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Effect of moringa leaves on lipid content of table eggs in layer hens

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Abstract

This study aimed to evaluate the effect of low dose levels of Moringa oleifera leaves powder (MOLP) as a natural feed additive on feed efficiency, egg productivity and quality as well as lipid profile in both serum and egg yolk of sixty Lohmann Brown laying hens aged 32 weeks. Birds were assigned to four dietary groups. The dietary treatments containing varying MOLP levels, 0, 1, 1.5 or 2 g/kg diet for 45 days. It was revealed that inclusion of MOLP in layers diet at all dose levels improved egg quality indices as manifested by a significant increase in whole egg weight, shell weight and yolk colour, while induced a significant decrease in yolk weight at all dose levels and increase albumin weight at a dose level of 1.5 and 2mg/kg diet without any adverse effect on laying percentage, egg mass production,feed intake (FI) and feed convertion ratio (FCR). In addition, MOLP evoked a significant decrease in total lipid, total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL) and atherogenic index (AI) in both yolk and serum all dose levels, while induced a significant increase in high density lipoprotein (HDL) in serum from all treated groups and in yolk at dose levels of 1.5 and 2 g/kg diet. In this study, MOLP effects were dose dependent. The increased shell and albumin and the decreased the yolk weight in the eggs could imply lower cholesterol content. Moringa leaves as natural feed additives was found to be a good and cheap alternative sources of protein and reduced consumption of imported synthetic supplements and medicine and thus the demand for low fat organic eggs production for consumers health was achieved.

Keyword: Moringa oleifera- feed convertion rate- layers-egg-lipids-cholesterol

Introduction

Moringa oleifera Lamarck regarded as a miracle tree is reputed to have many medicinal properties. It is well distributed in Africa and Asia and possesses uncountable uses due to its various nutritional and pharmacological applications **(Mbikay, 2012).** Feeding of poultry with a meal formulated with M. oleifera leaf powder can lead to increase in weight and activity of the birds. M. oleifera leaves are free from heavy metals which are potentially toxic, thus making their incorporation into poultry diet safe **(Donkor et al., 2013)**. Moringa leaves act as a good source of natural antioxidants such as vitamins, selenium, flavonoids, phenolics and carotenoids, which makes it suitable to be utilized as a nutritionally valuable and healthy additive ingredient for poultry diet and could be valuable in preventing diseases and enhance the shelf-life of fat containing foods. It has multimedicinal activity includes, antimicrobial, antihyperlipidemic and water purification. Moringa oleifera leaves diet content is superior to vitamins and more than milk in protein content **(Toma and Deyno, 2014)** and has been reported to have hypocholesterolemic properties **(Olugbemi et al., 2010)**. Moringa oleifera leaves contained 27.2% protein and as a natural source of high-quality protein and antioxidant compound in diets for poultry can be used as cheap alternative to soybean meal and sunflower seed cake **(Yameogo et al., 2011)**. Moringa is not only concentrated in nutrients such as calcium, iron, sulfur, but in the raw form. It absorbs and neutralizes toxic elements in food. Thus, effect of Moringa oleifera leaf powder inclusion on layer diet was evaluated on laying performance, economic gains and egg quality of chicken **(Mathew et al., 2001).** M.oleifera foliages generally contain certain bioactive chemical compounds (such as saponins, tannins, phytates and trypsin inhibitors), which are known as secondary metabolites of plants are said to have pharmacologically active agents**.** The presence of essential nutrients and minerals in Moringa leaves imply they could be utilized to improve growth performance and health status of poultry **(Ogbe and John, 2012)**.

Therefore, the main objective of the present study was to evaluate the potential effectiveness of a natural products such as Moringa oleifera leaf powder (MLP) and its bioactive phytochemical constituents on production of low fat organic eggs with high quality and mass production.

Materials and Methods

Moringa oleifera leaf powder

Moringa oleifera Lamarck (synonym: Moringa pterygosperma Gaertner) is one of the 13 species of monogeneric family Moringaceae, commonly called horseradish tree or drumstick tree **(Mbikay, 2012).** Moringa oleifera leaves powder are brown yellow fine powder obtained from Marrakecch import and export Agriculture Research Center.

