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Borassus aethiopum Ripe Fruits' Dried Pulp as Egg Yolk Coloration Agent

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Abstract: The essay assessed *Borassus aethiopum* (*B.a*) ripe fruits' dried pulp effect on egg yolk coloration. First, thirty-six Harco laying hens, 42 weeks old with similar weight (α=0.05) were allotted in 12 cages of $4m^2$ (2m x 2m, respectively for length x width) each. Second, 4 laying hens' diets were formulated; a yellow corn-based diet (YC), a white corn-based diet (WC), a white corn-based diet supplemented with 10% of *B.a* mature fruits' dried pulp (WC+10%*B.a*), and finally a white corn-based diet supplemented with 20% of *B.a* ripe fruits' dried pulp (WC+10%*B.a*). During the evaluations, similar eggs for weights were analyzed. As a result, rich pigment diets formed a group and produced the heaviest egg yolk compared to the WC diet, for 13.94+0.16 g versus 13.64+0.16 g. Whereas, WC diet delivered the heaviest albumin weights, 31.92+0.33 g versus 29.27+0.33 g for WC+10%*B.a* which had the lightest weight. Looking at eggs' yolks color, Roche yolk color fan distinguished just 2 levels whose were the pale egg yolk from WC graded 1, and the intense colored yolks group graded 6. A spectrophotometer reflectance method helped for better yolks color distinctions. The yolks' yellow colors from WC+10%*B.a* (b*=52.29), WC+20%*B.a* (b*=52.49) and YC (b*=52.22) were similar (p=0.7278). But, the WC+10%*B.a* diet egg yolk color was significantly higher than that of WC (b*=20.70; p<0.0001). In conclusion, *Borassus aethiopum* mature fruits' dried pulp can be incorporated into laying hens feed as natural pigments' source.

Keywords: *Borassus aethiopum*, Natural Pigment, White Corn-Based Diet, Yolk Color, Yolk Weight

1. Introduction

First, *Borassus flabellifer* specie trees are widely spread in Asia and America, while *Borassus aethiopum* specie trees are alongside the equator in Africa. In these continents, people are collecting sap wine from these Palmyra palms [1], doing business with growing young shoots and whole ripe fruits, and making some edible flour with mature fruits' dried pulp [2]. In addition, the yellow fibrous pulp is very energetic because it exhibits more than 3,600 kcal/kg (DM) [3, 4]. Equally important, this pulp is a functional food due to its high polyphenols' contents for 447.87 mg GAE/g, whose induce 0.44 µmol TE/g antioxidant activity [5] and more than 76% of its fatty acids are unsaturated [4]. Also, because these dried pulps contain 15.5 to 35.4 mg/100g (DM) of total carotenoid [6], they are considerable pigment sources. These pulps may be better pigment sources than yellow and red corns which contain 8.86 and 9.81 mg/100g (DM), respectively [7]. Second, egg yolk industry is very diverse and its color remains one of the best egg quality parameters for consumers [8, 9]. Since egg yolk color strongly depends on the laying hens diet pigment contents [7, 10], many pigments including synthetic and natural are used [9, 11]. Among the synthetics, β-apo-8'-carotenal ethyl ester and β-apo-8'-carotenoic acid are among the most used [12]. First of all, taking into account 40 mg per kg incorporation rate in feed for β-apo-8'-carotenal as the only pigment source, and lastly 10 mg per kg supplementation rate in feed when a red pigmentation carotenoid such as canthaxanthin is also used; European food safety authority recommends minimum possible doses addition in poultry diets [12]. Consequently, laying hens diets contain more than 50% of yellow corn. This corn is the main source of natural pigments [13]. In addition to its yellow pigments, yellow corn provides energy for more than 3,600 kcal/kg (DM) [14].

So, considering the importance of egg yolk color for consumers, the uses of synthetics coloration agents, and finally due to *Borassus* trees abundance in Africa and Asia, this experiment aimed to test *Borassus aethiopum* ripe fruits' dried pulp acceptability by laying hens and its effects on egg yolks coloration. The hypothesis was that ''Supplementing *Borassus aethiopum* ripe fruits' dried pulp in a white corn-based diets, laying hens would peck. Subsequently, the dried pulp would color the egg yolk''. For this purpose, *Borassus aethiopum* ripe fruits' dried pulp was incorporated into a white corn-based diets for laying hens. Finally, the induced effects on egg yolk weights and colors were assessed.

