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Effect of feeding *Euphorbia heterophylla* seeds on egg production, egg quality, lipid composition and sensory evaluation of eggs

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1 ABSTRACT

Euphorbia heterophylla **(poison milk) diet has a significant beneficial effect on the nutritional quality of animal products with the enrichment in polyunsaturated fatty acids omega 3. The present study was conducted to investigate the effect of feeding *Euphorbia heterophylla* seeds on egg production, egg quality, lipid composition and sensory evaluation of eggs. For this purpose, twenty four ISA Warren laying hens (1.45 kg initial body weight) were kept in cages and fed a control diet (RC) or an experimental diet (R15) containing (80% RC + 15% of *Euphorbia heterophylla*'s seeds and 5% vitamin and mineral supplement) for 28 days. There was no diet effect (P>0.05) on egg characteristics (weights of eggs, shells, yolk, albumen, shell thickness, yolk colour score, dry matter, lipid proportions and egg preferences). All the n-3 PUFA contents were increased (P<0.001) by the experimental diet (proportions of ALA, EPA, DPA and DHA were respectively multiplied by 31, 10, 4 and 3). Feeding the experimental diet led to an increase (P<0.001) in the PUFA/SFA ratio and to a robust decrease (P<0.001) in the C18:2 n-6/C18:3 n-3 and Σn-6/Σn-3 ratios. A significant reduction of 18% in egg yolk cholesterol level (P<0.05) from laying hens fed the experimental diet was observed at the end of the experiment. Inclusion of *Euphorbia heterophylla* seeds in the diets of laying hens is a valid method of improving the nutritional value of the eggs. However, the level of incorporation of *Euphorbia heterophylla* seeds seems higher and not optimal. The consumption of this type of egg will allow an improvement of the prevention of cardiovascular disease, leading cause of death in low- and middle-income.**

2 INTRODUCTION

Originated from tropical and subtropical America, *Euphorbia heterophylla* (Picture 1) is a weed that also occurs in Africa and Asia. It is considered as a toxic plant for humans. In West Africa (Ivory Coast, Ghana, southern Togo, southern Nigeria), it is a serious problem because it can grow among many cultivations. In the Ivory Coast, it is found in 70% of cotton fields (Ipou *et al.*, 2004). The duration of *Euphorbia heterophylla* life is about 45 to 50 days and consequently it can have many reproductive cycles per year. Its high αlinolenic acid content (ALA, C18:3 n-3) is higher than in flax (*Linum usitatissimum*) (Earle *et al.*, 1960) and it's used to improve significantly the n-3 PUFA content of Guinea pig meat and carcass (Kouakou *et al.*, 2013). Food rich in long chain n-3 polyunsaturated fatty acids (n-3 PUFA), such as eicosapentaenoic (EPA, C20:5 n-3) and docosahexaenoic (DHA, C22:6 n-3) acids, have antiatherogenic, anti-thrombotic and anti-inflammatory effects and overall, increased intake leads to a reduced risk of coronary heart disease (CHD) (Visentainer *et al.*, 2000; ANSES, 2011), first cause of death in the world (OMS, 2014) particularly in low or middle-income countries.

Picture 1: Poison Milk (*Euphorbia heterophylla* L.)

Due to the limited intake of n-3 PUFA in the human diet, different strategies to produce animal products with higher concentrations of these fatty acids have been undertaken in the past decade (Cherian, 2002; Kouba *et al.*, 2003 and 2008; Hayat *et al.*, 2010; review of Kouba and Mourot, 2011). Hens have the ability to deposit dietary lipids into the egg yolks, which makes the egg a potential source of unsaturated fatty acids (Ferrier *et al.*, 1995; Scheideler and Froning, 1996). Among the different animal products investigated for n-3 fatty acid enrichment, the hen egg has been the most reported as a realistic and successful way to incorporate n-3 fatty acids into the human diet (Cherian and Sim, 1991; Caston *et al.*, 1994; Cherian *et al.*, 1995; Nash *et al.*, 1995; Hayat *et al.*, 2010). These eggs are a natural, healthy, inexpensive, and highly nutritious alternative to direct supplementation (by tablets for example) for providing essential fatty acids to humans, especially to infants (Galobart *et al.*, 2002; Schreiner *et al.*, 2004; Oliveira *et al.*, 2010). The objectives of this study are to investigate the effect of feeding *Euphorbia heterophylla* seeds on egg production, egg quality, lipid composition and sensory evaluation of eggs.

