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Research Article
Bixa orellana Grains and *Curcuma longa* Rhizomes Powders for *Coturnix coturnix japonica* Egg Yolks' Coloration

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Abstract
Background and Objective: Because of a severe yellow corn shortage in Côte d'Ivoire in 2021, the study assessed *Bixa orellana* (B.o) grains and *Curcuma longa* (C.l) rhizomes powder effect on *Coturnix coturnix japonica* hens egg yolks colouration. **Materials and Methods:** First, two control diets were made of yellow corn (YC) and white corn (WC). Additionally, 10 diets were made by enriching the white corn-based diet with B.o grains and C.l rhizomes powders, at different incorporation rates. Second, three hundred sixty *Coturnix coturnix japonica* laying hens, 51 weeks old weighing 227.68±16.68 g, were randomly allotted to the 12 diets, with 10 birds in each, with 3 replications. Following, on the last day of week 8 (59 weeks old), the eggs were weighed. Then, three eggs of similar weights per diet were collected for analysis. To end, the eggs and their constituents were weighed and the yolks' colours were assessed through L*, a* and b* measurements. **Results:** First, the best egg weights were obtained with WC+1% B.o, WC+0.15% B.o+0.45% C.l and WC+0.6% B.o diets for 11.31±0.21, 11.10±0.12 and 11.06±0.12 g, respectively. Second, following the YC diet that performed 98.34±061 ($p<0.0001$), white corn-based diets containing natural carotenoid sources best egg yolk yellowness (b*) were obtained with WC+0.75% B.o+0.25% C.l and WC+1% B.o for 90.11±1.21 and 80.55±1.21 ($p<0.0001$). Thirdly, the total cholesterol lowest content with well-coloured egg yolk was obtained with WC+1% B.o for 18.60±3.47 mg per g of fresh yolk. The WC+1% B.o result was far away from that of the YC diet (32.58±1.74 mg/g, $p<0.0001$). **Conclusion:** White corn-based diets can be beneficially enriched with 1% of *Bixa orellana* grains' powders for *Coturnix coturnix japonica* hens egg yolks colouration.

Key words: L*a*b*, total cholesterol, white corn, yellow corn, carotenoids, drought, human nutrition

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

The cake industry consumes a lot of eggs because the eggs' yolks' carotenoids influence the overall cake colour[1]. So, due to their important carotenoid content coupled with an important antioxidant property, many plant-coloured grains are added to poultry feed[2]. Thus, the grains' carotenoids colour the egg yolk, improve the hens' health and allow enhancing the cakes' colour[2]. The egg yolk colouration concept with diverse carotenoids source is supported by many works. For example, the pigment type and its density severely impact the egg yolk colour intensity[3,4]. In laying hens' diets, yellow corn is used for two reasons. It is used as a natural carotenoid and energy source. Competition arises between laying hens and human beings because this yellow corn is recommended for human nutrition. In addition, in West African countries the drought causes unpredictable crop yields. Unfortunately, the yields are decreasing, while the population is increasing in each living place. For example, in Côte d'Ivoire, a severe yellow corn lack in 2021 induced a high purchasing cost. The cost of a bag of 120 kg yellow corn moved from 12000-35000 Fr. CFA, meaning almost a 3 folds increase. Fortunately, there are many other native bowls of cereal such as sorghum, millet and brown and white corn. But, because of the lack of carotenoids, these local agricultural products' incorporation in laying hens' diets is limited. So, the hypothesis of this work was that "a white corn-based diet may be associated with *Bixa orellana* grains and/or *Curcuma longa* rhizomes powders and achieve the same results as a yellow corn-based diet for egg yolk colouration". So, the subsequent objective was the assessment of the effect of *Bixa orellana* grains' powder and *Curcuma longa* rhizomes powder on the egg yolk colouration with a white corn-based diet. Moreover, an associated objective was to follow up on the induced total cholesterol content in these egg yolks.

MATERIALS AND METHODS

Experimental site situation, *Coturnix coturnix japonica* and treatments: The experiment was carried out in Côte d'Ivoire (Ivory Coast), at National Polytechnic Institute Félix Houphouet-Boigny of Yamoussoukro (INP-HB). Specifically, the experiment was set at the research station of the graduate school of agriculture, at the laboratory of animal science. The experiment lasted from mid-July, to mid-September, 2022. Twelve diets were made for laying *Coturnix coturnix japonica* (Table 1). Namely, two reference diets within were a good reference made of yellow corn (YC) and a bad reference made of white corn (WC). So, the good reference had some carotenoids provided by the yellow corn, while the bad reference had no carotenoids from the white corn. Following, some combinations were made with *Bixa orellana* (B.o) grains and some *Curcuma longa* (C.l) rhizome powders. Furthermore, 10 layings of *Coturnix coturnix japonica*, 51 weeks-old with 227.68±16.68 g average weight, were fed on a given diet. There were three replicates, so, we had 30 layings of *Coturnix coturnix japonica* per diet. Thus, 360 laying *Coturnix coturnix japonica* were used in total. The adaptation period lasted a week during which the birds were fed on mixed transition diets. Thereafter, the birds received test diets for 7 additional weeks. At the end of week 8, the egg yolk colourations were assessed by evaluating their lightness (L*) and yellowness (b*)[4]. In addition, the total cholesterol contents were assessed according to the diets[5]. The 10 quails were placed in cages measuring 0.5×0.5×1 m (width×height×length). Moreover, the cages were made of wooden supports and covered with nets of 3 cm squared openings. The cage bottom was 0.5 m above the ground. Daily, each quail received 35 g of feed. Also, the drinking water was *ad-libitum* available. Each animal's group had 3 replicates. The daily ratio was distributed half at 8 AM and the second half was given at 4 PM. No lighting program management was set. Thus, the quails had the sunshine light and were in the dark during the night.

