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Effect of *Moringa oleifera* (Lam) Leaf meal on Egg production, Blood and Serum profile of laying Japanese quail (*Coturnix coturnix japonica*)

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Target Audience: Poultry farmers, Extension agents, crop farmers, Researchers

Abstract

Ninety 12-wk old Japanese quail hens were allocated in a completely design (CRD)to 3 treatments with 3 replicates of 10 birds each which were fed ad libitum for forty two days. The diets were formulated to contain 19.8% CP and 2856.2 Kcal/kg ME and (diet 1) T_1, (diet 2) T_2 and (diet 3) T_3 contained 0, 1.0 and 2.0 % Moringa oleifera leaf meal (MOLM), respectively. Feed intake and egg production were recorded daily. At 42nd day of the feeding trial, blood samples from nine birds per treatment at three birds per replicate were collected for haematology, serum cholesterol, total protein, alanine amino transferase, creatinine, albumin, globulin, glucose, high density lipoprotein, low density lipoprotein, triglyceride and aspartate amino transferase. Feed intake revealed that hens fed diet T_1 (control, without leaf meal) consumed significantly ($p<0.05$) higher feed (25.37g/day) compared with those of birds fed diet T_2 (1.0% leaf meal) (23.75g/day) which was similar ($p < 0.05$) to the feed intake of birds fed diets T_3 (2.0% leaf meal) (22.78g/day).The hen-day production did not differ significantly ($p<0.05$) among the treatments. Average egg weight of T_2 (9.79unit) was significantly ($p<0.05$) different from T_3 (9.38g), however, T_1 (9.67g) was not significantly different from T_2 and T_3. The feed conversion ratio followed similar trend as the feed intake and hen-day production. The Results of serum biochemistry parameters examined were normal and not significantly ($p>0.05$) different with Moringa oleifera inclusion levels except cholesterol and HDL. The Packed cell volume, Haemoglobin (Hb), RBC, White blood cell (WBC), Platelet, Lymphocytes, heterocytes, monocytes and eosinophils were not affected ($P>0.05$) by different inclusion levels of Moringa oleifera leaf meal. However cholesterol and HDL were lowered at 1% inclusion of leaf meal while blood glucose and creatinine levels reduced with the inclusion of moringa leaf meal.

Keywords: *Moringa oleifera* leaf meal, Japanese quail hens, egg production, Blood and Serum profiles.

Description of Problem

Plants have been reported to contain large varieties of chemically active substances that possess important preventative and curative properties (1). Feed additives with growth promoting

activity increase stability of feed and beneficially influence the gastrointestinal ecosystem mostly through growth inhibition of pathogenic microorganisms (2) and antioxidant property (3) and anti stress (4). Antibiotic growth promoters (AGPs) have been used as a feed additive in poultry industry to enhance gut health and to control sub-clinical diseases. With increasing public concerns about bacterial resistance to synthetic antibiotics, the use of antibiotics in therapeutic or sub-therapeutic doses in poultry feed has been severely limited or eliminated in many countries. The European Union has preventively banned the use of antibiotics as growth promoters since 1st January 2006 (5). *Moringa oleifera* leaf contains bio-components whose antibacterial potentials are highly comparable with that of the antibiotic tetracycline (6).*Moringa* is a multipurpose tree, various parts which are used as fodder, herbal medicine otherwise regarded as a "miracle tree" (7), spices, food, natural coagulants, nectar for bees, fuel and fertilizer. *Moringa* contains very high antioxidants and anti-inflammatory compounds (8). The leaves, flowers and pods are used as good sources of vitamins A, B and C, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, beta-carotene, calcium, iron, and α-tocopherol (9). The pods are considered as an important source of the essential amino acids. The root bark contains two alkaloids: moringine and moringinine. Moringinine acts as cardiac stimulant, increases blood-pressure, acts on sympathetic nerve-endings as well as smooth muscles all over the body, and depresses the sympathetic motor fibers of vessels in large doses only. Aqueous leaf extracts are being used to treat hyperthyroidism as they help regulating thyroid hormone (10). Leaf extracts are also used to treat ulcer (11). *Moringa* leaves and pods also have a positive effect in reducing blood cholesterol (7), and anti-tumor promoting activity (12). It is an important source of the glucosinolate precursors of the isothiocyanate group of chemo preventives (13) that can inhibit carcinogenesis and hence *Moringa oleifera* leaf has a great potential in improving nutrition and strengthening immune functions of broiler chicken. Dietary supplementation of Moringa leaf in the broiler diets was effective in enhancing the oxidative stability of chicken meat (14). The replacement of antibiotic growth promoters with moringa leaf powder of 0.1 or 0.05% or Moringa fruit powder of 0.1 or 0.05% has beneficial effects on the growth performance and carcass yield of broiler chickens (15). The hormonal profiles of laying chicken were enhanced when fed with *Moringa oleifera* leaf meal with full fat soya bean meal at 2% compared to full-fat soya bean alone (16). *Moringa oleifera* could therefore become promising natural growth promoting, anti-oxidizing, hormonal influencing and anti-microbial agent. Therefore, alternatives to antibiotic growth promoters need to be proposed to livestock producers in order to maintain animal health, productivity and carcass quality. This study was therefore conducted to evaluate the *Moringa oleifera* leaf meal at 0, 1 and 2% inclusion as feed additive

