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RESEARCH ARTICLE

EVALUATION OF EGG YOLK QUALITY IN LAYING HENS FED DIETS BASED ON *HIBISCUS SABDARIFFA* FLOWERS

Atchadé Ghislaine Sègbédji Théodora, Behingan Boris Milognon, Houndonougbo Mankpondji Frédéric, Chrysostome Christophe Archille Armand Mahussi and Mensah Guy Apollinaire

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Abstract

This study evaluated the pigmenting effect of *Hibiscus sabdariffa* flowers on egg yolk coloration and laying performance in hens. Ninety-six ISA-Brown hens, 50 weeks old, housed in pairs in Californian-type cages, were evenly allocated into four groups of 24 birds each corresponding to four dietary treatments: a negative control (R0, no pigment), a positive control (RJ, synthetic pigment), a diet supplemented with dried *Hibiscus* flowers (RF), and a diet combined with an aqueous extract of *Hibiscus* flowers (RE). Results showed that RJ produced the highest yolk color score (9.4), followed by RF (7.6), while RE (3.5) did not differ significantly ($p > 0.05$) from R0 (2.0). In addition to pigmentation, supplemented diets improved laying rate (82.7-87.4 %) compared with R0 (76.8 %) and reduced Feed Conversion Ratio (2.09-2.19 vs. 2.36). Economic analysis revealed that RF achieved an intermediate feed cost (30.4 FCFA/egg) and satisfactory efficiency, whereas RE was the most expensive (32.8 FCFA/egg) and least efficient. These findings indicate that *Hibiscus sabdariffa* flowers, when incorporated into diet, represent a sustainable natural alternative to synthetic pigments, enhancing yolk quality and supporting economic viability in laying hen production.

Introduction:-

Egg yolk color is a major quality criterion for consumers and strongly influences market acceptability. Although it does not directly affect the nutritional value of the egg, this characteristic depends essentially on the diet of laying hens. Yolk pigmentation is mainly provided by carotenoids, particularly xanthophylls such as lutein and zeaxanthin, which must be supplied through feed (Blount *et al*., 2000; Zongo *et al*., 1997). Therefore, the incorporation of pigmenting ingredients into laying hen diets is a common practice aimed at improving the visual quality of eggs. Several studies have highlighted the interest of using natural pigments (Zongo *et al*., 1997; Kang *et al*., 2003; Magnin *et al*., 2009; Aureli *et al*., 2009; Kijparkorn *et al*., 2010; Kljak *et al*., 2012). Among the reported sources, Sauveur (1988) distinguished raw materials with varying pigment concentrations, such as alfalfa meal (250 mg/kg), yellow maize (20-25 mg/kg), algae (2200-4000 mg/kg), *Leucaena leucocephala* leaves (440-660 mg/kg), marigold and tagetes petals (6000-10,000 mg/kg), as well as kola, palm oil, and sorghum. Recent research has expanded the range of natural pigment sources. Matache *et al*. (2024) demonstrated that marigold and paprika extracts significantly increased yolk pigmentation (DSM YolkFan™ scores >12) and improved oxidative stability during

Corresponding Author:-Atchadé Ghislaine

storage, highlighting their dual role as colorants and antioxidants. Similarly, Anam *et al*. (2025), through a meta-analysis of 36 studies, confirmed that microalgae supplementation enhanced yolk pigmentation, enriched eggs with n-3 fatty acids (EPA and DHA), and improved laying performance, although economic feasibility varied depending on species and inclusion level. These findings emphasize the growing interest in natural pigments that combine consumer appeal with functional benefits. However, in many Sub-Saharan African countries, the use of synthetic pigment additives remains limited due to their high cost, linked to importation (Zongo *et al*., 1997). Feed accounts for up to 70 % of total production costs in poultry farming (Omole *et al*., 2005). In this context, the search for alternative local sources, available at low cost and capable of ensuring effective yolk pigmentation, is a major challenge for the viability of production systems.

Among these resources is *Hibiscus sabdariffa*, commonly known as "Bissap." It is widely cultivated in tropical and subtropical regions. Its calyces, particularly the red varieties, are rich in anthocyanins, mainly delphinidin and cyanidin, which are responsible for their intense coloration and recognized for their antioxidant properties (Cissé *et al*., 2009). In addition, *Hibiscus sabdariffa* calyces contain various nutrients such as organic acids, minerals, and vitamins, reinforcing their potential use in animal feeding (Babalola *et al*., 2001). Nevertheless, despite this pigmenting and nutritional potential, the use of Bissap in laying hen diets remains limited by several factors. On the one hand, anthocyanins are highly sensitive to storage and processing conditions, which compromises their stability (Da-Costa-Rocha *et al*., 2014). On the other hand, their water-soluble nature limits their bioavailability and transfer to the yolk, which is essentially lipid-based. Finally, the absence of standardized protocols for the use of this resource contributes to inconsistent yolk coloration. The present study therefore aims to evaluate the quality of egg yolk in laying hens fed diets based on *Hibiscus sabdariffa* flowers, by comparing the efficiency of administration through feed and drinking water in order to overcome these technical constraints.