Experimental design and treatment :

Sixty Lohmann brown laying hens aged 32 weeks were provided daily with equal quantities of preweighed feeds given in mash form and supplements were equally mixed in the diets. Hens were assigned into four dietary groups containing varying levels of moringa leaf powder (MLP) (0, 1, 1.5 or 2 g/kg diet) for 45 days.

Feed efficiency and egg quality measurement :

Eggs were collected daily three times a day at 9h, 13h and 16h. Daily egg production (EP) (%) was used to calculate daily laying percentage (LP) in each dietary treatment at 6:00 PM following standard procedures. During the experiment, feed intake (FI) (g) (the differences between feed given and left over), average egg weight (EWT) (g), egg mass production (g/hen /day) a factor of egg weight and egg production, and feed conversion rate (FCR) [feed intake (kg) divided by total egg mass production (kg)] were calculated daily per bird in each dietary treatment. Ten eggs were collected from each dietary treatment group of birds three days before the end of the feeding period for measuring egg quality (egg weight, shell weight, yolk weight, yolk colour) as well as for chemical analysis of yolk fat content. Yolk color was scored using a colorimetric fan (Roch). Freshly laid eggs were were individually weighed using digital balance, carefully broken and different components were carefully separated and weighed. Percentages of egg components (shell, yolk and albumin materials) as a ratio to total egg weight were determined according to **Stadelman and Cotterill (1995)** using the equation: Egg components percentages (%) = component weight (gm)/ egg weight (gm)×100.

Chemical analysis:

Blood was collected from the brachial wing vein and left for an hour at room temperature, serum was isolated by centrifugation at 3000× g for 10 min. Yolks were carefully separated into a container. For lipid extraction, one gram of yolk was placed into a centrifuge tube, homogenized with 15 ml of polar solvents chloroform: methanol mixture, 2:1 (v/v), vortexed, filtered and evaporated according to **Folch et al. (1957).** and then lipid extract and serum samples were stored at −20 C° until chemical analysis. Total lipids was estimated according to **Frings *et al.* (1972)**, triglycerides by method described by **Fossati and Prencips (1982),** total cholesterol concentration was determined according to **Artiss and Zak (1997)**. Low density lipoprotein (LDL) cholesterol was determined according to **Wieland and Seidel (1983)**, while high density lipoprotein (HDL) cholesterol was determined enzymatically as described by **Dernacker and Hifrnans (1980)**. Very low density lipoprotein (VLDL) was determined by the formula: VLDL=Triglycerides/5 and the Atherogenic index (AI) was calculated by the formula: AI= (Total cholesterol−HDL)/HDL **(Norbert , 1995)**.

Statistical analysis :

The influence of dietary treatments were examined using analysis of variance while differences among means were evaluated using Duncan's multiple range test at 5% probability with the aid of software **SPSS (2006)**.

