Original Research Article

**Haematological and Biochemical Profiles in Broiler Chickens Fed Diets Containing Different Levels of *Moringa oleifera* Leaf Meal**

ABSTRACT

|  |
| --- |
| **Aims:** A study was conducted to identify the Haematological and Biochemical Profiles of Broiler Chickens Fed at Different Levels of *Moringa oleifera* leaf meal.Place and Duration of Study: The experimental study included the rearing of broiler chickens were conducted at the National Animal Health Research Centre (NAHRC), Khumaltar, Lalitpur, Nepal. Laboratory examination was conducted at, NAHRC, Pathology lab. The feeding trial lasted for 42 days.**Methodology:** A total of 144 birds were divided into 4 treatment groups with 3 replications in each group, each replication containing 12 birds. Experimental broiler diets were comprised of no *Moringa oleifera* (T1); *Moringa oleifera* 0.5% (T2); *Moringa oleifera* 1% (T3), *and Moringa oleifera* 1.5% (T4). Haemoglobin, haematocrit and differential leukocyte count was determined. In the biochemical test, total serum protein (Albumin and Globulin), Glucose and Cholesterol, AST (Aspartate Transaminase), ALT (Glutamate Pyruvate Transaminase), Calcium, Urea and Blood Urea Nitrogen (BUN) were determined. The obtained values were statistically analysed by one-way analysis of variance (ANOVA).**Result:** Haematocrit, heterophil, monocyte, lymphocyte and eosinophil, albumin, glucose, cholesterol, calcium, AST, ALT, urea and BUN were found to be statistically nonsignificant (p>0.05) among the treatment and control groups throughout the observation period. But Haemoglobin, total protein and globulin was found to be significantly (p< 0.05) affected. Haemoglobin, haematocrit, heterophil, monocyte, lymphocyte, and eosinophil values were higher in broiler feeding *Moringa oleifera* treated diet. Serum glucose level increases in the *Moringa oleifera* treated diet. The result shows a significant reduction in total serum cholesterol level in *Moringa oleifera* treated diet. **Conclusion:** It could be concluded that Moringa oleifera leaf meal with levels of 0.5, 1 and 1.5% has a beneficial effect on some haeamatological and serum biochemical indices. The best level occurred by 1% in broiler chicken diets. |

*Keywords:**Broiler; Diets; Haematological; Biochemical; Moringa oleifera.*

1.INTRODUCTION

A Miracle tree, *Moringa oleifera,* known as the drumstick tree, is a multipurpose tree that thrives in both tropical and sub-tropical conditions. It has high nutritional and medicinal value.  In Nepal, it is called Sheetal Chini. Its different parts are sources of proteins, vitamins and minerals and present different medicinal and nutritional properties. Due to the scarcity and rising costs of conventional feed ingredients, researchers in developing countries are increasingly exploring non-traditional feed sources, with a focus on protein alternatives such as leguminous multipurpose trees and shrubs, which offer a rich supply of proteins, vitamins, and minerals for poultry nutrition (Elebha et al., 2018).

 *Moringa oleifera* leaf is reported to contain 25–27% crude protein (Gadzirayi et al., 2012). Its medicinal properties derive from  [alkaloids](https://www.sciencedirect.com/topics/medicine-and-dentistry/alkaloid), [tannins](https://www.sciencedirect.com/topics/medicine-and-dentistry/tannin-derivative), [flavonoids](https://www.sciencedirect.com/topics/medicine-and-dentistry/flavonoid), steroids, [saponins](https://www.sciencedirect.com/topics/medicine-and-dentistry/saponin), [coumarins](https://www.sciencedirect.com/topics/medicine-and-dentistry/coumarin-derivative), [quinones](https://www.sciencedirect.com/topics/medicine-and-dentistry/quinone-derivative) and resins contained in it (Anwar et al., 2007). The chemical constituents of *M. oleifera* have bioactive compounds, secondary metabolites such as phenolic acids, gallic acid, ellagic acid, chlorogenic acid, ferulic acid, glucosinolates, [quercetin](https://www.sciencedirect.com/topics/medicine-and-dentistry/quercetin), [vanillin](https://www.sciencedirect.com/topics/medicine-and-dentistry/vanillin) and [kaempferol](https://www.sciencedirect.com/topics/medicine-and-dentistry/kaempferol), which have nutritional, pharmaceutical and/or [antimicrobial properties](https://www.sciencedirect.com/topics/medicine-and-dentistry/antimicrobial-activity) (Mbikay, 2012 and Brilhante et al., 2017). Supplementation of *Moringa oleifera* leaf meal also helped in improving immunocompetence and gut health of broilers.The study aimed to investigate the effects of *Moringa oleifera* leaf meal as dietary supplementation on haematological and biochemical parameters of broiler chickens.

