**Influence of drumstick (*Moringa oleifera Lam.*)**  **leaf meal as functional feed additive on the haematological, total immunoglobulin and serum biochemical profile of laying Japanese Quail**

**ABSTRACT**

A feeding trial was conducted to discern the influence of drumstick(*Moringa oleifera* Lam.)leaf mealas functional feed additive on the haematological parameter, serum total immunoglobulin concentration andserum biochemical profile on layer Japanese quail *(Coturnix* *coturnix japonica*). A total 230 Japanese quails (182 hens and 48 males) at 6th weeks of age in laying phase were randomly allocated into eight treatment groups having three replicates in a complete randomized design. Laying Japanese quail of treatment T1 (control group) were fed a basal diet (starter and finisher), whereas in treatment group T2 basal diet was incorporated with Vitamin C @200 mg/kg, T3 basal diet was incorporated with Vitamin E @10 IU/kg, and in diets T4, T5, T6, T7, and T8, the basal diet was incorporated with 1.0%, 3.0%, 5.0%, 7.0% and 9.0% *Moringa oleifera* leaf meal, respectively. Results showed that hematological indices viz., Hb, PCV, TEC, TLC, MCH, MCHC, heterophil, and H/L ratio were significantly (P≤0.05) highest in T6 (5%MOLM) and lowest in T8 (9%MOLM) treatment group. A significant (P≤0.05) difference in serum total immunoglobulin (g/L) was observed amongst all the treatment groups of laying birds. The serum biochemical parameters showed significant (P≤0.05) reduction in serum glucose, cholesterol, triglycerides, LDL, andVLDL cholesterol in T6 (5%MOLM) treatment groups of laying birds. It can be concluded that 5.0 % drumstick (*Moringa oleifera*) leaf meal can be incorporated as a functional feed additive for improvement in hematological profile, serum total immunoglobulin concentration and serum biochemical profile on laying Japanese quail.

***Keywords****: Moringa oleifera leaf meal, haematological parameter, serum total immunoglobulin, biochemical parameter, laying Japanese quail*

**1. INTRODUCTION**

Poultry production plays a major role in bridging the protein gap in developing countries where average daily consumption is far below recommended standards (Onyimonyi *et al*. 2009). For poultry production quail, also known as "bater" in hindi, is a small bird that belongs to the family Phasianidae, genus *Coturnix bonnaterre* and species *Coturnix* *coturnix japonica*. Commercial quail farming is becoming more popular and is being increasingly promoted in number of Asian and European countries and recently in Africa **(RSPCA, 2011)**. Quail birds have, since introduction to Ghana, helped in diversifying the poultry sub-sector, supplemented conventional poultry production and also helped in bridging the gap of protein malnutrition **(Omane *et al*., 2020)**. Quail farming has been growing in popularity in Ghana as the years went by. Quail birds possess the unique characteristics of fast growth (they can be sold at five weeks of age as table birds), early sexual maturity (they lay their first egg at 40 days of age), high rate of egg production (up to 250 eggs a year) and shorter incubation period (16-17 day) **(Poynter *et al.,* 2009)**. Productivity of poultry in tropics has been limited by scarcity and consequent high prices of the conventional protein sources which are limiting factors for poultry feed production **(Atawodi *et al*. 2008)**. Hence, there is a need to search for alternate protein sources for use as feed supplement for sustainable poultry production. One possible source of cheap protein for poultry is the leaf meal of some tropical legume and plants **(Iheukwumere *et* *al*. 2008)**.

The leaves and green fresh pods of drumstick (*Moringa oleifera* Lam.) are used as vegetables by humans and are rich in carotene and ascorbic acid with a good profile of amino acids **(Makkar and Becker 1996)**.Drumstick (*Moringa oleifera* Lam.)is a *“Miracle trees”* with all the essential nutritional elements that are essential for livestock and human beings as well **(Fahey, 2005; Anjorin *et al.,* 2010)**. *Moringa oleifera* leaf meal (MOLM) can substitute protein source like sunflower seed meal partially (at 10% on protein basis) which suggest that the shrub have a potential for poultry feeding particularly for laying hens. This is exhibited through its protein content, relatively low fibre and high mineral contents, crude protein ranging from 22% to 30%, amino acids, carotenoids, and vitamins **(Lata *et al.* 2024 and Lu *et al.* 2016)**. The essential nutrient contents of *Moringa* leaves/ twigs such as vitamin A & B, calcium, iron, copper, sulphur and protein and its ability to absorb and neutralize toxic elements in food could justify its significance in developing the plant as one of the major local feed stuffs **(Lannaon 2007)**. *Moringa* leaves are highly nutritious and contain significant quantities of vitamins (A, B and C), calcium, iron, phosphorus and protein **(Murro *et al*. 2003)**. Furthermore, heavy metals such as mercury, arsenic and cadmium which are potentially toxic are absent from leaves of *Moringa oleifera*, thus making their incorporation in to poultry diets safe **(Donkor *et al*. 2013)**. Dietary inclusion of 10% in layer diets have been found to improve bird performance in terms of growth rate and egg production including egg size **(Cassius and Kenaleone 2014)**. Large amounts of *Moringa* forage can be obtained from easily established plots in the field without expensive inputs. *Moringa* is also a perennial plant that can be harvested several times in a year.