Materials and Methods:-

Plant and animal materials:-

The plant material used in this study consisted of dried flowers of *Hibiscus sabdariffa*, purchased from the local market in Abomey-Calavi, Atlantique Department. To preserve pigment integrity, the flowers were stored at ambient temperature, protected from direct light, in jute bags until use. They were incorporated directly as a feed ingredient in the centesimal formulation of the experimental diets (Table 1).

The RJ diet (positive control) was formulated by adding a commercial yellow pigment, identified as canthaxanthin 10 %, a carotenoid compound ($C_{40}H_{52}O_2$; CAS 514-78-3). This pigment is supplied as red-violet to reddish-brown powder or granules containing 10 % active canthaxanthin, insoluble in water but slightly soluble in oils. The aqueous extract of *Hibiscus sabdariffa* (RE) was prepared daily following a standardized procedure. It was obtained by cold maceration of dried flowers for twelve (12) hours at a rate of 40 g of plant material per liter of water. Preliminary tests indicated no difference in coloration between whole and ground flowers, and economic evaluation showed that 40 g/L provided coloration comparable to 50 g/L while reducing production cost to 280 FCFA/L. The mixture was then filtered and distributed daily to the hens, with feed and water provided *ad libitum* throughout the experimental period.

Table 1. Ingredient and nutrient composition of the experimental diets

Pigment source	**Traitements**			
	R0 (Negative control)	**RJ (Positive control)**	**RF (Diet with *Hibiscus sabdariffa*)**	**RE (Aqueous extract of *Hibiscus sabdariffa*)**
Incorporation rate	0 %	3 %	3 %	40 g/L of water
Ingredients (per 100 kg diet)				
Maize	55.4	55.4	55	55.4
Soybean meal	24	24	24	24
Cottonseed meal	9	9	6	9
Red palm oil[1]	0	0	1	0
Oyster shell	10	10	9.5	10
Lysine	0.05	0.05	0.05	0.05
Methionine	0.15	0.15	0.15	0.15
Dicalcium phosphate	0.8	0.8	0.9	0.8
Salt (NaCl)	0.3	0.3	0.3	0.3
Premix (CMV)[2]	0.25	0.25	0.25	0.25

Ferrous sulfate	0.025	0.025	0.03	0.025
Synthetic yellow pigment	0	3	0	0
Dried *Hibiscus sabdariffa* flowers	0	0	3	0
Nutrient composition				
Dry matter (%)	88.6	88.6	86	88.6
Crude fiber (%)	4.16	4.16	4.19	4.16
Crude protein (%)	18.85	18.85	17.83	18.85
Lysine (%)	0.97	0.97	0.93	0.97
Methionine (%)	0.46	0.46	0.44	0.46
Sulfuraminoacids (%)	0.78	0.78	0.74	0.78
Sodium (Na, %)	0.16	0.16	0.16	0.16
Calcium (Ca, %)	4.05	4.05	3.96	4.05
Phosphorus (P, %)	0.57	0.57	0.57	0.57
Ca/P ratio	7.11	7.11	6.95	7.11
Metabolizableenergy (MJ/kg)	10.85	10.85	11.24	10.85

[1].Unrefined and untreated oil obtained from artisanal processing

[2].Premix composition per kg: Vitamins: A 4,000,000 IU; D3 800,000 IU; E 2000 mg; K 800 mg; B1 600 mg; B2 2000 mg; Niacin 3600 mg; B6 1200 mg; B12 4 mg; Choline chloride 80,000 mg. Minerals: Cu 8000 mg; Mn 64,000 mg; Zn 40,000 mg; Fe 32,000 mg; Se 160 mg.

The experiment involved ninety-six (96) ISA-Brown laying hens, aged fifty (50) weeks at the beginning of the trial. The birds were housed in pairs in Californian-type cages and evenly distributed into four groups of 24 hens each, corresponding to the different dietary treatments tested (R0, RJ, RF, and RE) (Table 1).

Study site and experimental design:-

The experiment was conducted over a period of eight (08) weeks at the Poultry Research and Zoo-Economics Laboratory (LaRAZE) of the University of Abomey-Calavi, Benin. The experimental design was a completely randomized block design, in which each cage (containing two hens) represented an experimental unit, yielding a total of twelve (12) replicates per dietary treatment.

