



23 **Introduction:**

24 Egg yolk color is a major quality criterion for consumers and strongly influences  
25 market acceptability. Although it does not directly affect the nutritional value of the  
26 egg, this characteristic depends essentially on the diet of laying hens. Yolk  
27 pigmentation is mainly provided by carotenoids, particularly xanthophylls such as  
28 lutein and zeaxanthin, which must be supplied through feed (Blount *et al.*, 2000;  
29 Zongoet *al.*, 1997). Therefore, the incorporation of pigmenting ingredients into laying  
30 hen diets is a common practice aimed at improving the visual quality of eggs. Several  
31 studies have highlighted the interest of using natural pigments (Zongoet *al.*, 1997;  
32 Kang *et al.*, 2003; Magninet *al.*, 2009; Aureliet *al.*, 2009; Kijparkornet *al.*, 2010;  
33 Kljaket *al.*, 2012). Among the reported sources, Sauveur (1988) distinguished raw  
34 materials with varying pigment concentrations, such as alfalfa meal (250 mg/kg),  
35 yellow maize (20-25 mg/kg), algae (2200-4000 mg/kg), *Leucaenaleucocephala* leaves  
36 (440-660 mg/kg), marigold and tagetes petals (6000-10,000 mg/kg), as well as kola,  
37 palm oil, and sorghum.

38 However, in many Sub-Saharan African countries, the use of synthetic pigment  
39 additives remains limited due to their high cost, linked to importation (Zongoet *al.*,  
40 1997). Feed accounts for up to 70% of total production costs in poultry farming  
41 (Omoleet *al.*, 2005). In this context, the search for alternative local sources, available  
42 at low cost and capable of ensuring effective yolk pigmentation, is a major challenge  
43 for the viability of production systems.

44 Among these resources is *Hibiscus sabdariffa*, commonly known as “Bissap.” It is  
45 widely cultivated in tropical and subtropical regions. Its calyces, particularly the red  
46 varieties, are rich in anthocyanins, mainly delphinidin and cyanidin, which are  
47 responsible for their intense coloration and recognized for their antioxidant properties  
48 (Cisséet *al.*, 2009). In addition, *Hibiscus sabdariffa* calyces contain various nutrients  
49 such as organic acids, minerals, and vitamins, reinforcing their potential use in animal  
50 feeding (Babalolaet *al.*, 2001). Nevertheless, despite this pigmenting and nutritional  
51 potential, the use of Bissap in laying hen diets remains limited by several factors. On  
52 the one hand, anthocyanins are highly sensitive to storage and processing conditions,  
53 which compromises their stability (Da-Costa-Rocha *et al.*, 2014). On the other hand,  
54 their water-soluble nature limits their bioavailability and transfer to the yolk, which is

55 essentially lipid-based. Finally, the absence of standardized protocols for the use of  
 56 this resource contributes to inconsistent yolk coloration.

57 The present study therefore aims to evaluate the quality of egg yolk in laying hens fed  
 58 diets based on *Hibiscus sabdariffa* flowers, by comparing the efficiency of  
 59 administration through feed and drinking water in order to overcome these technical  
 60 constraints.

61 **Materials and methods:**

62 **Plant and animal materials**

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

68 **Table 1.** Ingredient and nutrient composition of the experimental diets

Pigment source	Traitements			
	R0 (Negative control)	RJ (Positive control)	RF (Diet with <i>Hibiscus sabdariffa</i> )	RE (Aqueous extract of <i>Hibiscus sabdariffa</i> )
<b>Incorporation rate</b>	0 %	3%	3%	40 g/L of water
<b>Ingredients (per 100 kg diet)</b>				
Maize	55.4	55.4	55	55.4
Soybean meal	24	24	24	24
Cottonseed meal	9	9	6	9
Red palm oil <sup>1</sup>	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) <sup>2</sup>	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 <i>Hibiscus sabdariffa</i> flowers	0	0	3	0
<b>Nutrient composition</b>				
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
Metabolizable energy (MJ/kg)	10.85	10.85	11.24	10.85

69 <sup>1</sup>Unrefined and untreated oil obtained from artisanal processing

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

74

75 The experiment involved ninety-six (96) ISA-Brown laying hens, aged fifty (50)  
76 weeks at the beginning of the trial. The birds were housed in pairs in Californian-type  
77 cages and evenly distributed into four (04) groups of twenty-four (24) hens,  
78 corresponding to the different dietary treatments tested (R0, RJ, RF, and RE) (Table  
79 1).