Keeping in view the potential feed value of *Moringa oleifera* leaf meal (MOLM), an experiment was conducted to study the effect of dietary incorporation of MOLM on the haematological, total immunoglobulin and serum biochemical profile of laying Japanese Quail.

**2. MATERIALS AND METHODS**

**2.1 Experimental Location**

The experimental trial was conducted to discern the influence of dietary incorporation of drumstick (*Moringa oleifera* Lam*.*) leaf meal on haemato-biochemical, serum total immunoglobulin efficacy on performance of laying Japanese quail. The entire study was conducted at Instructional Poultry Farm (I.P.F.) and Department of Animal Nutrition, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar located at latitude of 28053’24” North, longitude of 77034’27” East at an altitude of 243.84 meters above mean sea level.

**2.2 Experimental Birds and Housing Management**

In total 230 Japanese quails (182 hens and 48 males) at 6th weeks of age in laying phase were randomly allocated into eight treatment groups having three replicates in a complete randomized design. The experiment was carried out at Instructional Poultry Farm (I.P.F.), Nagla, and Department of Animal Nutrition, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. All birds were individually weighed and the birds were housed in cages. All birds received feed and water *ad-libitum*. Throughout the trial, adequate light (for 18 hours) and proper ventilation was provided to the all laying quail birds. The experiment was conducted in strict compliance with the guidelines of ‘Institutional Animal Ethics Committee (IAEC)’, GBPUAT, Pantnagar, India.



**Fig. 1: Laying Japanese quails**

**2.3 Preparation of *Moringa oleifera* leaf meal**

*Moringa oleifera* leaves also known as Drumstick was collected from farmers field. After removing twigs and leaflets by hand, the cut branches were spread out on a concrete floor and allowed to dry for a period of 5 days under shady conditions. The leaves were then kept into hot air oven at 500C for 3-4 hours to make them crispy for easy blending. The dried leaves were then run through a hammer mill sieve with a size of 4-5mm to produce the *Moringa oleifera* leaf meal (MOLM) and then stored in air tight polythene bags.

**2.4. Experimental treatment and diet**

There were eight dietary treatments where in, basal diet was supplemented with Vitamin C (200 mg/kg), Vitamin E (10 IU/kg), and varying level of *Moringa oleifera* leaf meal @ 1.0, 3.0, 5.0, 7.0, 9.0 % in diet of Japanese quail layer birds. The corn-soybean diet was in a mash form and was calculated as per **ICAR (2013)** specification**.** Feed ingredients required for the formulation of the experimental diet were procured from the feed unit and all the ingredients were ground at feed mill before mixing at feed unit of the Instructional Poultry Farm (IPF), GBPUAT, Pantnagar. α- tocopherol (Evion-400) and Vitamin C required for the trial was procured from local market of Pantnagar.

**2.5 Chemical analysis of drumstick leaves**

The proximate analysis of drumstick leaves was performed as per **AOAC (2005)**. Mineral estimation (Calcium, Phosphorus, Potassium, Zinc, Copper, and Manganese) of drumstick (*Moringa oleifera*) leaf meal was done by **AOAC (2005)** method and also by Atomicabsorption spectrophotometry. Vitamin C and Vitamin E was estimated by **(AOAC, 2005)**. Phenolics compound i.e. total tannin and phenolics acid was estimated by DPPH method. Amino acids such as Arginine, Histidine, Lysine, Methionine, Leucine, Tryptophan and Valine were estimated by DPPH method.

**2.6**  **Blood Collection and Analysis**

Blood samples were collected at the end of the trial, i.e., at 20th week of the experimental feeding trial for haematological parameters, serum biochemical profile and serum total immunoglobulin parameters. At the 20th week of the feeding trial, two birds were randomly selected from each replicate, making a total of 48 birds.Blood samples were collected for haematological analysis on the 20th week of the experiment. Three ml of blood was collected into well labeled blood collecting vials containing EDTA which act as anticoagulant for haematological analysis. Simultaneously, 1.5 ml blood was dispensed into another set of vials (without anticoagulant) and allowed to stand for three to four hours at room temperature in slanting position for clotting. After clotting of blood, serum samples were separated by centrifuging at 3000 rpm for 10-15min, and collected into Eppendorf tube and was kept at -200C in a deep freeze with date and sample number for serum total immunoglobulin concentration parameters analysis.

