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Effects of *Moringa oleifera* Leaf on Laying Rate, Egg Quality and Blood Parameters

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Abstract: Protein sources diversification and antibiotic growth promoter ban have led to the use of plants. Through production improving, leaves such as *Moringa oleifera* leaves were often used to ameliorate broilers and layers growth. Attempt to improve egg production led us to undertake this study with 600 day-old ISA Brown chicks (layer-type) divided into three groups (M0, M1 and M2) of 200 birds each. They were reared up to 40 weeks. Daily egg and feed intake were collected to determine laying rate and conversion ratio. Weekly, 30 eggs per group were weighed and broken to determine egg components ratio. At 20 and 40 weeks, 40 birds per group were slaughtered to collect blood, ovarian grape and oviduct. So, total protein, glucose and triglycerides concentrations, ovarian grape weight, oviduct weight and follicles number were determined. Data collected showed higher egg production, heavier eggs and higher triglycerides concentration in M1 compared to M2. High level of triglycerides can be linked to oestrogens synthesis from sterols contained in *Moringa oleifera* leaves in M1 while high intake of oestrogen substances by hens of M2 reduces their production although antinutritive substances effect on them cannot be excluded.

Key words: *Moringa oleifera* leaf, egg, ovarian grape, oviduct, blood parameters

INTRODUCTION

Egg and poultry meat account for 35.56% of human animal protein requirements in the world with 13.12% for eggs only. Egg consumption and its high nutritive value result in an important production estimated to 1,140 billion in 2008 (Nys *et al*., 2011). According to Sparks (2006), egg nutritive value depends on geography and feed composition which influences egg size, egg weight, albumen ratio, yolk ratio and shell ratio. Poultry farmers try to manipulate poultry feed through its composition and quality in the goal to improve egg productivity and profitability. Antibiotic growth promoters are used to inhibit the multiplication of pathogens and, subsequently, to favour digestion and nutrients absorption by bird's digestive tract. However, antibiotic residues and transfer of antibiotic-resistant genes to human pathogens result in several billion dollars loss every year (Farhad and Farida, 2011). Financial and public health damages have led researchers to focus their attention on probiotics and prebiotics especially the natural ones such as plant as alternative after the ban in 2006 of the use of antibiotic as growth promoter. Other considerations that encourage the use of plant substances in poultry feeding are the improving of yolk colouration and their high content in macronutrients which is the key element of protein source diversification policy (FAO, 2004). *Leucena leucocephala*, *Gliceridia sepium*, *Sesbania sesban* etc. have been widely used to feed poultry resulting in improvement of productivity (D'Mello and Acamovic, 1989). In addition, the incorporation of *Curcuma longa* at 0.5 and 1% in layer mash has improved egg weight, shell thickness and yolk ratio (Radwan *et al*., 2008). Studies of Aktar *et al*. (2003) pointed out the increasing of laying rate, albumen ratio and significant reduction of yolk cholesterol content with layers consuming feed containing 1.5% of *Nigella sativa* roots. Dong *et al*. (2007) found low laying rate and high shell, high albumen and high yolk ratio when layers have consumed *Medicagosativa* leaves. Using dried leaves of *Manihot esculenta* in layer mash, Houndonougbo *et al*. (2012) obtained significant improving of egg weight, albumen and yolk ratio at 21 and 32 weeks of age. Regarding *Moringa oleifera*, a plant largely used in traditional medicine in the tropical zone, most studies are undertaken about its effect on broiler growth (Portugaliza and Fernandez, 2012; Banjo, 2012; Gadzirayi *et al*., 2012; Teteh *et al*., 2013) while few are devoted to laying rate, egg mass and yolk colour (Kakengi *et al*., 2007). Effects of *Moringa oleifera* leaves on egg quality and laying rate as well as reproductive organs of hens were not yet investigated. The aim of this study was to determine optimum inclusion level of *Moringa oleifera* leaves in layer diet for egg production and egg quality improvement in relationship with blood parameters concentrations.