2. materials and methods

**2.1 Experimental trial**

The experimental study which included the rearing of broiler chickens was conducted at the National Animal Health Research Centre (NAHRC), Khumaltar, Lalitpur, Nepal. Laboratory examination was conducted at, NAHRC, Pathology lab.

**2.2 Experimental design**

One hundred and forty-four day old Cobb 500 broiler chickens (with an average body weight 43.06 ± 2.39) from a commercial hatchery were procured and divided into four (4) treatments groups each consisting of thiry-six birds. Initially, birds were kept for one week in the experimental house. Each group was replicated three times with each replication consisting of 12 birds. Experimental groups were given different treatments which are mentioned in [Table 1](https://www.sciencedirect.com/science/article/pii/S2451943X18300887#tbl0001) below. The birds were allocated randomly to the different groups.

 **Table 1: Experimental trial**

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Replication** | **Feed** |
| T1 | 3 | Basal diet with no *Moringa oleifera* |
| T2 | 3 | Basal diet with *Moringa oleifera* 0.5% (5gm/kg feed) in feed |
| T3 | 3 | Basal diet with *Moringa oleifera* 1% (10gm/kg feed) in feed |
| T4 | 3 | Basal diet with *Moringa oleifera* 1.5% (15gm/kg feed) in feed |
| **Total:** 4 | **Total:** 12 |  |

### 2.2 General management

The birds were reared on a [deep litter](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/deep-litter) system open-sided wire mesh constructed poultry house to allow for adequate ventilation and maintained with all recommended husbandry norms in relation to light, feed and water. Commercial mash feeds were acquired from a commercial feed mill. The *Moringa oleifera* leaf powder was sourced from a reputed company, Annapurna Organic Agricultural Udhoyug, Kapilvastu, Nepal. Feed and water were given to the birds *ad-libitum.*

All the groups were reared on commercial feeds with fulfillment of all its requirements according to the increasing life time and days of life. They were fed with broiler starter feed (B1) from zero day to 3rd weeks, broiler grower feed (B2) after 3rd week up to 5th weeks and broiler finisher (B3) from 5th week onwards. Frequent changes of water with proper arrangement of space and light were managed for all the birds. Lighting source was provided using electricity bulbs during the night. Medications, vaccinations and other routine management practices were strictly followed.

**2.2 Laboratory examination**

 **2.2.1 Haematological & Biochemical estimation**

One bird from each replication was selected randomly at the end of trial at 42 days. The blood samples was collected from wing vein and kept in EDTA and Non-EDTA vials. The serum was immediately separated from blood and stored at −20 °C. Haematological & biochemical parameters of poultry were analysed. Haemoglobin, haematocrit and differential leukocyte count was determined. In the biochemical test, total serum protein (Albumin and Globulin), Glucose and Cholesterol, AST (Aspartate Transaminase), ALT (Glutamate Pyruvate Transaminase), Calcium, Urea and Blood Urea Nitrogen (BUN) were determined by by Reitman & Frankel's method as per Erba Mannheim test kit and Accurex biomedical, Pvt. Ltd test kit with the help of a spectrophotometer (Sharma et al., 2018).

**2.3 Bio-statistics and data analysis:**

Statistical analysis was carried out using Microsoft Excel-2007 and ANOVA using SPSS-20 version, at a significance level of 0.05. The treatments were assigned randomly following the Complete Randomized Design (CRD).

3. results and discussion

**3.1 Effect of feeding *Moringa oleifera* leaf meal on haemoglobin, haematocrit and Differential leukocyte count**

Table 2 below presents changes in the haemoglobin, haematocrit and differential leukocyte count in broiler chickens under experimentation at different levels of *Moringa oleifera* leaf meal inclusion. Haematocrit, heterophil, monocyte, lymphocyte and eosinophil were found to be statistically nonsignificant (p>0.05) among the treatment and control groups throughout the observation period.