***Bixa orellana* grains and *Curcuma longa* rhizome powders:** Some *Bixa orellana* (B.o) grains and *Curcuma longa* (C.l) rhizome were purchased from producers. The grains and rhizomes were cleaned to take off all stones and impurities. Thereafter, *Curcuma longa* rhizomes were dried in an oven at 55°C for 5 days. On the contrary, *Bixa orellana* grains were not dried anymore. Later, the products were crushed in animal feed-making machinery. Immediately, they were used to make the diets for laying *Coturnix coturnix japonica* birds.

Egg selection for analysis: Week 51 was used for the transition feed toward the experimental diets. From week 52 to week 59, the eggs were weighed every day. So, egg weights' mean (μ) was computed on the total eggs laid (x_i) during the 7 weeks (Eq. 1). But, on the last day of week 8 (59 weeks old), each egg was weighed considering the diet. Next, the absolute intervals (I) by subtracting the mean from each weight were computed (Eq. 2) per diet. To end, the 3 eggs that had the smallest interval values (Eq. 3) were selected for egg constituents' assessment, colour (L*, a*, b*) and cholesterols analysis:

$$\mu = \frac{1}{n}\sum_{1}^{n}(x_i) \quad (1)$$

$$I = |x_i - \mu| \quad (2)$$

In addition, during the experiment, no quail dies and the eggs' laying rates were around 86.42%. On average, the number of eggs weighed per diet was 1,176 with the white corn-based diet and 1,279 with each other diet (Eq. 4):

$$n_{wc} \approx \text{Quails-number} \times \text{average-laying-rate} \times 7 \text{ days} \times 7 \text{ weeks} = 1{,}176 \text{ eggs} \quad (3)$$

$$n_{other\text{-}diets} \approx \text{Quails-number} \times \text{average-laying-rate} \times 7 \text{ days} \times 7 \text{ weeks} = 1{,}279 \text{ eggs} \quad (4)$$

Egg yolk weight and colour: After selecting the 3 closest eggs to the average, they were broken and the constituents were weighed. Indeed, the shell and the yolk were weighed. Subsequently, the albumen weight was derived by subtracting albumen, yolk and shell weights from the whole egg weight. Continuing, the yolk colouration was assessed according to the international colourimetric system[4]. So, the colour components (L*, a* and b*) were read on the raw egg yolk with a colourimeter spectrophotometer (Precise Colour Reader, CHN Spec, CS-10, Hangzhou, China). The L* is about lightness, its values have no limit. When L* is positive, the product is bright. But, when L* tends to zero, the product tends to be black.

Egg yolk's total cholesterol contents: In the beginning, the raw egg yolk was diluted according to Pasin *et al.*[5] protocol. Afterwards, for each diet, the 3 selected egg yolks were mixed to get a pool. Then, 1 g of raw egg yolk was collected and placed in a 25 mL glass beaker and 9 mL NaCl solution (2%) was added[5]. A bar magnet was immersed in the mixture and it was covered with aluminium paper. The beakers were magnetically stirred for 30 min. From each pool, 3 samples were prepared similarly, thus 3 repetitions. Straightaway, each mixture was transferred to a test glass tube and centrifuged for 2 min to separate the yolk membranes from the liquid. Forthwith, 10 µL from a replicate was collected, put in a 5 mL glass test tube and 1 mL of the cholesterol kit "Cromatest, Linear Chemicals, S.L.U. Spain" was added. This final solution was vortexed and incubated under a shade for 10 min at ambient temperature. Finally, the egg yolk cholesterol contents were determined by reading the optical density at 500 nm wavelength, using a UV 1901 spectrophotometer and the total cholesterol content was computed (Eq. 5):

$$\text{Tot}_{\text{Chol}} \text{ (mg dL}^{-1}) = \frac{\text{Sample\_Abs}}{\text{Standard\_Abs}} \text{ x standard\_concentration} = \frac{\text{Sample\_Abs}}{\text{Standard\_Abs}} \text{ x 200} \quad (5)$$

This total cholesterol content computed in mg dL^{-1} can be converted in mg g^{-1} of egg yolk by timing by 0.1.