2. Material and Methods

2.1. Borassus Aethiopum Mature Fruits' Dried Pulp Preparation

After their falls following their physiological maturity, *Borassus aethiopum* ripe fruits were collected. They were sorted and the undamaged by fall chocs and unspoiled ones were kept at room temperature. Later on, the fruits' yellowish hard skin softened a bit. Thereafter, the fruits were peeled and the yellowish fibrous pulp was dried at 70°C in a thin layer in laboratory ovens during 5 days [5]. Finally, the dried product was grounded in a poultry feed mill.

2.2. Cages and Laying Hens' Diets

Thirty-six Harco laying hens of 42 weeks old with similar weight (α=0.05) were used. The birds were allotted in 12 well aerated cages of $4m^2$ (2m*2m, respectively for length*width) each, on a cemented ground. The cages were delimited by large mesh fishing nets. Moreover, the interiors were covered with white wood chips, and equipped with feeders and drinkers. A concentrated commercial premix for laying hens was purchased from an authorized poultry feed seller (*Koudijs, Advanced Nutritional Products, De Heus Animal Nutrition, NL 14841, Netherlands*). Four diets were formulated including one yellow corn-based diet (YC) which was the control, one white corn-based diet (WC), one white corn-based diet in which 10% of the white corn was substituted by *Borassus aethiopum* mature fruits' dried pulp (WC+10%*B.a*) and one white corn-based diet containing 20% dried pulp (WC+20%*B.a*) (Table 1). Each laying hen received 120 g of feed per day. Following, the diets were randomly assigned, resulting in a completely randomized block of 4 diets, 3 hens per diet and 3 repetitions.

Table 1. *Laying hens' diets.*

Ingredients	Diets			
	YC	WC	WC+10%*B.a*	WC+20%*B.a*
Yellow corn	55.2	-	-	-
Premix*	10	10	10	10
Soy bean meal	5	5	5	5
Fish meal	8	8	8	8
Cotton seed meal	15	15	15	15
Egg shell meal	6.8	6.8	6.8	6.8
White corn	-	55.2	45.2	35.2
B.a	-	-	10	20
Total	100	100	100	100
Analyzed nutrient contents				
M. E	2230	2230	2385	2460
Crude Protein (%)	18	17.5	17	15.15
Computed nutrient contents				
Calcium (%)	0.45	0.45	0.45	0.45
Lysine (%)	1.32	1.32	1.32	1.32
Methionine (%)	0.63	0.63	0.63	0.63
Meth + Cyst. (%)	0.9	0.9	0.9	0.9

B.a: *Borassus aethiopum* ripe fruits' dried pulp; M. E: Metabolizable energy given as kcal/kg (DM), YC: Yellow corn; WC: White corn; CP: Crude protein; Cyst.: Cysteine; *Premix: Koudijs, Advanced Nutritional Products, De Heus Animal Nutrition, NL 14841, Netherland; Metabolizable energy 2.230 kcal/kg (DM), Crude protein 43%, Fat 4.4%, Crude fiber 5%, Lysine 2.95%, Methionine 1.4%, Meth + Cysteine 2%, Calcium 1%, Sodium 0.7%, Phosphorus 1.05%

2.3. Egg Yolks Color Evaluation

To begin, 3 eggs of similar weight (μ±δ, α=0.05) were sorted from each day collect, and days 13, 15 and 17 were considered. Following, each egg was broken and the yolk color was assessed through 2 different methods, subjective and objective. The subjective method was done with the Roche yolk color fan, which presents a range of 15 different yellows, from pale to dark yellow. Then, after the visual quantifications, these colors were re-evaluated by a reflectance method by using a spectrophotometer (Shimadzu UV-1601 PC, Kyoto, Japan) equipped with a "D65" system to determine L*, a* and b* components [15]. Its light beams are normalized from 100 to 560 nm values, and the integration time is 10 milliseconds. Some pure egg yolk was poured in the reading tank and the values L*, a* and b* were read. To conclude, some parameters were computed, such as the distance Chroma (C*, (1)) in the plane a*, b*, the angle (H^o, (2)) in degrees in the plane a*, b*, and the distance (h, (3)) in the sphere L*, a*, b*.