Blood samples were analyzed within 3 hours of their collection for total erythrocyte, haematocrit (PCV), haemoglobin (Hb) and differential leucocytes count. Haemoglobin concentration (g/dl) was estimated by **Sharma and Singh (2000)** using Sahil’s haemoglobinometer with acid haematin method. The brown colour was matched with glass standard and haemoglobin concentration (g/ dl) was recorded. Micro haematocrit method was used to estimate PCV as described by **Sharma and Singh (2000).** Fresh anticoagulant added blood was drawn into micro capillaries and sealed with wax at one end. Capillaries were centrifuged at 10,000 rpm for 30 minutes. PCV was directly measured using Citro Cap Microhematocrit tube reader and expressed in percent. For total erythrocytes count (TEC) Neubauer's chamber was used for counting the red blood cells, as described by **Jain (1986)**. The blood samples containing anticoagulant were diluted with Natt and Herrick’s diluting solution (1952). In a diluting pipette, well mixed blood was sucked until it reached the 1.0 mark. Natt and Herrick's dilution fluid was sucked up to the 101 mark. For 1-2 minutes, the mixture was allowed to stand in the pipette. Neubauer’s chamber was charged and RBCs were counted in the central primary square (four corner squares and one central square within the large primary square). The TEC (total erythrocyte counts) were expressed in million per microlitre (106/µl).

The immune response of Japanese layer quail was evaluated by total serum immunoglobulin concentration (g/L) by zinc sulphate turbidity test **(Mc Evans *et al.*, 1969)**.

**2.7 Statistical Analysis**

The data were presented as means ± standard error (SE). Statistical analysis was conducted using SPSS version 20. ANOVA, followed by Duncan’s Multiple Range test was used for multiple comparisons. Statistical differences were determined at the 5% level of significance.

**3. Results and Discussion**

**3.1 Nutrient Profile of Drumstick (*Moringa oleifera*) leaf meal (MOLM)**

Data pertaining to proximate and mineral composition of MOLM are presented in **Table 1** and vitamins, amino acids and phenolics compounds of *Moringa oleifera* leaf meal are presented in **Table 2.**

In current studyMOLM contained 92.40% organic matter (OM), 22.47% crude protein (CP), 9.67% ether extract (EE), 12.14% crude fibre (CF), 7.6% total ash (TA), 48.13% nitrogen free extract (NFE), 1.13% acid insoluble ash (AIA) and 60.26% total carbohydrate on dry matter basis. Mineral contents noted as 1.95% calcium, 0.56% phosphorus, 0.16% sodium, 0.68% potassium, 0.21%zinc, 0.18%copper, 0.24% manganese, 0.28% sulphur and 0.49% iron on DM basis

**Table 1 Proximate and mineral composition of drumstick (*Moringa oleifera*) leaf meal**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Moisture (%)** | **Proximate composition (%)** | | | | | | | | | |
| **OM** | **CP** | **EE** | **CF** | **TA** | **AIA** | **NFE** | | **Total carbohydrate** | |
| 72.63 | 92.40 | 22.47 | 9.67 | 12.14 | 7.6 | 1.13 | 48.13 | | 60.26 | |
| **Minerals composition (%)** | | | | | | | | | | |
| **Mineral (%)** | **Calcium** | **Phosphorus** | **Sodium** | **Potassium** | **Zinc** | **Copper** | **Manganese** | **Sulphur** | | **Iron** |
| 1.95 | 0.56 | 0.16 | 0.68 | 0.21 | 0.18 | 0.24 | 0.28 | | 0.49 |

Vitamins content were recorded as 0.38% vitamin C (Ascorbic acid) and 0.26% vitamin E (Tocopherol acetate). Amino acids (%) content in MOLM was as 0.24% arginine, 0.34% histidine, 0.25% lysine, 0.12% methionine, 0.08% leucine, 0.19% tryptophan, 0.27% valine, 0.23% glycine and 0.18% glutamic acid. Phenolics compounds such as total tannin 0.4602% and phenolics acid 0.8217% was noticed in *Moringa oleifera* leaf meal on dry matter basis.

**Table 2 Vitamins, amino acids and phenolics status (% DM basis) of *Moringa olifera* leaf meal**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Amino acids composition (%)** | | | | | | | | |
| **Arginine** | **Histidine** | **Lysine** | **Metheonine** | **Leucine** | **Tryptophan** | **Valine** | **Glycine** | **Glutamic acid** |
| 0.24 | 0.34 | 0.25 | 0.12 | 0.08 | 0.19 | 0.27 | 0.23 | 0.18 |
| **Vitamins composition (%)** | | | | | | | | |
| **Vitamins(%)** | **Vitamin C (Ascorbic Acid)** | | | **Vitamin E ( Tocopherol acetate)** | | | | |
| 0.38 | | | 0.26 | | | | |
| **Phenolics compounds (%)** | | | | | | | | |
| **Phenolics compounds** | **Total tannin** | | | **Phenolics acid** | | | | |
| 0.4602 | | | 0.8217 | | | | |

The present findings are in accordance with the report of **Macambira *et al.* (2022)** who observed 25.87% crude protein, 6.31% ether extract, and 15.94% mineral matter in *Moringa oleifera* leaf meal on DM basis respectively. Likewise, **Baloch *et al.* (2021)** found that *Moringa oleifera* leaf meal contains 9.13% crude fiber, 6.30% ether extract, 27.4% crude protein, 11.12% total ash, 44.31% nitrogen free extract, 1.42% calcium, and 0.35% phosphorus on DM basis.