Results and Discussion

Table (1) revealed that M.oleifera leaf powder (MOLP) at all dose levels improved egg quality which is emphasized by a significant increase ($p<0.05$) in whole egg weight, shell weight and yolk colour, while induced a significant decrease ($p<0.05$) in yolk weight at all dose levels and increase albumin weight ($p<0.05$) at a dose level of 1.5 and 2mg/kg diet without any adverse effect on laying percentage, egg mass production,feed intake (FI) and feed convertion ratio (FCR). This study showed slightly lower yolk proportion and higher shell and albumin percentage than the proportions of components for fresh egg reported by **Encyclopedia Britannica (2012)** which are 32% yolk, 58% albumen and 10% shell**.** Nearly similar to our results, layers fed with 0.2% MLP had the higher egg production. Relatively better egg production percent were obtained from the enzyme enriched moringa leaf meal treated groups. The addition of 0.5 % MLP in the ration obtained heavier eggs weight of the force-molted hens while the use of MLP in excess of 1.0 percent adversely affected the egg weight. MLP in the layer ration has no direct influence on shell thickness quality of the egg. However, the egg yolk color of the treatment with 0.6% MLP had a better yolk color intensity while the control had the least mean value of 6.167. However, there was a marked reduction in the feed consumption of birds fed 0.75% MLP diet due to reduced palatability of the diet **(Paguia et al., 2012).** The slightly higher egg weights are probably due to the relatively higher feed intake and high nutritive value of Moringa leaf meal **(Etches, 1996)**. In coincidence with our result, **Abou-Elezz et al. (2011)** found that all yolks obtained from (0% leaf meal) group were whiter than the lowest degree (6) of the yolk color. MOLM are a good source for yolk pigments. Significantly highest roche colour score was observed in eggs obtained from MOLM inclusion in layers ration indicating improved yolk colouration demanded by consumers due to its rich beta-carotene, oxycaretenoids and xanthophyl content. MOLM. Leaf meals are a good source for yolk pigments. The inclusion of MOLM in the laying hen diets increased the yolk color, which are desirable quality traits as it contains high carotene content (16.3 mg/100g). Egg weight significantly increased as a result of the supplementation of Moringa oleifera leaf. MOLM increased the albumin and decreased the yolk proportions in the eggs. Having eggs with higher albumens and lower yolk proprtion is implying relatively lower concentrations of cholesterol which is a good quality for egg consumers who prefer eggs obtained from birds on the MOLM treatment. The values of Roche color scores and albumen percentages increased, while the yolk proportions decreased accompanying the increase in MOLM levels in the diet. Moringa inclusion enhanced utilization of Ca or P and its deposition**.** Shell weight percentage and thickness which are important for the transportation of the eggs was the highest being from the 10%

MOLM diet. Feed cost/kg egg produced declined with inclusion of MOLM. MOLM could be acceptable as feed resource for commercial egg production up to 10 % diets had no adverse effect on the productive performance of laying hens, feed intake and FCR but higher levels above 10% to reduced markedly the laying percent (LP), egg weight (EWT) and egg mass (EMP). The use of M. oleifera as a supplement can improve feed intake, digestibility and bird performance. The significant increased in feed consumption with inclusion of MOLM may be due to total soluble protein and high pepsin, the natural enzyme aids feed digestibility making it more suitable to monogastric birds. The decrease in, egg production, laying percentage and egg weight, egg mass production and eventually feed conversion ratio (Kg feed/Kg eggs) when M. oleifera leaves were high in diet, are mainly due to the increased bulkiness accompanying the high levels of leaf meals which might be made it difficult for the hens to increase their FI and could result in inadequate nutrients availability for the hens and low available energy as a result of low digestibility of crude fibre (CF), curd protein (CP) component of leaves and reduced utilisation capacity of the feed. Hens' digestive system is simple and lacks the enzymes necessary to digest high fibrous ingredients efficiently **(Abou-Elezz et al., 2011)**. Inclusion of Moringa at low levels improved egg production and egg quality but higher levels of inclusion resulted in lower productivity and poorer egg quality indices. Chicks fed on MOLM at 5% maintained better performance and feed conversion ratio (FCR) up to 10%. **(Ebenebe et al., 2013)**. Dietary inclusion levels of 10% in layer diets have been found to improve bird performance in terms of egg production (including egg size) **(Moreki and Gabanakgosi, 2014).** Moringa oleifera is found to be a very good indigenous source of highly digestible protein and essential sulfur-containing amino acid contents (methionine and cysteine), iron, potassium, calcium, phosphorus, magnesium and vitamins and phenolic compounds **(Ferreira et al., 2008).** A positive influence of sulphur containing amino acids on egg weight was reported by **(North, 1990)**. Rhode Island Red hens' feed supplemented with MOL had higher egg laying rate, higher daily egg mass production, lower feed intake and better feed conversion ratio and yolk color was improved significantly and had eggs with lower yolk and higher albumen percentages than the control group. Shell weight percentage averaged 19% and the higher shell percentage caused a decrease in the percentage of albumin and yolk **(Mohammed et al,, 2012)**. **Araujo et al. (2006)** obtained higher specific egg weight with higher dietary Ca levels. M. oleifera leaves may be regarded as a protein and calcium supplement **(Rajangam et al., 2001)**. Layer ration should be formulated with correct amount of calcium and phosphorus and Vit. D that is critical for proper egg production and eggshell quality of commercial layers. The increase in eggshell percentage as dietary Ca level may have resulted from the possible increase in eggshell Ca content, thereby increasing egg shell weight **(Abbas, 2013).**