on egg production, serum and haematological changes in Japanese quail hens.

Materials and Methods

Experimental site

The experiment was carried out at the Quail Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria.

Preparation of Test ingredient

The *Moringa oleifera* leaf meal used for this study was collected from University of Ibadan, Oyo State, Nigeria. The leaves were air dried under shade until they were crispy to touch while retaining their greenish coloration. The dried leaves were milled and then incorporated into quail layer diets. The diets contain 0, 1 and 2% of *moringa leaf meal* in treatments T_1, T_2 and T_3, respectively. The experimental diets are shown in table 1 as earlier reported (17).

Experimental Design

Completely Randomized Design was adopted for the experiment. Ninety 12-wk old quail hens were assigned randomly to three dietary treatments of 30 birds per treatment. Each treatment was replicated thrice and ten birds were allotted to each replicate. The hens were fed and given water *ad libitum* for six weeks.

Management

Housing and Management of birds

The pens were clean and disinfected before wood shavings served as litter material. The birds were reared in deep litter system and the floor was covered with wood shavings. Wood shavings were replaced at the 3rd week and It was ensured that water spillage on the litter was minimized. For the first 7 days, the birds were fed control diet for acclimatisation and from the 8th day each group received its respective feed treatment.

Experimental diet layout

T_1 –0.0% *Moringa* leaf meal
T_2 – 1.0% *Moringa* leaf meal
T_3 – 2.0% *Moringa* leaf meal

Parameters Measured

Performance characteristics:

Daily Feed Intake (g)

Daily feed intake was taken by deducting the left over for the day from the total feed served for the day.

Feed Intake = Total Feed – Left over

Hen –Day Production

Hen –day production : Egg were collected daily and the average weekly hen – day production was obtained by dividing the number of eggs collected per week per treatment by the product of number of birds per treatment and numbers of the day in a week.

Hen day production (%) =

$$\frac{\text{Numbers of egg collected per week}}{\text{Numbers of birds x No of days in a week}} \times 100$$

Average Weight Gain

Egg Weight gained (W) was taken by deducting the initial egg weight (W_1) from the new egg weight (W_2).

That is,

Egg weight gained (W) =

Final egg weight (W_2) – Initial egg weight (W_1)

Average egg weight (g)=

$$\frac{\text{Total weight of eggs per week (g)}}{\text{Numbers of egg laid per week}}$$

Body Weight gained (W_g) was taken by deducting the initial body weight (W_1)

Table 1: Composition of Experimental Diets for laying quail hens fed moringa leaf meal

Ingredients	**T1***(0.0% MOLM)*	**T2***(1.0% MOLM)*	**T3***(2.0% MOLM)*
Maize (white)	57.00	56.00	55.00
Soyabean meal	26.00	26.00	26.00
Wheat offal	7.00	7.00	7.00
Fish meal	2.00	2.00	2.00
Dicalcium phosphate	1.25	1.25	1.25
Limestone	6.00	6.00	6.00
Moringa leaf meal	0.00	1.00	2.00
L-Lysine	0.15	0.15	0.15
DL-Methionine	0.10	0.10	0.10
Premix*	0.25	0.25	0.25
Table salt	0.25	0.25	0.25
Total	**100**	**100**	**100**
Calculated Nutrients			
Crude Protein (%)	19.30	19.41	19.52
Crude Fibre (%)	3.50	3.53	3.64
Calcium (%)	2.50	2.65	2.71
Total P (%)	0.55	0.61	0.62
Fat (%)	3.60	3.70	3.80
Lysine (%)	1.20	1.30	1.40
Methionine (%)	0.40	0.41	0.42
ME (kcal/kg)	2883.30	2852.30	2821.40