Dietary treatments and experimental design:-

Four (04) dietary treatments (R0, RJ, RF, and RE) were tested. Diet R0 served as the negative control and contained no pigment additive. Diet RJ, considered the positive control, was supplemented with a commercial synthetic pigment. Diet RF included dried *Hibiscus sabdariffa* flowers as a natural pigment source. Finally, hens in the RE group received the negative control diet (R0), but their drinking water was replaced with an aqueous extract of *Hibiscus sabdariffa*. The diets and aqueous extract of Bissap were provided *ad libitum* throughout the experimental period.

Data collection and parameters studied:-

Data collection focused on zootechnical and economic performance as well as egg quality traits. Zootechnical parameters included feed intake, water consumption, Feed Conversion Ratio, and laying rate. Economic performance was assessed through feed cost and Economic Feed Efficiency of four dietary treatments. Egg quality was evaluated using several criteria: average egg weight, yolk color, Haugh unit, shell weight and thickness, shape index, yolk index, and albumen index. Yolk pigmentation was quantified by visual comparison with the Roche color fan (scale 1-15), a standardized method widely recognized for assessing yolk pigmentation in poultry.

Statistical analysis:-

Data were analyzed using the General Linear Model (GLM) procedure in SAS software (version 9.1). Mean values accompanied by standard errors (SE) and probability levels (p-value) were presented in tables. Treatment effects were considered significant at p-value < 0.05.

The statistical model used was as follows:

$$\mathbf{Yi = \mu + Ri + \varepsilon i}$$

Where:

- Yi = observation of the dependent variables
- μ = overall mean
- Ri = fixed effect of dietary treatment or drinking water

- εi = residual error

Ethical statement:
All experimental procedures involving laying hens were conducted in accordance with good poultry husbandry practices and national recommendations for animal welfare. Birds were housed in pairs in Californian-type cages under controlled conditions, with *ad libitum* access to feed and water. No invasive procedures were performed beyond routine zootechnical monitoring, and care was taken to minimize stress throughout the trial.

Results:-

Feed and water intake and egg production:-
Table 2 presents Feed Intake, Water Intake, Laying rate, and Feed Conversion Ratio (FCR) obtained during the experiment.

Table 2. Feed and water intake, Laying rate, and Feed Conversion Ratio of laying hens

Parameters	Traitements				SE	p-value
	R0	RJ	RF	RE		
Feed intake (g/hen/day)	119.9	120.0	120.0	119.8	0.0855	0.3241
Water intake (ml/hen/day)	337^{a}	286^{b}	333^{a}	273^{b}	0.0088	<0.0001
Laying rate (%)	76.8^{c}	87.4^{a}	82.7^{b}	83.9^{b}	2.256	0.0127
FCR (kg feed/kg egg)	2.36^{a}	2.09^{c}	2.19^{b}	2.14^{b}	0.0623	0.0179

R0 = Negative control; RJ = Positive control; RF = Diet with *Hibiscus sabdariffa*; RE = Aqueous extract of *Hibiscus sabdariffa*; FCR = Feed Conversion Ratio; Values within the same row bearing different superscript letters (a, b, c) differ significantly ($P < 0.05$); SE = Standard error.

Feed intake recorded in laying hens was not significantly affected ($P > 0.05$) by the incorporation of *Hibiscus sabdariffa* flowers into their diets. In contrast, hens in groups R0 and RF consumed significantly higher amounts of water (337 and 333 ml/hen/day, respectively) compared to those in groups RE and RJ (273 and 286 ml/hen/day, respectively; $P < 0.05$). The laying rate of hens in R0 (76.8 %) was significantly lower ($P < 0.05$) than that observed in RJ, RF, and RE groups. Supplemented diets (RJ, RF, RE) improved laying rate to values ranging between 82.7 % and 87.4 %, confirming the positive effect of pigment inclusion. Similarly, the Feed Conversion Ratio was significantly better in hens receiving diets supplemented with pigments (RE: 2.14; RF: 2.19) compared to the absolute control diet R0 (2.36). The best Feed Conversion Ratio (2.09) was obtained in hens of the RJ group, highlighting the efficiency of the synthetic pigment.

Egg quality:-
The results of egg quality traits obtained during the experiment are presented in Table 3.