C*: the distance in the plane a*, b* and the vertical line L* passes through the origin 0.

$$C^*(\text{Chroma}) = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

H^o: the angle in degrees in the plane a*, b*

$$H^o(\text{hue}) = \frac{[\text{Acot}(a^*/b^*)]*180}{\pi} \quad (2)$$

h: the distance in the sphere L*, a*, b*

$$h = \sqrt{L^{*2} + {a^*}^2 + b^{*2}} \quad (3)$$

2.4. Eggs' Albumens and Yolks Weights

The eggs were collected daily and weighed using a laboratory scale (Radwag, maximum load 2100 g; minimum load 0.5 g; precision 0.01 g). Singularly, the eggs from day 13 to day 18 were stored in a refrigerator at 4°C. Then, on day 19, 3 eggs of similar weight (α=0.05) from each diet were randomly selected. When an egg was broken, its yolk and albumen were weighed before going for color analysis.

2.5. Statistical Analyses

For each analysis, the data were generated in triplicate and were subjected to an analysis of variance using XLSTAT version 2014. The least squares method was used to discriminate the means according to Duncan method. The confidence interval was set at 99% (α=0.01) for egg yolk L*, a* and b* color components. For manipulations generated data such as eggs albumins and yolks weights, the confidence interval was set at 95% (α=0.05).

3. Results and Discussion

3.1. Egg Yolk Color Evaluation with Roche Yolk Color Fan

The egg yolk color first evaluation was assessed with Roche yolk color fan (Figure 1). Overall scores fluctuated between 1 for the pale egg yolk from WC diet and 6 for relatively dark yolks from YC, WC+10%*B.a* and WC+20%*B.a* diets. Apart WC diet egg yolk color, which egg yolk color required a score less than 1 on Roche yolk color fan scale, other egg yolks notations were not easy. Egg yolks from YC, WC+10%*B.a* and WC+20%*B.a* diets were all graded 6.

As paprika pepper (*Capsicum annuum*) [7], and marigold (*Calendula officinalis*) powders [10], the hens assimilated and transferred *Borassus aethiopum* ripe fruits' dried pulp natural pigments to egg yolks. Indeed, *Daucus carota* orange pigments were also accurately transferred to the egg yolks [11, 16]. Furthermore, depending on the pigments source and its concentration in the feed, some variants appear in egg yolk colors.

Thus, the egg yolk obtained from a diet enriched with *Calendula officinalis* is different from another enriched with dried orange peels [8]. Even if, orange peels are yellow-orange as much as the marigold flowers, the grades obtained with the Roche yolk color fan made it possible to differentiate their derived egg yolk colors. Indeed, marigold diet egg yolk was graded 10, while orange peels diet egg yolk was noted 4.5 [8]. On the contrary, sometimes egg color differentiation becomes a tough exercise. For example, when reference [16] mixed dried carrot (*Daucus carota*) and paprika pepper (*Capsicum annuum*) at different rates, trained panelist was not able to make the differences.

