**Effects of Drumstick (Moringa oleifera Lam.) Leaf Meal on haematology, Immunity, and Serum Biochemistry in Laying Japanese Quail**

**Abstract**

The experiment aims to discern the effect of dietary incorporation of drumstick (Moringa oleifera Lam.) leaf meal on haemato-biochemical, serum total immunoglobulin efficacy on the performance of laying Japanese quail *(Coturnix* *coturnix japonica*). The 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, India. A total of 230 Japanese quails (182 hens and 48 males) at 6th weeks of age in the laying phase were randomly allocated into eight treatment groups having three replicates in a complete randomized design. Statistical analysis was conducted using SPSS version 20. ANOVA, followed by Duncan’s Multiple Range test was used for multiple comparisons. 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 (MOLM), respectively. The results showed that haematological 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 among 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 haematological 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*. The Japanese quail (Coturnix coturnix japonica) is one of the enterprises gaining attention as the ideal poultry species for meeting the animal protein needs in most of the developing countries (Longjam et al. 2024; Jahan et al. 2024). Commercial quail farming is becoming more popular and is being increasingly promoted in a number of Asian and European countries and recently in Africa (RSPCA, 2011). Quail is suited for commercial rearing for meat and egg production under intensive management, due to its early maturity, high laying intensity, diseases resistance, low space requirement, high nutritional value of egg and cheaper production cost, making commercial quail farming a choice of the farmers ((Abang et al. 2017; Kumari et al. 2007; Longjam et al. 2024). Quail birds have, since introduction to Ghana, helped diversify 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). The productivity of poultry in the tropics has been limited by scarcity and consequent high prices of 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 supplements for sustainable poultry production. One possible source of cheap protein for poultry is the leaf meal of some tropical legumes 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 sources like sunflower seed meal partially (at 10% on a protein basis) which suggests that the shrub has a potential for poultry feeding particularly for laying hens (Su& Chen, 2020). 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; Lu *et al.* 2016). The essential nutrient contents of *Moringa* leaves/ twigs such as vitamins A & B, calcium, iron, copper, sulphur and protein and their ability to absorb and neutralize toxic elements in food could justify its significance in developing the plant as a major local feedstuffs (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 into poultry diets safe (Donkor *et al*. 2013). Dietary inclusion of 10% in layer diets has 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 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 the performance of laying Japanese quail. The 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 a 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 the 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 the 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 were provided to all laying quail birds. The experiment was conducted in strict compliance with the guidelines of ‘The Institutional Animal Ethics Committee (IAEC)’, GBPUAT, Pantnagar, India.



**Pic 1: Laying Japanese quails**

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

*Moringa oleifera* leaves, also known as Drumstick, were collected from farmers’ fields. 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 in a 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, the basal diet was supplemented with Vitamin C (200 mg/kg), Vitamin E (10 IU/kg), and varying levels 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 the feed mill before mixing at the feed unit of the Instructional Poultry Farm (IPF), GBPUAT, Pantnagar. α- tocopherol (Evion-400) and Vitamin C required for the trial were procured from the local market, 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 Atomic absorption spectrophotometry. Vitamins C and E were estimated using the guidelines provided by AOAC (2005). Phenolics compound i.e. total tannin and phenolics acid was estimated by the DPPH method. Amino acids such as Arginine, Histidine, Lysine, Metheonine, Leucine, Tryptophane and Valine were estimated by the DPPH method.

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

Blood samples were collected at the end of the trial, i.e., in the 20th week of the experimental feeding trial for haematological parameters, serum biochemical profile and serum total immunoglobulin parameters. In 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 acts as an 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 a 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 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 leucocyte 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 the glass standard and haemoglobin concentration (g/ dl) was recorded. The 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 a Citro Cap Microhematocrit tube reader and expressed in per cent. 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 anticoagulants 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 millions 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).

**3. 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.

**4. Results and Discussion**

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

Results on the proximate and mineral composition of MOLM are presented in Table 1 while those on vitamins, amino acids and phenolic compounds of *Moringa oleifera* leaf meal are presented in Table 2.