Table 3. Egg quality traits of laying hens

Parameters	Traitements				SE	p-value
	R0	RJ	RF	RE		
Yolk color score	2.0^{c}	9.4^{a}	7.6^{b}	3.5^{c}	0.6054	<0.0001
Average egg weight (g)	64.8^{a}	63.8^{a}	63.7^{a}	62.1^{b}	0.4063	<0.0001
Shell weight (g)	8.24	8.30	8.65	8.01	0.168	0.066
Shell thickness (mm)	0.377	0.378	0.382	0.374	0.0046	0.702
Shape index	0.783	0.778	0.791	0.788	0.0070	0.566
Haugh units	88.6	84.1	88.0	84.5	2.064	0.294
Yolk index	0.449	0.436	0.434	0.434	0.0071	0.347
Albumen index	0.096	0.086	0.097	0.088	0.0048	0.238

R0 = Negative control; RJ = Positive control; RF = Diet with *Hibiscus sabdariffa*; RE = Aqueous extract of *Hibiscus sabdariffa*; Values within the same row bearing different superscript letters (a, b, c) differ significantly ($P < 0.05$); SE = Standard error.

Analysis of egg quality traits showed that the incorporation of *Hibiscus sabdariffa* significantly influenced ($P < 0.0001$) yolk pigmentation and egg weight, without affecting ($P > 0.05$) other internal or external quality parameters.

About yolk pigmentation, diet RJ exhibited the highest score (9.4). Diet RF markedly improved yolk coloration (7.6), reaching consumer-acceptable levels (>7 on the Roche scale). In contrast, the aqueous extract RE (3.5) did not differ significantly from the absolute control R0 (2.0), highlighting the inefficiency of the liquid route for pigment transfer. Furthermore, although egg weights from R0, RJ, and RF diets were comparable (63.7-64.8 g), a significant reduction was observed in RE (62.1 g). Finally, other parameters such as shell weight, shell thickness, Haugh units, and indices (shape, yolk, albumen) remained stable across treatments, confirming that pigment supplementation did not compromise egg integrity.

Feed cost and Feed Efficiency Indice:-

The comparative analysis of feed cost, efficiency, and yolk pigmentation are presented in Table 4.

Table 4. Comparative feed cost, efficiency and yolk pigmentation of laying hens

Parameters	Traitements				SE	p-value	Economic interpretation
	R0	RJ	RF	RE			
Feed cost (FCFA/kg egg)	492.0^{b}	454.4^{c}	477.6^{bc}	528.6^{a}	8.994	<0.0001	RJ lowest cost; RF intermediate; RE highest
Feed cost (FCFA/egg)	31.9^{ab}	29.0^{c}	30.4^{bc}	32.8^{a}	0.583	<0.0001	RJ most profitable; RF close alternative; RE unfavorable
FEI (FCFA egg produced / FCFA feed)	2.01^{ab}	2.20^{a}	2.09^{b}	1.94^{c}	0.035	<0.0001	RJ most efficient; RF acceptable; RE least
Yolk color score	2.0^{c}	9.4^{a}	7.6^{b}	3.5^{c}	0.6054	<0.0001	RJ optimal pigmentation; RF satisfactory; RE poor

R0 = Negative control; RJ = Positive control; RF = Diet with *Hibiscus sabdariffa*; RE = Aqueous extract of *Hibiscus sabdariffa*; Values within the same row bearing different superscript letters (a, b, c) differ significantly ($P < 0.05$); SE = Standard error.

The feed cost per kilogram of egg was significantly lower in the RJ and RF dietary treatments (454.4 and 477.6 FCFA/kg egg, respectively); however, the feed costs of R0 and RF were statistically similar ($P < 0.0001$) (492.0 and 477.6 FCFA/kg egg, respectively). The feed cost expressed per egg was also significantly lower ($P < 0.0001$) with RJ and RF diets (29.9 and 30.4 FCFA/egg) compared to R0 and RE (31.9 and 32.8 FCFA/egg). The Feed Efficiency Indice, which reflects the revenue generated per monetary unit invested in feed, was significantly higher with the positive control diet RJ compared to the indices obtained with the other treatments (Table 4). The lowest values ($P < 0.0001$) were recorded with RE and R0 (1.94 and 2.01, respectively).

In terms of yolk pigmentation, RJ achieved the highest score (9.4), followed by RF (7.6), which provided satisfactory coloration. RE and R0 recorded poor pigmentation (3.5 and 2.0, respectively). Overall, RJ remains the most profitable option, but RF emerges as a viable local alternative, combining acceptable costs, good efficiency, and satisfactory yolk pigmentation.

Discussion:-

The incorporation of Roselle (*Hibiscus sabdariffa*) flowers into laying hen diets influences egg quality, particularly by improving yolk coloration and potentially modifying its nutritional profile (Sukkhavanit *et al*., 2011; Simon *et al*., 2024; Nomagugu *et al*., 2025).