**3.2 Effect of dietary inclusion of *Moringa oleifera* leaf meal on haematological indices**

Data pertaining to hematological indices of laying Japanese quail are exhibited in **Table 3 and Fig. 2.**

**3.2.1 Hemoglobin**

During this period, significantly (P≤0.05) higher Hb (%) was noted in T6 (15.64±0.24) and lower in T1 (11.18±0.37) treatment group. Hb percentage content was noticed as 11.18±0.37, 11.89±0.59, 11.84±0.32,13.23±0.79, 14.38±0.70,15.64±0.24,13.99±0.10 and 13.86±0.20 in different treatment groups viz., T1, T2, T3, T4 T5, T6, T7 and T8 respectively.

**3.2.2 Packed cell volume**

During this period, packed cell volume (%) was recorded as 35.16±1.62,33.71±1.04,35.57±0.62,32.62±1.01,31.74±1.07,30.85±0.16,31.39±0.73,and 31.83±0.33in different treatment group’s viz., T1, T2, T3, T4, T5, T6, T7 and T8 respectively. Significantly (P≤0.05) higher packed cell volume was in T3 (35.57±0.62) and lower in T6 (30.85±0.16) group. The PCV (%) was noticed statistically similar (P≥0.05) inT2, T4,T5, T6, T7 and T8 treatment group respectively.

**Table 3: Average values of haematological parameters in laying Japanese quail during 20th weeks period fed diets incorporated with *Moringa olifera* leaf meal**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | | | | | | | | | | |
| **Attributes** | **T1** | **T2** | **T3** | **T4** | **T5** | **T6** | **T7** | **T8** | **SEm** | **P- Value** |
| **Haemoglobin (%)** | 11.18±0.37**d** | 11.89±0.59**cd** | 11.84±0.32**cd** | 13.23±0.79**bc** | 14.38±0.70**ab** | 15.64±0.24**a** | 13.99±0.10**b** | 13.86±0.20**b** | 0.327 | 0.001 |
| **PCV(%)** | 35.16±1.62**a** | 33.71±1.04**ab** | 35.57±0.62**a** | 32.62±1.01**ab** | 31.74±1.07**b** | 30.85±0.16**b** | 31.39±0.73**b** | 31.83±0.33**b** | 0.441 | 0.016 |
| **TEC(106 μl)** | 3.74±0.55**a** | 2.96±0.29**ab** | 2.70±0.11*b* | 2.68±0.09**b** | 2.49±0.32**b** | 2.56±0.30**b** | 2.62±0.24**b** | 2.68±0.25**b** | 0.119 | 0.015 |
| **TLC (103/mm3)** | 23.78±0.29**a** | 23.31±0.03**ab** | 23.37±0.05**ab** | 23.26±0.01**ab** | 22.61±0.36**b** | 21.46±0.34c | 22.85±0.35**ab** | 22.83±0.43**ab** | 0.160 | 0.002 |
| **MCV (fl)** | 96.98±11.60 | 115.85±11.40 | 132.09±7.96 | 121.62±3.95 | 132.24±19.01 | 123.34±12.67 | 121.77±11.40 | 120.90±11.97 | 4.129 | 0.557 |
| **MCH (Pg)** | 31.19±4.63**c** | 41.04±5.04**bc** | 43.84±1.53**abc** | 49.62±4.90**abc** | 60.02±9.16**ab** | 62.47±6.09**a** | 54.38±5.58**ab** | 52.79±5.90**ab** | 2.630 | 0.023 |
| **MCHC (%)** | 32.05±2.65**d** | 35.44±2.71**cd** | 33.33±1.49**d** | 40.69±3.09**bc** | 45.29±1.26**ab** | 50.71±0.66**a** | 44.60±0.77**b** | 43.55±0.64**b** | 1.398 | 0.001 |
| **Heterophil (%)** | 24.57±0.11a | 24.25±0.05a | 24.21±0.03a | 23.85±0.47ab | 22.65±0.32**c** | 22.41±0.08**c** | 23.08±0.46**bc** | 23.02±0.43**bc** | 0.183 | 0.001 |
| **Lymphocyte (%)** | 70.50±0.28 | 70.51±0.29 | 70.54±0.31 | 70.71±0.02 | 70.87±0.08 | 70.97±0.40 | 70.82±0.29 | 70.84±0.65 | 0.108 | 0.952 |
| **Basophil+ Eosinophil (%)** | 4.23±0.28 | 4.25±0.31 | 4.23±0.03 | 4.21±0.03 | 4.21±0.03 | 4.23±0.02 | 4.22±0.02 | 4.23±0.02 | 0.044 | 1.000 |
| **Monocyte (%)** | 2.56±0.05 | 2.51±0.03 | 2.52±0.03 | 2.57±0.01 | 2.59±0.01 | 2.63±0.01 | 2.58±0.01 | 2.57±0.07 | 0.013 | 0.383 |
| **H/L Ratio** | 0.35±0.00**a** | 0.34±0.00**a** | 0.34±0.00**a** | 0.33±0.00**ab** | 0.32±0.00**c** | 0.31±0.00**c** | 0.32±0.00**bc** | 0.32±0.00**bc** | 0.003 | 0.001 |

**{T1 :**Control **;T2** :Basal diet with Vitamin C (200 mg/kg); **T3** : Basal diet with Vitamin E (10 IU/kg); **T4** :Basal diet with 1% MOLM;**T5**: Basal diet with3% MOLM; **T6** : Basal diet with 5% MOLM; **T7** : Basal diet with 7% MOLM; **T8** : Basal diet with 9%MOLM**}**