Results reveal that MOLM 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 were recorded 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 dry matter 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 | 7.60 | 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 |

As shown in Table 2, 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% metheonine, 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 fibre, 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 a DM basis.

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

Results on the hematological indices of laying Japanese quail are presented in **Table 3 and Figure 1.**

**a) 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 groups. 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.

**b) 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±0.33 in different treatment group’s viz., T1, T2, T3, T4, T5, T6, T7 and T8 respectively. Significantly (p≤0.05) higher packed cell volume was recorded in T3 (35.57±0.62) and lower in T6 (30.85±0.16) group. The PCV (%) was noticed to be statistically similar (p≥0.05) in treatments T2, T4, T5, T6, T7 and T8 .

**Table 3: Average values of haematological parameters in laying Japanese quail during the 20th week 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

**c) 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) highest TEC (106/µl) was noticed in T1 (3.74±0.55) and least in T5 (2.49±0.32) group.

**Figure 1: Average values of haematological parameters in laying Japanese quail during the 20thweeks period fed diets incorporated with *Moringa olifera* leaf meal**

**d) 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, and 22.83±0.43 in different treatment group’s viz., T1, T2, T3, T4 T5, T6, T7 and T8 respectively. A significant difference (p≤0.05) was noted among total leucocyte count (×103/µl) in different treatment groups. The highest TLC (×103/µl) value was observed in T1 (23.78±0.29) and the least in T6 (21.46±0.34) group.

**e) 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) were 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.

**f) 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.

**g) Mean corpuscular hemoglobin concentration**

Significantly difference (P≤0.05) was observed among mean corpuscular hemoglobin concentration in different treatment groups. A 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.

**h) 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. However no significant (P≥0.05) difference was observed among lymphocytes in different treatment groups.

**i) 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.

**j) Monocyte**

A non-significant (P≥0.05) difference was observed among monocytes 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.

**k) 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, and0.32±0.00in different treatment groups viz., T1, T2, T3, T4, T5, T6, T7 and T8 respectively. Significantly (P≤0.05) lower was noticed in the T6 (0.31±0.00) group and higher in the T1 (0.35±0.00) followed by the 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. These findings are in consonance with the report of Akinola and Ovotu (2018) who 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 all levels of MOLM (0.2, 0.4 and 0.6%) had significantly (p≤0.05) higher WBCs, RBC, Hb and Hematocrite than the control. Similarly Meel *et al.* (2022) noticed significantly higher (p˂0.01) levels 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 MOLM level across the treatment. Also, a significant reduction (p˂0.05) in albumin, globulin and total protein upon *Moringa* supplementation was observed as compared to the control diet. In contrast, Abu and Akangbe (2017)observed that 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.

**4.3 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 the 20th week feeding period in Table 4 and Figure 2. 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, and 2.38±0.51 for T1, T2, T3, T4, T5, T6, T7 and T8 treatments respectively. A significant difference (p≤0.05) was observed among total immunoglobulin concentrations of different treatment groups. Significantly higher total immunoglobulin concentration (p≤0.05) was observed 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 improved intestinal health of birds.

**Table No. 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. 2 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**

**4.4 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 the 20th week feeding period in **Table 5 and Figure 3.**

**a) 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 recorded 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.

**b) 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) least value of serum globulin was noticed in T2 (1.80±0.37g/dl) group and highest in T6 (3.26±0.03 g/dl) group.

**c) 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.

**d) 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 were 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 the T8 (189.36±1.86 mg/dl) group as compared to the T1 (224.51±5.10 mg/dl) treatment group.

**Table No. 5. Average values of serum biochemical parameters in laying Japanese quail during the 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

**e) 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. 3: Average values of serum biochemical parameters in laying Japanese quail during 20thweeks period fed diets incorporated with *Moringa olifera* leaf meal**

