Effect of Hevea brasiliensis seed meal or Euphorbia heterophylla seed supplemented diets on performance, physicochemical and sensory properties of eggs, and egg yolk fatty acid profile in guinea fowl (Numida meleagris)

G. F. Kouassi, G. A. Koné, M. Good, N.E. E Assidjo, Maryline Kouba

Effect of *Hevea brasiliensis* seed meal or *Euphorbia heterophylla* seed supplemented diets on performance, physicochemical and sensory properties of eggs, and egg yolk fatty acid profile in guinea fowl (*Numida meleagris*)

G. F. Kouassi,[*] G. A. Koné,[*] M. Good,[†] N. E. Assidjo,[*] and M. Kouba[‡,1]

[*]*Institut National Polytechnique Felix Houphouët Boigny, BP 1313 Yamoussoukro, Cote d'Ivoire;* [†]*Independant Researcher and Private Consultant, A96 Dun Laoghaire, Ireland; and* [‡]*UMR 1348 PEGASE, INRA-AGROCAMPUS, 65 rue de Saint Brieuc, 35042 Rennes Cedex, France*

ABSTRACT A total of 144 French selected breed (Galor) female guinea fowl (GF) of 42 wk of age were enrolled for a feeding trial of 15, 30, and 45 D duration. The birds were randomly assigned to 18 cages, each containing 8 birds. A total of 3 isonitrogenous and isocaloric dietary treatments were trialed, each diet comprising 6 replications (cages), which meant a total of 48 birds per diet. The GF were fed either a control diet C (commercial diet "FACI ponte 20", SIPRA, Ivory Coast, usually used for all poultry species) or the diet C supplemented with 5% *Euphorbia heterophylla* seeds (diet E) and the diet C supplemented with 5% Hevea seed meal (*Hevea brasiliensis*) (diet H). Animal performance were assessed for 3 periods (days 0 to 15, 0 to 30, and 0 to 45), and egg quality and composition were assessed at 15, 30, and 45 D of the trial. The results indicated no mortality during the trial. The laying rate was the highest (43.9%) with diet E and the lowest with diet C (32.5%), the laying rate with diet H being intermediate (38.5%). Diet E containing Euphorbia seeds led to a reduced cholesterol content of the eggs. Additionally, inclusion of Euphorbia seeds and, to a lesser extent, of the Hevea seed meal in the diet led to n-3 polyunsaturated fatty acid enriched GF eggs, with thereby, improved nutritional value. A sensory test did not find any difference between the 3 diets on trial.

Key words: guinea fowl, egg, *Hevea brasiliensis*, *Euphoria heterophylla*, n-3 polyunsaturated fatty acid

2020 Poultry Science 99:342–349
http://dx.doi.org/10.3382/ps/pez500

INTRODUCTION

According to the Ivorian poultry farmer association (IPRAVI) data, 1.48 billion eggs were produced for consumption in 2015 in the Ivory Coast. This represented an egg-consumption rate of 64 kg per inhabitant. Despite the importance of the hens (*Gallus domesticus*) in poultry production, guinea fowl (**GF**) (*Numida meleagris*) are increasingly valued for their meat and eggs in West Africa (Sanfo et al., 2007). Indeed, GF consume less feed than chickens, and are more resistant to the most common poultry diseases (Moreki and Seabo, 2012). The increasing cost of feed ingredients such as corn and soybean meal consequent to their increased demand in the biofuel industry, justifies the need to evaluate less-conventional feed ingredients.

With 3% of the world rubber production in the Ivory Coast, Hevea seeds (*Hevea brasiliensis*), which are usually discarded, are in abundant supply (Ducroquet et al., 2017). Hossain et al. (2015) studied the nutritive value of decorticated Hevea seeds in Bangladesh and they assumed that these seeds could be used in animal nutrition. Another local product (an adventitious herb *Euphorbia heterophylla*) can be used to improve animal product quality, like laying hen eggs (Kouakou et al., 2015, 2016). The regular accessibility of Hevea and Euphorbia seeds in the Ivory Coast makes them potential sources of energy and protein in GF diets. Both plants also contain omega-3 fatty acids (Abedi and Ali Sahari, 2014; Kouakou et al., 2015). In monogastric animals, the composition of fatty acids stored in adipose tissue, muscle, or animal products such as eggs largely reflects the ingested lipids (Kouba and Mourot, 2011). Nutritionists recommend that humans increase n-3 polyunsaturated fatty acid (**PUFA**) intake, because, whatever the country, the human diet is ordinarily very low in n-3 PUFA (Stark et al., 2016). Indeed, n-3 fatty acids decrease serum triglyceride and cholesterol content, 2 factors implicated in the risk of coronary heart disease, hence, increasing n-3 PUFA dietary intake reduces coronary heart disease risk (Conquer and Holub, 1998).

The aim of this research was: (1) to evaluate the effect of supplementing GF diet with Hevea seed meal or Euphorbia seeds on laying performance and egg characteristics of French Galor GF; and (2) to evaluate the

Received May 20, 2019.
Accepted August 15, 2019.
[1]Corresponding author: maryline.kouba@agrocampus-ouest.fr

effect of supplemented diets on the quality of the eggs, particularly on their omega-3 fatty acid content.

MATERIALS AND METHODS

Ethics Statement

The animals used were reared and slaughtered in compliance with regulations for the humane care and use of animals in research, according to EU directive 86/6096. (National Authorization to Experiment on alive animals n°3502 delivered by the French Minister of Agriculture).

Trial Site Location

The study was carried out in the Ivory Coast, at the National Polytechnic Institute Félix Houphouët-Boigny of Yamoussoukro (INP-HB), from April to June 2017 in collaboration with the National Institute for Agronomic Research (INRA), France.

Animals and Diets

A total of 144 female GF from French selected breed (Galor) were divided into 3 groups of 48 birds when 42 wk old. Each group of 48 birds were randomly penned, 8 per 6 m^2 cage to provide 6 groups of 8 birds, and received 1 diet (6 replicates per diet). The GF of each group received a control diet C (commercial diet, FACI ponte 20, SIPRA, Ivory Coast) or diet C supplemented with 5% *Euphorbia heterophylla* seeds (Diet E) or diet C supplemented with 5% Hevea (*Hevea brasiliensis*) seed meal (diet H). During the 45-day study, the birds received diet and water ad libitum. The experimental diets E and H were formulated to meet the requirements of the birds. They were manufactured at the Animal Science Laboratory of INP-HB, Yamoussoukro, Ivory Coast. Meals were prepared from decorticated Hevea seeds (diet H), by using a screw press to extract the maximum amount of oil. The Hevea seed meal was sun-dried for 5 D and heated with wood fire half an hour to reduce the cyanide content. The 3 diets (C, E, and H) were designed to be isonitrogenous and isocaloric (Table 1).