#### 80 **Study site and experimental design**

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

#### 86 **Dietary treatments and experimental design**

87 Four (04) dietary treatments (R0, RJ, RF, and RE) were tested. Diet R0 served as the  
88 negative control and contained no pigment additive. Diet RJ, considered the positive  
89 control, was supplemented with a commercial synthetic pigment. Diet RF included  
90 dried *Hibiscus sabdariffa* flowers as a natural pigment source. Finally, hens in the RE  
91 group received the negative control diet (R0), but their drinking water was replaced  
92 with an aqueous extract of *Hibiscus sabdariffa*.

93 The aqueous extract of *Hibiscus sabdariffa* was obtained by cold maceration of the  
94 dried flowers for twelve (12) hours at a rate of 40 g of plant material per liter of water.  
95 The mixture was then filtered and distributed to the hens. Feed and water were  
96 provided *ad libitum* throughout the experimental period.

#### 97 **Data collection and parameters studied**

98 Data collection focused on zootechnical and economic performance as well as egg  
 99 quality traits. Zootechnical parameters included feed intake, water consumption, feed  
 100 conversion ratio, and laying rate. Economic performance was assessed through feed  
 101 cost and Economic Feed Efficiency of four dietary treatments.

102 Egg quality was evaluated using several criteria: average egg weight, yolk color,  
 103 Haugh unit, shell weight and thickness, shape index, yolk index, and albumen index.  
 104 Yolk pigmentation was quantified by visual comparison with the Roche color fan  
 105 (scale 1-15), a standardized method widely recognized for assessing yolk  
 106 pigmentation in poultry.

107 **Statistical analysis**

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

112 The statistical model used was as follows:

113 
$$Y_i = \mu + R_i + \epsilon_i$$

114 Where:

- 115 •  $Y_i$  = observation of the dependent variables
- 116 •  $\mu$  = overall mean
- 117 •  $R_i$  = fixed effect of dietary treatment or drinking water
- 118 •  $\epsilon_i$  = residual error

119  
 120 **Results:**

121 **Feed and water intake and egg production**

122 Table 2 presents Feed Intake, Water Intake, Laying rate, and Feed Conversion Ratio  
 123 (FCR) obtained during the experiment.

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

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

126 R0 = Negative control; RJ = Positive control; RF = Diet with *Hibiscus sabdariffa*; RE = Aqueous  
 127 extract of *Hibiscus sabdariffa*; FCR = Feed Conversion Ratio; Values marked with different letters (a,  
 128 b, c) within the same row indicate significant differences at the 5% level; SE = Standard error.

129  
 130 Feed intake recorded in laying hens was not significantly affected ( $P > 0.05$ ) by the  
 131 incorporation of *Hibiscus sabdariffa* flowers into their diets. In contrast, hens in  
 132 groups R0 and RF consumed significantly higher amounts of water (337 and 333  
 133 ml/hen/day, respectively) compared to those in groups RE and RJ (273 and 286  
 134 ml/hen/day, respectively;  $P < 0.05$ ).

135 The laying rate of hens in R0 (76.8%) was significantly lower ( $P < 0.05$ ) than that  
 136 observed in RJ, RF, and RE groups (Table 2). The inclusion of pigmenting products  
 137 (commercial synthetic or *Hibiscus sabdariffa*) in diet or drinking water therefore  
 138 improved egg production. Similarly, the Feed Conversion Ratio was significantly  
 139 better in hens receiving diets supplemented with Bissap (RE: 2.14; RF: 2.19)  
 140 compared to the absolute control diet R0 (2.36). Moreover, the best Feed Conversion  
 141 Ratio recorded during the experiment (2.09) was obtained in hens of the positive  
 142 control group RJ (Table 2).