Means with different superscripts **(a, b, c, d )** in a row differ significantly (P≤ 0.05) from each other

**3.2.3 Total erythrocyte count**

During this feeding period, total erythrocyte count (106/µl) was noted as 3.74±0.55, 2.96±0.29, 2.70±0.11, 2.68±0.09, 2.49±0.32, 2.56±0.30, 2.62±0.24, 2.68±0.25 (×106 /µl) in different treatment group’s viz., T1, T2, T3, T4 T5, T6, T7 and T8 treatments respectively. Significantly (P≤0.05) higher TEC (106/µl) was noticed in T1 (3.74±0.55) and lower in T5 (2.49±0.32) group.

**Fig. 2: Average values of haematological parameters in laying Japanese quail during 20thweeks period fed diets incorporated with *Moringa oleifera* leaf meal**

**3.2.4 Total leucocytes count**

The total leucocytes count (×103/µl) value was noted as 23.78±0.29, 23.31±0.03, 23.37±0.05,23.26±0.01,22.61±0.36,21.46±0.34,22.85±0.35,22.83±0.43 in different treatment group’s viz., T1, T2, T3, T4 T5, T6, T7 and T8 treatments respectively. Significantly difference (P≤0.05) was noted among total leucocytes count (×103/µl) in different treatment groups. Higher TLC (×103/µl) value was observed in T1 (23.78±0.29) and lower in T6 (21.46±0.34) group.

**3.2.5 Mean corpuscular volume**

The non significant (P≥0.05) difference was noted among mean corpuscular volume in different treatment groups. The values of mean corpuscular volume (fl) was noted as 96.98±11.60, 115.85±11.40, 132.09±7.96, 121.62±3.95, 132.24±19.01, 123.34±12.67, 121.77±11.40, and 120.90±11.97 in different treatment group’s viz., T1, T2, T3, T4 T5, T6, T7 and T8 group respectively.

**3.2.6 Mean corpuscular hemoglobin**

Significantly difference (P≤0.05) was noted among mean corpuscular hemoglobin in different treatment groups. Higher (P≤0.05) MCH value was noticed in T6 (62.47±6.09) and lower in T1 (31.19±4.63) group.

**3.2.7 Mean corpuscular hemoglobin concentration**

Significantly difference (P≤0.05) was observed among mean corpuscular hemoglobin concentration in different treatment groups. Higher (P≤0.05) MCHC value was noticed in T6 (50.71±0.66) and lower in T1 (32.05±2.65) group. The value of mean corpuscular hemoglobin concentration (%) was noted as 32.05±2.65, 35.44±2.71,33.33±1.49,40.69±3.09, 45.29±1.26, 50.71±0.66, 44.60±0.77, 43.55±0.64 in different treatment group’s viz., T1, T2, T3, T4 T5, T6, T7 and T8 group respectively.

**3.2.8 Heterophil and Lymphocyte**

Significantly (P≤0.05) higher value of heterophil was in T1 (24.57±0.11) group and lower in T6 (22.41±0.08) group. But no significant (P≥0.05) difference was observed among lymphocyte in different treatment groups.

**3.2.9 Basophil plus Eosinophil**

The basophil plus eosinophil (%) did not differ significantly between different dietary treatment groups. The value of basophil plus eosinophil was noted as4.23±0.28, 4.25±0.31, 4.23±0.03, 4.21±0.03, 4.21±0.03, 4.23±0.02, 4.22±0.02, and 4.23±0.02 in different treatment group’s viz., T1, T2, T3, T4, T5, T6, T7 and T8 treatments respectively.

**3.2.10 Monocyte**

A non-significant (P≥0.05) difference was observed among monocyte in different treatment groups. The value of monocyte (%) was found as 2.56±0.05, 2.51±0.03, 2.52±0.03, 2.57±0.01, 2.59±0.01, 2.63±0.01, 2.58±0.01, and 2.57±0.07 in different treatment groups viz., T1, T2, T3, T4, T5, T6, T7 and T8 respectively.

**3.2.11 H/L Ratio**

The value of H/L ratio was noted as0.35±0.00, 0.34±0.00, 0.34±0.00, 0.33±0.00, 0.32±0.00, 0.31±0.00, 0.32±0.00, and 0.32±0.00 in different treatment groups viz., T1, T2, T3, T4, T5, T6, T7 and T8 respectively. Significantly (P≤0.05) lower was noticed in T6 (0.31±0.00) group and higher in T1 (0.35±0.00) followed by T2 (0.34±0.00) treatment group.