Diet Analyses

Samples of the 3 diets were analyzed for dry matter, mineral matter, starch, and crude proteins (N × 6.25) according to Association of Official Analytical Chemists (AOAC, 2007). Lipids were extracted according to Folch et al. (1957). Total fibers were determined using Fibertec System 1021 Cold extractor (Saint André de Cubzac, France). Dietary hydrogen cyanide content was analyzed in diet H, according to the procedure of Deniges (1893). Mineral macro-element calcium and phosphorus were analyzed by Atomic Absorption Spectrometer (Varian Spectraa 20, IET, Mundelein, USA).

Table 1. Ingredient and chemical composition of diets (control diet (C), *Euphorbia heterophylla* seed supplemented diet (E), *Hevea brasiliensis* seed meal supplemented diet (H)).

	Diet		
	C	E	H
Ingredients (%)			
Commercial feed	100	95	95
Euphorbia seeds	0	5	0
Hevea seed meal	0	0	5
Analyses (%)			
Dry matter	92.7	92.8	91.5
Ash	11.9	12.2	12.1
Lipid	8.4	8.6	9.0
…CP	15.5	15.7	15.9
…Starch	11.7	11.6	11.7
…Crude fiber	5.5	7.0	6.2
Hydrogen cyanide (µg/kg)	0	0.02	0.05
Metabolizable energy (MJ/kg)*	14.5	14.2	14.7
Minerals (%)			
Calcium	3.2	3.5	3.3
Phosphorus	1.5	2.1	2.2
Calcium/Phosphorus ratio	2.0	1.7	1.5
Fatty acids (FA), % of total FA			
SFA	40.4	32.9	41.8
MUFA	31.4	25.8	35.1
PUFA	28.2	41.3	28.1
n-6	26.3	24.9	17.4
n-3	1.83	16.4	5.50
PUFA/SFA	0.81	1.42	0.41
n-6n/n-3	14.4	1.52	3.16
18:2 n-6/18:3 n-3	18.9	1.53	7.48
Fatty acids (FA), % of total FA in seeds	Hevea		Euphorbia
n-6	37.1		15.5
n-3	21.2		56.0

*ME = Metabolisable energy (ME) calculated according to Fisher and McNab method, ME (MJ/kg) = (0.155xCP) + (0.343xFat) + (0.167xStarch) + (0.130xSugar) (Fisher and McNab, 1987).

SFA = sum of saturated fatty acids, MUFA = sum of monounsaturated fatty acids, PUFA = sum of polyunsaturated fatty acids, n-6/n-3 = sum of n-6 fatty acids/sum of n-3 fatty acids ratio.

PUFA/ SFA ratio = (18:2n-6 + 18:3n-3)/(14:0 + 16:0 + 18:0) (Kouba et al., 2003).

Animal Performance

Health status and mortality were monitored throughout the trial. Individual BW of the animals was measured at the start, at 15 D, 30 D, and 45 D of trial and the daily weight gain was calculated. Feed intake was measured each day per cage throughout the trial and eggs were collected daily. Feed to egg ratios (F/E) and laying rates (**LR**) were calculated for each diet for the 3 periods (days 0 to 15, 0 to 30, and 0 to 45).

At the end of the trial (45 D), a blood sample was collected from 2 fasting birds per cage (12 animals per diet) for liver enzyme analysis. Plasma was separated by centrifugation at 2,000 × *g* for 15 min at 4°C, and then frozen at −20°C until analysis. The birds were then stunned, slaughtered, scalded (water at 60°C) and plucked manually.

Egg Physical and Chemical Parameter Determination

On trial-day 15, 30, and 45, 12 eggs per diet (2 eggs/cage/diet) were kept for 1 D in the hatchery cooler at 15°C and 70% RH. Analysis the following morning measured the length and the diameter of each egg at the equator with a micrometer with a 0.01 mm accuracy (Tenessee Speed Sport, Goodlettsville, USA). The egg index I (which determines the egg solidity), was calculated as the ratio: I = D/L with D = diameter at the equator of each egg and L = length of each egg.

The eggs were weighed and broken open to determine yolk, albumen, and shell characteristics. Entire egg, shell (with its internal membranes), yolk, and albumen weights were recorded. Eggshell (with its internal membranes) thickness was measured with a micrometer with a 0.01 mm accuracy (Tenessee Speed Sport, Goodlettsville, USA). Yolk color was determined under natural light using a DSM Yolk color fan (scale from 1 to 15) (DSM, Basel, Switzerland). The height of the albumen was measured, using a micrometer with a 0.01 mm accuracy (Tenessee Speed Sport, Goodlettsville, USA). The Haugh unit (HU) which denotes egg freshness was determined by the formula $HU = 100 \times \log(h - 1.7 \times W^{0.37} + 7.6)$ with h = height of the thick albumen at 1 cm from the yolk and W = egg weight.

Selected eggs were analyzed for chemical composition. Dry matter, ash, and crude proteins (N × 6.25) were determined in the yolk and albumen of each egg, according to Association of Official Analytical Chemists (AOAC, 2007). Mineral macro-element Ca and P were analyzed in yolk by Atomic Absorption Spectrometer (Varian Spectraa 20, IET, Mundelein, USA). Yolk total cholesterol content was determined using commercial kits (Cypress Diagnostics SRL, Italy). Lipids were extracted from egg yolk and samples of diets according to Folch et al. (1957). Fatty acid composition was measured after methylation of samples. Fatty acid methyl esters were prepared with brome trifluorid methanol according to Morrison and Smith (1964). They were analyzed on an Agilent Technologies 6890N gas chromatograph (Bios Analytic, Toulouse France), with an internal standard (C21:0, Sigma-Aldrich, France) used to quantify fatty acids (g/100 g of total fatty acids). Nutritional quality was described by the polyunsaturated fatty acids (PUFA)/ saturated fatty acids (**SFA**) ratio expressed as: (18:2n-6 + 18:3n-3)/(14:0 + 16:0 + 18:0) (Kouba et al., 2003). The 18:2n-6/18:3n-3 ratio is relevant to the competition for synthesis of longer-chain PUFA. Because this ratio ignores the existence of longer-chain PUFA in the egg, the results were also expressed as sum of n-6 PUFA/sum of n-3 PUFA.

