Original Research Article

**Haematological and Biochemical Profiles of Broiler Chickens Fed at Different Levels of Moringa oleifera leaf meal**

ABSTRACT

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| --- |
| **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 rearing of broilers chicken was conducted at National Animal Health Research Center (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). Hemoglobin, hematocrit 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) was determined. The obtained values were statistically analyzed by one way analysis of variance (ANOVA).**Result:** Hemoglobin, hematocrit heterophil, monocyte, lymphocyte and eosinophil value was higher in broiler feeding *Moringa oleifera* treated diet. The result of total protein and globulin levels showed statistically significant (p<0.05). But albumin level showed non- significant (p>0.05) differences among the treatment and control group. 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% have beneficial effect on some heamatological and serum biochemical indices. The best level occurred by 1% in broiler chickens diets. |

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

1.INTRODUCTION

A Miracle tree *Moringa oleifera* known as 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. *Moringa oleifera* leaf is reported to contain 25–27% crude protein (Gadzirayi et al., 2012). Its medicinal properties derives 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 (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 aim of this study was to investigate the effects of *Moringa oleifera* leaf meal as dietary supplementation on haematological and biochemical parameters of broiler chickens.

2. material and methods

**2.1 Experimental trial**

The experimental study included rearing of broilers chicken was conducted at National Animal Health Research Center (NAHRC), Khumaltar, Lalitpur, Nepal. Laboratory examination was conducted at, NAHRC, Pathology lab.

One hundred and forty four day old Cobb - 500 broilers chicken from commercial hatchery were procured. Initially, birds were kept for one week in experimental house. Each group was replicated and given different treatments which are mentioned in Table 1 below. Commercial mash feeds were acquired from a commercial feed mill. The *Moringa oleifera* leaf powder was from a reputed company Annapurna Organic Agricultural Udhoyug, Kapilvastu, Nepal. One bird from each replication was selected randomly, their blood were collected. The serum was immediately separated from blood and stored at −20 °C. Hematological & biochemical parameters of poultry were analyzed.

 **Table 1: Experimental trial**

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Replication** | **Feed** |
| T1 | 3 | Basal diet with no *Moringa oleifera* |
| T2 | 3 | Basal diet with *Moringa oleifera* 0.5% (i.e 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 Laboratory examination**

 **2.2.1 Hematological & Biochemical estimation**

Hemoglobin, hematocrit 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) was determined by Erba Mannheim test kit and Accurex biomedical, Pvt. Ltd test kit with the help of a spectrophotometer.

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

Statistical analysis was carried out using the Microsoft Excel-2007 and SPSS-20 version.

3. results and discussion

**3.1 Effect of *Moringa olifera* feeding on hemoglobin, hematocrit and**

 **Differential leukocyte count**

The Table 2 below presents changes in the hemoglobin, hematocrit and differential leukocyte count of broiler chickens under experimentation at different levels of *Moringa oleifera* treatment. Hematocrit, heterophil, monocyte, lymphocyte and eosinophil was found to be statistically non- significant (p>0.05) differences among the treatment and control group throughout the observation period.

But Hemoglobin was found to be significant (p< 0.05) difference. Hemogloblin, hematocrit, heterophil, monocyte, lymphocyte and eosinophil value was higher in broiler feeding *Moringa oleifera* treated diet 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 (Moyo et al., 2016, Sebola et al., 2017, Hurrell, 1997, McDowell, 2003).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).

There was no any basophil found on any treatments. 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 white blood cell (WBC) counts 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 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 defense 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 of Moringa olifera 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** | **Hemogloblin%** | **Hematocrit%** | **Heterophil%** | **Monocyte%** | **Lymphocyte%** | **Eosinophil%** | **Basophil%** |
| T1 | 8±0.00 | 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±0.50 | 37±5.00 | 26±6.00 | 16.50±0.50 | 68.50±0.50 | 5±1.00 | 0.00±0.00 |
| T3 | 8.50±0.50 | 37±2.00 | 26.50±6.50 | 14±3.00 | 72.50±1.50 | 4.50±1.50 | 0.00±0.00 |
| T4 | 10±0.00 | 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 |

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

**3.2 Effect of *Moringa olifera* feeding on total protein, albumin & globulin**

 **levels**

The 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* treatment. In the T3 group, the highest value of total protein was observed 3.38±0.11 followed by 3.09±0.09, 3.02±0.00 in T4 and T1 group. While the lowest value was found 2.89±0.048 in T2 group. Similarly, the highest value of albumin was observed 1.74±0.04 in T4 followed by 1.69±0.04, 1.68±0.06 in T3 and T1 group .While the lowest value was found 1.68±0.00 in T2 group. The highest value of globulin was observed 1.69±0.15in T3 followed by1.35±0.08, 1.33±0.59 in T4 and T1 group .While the lowest value was found 1.21±0.04 in T2 group. Total protein and globulin was found to be statistically significant (p< 0.05). But albumin was found to be statistically non- significant (p>0.05) differences among the treatment and control group throughout the observation period.