With the exception of Hb, PCV, TEC, TLC, MCH, MCHC, heterophil, and H/L ratio, the other blood parameters (MCV, Lymphocyte, Basophil plus Eosinophil, Monocyte) did not change significantly with inclusion of *Moringa oleifera* leaf meal (1.0,3.0,5.0,7.0 and 9.0 %) in Japanese quail chicks diets during 20th weeks feeding period in present study. The present findings are accordance with the report of Akinola and Ovotu (2018) found that Hb, PCV, MCV and MCH of the blood were highly (P≤0.05) influenced by supplementation of MOLM @ of 0.5, 1.0 and 1.5% in laying hens. Similarly Mousa *et al.* (2017) noted that there were of all levels of MOLM (0.2, 0.4 and 0.6%) had significantly (P≤0.05) higher WBCs, RBC, Hb and Hematocrite than control. Similarly Meel *et al.* (2022) noticed significantly higher (P˂0.01) level of Hb, PCV, TEC and lymphocytes in birds fed with 1.5% MOLM. However, the heterophils and H/L ratio decreased significantly (P<0.01) with an increased in MOLM levels across the treatment also significant reduction (P˂0.05) in albumin, globulin and total protein upon *Moringa* supplementation as compare to control diet. In contrast, Abu and Akangbe (2017)observed PCV, Hb, White blood cell, Platelet, lymphocytes, heterocytes, monocytes and eosinophils were not affected (P≤0.05) by 2.0% inclusion level of *Moringa oleifera* leaf meal in diet of laying Japanese quail.

**3.3 Effect of dietary inclusion of MOLMon serum total immunological concentration**

Data pertaining to total immunoglobulin concentration (g/L) in different treatment groups of laying Japanese quail are recorded during 20th weeks feeding period in Table 4 and Fig. 3. During this period, the total immunoglobulin concentration (g/L) was recorded as 3.34±0.32, 3.40±0.53,3.53±0.25,3.58±0.21,3.52±0.02,3.45±0.13,2.34±0.13, and2.38±0.51for T1, T2, T3, T4, T5, T6, T7 and T8 treatments respectively. Significantly (P≤0.05) difference was noted among total immunoglobulin concentration of different treatment groups. Significantly (P≤0.05) higher total immunoglobulin concentration was noticed in T4 (3.58±0.21) group and lower in T7(2.34±0.13) group. This result is also supported by the works of Olugbemi *et al*. (2010) who reported that *Moringa* leaves had a beneficial effect on the immune responses and improve intestinal health of birds.

**Table 4. Average values of total immunoglobulin concentration (g/L) in serum of laying Japanese quail during 20thweeks period fed diets incorporated with *Moringa olifera* leaf meal**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | | | | | | | | | | |
| **Attributes** | **T1** | **T2** | **T3** | **T4** | **T5** | **T6** | **T7** | **T8** | **SEm** | ***P- Value*** |
| **Total immunoglobin(g/L)** | 3.34±0.32**ab** | 3.40±0.53**a** | 3.53±0.25**a** | 3.58±0.21**a** | 3.52±0.02**a** | 3.45±0.13**a** | 2.34±0.13**b** | 2.38±0.51**b** | 0.138 | 0.043 |

**{T1 :**Control **; T2** :Basal diet with Vitamin C (200 mg/kg); **T3** : Basal diet with Vitamin E (10 IU/kg); **T4** : Basal diet with 1% MOLM; **T5**: Basal diet with 3% MOLM; **T6** : Basal diet with 5% MOLM; **T7** : Basal diet with 7% MOLM; **T8** : Basal diet with 9% MOLM**}**

Means with different superscripts **(a, b )**in a row differ significantly (P≤ 0.05) from each other

**Fig. 3 Average values of total immunoglobulin concentration (g/L)in serum of laying Japanese quail during 20thweeks period fed diets incorporated with *Moringa olifera* leaf meal**

**3.4 Effect of dietary inclusion of MOLMon Serum biochemical parameters**

Data pertaining to the biochemical parameters viz., glucose, total protein, albumin, globulin cholesterol, triglycerides, HDL, LDL, VLDL, in different treatment groups of laying Japanese quail are recorded during 20th weeks feeding period in **Table 5. and Fig. 4.**

**3.4.1 Serum total protein**

During this feeding period, mean serum total protein (g/dl) values for T1, T2, T3, T4, T5, T6, T7 and T8 dietary treatment incorporated with *Moringa oleifera* leaf meal was noted to be 5.82±0.21, 5.58±0.33, 5.75±0.09, 6.07±0.03, 6.42±0.32, 7.15±0.01, 6.33±0.09, and 6.37±0.09 (g/dl) respectively. Significantly (P≤0.05) lower value of total protein (g/dl) was in T2(5.58±0.33)group and higher in T6(7.15±0.01) followed by T5 (6.42±0.32) and T8(6.37±0.09g/dl)treatment group. **3.4.2 Serum albumin and Serum globulin**

There was no significant (P≥0.05) difference was observed among serum albumin in different treatment groups. But significantly (P≤0.05) lower value of serum globulin was noticed in T2 (1.80±0.37g/dl) group and higher in T6 (3.26±0.03 g/dl) group.

**3.4.3 Albumin/ Globulin ratio**

The value of A/G ratio was calculated as 1.92±0.16,2.28±0.43,2.07±0.50, 1.72±0.01,1.57±0.19, 1.19±0.02,1.48±0.15, and 1.44±0.13 in different treatment group’s viz., T1, T2, T3, T4, T5, T6, T7 and T8 treatment group respectively. No significant (P≥0.05) difference was observed among A/G ratio in different treatment groups.