### 143 Egg quality

144 The results of egg quality traits obtained during the experiment are presented in Table  
 145 3.

146 **Table 3.** Egg quality traits of laying hens

Parameters	Traitements				SE	p-value
	R0	RJ	RF	RE		
Yolk color score	2.0 <sup>c</sup>	9.4 <sup>a</sup>	7.6 <sup>b</sup>	3.5 <sup>c</sup>	0.6054	<0.0001
Average egg weight (g)	64.8 <sup>a</sup>	63.8 <sup>a</sup>	63.7 <sup>a</sup>	62.1 <sup>b</sup>	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 (mm)	0.096	0.086	0.097	0.088	0.0048	0.238

147 R0 = Negative control; RJ = Positive control; RF = Diet with *Hibiscus sabdariffa*; RE = Aqueous  
 148 extract of *Hibiscus sabdariffa*; Values marked with different letters (a, b, c) within the same row  
 149 indicate significant differences at the 5% level; SE = Standard error.

150  
 151 Analysis of egg quality traits showed that the incorporation of *Hibiscus sabdariffa*  
 152 significantly influenced ( $P < 0.0001$ ) yolk pigmentation and egg weight, without  
 153 affecting ( $P > 0.05$ ) other internal or external quality parameters. About yolk  
 154 pigmentation, diet RJ exhibited the highest score (9.4). It was followed by diet RF, in  
 155 which the inclusion of Bissap markedly improved yolk coloration (7.6), compared to

156 the aqueous extract RE (3.5) and the absolute control R0 (2.0). The scores obtained  
 157 with RE and R0 were significantly similar, highlighting the inefficiency of the liquid  
 158 route for pigment transfer.

159

160 Furthermore, although egg weights from R0, RJ, and RF diets were comparable (63.7-  
 161 64.8 g), a significant reduction was observed in RE (62.1 g). Finally, the stability of  
 162 parameters such as shell weight and thickness, Haugh units (84.1-88.6), and the  
 163 different indices (shape, yolk, and albumen) demonstrates that the use of dried  
 164 *Hibiscus sabdariffa* flowers, particularly when incorporated into diet, preserves the  
 165 structural integrity and freshness of the eggs produced.

166 **Feed cost and Feed Efficiency Indice**

167 The feed costs and Feed Efficiency Indice (FEI) obtained with the different dietary  
 168 treatments are presented in Table 4.

169 **Table 4.** Feed cost and Feed Efficiency Indice of laying hens

Parameters	Traitements				SE	p-value
	R0	RJ	RF	RE		
Feed cost (FCFA/kg egg)	492.0 <sup>b</sup>	454.4 <sup>c</sup>	477.6 <sup>bc</sup>	528.6 <sup>a</sup>	8.994	<0.0001
Feed cost (FCFA/egg)	31.9 <sup>ab</sup>	29.0 <sup>c</sup>	30.4 <sup>bc</sup>	32.8 <sup>a</sup>	0.583	<0.0001
FEI (FCFA egg produced / FCFA feed)	2.01 <sup>ab</sup>	2.20 <sup>a</sup>	2.09 <sup>b</sup>	1.94 <sup>c</sup>	0.035	<0.0001

170 R0 = Negative control; RJ = Positive control; RF = Diet with *Hibiscus sabdariffa*; RE = Aqueous  
 171 extract of *Hibiscus sabdariffa*; Values marked with different letters (a, b, c) within the same row  
 172 indicate significant differences at the 5% level; SE = Standard error.

173

174 The feed cost per kilogram of egg was significantly lower in the RJ and RF dietary  
 175 treatments (454.4 and 477.6 FCFA/kg egg, respectively); however, the feed costs of  
 176 R0 and RF were statistically similar ( $P < 0.0001$ ) (492.0 and 477.6 FCFA/kg egg,  
 177 respectively). The feed cost expressed per egg was also significantly lower ( $P <$   
 178  $0.0001$ ) with RJ and RF diets (29.9 and 30.4 FCFA/egg) compared to R0 and RE  
 179 (31.9 and 32.8 FCFA/egg).

180 The Feed Efficiency Indice, which reflects the revenue generated per monetary unit  
 181 invested in feed, was significantly higher with the positive control diet RJ compared  
 182 to the indices obtained with the other treatments (Table 4). The lowest values ( $P <$   
 183  $0.0001$ ) were recorded with RE and R0 (1.94 and 2.01, respectively).