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MATERIALS AND METHODS

Experimental design: The experiment was carried out at the Centre d'Excellence Regional sur les Sciences Aviaires (CERSA) of University of Lome (Togo). During 40 weeks, day-old chicks ISA brown (layer-type) produced by CERSA were fed with three types of diets containing *Moringa oleifera* leaves. Leaves were collected from rural areas of Togo and dried under air conditioning system and pulverized to be incorporated in poultry feed.

Chicken feeding treatment: A total of 600 day-old ISA Brown chicks (layer-type), divided into three groups (M0, M1 and M2) with 4 replications of 50 layers for each group. The three groups were, respectively fed with diet 1, 2 and 3 from day-old to d 280. Diet 1 is the basal diet with 0% of *Moringa* leaves while diets 2 and 3 contain, respectively 1 and 2% of *Moringa* leaves. Each diet was formulated to fit crude protein (CP) and metabolizable energy (ME) of birds according to the developmental stage (Table 1). Data were collected from 20 weeks when birds started consuming layer diet.

Determination of laying rate and feed conversion ratio: Eggs were collected daily and laying rate registered weekly (sum of daily egg number x 100/sum of daily bird number). Also, weekly feed intake was determined. At the end of every week, 30 eggs per group were weighed to determine feed conversion ratio (weekly feed intake /weekly eggs weight).

Determination of egg components ratio and yolk colour: Weekly, 30 eggs per group were weighed and broken to collect meticulously shell, albumen and yolk. These components were also weighed to determine their ratio (component weight x 100/absolute egg weight). After weighing, the intensity of yolk colour was determined using Roche Scale.

Determination of relative weights of different parts of reproductive system and blood parameters concentrations: At 20 and 40 weeks, 40 hens per group were slaughtered to collect blood and to isolate reproductive organs. Blood samples were immediately centrifuged at 3000 tours/min and serum was collected for glucose, total protein and triglycerides levels measuring. Blood parameters levels were determined by colorimetric method with a spectrometer type Climax Plus Version RAL. Chemical reagents used were Glucose Liquicolor® produced by Human Gmbh-65205, Triglycerides® and Total Protein® produced by Sprinreact SA-ctra Santa Coloma 7-E-17176. Ovarian grape and oviduct were collected and weighed, only at 40 weeks, to determine their relative weight (ovarian grape or oviduct weight x 100/reproductive system weight). Also, follicles around each ovary were numbered.

Statistical analysis: Data obtained were expressed as mean±standard error (SE) of mean and processed with the statistical software package Systat 11. ANOVA model was used to analyse the effects of experimental diets on laying rate, egg weight, egg components ratio, ovarian grape and oviduct relative weights and glucose, total protein and triglycerides levels. If the overall F-value was statistically significant ($p<0.05$), further comparisons among groups were made according to Tuckey's test.

RESULTS

Effects of *Moringa oleifera* leaves on laying rate and feed conversion ratio: In general, Fig. 1 shows a regular increasing of laying rates of groups up to 28 weeks where each group reaches its peak (80% for M0, 90.69% for M1 and 83.02% for M2). From 21th week, age of the first lay in the three groups, to 24th week, M1 had the highest laying rate compared to M0 and M2 ($p<0.05$) while from 24 to 40th week this difference remained and become significantly more important than previously ($p<0.01$). Regarding M0 and M2, laying rates were comparable until 31th week where M2 become significantly less productive than M0 ($p<0.05$). In Table 2, M0, M1 and M2 had similar feed intake while egg weight was higher in M1 than M0 ($p<0.05$)and M2 ($p<0.01$). Therefore, feed conversion in M2 was higher if compared to M1 ($p<0.05$). Groups M0 and M2 were statistically similar, although M2 had numerically the highest feed conversion ratio.

Effects of *Moringa oleifera* leaves on egg components and yolk colour intensity: Table 3 shows shell ratio, yolk ratio, albumen ratio and yolk colour intensity. Shell ratio (12.8±0.25 for M0, 13±0.01 for M1 and 12.56±0.34 for M2) and yolk ratio (23.8±0.18 for M0, 23.9±0.17 for M1 and 23.51±0.4 for M2) were comparable. But albumen was heavier in M1 than M0 ($p<0.05$) and M2 ($p<0.01$). Concerning yolk colour intensity, eggs of M2 had more coloured yolk than those of M1 ($p<0.05$) and M0 ($p<0.01$).