But Haemoglobin was found to be significantly (p< 0.05) affected. There were no basophils found on any treatments. Haemoglobin, haematocrit, heterophil, monocyte, lymphocyte and eosinophil value were higher in broilers receving *Moringa oleifera* leaf meal than control. *Moringa oleifera* leaves contain iron at levels of 250, 5 dpm, and 490 mg/kg, which may contribute to increased iron intake that supports haemoglobin synthesis and red blood cell production within safe limits (Hurrell, 1997; McDowell, 2003; Moyo et al., 2016; Sebola et al., 2017).Chickens with a greater number of functional heterophils may be more capable of resisting bacterial infections, and higher heterophil counts could therefore be indicative of a stronger innate immune response (Bilková et al., 2017). The obtained data were agreed with those reported by Allam et al., (2016) who found that effect of *Moringa oleifera* in blood picture (HB and PCV which were 14.23g/dl and 37.22%), respectively. Compared with of control broilers supplemented, which were (13.24g/dl and 34.28%), respectively. This increase may be due to iron and beta-carotene contains in *Moringa oleifera* leaves. Iron is necessary for many functions in the body including the formation of hemoglobin and myoglobin. Red blood cells are responsible for the transportation of oxygen and carbon dioxide in the blood as well as the manufacture of haemogloblin, hence higher values indicates a greater potential for these function and a better state of health.

The inclusion of *Moringa oleifera*-treated meal led to an increase in differential leukocyte count (heterophil, monocyte, lymphocyte and eosinophil) compared to the control diet. Such increases, remaining within the normal range, could enhance the animal's immune function by promoting antibody production and improving overall immunity. Similarly, Gupta et al. (2010) reported an increase in the WBC count and neutrophils in mice treated with *Moringa oleifera* extract. This reaction could be attributed to dietary phytochemicals with antioxidant properties, such as flavonoids, which are known to improve the immune system response in all taxa of vertebrates. The *Moringa oleifera*-treated meal causes elevated eosinophil counts compared to the control group. According to Schalm et al. (1975), white blood cells play a crucial role in the body’s defence system, significantly contributing to immune responses and the ability to combat infections. Therefore, higher WBC levels may provide birds with enhanced resistance to infections compared to those with lower WBC counts.

**Table2: Effect feeding Moringa oleifera feeding on Hemoglobin, Heterophil & differential Leukocyte count (Mean± SE) in blood at 42 days of broilers.**

This increase may be due to iron

contains (23mg/100g) in Moringa oleifera leaves. In this

regard, Lutz and Prytulski (2008); Elbashier and Ahmed

(2016) reported that iron is necessary for many functions

in the body including the formation of hemoglobin and

myoglobin. According to Olugbemi et al (2010) red blood

cells are responsible for the transportation of oxygen and

carbon dioxide in the blood as well as the manufacture of

haemoglobin, hence higher values indicates a greater

potential for these function and a better state of health. A

marked improvement in the number of RBC and Hb value

may be attributed to the influence of Moringa oleifera

protein content, which is rich in nutrients such as protein

and minerals (Elbashier and Ahmed, 2016). Jiwuba et al

(2016) proposed that haemoglobin improvement by

Moringa oleifera leaf meal inclusion was due to the higher

quality of the protein in leaves, a view confirmed by Fuglie

(2009) and with Elbashier and Ahmed (2016), who

suggested that Moringa oleifera has a blood boosting

effect because for the high protein content, with significant

quantities of most essential amino acids (Foidland Paull,

2008). Conversely, the higher inclusion level of Moringa

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|  |  |
| --- | --- |
|  | **Differential leukocyte count** |
| **Treatment** | **Haemogloblin%** | **Haematocrit%** | **Heterophil%** | **Monocyte%** | **Lymphocyte%** | **Eosinophil%** | **Basophil%** |
| T1 | 8±0.00a | 29±3.00 | 18.50±0.50 | 13.50±1.50 | 67.50±0.50 | 4±0.00 | 0.00±0.00 |
| T2 | 9.50±0.50b | 37±5.00 | 26±6.00 | 16.50±0.50 | 68.50±0.50 | 5±1.00 | 0.00±0.00 |
| T3 | 8.50±0.50ab | 37±2.00 | 26.50±6.50 | 14±3.00 | 72.50±1.50 | 4.50±1.50 | 0.00±0.00 |
| T4 | 10±0.00c | 33±1.00 | 18.50±1.50 | 14.50±1.50 | 70.50±4.50 | 4.50±0.50 | 0.00±0.00 |
| p- value | 0.05s | 0.34ns | 0.48ns | 0.70ns | 0.53ns | 0.89ns | N/A |
| CV% | 0.93 | 4.87 | 6.35 | 2.33 | 3.28 | 1.07 | N/A |
| SEM | 1.62 | 1.40 | 0.04 | 1.43 | 2.83 | 0.06 | N/A |

