = 0.972; p = 0.152) in the present work. These findings suggested that approximately 91, 99, and 94% of the variations in consumer preference for egg yolks were explained by their association with redness, yellowness, and the β carotene content of the egg yolk, respectively. However, there was a moderately negative correlation between yolk colour preference and lightness (r = -0.594; p = 0.595) in the present work, which accounted for only 35% of the variation in yolk preference.

Colour chart

Hues and appearance play vital roles for consumers, as consumers often make assumptions about the nutritional quality of eggs based on the colour of the yolks. The human eye cannot determine nutrient levels in raw egg yolks (laboratory analysis is needed for this), let alone accurately discern specific β -carotene contents. To improve consumer communication and understanding, standardised colours are necessary; since verbal descriptions such as "deep yellow" or "light yellow" can be ambiguous, the colour chart was developed (Figure 3) to empower ordinary consumers who may lack expertise in food analysis to recognise varying β -carotene levels by comparing yolk hues to the chart.

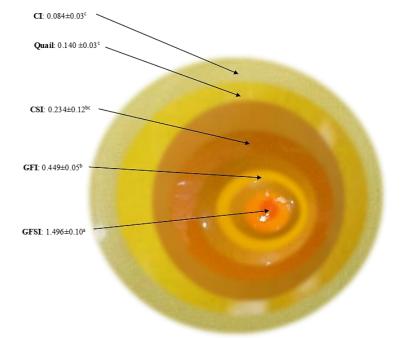


Figure 3. Colour chart showing bird type and corresponding β -carotene value (mg/100 g). CI: chicken (intensive); CSI: chicken (semi-intensive); GFSI: guinea fowl (semi-intensive); and GFI: guinea fowl (intensive).

Although this colour chart could be a good proxy for the carotenoid level at purchase points, its accuracy must be validated in field studies. This will be considered in a follow-up study. In summary, after comparing consumer preferences for β -carotene and colour parameters, we can conclude that the common notion among everyday consumers that yellow yolks are more nutritious than pale yellow yolks was justified. The low β -carotene content of yolks in the present work could be the reason for the low consumption of eggs in Ghana. To address this, Ghanaian poultry farmers are encouraged to supplement laying bird diets with carotenoid-rich foods such as green leaves, orange-fleshed sweet potato feed, and other nutritious pigmented feeds, as feed significantly contributes to yolk hue intensity. This approach could ensure that the yolks meet consumers' colour preferences. Furthermore, eggs from intensively reared quail did not have substantially higher β -carotene contents, so Ghanaian regulatory agencies should ensure the strict regulation of feed quality to guarantee the production of nutritious eggs.

Limitations of the study

It was difficult to obtain eggs from quail raised extensively or semi-intensively, which prevented direct comparisons with intensively raised quail yolks. Additionally, data on the nutritional composition of feed in various poultry farms during the sampling phase was lacking; therefore, assumptions were made based on existing scientific studies.

Conclusion

Guinea fowl egg yolks exhibited the deepest yellow hues and highest β -carotene contents among the bird types. Compared with intensive rearing systems, semi-intensive rearing systems produced yolks with deeper yellow colours, and greater β carotene contents. Strong positive correlation was observed between yolk colour (yellowness and redness), β -carotene content, and consumer preference. Therefore, to increase egg yolk β carotene content and consumer demand, efforts should be made to enhance yolk yellowness. A colour chart was developed to help consumers visually match yolk colour and β -carotene content, but this must be validated in future studies before implementation.

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