Effects of *Moringa oleifera* leaves on ovarian grape and oviduct: Results are mentioned in Table 4. M2 had the lowest ovarian grape compared to M0 ($p<0.05$) and M1 ($p<0.01$). M2 had also fewer follicles than M0 ($p<0.05$) and M1 ($p<0.01$). About oviduct, M0 (4.2±0.15) and M2 (4±0.31) were comparable on the one hand and had lighter oviducts than M1 ($p<0.05$) on the other hand.

Effects of *Moringa oleifera* leaves on blood parameters concentrations: Blood parameters concentrations are summarized in Table 5. Glucose concentrations at 20 and 40 weeks of age were comparable between M0, M1 and M2. Conversely, besides the regular increasing of blood parameters levels observed from 20 to 40 weeks, significant differences appear between total protein levels on one

Table 1: Gross composition of experimental diets (%)

	Feed composition according to age and group								
	Starter mash			Grower mash			Layer mash		
Feed stuffs	M0	M1	M2	M0	M1	M2	M0	M1	M2
Maize	58	57	57.	52	52	51	58	57	57
Wheat bran	9	8.90	8.82	25	25	25	9	8.91	8.82
Cotton cake	4	3.96	3.92	0	0	0	0	0	0
Fish meal	10	9.90	9.80	8	7.92	7.84	9	8.91	8.35
Soya seed	15	15	15	10	9.90	9.25	15	15	15
Concentrate	3	3.25	2.47	3	2.20	2.94	3	3.24	2.94
Oyster shell	0.750	0.740	0.740	1.75	1.73	1.72	5.75	5.69	5.64
Salt	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Moringa leaf (%)	0	1	2	0	1	2	0	1	2
Total	100	100	100	100	100	100	100	100	100
Calculated analysis									
CP (%)	20	20.	20	17	17	17	18	18	18
ME (Kcal/kg)	2923	2923	2923	2706	2708	2711	2839	2840	2841
CF (%)	4.89	5.69	5.85	5.60	5.75	5.91	4.58	4.74	4.91

CP: Crude Protein, ME: Metabolizable Energy, CF: Crude Fibre

Table 2: Feed intake, egg weight and feed conversion ratio according to the treatment

	Groups		
Parameters	M0	M1	M2
Feed intake (g)	108±1.9^{a}	107±1.7^{a}	109±0.030^{a}
Egg weight (g)	54±1.05^{b}	56±1.11^{a}	52±0.930^{c}
Conversion ratio	2.61±0.200^{a}	2.02±0.010^{b}	2.93±0.030^{a}

Values are means ± SEM.
^{abc}Within row, values sharing the same letter are not significantly different

Table 3: Egg components ratio and yolk colour intensity

	Groups		
Parameters	M0	M1	M2
Shell ratio	13±0.25^{a}	13±0.01^{a}	13±0.34^{a}
Yolk ratio	24±0.18^{a}	24±0.17^{a}	24±0.4^{a}
Albumen ratio	61±0.200^{b}	63±0.210^{a}	60±0.200^{c}
Yolk colour intensity	2.40±0.220^{c}	5.50±0.230^{b}	6.60±0.140^{a}

Values are means ± SEM.
^{abc}Within row, values sharing the same letter are not significantly different

Table 4: Oviduct and ovarian grape relative weights according to the treatment

	Groups		
Reprod. segment	M0	M1	M2
Ovarian grape			
Relative weight	2.64±0.080^{b}	2.89±0.070^{a}	2.36±0.120^{c}
No. of follicles	10±0.36^{b}	14±0.750^{a}	8.00±0.310^{c}
Oviduct			
Relative weight	4.20±0.150^{b}	4.82±0.210^{a}	4.00±0.310^{b}

Values are means ± SEM.
^{abc}Within row, values sharing the same letter are not significantly different. Reprod.: Reproductive. No: Number

hand and triglycerides levels on the other hand. At 20 and 40 weeks, M0 showed low total protein level as compared with those of M1 and M2 ($p<0.05$). Regarding triglycerides levels, M1 had the highest level if compared to M2 ($p<0.05$) and M0 ($p<0.01$) at 20th week. At 40 weeks of age, triglycerides concentration of M2 was lower than those of M0 ($p<0.01$) and M1 ($p<0.001$).