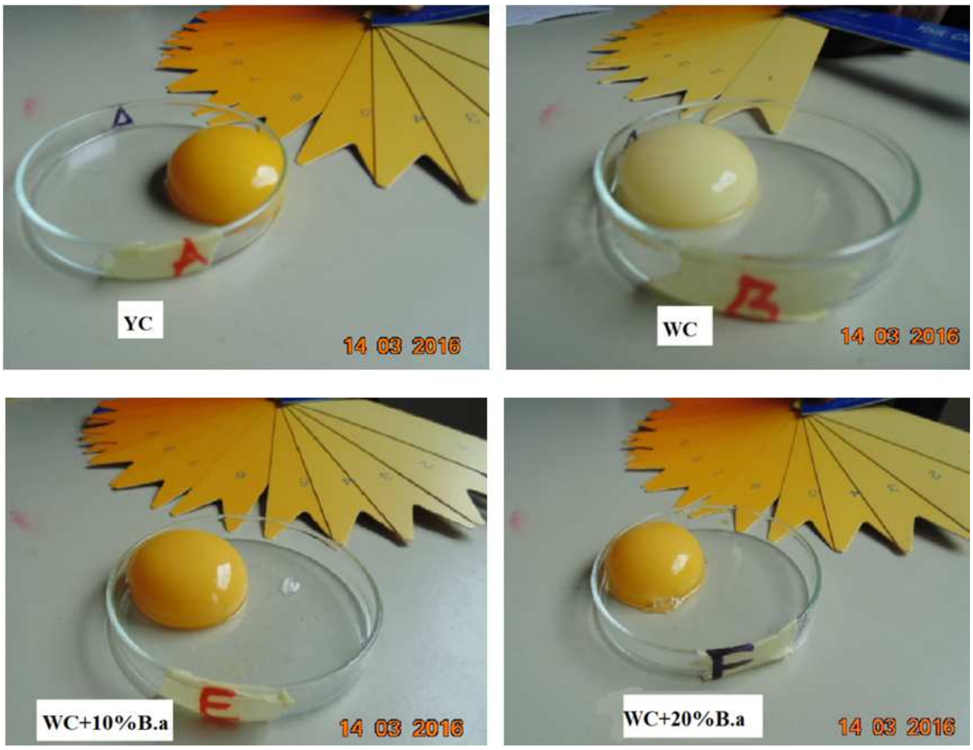

Figure 1. *Egg yolks color evaluation using the Roche yolk color fan.*

Facing diets (D) compositions at different ratios (%) (*Daucus carota*/*Capsicum annuum*, g/g), D1: 1.5/0; D2: 1/0.5; D3: 0.5/1; D4: 0/1.5, the panelist attributed 8.64 to D1 and ended with 14.71 for D4 when they used Roche yolk fan. Obviously, *Capsicum annuum* powder is not more yellow than *Daucus carota* one. These errors were corrected through L*, a* and b* reflectance. Similarly, herein results put the panelist in the same troubles because clear differences were not made between YC, WC+10%*B.a* and WC+20%*B.a* diets derived egg yolks colors.

3.2. Egg Yolk L, a* and b* Color Evaluation*

Facing human eyes' limit for egg yolks colors appreciation with Roche yolk fan color, this method was substituted by a reflectance colorimetric spectrophotometer L*, a* and b* system. Table 2 summarizes the color parameters following diets and days. For the comments, the red-green trend (a*) was ignored because the feed ingredients had no apparent red pigments. Moreover, a* values were negligible compared to those of yellow-blue (b*) trend. Looking at the brightness (L*) on diets level, WC whose L* equaled to 83.20±0.38 was similar to those of WC+10%*B.a* and WC+20%*B.a* which displayed 82.19±0.38 and 82.04±0.38, respectively ($p \geq 0.1006$). Following, WC+10%*B.a* and WC+20%*B.a* eggs yolks brightness was similar to that of YC (81.01±0.38, $p=0.1979$). However, with 2.7% higher gap, WC diet egg yolks brightness was significantly higher than that of YC diet ($p<0.0001$). Following with the yellowness (b*), the comparisons between WC+10%*B.a* (52.29±0.13) versus WC+20%*B.a* (52.49±0.13) ($p=0.3526$), WC+10%*B.a* versus YC (52.22±0.13) ($p=0.7278$), and WC+20%*B.a* versus YC ($p=0.3526$) show that these 3 diets formed a homogeneous group, leading to 52.33±0.13 overall average. This group formed by rich natural pigments diets was highly distinct to WC diet (20.70±0.13, $p<0.0001$).

Table 2. *Diets and days effect on L*, a*, b*, C*, Ho and h.*

Variables	L*	a*	b*	C*	Ho	h
Diets						
WC	83.20±0.38^{a}	0.22±0.07	20.70±0.13^{b}	20.70±0.13^{b}	89.40±0.09^{b}	85.74±0.32^{b}
WC+10%*B.a*	82.30±0.38^{ab}	0.29±0.07	52.29±0.13^{a}	52.29±0.13^{a}	89.68±0.09^{a}	97.42±0.32^{a}
WC+20%*B.a*	82.04±0.38^{ab}	0.20±0.07	52.49±0.13^{a}	52.49±0.13^{a}	89.78±0.09^{a}	97.40±0.32^{a}
YC	81.34±0.38^{b}	0.14±0.07	52.22±0.13^{a}	52.22±0.13^{a}	89.84±0.09^{a}	96.39±0.32^{a}
μ±δ		0.21±0.07				
Days						
13	82.83±0.33	0.32±0.06	44.25±0.12	44.25±0.12	89.54±0.08	94.76±0.28
15	81.86±0.33	0.13±0.06	44.60±0.12	44.60±0.12	89.77±0.08	94.12±0.28
17	81.98±0.33	0.19±0.06	44.43±0.12	44.43±0.12	89.72±0.12	94.12±0.28
μ±δ	82.22±0.33	0.21±0.06	44.43±0.12	44.42±0.12	89.68±0.12	94.33±0.28

Results are given as means (μ) ± standard error (δ)
WC: White corn-based diet;
WC+10%*B.a*: White corn-based diet + 10% of *Borassus aethiopum* ripe fruits' dried pulp;
WC+20%*B.a*: White corn-based diet + 20% of *Borassus aethiopum* ripe fruits' dried pulp;
YC: Yellow corn based-diet;
In the same category [Diets, Days], in the same column, the means with the same exponent are not statistically different, according to Duncan's multiple means ranking test (α=0.01).