Plasma Analyses

Serum glutamic-pyruvic acid transaminase (**SGPT**) and serum glutamic-oxaloacetic transaminase (**SGOT**) activities were measured using commercial kits (Cypress Diagnostics SRL, Italy)

Organoleptic Tests

A taste test was conducted to evaluate preference. 18 eggs per diet were collected (3 eggs/cage) at 15, 30, and 45 D of experiment (a total of 54 eggs for each time on feed). All the eggs were prepared in the same manner, 2 h before the organoleptic test started. Eggs from each treatment were placed in water at room temperature, brought to boiling for 5 min, then taken from the pot and placed under cold running water for 10 min. The eggs were shelled, cut longitudinally in 2 halves, and placed in separate containers. Based on their egg consumption frequency (at least twice monthly) a panel of 9 men and 9 women was recruited. All panelists were staff members and students from the University of Agronomy (Ecole Supérieure d'Agronomie de Yamoussokro, Ivory Coast), and Ph.D. students from the National Polytechnic Institute Félix Houphouët-Boigny of Yamoussoukro. Relying on these criteria, panelists received 3 h training per day for 3 D focusing on the sensory characteristics of the GF egg: the texture, flavor, odor, and yolk color. Scales were also developed during the training sessions. The scale ranged from 1 (low expression of the attribute, i.e., not acceptable) to 10 (high expression of the attribute, i.e., excellent).

Sensory assessments were performed using the 18 trained adult panelists. A total of 3 half eggs were presented to each panelist on a small plate. Each of the plates (at 15, 30, and 45 D) comprised eggs from the 3 diets (control, Euphorbia seeds supplemented diet, and Hevea seed meal supplemented diet), 1 half egg per diet in the plate. Panelists rated eggs on the previously developed ten-point scale for texture, flavor, odor, yolk color, and overall liking (1 = extremely weak flavor, no odor, extremely weak yolk color, and dislike extremely; 10 = extremely strong flavor, extremely good odor, extremely high yolk color, and like extremely).

Statistical Analyses

Data were analyzed by the ANOVA option of the LMG of R 3.4.2 software (Copyright 2016, R Foundation for Statistical Computing Platform) as a 3 × 3 factorial arrangement of dietary treatments with times on feed as main effects. The statistical model used was $Yijkl = \mu + Di + Sj + (DS)ij + Rijk + \gamma ijkl$, where Yijkl = response variables from each individual replication or cage, μ = the overall mean; Di = the effect of dietary; Sj = the effect of time on feed; (DS)ij = the effect due to interactions between dietary and time on feed; Rijk = the inter-experimental unit (replications) error term; and γijkl = the intra-experimental unit error term. Two-way interactions between dietary

Table 2. Performance of guinea fowl fed control diet (C), *Eupohorbia heterophylla* seed supplemented diet (E), *Hevea brasiliensis* seed meal supplemented diet (H). Diets for 15, 30, or 45 D.

	Diet				Time on feed (D)				Probability		
Parameters	C	E	H	SEM	15	30	45	SEM	Diet	Time	Diet × Time
BW (g)	2,386	2,448	2,496	36.0	2,456	2,414	2,461	36.0	$1.1e^{-1}$	$6.0e^{-1}$	NS
FER	9.3[b]	5.2[a]	6.4[a]	0.98	7.2	7.3	6.4	0.98	$2.3e^{-2}$	$7.9e^{-1}$	NS
Egg production (%)	32.5[a]	43.9[c]	38.5[b]	1.8	43.1[b]	37.7[a,b]	34.1[a]	1.8	$<1e^{-4}$	$6e^{-4}$	NS

[a–c]Parameter means within rows of diet or strain with no common superscript differ ($P < 0.05$).
FER: feed to egg ratio.

Table 3. Characteristics of eggs from guinea fowl fed control diet (C), *Euphorbia heterophylla* seed supplemented diet (E), Hevea brasiliensis seed meal supplemented diet (H). Diets for 15, 30, or 45 D.

	Diet				Time on feed (D)				Probability		
Parameters	C	E	H	SEM	15	30	45	SEM	Diet	Time	Diet × Time
Egg weight (g)	45.8	44.2	44.8	0.70	43.5[a]	45.1[a,b]	46.2[b]	0.70	0.27	$3e^{-2}$	NS
Shell thickness (mm)	0.67	0.68	0.64	0.01	0.69[b]	0.67[b]	0.63[a]	0.01	$0.87e^{-1}$	$1e^{-2}$	NS
Egg shape index	77.1	76.2	76.1	1.6	77.1	75.2	77.1	1.7	$7.24e^{-1}$	$5.13e^{-1}$	NS
Shell proportion (%)	18.0	18.3	17.6	0.30	17.5[a]	18.9[b]	17.6[a]	0.30	$2.97e^{-1}$	$3e^{-3}$	NS
Albumen proportion (%)	50.1	49.3	49.8	0.56	49.1	49.9	50.3	0.56	$5.88e^{-1}$	$3.25e^{-1}$	NS
Yolk proportion (%)	31.8	32.4	32.6	0.46	33.4[b]	31.3[a]	32.1[a]	0.46	$5.09e^{-1}$	$7e^{-3}$	NS
Haugh	81.1	82.8	86.5	1.79	77.8[a]	85.4[b]	87.2[b]	1.83	$1.11e^{-1}$	$2e^{-3}$	NS
Yolk color	8.7	9.0	9.0	0.22	9.2	8.9	8.7	0.22	$4.11e^{-1}$	$2.86e^{-1}$	NS

Egg shape index = egg width/egg length; yolk color: measured with the DSM Yolk Fan with shades 1 to 15.
[a,b]Parameter means within rows of diet or strain with no common superscript differ ($P < 0.05$).

and time on feed were not significant, thus, data were analyzed for main effects. Least significant difference comparisons were made between treatment means for main effects when there was a significant F-value. Significance implies $P < 0.05$, unless stated otherwise.