*abcdMeans in columns with different superscripts are significantly different. ssignificant at 5% (p < 0.05); nsnon significantly different.*

**3.2 Effect of feeding *Moringa oleifera* leaf meal on total protein, albumin & globulin levels**

Table 3 below presents changes in the serum total protein, albumin and globulin levels (g/dL) of broiler chickens under experimentation at different levels of *Moringa oleifera* leaf meal treatment.Total protein and globulin were found to be statistically significant (p< 0.05). But albumin was found to be statistically nonsignificant (p>0.05) differences among the treatment and control group throughout the observation period.

In the T3 group, the higher value of total protein was observed to be 3.38±0.11, followed by 3.09±0.09, 3.02±0.00 in the T4 and T1 groups. The lowest value was found to be 2.89±0.048 in the T2 group. The highest value of globulin was observed at 1.69±0.15in T3, followed by 1.35±0.08, 1.33±0.59 in T4 and T1 group .The lowest value was found to be 1.21±0.04 in the T2 group. The values of serum total protein (2.89 -3.38 g/dL) obtained in current study however fell within the normal range (2.5 and 4.5 g/dL) for healthy broilers as reported by Campbell (2004), an indication of nutritional adequacy of the dietary protein of *Moringa oleifera* in this experiment. Makanjuola et al. (2014) reported that 0.2%, 0.4% and 0.6% Moringaleaf meal did not influence the serum total protein, albumin and globulin of broilers, which supports our findings. Elkloub et al. (2015), did study *of* Moringa leaf meal inclusion on quail diets, causing a significant increase in total protein and globulin.

**Table 3: Effect of feeding *Moringa oleifera* leaf meal on total protein, albumin & globulin levels, g/dL (Mean ± SE) in serum at 42 days of broilers**

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment** | **Total protein** | **Albumin** | **Globulin** |
| T1 | 3.02±0.00a | 1.68±0.06 | 1.33±0.59b |
| T2 | 2.89±0.048b | 1.68±0.00 | 1.21±0.04a |
| T3 | 3.38±0.11c | 1.69±0.04 | 1.69±0.15bc |
| T4 | 3.09±0.09ab | 1.74±0.04 | 1.35±0.08a |
| p value | 0.01s | 0.72ns | 0.03s |
| CV% | 7.08 | 3.99 | 16.65 |
| SEM | 0.06 | 0.02 | 0.07 |

*abcdMeans in column with different superscripts are significantly different. ssignificant at 5% (p < 0.05); nsnon significantly different.*

**3.3 Effect of feeding *Moringa oleifera* leaf meal on glucose, cholesterol & calcium levels**

Table 4 below presents changes in the serum glucose (mg/dL), cholesterol (mg/dL) & calcium (mg%) levels of broiler chickens under experimentation at different levels of *Moringa oleifera* leaf meal treatment. Glucose, cholesterol & calcium were found to be statistically non-significant (p<0.05) differences among the treatments and the control group throughout the observation period.

Serum glucose level increases in *Moringa oleifera* treated diet than control group. There is significant reduction in total serum cholesterol level in *Moringa oleifera* treated diet as compare to control. The reduction in the levels of cholesterol shows reflects the beneficial effects of *M. oleifera* in the diets. *M. oleifera* has a high content of phytosterols, which decrease the cholesterol levels of serum and eggs (Hussain et al., 2014). Calcium was found to be statistically non- significant (p>0.05) among the treatment and control group. Blood calcium level was almost similar in all *Moringa oleifera* treated diet and control group. The serum calcium values obtained in this study were higher than the normal value of 7.10 mmol/L reported by Kaneko et al. (1997) for chickens, but lower than the range of 16.03 to 19.12 mmol/L reported by Egu (2016) in mature male turkeys.