**f) Serum HDL and Serum LDL**

There was a non-significant (P≥0.05) difference 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) 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 the 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 the present study. Similarly, Mousa *et al.* (2017) reported that the total plasma protein and globulin were significantly increased by using 0.2 and 0.4% MOLM and that the lowest value of A/G ratio was in the 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 the 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 significant 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 levels of *Moringa oleifera* leaf meal had a significant (p≤0.05) effect on serum cholesterol, and triglyceride content of laying Japanese quail. This agrees with El-Sheikh *et al.* (2015) who reported that *Moringa oleifera* leaf powder inclusion in layer diets was active in cholesterol reduction in serum. Improvement in cholesterol parameters may be due to *Moringa oleifera* containing hypocholesterolemic agents such as phytoconstituent, β-sitosterol (Kumar *et al.,* 2010). Likewise Sharmin *et al.* (2021) noticed that 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 parameters (total protein, total cholesterol, triglyceride, LDL, were not affected by *Moringa oleifera* leaf meal @0.5, 1.0 and 1.5% supplementation in the 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 a heart attack. Furthermore, Garcia *et al.* (2021) observed total cholesterol and triglycerides, a diet fed with 6% *Moringa* inclusion was lower as compared to a 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 the control group.

From the present study, it can be inferred that the incorporation of vitamins C and E decreased serum concentrations of glucose, cholesterol, and triglyceride in the treated group of the Japanese quail layer. This finding was in line with Kucuk *et al.* (2003) which showed that supplementation of vitamins C and E in the 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 that treatment with vitamin E caused a rise in serum concentrations of total protein and albumin in broilers during heat stress conditions. On the other hand, El-Mallah *et al.* (2011) reported that hens fed a diet added with vitamin E @0.50mg/kg had a significant (p<0.05) increase on serum total protein, albumin, and globulin, whereas no effect on serum triglycerides compared to the control.

**5. CONCLUSION**

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

**6. REFERENCES**

**Abu, O, A., & Akangbe, E, E. (2017).** Effect of *Moringa oleifera* (Lam) leaf meal on egg production, blood and serum profile of laying Japanese quail (Coturnix coturnix japonica). *Nigerian Journal of Animal Science*, *19*(2), 165-176.

**Akinola, L,A,F., & Ekine, O, A. (2018).** Evaluation of commercial layers feeds and their impact on performance and egg quality traits. *Nigerian Journal of Animal Production,* 20(2): 222-231.

**Anjorin, T, S., Ikokoh, P., & Okolo, S. (2010).** Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja and Nigeria. *International Journal of Agriculture and Biology,* 12, 431-434.

**AOAC. (2005).** Official Methods of Analysis. Hornitz, W. Ed. Washington, Association of Analytical Chemists. 18th Edn. Washington, D.C., USA.

**Ashour, E. A., El-Kholy, M. S., Alagawany, M., Abd El-Hack, M. E., Mohamed, L. A., Taha, A. E., & Tufarelli, V. (2020).** Effect of dietary supplementation with *Moringa oleifera* leaves and/or seeds powder on production, egg characteristics, hatchability and blood chemistry of laying Japanese quails. *Sustainability*, *12*(6), 2463.

**Atawodi, S. E., Mari, D., Atawodi, J. C., & Yahaya, Y. (2008).** Assessment of *Leucaena leucocephala* leaves as feed supplement in laying hens. *African Journal of Biotechnology* , 7, 317–21.

**Baloch, F. N., Baloch, H. N., & Mughal, G. A. (2021).** Replacement of sunflower meal with *Moringa oleifera* leaves on growth performance of broiler. *Approaches in Poultry, Dairy & Veterinary Sciences, 8*(2), 756-762.

**CARI, Izatnagar. (2013).** CARI Perspective Plan Vision 2050*.*Annual Report 2013-14, Published by *Central Research Institute Izatnagar*, Bareilly, Uttar Pradesh.32 p.

**Cassius Moreki John & Kenaleone Gabanakgosi. (2014).** Potential use of *Moringa olifera* in poultry diets. *Global Journal of* *Animal Scientific Research*, 2 (2), 109–115.

**Donkor, A. M., Kwame Glover, R. L., Addae Daniel., & Kubi , K. A. ( 2013).** Estimating the nutritional value of the leaves of *Moringa* *oleifera* on poultry. *Food and Nutrition Sciences,* 4, 1077–83.

**El-Mallah, G. M., Yassein, S. A., Magda, M. A. F., & El-Ghamry, A. A. (2011).** Improving performance and some metabolic response by using some antioxidants in laying diets during summer season. 217- 224.