MOLP provoked a significant reduction ($p<0.05$) in total lipid, triglycerides, total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (VLDL) cholesterol and atherogenic index (AI) in both serum and yolk while induced a significant increase in high density lipoprotein (HDL) in serum from all treated groups and in yolk at dose levels of 1.5 and 2 g/kg diet Moringa leaf powder (MOLP) (Table 2&3). These results were in accordance with **Mehta et al. (2003)** who found that moringa has been found to reduce the lipid profile, lower the serum cholesterol, phospholipids, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL) cholesterol, atherogenic index.

In laying hens, egg cholesterol is synthesized in the liver and secreted into the blood as very low density lipoprotein (VLDL) particles, the main yolk cholesterol carrying macromolecules. Plasma VLDL particles are then internalized by the oocyte vitellogenin receptor in the rapidly growing follicles and deposited to yolk. Thus, cholesterol is mainly excreted through the egg in the hen. Faecal neutral and acidic sterol represents a second major pathway for elimination of cholesterol **(Hall and Mckay, 1993)**. It has been suggested that either selective inhibition of liver cholesterol biosynthesis or increased excretion of cholesterol from the body would result in reduction of egg cholesterol. **Fahey (2005)** reported that moringa is an important source of naturally occurring plant chemicals (phytochemicals) such as alkaloids, glucosinolates and isothiocyanates that provide cholesterol lowering activities. These phytochemicals are the major secondary metabolites present in the M. oleifera leaves. M. oleifera leaf powder meal holds some therapeutic potential for chronic hyperlipidemia and enhances colour pigmentation because of carotinoids presence in it. The presence of some phytochemicals like tannins, saponins, phenols, alkaloids, phlobatannins and flavonoids explained the medicinal action of M. oleifera leaves encountered in its therapeutic uses (**Bamishaiye et al., 2011)**. Yolk colour and high density lipoprotein content increased in the eggs of layers fed MOML supplemented diets. The egg cholesterol and low density lipoprotein reduced with MOML supplementation in the diets of the layers **(Lala et al., 2012).** MOL lowered serum triglyceride level and increased serum calcium. High dietary Ca levels may have influenced metabolic processes that resulted in the more intense yolk color and shell weight observed in the present study. Most of the phosphorus used during egg formation is incorporated to the yolk as phospholipids and phosphoproteins **(Donkor et al., 2013).** The mechanism of cholesterol reduction is thought to be through the lowering of plasma concentrations of LDL by phytosterols such as β-sitosterol, the bioactive phytoconstituent isolated from M. oleifera which account for the decrease of plasma cholesterol and and increased the excretion of fecal cholesterol. These compounds can reduce intestinal uptake of dietary cholesterol **(Lin et al., 2010)**. M. oleifera leaf powder also contain about 12% (w/w) fibers and high fiber diet reduces cholesterol levels. Fibre (soluble and insoluble) is a complex carbohydrate; The soluble fibre binds cholesterol and inhibits its intestinal absorption until it is eliminated, thus reducing plasma cholesterol level and decrease low-density lipoprotein (LDL) cholesterol while the main function of insoluble fibre is to bind bile acids, which reduces fat and cholesterol absorption **(Joshi and Mehta, 2010).** MOLM inclusion in layer diets was instrumental in cholesterol reduction in serum and yolk. The fact that inclusion of MOLM resulted in a decrease in cholesterol levels, affirms its potential as a hypocholesterolemic agent which might be attributed to the presence of a bioactive phytoconstituent, i.e. β-sitosterol and its natural amino acids, vitamins and alkaloids **(Kumar et al. 2010).** Moringa oleifera leaves and that it can be included in layers diets to facilitate reductions in egg cholesterol content. The supplement (MOLM) has an influence on expulsion of fat from the body and impairment of fat synthesis **(Olugbemi et al., 2010)**. Low cholesterol and triglyceride content were observed in the serum of birds on MOLM **(Ogbe and John, 2012)**. Its inclusion in the form of powder might enhance its hypocholesterolemic properties. 8g of the leaf powder daily over the course of 40 days is able to reduce total cholesterol (14%), LDL-C (29%), VLDL -C (15%), and triglycerides (14%) with an increase in HDL-C by 9% **(Mbikay, 2012)**. M. oleifera leaves have appreciable amounts of saponins (80g/kg), besides low quantity of phytates (21g/kg) and tannins (12g/kg) **(Ferreira et al., 2008).** Saponins are glycosides, which include steroid saponins and triterpenoid saponins. Tannins are plant polyphenols, which have ability to form complexes with macro-molecules such as proteins and interfere with the biological utilization of protein and to a lesser extent available lipids **(Dei et al., 2007)**. Saponins from different sources reduce circulating cholesterol levels in a variety of animals, including man. Saponins are used medically for the treatment of increased blood cholesterol. The hypocholesterolemic action of these compounds may be primarily mediated via cholesterol binding and bile acids in the intestinal lumen, thus hampering the absorption and enhancing the excretion of these steroids in the faeces and that they could reduce the accumulation of cholesterol**.** Cholesterol metabolism is accelerated and its circulating levels are decreased **(Francis et al., 2002)**.