3 METHODOLOGY

3.1 Animals, diets and housing: The animals were reared in compliance with the regulations for the care and use of animals in research. A total of 24 eight months laying hens ISA Warren, with an average initial body weight of 1.45±0.1 kg, were obtained from the experimental farm of the Institut National Polytechnique Félix Houphouët-Boigny, Yamoussoukro (Ivory Coast). Hens were randomly allocated to two treatment groups. There were 3 replicates per treatment with 4 laying hens per cage of 3.375 m^3 (1.5 m x 1.5 m x 1.5 m) (width x length x height). The hens were fed with one of two following diets: a control diet (R0) (industrial feed IVOGRAIN-ponte 20 SIPRA) and an experimental diet (R15) composed by 80% of R0 and 15% *Euphorbia heterophylla* seeds with 5% vitamin and mineral supplement (Table 2). Diet composition was analyzed according to the Association of Official Analytical Chemists (AOAC) (2010) (Table 1). All the 24 hens were fed R0 for 30 days prior the start of the experiment. The trial lasted 28 days, with the hens receiving 120 g of feed from R0 or R15 per day. Water was provided *ad libitum*. Feed intake was recorded per cage weekly. At the end of the experiment, all the animals were weighed just before being killed.

3.2 Assessing Egg production and Egg quality: Egg production was recorded throughout the trial, with all the eggs collected and counted daily. Each week of the experiment, a total of 18 eggs (three eggs from each cage) were randomly selected in each cage during 3 days, individually weighed and then broken open. Shell thickness was recorded using a micrometer screw gauge, after removing the shell membranes of the 18 eggs. Egg, shell, yolk and albumen weights were recorded weekly for the 18 eggs.

3.3 Sensory evaluation: Egg yolk colour was assessed immediately post-brooked for each of the 18 eggs by using the DSM Yolk Color Fan (DSM, Basel, Switzerland) with a scale (1-15) under natural light. Yolks were placed in storage at – 8°C prior to analyses. A taste test was conducted to evaluate preference. The eggs were randomly selected from those collected during the two last weeks of the trial from the control and Euphorbia diets. All the eggs were prepared in the same manner, 2 h before the taste test began. Separately, 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 and placed in separate containers. Nineteen untrained, unpaid adult panelists from INP-HB were chosen for the test. Each panelist individually received one small plate containing two half-eggs from the control diet (A) and Euphorbia diet (B). Panelists sat at a long table, 1 m apart, and received a plate. Each panelist had a sheet of paper with 2 numbered boxes (A and B) (one for each half-egg) to fill in using the following alternatives for preference: like or dislike. Cold tap water was provided for panelists to rinse their mouths between samples (Ayerza and Coates, 2000a).

3.4 Total lipid, cholesterol and fatty acid analysis: Lipids were extracted from samples of the egg yolks and the diets, using the procedure of Delsal (1944). Fatty acid methyl esters (FAME) were prepared with boron trifluoridemethanol according to Morrison and Smith (1964) and analyzed by gas chromatography (Agilent Technologies 6890 N gas chromatograph (Bios Analytic, Toulouse, France)). Identification of FA methyl esters was based on retention times obtained for FA methyl esters prepared from FA standards. Results were expressed as percentages of total fatty acids. Yolk cholesterol from the eggs was determined by an enzymatic method using cholesterol esterase and cholesterol oxidase with a cholesterol reagent kit (Cat. No., 11491458, Roche Cholesterol assay). A known concentration of yolk cholesterol was confirmed by this method (Elkin, 2007). The total cholesterol of the egg yolk was calculated and expressed in $mg.g^{-1}$ of egg yolk.