RESULTS

Composition of Diets

The characteristics of the 3 diets are presented in Table 1. The diets were isonitrogenous and isocaloric. The proportion of dry matter was similar. The hydrogen cyanide content of diets E and H was very low. Diet E and to a lesser extent diet H were rich in PUFA among which n-3 fatty acids represented respectively more than 16% and 5.5% of the total fatty acids. Control diet C exhibited the lowest n-3 fatty acid proportion (1.83%).

Animal Performance

The GF performances are presented in Table 2. There was no mortality during the trial. There was no effect of diet or time duration on the diet on the GF weights. Diets E and H significantly improved the feed to egg ratio. The LR was the highest (43.9%) with diet E, the lowest with diet C (32.5%), and with diet H intermediary (38.5%). There was no feed to egg ratio time duration effect; however, there was an overall decrease of the LR, with time.

Egg Characteristics and Composition (Tables 3 and 4)

None of the egg characteristics (weight, proportions of albumen, yolk and shell, yolk-color, shell-thickness, shape index, and HUs) were affected by the diet. There was an increase of egg weight and HUs with time as well as a decrease of shell thickness with time; the proportions of albumen and shell, the yolk color, and the shape index remained consistent over time. Yolk proportion however, decreased with time.

In egg yolk, the dry matter proportion was higher from animals fed diet H than from birds fed diets C or E; the protein proportion was the highest from animals fed diet E whereas the lipid proportion was the lowest with diet E; the cholesterol content was the lowest from birds fed diet E compared to diets C or H. The dry matter proportion of egg yolks increased with time. Proportions of lipids and protein fluctuated over time, with the highest lipid and the lowest protein proportions at 30 D of trial; proportions of ash and cholesterol in egg yolk did not vary with time.

In albumen, the protein proportion was lower from both supplemented diets compared to the control diet. The protein proportion increased with time, whereas

Table 4. Composition of eggs from guinea fowl fed control diet (C), *Euphorbia heterophylla* seed supplemented diet (E), *Hevea brasiliensis* seed meal supplemented diet (H). Diets for 15, 30, or 45 D.

	Diet				Time on feed (D)				Probability		
Parameters	C	E	H	SEM	15	30	45	SEM	Diet	Time	Diet × Time
Yolk composition (%. w/w)											
Dry matter	52.6[a]	52.6[a]	53.7[b]	0.22	52.2[a]	53.3[b]	53.4[b]	0.22	$3e^{-3}$	$2e^{-3}$	NS
Ash	2.22	1.97	1.96	0.11	2.03	1.94	2.18	0.11	$1.89e^{-1}$	$3.25e^{-1}$	NS
Lipids	29.6[b]	27.2[a]	29.9[b]	0.23	28.3[a]	30.4[b]	28.3[a]	0.23	$<1e^{-4}$	$<1e^{-4}$	NS
Proteins	20.8[a]	23.4[c]	21.8[b]	0.06	21.9[b]	21.0[a]	22.9[c]	0.06	$<1e^{-4}$	$<1e^{-4}$	NS
Cholesterol per egg (g)	0.32[b]	0.27[a]	0.31[b]	0.01	0.30	0.30	0.30	0.13	$<1e^{-4}$	$2.98e^{-1}$	NS
Calcium	1.30[c]	0.98[a]	1.08[b]	0.01	1.31[c]	1.1[b]	0.95[a]	0.01	$<1e^{-4}$	$<1e^{-4}$	NS
Phosphorus	0.79[a]	0.90[c]	0.84[b]	0.01	0.78[a]	0.83[b]	0.92[c]	0.01	$<1e^{-4}$	$<1e^{-4}$	NS
Albumen composition (%. w/w)											
Dry matter	15.1	14.4	14.5	0.23	14.4	14.7	14.8	0.23	$9.1e^{-2}$	$5.74e^{-1}$	NS
Ash	0.75	0.74	0.75	0.01	0.72[a]	0.78[b]	0.74[a]	0.01	0.31	$<1e^{-4}$	NS
Proteins	13.7[c]	13.2[b]	13.0[a]	0.02	13.2[a]	13.4[b]	13.4[b]	0.02	$<1e^{-4}$	$<1e^{-4}$	NS

[a–c]Parameter means within rows of diet or strain with no common superscript differ ($P < 0.05$).

there was no diet or time duration effect on dry matter and ash proportions.

Fatty Acid Profiles of Egg Yolks

The fatty acid compositions of egg yolks of GF fed the different diets are presented in Table 5. The SFA percentages were not affected by the diet nor by the time duration on the diet. The proportions of the monounsaturated fatty acids (**MUFA**) (oleic acid representing 90% of total MUFA for all the diets) were the highest from diets C and E (35.3 and 35.0%, respectively), and the lowest with diet H (34.2%). The MUFA proportion decreased with the duration of time on the diet. The percentage of total PUFA was the highest with diet H. Among these PUFA, the proportion of n-6 PUFA was the highest with diet H, and this proportion decreased with the duration of time on the diet. Egg yolks of birds fed the Euphorbia seeds supplemented diet (diet E) exhibited 2.4 and 1.4 times more total n-3 PUFA percentage than eggs from birds fed respectively diets C or H. This much higher proportion of n-3 PUFA in the eggs of diet E fed birds was observed for all the n-3 PUFA, the precursor linolenic acid (LA) C18:3 n-3 as well as the long-chain n-3 PUFA. To a lesser extent, eggs from diet H fed GF also exhibited 1.7 times more total n-3 percentage than eggs from birds fed the control diet C. The PUFA/SFA ratio was the lowest in eggs of birds fed the control diet C. The ratios n-6/n-3 and C18:2 n-6/C18:3 n-3 were the lowest in eggs of birds fed diet E (3.4 and 7.6, respectively). The ratios n-6/n-3 and C18:2 n-6/C18:3 n-3 obtained in the eggs of GF fed diets C and H were higher (9.0 and 5.4 for the n-6/n-3 ratio and 35.5 and 12.4 for the C18:2 n-6/C18:3 n-3 ratio, respectively).

Blood Biochemistry

SGPT and SGOT activities were not affected by the diet (Table 6).