Conclusion and Recommendations

It was concluded that low dose levels of Moringa leaves powder in layers' diets improved egg quality indices and reduced lipid content of eggs without any adverse effect on laying percentage, egg mass production,feed intake (FI) and feed convertion ratio (FCR) and these effects were dose dependent. The increased shell and albumen and the decreased the yolk weghts in the eggs could imply lower cholesterol content. The demand for less expensive organic eggs was attained since utilization of imported synthetic supplements and medicine were reduced when Moringa included in the diets. We recommended inclusion of MOLP to obtain low fat eggs of high grade without any adverse effect on mass production. Traditional farmers must be encouraged to to grow fast, easy growing moringa trees and use leaves in poultry feed because of its rich nutrients and bioactive phytochemical compounds.

Table (1): Effect of 0, 1, 1.5, 2 g/kg diet Moringa leaf powder (MLP) for 45 days on productivity, feed efficiency and eggs quality of layers.

Treatments / Parameters	MLP (0 g/kg diet)	MLP (1 g/kg diet)	MLP (1.5 g/kg diet)	MLP (2 g/kg diet)	F-Prob
Daily laying percentage	72.14± 6.33	82.86± 2.08	71.41±5.41	82.15±6.78	0.312
Daily egg mass production (g/hen)	41.46±3.46	46.78±0.53	37.29±3.58	48.46±4.54	0.118
Daily feed intake (g/hen)	127.29±8.28	131.00± 7.92	120.86± 13.96	129.14± 4.45	0.878
Feed conversion Ratio (Kg feed/kg eggs)	4.33± 0.57	3.41± 0.28	4.75± 0.64	3.46±0.47	0.207
Egg weight, g	52.74±0.62 **a**	56.01±0.83**b**	56.07±1.03 **b**	57.36±0.57 **b**	0.000*
Shell weight, g	7.06±0.20 **a**	7.94±0.23 **b**	7.95±0.23 **b**	7.95±0.20 **b**	0.006*
Yolk weight, g	15.79±0.32 **a**	14.65±0.18 **b**	14.46±0.34 **b**	14.29±0.36 **b**	0.007*
Albumen weight, g	30.32±0.34 **a**	32.41±0.88 **ab**	32.96±0.81 **b**	33.06±0.79 **b**	0.043*
Shell Percentage	13.67±0.46	13.89±0.42	14.17±0.37	15.02±0.60	0.210
Yolk Percentage	27.68±0.67	27.55±0.63	26.20±0.57	25.74±0.82	0.126
Albumen Percentage	57.64±0.96	57.73±1.61	58.64±0.64	58.88±1.39	0.934
Roche Colour	5.40±0.27 **a**	6.20±0.13 **b**	6.60±0.22 **b**	7.50±0.22 **c**	0.000*