3.5 Statistical analysis: Differences between treatments were examined with one way ANOVA analysis using STATA (2008). All the hens by treatment were the experimental unit. Fixed factor was *Euphorbia heterophylla* supplementation (without *Euphorbia heterophylla* vs. + *Euphorbia heterophylla*). When a significant ($P<0.05$) treatment effect was observed, Bonferroni's multiple comparison test was performed. Evaluations of preference were performed using the chi-square test, each panelist displaying a single preference.

4 RESULTS AND DISCUSSION

4.1 Chemical and fatty acid composition of the experimental feeds: The chemical composition of the diets is presented in Table 1. The very high percentage of ALA in *Euphorbia heterophylla* was already demonstrated by Earle *et al.* (1960) and Kouakou *et al.* (2013). The diets were iso-proteic and iso-energetic. Their fatty acid profiles were very different.

Table 1: Chemical composition of diets

	Laying hens diets	
	R0	R15
Ingredients (%)		
Hens diet "Ivograin Ponte 20"	100	80.05
Euphorbia heterophylla (seeds)	-	15.00
Dicalcium phosphate	-	4.78
Lysine	-	0.17
Total	100	100
Analysed		
Metabolizable energy (kcal/kg MS)*	3390.13	3389.10
Crude protein (%)	18.93	18.93
Lipids (%)	1.89	6.38
Mineral (%)	12.83	15.72
Fiber (%)	1.58	2.89
Calcium (%)	3.58	4.64
Phosphorus (%)	1.79	2.76
Calcium/Phosphorus	2.01	1.68
Fatty acids profile (g/kg fatty acids)		
Analysed		
Myristic	0.25	0.12
Palmitic	18.51	11.99
Palmitoleic	0.47	0.19
Stearic	4.59	5.71
Oleic	29.43	14.93
Linoleic	42.05	25.38
α-Linolenic	2.46	40.14
Arachidic	0.54	0.29
Calculated		
ΣSFA	23.96	18.16
ΣMUFA	31.52	16.33
Σ PUFA	44.51	65.52
Σn-6 FA	42.05	25.38
Σn-3 FA	2.46	40.14
C18:2 n-6/C18:3 n-3	17.07	0.63
Σn-6 FA/Σn-3 FA	17.07	0.63
PUFA/SFA	1.86	3.61

* calculated

The proportions of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were lower in the R15 diet. The control diet had higher contents of oleic (C18:1 n-9) and linoleic acids (C18:2 n-6) than the R15 diet. The proportion of polyunsaturated fatty acids (PUFAs) in the diet R15 was higher, particularly linolenic acid (C18:3 n-3) proportion which was sixteen times higher than in the control diet.

4.2 Assessing Feed Intake, Egg production and Egg quality: After the first week, daily feed intake of the experimental diet was reduced to 16% in comparison at control diet, against 8% during first week. Feeding the experimental diet led to a 6% lower hen body weight at the end of the experiment, and to a 27% and 32% lower egg production for respectively the third and the last week of the study. Diet had not effect (P>0.05) on feed conversion (Table 2). The lower feed consumption of the hens fed the experimental diet at the end of the experiment could be caused by the presence of cyanogenic glucosides and others toxics substances in *Euphorbia heterophylla* (Okolie *et al.*, 2015; Adedapo *et al.*, 2004). These results are in agreement with those of Bean and Leeson (2003) who showed that feed intake was reduced (P<0.05) for the hens fed 10% flaxseeds in their diet. They also confirm the results of Najib and al-Khateeb (2004), which mentioned a decrease in feed consumption with the increasing proportions (20 to 30%) of canola seed in the hen diets. Consequently, the lowest body weight and egg production of hens fed the experimental diet may partially be attributed to a lower feed consumption. Egg characteristics (egg, shell, yolk and albumin weights, yolk colour, shell thickness, and dry matter proportion) were not affected by the diet (P>0.05). The lack of significant difference in egg weight, albumen and yolk eggs, eggshell quality (weight and thickness) in the present study, is conform to those results of numerous studies (Basmacioglu *et al.*, 2003; Bean and Leeson, 2003; Schreiner *et al.*, 2004; Filardi *et al.*, 2005; Augustyn *et al.*, 2006; Sosin *et al.*, 2006; Rowghani *et al.*, 2007).