Sensory Results

Sensory results from the trained taste-panel are given in Table 7. There was no diet effect on texture, flavor, and overall liking. However, eggs from diet E had higher odor liking scores and higher yolk color score than those from diets C or H. There was a time effect on all the sensory attributes. At 30 D of trial the scores were better for texture and flavor parameters than scores at days 15 or 45 of trial. The odor liking scores were better for eggs at day 15 of trial and the color obtained better scores at days 30 and 45 of trial. The scores of overall liking were the best for eggs at days 15 and 45 of trial.

DISCUSSION

The hydrogen cyanide content in diets E and H was very low and consequently HCN levels in eggs would be extremely low and would pose no risk to consumers. Egg production and its decrease with time were similar to results on GF of Bernacki et al. (2013). However contrary to the study of Huang et al. (2018) on laying hens, in our study egg production was affected by the diet, being the highest with diet E and intermediary with diet H, and the lowest with control diet. Dudusola (2010) found egg weight (46.7 g) from GF from Benin, similar to the weight we reported in the present study. However, lower egg weight in Ghanaian GF was found by Sarfo et al. (2018), and by Bashir et al. (2015) in Nigerian GF, compared to the weights we observed in the present study. All these studies were not using the diets used in this study but served as a reference for the range of normal parameters found in Africa. The increase of egg weight with time that we observed in the present study has been reported earlier in laying hens. Authors showed that egg weight generally increased with hen's age (Van den Brand et al., 2004). The egg shape index was similar to results of Bernacki et al. (2013), Kgwatalaia et al. (2013), and Obike et al. (2011). The albumen proportion was lower than results of Song et al. (2000) and Dudusola (2010)

Table 5. Effect of dietary and time on feed on the fatty acid (FA) composition (% of total FA) of egg yolks from guinea fowl fed: control diet C, *Euphorbia heterophylla* seed supplemented diet E, or *Hevea brasiliensis* seed meal supplemented diet H. for 15, 30, or 45 D.

	Diet				Time on feed (D)				Probability		
Parameter	C	E	H	SEM	15	30	45	SEM	Diet	Time	Diet × Time
C12:0	0.08	0.07	0,08	0.03	0.06	0.07	0.08	0.02	$5.51e^{-2}$	$5.11e^{-2}$	NS
C14:0	1.51	1.26	1.24	0.27	1.25	1.31	1.33	0.28	$6.04e^{-2}$	$5.73e^{-2}$	NS
C14:1 (n-5, cis-9)	0.09	0.11	0.08	0.03	0.09	0.09	0.10	0.03	$5.39e^{-2}$	$6.72e^{-2}$	NS
C15:0	0.13	0.11	0.12	0.05	0.12	0.12	0.13	0.04	$8.32e^{-2}$	$7.58e^{-2}$	NS
C16:0	26.6	26.9	26.0	0.92	26.5	26.2	26.8	0.82	$5.16e^{-2}$	$5.33e^{-2}$	NS
C16:1 (n-9, cis-9	0.57	0.51	0.52	0.07	0.59	0.51	0.50	0.09	$7.47e^{-2}$	$5.86e^{-2}$	NS
C16:1 (n-7, cis-9)	1.54	1.61	1.41	0.20	1.66	1.45	1.39	0.27	$5.82e^{-2}$	$6.69e^{-2}$	NS
C18:0	14.3	13.8	14.3	0.6	13.9	14.6	13.9	0.73	$6.42e^{-2}$	$5.27e^{-2}$	NS
C18:1 (n-9, cis-9)	31.8	31.6	30.9	0.85	32.4^{b}	31.0^{a}	30.8^{a}	0.81	$6.84e^{-2}$	$4.46e^{-2}$	NS
C18:1 (n-7, trans-9)	1.09	1.02	1.05	0.14	1.07	1.10	0.97	0.14	$6.12e^{-2}$	$5.41e^{-2}$	NS
C18:2 (n-6, cis-9,12)	16.7^{b}	15.3^{a}	17.7^{c}	0.55	15.9^{a}	16.5^{a}	17.4^{b}	0.53	$8.34e^{-4}$	$4.05e^{-2}$	NS
C18:3 n-6, (cis-6,9,12)	0.16	0.16	0.15	0.07	0.16	0.15	0.16	0.06	$6.73e^{-2}$	$5.82e^{-2}$	NS
C18:3 (n-3, cis-6,9,12)	0.47^{a}	2.01^{c}	1.43^{b}	0.2	1.21	1.25	1.44	0.22	$2.13e^{-3}$	$6.5e^{-2}$	NS
C20:0	0.07	0.10	0.13	0.06	0.03^{a}	0.15^{b}	0.13^{b}	0.07	$8.23e^{-2}$	$3.7e^{-2}$	NS
C20:1 (n-9, cis-11)	0.20	0.18	0.21	0.05	0.21	0.19	0.20	0.05	$5.64e^{-2}$	$6.42e^{-2}$	NS
C20:2 (n-6, cis-11,14)	0.15	0.16	0.18	0.03	0.16	0.16	0.15	0.03	$5.88e^{-2}$	$7.51e^{-2}$	NS
C20:3 (n-6, cis-8,11,14)	0.23	0.24	0.27	0.15	0.25	0.29	0.20	0.15	$5.12e^{-2}$	$6.06e^{-2}$	NS
C20:4 (n-6, cis-5,8,11,14)	2.01	1.50	1.74	0.53	1.67	1.99	1.58	0.49	$6.36e^{-3}$	$1.73e^{-1}$	NS
C20:3 (n-3, cis-11,14,17)	0.00^{a}	0.06^{b}	0.05^{b}	0.03	0.03	0.04	0.04	0.03	$4.95e^{-2}$	$5.16e^{-2}$	NS
C20:5 (n-3, cis-5,8,11,14,17)	0.06	0.13	0.09	0.07	0.06	0.11	0.10	0.05	$5.22e^{-2}$	$5.83e^{-2}$	NS
C22:4 (n-6, cis-7,10,13,16)	0.13^{b}	0.05^{a}	0.02^{a}	0.03	0.03^{a}	0.10^{b}	0.05^{a}	0.03	$3.34e^{-2}$	$3.69e^{-2}$	NS
C22:5 (n-6, cis-4,7,10,13,16)	0.41^{b}	0.08^{a}	0.10^{a}	0.02	0.21	0.20	0.18	0.03	$4.21e^{-2}$	$5.27e^{-2}$	NS
C22:5 (n-3, cis-7,10,13,16, 19)	0.06	0.21	0.12	0.09	0.13	0.15	0.18	0.1	$5.46e^{-2}$	$7.53e^{-2}$	NS
C22:6 n-3, cis-4,7,10,13,16,19)	1.52^{a}	2.75^{b}	2.00^{c}	0.15	2.01	2.11	2.16	0.15	$2.26e^{-3}$	$6.07e^{-2}$	NS
SFA	42.7	42.2	41.8	0.84	41.9	42.5	42.3	0.62	$5.21e^{-2}$	$5.86e^{-2}$	NS
MUFA	35.3^{b}	35.0^{b}	34.2^{a}	0.45	36.1^{b}	34.4^{a}	33.9^{a}	0.44	$4.63e^{-2}$	$4.22e^{-2}$	NS
PUFA	22.0^{a}	22.7^{a}	23.9^{b}	0.70	21.9^{a}	23.1^{b}	23.7^{b}	0.61	$3.55e^{-2}$	$2.83e^{-2}$	NS
n-6	19.7^{b}	17.4^{a}	20.1^{b}	0.37	18.3^{a}	19.3^{b}	19.6^{b}	0.37	$6.02e^{-3}$	$5.03e^{-3}$	NS
n-3	2.11^{a}	5.17^{c}	3.69^{b}	0.51	3.44	3.66	3.93	0.50	$1.74e^{-3}$	$6.9e^{-2}$	NS
PUFA/SFA	0.24^{a}	0.41^{b}	0.46^{b}	0.1	0.41	0.42	0.45	0.6	$1.2e^{-2}$	0.2	NS
n-6/n-3	9.04^{c}	3.37^{a}	5.44^{b}	0.33	5.33	5.27	5.00	0.33	$1.83e^{-3}$	$5.32e^{-2}$	NS
18:2 n-6/18:3 n-3	35.5^{c}	7.61^{a}	12.4^{b}	3.9	13.1^{b}	13.2^{b}	12.1^{a}	0.30	$2.1e^{-2}$	$1.73e^{-2}$	NS