**El-Sheikh, N. I., El-Shazly, E. S., Abbas, E. A., & El-Gobary, G. I. (2015).** Effect of *Moringa l*eaves on lipid content of table eggs in layer hens. *Egyptian Journal of Chemistry and Environmental Health*, *1*(1), 291-302.

**Fahey, J. W. (2005).** *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1.*Trees for life Journal*, 1(5), 1-15.

**Garcia, R, G., Gandra, E, R, S., Burbarelli, M, F, C., Valentim, J, K., Felix, G, A., Lopes, B, A. & Caldara, F, R. (2021).** *Moringa oleifera*: an alternative ingredient to improve the egg quality of Japanese quail. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*,  *73*(03), 721-732.

**ICAR. (2013).** Nutrient requirement of Japanese quail. Central Avian Research Institute (CARI), Izatnagar, Bareilly, Uttar Pradesh, India.

**Iheukwumere, F. C., Ndubuisi, E. C., Mazi, E. A., & Onyekwere, M. U. (2008).**  Performance, nutrient utilization and organ characteristics of broilers fed cassava leaf meal (*Manihot esculenta Crantz*). *Pakistan Journal of Nutrition*, 7, 13–16.

**India, Ministry of Fisheries, Animal Husbandry and Dairying (2022).** Annual report 2021-22, published by department of Animal Husbandry & Dairying, New Delhi*,* 4 p.

**Jain, N, C. (1986)*.*** Schalm’s Veterinary Haematology. Lea and Febiger, Philadelphia, USA, pp. 56-61.

**Kucuk, O., Sahin, N., Sahin, K., Gursu, M. F., Gulcu, F., Ozcelik, M., & Issi, M. (2003).** Egg production, egg quality, and lipid peroxidation status in laying hens maintained at a low ambient temperature (6 C) and fed a vitamin C and vitamin E-supplemented diet. *Veterinární Medicína*, *48*(12), 33.

**Kumar, P, S., Mishra, D., Ghosh, G., & Panda, C, S. (2010).** Medicinal uses and pharmacological properties of *Moringa oleifera*.  *International Journal of Phytomedicine*, *2*(3), 210-216.

**Lannaon , W. J. (2007).** Herbal plants as source of antibiotics for broilers. *Agriculture Magazine,* 11, 55.

**Lata, M. & Mondal, B, C. (2024).**  *Moringa oleifera* Leaf Meal: A sustainable approach for poultry production: A review. *Archives of Current Research International*, *24* (11), 176-185.

**Lu, W., Wang, J., Zhang, H. J., Wu, S. G. & Qi, G. H. (2016).** Evaluation of *Moringa oleifera* leaf in laying hens: Effects on laying performance, egg quality, plasma biochemistry and organ histopathological indices. *Italian Journal of Animal Science,* 15, 658–665.

**Macambira, G. M., Rabello, C. B. V., Navarro, M. I. V., Lopes, C. D. C., Lopes, E. C., Nascimento, G. R. D., & Silva, J. D. C. R. D. (2022).** Effects of *Moringa oleifera* leaf meal on performance and carcass yield of broilers. *Revista Brasileira de Zootecnia*, *51*, e20210203.

**Makanjuola, B. A., Obi, O. O., Olorungbohunmi, T. O., Morakinyo, O. A., Oladele-Bukola, M. O., & Boladuro, B. A. (2014).**  Effect of *Moringa oleifera* leaf meal as a substitute for antibiotics on the performance and blood parameters of broiler chickens. *Livestock Research for Rural Development*, *26*(8), 144.

**Makkar, H. P. S., & Becker, K. (1996).** Nutritional value and nutritional components of whole and extracted *Moringa* *oleifera* leaves. *Animal Feed Science and Technology,* 63, 211–228.

**Mc Evans, A. D., Fischer, W., Selman, I. F. & Perihale, W. J. (1969).**  A turbidity test for estimation of immunoglobulin levels in neonatal calf serum. *Clinica Chima Acta*, 27, 155-163.