According to Campbell (2004) the normal value of serum total protein of healthy broiler is (2.5 and 4.5 g/dl). The values of serum total protein obtained in current study however fell within the normal range, an indication of nutritional adequacy of the dietary proteins in this experiment. Makanjuola et al. (2014) reported that 0.2%, 0.4% and 0.6% *Moringa* leaf meal did not influence the serum total protein, albumin and globulin of broilers which support our findings. Elkloub et al. (2015), did study on *Moringa* leaf meal inclusion on quail diets causes significant increase in total protein and globulin but albumin/ globulin ratio in all dietary treatments appeared to be decreased in *Moringa* supplemented quail diet.

**Table 3: Effect of *Moringa olifera* feeding 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±0.00 | 1.68±0.06 | 1.33±0.59 |
| T2 | 2.89±0.048 | 1.68±0.00 | 1.21±0.04 |
| T3 | 3.38±0.11 | 1.69±0.04 | 1.69±0.15 |
| T4 | 3.09±0.09 | 1.74±0.04 | 1.35±0.08 |
| p value | 0.01s | 0.72ns | 0.03s |
| CV% | 7.08 | 3.99 | 16.65 |

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

**3.3 Effect of *Moringa olifera* feeding on glucose, cholesterol & calcium levels**

The 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* treatment. Glucose, cholesterol & calcium was found to be statistically non-significant (p<0.05) differences among the treatment and the control group throughout the observation period.

Serum glucose level increases in *Moringa oleifera* treated diet than control group. Which do not match the findings of Egu, U.N (2019). Serum glucose content was higher in birds fed control diet than those fed 6%, 8% and 10% *Moringa olifera* leaf meal. According to Egu (2010) normal glucose value in broiler fed slam weed leaf meal was 75.60 to 100.50 mg/100ml, however glucose values obtained in our study were within the range except in control group (56.67±21.31 mg/dl).

There is significant reduction in total serum cholesterol level in *Moringa oleifera* treated diet as compare to control. This result support the finding of Akpet et al (2012) on broiler, who stated decreased in the TC and LDL-c concentration in those fed different levels of *Moringa oleifera* leaf meal. 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. Which do not match the finding of Egu, (2019) who did research in effect of graded levels of *Moringa oleifera* leaf meal on performance and serum biochemical parameters of broiler. According to him serum calcium content was significantly increased as the level of *Moringa oleifera* leaf meal increased in the diets & lowest value was observed in broiler chicken fed control diet (T1) (7.40 mmol/L). 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 *Moringa olifera* feeding 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 |

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

### 3.4 Effect on liver function tests

The 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. In the T1 group, the highest value of AST was observed 138.17±14.67 followed by 105.52±6.17 & 103.19±15.15 in T4 and T2 group. The lowest value was found 99.11±11.79 in T3 group. Similarly, the highest value of ALT was observed 12.88±1.83 in T1 (control) and in T2, T3 and T4 group. AST & ALT value were lower in *Moringa oleifera* treated diet than control. Both ALT and AST was found to be statistically non-significant (p<0.05) differences among the treatment and the control group throughout the observation period. 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 |

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

### 3.5 Effect on kidney function tests

The 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. In the T1 group, the highest value of urea was observed 8.08±0.40 followed by 7.68±1.07 & 6.87±0.40 in T2 and T4 group. The lowest value was found 6.06±0.00in T3 group. Similarly, the highest value of blood urea nitrogen (BUN) was observed 3.78±0.19 in T1 followed by 3.59±0.5, 3.21±0.19 in T2 and T4 group .While the lowest value was found 2.83±0.00 in T3 group. Urea & BUN was 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 (Iheukwumere 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 *Moringa olifera* feeding 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 |

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

4. Conclusion

Hemogloblin, hematocrit, differential leukocyte value was higher in broiler feeding *Moringa oleifera* treated diets. The result of total protein and globulin levels showed statistically significant (p<0.05). But albumin level showed non- significant (p>0.05) differences among the treatment groups as compared to the control group. Serum glucose level increases in the *Moringa oleifera* treated diet then the control group. The result shows a significant reduction in total serum cholesterol level in *Moringa oleifera* treated diet as compare to control. Blood calcium level was almost similar in all *Moringa oleifera* treated diet and control group. AST & ALT, Urea and BUN value were lower in *Moringa oleifera* treated diet than control. It could be concluded that Moringa oleifera leaf meal with levels of 0.5, 1 and 1.5% have beneficial effect on some heamatological and serum biochemical indices. The best level occurred by 1% in broiler chickens 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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