Statistical analysis: During the tests, the results were generated in triplicate. Thereafter, for the statistical tests, the results were submitted to an Analysis of Variance (ANOVA), using XLSTAT 2014. The least-squares means were separated according to Newman-Keuls (SNK) multiple range tests in a confidence interval of 95% ($\alpha = 5\%$).

RESULTS AND DISCUSSION

Egg weights: The eggs' weights fluctuated between 11.31±0.21 and 9.61±0.11 g (Table 2), within 3 different groups, following the different superscripts on averages. The egg weight decreased from the highest *Bixa orellana* content diet to the white corn diet. In another word, the egg weight dropped from the most coloured diet to the least coloured one. Notably, the white corn-based (WC) diet led to the worst egg weights with 9.61±0.11 g. Indeed, white corn-based diet egg weights decreased significantly, though the egg weights should positively correlate with the quails' age[6]. Despite an energetic diet of 3.200 kcal kg^{-1}, bearing 24% crude protein (Table 1), coupled with birds of 54 weeks old, the white corn-based diet delivered eggs of 9.61±0.11 g. This weight was close to 9.67±0.08 g, reported for eggs weight from quails of 8 weeks old[6].

On the contrary, the very rich carotenoid diets formed one group. Truly, from a white corn-based diet enriched with *Bixa orellana* (WC+1% B.o) to a yellow corn-based diet (YC), this block had a 10.87±0.15 g average egg weight ($0.0583 \leq p \leq 0.2418$). But this average was less than 12.23±0.27 g with quails of 49 weeks old[7], 12.53±0.18 g with quails of 20 weeks old[8] and 12.88±0.16 g with quails of 45 weeks old[9]. Comparatively, these egg weights were still less than those obtained by Canogullari *et al.*[10], because they announced 13.50-13.62 g when they used some garlic in quails' diets. These egg weight gaps between experiments are due to birds' daily feed, the atmospheric temperature and the quail's age and weight. In addition to the positive correlation between quails' age and their egg weights, Hanafy *et al.*[9] also observed that the diet fat content had a positive correlation with these factors. So, the quails' age and the diet fat content are important factors that significantly affect quails egg

Table 1: Diet composition and their nutritional value

	Diet											
Constituent	YC	WC	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
Yellow corn	55											
White corn	-	55	54	54	54	54	54	54.4	54.4	54.4	54.4	54.4
Bixa orellana	-	-	1	-	0.5	0.75	0.25	0.3	0.6	-	0.15	0.45
Curcuma longa	-	-	-	1	0.5	0.25	0.75	0.3	-	0.6	0.45	0.15
Soya meal	15	15	15	15	15	15	15	15	15	15	15	15
Wheat meal	8	8	8	8	8	8	8	8	8	8	8	8
Fish meal	16	16	16	16	16	16	16	16	16	16	16	16
Egg shell powder	2	2	2	2	2	2	2	2	2	2	2	2
Premix nutri-A	4	4	4	4	4	4	4	4	4	4	4	4
Total	100	100	100	100	100	100	100	100	100	100	100	100
Analysis												
Dry matter (%)	90.00	90.40	94.75	95.28	93.68	95.40	95.45	91.34	91.19	90.10	92.04	91.84
Fat (%)	9.81	8.20	5.65	6.25	6.20	8.52	7.57	9.02	6.14	8.49	11.20	9.71
Protein (%)	23.89	24.53	22.40	22.90	22.23	22.95	22.48	22.32	22.57	22.52	22.17	22.00
Tot_Carb (%)	36.60	38.46	59.08	57.40	58.75	55.78	57.62	56.00	58.51	56.44	53.63	55.63
M E. (kcal/kg.DM)	3,253.24	3,220.45	3,128.74	3,131.18	3,158.61	3,264.45	3,239.16	3,298.78	3,153.43	3,275.01	3,392.98	3,335.52

B.o: *Bixa orellana*, C.l: *Curcuma longa*, YC: Yellow corn, WC: White corn, Diets: YC: Yellow corn, WC: White corn, T_1: WC+1% B.o , T_2: WC+1% C.l , T_3: WC+0.5% B.o+0.5% C.l, T_4: WC+0.75% B.o+0.25% C.l, T_5: WC+0.25% B.o+0.75% C.l, T_6: WC+0.3% B.o+0.3% C.l, T_7: WC+0.6% B.o, T_8: WC+0.6% C.l, T_9: WC+0.15% B.o+0.45% C.l, T_{10}: WC+0.45% B.o+0.15% C.l, M.E. (kcal/kg.DM): Metabolizable energy, Total carbohydrate (Tot_Carb) = 100%-(Protein (%)+Fat (%)+Ash (%)) (FAO, 2003) and M.E. (kcal/kg DM) = [2.44×Protein (%DM)+8.37×Fat (%DM)+3.57×Tot_Carb (%DM)]×10 (FAO, 2003)