184 **Discussion:**

185 The incorporation of Roselle (*Hibiscus sabdariffa*) flowers into laying hen diets  
 186 influences egg quality, particularly by improving yolk coloration and potentially

187 modifying its nutritional profile (Sukkhavanitet *al.*, 2011; Simon *et al.*, 2024;  
188 Nomaguguet *al.*, 2025).

189

190

### 191 **Zootechnical performance: intake and metabolism**

192 The use of *Hibiscus sabdariffa* flowers in the diet had no significant effect ( $P > 0.05$ )  
193 on Feed Intake, which remained stable at approximately 119-120 g DM/hen/day.

194 These results are consistent with those of Sukkhavanitet *al.* (2011), who reported no  
195 evidence of reduced palatability at the inclusion levels tested in their study on the  
196 effects of *Hibiscus sabdariffa* calyces in laying hen diets on production performance  
197 and egg quality. Our values are slightly higher than those reported by Sukkhavanitet  
198 *al.* (2011), who observed no decline in Feed Intake over an 8-week period, ranging  
199 between 111.39 g (1% extract) and 115.24 g (4% powder). Similarly, Kijparkornet *al.*  
200 (2010), in their study on *Sesbania javanica* flowers as a natural yolk pigment source,  
201 confirmed that floral raw materials do not impair feed palatability. In contrast, Kaya  
202 and Yildirim (2011) reported that the use of dried sweet potato (*Ipomea batatas*) stems  
203 as a natural pigment source reduced feed intake to as low as 98 g DM/hen/day.

204 Our results also highlight a significant reduction ( $P < 0.0001$ ) in water consumption in  
205 RE (273 ml/hen/day) and RJ (286 ml/hen/day) compared to the control R0 (337  
206 ml/hen/day). Compared to physiological standards in tropical climates, which range  
207 between 250 and 350 mL/day (Sauveur, 1988), only hens fed with R0 and RF (333  
208 mL/day) displayed optimal hydration. In contrast, the water intake recorded for those  
209 under RE and RJ dietary treatments was at the lower limit of these standards. This  
210 reduction in water intake observed with RE may be explained by the high content of  
211 organic acids (malic and citric) in *Hibiscus sabdariffa* calyces, which, according to  
212 Cissé *et al.* (2009), impart marked acidity and astringency that can reduce voluntary  
213 drinking. Although the commercial synthetic yellow pigment used in the RJ treatment  
214 was incorporated into the mash feed, the recorded decrease suggests that the chemical  
215 nature or concentration of this standardized commercial additive modifies the osmotic  
216 balance or the overall palatability of the diet.

217 Consequently, the incorporation of Roselle flowers in dried form (RF) appears to be  
218 the safest method to ensure effective yolk pigmentation while maintaining water  
219 homeostasis in hens and preventing risks of water stress under tropical conditions.

### 220 **Laying dynamics and feed efficiency**

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

231 The absence of significant differences between laying rates obtained with Roselle in  
232 flour form (RF) and in aqueous extract form (RE) indicates that the active compounds  
233 of Roselle, most likely anthocyanins (Cissé *et al.*, 2009), retain their biological  
234 efficacy regardless of the administration method. This performance was reinforced by  
235 the reduction in feed conversion ratio (FCR), which decreased from 2.36 (R0) to an  
236 average of 2.16 in Roselle-based diets. Such improved feed efficiency, also reported  
237 by Kijparkorn *et al.* (2010) with other plant pigments (84.9% laying rate and FCR of  
238 2.15), suggests that these antioxidant compounds optimize intestinal health and  
239 sustain laying persistence.

240 However, our conclusions diverge sharply from those of Santos-Bocanegra *et al.*  
241 (2004) and Rowghaniet *et al.* (2006). The incorporation of xanthophylls extracted from  
242 *Tagetes erectus* and *Capsicum* sp. as yolk pigment sources did not affect production,  
243 feed intake, or feed efficiency in laying hens (Santos-Bocanegra *et al.*, 2004).  
244 Similarly, the use of *Tagetes erectus* flowers, *Carthamus tinctorius* petals, or red  
245 pepper had no significant effect on laying rate (Rowghaniet *et al.*, 2006). Thus, unlike  
246 these other plant pigments, *Hibiscus sabdariffa* flowers demonstrated a genuine bio  
247 stimulant effect. This specificity may be attributed to the richness of Roselle in  
248 micronutrients, which, beyond simple pigmentation, acted favorably on the  
249 homeostasis of hens under tropical conditions.