Zootechnical performance: intake and metabolism:-

The use of *Hibiscus sabdariffa* flowers in the diet had no significant effect ($P > 0.05$) on Feed Intake, which remained stable at approximately 119-120 g DM/hen/day. These results are consistent with those of Sukkhavanit *et al*. (2011), who reported no evidence of reduced palatability at the inclusion levels tested in their study on the effects of *Hibiscus sabdariffa* calyces in laying hen diets on production performance and egg quality. Our values are slightly higher than those reported by Sukkhavanit *et al*. (2011), who observed no decline in Feed Intake over an

8-week period, ranging between 111.39 g (1 % extract) and 115.24 g (4 % powder). Similarly, Kijparkorn *et al*. (2010), in their study on *Sesbania javanica* flowers as a natural yolk pigment source, confirmed that floral raw materials do not impair feed palatability. In contrast, Kaya and Yildirim (2011) reported that the use of dried sweet potato *(Ipomea batatas*) stems as a natural pigment source reduced feed intake to as low as 98 g DM/hen/day.

Our results also highlight a significant reduction ($P < 0.0001$) in water consumption in RE (273 ml/hen/day) and RJ (286 ml/hen/day) compared to the control R0 (337 ml/hen/day). Compared to physiological standards in tropical climates, which range between 250 and 350 mL/day (Sauveur, 1988), only hens fed with R0 and RF (333 mL/day) displayed optimal hydration. In contrast, the water intake recorded for those under RE and RJ dietary treatments was at the lower limit of these standards. This reduction in water intake observed with RE may be explained by the high content of organic acids (malic and citric) in *Hibiscus sabdariffa* calyces, which, according to Cissé *et al*. (2009), impart marked acidity and astringency that can reduce voluntary drinking. Although the commercial synthetic yellow pigment used in the RJ treatment was incorporated into the mash feed, the recorded decrease suggests that the chemical nature or concentration of this standardized commercial additive modifies the osmotic balance or the overall palatability of the diet. Consequently, the incorporation of Roselle flowers in dried form (RF) appears to be the safest method to ensure effective pigmenting capacitywhile maintaining water homeostasis in hens and preventing risks of water stress under tropical conditions.

Laying dynamics and feed efficiency:-

The significant productivity improvement observed with supplemented diets suggests that the inclusion of pigment additives, whether synthetic (RJ) or natural (RF, RE), exerts a positive influence on laying rate. This superiority compared to the absolute control diet without pigment (R0) highlights the zootechnical relevance of *Hibiscus sabdariffa*. Although our laying performances (82.7-83.9 %) were evidently higher than the 65.8-69.5 % reported by Sukkhavanit *et al*. (2011), they follow the same trend of improved laying rate associated with Roselle supplementation. This divergence in laying levels may be justified by a better synergy between the basal diet and the genetic potential of the ISA-Brown strain used in our study, unlike the CP Brown strain employed by Sukkhavanit *et al*. (2011).

The absence of significant differences between laying rates obtained with Roselle in flour form (RF) and in aqueous extract form (RE) indicates that the active compounds of Roselle, most likely anthocyanins (Cissé *et al*., 2009), retain their biological efficacy regardless of the administration method. This performance was reinforced by the reduction in Feed Conversion Ratio (FCR), which decreased from 2.36 (R0) to an average of 2.16 in Roselle-based diets. Such improved feed efficiency, also reported by Kijparkorn *et al*. (2010) with other plant pigments (84.9 % laying rate and FCR of 2.15), suggests that these antioxidant compounds optimize intestinal health and sustain laying persistence. However, our conclusions diverge sharply from those of Santos-Bocanegra *et al*. (2004) and Rowghani *et al*. (2006). The incorporation of xanthophylls extracted from *Tagetes erectus* and *Capsicum* sp. as yolk pigment sources did not affect production, feed intake, or feed efficiency in laying hens (Santos-Bocanegra *et al*., 2004). Similarly, the use of *Tagetes erectus* flowers, *Carthamus tinctorius* petals, or red pepper had no significant effect on laying rate (Rowghani *et al*., 2006). Thus, unlike these other plant pigments, *Hibiscus sabdariffa* flowers demonstrated a genuine bio stimulant effect. This specificity may be attributed to the richness of Roselle in micronutrients, which, beyond simple pigmentation, acted favorably on the homeostasis of hens under tropical conditions.

Egg quality and commercial compliance:-

The use of *Hibiscus sabdariffa* flowers in laying hen diets revealed notable variations in the physical characteristics of eggs. A significant decrease ($P < 0.05$) in average egg weight was recorded with the RE diet (62.1 g), which differs from the observations of Zongo *et al*. (1997), who reported no effect on egg weight (≈ 54 g across all groups) when using *Leucaena leucocephala* meal. The reduction in average egg weight observed in RE is likely related to the water restriction previously discussed, although our values remain higher than those reported by Sukkhavanit *et al*. (2011), who recorded 59.3-60.2 g.