Table 2: Eggs weight (g) according to the diet

Diet	μ±SE	Comparison	p-values
(1) WC+1% B.o	11.31 ± 0.21^{a}		
(2) WC+0.15% B.o+0.45% C.l	11.10 ± 0.12^{a}	(1) vs (2)	0.0726
(3) WC+0.6% B.o	11.06 ± 0.12^{a}	(2) vs (3)	0.0583
(4) WC+0.45% B.o+0.15% C.l	10.75 ± 0.12^{ab}	(3) vs (4)	0.0687
(5) WC+0.50% B.o+0.50% C.l	10.73 ± 0.21^{ab}	(4) vs (5)	0.0796
(6) WC+0.30% B.o+0.30% C.l	10.62 ± 0.12^{ab}	(5) vs (6)	0.2418
(7) YC	10.53 ± 0.11^{b}	(6) vs (7)	0.1672
(8) WC+0.6% C.l	10.44 ± 0.12^{b}	(7) vs (8)	0.2271
		(3) vs (8)	0.0097
(9) WC+1% C.l	10.11 ± 0.2^{bc}	(8) vs (9)	0.3414
(10) WC+0.25% B.o+0.75% C.l	10.06 ± 0.21^{bc}	(9) vs (10)	0.1572
(11) WC+0.75% B.o+0.25% C.l	10.03 ± 0.21^{bc}	(10) vs (11)	0.1572
(12) WC	09.61 ± 0.11^{c}	(11) vs (12)	0.1572
		(8) vs (12)	<0.0001
		(3) vs (12)	<0.0001

B.o: *Bixa orellana*, C.l: *Curcuma longa*, YC: Yellow corn, WC: White corn, Diets: YC: Yellow corn, WC: White corn, T_1: WC+1% B.o , T_2: WC+1% C.l , T_3: WC+0.5% B.o+0.5% C.l, T_4: WC+0.75% B.o+0.25% C.l, T_5: WC+0.25% B.o+0.75% C.l, T_6: WC+0.3% B.o+0.3% C.l, T_7: WC+0.6% B.o, T_8: WC+0.6% C.l, T_9: WC+0.15% B.o+0.45% C.l, T_{10}: WC+0.45% B.o+0.15% C.l and ^{a,b,c}: Means bearing different superscripts in the column differ significantly ($p<0.05$)

weights. Nonetheless, atmospheric conditions such as air humidity, airflow speed and temperature influence laying hens' performances by modifying egg weight over time[6]. So, from 12-39 weeks old, the quail laid similar egg weights[6]. Shortly, the first group was followed by the second, with a large covered zone from WC+0.45% B.o+0.15% C.l to WC+0.75% B.o+0.25% C.l diets. But the significant difference between egg weights began with WC+0.6% C.l (10.44±0.12 g) and ended with WC+0.75% B.o+0.25% C.l (10.03±0.21 g). This section shows that high *Curcuma longa* contents (0.6, 0.75, 1%) in white corn-based diets did not greatly influence the egg weights.

Eggs yolks and albumens weights: The yolks' weights displayed 2 distinct groups (Table 3). Surely, well-coloured diets lead to heavy yolks. Oddly, at an equal incorporation rate of *Bixa orellana* and *Curcuma longa*, the mixture led to poor results. For example, with 0.30% B.o+0.30% C.l and 0.50% B.o+0.50% C.l, the egg yolks weighed 3.27±0.09 and 3.24±0.16 g, respectively. Unfortunately, they belong to the poorest group. On the contrary, the white corn-based diets enriched with 0.45% B.o+0.15% C.l, 0.15% B.o+0.45% C.l, 1% B.o, 0.6% B.o, 0.25% B.o+0.75% C.l, 0.6% C.l and 1% C.l formed a homogenous group ($0.2420\leq p\leq 0.9086$). When a white corn-based was enriched by *Bixa orellana* combined