#### 250 **Egg quality and commercial compliance**

251 The use of *Hibiscus sabdariffa* flowers in laying hen diets revealed notable variations  
252 in the physical characteristics of eggs. A significant decrease ( $P < 0.05$ ) in average  
253 egg weight was recorded with the RE diet (62.1 g), which differs from the  
254 observations of Zongo *et al.* (1997), who reported no effect on egg weight ( $\approx 54$  g

255 across all groups) when using *Leucaenaleucocephala* meal. The reduction in average  
256 egg weight observed in RE is likely related to the water restriction previously  
257 discussed. Nevertheless, our results remain higher in absolute value than those  
258 reported by Sukkhavanit *et al.* (2011), who recorded average egg weights ranging  
259 between 59.3 and 60.2 g.

260 Regarding the internal and external quality of the eggs, the stability observed in the  
261 parameters (Haugh Units, yolk index, shell strength) confirms the safety of Bissap.  
262 This stability is consistent with the findings of Sukkhavanit *et al.* (2011) for hibiscus  
263 and those of Kijparkorn *et al.* (2010) for *Sesbaniajavanica* flowers, demonstrating that  
264 natural pigments preserve egg integrity. Similarly, Simon *et al.* (2024), in organic  
265 production systems, emphasized that although natural pigment sources vary in their  
266 coloring power, they do not compromise the fundamental quality traits of eggs, which  
267 corroborates our results.

268 In terms of market compliance, despite the slight decrease in average egg weight  
269 recorded with the RE diet, the overall average weight of our production (between 62.1  
270 and 64.8 g) falls perfectly within the weight category ranging from 55 to 65 g. This  
271 class is the most sought after according to European regulatory standards (Aureliet *et al.*,  
272 2009), thereby ensuring excellent market value for the eggs produced in the present  
273 study. In contrast, the results of Zongoet *et al.* (1997), with average egg weights of  
274 approximately 54.1 g, remained within a lower commercial grade.

### 275 **Yellowing capacity and pigment bioavailability**

276 Based on the Roche color fan score increasing from 2.0 (R0) to 7.6 with the RF diet,  
277 the pigmentation power of *Hibiscus sabdariffa* flowers is evident. This performance is  
278 comparable to recently tested natural sources such as nettle or kale, which yielded  
279 scores between 7.1 and 8.2 at single doses (Simon *et al.*, 2024). However, both these  
280 values and the RF score remain below the 9.4 obtained with the synthetic commercial  
281 pigment. In contrast, the yolk coloration achieved with the aqueous extract (RE)  
282 proved ineffective, with a score of only 3.5. The low efficiency of the aqueous extract  
283 compared to the flour form highlights a likely issue of pigment bioavailability or  
284 stability. Indeed, Baiaoet *et al.* (1999) and Hencken (1992) reported that the  
285 effectiveness of natural extracts is often reduced by chemical transformations, such as  
286 oxidation, during digestive transit. The flour form (RF) appears to better protect  
287 Roselle pigments from such degradation than the aqueous extract.