**composition of premix/kg diet: Vit A 10,000.00iu, Vit D 3 20,000.00u, Vit E 20,0000mg, Vit k 3 2,000mg, Vit B 1 3,000mg, Vit B 2 5,000mg, Niacin 4,5000mg, Calcium Pantothenate 10,000mg, Vit B6 4000mg, Vit B12 20mg, Choline Chloride 300,00mg, Biotin 100mg, Manganese 50mg, Iron 300,000mg, Zinc 120,000mg, Copper 80,000mg, Iodine 15000mg, Cobalt 300mg, Selenium 120mg, Anti-oxidant 120,000mg.*

(17)(Abu, 2017)

from the new body weight (W_2).

Body weight gain (W_g)= Final body weight (W_2) – Initial body weight (W_1)

Feed Conversion Ratio

The feed conversion rate (FCR) was calculated by taking ratio of feed intake (FI) against the product of the egg weight per week and number of egg laid per week.

$$\text{FCR} = \frac{\text{Feed Intake per week (g)}}{\text{Average egg weight per week} \times \text{Number of egg laid per week}}$$

Egg Number

This was calculated by pooling the eggs collected on a daily basis together and the average obtained on a weekly basis per replicate.

Collection of blood samples for serum biochemistry variables

At the end of feeding trial, twenty seven quail birds (9 birds per dietary treatment) were selected and bled by the jugular vein using hypodermic needle with syringe, Blood was drained into two different carefully labeled bottles for haematological and serum metabolite investigation. Blood samples for biochemical variables were collected into sample bottles containing no Ethylene Diamine Tetra Acetic acid (EDTA), an anti-coagulant.

Haematology and Serum profile

The blood samples were collected into bottles pre-treated with Ethylene Diamine Tetra Acetic acid (EDTA), an anti-coagulant. Packed cell volume was determined using micro heamatocrit method, Haemoglobin concentration using cyanometaemoglobin method

(18). Erythrocytes (RBC) and leukocytes (WBC) count were determined using the improved Neubrauer haemocytometer after appropriate dilution and Lymphocytes, Monocytes, Eosinophils and Basophils were determined by scanning Gresham's stained slide (19). A commercial diagnostic cholesterol reagent kit (Erba Diagnostic MannasmGmbh) was used for cholesterol determination, Total protein and Albumin, while Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) were determined using Spectrophotometric methods (20), Alkaline phosphatase (ALP) (21). Total protein, Albumin and Globulin (22).Cholesterol (23). Total Triglycerides, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL). Serum cholesterol was calculated as;

$$\text{Cholesterol, mg/dL} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of calibrator x concentrate of calibrator}}$$

Statistical analysis

Data were analysed with the standard procedures of analysis (ANOVA) Using completely randomized design. Mean were separated using Duncan multiple Range Test (Gomez and Gomez, 1984) (24) to determine the significant differences.

Results and Discussion

Performance characteristics:

Feed intake

The result revealed (table 2) that quail in T_1(25.37g) had significantly higher feed intake compared to its counterparts on T_2(23.75g) and T_3(22.78g)

Average egg weight

Results in average egg weight shows in table 2 that there was significant difference ($p<0.05$) in T_2 (9.79) and T_3 (9.38) in which T_2 (9.79) and T_1 (9.67) are significantly ($p>0.05$) similar.

Numbers of egg laid

It was observed that there was no significantly difference ($p<0.05$) in numbers of egg laid across the treatments. The highest value was observed in T_3 (5.07) to T_1 (5.02) and T_2 (4.96).

Hen day production

Results in hen day production shown in table 2 that there was no significant difference ($p<0.05$) in the hen day production of layer quail fed Moringa leaf meal as feed additive.. The highest value was observed in T_3 (24.26) to T_1 (23.88) and T_2 (23.77).

Feed conversion ratio

Results of the feed conversion ratio (FCR) in table 2 revealed that birds fed diet T_1 (3.67) had the highest value while the lowest was observed in T_3 (3.37). T_1 (3.67)was significantly difference ($p<0.05$) from T_2 (3.45) and T_3 (3.37) but T_2 and T_3 was not significantly difference ($p<0.05$).