DISCUSSION

According to Swennen *et al*. (2007), productivity depends on macronutrient contents of feed, composition of diet and feed intake as well as digestion and absorption processes. In this study, although the three types of feed fit with layers nutritional requirements on the one hand and feed intakes were similar between M0, M1 and M2, egg production was variously influenced by *Moringa oleifera* leaves incorporated in layer mash at 0, 1 and 2%. During laying period, group M1 produced more eggs and heavier eggs than the others. These performances confirm the results of Kakengi *et al*. (2007) who showed that substitution of sunflower cake with 5% of *Moringa oleifera* leaves in layer diet improves egg number and egg mass in opposite to 10 and 20% substitutions. These authors attributed these results to the high content of *Moringa oleifera* leaf in amino acids without clarifying the causes of low production observed with high substitution levels. So, besides amino acid contents, other factors related to the impairing effect on growth of feed containing 2% of *Moringa oleifera* leaves as shown by Teteh *et al*. (2013) could play a role in the observations on laying rate and egg weight. Effects of antinutritional factors are not observed about glucose concentrations confirming results of Teteh *et al*. (2010) who claimed the strict regulation of carbohydrates metabolism whatever the diet. Concerning shell ratio, it appears that antinutritional factors such as phytates and oxalates, found in these leaves have less chelating effect on feed calcium as reported by Gupta *et al*. (1989) and Foidl *et al*. (2001). As pointed out by Wright *et al*. (1990), up to 40 weeks, hens have still the capacity to move calcium from bones completing feed calcium intake for shell calcification. In opposite, triglycerides concentrations obtained at d 280, confirm the hypolipidemic effect of *Moringa oleifera* as reported by Mehta *et al*. (2003). According to Dong *et al*. (2007), Dei *et al*. (2007) and Francis *et al*. (2002), this hypolipidemic effect is due to the presence of saponins found in high amount in Moringa leaves (Gupta *et al*., 1989 and Foidl *et al*., 2001). As claimed by Teteh *et al*. (2013), saponins seem to be the most active antinutritional substance in

Table 5: Blood parameters concentration according to the group and the age

	Blood parameters								
	Glucose			Total protein			Triglycerides		
Age (w)	M0	M1	M2	M0	M1	M2	M0	M1	M2
20	136±2.20^{a}	131±5.20^{a}	136±5.2^{a}	60.83±2.30^{b}	72±1.30^{a}	69±3.20^{a}	97±2.20^{c}	189±1.30^{a}	107±3.30^{b}
40	155±1.10^{a}	154±2.30^{a}	154±2.30^{a}	77±1.10^{b}	83±3.10^{a}	86±1.50^{a}	702±1.20^{b}	911±1.30^{a}	397±2.50^{c}

Values are means±SEM. ^{abc}Within row and according to blood parameter, values sharing the same letter are not significantly different