Regarding the internal and external quality of the eggs, the stability observed in the parameters (Haugh Units, yolk index, shell strength) confirms the safety of Bissap. This stability is consistent with Sukkhavanit *et al*. (2011) for *Hibiscus* and with Kijparkorn *et al*. (2010) for *Sesbania javanica* flowers, and more recently with Matache *et al*. (2024), who showed that marigold and paprika extracts improved yolk color without compromising egg integrity. Similarly, Simon *et al*. (2024), in organic production systems, emphasized that although natural pigment sources vary in their coloring power, they do not compromise the fundamental quality traits of eggs, which corroborates our results. In terms of market compliance, despite the slight decrease in average egg weight recorded with the RE diet, the overall average weight of our production (between 62.1 and 64.8 g) falls perfectly within the weight category ranging from 55 to 65 g. This class is the most sought after according to European regulatory standards (Aureli *et al.*, 2009), thereby ensuring excellent market value for the eggs produced in the present study. In contrast, the results

of Zongo *et al*. (1997), with average egg weights of approximately 54.1 g, remained within a lower commercial grade.

Pigmenting capacity and pigment bioavailability:-

Based on the Roche color fan score increasing from 2.0 (R0) to 7.6 with the RF diet, the pigmenting capacity of *Hibiscus sabdariffa* flowers is evident. This score is higher than nettle (7.1) and close to kale (8.2) reported by Simon *et al*. (2024), and falls within the same range as marigold and paprika supplementation (7.1-8.2) (Matache *et al*., 2024). However, both these values and the RF score remain below the 9.4 obtained with the synthetic commercial pigment. In contrast, the yolk coloration achieved with the aqueous extract (RE) proved ineffective, with a score of only 3.5. The low efficiency of the aqueous extract compared to the flour form highlights a likely issue of pigment bioavailability or stability. This inefficiency is consistent with Anam *et al*. (2025), who showed that microalgae supplementation increased yolk redness and intensity but required liposoluble carotenoids for effective deposition, whereas anthocyanins from Roselle are water-soluble and less efficiently transferred to the yolk. Indeed, Baiao *et al*. (1999) and Hencken (1992) reported that the effectiveness of natural extracts is often reduced by chemical transformations, such as oxidation, during digestive transit. The flour form (RF) appears to better protect Roselle pigments from such degradation than the aqueous extract.

Our results corroborate the observations of Sukkhavanit *et al*. (2011), who also noted that the incorporation of hibiscus calyx powder (2 to 4 %) significantly improved yolk color, although their scores peaked around 4.0, which is markedly lower than the 7.6 obtained with the RF diet. This difference suggests that the drying method and concentration of our *Hibiscus* flowers allowed better preservation of pigment precursors compared to earlier trials. Furthermore, the biochemical nature of *Hibiscus sabdariffa* pigments played a key role in the results obtained. Cissé *et al*. (2009) identified Roselle anthocyanins as water-soluble molecules, which complicates their deposition in the lipid matrix of the yolk compared to strictly liposoluble xanthophylls. This explains why the RF diet did not reach the scores reported with kale (8.2) and nettle (7.1) (Simon *et al*., 2024), marigold and paprika (7.1-8.2) (Matache *et al*., 2024), or microalgae (Anam *et al*., 2025), all of which rely on carotenoids with stronger affinity for yolk lipids. In the same way, this limitation may also account for the fact that RF did not achieve the higher scores of 10-12 reported by Kijparkorn *et al*. (2010) with *Sesbania javanica* flowers or by Zongo *et al*. (1997) with *Leucaena leucocephala*. These authors worked with pigment-rich plant materials containing lutein and zeaxanthin, xanthophylls with greater lipid affinity than hibiscus anthocyanins.

Finally, our direct observations during the experiment, particularly the red coloration of the droppings specifically in the group of hens fed the RF diet, provide an essential key to interpreting our numerical results regarding the degree of yolk coloration. This visual evidence may be linked to substantial pigment transit coupled with incomplete intestinal absorption, explaining why yolk coloration intensity remained significantly lower than that conferred by synthetic pigment, despite high feed intake. This link between visual observations and measured performance supports the conclusions of Simon *et al*. (2024) regarding the complexity of plant matrices, which can hinder pigment release and limit bioavailability. Nevertheless, the yolk color score of 7.6 obtained with the flour form (RF) positions Roselle as a viable natural alternative to meet consumer preferences for golden yolks (score > 7), while reducing reliance on synthetic pigments.