Table 2: Performance characteristics of layer quails fed graded levels of moringa leaf meal

Parameters	T_1	T_2	T_3	SEM
Feed Intake/bird/week (g)	25.37^{a}	23.75^{b}	22.78^{b}	0.44
Egg Weight/bird/day (g)	9.67^{ab}	9.79^{a}	9.38^{b}	0.10
No of eggs/bird/week	5.02	4.96	5.07	0.17
Hen day production (%)	23.88	23.77	24.26	0.79
Feed Conversion Ratio (g)	3.67^{a}	3.45^{ab}	3.37^{b}	0.09

^{abc}Means with the same superscript are not significantly different ($p<0.05$)
SEM = Standard error of mean

Table 3 shows the haematological indices of laying quails fed levels of *Moringa oleifera* leaf meal. It was observed that the Packed cell volume (PCV), Haemoglobin (Hb), White blood cell (WBC), Platelet, Lymphocytes, heterocytes, monocytes and eosinophils were not affected (P>0.05) by different inclusion levels of *moringa oleifera* leaf meal. The PCV ranged from 41.56±8.79 to 47.56±3.91. The Hb and WBC ($\times 10^3$) ranged from 14.17±3.29 to 15.88±1.23 and 1.06±3.50 to 1.13±4.43 respectively. The lymphocytes ranged from 64.11 to 65.56 and heterocytes 30.00±5.79 to 31.78, respectively. The monocytes and eosinophils ranged from 1.78±0.83 (control) to 2.22±0.83 (2% MOLM) and 1.78±1.20 (2% leaf meal) to 2.67±0.87 (control), respectively. However, the RBC ($\times 10^6$) was not affected (P>0.05) by different levels of *Moringa oleifera* leaf meal.

Table 3: Haematological Indices of laying quail fed *Moringa oleifera* leaf meal

Parameters	0%MOLM	1% MOLM	2% MOLM	SEM
PCV (%)	47.56	45.44	41.56	1.99
Hb (g/100mL)	15.88	15.22	14.17	0.72
RBC ($\times 10^6$)	7.98^a	7.60^{ab}	6.88^b	0.35
WBC ($\times 10^3$)	1.13	1.11	1.06	1.25
Platelets ($\times 10^5$)	1.86	1.78	1.93	0.53
Lymphocytes (%)	65.56	64.11	65.44	2.22
Heterocytes (%)	30.00	31.78	30.00	2.41
Monocytes (%)	1.78	1.89	2.22	0.27
Eosinophils (%)	2.67	2.11	1.78	0.34

ab: Means in the same row with different superscripts are statistically (P<0.05) different
SEM: Standard Error of Mean

Serum biochemical indices

Total protein

Results of total protein in Table 4 shows that there was no significant (p<0.05) difference in total protein across the treatments.

Albumin

Results of albumin in table 4 show that there was no significant (p<0.05) difference among all treatments in this study. The highest value was observed in T_1(2.44) to T_3 (2.30) and T_2 (2.29). Globulin, albumin-globulin ratio, AST, ALT, ALP, triglycerides and creatinine did not also show significant differences among treatments. Serum glucose however showed some variations as it was lowered in the presence of moringa leaf meal

Feed intake was reduced with inclusion of moringa leaf meal and result obtained was in line with the results obtained by various authors using different leaf meal as feed supplements in poultry feeds (25, 26). These authors attributed the significant decrease in feed intake as the level of leaf meal increased to the high fibre content of the moringa leaf meal and also due to the stringent bitter taste posed by the moringa leaf meal and different in the leaf meal used. It was suggested that low palatability, nutrient imbalance and bitterness of the diets as imparted by the neem leaf meal could be responsible for the low feed intake (26). These observations were similar to (27) who used neem leaf meal as a hypocholesterolemic dietary additive in

Table 4: Serum profile of Japanese quail hens fed moringa leaf meal as feed additive

PARAMETERS	T1	T2	T3	SEM
Total protein (g/dL)	5.71	5.34	5.49	0.39
Albumin (g/dL)	2.44	2.29	2.30	0.19
Globulin (g/dL)	3.27	3.06	3.19	0.24
A:G ratio	0.74	0.70	0.69	0.05
AST(iu/L)	44.11	44.00	43.33	1.65
ALT(iu/L)	31.67	32.00	32.33	0.98
ALP(iu/L)	124.11	120.00	123.67	2.06
Creatinine (mg/dL)	0.72[a]	0.66[b]	0.68[b]	0.07
Glucose (mg/dL)	128.22[a]	123.33[b]	121.44[b]	3.53
Cholesterol (mg/dL)	60.22[a]	50.78[b]	59.22[a]	4.90
Triglycerides (mg/dL)	48.44	37.67	46.78	4.84
HDL (mg/dL)	28.56[a]	19.89 [b]	26.56[a]	3.75
LDL (mg/dL)	21.98	23.36	23.31	0.65