**Meel, M. S., Sharma, T., Dhuria, R. K., Joshi, M., Shende, K. A., Kumari, M., & Mishra, G. (2022).** Influence of dietary inclusion of *Moringa oleifera* leaf meal on blood chemistry of broiler chicks. *Scientist*, *1*(3), 4300-4305.

**Mousa, M. A. M., Moustafa, K. E. M., Shata, R. F., Alghonimy, H. A., & Youssef, S, F. (2017).** Effect of using *Moringa oleifera* leaf meal as feed additives on Japanese quail during lying period. *Egyptian Journal of Nutrition and Feeds*, *20*(2), 203-212.

**Murro, J. K., Muhikambele, V. R. M., & Sarwatt, S, V. (2003).**  *Moringa oleifera* leaf meal can replace cottonseed cake in the concentrate mix fed with Rhodes grass (*Chloris gayana*) hay for growing sheep. *Livestock Research for Rural* *Development.* 15, 84.

**Olugbemi, T. S., Mutayoba, S. K. and Lekule, F. P. (2010).** *Moringa oleifera*  leaf meal as a hypocholesterolemic agent in laying hen diets. *Livestock Research Rural Development*, 84, 27–37.

**Omane, O. K., Aikins, T. K., & Imoro, Z. A. (2020).** Reproductive potential and egg laying performance of quail in savannah ecological zone of GHANA. *UDS, International* *Journal of Development*, *7*(1), 246-255.

**Onyimonyi, A. E., Olabode, A ., & Okeke, G. C. (2009).** Performance and economic characteristics of broilers fed varying dietary levels of neem leaf meal (*Azadiracta indica*). *International* *Journal of Poultry Science,* 8, 256–259.

**Poynter, G., Huss, D. & Lansford, R. (2009).** Japanese quail: an efficient animal model for the production of transgenic avians. *Cold Spring Harbor Protocols*, 3 (1), 112-123.

Royal Society for the Prevention of Cruelty to Animals (**RSPCA). (2011).** Quail: Good practice for housing and care. *Research Animals Department* .4th edition. West Sussex.

**Sahin, K., Kucuk, O., Sahin, N., & Gursu, M. F. (2002).** Optimal dietary concentration of vitamin E for alleviating the effect of heat stress on performance, thyroid status, ACTH and some serum metabolite and mineral concentrations in broilers. *Veterinarni Medicina- Praha*, *47*(4), 110-116.

**Sharma, I. J. & Singh, H. S. (2000).** *Student’s Laboratory Manual of Veterinary Physiology*. Kalyani Publishers, New Delhi. pp. 26-28.

**Sharmin, F., Sarker, M. S. K., Sarker, N. R., & Faruque, S. (2021).** Dietary effect of *Moringa oleifera* on native laying hens’ egg quality, cholesterol and fatty-acid profile. *Italian Journal of Animal Science*, *20*(1), 1544-1553.

Su, B., & Chen, X. (2020). Current status and potential of Moringa oleifera leaf as an alternative protein source for animal feeds. Frontiers in veterinary science, 7, 53.

Longjam, S. D., Goswami, R., Kalita, G., Samanta, A. K., & Ahmed, F. A. (2024). Effect of Turmeric Powder Supplementation on Egg Production, Hatchability and Internal Egg Quality Characteristics of Quail Eggs. Archives of Current Research International, 24(5), 769–776. <https://doi.org/10.9734/acri/2024/v24i5752>

Jahan, N., Antora, F. H., Mim, M. M. A., Tuhin, M. K. H., Siddiqi, M. N. H., & Nasrin, M. (2024). Prospects and problems of quail farming at Jhenaidah Sadar Upazila, Bangladesh. International Journal of Natural and Social Sciences, 11(1), 39-48.

Abang FB, Oko OK, Yelwa JT. Carcass and organ characteristics of growing Japanese quails (Coturnix coturnix japonica) fed sun-dried mango (Mangifera spp) kernel meal as a replacement for maize. Annual Research and Review in Biology. 2017;20(6):1-7

Kumari P, Gupta MK, Ranjan R, Singh KK, Yadava R. Curcuma longaas feed additive in broiler bird and its patophysiological effects. Indian Journal of Experimental Biology. 2007;45(3):272.277.