Table 3: Eggs yolk and albumens weight (g) according to the diet

	Yolk (g)			Albumen (g)		
Diet	μ±SE	Comparison	p-values	μ±SE	Comparison	p-values
(1) WC+0.45% B.o+0.15% C.l	3.78±0.09[a]			5.67±0.12[ab]		
(2) WC+0.15% B.o+0.45% C.l	3.76±0.09[a]	(1) vs (2)	0.2534	5.93±0.12[ab]	(1) vs (2)	0.1373
(3) WC+1% B.o	3.65±0.16[ab]	(2) vs (3)	0.2514	6.31±0.21[a]	(2) vs (3)	0.0700
(4) WC+0.6% B.o	3.54±0.09[ab]	(3) vs (4)	0.5015	6.12±0.12[a]	(3) vs (4)	0.0700
(5) WC+0.25% B.o+0.75% C.l	3.50±0.16[ab]	(4) vs (5)	0.2420	5.27±0.21[bc]	(4) vs (5)	0.0277
(6) WC+0.6% C.l	3.42±0.09[ab]	(5) vs (6)	0.7708	5.72±0.12[ab]	(5) vs (6)	0.3493
(7) WC+1% C.l	3.36±0.16[ab]	(6) vs (7)	0.9086	5.38±0.21[abc]	(6) vs (7)	0.3493
(8) YC	3.35±0.08[b]	(7) vs (8)	0.9882	5.84±0.10[ab]	(7) vs (8)	0.2043
		(2) vs (8)	0.0177			
(9) WC	3.28±0.08[b]	(8) vs (9)	0.9882	5.13±0.10[c]	(8) vs (9)	0.0003
(10) WC+0.30% B.o+0.30% C.l	3.27±0.09[b]	(9) vs (10)	0.9882	5.81±0.12[ab]	(9) vs (10)	0.0012
(11) WC+0.50% B.o+0.50% C.l	3.24±0.16[b]	(10) vs (11)	0.9882	6.06±0.21[ab]	(10) vs (11)	0.1846
(12) WC+0.75% B.o+0.25% C.l	3.22±0.16[b]	(11) vs (12)	0.9882	5.53±0.21[abc]	(11) vs (12)	0.1846

B.o: *Bixa orellana*, C.l: *Curcuma longa*, YC: Yellow corn, WC: White corn, Diets: YC: Yellow corn, WC: White corn, T_1: WC+1% B.o , T_2: WC+1% C.l , T_3: WC+0.5% B.o+0.5% C.l, T_4: WC+0.75% B.o+0.25% C.l, T_5: WC+0.25% B.o+0.75% C.l, T_6: WC+0.3% B.o+0.3% C.l, T_7: WC+0.6% B.o, T_8: WC+0.6% C.l, T_9: WC+0.15% B.o+0.45% C.l, T_{10}: WC+0.45% B.o+0.15% C.l and [a,b,c]: Under the same item (Yolks or Albumens), means bearing different superscripts in the column differ significantly (p<0.05)

with *Curcuma longa* around 0.6% altogether, the absorption of the carotenoids was better than that of the yellow corn-based diet. While WC+0.45% B.o+0.15% C.l and WC+0.15% B.o+0.45% C.l allowed 3.78±0.09 and 3.76±0.09 g, respectively, the yellow corn-based got 3.35±0.08 g. So, the yellow corn-based diet output was significantly lower (p = 0.0177).

The small egg yolks from the YC diet compared to those from 0.45% B.o+0.15% C.l, 0.15% B.o+0.45% C.l, 1% B.o, 0.6% B.o, 0.25% B.o+0.75% C.l, 0.6% C.l and 1% C.l follow Skřivanová *et al.*[11] remarks. They reported that Lutein retention in egg yolks is better than that of zeaxanthin. Remarkably, these two carotenoids remain the most important in egg yolk[11,12]. Also, increasing the natural carotenoid source incorporation rate in the diet leads to a decrease in the yolks' weights. So, the egg yolk weights tend to be smaller alongside an increasing dietary carotenoid content elevation in the diets[4,12]. Herein the experiment, from WC+0.6% C.l-WC+1% C.l, the egg yolk weights dropped from 3.42±0.09-3.36±0.16 g, respectively. Of course, this gap was not significant (p = 0.9086) but represented 1.75% weight loss. Similarly, when Isa Brown hens' diets were supplemented with 10 and 20% of *Borassus aethiopum* dried pulp, the yolk weights dropped from 14.16-13.91 g[4], thus 1.76% weight loss. Again, when 1 and 2% Chlorella were used to supplement hens' diets, the yolks' weights decreased from 17.3-16.6 g, thus 4.05% weight loss[12].

Though the hens fed on white corn-based diets enriched by 0.50% B.o+0.50% C.l and 0.30% B.o+0.30% C.l gave big eggs (Table 3), the derived egg yolks were small, putting them at the queue. For instance, in this order, the egg yolk weight averages were 3.24±0.16 and 3.27±0.09 g, respectively. These light egg yolk weights were compensated by relatively heavy albumen for 6.06±0.21 and 5.81±0.12 g, respectively. Alongside, these results revealed that the correlation coefficient (r) between the egg weights and their corresponding yolk weights and albumen weights were 66.60 and 79.70%, respectively. These tendencies were alike Seker[13] observations. Similarly, Seker[13] announced 0.94, 0.77 and 0.61 for the correlation coefficients between the egg weights and albumen, yolk and shell weights, respectively. So, the big eggs don't surely give relatively big yolks because, in a linear correlation, the coefficient is not good ($R^2 \leq 0.70$)[13]. Nonetheless, yolk and albumen are both equally important in cake making. For instance, Hwang *et al.*[1] showed that the uses of chicken, duck and ostrich eggs led to different foaming for sponge cakes. These eggs differ in egg weight, yolk colour and albumen quantities[6]. Just the yolk colour made a difference in the cake's colour, but the foam stability depends on both yolk and albumen[6].