SFA = sum of saturated fatty acids, MUFA = sum of monounsaturated fatty acids, PUFA = sum of polyunsaturated fatty acids, n-6/n-3 = sum of n-6 fatty acids/sum of n-3 fatty acids ratio.

PUFA/ SFA ratio = (18:2n-6 + 18:3n-3)/(14:0 + 16:0 + 18:0) (Kouba et al., 2003).

[a–c]Parameter means within rows of diet or breed with no common superscript differ ($P < 0.05$).

Table 6. Blood parameters of guinea fowl fed diets control diet (C), *Euphorbia heterophylla* seed supplemented diet (E), *Hevea brasiliensis* seed meal supplemented diet (H) (n = 12 animals per diet).

	Diets			
Hepatic enzymatic activities	C	E	H	*P*-value
SGPT (U/L)	3.1	3.5	3.5	0.3
SGOT (U/L)	157.5	157.8	159	0.9

SGPT = serum glutamic-pyruvic transaminase.

SGOT = serum glutamic-oxaloacetic transaminase.

while yolk proportion was similar. Haugh units were in accordance with results of Kgwatalaia et al. (2013) and Sarfo et al. (2018). Eggs of the present study exhibited less intensive yolk color than eggs in the study of Bernacki et al. (2013). However, this trait depends mainly on the content of carotenoid pigments in GF diets.

The egg yolk in the present study exhibited a higher dry matter content than results of studies of Bashir et al. (2015), Dudusola (2010), and Song et al. (2000), with a much higher content of protein and a lower fat content. The ash content was in the range of the results of these authors.

The egg cholesterol content (570 mg for 100 g of eggs) in the present study was slightly higher than results of Maurice et al. (1994) and Oguntona and Hughes (1988) who found a cholesterol content of 550 and 507 mg for 100 g of eggs, respectively. The egg cholesterol content can be reduced by dietary means. Indeed feeding laying hens n-3 supplemented diets can lead to a reduction of cholesterol in egg yolk. This results was in accordance with data of several authors who used Chia (Ayerza and Coates, 2000), canola oil (Ismail et al., 2013), linseed or fish oil (Dalle Zotte et al., 2015), or *Euphorbia heterophylla* (Kouakou et al., 2015). However, other authors found no effect of n-3 supplemented diets on the egg cholesterol content (Carillo-Dominguez et al., 2005; Faitarone et al., 2013). In the present study, there was a strong effect of diet E containing Euphorbia seeds that decreased the egg cholesterol content by 19% as

Table 7. Eating quality of boiled eggs (1 to 10 scale)[*] of guina fowl fed trial diets: control diet (C), *Euphorbia heterophylla* seed supplemented diet (E), or *Hevea brasiliensis* seed meal supplemented diet (H).

	Diets				Time on feed (D)				Probability		
Parameterss	C	E	H	SEM	15	30	45	SEM	Diet	Time	Diet × Time
Texture	3.5	3.4	3.4	0.11	2.1[a]	4.5[c]	4.0[b]	0.11	$3.39e^{-1}$	$<1e^{-4}$	NS
Flavor	6.1	6.1	6.2	0.12	5.2[a]	6.7[c]	6.3[b]	0.11	$8.56e^{-1}$	$<1e^{-4}$	NS
Odor	3.6[a]	3.9[b]	3.4[a]	0.10	4.7[b]	3.0[a]	3.2[a]	0.09	$2.53e^{-2}$	$<1e^{-4}$	NS
Yolk color	6.9[a]	7.6[b]	6.9[a]	0.08	6.7[a]	7.4[b]	7.3[b]	0.07	$<1e^{-4}$	$<1e^{-4}$	NS
Overall liking	6.1	6.3	6.2	0.12	6.7[b]	5.5[a]	6.4[b]	0.11	$3.59e^{-1}$	$<1e^{-4}$	NS

[*]1 = extremely weak flavor, no odor, extremely weak yolk color, and dislike extremely; 10 = extremely strong flavor, extremely good odor, extremely high yolk color, and like extremely.