**Table 4: Effect of feeding *Moringa oleifera* leaf meal on glucose (mg/dl), cholesterol (mg/dl) & calcium (mg %) levels, (Mean ± SE) in serum at 42 days of broilers**

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment** | **Glucose** | **Cholesterol** | **Calcium** |
| T1 | 56.67±21.31 | 184.73±10.97 | 10.86±0.68 |
| T2 | 96.48±16.02 | 176.93±1.26 | 10.53±0.74 |
| T3 | 78.68±12.17 | 162.84±5.35 | 10.71±0.36 |
| T4 | 83.59±3.98 | 166.56±7.17 | 10.38±1.04 |
| p value | 0.35ns | 0.19ns | 0.97ns |
| CV% | 33.6 | 8.01 |  10.52 |
| SEM | 1.78 | 7.17 |  1.32 |

*abcdMeans in columns with different superscripts are significantly different. ssignificant at 5% (p < 0.05); nsnon significantly different.*

### 3.4 Effect on liver function tests

 Table 5 below presents changes in the serum aspartate aminotransferase (AST) & alanine aminotransferase (ALT) levels (IU/l) of broiler chickens under experimentation at different levels of *Moringa oleifera* treatment. AST and ALT were found to be statistically non-significant (p<0.05) differences among the treatments and the control group throughout the observation period.In the experimental trial AST & ALT value were lower in *Moringa oleifera* treated diet than control. Lowest values of AST and ALP were observed on *Moringa oleifera* treated diet (Seboya et al., 2019). This indicates that *Moringa* had no toxic effect within the liver parenchyma of the birds, thus resulting in improved immune response of the birds.

**Table 5: Effect of *Moringa olifera* feeding on AST & ALT, IU/l (Mean ± SE) in serum at**

 **42 days of broilers**

|  |  |  |
| --- | --- | --- |
| **Treatment** | **AST IU/l** | **ALT IU/l** |
| T1 | 138.17±14.67 | 12.88±1.83 |
| T2 | 103.19±15.15 | 11.43±1.98 |
| T3 | 99.11±11.79 | 11.19±0.66 |
| T4 | 105.52±6.17 | 11.54±0.89 |
| p value | 0.84ns | 0.84ns |
| CV% | 22.03 | 19.25 |
| SEM | 1.76 | 2.04 |

*abcdMeans in columns with different superscripts are significantly different. ssignificant at 5% (p < 0.05); nsnon significantly different.*

### 3.5 Effect on kidney function tests

Table 6 below presents changes in the serum urea & blood urea nitrogen (BUN) levels (mg%) of broiler chickens under experimentation at different levels of *Moringa oleifera* treatment. Urea & BUN were found to be statistically non- significant (p>0.05) among the treatment and control group throughout the observation period. Urea & blood urea nitrogen was lower in *Moringa oleifera* treated diet than control group. However, the urea values in our study were within normal range as reported by Kaneko et al. (1997) i.e. 4.80 to 19.80 mg/dl in birds. It has been observed that serum urea content depends on both the quantity and quality of protein supplied in the diet (Lheukwumere and Herbert, 2002). Higher blood urea levels have been associated with poor protein quality or excess tissue protein catabolism associated with protein deficiency (Eggum, 1970).

Incremental levels of *Moringa oleifera* leaf meal resulted in reduction of urea and BUN level in blood. From this we can conclude that there was better absorption and efficient utilization of dietary protein as compared to control diet (Seboya et al., 2019).

**Table 6: Effect of feeding *Moringa olifera* leaf meal on Urea & BUN, mg% (Mean ± SE) in serum at 42 days of broilers**

|  |  |  |
| --- | --- | --- |
| **Treatment** | **Urea mg%** | **BUN mg%** |
| T1 | 8.08±0.40 | 3.78±0.19 |
| T2 | 7.68±1.07 | 3.59±0.5 |
| T3 | 6.06±0.00 | 2.83±0.00 |
| T4 | 6.87±0.40 | 3.21±0.19 |
| p value | 0.17ns | 0.17ns |
| CV% | 16.84 | 16.84 |
| SEM | 1.04 | 1.07 |

*abcdMeans in columns with different superscripts are significantly different. ssignificant at 5% (p < 0.05); nsnon significantly different.*

4. Conclusion

It could be concluded that *Moringa oleifera* leaf meal with levels of 0.5, 1 and 1.5% has beneficial effect on some haematological and serum biochemical indices. The best level occurred by 1% in broiler chicken diets.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

All authors hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

Ethical approval

All experiments have been examined and approved by Nepal Agriculture Research Council (NARC).

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