Economic analysis and feed efficiency:-

The evaluation of the profitability of using Roselle as a natural pigment in laying hen diets revealed that feed cost and Feed Efficiency Indice (FEI) varied significantly among treatments. The highest production cost per egg (32.8 FCFA) was recorded in hens fed the RE dietary treatment. In parallel, the FEI indicated that for each monetary unit invested, the revenue generated by RE was significantly lower than that of RF and RJ diets. This reduced profitability may be explained by a dual constraint: on the one hand, the high market price of *Hibiscus sabdariffa* in Benin, driven by strong competition from human consumption; on the other hand, the stability of feed intake across all dietary treatment, with the aqueous extract failing to induce sufficient improvement in laying rate to offset the additional ingredient cost.

It should also be noted that the majority of studies conducted on natural pigments, such as those by Simon *et al*. (2024), Zongo *et al*. (1997), Kijparkorn *et al*. (2010), or Sukkhavanit *et al*. (2011), have been limited to biological efficacy (weight, laying rate, coloration) without integrating the technico-economic aspect. In contrast, our study provides an original decision-making perspective by combining zootechnical performance with economic viability, thereby addressing a gap in the literature. Despite the relatively high cost of Roselle, the feed costs obtained in our trial remain lower than the 47.3-57.2 FCFA/egg reported by Houndonougbo *et al*. (2012) in Benin. This comparison demonstrates that our tested diets remain competitive, with the This comparison demonstrates that the tested diets remain competitive, with Roselle flour (RF) emerging as the most efficient option, balancing yolk pigmentation performance with financial profitability.

Conclusion:-

The study shows that the incorporation of *Hibiscus sabdariffa* flowers in flour form (RF) constitutes an effective local alternative to the widely used imported synthetic pigments. Although synthetic pigments are efficient, they involve foreign currency costs and raise concerns regarding potential side effects on consumer health. The results prove that the incorporation of Bissap in flour form achieves a laying rate of 83.9 % and a highly competitive golden-yellow coloration (score of 7.6), thus meeting market requirements without resorting to chemical pigments. From an economic perspective, the RF diet ensured superior feed efficiency compared to the aqueous extract and proved to be the most cost-effective option. In summary, the valorization of *Hibiscus sabdariffa* flowers in poultry production provides a sustainable solution for Beninese producers: it guarantees safe eggs with desirable chromatic qualities, while ensuring economic profitability and improved consumer health protection. Roselle flour therefore represents a natural method to obtain darker, antioxidant-rich yolks without compromising internal egg quality*Hibiscus sabdariffa*.

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Authors' Contributions:-

All authors contributed equally to the design, implementation of the research, analysis of the results, and the writing of the manuscript.

Conflict of interest:-

We declare that we have no conflict of interest.

References:-

1. Anam, M.S., Muslykhah, U., Sadid, M.M., Noviandi, C.T., Astuti, A., Paradhipta, D.H.V. and Agus, A. (2025). Effects of microalgae supplementation on performance, egg quality, yolk fatty acid levels, and blood parameters in laying hens: a meta-analysis. Sci. Rep., 15, 44877. doi:10.1038/s41598-025-28515-3.
2. Aureli, R., Philipps, P., Fru, F., Schierle, J. and Gadient, M. (2009). Comparer l'efficacité de différents pigments rouges sur la pigmentation du jaune d'œuf. 8eme Journée de la Recherche Avicole, Saint Malo. Disponible à: http://www.cabi.org/animalscience/Uploads/File/AnimalScience/additionalFiles/WPSAStMalo2009/34_fpd2009_aurelia.pdf.
3. Babalola, S.O., Babalola, A.O. and Aworh, O.C. (2001). Compositional attributes of the calyces of roselle (*Hibiscus sabdariffa*). J. Food Technol. Afr., 6(4), 133–134.
4. Baiao, N.C., Mandez, J., Mateos, J., Garcia, M. and Mantos, G.G. (1999). Pigmenting efficacy of several oxycarotenoids on egg yolk. J. Appl. Poult. Res., 8, 472–479. Disponible à: http://japr.fass.org/content/8/4/472.full.pdf+html.
5. Blount, J.D., Houston, D.C. and Møller, A.P. (2000). Why egg yolk is yellow. Trends Ecol. Evol., 15, 47–49. Disponible à: http://www.sciencedirect.com/science/article/pii/S0169534799017747.
6. Cissé, M., Dornier, M., Sakho, M., Ndiaye, A. and Reynes, M. (2009). Le Bissap (*Hibiscus sabdariffa* L.): composition et principales utilisations. Fruits, 64(3), 179–193. doi:10.1051/fruits/2009013.
7. Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I. and Heinrich, M. (2014). *Hibiscus sabdariffa* L. – A phytochemical and pharmacological review. Food Chem., 165, 424–443. doi: 10.1016/j.foodchem.2014.05.002.
8. Hencken, H. (1992). Chemical and physiological behavior of feed carotenoids and their effects on pigmentation. Poult. Sci., 71, 711–717.
9. Houndonougbo, M.F., Chrysostome, C.A.A.M., DagaDadjo, F. and Adjaho, S.L. (2012). Bioeconomic performance of pullets and layer hens fed soybean grains-based diets in hot and humid climate. ISRN Vet. Sci., Vol. 2012, Article ID 812564, 6 pp. doi:10.5402/2012/812564.
10. Kang, D.K., Kim, S.I., Cho, C.H., Yim, Y.H. and Kim, H.S. (2003). Use of lycopene, an antioxidant carotenoid, in laying hens for egg yolk pigmentation. Asian-Australas. J. Anim. Sci., 16(12), 1799–1803.
11. Kaya, Ş. and Yildirim, H. (2011). The effect of dried sweet potato (Ipomeabatatas) vines on egg yolk color and some egg yield parameters. Int. J. Agric. Biol., 15, 766–770.
12. Kijparkorn, S., Plaimast, H. and Wangsoonoen, S. (2010). Sano (SesbaniajavanicaMiq.) flower as a pigment source in egg yolk of laying hens. Thai J. Vet. Med., 40(3), 281–287.