Eggs yolks yellowness (b*) and lightness (L*): On an overall view, a high egg yolk yellowness (b*) value is followed by a low lightness (L*) one. Moreover, the yellowness level depends on the carotenoid type and its content in the yolk. Comparatively, when a yolk is densely coloured, its yellowness level is high, while its associated lightness is low. The reference diets, good and bad, were made of 55% yellow and white corn, respectively. While the yellow corn contains some zeaxanthin, the white corn has no carotenoid. The colouration tests showed that, while the white corn-based diet (WC) gave a very bad yellowness (b* = 45.31, Table 4). The YC diet gave the best yellowness (b* = 98.34). These two groups' yolk

Table 4: Eggs yolk yellowness (b*) and lightness (L*) according to the diet

	Yellowness (b*)			Lightness (L*)		
Diet	μ±SE	Comparison	p-values	μ±SE	Comparison	p-values
(1) YC	98.34±0.61[a]			80.16±0.09[f]		
(2) WC+0.75% B.o+0.25% C.l	90.11±1.21[b]	(1) vs (2)	<0.0001	80.01±0.17[f]	(1) vs (2)	0.9996
(3) WC+1% B.o	80.55±1.21[c]	(2) vs (3)	<0.0001	80.53±0.17[ef]	(2) vs (3)	0.6084
(4) WC+0.50% B.o+0.50% C.l	78.04±1.21[c]	(3) vs (4)	0.9441	81.03±0.17[de]	(3) vs (4)	0.6544
(5) WC+0.6% B.o	77.56±0.70[c]	(4) vs (5)	1	80.82±0.10[e]	(4) vs (5)	<0.0001
					(2) vs (5)	0.0008
(6) WC+0.45% B.o+0.15% C.l	72.31±0.70[d]	(5) vs (6)	<0.0001	81.01±0.10[e]	(5) vs (6)	0.9751
(7) WC+0.30% B.o+0.30% C.l	68.64±0.70[e]	(6) vs (7)	0.0215	81.52±0.10[d]	(6) vs (7)	0.0286
(8) WC+0.15% B.o+0.45% C.l	63.25±0.70[f]	(7) vs (8)	<0.0001	82.60±0.10[c]	(7) vs (8)	<0.0001
(9) WC+0.25% B.o+0.75% C.l	58.29±1.21[g]	(8) vs (9)	0.0350	83.68±0.17[b]	(8) vs (9)	<0.0001
(10) WC+0.6% C.l	48.87±0.70[h]	(9) vs (10)	<0.0001	84.25±0.10[ab]	(9) vs (10)	0.1916
(11) WC	45.31±0.61[i]	(10 vs (10)	0.0146	84.35±0.09[a]	(10) vs (11)	0.9998
		(1) vs (11)	<0.0001		(9) vs (11)	0.0451
(12) WC+1% C.l	40.32±1.21[j]	(10) vs (11)	0.0232	84.88±0.17[a]	(11) vs (12)	0.2452

B.o: *Bixa orellana*, C.l: *Curcuma longa*, YC: Yellow corn, WC: White corn, Diets: YC: Yellow corn, WC: White corn, T_1: WC+1% B.o , T_2: WC+1% C.l , T_3: WC+0.5% B.o+0.5% C.l , T_4: WC+0.75% B.o+0.25% C.l, T_5: WC+0.25% B.o+0.75% C.l, T_6: WC+0.3% B.o+0.3% C.l, T_7: WC+0.6% B.o, T_8: WC+0.6% C.l, T_9: WC+0.15% B.o+0.45% C.l, T_{10}: WC+0.45% B.o+0.15% C.l and [a,b,c,d,e,f,g,h,i,j]: Under the same item (b* or L*), means bearing different superscripts in the column differ significantly (p<0.05)

yellowness was highly different (p<0.0001). Following, the yellowness heavily relied on the carotenoid source and its incorporation rate. After the YC diet, the second class depended on *Bixa orellana* and *Curcuma longa* incorporation rates.

First, a relatively high *Bixa orellana* incorporation rate led to a relatively good yellowness compared to *Curcuma longa*. So that, WC+0.75% B.o+0.25% C.l, WC+1% B.o, WC+0.50% B.o+0.50% C.l and WC+0.6% B.o delivered b* values were 90.11, 80.55, 78.04 and 77.56, respectively. In this order, from the white corn-based diet, b* values were improved by 98.87, 77.77, 72.24 and 71.78%. Just 1% of *Bixa orellana* powder rose b* value from 45.31-80.55. Following Martínez *et al.*[14] work, *Bixa orellana* grains powder can enhance eggs' yolks yellowness tendencies. But, instead of reinforcing a yellow corn-based diet[14], *Bixa orellana* grains powder can deliver well-coloured egg yolks with a white corn-based diet at a 0.6% incorporation rate.