Table 2: Physical characteristics of eggs and egg production (n=12 per diet)

Parameters	Weeks	Laying hens diets	
		R0	R15
Weight laying hens (g)	0	1447.42±150.70aA	1448.33±134.79aA
	2	1541.42±114.98aB	1522.25±111.83aB
	4	1511.08±120.21aA	1421.42±133.30bB
Daily feed intake (g)	1	108±5aA	99±9aA
	2	117±1aB	102±9bA
	3	118±2aB	95±13bA
	4	119±1aB	101±5bA
Egg production (%)	1	92,86±12,20aA	82,14±7,50aA
	2	95,24±4,45aA	82,14±10,12aA
	3	95,24±4,45aA	75,00±6,80bA
	4	94,05±10,45aA	71,43±8,13bA
Yolk colour	1	8,33±1,51aAB	8,50±1,05aA
	2	7,33±0,82aAB	7,50±0,55aB
	3	8,67±0,52aA	8,50±0,55aA
	4	7,17±0,41aB	7,00±0,00aB

means ± SEM,
[a,b]Means within line and diet group with no common superscript differ significantly (P<0.05).
[A,B]Means within column and diet group with no common superscript differ significantly (P<0.05).

4.3 Sensory evaluation: The results of the preference test show that 45 people preferred eggs from the control diet against 39 for R15 diet. Six answers (multiple choices and no answer) were cancelled. There was not diet effect on the preference test (P>0.05).

4.4 Total lipid, cholesterol and fatty acid of Egg Yolk: There was no dietary effect on dry matter and the total lipid proportion of the yolk (Table 3). The lack of significant difference in yolk lipid content between dietary treatments could be explained by the lack of diet effect on lipogenic enzymes (Rodriguez *et al.*, 2001). Similar results were reported for egg yolks obtained from laying hens fed flaxseed supplemented diets (Cherian and Sim, 1991; Cherian *et al.*, 1995; Augustyn *et al.*, 2006).

Table 3: Total lipid content and fatty acid composition of liver of yolk eggs fed control diet or euphorbia diet

	Control diet	Euphorbia diet	P-value
Dry matter (%)	51,32	49,09	
Total lipids (g/100g)	30,51	28,21	NS
Fatty acid, % of total fatty acids			
total (mg/g)	355,0 ±12,5	299,9 ±9,7	**
ΣAGS	35,8	34,8	*
ΣAGMI	48,8	41,5	***
ΣAGPI n-3	1,05	11,4	***
C18:3 n-3	0,29	9,06	***
C20:5 n-3	0,01	0,10	***
C22:5 n-3	0,08	0,31	***
C22:6 n-3	0,58	1,77	**
ΣAGPI n-6	14,0	12,2	**
C18:2 n-6	12,1	10,9	*
C20:4 n-6	1,40	0,89	*
C22:4 n-6	0,14	0,07	**
ΣAGPI/ ΣAGS	0,42	0,68	***
18:2n-6/18:3n-3	41,7	1,20	***
ΣAGS	35,8	34,8	*

Σ SFA: sum of saturated fatty acids (C14:0+C15:0+C16:0+C18:0+C20:0).
Σ MUFA: sum of monounsatured fatty acids (C14:1+C16:1+C18:1+C20:1).
Σ PUFA: sum of polyunsatured fatty acids (Σ n-3 FA+Σ n-6 FA).
Σ n-3 FA: sum of (n-3) fatty acids (C18:3+C18: 4+C20:3+C20:5+C22:5+C22:6).
Σ n-6 FA: sum of (n-6) fatty acids (C18:2+C18:3+C20:2+C20:4+C22:4).