13. Kljak, K., Drdić, M., Karolyi, D. and Grbeša, D. (2012). Pigmentation efficiency of Croatian corn hybrids in egg production. Croat. J. Food Technol. Biotechnol. Nutr., 7 (Special Issue), 23–27.
14. Magnin, M., Jeanmichel, P. and Mathieu, A. (2009). Comparaison de l'efficacité de trois sources de pigments rouges naturels et de la canthaxanthine pour la coloration du jaune d'œuf de poule. 8eme Journée de la Recherche Avicole, Saint Malo, pp.459–463.
15. Matache, C.C., Cornescu, G.M., Drăgotoiu, D., Cișmileanu, A.E., Untea, A.E., Saracila, M. and Panaite, T.D. (2024). Effects of marigold and paprika extracts as natural pigments on laying hen productive performances, egg quality and oxidative stability. Agriculture, 14, 1464. doi:10.3390/agriculture14091464.
16. Nomagugu, N., Kennedy, H.E. and Eliton, C. (2025). Effect of dietary fortification with *Hibiscus sabdariffa* calyces meal on egg production and egg quality of Japanese quail (Coturnixcoturnix japonica). Vet. Med. Sci., 11: e70141, 10 pp. doi:10.1002/vms3.70141. PMCID: PMC11610158. PMID: 39620906.
17. Omole, A.J., Ogbosuka, G.E., Salako, R.A. and Ajayi, O.O. (2005). Effect of replacing oyster shell with gypsum in broiler finisher diet. J. Appl. Sci. Res., 1(2), 245–248.
18. Rowghani, E., Maddahian, A. and Arab Abousadi, M. (2006). Effects of addition of marigold flower, safflower petals, red pepper on egg-yolk color and egg production in laying hens. Pak. J. Biol. Sci., 9(7), 1333–1337.
19. Santos-Bocanegra, E., Ospina-Osorio, X. and Oviedo-Rondon, E.O. (2004). Evaluation of xanthophylls extracted from Tagetes erectus (Marigold Flower) and Capsicum sp. (Red Pepper Paprika) as a pigment for egg-yolks compared with synthetic pigments. Int. J. Poult. Sci., 3(11), 685–689.
20. Sauveur, B. (1988). Reproduction des volailles et production d'œufs. Institut National de la Recherche Agronomique (INRA), Paris, France, 449 pp. ISBN 2-85340-961-9.
21. Simon, E., Beyer, E., Remiot, P. and Faouen, A. (2024). La gestion de la pigmentation du jaune d'œuf en élevage biologique. Quinziemes Journées de la Recherche Avicole et Palmipedes à Foie Gras, Tours, 20–21 mars 2024, 5 pp. Disponible à: https://www.itavi.fr/publications/
22. Sukkhavanit, P., Angkanaporn, K. and Kijparkorn, S. (2011). Effect of roselle (*Hibiscus sabdariffa* Linn.) calyx in laying hen diet on egg production performance, egg quality and TBARS value in plasma and yolk. Thai J. Vet. Med., 41(3), 337–344.
23. Zongo, D., Ba, C., Diambra, O. and Coulibaly, M. (1997). Essai d'évaluation du pouvoir colorant d'une source naturelle de jaunissant (Leucaenaleucocephala) sur la pigmentation du jaune d'œuf chez la poule. Ann. Zootech., 46, 185–190.