Secondly, without any doubt, increasing *Curcuma longa* rhizome powder incorporation rates did not improve egg yolk yellowness. On the contrary, increasing *Curcuma longa* rhizome powder incorporation rates decreased the yellowness tendency. Coupled with the egg yolks' yellowness decrease, their lightness significantly rose. So, regarding carotenoid concentration, b* and L* values were inversely proportional. By the way, mixing 1% of *Curcuma longa* rhizome powder in a 54% white corn-based diet, the egg yolks' yellowness worsens. In this case, from WC to WC+1% C.l, egg yolk yellowness significantly dropped from 45.31-40.32 (p = 0.0232), respectively.

Looking at the egg yolk lightness, the diet WC+0.6% C.l, WC and WC+1% C.l delivered similar results. Indeed, L* values were 84.25, 84.35 and 84.88, respectively ($0.2452 \leq p \leq 0.9998$). Bearing poor b* results, these three diets had the best L* outputs group and their eggs' yolks' overall colour was similar (Table 4). Similarly, when Mirbod *et al.*[15] added *Curcuma longa* powder to laying hens' diets, the egg yolk colour was barely improved. Though the yolk colour was enhanced, increasing *Curcuma longa* in Hy-line laying hens' diets led to a stronger eggshell.

Egg yolks' cholesterol content: Remarkably, from the yellow corn-based diet to the white corn-based diet, the total cholesterol content decreased from 32.58±1.74-26.02±1.74 mg g^{-1} of egg yolk, a reduction of 6.56 mg g^{-1}, representing 20.13% loss (Table 5). Instead, this gap was not significantly different, so neither least square averages were statistically different (p = 0.0760). Thereafter, while a natural carotenoid source (*Bixa orellana* and *Curcuma longa*) was mixed in the white corn-based diet, the mixture allowed more total cholesterol reduction compared to the yellow corn-based and white corn-based diets. In an overall view, two groups were observed on cholesterol contents. Admittedly, the incorporation of low natural carotenoid sources (0.6%) led to a very high total cholesterol content in egg yolks. While the incorporation of a 1% natural carotenoid source in a white corn-based diet led to low total cholesterol contents. The first group covered the interval from YC to WC+0.75% B.o+0.25% C.l diets. Correspondingly, the total cholesterol content declined from 32.58±1.74-20.71±3.47 mg g^{-1}.

Table 5: Total cholesterol contents (mg) per gram of egg yolk

Diet	μ±SE (mg g^{-1})	Comparison Diet	gap	p-value
(1) YC	32.58±1.74[a]			
(2) WC	26.02±1.74[ab]	(1) vs (2)	6.56	0.0760
(3) WC+0.45% B.o+0.15% C.l	25.30±2.00[ab]	(2) vs (3)	0.72	0.7888
(4) WC+0.25% B.o+0.75% C.l	23.82±3.47[ab]	(3) vs (4)	1.48	0.7888
(5) WC+0.15% B.o+0.45% C.l	23.80±2.00[ab]	(4) vs (5)	0.02	0.7888
(6) WC+0.30% B.o+0.30% C.l	23.62±2.00[ab]	(5) vs (6)	0.18	0.7888
(7) WC+0.6% C.l	23.06±2.00[ab]	(6) vs (7)	0.56	0.7888
(8) WC+0.6% B.o	22.70±2.00[ab]	(7) vs (8)	0.36	0.7888
(9) WC+0.75% B.o+0.25% C.l	20.71±3.47[ab]	(8) vs (9)	1.99	0.7888
(10) WC+1% C.l	19.73±3.47[b]	(9) vs (10)	0.98	0.7888
		(1) vs (10)	12.85	0.0477
(11) WC+0.50% B.o+0.50% C.l	19.14±3.47[b]	(10) vs (11)	0.59	0.7481
		(1) vs (11)	13.44	0.0374
(12) WC+1% B.o	18.60±3.47[b]	(11) vs (12)	0.54	0.7073
		(1) vs (12)	13.98	0.0297

B.o: *Bixa orellana*, C.l: *Curcuma longa*, YC: Yellow corn, WC: White corn, Diets: YC: Yellow corn, WC: White corn, T_1: WC+1% B.o , T_2: WC+1% C.l , T_3: WC+0.5% B.o+0.5% C.l , T_4: WC+0.75% B.o+0.25% C.l, T_5: WC+0.25% B.o+0.75% C.l, T_6: WC+0.3% B.o+0.3% C.l, T_7: WC+0.6% B.o, T_8: WC+0.6% C.l, T_9: WC+0.15% B.o+0.45% C.l, T_{10}: WC+0.45% B.o+0.15% C.l, μ±SE: mean± Standard Error and [a,b]: Means bearing different superscripts in the column differ significantly ($p<0.05$)