[a–c]Parameter means within rows of diet or strain with no common superscript differ ($P < 0.05$).

was the case for the eggs of laying hens fed *Euphorbia heterophylla* seeds supplemented diet (Kouakou et al., 2015).

Hepatic serum active levels of SGOT and SGPT are routinely used as biomarkers of liver injury. These enzymes are usually present in negligible concentration. An increase in the concentration of these enzymes may be the sign of damaged or diseased cells, which denotes the status of the liver function. SGOT is a very sensitive, nonspecific biomarker of bird liver disease. However, SGPT has poor specificity for liver disease in birds due to its existence in many tissues. Our results of SGOT activity (157.5 to 159 U/L) in GF were low compared to results in chickens of Wang et al. (2017) (174 to 190 U/L). SGPT activity (3.1 to 3.5 U/L) in GF was in the range of results of these authors (1.83 to 3.17). There was no diet effect on both enzyme activities, which confirmed the lack of GF liver injury whatever the diet.

In monogastric animals, the composition of fatty acids stored largely reflects that of the ingested lipids and the fatty acid composition of hen eggs can be changed by dietary means (Kouba and Mourot, 2011). Therefore, eggs can be a significant source of n-3 PUFA when poultry are fed high n-3 PUFA supplemented diets. These enriched eggs can therefore serve as a vehicle for supplying nutrients such as the n-3 fatty acids (FA). In our study, feeding Euphorbia seeds (diet E) was associated with a reduction of n-6 PUFA proportion and a strong increase in n-3 PUFA proportion of GF egg compared to the control diet C. To a lesser extent, eggs from diet H fed GF also exhibited a higher n-3 PUFA content, compared to the control diet C. This increase was largely attributable respectively to Euphorbia seeds and to Hevea seeds that are a source of n-3 PUFA (Abedi and Ali Sahari, 2014; Kouakou et al., 2015). There is no study on the effect of n-3 supplemented diet on fatty acid composition of GF eggs; so, our comparisons will be with other poultry species.

The increase in n-3 PUFA proportion in the egg yolk of birds fed diet E or H was due to an increase of all the n-3 PUFA proportions [C18:3 n-3, Linolenic acid (**LA**); C20:5 n-3, Eicosapentaenoic acid (**EPA**); C22:5 n-3, Docosapentaenoic acid (**DPA**); and C22:6 n-3, Docosahexanoic acid (**DHA**)]. The results were in agreement with another study showing that LA, EPA, DPA, and DHA proportions were increased when laying hens received linseed supplemented diet (Petrovic et al., 2012). In general, the use of extruded linseed supplemented diet to feed hens increases the n-3 PUFA content of the egg (Huang et al., 2018; Kouba and Mourot, 2011). Enriching eggs with EPA and DHA is important for human nutrition. EPA and DHA present many health benefits for humans, such as protection against cardiovascular disease, proper fetal development and healthy aging. Concerns about excess SFA and a deficiency of n-3 PUFA in the human diet have led to recommendations that the ratio of C18:2 n-6/C18:3 n-3 in the diet be lowered under 5 (ANSES, 2011). Feeding GF the Euphorbia seeds or Hevea seed meal supplemented diet produced a C18:2 n-6/C18:3 n-3 ratio in the egg yolk of 7.6 and 12.4, respectively, compared to egg yolk of control GF (35.5). However, these ratios were higher than the recommendations. Furthermore, feeding diets E or H caused a large decrease in the n-6/n-3 ratio in egg yolk (3.4 and 5.4, respectively) compared to egg yolks of control GF (9.0). This ratio in eggs of GF fed diet E was better than results of Huang et al. (2018). These authors found a ratio of 5.3 (comparable to the ratio of egg yolks from diet H), with 7.5% of extruded linseed, in eggs from laying hens. However, Dalle Zotte et al. (2015) found a much better ratio with 10% extruded linseed in diets of laying hens (0.86).

In addition to nutritional properties, n-3 fatty acid-enriched eggs must be of acceptable sensory quality to meet with consumer approval. In the present study, the consumer preference did not find a diet effect, which was in accordance with previous work on laying hen eggs. Indeed Hayat et al. (2010) found that consumer acceptance was not different between control and flaxseed-fed eggs presented to consumers as hard-boiled eggs. We also used hard-boiled eggs and according to Hayat et al. (2010), consumers cannot detect a flavor difference in hard-boiled eggs.

In conclusion, these results demonstrated that *Euphorbia heterophylla* seeds or *Hevea brasiliensis* seed meal could be used in GF diet and had a positive effect on GF laying performance. The use of Euphorbia seeds supplemented diet led to a healthy egg with less cholesterol and more n-3 fatty acids.

REFERENCES

Abedi, E., and M. Ali Sahari. 2014. Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. Food Sci. Nutr. 2:443−463.

ANSES 2011. Actualisation Des Apports Nutritionnels Conseillés Pour Les Acides Gras. ANSES, Paris, France.

AOAC. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 2007. Official Methods of Analysis. 18th ed. AOAC International, Rockville, MD.

Ayerza, R., and W. Coates. 2000. Dietary levels of Chia: influence on yolk cholesterol, lipid content and fatty acid composition for two strains of hens. Poult. Sci. 79:724−739.

Bashir, L., P. C. Ossai, O. K Shittu, A.N Abubakar, and T. Caleb. 2015. Comparison of the nutritional value of egg yolk and egg albumin from domestic chicken, guinea fowl and hybrid chicken. Am. Exper. Agric. 6:310−316.

Bernacki, Z., D. Kokoszynski, and M. Bawe. 2013. Laying performance, egg quality and hatching. Results in two guinea fowl genotypes. Arch. Geflugelk. 77:109−115.

Carrillo-Dominguez, S., M. E. Carranco-Jauregui, M. I. Castro-Gonzalez, E. Avila-Gonzalez, and F. Perez-Gill. 2005. Cholesterol and n-3 fatty acid content in eggs from laying hens fed with red crab meal (*Pleuroncode planipes*). Poult. Sci. 84:167−172.

Conquer, J. A., and B. J. Holub. 1998. Effect of supplementation with different doses of DHA on the levels of circulating DHA as non-esterified fatty acid in subjects of Asian Indian background. J. Lipid Res. 39:286−292.