288 Our results corroborate the observations of Sukkhavanit *et al.* (2011), who also noted  
289 that the incorporation of hibiscus calyx powder (2 to 4%) significantly improved yolk  
290 color, although their scores peaked around 4.0, which is markedly lower than the 7.6  
291 obtained with the RF diet. This difference suggests that the concentration or drying  
292 method of our hibiscus flowers allowed better preservation of pigment precursors.  
293 Furthermore, the biochemical nature of *Hibiscus sabdariffa* pigments played a key role  
294 in the results obtained. Cissé *et al.* (2009) identified Roselle anthocyanins as  
295 water-soluble molecules, which complicates their deposition in the lipid matrix of the  
296 yolk compared to strictly liposoluble xanthophylls. This may explain why RF did not  
297 reach the scores of 10-12 reported by Kijparkorn *et al.* (2010) with *Sesbania javanica*  
298 flowers or by Zongo *et al.* (1997) with *Leucaena leucocephala*. These authors worked  
299 with pigment-rich plant materials containing lutein and zeaxanthin, xanthophylls with  
300 higher affinity for yolk lipids than hibiscus anthocyanins.  
301 Finally, our direct observations during the experiment, particularly the red coloration  
302 of the droppings specifically in the group of hens fed the RF diet, provide an essential  
303 key to interpreting our numerical results regarding the degree of yolk coloration. This  
304 visual evidence may be linked to substantial pigment transit coupled with incomplete  
305 intestinal absorption, explaining why yolk coloration intensity remained significantly  
306 lower than that conferred by synthetic pigment, despite high feed intake. This link  
307 between visual observations and measured performance supports the conclusions of  
308 Simon *et al.* (2024) regarding the complexity of plant matrices, which can hinder  
309 pigment release and limit bioavailability.  
310 Nevertheless, the yolk color score of 7.6 obtained with the flour form (RF) positions  
311 Roselle as a viable natural alternative to meet consumer preferences for golden yolks  
312 (score >7), while reducing reliance on synthetic pigments.

### 313 **Economic analysis and feed efficiency**

314 The evaluation of the profitability of using Roselle as a natural pigment in laying hen  
315 diets revealed that feed cost and Feed Efficiency Indices (FEI) varied significantly  
316 among treatments. The highest production cost per egg (32.8 FCFA) was recorded in  
317 hens fed the RE dietary treatment. In parallel, the FEI indicated that for each  
318 monetary unit invested, the revenue generated by RE was significantly lower than that  
319 of RF and RJ diets. This reduced profitability may be explained by a dual constraint:  
320 on the one hand, the high market price of *Hibiscus sabdariffa* in Benin, driven by  
321 strong competition from human consumption; on the other hand, the stability of feed

322 intake across all dietary treatment, with the aqueous extract failing to induce sufficient  
323 improvement in laying rate to offset the additional ingredient cost.

324 It should also be noted that the majority of studies conducted on natural pigments,  
325 such as those by Simon *et al.* (2024), Zongoet *al.* (1997), Kijparkornet *al.* (2010), or  
326 Sukkhavanitet *al.* (2011), have been limited to biological efficacy (weight, laying rate,  
327 coloration) without integrating the technico-economic aspect. Our study therefore  
328 provides a crucial decision-making perspective for local poultry production. Despite  
329 the relatively high cost of Roselle, the feed costs obtained in our trial remain lower  
330 than the 47.3-57.2 FCFA/egg reported by Houndonougboet *al.* (2012) in Benin. This  
331 comparison demonstrates that our tested diets remain competitive, with the RF diet  
332 appearing to be the most efficient in balancing coloring performance and financial  
333 profitability.

#### 334 **Conclusion:**

335 The study shows that the incorporation of *Hibiscus sabdariffa* flowers in flour form  
336 (RF) constitutes an effective local alternative to the widely used imported synthetic  
337 yellowing pigments. Although synthetic pigments are efficient, they involve foreign  
338 currency costs and raise concerns regarding potential side effects on consumer health.  
339 The results prove that the incorporation of Bissap in flour form achieves a laying rate  
340 of 83.9% and a highly competitive golden-yellow coloration (score of 7.6), thus  
341 meeting market requirements without resorting to chemical yellowing pigments. From  
342 a financial perspective, the RF diet ensured better feed efficiency than the aqueous  
343 extract and proved to be the most economical option.

344 In summary, the valorization of *Hibiscus sabdariffa* flowers in poultry production  
345 offers a sustainable solution for Beninese producers: it guarantees the production of  
346 safe eggs with desirable chromatic qualities, while ensuring optimal economic  
347 profitability and improved consumer health security. The use of *Hibiscus sabdariffa*  
348 flowers is a natural method to obtain darker egg yolks with higher antioxidant content,  
349 while maintaining the internal quality of the egg.

#### 350 **Acknowledgement:**

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#### 353 **Authors' Contributions:**

354 All authors contributed equally to the design, implementation of the research, analysis  
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356 **Conflict of interest:**

357 We declare that we have no conflict of interest.

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