Despite an 11.87 mg decrease of total cholesterol per gram of egg yolk, representing a 36.43% reduction, this interval constituted one group ($0.0760\leq p\leq 0.7888$). However, the WC+0.25% B.o+0.75% C.l diet, containing 1% of natural carotenoids source was among the first group, disrupting the tendance. Anyhow, the incorporation of 0.75% C.l may be responsible for this increase in total cholesterol content. Finally, the second group was composed of three diets statistically different from the yellow corn-based diet. Notably, WC+1% C.l, WC+0.5% B.o+0.5% C.l and WC+1% B.o diets, whose total cholesterol contents were 19.73±3.47, 19.14±3.47 and 18.60±3.47 mg g^{-1} of egg yolk, significantly induced the total cholesterol contents compared to YC diet. Thus, in this same order, the cholesterol contents were notably lessened by 12.85, 13.44 and 13.98 mg g^{-1}, (p = 0.0477, 0.0374 and 0.0297). Singularly, the supplementations between 0.5 and 1% of *Bixa orellana* powders in white corn-based diets were associated with very good egg yolks colours (Table 4, b* values). Still, the WC+1% B.o diet delivered the lowest total cholesterol content. Similarly, Canogullari *et al.*[10] obtained the lowest egg yolk cholesterol content (15.49 mg g^{-1}) by adding 4% of garlic to the diet. Admittedly, herein results (WC+0.6% C.l for 23.06±2.00 and WC+0.6% B.o for 22.70±2.00 mg g^{-1}) were similar to their outputs at 1 and 2% diets supplementation with garlic, 23.30 and 20.35 mg g^{-1}, respectively (Canogullari *et al.*[10]). Also, Mirbod *et al.*[15] obtained significant total cholesterol reduction in egg yolk, after adding 0.25 and 0.5% of *Curcuma longa* powder in the diets.

Good dietary cholesterol sources such as natural carotenoid sources are very important ingredients in the human body. Because our health depends on what we eat, a good diet is a primordial issue. For example, according to Tziakas *et al.*[16], red blood cell membranes are 1.5-2 times richer in cholesterol compared with any other cell in the body. These cells are the main transporters of Dioxygen (O_2), Dioxide of Carbon (CO_2) and nutrients all over the body. Conversely, all patients at risk of vascular disease should carefully consume eggs. Indeed, these persons should not consume more than 200 mg of total cholesterol per day[17]. So, combining the human body's needs and its health regarding cholesterol consumption, egg yolks with low total cholesterol content are required. Moreover, dietary cholesterol through natural carotenoid sources is essential for animal offspring's immunology resistance and their future growth[18]. As an illustration, carotenoid supplementation of hens' diets strengthened *Coturnix coturnix japonica* offspring growth and their immune resistance[18].

Due to the yellow corn shortage in Côte d'Ivoire and the competition for this ingredient for laying hens feed and human nutrition, many other available colourless grains could be used. So, in the aim to get a well-coloured egg yolk and considering all patients at risk of vascular disease, some natural carotenoid sources such as *Bixa orellana* grains could be used. In fact, by mixing 1% of *Bixa orellana* grains in any colourless grains (white corn, *Sorghum* sp., grains and millets), the egg yolk will be improved, making this egg yolk acceptable by cake makers and take care of all patients at risk of vascular disease.

CONCLUSION

The experiment aimed to find some alternatives to the yellow corn shortage in Côte d'Ivoire, by using available natural carotenoid sources such as *Bixa orellana* grains and

Curcuma longa rhizomes. So, yellow corn based-diet and white corn-based diets were made. Reconsidering the white corn-based diet, it was enriched with some *Curcuma longa* rhizome and *Bixa orellana* grains powders, at different incorporation rates. After 7 weeks of experiment with 360 laying *Coturnix coturnix japonica*, it can be concluded that *Bixa orellana* grains are better than *Curcuma longa* rhizomes for egg yolk colouration. Better, using 1% of *Bixa orellana* grain powder significantly coloured the egg yolk and importantly decreased the egg yolk's total cholesterol content.

SIGNIFICANCE STATEMENT

The study discovered that while using colourless grains such as white corn in *Coturnix coturnix japonica* laying hens' diets, adding some *Bixa orellana* grains' powder and *Curcuma longa's* rhizome powder can affect the egg yolk colour and their total cholesterol content. Specifically, without natural carotenoids in grains, *Bixa orellana* grains powder is better than that *Curcuma longa* rhizome for yolks yellowness improvement. Furthermore, adding 1% of *Bixa orellana* powder to the diet significantly decreases egg yolk total cholesterol. So, colourless grains such as millet, *Sorghums* and white corn can be used in *Coturnix coturnix japonica* laying hens' diets, by adding 1% *Bixa orellana* grains powder to maintaining good egg yolk yellowness and reducing its total cholesterol content.

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