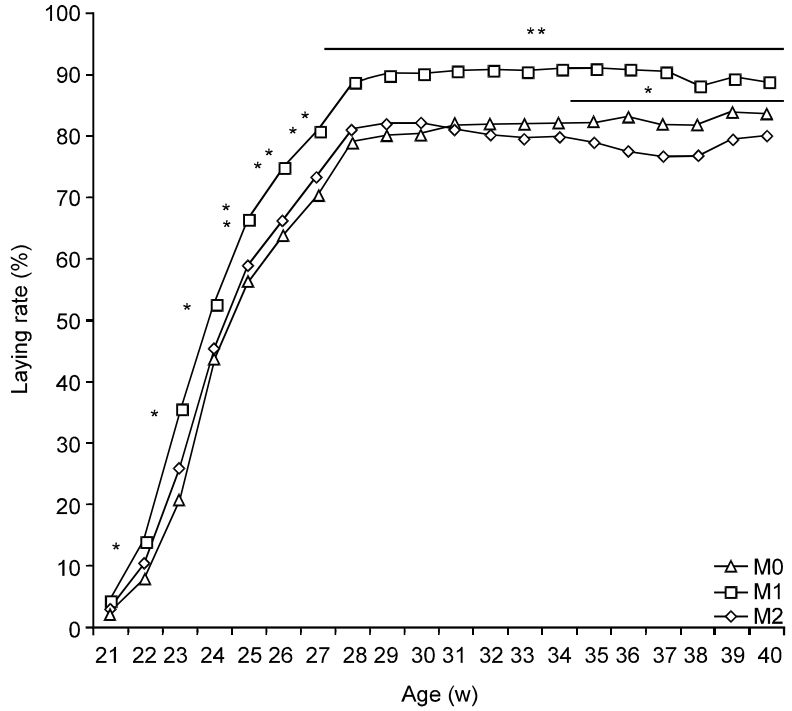

Fig. 1: Laying rate according to age and treatment. At each age, significant differences are indicated by *($p<0.05$) and **($p<0.01$)

these leaves. In spite of this chelating effect on triglycerides, those are main components of yolk, yolk ratio were similar up to 40 weeks of age. This result can be linked to yolk production process lasting for 8-10 days. During this process, lipoproteins are transported by Lipoprotein Receptors 8 (LR8) from liver to ovary where they are deposited in oocytes to form follicles. For instance, any feeding problem in laying hens takes more time to affect significantly yolk ratio as demonstrated by Nau *et al*. (2010) and Nys *et al*. (2011). The high level of triglycerides in M1 compared to M0 could be linked to oestrogenic effects on lipid metabolism as pointed out by Aftergood and Alfin-Slater (1965) and Mandour *et al*. (1977). Titi *et al*. (2013), feeding female rat with *Moringa oleifera* leaves, demonstrated that sterols contained in these leaves are used as precursor for oestrogens synthesis those stimulate gonads functioning. Hence, hens of M1 might benefit from oestrogen effect resulting in high number of follicles around ovaries. Our finding corroborates with results obtained with rat fed with *Moringa oleifera* by Cajuday and Pocsidio (2010) and Zade *et al*. (2013) about spermatogenesis and testes development. The high relative weight of oviduct of M1 could also be related to these oestrogen effects on tubular glands and epithelial cellular of magnum mucosa where albumen is produced. So, through oviduct development, sexual hormones could induce in group M1 higher production of albumen which is responsible for their heavier egg weight. In opposite, the lowest laying performance of M2 could be related to the high level of *Moringa* leaves which might increase too much oestrogens level resulting in reproductive organs development impairing. These results confirm the reports of Musa-Azara *et al*. (2014) who claimed that the intake of some amount of oestrogenic substances in *Moringa oleifera* inhibits the secretion of LH and FSH and reduces, subsequently, endogenous oestrogen and progesterone levels. The reduction of these sexual hormones levels may play a role in the low number of follicles and the low weight of ovarian grape and oviduct of M2. However, relying on studies of Nau *et al*. (2010) and Nys *et al*. (2011) who showed that the rapidity of egg albumen production process (less than 2 days) makes its synthesis very sensitive to any nutritional problem,

possible effects of antinutritional factors on M2 albumen ratio. In contrary, the intense colouration of yolk in M2 compared to M1 and M0 may be due to the high content of diet 3 in xanthophyll that escapes from the chelating effect of antinutritional substances especially saponins. This xanthophyll effect is in line with Surai *et al*. (2001) who pointed out a positive correlation between intensity of yolk colour and xanthophyll content of diet. Study of Galobart *et al*. (2004) showed that saponins, in opposite to their chelating effect on macronutrients, improve xanthophyll absorption through intestinal mucosa.

Conclusion: In conclusion, *Moringa oleifera* leaves incorporated at 1% in layer diet may increase oestrogens level and triglycerides serum concentration for improving laying rate and egg weight while 2% incorporation reduces egg production and strengthens yolk colour intensity.

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