[abc]Means with the same superscript are not significantly different ($p<0.05$)

SEM = Standard error of mean ; A: G RATIO = Albumin -Globulin ratio; AST = Aspartate amino transferase (AST); ALT = Alanine amino transferase (ALT); ALP = Alanine phosphate; HDL = High density lipoprotein; LDL = Low density lipoprotein

laying pullets and observed decreased feed consumption across the dietary groups. In this study the fibre level could not have significantly increased as to influence feed intake. Factor (s) other than fibre level of the diet may therefore be attributed to reduced feed intake but perhaps stringent bitter taste as observed in neem leaf (26). Although the feed intake results obtained in this study conformed to the reports of many authors but are not in line with the report of (28) who observed increased feed intake in layer hens fed diet containing 10 and 20% levels of *Moringa oleifera* leaf meal. Also the stage of growth of the moringa tree prior to harvesting of the leaves and processing (older leaves contains higher levels of fibre than younger leaves), the level of usage of the leaf meal in the diet of the birds and the health status of the birds when the experiment was conducted could affect the level of feed intake of the birds. The FCR reported in this study were in agreement with those reported by (25). But in disagreement with (28) observed low feed utilization in layers fed 20% levels of *M. oleifera* leaf meal. The contrast in values reported by different authors could be as a result of the type and breed of birds used in the study and the level of usage of the test ingredient. The hen day egg production was not affected perhaps because perhaps the levels moringine and moringinine were not significant to increase blood flow to the ovaries thereby causing more ovarian follicle formation which could have resulted in the increased egg lay by the birds in the diets incorporated with MOLM. Work on neem leaf meal in layers and suggested that at certain dietary inclusion level, there was the possibility of an increased blood flow to the ovaries thereby leading to more ovarian follicle formation which ultimately increased egg production (29). The results for serum cholesterol showed that the value obtained for birds were within the recommended range of 86-211mg/dl (30) (). From the present

study it can be inferred that the inclusion of moringa leaf meal had significant ($p < 0.05$) effect on the serum cholesterol levels of the birds but only at 1% inclusion level. No factor related reason could be adduced for this observation. Similarly, all other serum biochemical indices except glucose were affected by inclusion of moringa leaf meal. The serum creatinine showed that as the level of MOLM increased in the diet there was a decrease in the creatinine levels. The decrease in the levels of creatinine could be as a result of the relatively high protein level in the MOLM. (31, 32) had observed that the level of creatinine in the blood is dependent on the quantity and quality of the dietary protein. The normal range of values obtained for creatinine in this study suggests that the muscle of the birds were not affected negatively or depleted. Muscle wasting has been shown to be the source of excess creatinine in the blood of animals which is due to creatinine phosphate catabolism during this process (33). It was observed in this study that as the levels of creatinine and glucose decreased across the dietary groups. This suggests that serum creatinine and glucose levels are not negatively correlated as reported by (32) that animals will normally fall back on the stored energy in the muscles when there is reduction in blood glucose level. High density lipoprotein was only significantly reduced at 1.0% inclusion of leaf moringa leaf meal. High density lipoprotein (HDL), also known as good cholesterol, is responsible for carrying bad cholesterol away from arteries and may therefore help in lowering the risk of having heart attack (34). The reason for reduced HDL when birds were fed 1% leaf meal as opposed to 0 and 2% was no clear. The ALT and AST values were not affected but (35) observed increased in ALT and AST when neem leaf was fed to chickens. (36) reported low level of egg cholesterol in laying birds when *Moringa oleifera* leaf meal was used as a hypocholesterolemic agent in the diet of the birds at levels of 0, 5 and 10% inclusion. (37) also reported low cholesterol level in eggs of ISA Brown layers when moringa leaf meal was used as supplemental diet.

Conclusion and Recommendation

1 The present research was carried out to investigate the effect of incorporating *Moringa oleifera* leaf meal in quail hens' diet on egg production, blood profile and serum biochemistry.

2 *Moringa* leaf meal can be incorporated into the laying quail hens' diet up to 2% without declining effect on the egg production.

3 Cholesterol and HDL were reduced at 1% inclusion of leaf meal while glucose levels were reduced concomitantly as level of leaf meal increased.

4 The influence of *Moringa oleifera* leaf meal in quail hens may be somewhat different when fed to chickens. The high active nature and metabolism of the quail may be a possible cause.

5 The benefit of inclusion of moringa leaf meal in the diet of laying quail hens may be as a yolk colorant as investigated in

an earlier study.

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