Without doubt, WC+10%*B.a*, WC+20%*B.a* and YC diets b* values whose ranged between 52.22±0.13 and 52.49±0.13 were higher than those obtained by reference [7] ($43.7 \leq b^* \leq 49.7$) when they used *Calendula officinalis* flowers powder. Comparatively, herein results were similar to those obtained by reference [17]. For instance, when they used various pigments sources products and obtained 52.5 with the diet containing 35.5% of yellow corn, 49 with a synthetic carotenoid (Carophyll) added for 1.5 mg/kg, 54.6 with 90% lutein powder added for 100 mg/kg and finally 52.9 obtained with Mustard (*Brassica juncea*) powder incorporated for 10g/kg.

Finally, *Borassus aethiopum* ripe fruits' dried pulp strongly influenced egg yolks yellow tendency. The dried pulp yellow coloration could be due to a high content of lutein and zeaxanthin [7]. In contrast, the lack of pigments in white corn led to pale egg yolks. Due to the red tendency (a*) low values, Chroma (C*) distances from the origin in a* and b* plane, and hue angle (Ho) were strongly influenced by the yellow trends (b*). Likewise, h distances depended to L* and b*. In the same way as b* analyses, two distinctive blocks were observed. YC, WC+10%*B.a* and WC+20%*B.a* rich pigments diets delivered nice yellow egg yolks, while WC poor pigments diet which came up with whitish yolks. The WC diet Chroma distance (C*), hue angle (Ho) and distance h were 20.70±0.13; 89.40±0.09 and 85.74±0.32, respectively, and these values were significantly lower than their counterparts from other diets ($p<0.0001$). Again, WC+10%*B.a*, WC+20%*B.a* and YC diets constituted a homogeneous group. Coming back on the factor "Days", L*, a*, b*, C*, Ho and h values were all similar. This aspect indicates that chickens' organisms were adapted to the diets and metabolisms were stabilized after days 13.

3.3. Eggs' Albumins and Yolks Weights

Following the days, egg yolks and albumins weights remained statistically similar (Table 3). That is, egg yolks' weights fluctuated between 13.67±0.18 g and 14.12±0.18 g, a gap of 3.29%. This difference was not statistically significant ($p>0.3978$), so leading to 13.86±0.18 g overall mean. Continuing with the days, similarly, the albumins weights varied between 30.21±0.37 g and 30.82±0.37 g. Again, this additional 0.61 g, which represented 2.02% weight gain was not significant, leading to an overall average of 30.45±0.37 g ($p>0.6618$). In contrast, notable differences were observed within the diets. Accordingly, egg yolks weights fluctuated between 13.64±0.16 g for WC and 14.16±0.16 g for WC+10%*B.a*. This 0.52 g weight gain, 3.81% increase, was significant ($p<0.05$). In the same way, albumins' weights ranged between 29.27±0.33 g for WC+10%*B.a* and 31.92±0.33 g for

WC. This 2.65 g weight gain, which represents a 9.06% increase was highly significant (p<0.0001).

Table 3. *Eggs yolks and albumins weights according to diets and days.*

Egg parts	Diets				Days			
	WC	WC+10%*B.a*	WC+20%*B.a*	YC	15	16	17	18
Yolks (g)	13.64±0.16[b]	14.16±0.16[a]	13.91±0.16[ab]	13.75±0.16[ab]	13.67±0.18	13.71±0.18	13.87±0.18	14.12±0.18
Albumins (g)	31.92±0.33[a]	29.27±0.33[c]	30.18±0.33[bc]	30.41±0.33[b]	30.21±0.37	30.60±0.37	30.24±0.37	30.37±0.37

Results are given as mean (μ) ± standard error (δ)
WC: White corn-based diet; WC+10%*B.a*: White corn-based diet +10% of *Borassus aethiopum* ripe fruits' dried pulp;
WC+20%*B.a*: White corn-based diet+20% of *Borassus aethiopum* ripe fruits' dried pulp; YC: Yellow corn based-diet;
In the same category [Diets, Days], in the same row, the means with the same exponent are not statistically different, according to Duncan's multiple means ranking test (α=0.05).