Feeding the experimental diet decreased ($P<0.01$) the proportions of SFA and MUFA and increased ($P<0.001$) PUFA content. Among PUFA, total n-6 PUFA percentage was decreased ($P<0.01$) by the experimental diet whereas total n-3 PUFA proportion was eleven times higher than with the control diet. All the n-3 PUFA contents were increased ($P<0.001$) by the experimental diet (ALA percentage was multiplied by 24, EPA proportion by 10, DPA proportion by 4 and DHA percentage by 3). The increase in ALA (C18:3 n-3) and DHA (C22:6 n-3) of egg yolk laying hens fed with high n-3 PUFA supplemented diet has already been described by several authors (Ayerza and Coates, 2000b; Bean and Leeson, 2003). However, contrary to these authors, this study shows an increase in eicosapentaenoic acid content (C20:5 n-3 EPA) ($P<0.05$). According to Ayerza and Coates (2000b), the lack of EPA in the Euphorbia study could be attributed to an error in chromatographic interpretation, rather than to an actual absence, because the level of this fatty acid is very low (ANSES, 2011). Moreover, the small increase in DHA content compared to the increase of other n-3 PUFA has already been described in the hen (Kouba and Mourot, 2011; Fraeye *et al.*, 2012) and can be due to a limited ability to convert ALA to DHA of hen livers because *Euphorbia heterophylla* seeds do not contain DHA. Feeding the experimental diet led to an increase ($P<0.001$) in the PUFA/SFA ratio and to a robust decrease ($P<0.001$) in the C18:2n-6/C18:3n-3 and Σn-6/Σn-3 ratios. A significant reduction of 18% of egg yolk cholesterol level from laying hens fed the experimental diet was observed at the end of the test (P<0.05). The C18:3 n-3/C18:2 n-6 ratio found in the yolks from the hens fed the Euphorbia diet was lower than those from eggs produced in the study of Ayerza and Coates (2000b) who supplemented diet by using 21% chia seed (*Salvia hispanica L.*) during 30 days. These results confirm the nutritional advantage of *Euphorbia heterophylla* seed supplementation of laying hens diet, and is in total accordance with nutritional recommendations which suggest a dietary Σn-6 /Σn-3 PUFA ratio of less than 4 (ANSES, 2011). Another favorable health effect was the reduction in yolk cholesterol level for hens fed the *Euphorbia heterophylla* supplemented diet. This reduction of cholesterol by an n-3 PUFA supplemented diet has already been described by Lin and Pratt (1992) with menhaden oil and with flaxseed (Scheideler and Froning, 1996; Sari *et al.*, 2002). The results of this study show that Euphorbia seed could be an alternative to flaxseed and sea sources to produce eggs more acceptable for public health. Therefore, several studies must to be undertaken to find the optimal incorporation level of *Euphorbia heterophylla* seed in the hen diet. The consumption of this type of egg will participate to the prevention of cardiovascular disease, a major cause of death in low- and middle-income countries.

5 CONCLUSION

This study shows for the first time that adding *Euphorbia heterophylla* seeds to hen diets is an alternative way of increasing the concentration of n-3 PUFA in egg yolk. The present study also shows that *Euphorbia heterophylla* seeds when incorporated at 15% in laying hen diet have positive effects on yolk cholesterol content, which is reduced by 18%. However, this high incorporation (15%) has negative effects on production performance such as egg production, the weight and the feed consumption of the animals. It is therefore important to undertake other studies in order to determine the optimum level of incorporation of *Euphorbia heterophylla* into the diet to keep the beneficial effects of such a diet on n-3 PUFA and cholesterol contents in the egg without too much deleterious effect on production performances.

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