Data were represented as means±SE. * Significantly difference using ANOVA test at P <0.05

Mean in the same row with different letters are significantly different (Duncan multiple range test P < 0.05).

Table 2: Egg yolk lipid profile of layers fed 0, 1, 1.5, 2 g/kg diet Moringa leaf powder (MLP) for 45 days.

Treatments / Parameters	MLP (0 g/kg diet)	MLP (1 g/kg diet)	MLP (1.5 g/kg diet)	MLP (2 g/kg diet)	F-Prob
Total lipid (g/dl)	13.02± 0.15 **a**	11.86±0.03**b**	11.26±0.06**c**	9.12± 0.11**c**	0.000*
Triglyceride (mg/dl)	678.69± 1.72**a**	553.26± 37.80**b**	536.08± 2.07**b**	528..93±29.69**b**	0.002*
Total Cholesterol (mg/dl)	290.10± 1.97**a**	256.80± 0.60 **b**	353.05±1.15 **c**	238.60± 0.27 **d**	0.000 *
HDL (mg/dl)	64.20± 1.63 **a**	69,88± 0.09**a**	107.80± 0.09 **b**	154.65± 1.17**c**	0.000*
LDL (mg/dl)	199.99± 0.30**a**	178.98± 0.16 **b**	173.45± 2.15 **c**	157.15± 0.22 **d**	0.000*
VDL (mg/dl)	235.74± 0.34**a**	111.34± 7.79 **b**	107.22± 6.42**b**	105.79± 5.94 **b**	0.003*
Atherogenic index (AI)	3.55±0.15**a**	2.68±0.01 **b**	1.35±0.02 **b**	0.58±0.09 **c**	0.000 *

Data were represented as means±SE. * Significantly difference using ANOVA test at P <0.05

Mean in the same row with different letters are significantly different (Duncan multiple range test P < 0.05).

Table 3: Serum lipid profile of layers fed 0, 1, 1.5, 2 g/kg diet Moringa leaf powder (MLP) for 45 days.

Treatments / Parameters	MLP (0 g/kg diet)	MLP (1 g/kg diet)	MLP (1.5 g/kg diet)	MLP (2 g/kg diet)	F-Prob
Total lipid (g/dl)	2.57±0.08 **a**	2.25± 0.03 **b**	1.73±0.01 **c**	1.58±0.04**d**	0.000*
Triglyceride (mg/dl)	344.16± 7.27 **a**	306.18±2.63 **b**	234.71±2.41**c**	205.67±1.32**d**	0.000*
Total Cholesterol (mg/dl)	223.70± 0.63 **a**	195.70±4.27 **b**	197± 1.87 **b**	142.40±3.73**c**	0.000*
HDL (mg/dl)	29.27± 2.46 **a**	50.81±0.83 **b**	55.00± 0.57 **c**	57.67± 0.31**c**	0.000*
LDL (mg/dl)	148.60±0.43 **a**	144.18± 0.70 **a**	112.85± 1.22 **b**	79.15±5.85 **c**	0.000*
VDL (mg/dl)	68.83±1.55 **a**	61.24±0.53 **b**	59.35± 4.62 **b**	41.13± 0.26 **c**	0.000*
Atherogenic index (AI)	7.18±0.71**a**	2.85±0.02**b**	2.59±0.07 **b**	1.24±0.15 **c**	0.000*

Data were represented as means±SE. * Significantly difference using ANOVA test at P <0.05

Mean in the same row with different letters are significantly different (Duncan multiple range test P < 0.05).

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