WC diet egg yolks mean weight (13.64±0.16 g) was significantly smaller than that of WC+10%*B.a* (14.16±0.16 g, p<0.05). Remarkably, WC egg yolk light weights were compensated by heavy weight albumin production. In fact, while WC+10%*B.a* albumin weighed 29.27±0.33 g, that of WC was 31.92±0.33 g. This 2.65 g WC weight gain represented a 9.05% weight increase, and theses weights were different (p<0.0001). On one hand, while WC+10%*B.a* induced the heaviest egg yolks weight (14.16±0.16 g, p<0.05), conversely it produced lighter albumins (29.27±0.33 g, p<0.0001). On the other hand, while WC diet delivered the heaviest albumin (31.92±0.33 g, p<0.0026), on the contrary it produced the lightest egg yolk weight (13.64±0.16 g, p<0.05).

Naturally, rich pigment diets delivered similar egg yolk weights. Indeed, egg yolks from WC+10%*B.a* (14.16±0.16 g), WC+20%*B.a* (13.91±0.16 g) and YC (13.75±0.16 g) were not different (p≥0.2025). So, these high pigments potential diets egg yolks mean weight was 13.94±0.16 g. These results were in agreement with references [7, 10, 18, 19]. They argued that laying hens have the ability to transfer consumed pigments to egg yolks [7, 10] and a rich pigment diet contributes to intense egg yolk color production by increasing the pigment concentration in the yolk [18, 19]. Thus, due to their high pigment contents, YC, WC+10%*B.a* and WC+20%*B.a* diets led to more pigments stock in the corresponding egg yolks, thus leading to heavier egg yolk weights than WC diet.

After all, no significant difference was found following "Days''. The results showed that when the laying hens are fed on a stable diet, egg yolk weights do not greatly change after day 15. Moreover, it may not be necessary to formulate a diet with high natural pigment content. For example, from day 14 to day 18, even though WC+10%*B.a* and WC+20%*B.a* diets outputs were similar for egg yolk weights, numerically, WC+10%*B.a* egg yolks weights were heavier than those of WC+20%*B.a*. As a side result, this lowering egg yolk weight was reported [19], when they incorporated 1 and 2 g marigold (*Tagetes erecta*) extract per kilogram of feed. When they doubled the pigment content, the egg yolk weight dropped from 15.74 to 15.47 g, and that decrease represented 1.72% weight loss. Similarly, from WC+10%*B.a* (14.16±0.16 g) to WC+20%*B.a* (13.91±0.16 g), egg yolk weight was reduced by 0.25 g, this lessening equaled to 1.76% weight loss.

4. Conclusion

In Ivory Coast, looking for some off-farm additional financial resources, rural people are collecting the sap wine from *Borassus aethiopum* trees' apical apex for sales. Unfortunately, most of the time, bled trees die. Moreover, repeatedly, men fall from these smooth, branchless trees and die by looking for some off-farm additional financial resources. On the other hand, women are collecting the fell mature fruits after their physiological maturity, sow them, harvest and sell the hypocotyls a year later. While the men are destroying the trees through their unsustainable practices, women have to go farther and farther away in the bush to collect the fell fruits for their sustainable business. Anyhow, the abundant and yellow-orange fibrous pulp is not either exploited when they sow the ripe fruits. This paper initiates and promotes a valorization of the yellow-orange pulp without impairing the hypocotyls production. We assume that industrial uses of *Borassus aethiopum* ripe fruits' pulps in laying hens' diets could allow a stable market. This new off-farm resources opportunity could slow down the sap wine business, stop dying of farmers from falls from *Borassus aethiopum* trees, and promotes a sustainable new business. To end, the essay demonstrated that laying hens' diets can be supplemented with *Borassus aethiopum* ripe fruits' dried pulp up to 20% without any sanitary inconvenience. At a 10% incorporation rate in a white corn-based diet, *Borassus aethiopum* ripe fruits' dried pulp intensively colored the egg yolks. Thus, this dried pulp is a natural pigment source which could be used for egg yolk coloration. From the 10%, lower incorporation rates of *Borassus aethiopum* ripe fruits' dried pulp could be considered to optimize its uses, by taking a careful look at egg yolk coloration.

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