Dalle Zotte, A., I. Andrighetto, V. Giaccone, and G. Marchesini. 2015. Dietary enrichment of n-3 PUFA for laying hens: effect of different sources on production, composition and quality of eggs. Anim. Sci. Pap. Rep. 33:411−424.

Deniges, G. 1893. Titration of cyanide with silver using potassium iodide as indicator. Comptes Rendus 117:1078−1080.

Ducroquet, H., P. Tillie, K. Louchini, and S. Gomez-Y-Paloma. 2017. Les Filières animales et végétales. in L'agriculture de la Côte d'Ivoire à la Loupe: état des lieux des filières de productions végétales et animales et revue des politiques agricoles, Europe 28754 FR, JRC Science for Policy Report European Commission, Luxembourg. Pages 54−71.

Dudusola, I. O. 2010. Comparative evaluation of internal and external qualities of eggs from quail and guinea fowl. Int. Res. J. Plant Sci. 1:112−115.

Faitarone, A. B. G., E. A. Garcia, R. de, O. Roca, H. de, A. Ricardo, E. N. de Andrade, K. Pelicia, and F. Vercese. 2013. Cholesterol levels and nutritional composition of commercial layers eggs fed diets with different vegetable oils. Rev. Bras. Cienc. Avic. 15:31−37.

Fisher, C, and J. M McNab. 1987. Techniques for determining the ME content of poultry feed. Pages 3−17 in Recent Advances in Animal Nutrition, W. Haresign, and DJA Cole, eds. Butterworths, London, UK.

Folch, J, M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497−509.

Hayat, Z., G. Cherian, T. N. Pasha, F. M. Khattak, and M. A Jabbar. 2010. Sensory evaluation and consumer acceptance of eggs from hens fed flax seed and two different antioxidants. Poult. Sci. 89:2293−2298.

Huang, S., B. Baurhoo, and A. Mustafa. 2018. Effects of extruded flaxseed on layer performance, nutrient retention and yolk fatty acid composition. Br. Poult. Sci. 59:463−469.

Hossain, Md. E., M. H. Karim, S. Alam, and S. K. Nath. 2015. Nutritive value of rubber seed (*Hevea brasiliensis*). Online J. Anim. Feed Res. 5:18−21.

Ismail, I. B., K. A. Al-Busadah, and S. M. El-Bahr. 2013. Effect of dietary supplementation on canola oil on egg production, quality and biochemistry of egg yolk and plasma of laying hens. Int. J. Biol. Chem. 7:27−37.

Kgwatalaia, P. M., L. Bolebano, and S. J. Nsolo. 2013. Egg quality characteristics of different varieties of domesticated helmeted guinea fowl (*Numida meleagris*). Int. J. Poult. Sci. 12:245−250.

Kouakou, N. D. V., G. C. M. Traore, C. E. M. Angbo-Kouakou, K. B. Kouame, A. A. Adima, N. E. Assidjo, J. F. Grongnet, and M. Kouba. 2015. Essai preliminaire de production d'oeufs des poules pondeuses (ISA Warren) enrichis en acides gras polyinsaturés omega 3 avec les graines de *Euphorbia heterophylla L.* Int. J. Bio. Chem. Sci. 9:1902−1909.

Kouakou, N. D. V, G. C. M. Traoré, C. E. M. Angbo-Kouakou, J. F. Grongnet, and M. Kouba. 2016. Effect of feeding *Euphorbia heterophylla* seeds on egg production, egg quality, lipid composition and sensory evaluation of eggs. J. Anim. Plant Sci. 31:4889−4900.

Kouba, M., and J. Mourot. 2011. A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids. Biochimie 93:13−17.

Kouba, M., M. Enser, F. M. Whittington, G. R. Nute, and J. D. Wood. 2003. ffect of a high linolenic acid diet on lipogenic enzyme activities, fatty acid composition and meat quality in the growing pig. J. Anim. Sci. 81:1967–1979.

Maurice, D. V., S. F. Lightsey, K. T. Hsu, T. G. Gaylord, and R. V. Reddy. 1994. Cholesterol in eggs from different species of poultry determined by capillary GLC. Food Chem. 50:367−372.

Moreki, J. C., and D. Seabo. 2012. Guinea fowl production in Botswana. J. World Poult. Res. 2:01−04.

Morrison, W.R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5:600−608.

Obike, O. M., U. K. Oke, and K. E. Azu. 2011. Comparison of egg production performance and egg quality traits of pearl and black strains of guinea fowl in a humid rain-forest zone of Nigeria. Int. J. Poult. Sci. 10:547–551.

Oguntona, T., and B. L. Hughes. 1988. Cholesterol and fatty acid content of guinea fowl (*Numida meleagris*) egg. Food Biochem. 30:211−217.

Petrovic, M, M. Gacic, V. Karacic, Z. Gottstein, H. Mazija, H., and H. Medic. 2012. Enrichment of eggs in n-3 polyunsaturated fatty acids by feeding hens with different amount of linseed oil in diet. Food Chem. 135:1563−1568.

Sanfor, R., H. Boly, L. Sawadogo, and B. Ogle. 2007. Caractéristiques de l'élevage villageois de la pintade locale (*Numida meleagris*) au centre du Burkina Faso. Tropicultura 25:31−36.

Sarfo, G. K., J. A. Hamidu, A. Larbi, and A. Donkoh. 2018. Effect of direct fed microbial in layer diets, on egg laying performance and health response of indigenous Ghanaian guinea fowls (*Numida meleagris)*. Poult. Sci. 0:1−9.

Song, K. T., S. H. Choi, and H. R. Oh. 2000. A comparison of egg quality of pheasant, chukar, quail and guinea fowl. Asian Australas. J. Anim. Sci. 13: 986−990.

Stark, K. D., M. E. Van Elswyk, M. R. Higgins, C. A. Weatherford, and N. Salem, Jr. 2016. Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. Prog. Lipid Res. 63:132−152.

Van Den Brand, H., H. K. Parmentier, and B. Kemp. 2004. Effects of housing system (outdoor vs. cages) and age of laying hens on egg characteristics. Br. Poult. Sci. 45:745−752.

Wang, X., H. Zhang, H. Wang, J. Wang, S. Wu, and G. Qi. 2017. Effect of dietary protein sources on production performance, egg quality and plasma parameters of laying hens. Asian Australas. J. Anim. Sci. 30:400−409.