**3.4.4 Serum glucose**

During this feeding period, mean serum glucose values for T1, T2, T3, T4, T5, T6, T7 and T8 dietary treatment incorporated with *Moringa oleifera* leaf meal was noted to be 224.51±5.10, 224.17±2.59, 223.04±2.11, 221.84±9.09, 218.07±9.82, 219.33±6.01**,** 218.66±12.25, and 189.36±1.86 (mg/dl) respectively. Significantly (P≤0.05) lower value of glucose was in T8 (189.36±1.86 mg/dl) group as compared to T1 (224.51±5.10 mg/dl) treatment group.

**Table 5. Average values of serum biochemical parameters in laying Japanese quail during 20thweeks period fed diets incorporated with *Moringa olifera* leaf meal**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatments** | | | | | | | | | | |
| **Attributes** | **T1** | **T2** | **T3** | **T4** | **T5** | **T6** | **T7** | **T8** | **SEm** | **P- Value** |
| **Total protein (g/dl)** | 5.82±0.21**bcd** | 5.58±0.33**d** | 5.75±0.09**cd** | 6.07±0.03**bcd** | 6.42±0.32**b** | 7.15±0.01**a** | 6.33±0.09**bc** | 6.37±0.09**bc** | 0.112 | 0.001 |
| **Albumin (g/dl)** | 3.81±0.07 | 3.78±0.05 | 3.79±0.35 | 3.84±0.01 | 3.88±0.02 | 3.89±0.02 | 3.76±0.10 | 3.74±0.10 | 0.042 | 0.993 |
| **Globulin (g/dl)** | 2.01±0.17**bc** | 1.80±0.37**c** | 1.96±0.29**bc** | 2.23±0.01**bc** | 2.54±0.34**abc** | 3.26±0.03**a** | 2.57±0.18**abc** | 2.62±0.16**ab** | 0.115 | 0.041 |
| **A/G Ratio** | 1.92±0.16 | 2.28±0.43 | 2.07±0.50 | 1.72±0.01 | 1.57±0.19 | 1.19±0.02 | 1.48±0.15 | 1.44±0.13 | 0.104 | 0.138 |
| **Glucose (mg/dl)** | 224.51±5.10**a** | 224.17±2.59**a** | 223.04±2.11**a** | 221.84±9.09**a** | 218.07±9.82**a** | 219.33±6.01**a** | 218.66±12.25**a** | 189.36±1.86**b** | 3.088 | 0.045 |
| **Cholesterol (mg/dl)** | 142.01±1.41**a** | 138.07±1.15**ab** | 137.40±1.13**ab** | 130.07±0.87**bc** | 128.81±2.96**bc** | 125.07±3.51**c** | 124.74±7.29**c** | 127.07±1.91**c** | 1.659 | 0.043 |
| **Triglycerides (mg/dl)** | 89.54±0.06**a** | 89.48±0.04**a** | 88.11±0.66**a** | 79.81±0.88**b** | 78.38±0.89**b** | 71.71±3.63**c** | 77.37±1.19**b** | 77.51±0.36**b** | 1.383 | 0.001 |
| **HDL (mg/dl)** | 39.18±0.08 | 39.11±0.34 | 39.06±0.36 | 39.37±0.11 | 40.33±1.00 | 41.43±1.12 | 39.57±0.21 | 39.46±0.01 | 0.231 | 0.113 |
| **LDL(mg/dl)** | 84.91±1.31**a** | 81.01±0.92**ab** | 80.71±0.96**ab** | 74.74±0.93**abc** | 72.79±2.52**bc** | 69.29±4.26**c** | 69.69±7.71**c** | 69.10±1.90**c** | 1.565 | 0.022 |
| **VLDL Cholesterol (mg/dl)** | 17.90±0.01**a** | 17.95±0.01**a** | 17.62±0.13**a** | 15.96±0.17**b** | 15.67±0.17**b** | 14.34±0.72**c** | 15.47±0.23**b** | 15.50±0.07**b** | 0.276 | 0.001 |

**{T1 :**Control **; T2** :Basal diet with Vitamin C (200 mg/kg); **T3** : Basal diet with Vitamin E (10 IU/kg); **T4** : Basal diet with 1% MOLM; **T5**: Basal diet with 3% MOLM; **T6** : Basal diet with 5% MOLM; **T7** : Basal diet with 7% MOLM; **T8** : Basal diet with 9% MOLM**}**

Means with different superscripts **(a, b, c, d )** in a row differ significantly (P≤ 0.05) from each other

**3.4.5 Serum cholesterol and Serum triglycerides**

Significantly (P≤0.05) lower serum cholesterol (mg/dl) was noticed in T7 (124.74±7.29) group and higher in T1 (142.01±1.41) followed by T2 (138.07±1.15) and T3 (137.40±1.13) treatment group. Similarly, significantly (P≤0.05) lower serum triglyceride (mg/dl) was observed in T6 (71.71±3.63) as compared to T1 (89.54±0.06) group.

**Fig. 4: Average values of serum biochemical parameters in laying Japanese quail during 20thweeks period fed diets incorporated with *Moringa olifera* leaf meal**

**f) 3.4.6 Serum HDL and Serum LDL**

There was a non significant (P≥0.05) difference was observed among serum HDL in different treatment groups. But significantly (P≤0.05) higher serum LDL (mg/dl) was observed in T1 (84.91±1.31) followed by T2 (81.01±0.92) and T3 (80.71±0.96) treatment group. The value of serum LDL (mg/dl) was noticed as 84.91±1.31, 81.01±0.92, 80.71±0.96,74.74±0.93,72.79±2.52, 69.29±4.26, 69.69±7.71, and 69.10±1.90 in different treatment group’s viz., T1, T2, T3, T4, T5, T6, T7 and T8 respectively.

**g) 3.4.7 VLDL Cholesterol**

The value for serum VLDL cholesterol (mg/dl) was noticed for T1, T2, T3, T4, T5, T6, T7 and T8 treatment group was 17.90±0.01, 17.95±0.01, 17.62±0.13, 15.96±0.17, 15.67±0.17, 14.34±0.72, 15.47±0.23, and 15.50±0.07 respectively. Significantly (P≤0.05) lower serum VLDL Cholesterol (mg/dl) was observed in T6 (14.34±0.72) followed by T4(15.96±0.17) and T5 treatment group.

With the exception of glucose, total protein, globulin, total cholesterol, triglyceride, LDL and VLDL the other blood biochemical parameters (HDL, albumin) did not change significantly with inclusion of *Moringa oleifera* leaf meal (1.0,3.0,5.0,7.0 and 9.0 %) in laying Japanese quail diets during 20th weeks feeding period in present study. Similarly, **Mousa *et al.* (2017)** reported thetotal plasma protein and globulin were significantly increased by using 0.2 and 0.4% MOLM and lowest value of A/G ratio was in treated group of birds. Plasma albumin significantly decreased with all levels of MOLM as compared to control. Likewise, **Lu *et al.* (2016)** reported the *Moringa oleifera* leaf group had a lower concentration of albumen than those in the control group in laying hens. In contrast, **Makanjuola *et al.* (2014)** observed that *Moringa oleifera* leaf meal incorporation @ 0.2%, 0.4%, and 0.6% in diet of laying birds did not affect serum total protein, albumin, and globulin concentration. Similarly, **Ashour *et al.* (2020)** showed that albumin, total protein, globulin, and A/G ratio had no significantly difference among *Moringa leaf* and *Moringa* seed in comparison to the control group and MSL group.

From the present study it can be inferred that the inclusion of various level of *Moringa oleifera* leaf meal had significant (P≤0.05) effect on serum cholesterol, triglyceride content of laying Japanese quail. This report was agreement with **El-Sheikh *et al.* (2015)** reported *Moringa oleifera* leaf powder inclusion in layer diets was active in cholesterol reduction in serum. Improvement cholesterol parameters may be due to *Moringa oleifera* contained hypocholesterolemic agent such phytoconstituent, β-sitosterol **(Kumar *et al.,* 2010)**. Likewise, **Sharmin *et al.* (2021)** noticed dietary addition of 1.5% MOLMsignificantly reduced serum, total cholesterol and triglyceride content as compared to control in laying birds. In contrast, **Akinola and Ovotu (2018)** found that serum parameter (total protein, total cholesterol, triglyceride, LDL, were not affected by *Moringa oleifera* leaf meal @0.5, 1.0 and 1.5% supplementation in diet of laying hens. Significantly (P≤0.05) decreased total cholesterol, LDL and HDL content in serum. 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. Furthermore **Garcia *et al.* (2021)** observed total cholesterol and triglycerides, diet fed with 6% *Moringa* inclusion was lower as compared to control group of laying birds. Similarly, **Meel *et al.* (2022)** observed significantly (P≤0.05) reduced blood glucose and triglyceride with *Moringa* leaf meal as compared to control group.

From the present study, it can be inferred that incorporation of vitamin C and vitamin E had decreased serum concentrations of glucose, cholesterol, and triglyceride in treated group of the Japanese quail layer. This finding was in line with **Kucuk *et al.* (2003)** showed that supplementation of vitamin C and vitamin E in diet of laying hens significantly decreased (P≤0.05) serum glucose, cholesterol, and triglycerides concentration. Similarly, **El-Sebai (2000)** reported that plasma concentrations of total protein, total lipids and total cholesterol increased in experimental groups fed diets with vitamin E as compared to control. Furthermore, Sahin *et al.* (2002) observed treatment with vitamin E caused an elevation in serum concentrations of total protein and albumin of broilers during heat stress conditions. On the other hand, **El-Mallah *et al.* (2011)** reported that hens fed diet added with vitamin E @0.50mg/kg had significant (P<0.05) increased on serum total protein, albumin, and globulin, whereas no effect on serum triglycerides comparing to the control.

**4. CONCLUSION**

Based on the findings of the present study, it can be concluded that 3% drumstick (*Moringa oleifera)* leaf meal can be incorporated in feed for improvement of haematological parameter, serum total immunoglobulin concentration and serum biochemical profile on laying Japanese quail. Similar type of work will be conducted in different species of poultry.

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