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Research Article

Efficacy of Drumstick Tree (*Moringa oleifera*) Leaves Powder on Lipid Profile and Hematological Indices in Chickens on a High Fat Diet

Aisha Saleem¹, Irum Naureen^{1*}, and Muhammad Naeem^{1,2}

¹School of Zoology, Minhaj University Lahore, Pakistan ²Institute of Research and Information Mirpur AJK, Pakistan

Abstract: *Moringa oleifera* belongs to the Moringaceae family and genus Moringa. *Moringa oleifera* has many beneficial pharmaceutical and nutritional properties. The present study aimed to investigate the effects of dietary supplements of *Moringa oleifera* leaves powder after consumption of a high-fat diet on cholesterol TG, HDL, LDL, VLDL and hematological parameters such as Hb, MCV, MCH, MCHC, PCV, RBCs, WBCs, PLT neutrophil, heterophil and lymphocytes count in chicks. Golden Misri Chickens were divided into 4 groups: Group 1 (the Control group) was given Feed-13. Group 2 (Experimental Group) was given Feed-13 and mustard oil (high-fat diet) Group 3 (experimental Group) was given Feed-13 and 1.5% of *Moringa oleifera* leaves powder. Group 4 (experimental Group) was given Feed-13 and 1.5% of *Moringa oleifera* leaves powder. Group 3 showed a significant increase in levels of HDL, TG, RBCs, and Hb, while significantly decreased (p > 0.05) in LDL, TLC, WBCs, MCHC, MCV, MCH, TC, TP, Monocytes count, neutrophil count, and platelet counts. *Moringa oleifera* supplementation showed a significant reduction in cholesterol, LDL, and TC levels. The findings of this study demonstrated that powdered *Moringa oleifera* is a beneficial and effective dietary supplement that increases HDL, TG, Hb, and RBCs, and decreases cholesterol levels.

Keywords: Triglyceride, High-Density Lipoprotein, Low-Density Lipoprotein, Very Low-Density Lipoprotein, Hematological Indices.

1. INTRODUCTION

Poultry production is the most popular livestock for small and medium-scale farmers in both rural and urban areas. The main aim of the poultry is to attain the greatest earnings at the smallest amount of construction cost. It greatly contributes to the gross domestic production (1.3%) [1]. Commercial poultry Production in Pakistan began in the 1960s and has made significant contributions. Even though the business of poultry production is facing different problems, such as, high cost of feed and disease. Poultry meat production is increasing day by day, and consumers are very concerned about food safety issues, such as high-quality eggs and meat without any antibiotic residues [1]. Poultry meat is making a significant contribution to the delivery of the best protein. All poultry industries have played a central role in employment. Approximately 1.5 million individuals in Pakistan are involved in these enterprises for their income. Poultry enterprises supply approximately 18% of meat to the public. The annual growth rate 8-10% is very fast-growing rate in Pakistan. Poultry farming is a starting point of income in rural areas around the world, especially in Pakistan. Desi chicks are always a source of higher income [2]. Pakistan has not succeeded in producing specific-pathogen-free (SPF) chickens and poor-quality feed, contains high levels of mycotoxins. The industry needs to control fluctuating prices and maintain a constant income. Poultry health and production are major challenges for future poultry growth. A significant portion of the daily protein (meat and eggs) has been provided to the Pakistani people. Poultry products have been added, but more sustained efforts are needed to raise

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^{*}Corresponding Author: Irum Naureen <dr.irumnaureen@mul.edu.pk>

awareness among consumers regarding the overall quality and safety of value-added products [2].

Moringa oleifera is a fast-growing cultivated plant species that belongs to the order Brassicales, Moringaceae family and genus Moringa [3]. Due to its high nutritional values, content in amino acids, flavones, and other factors, *Moringa oleifera* has earned the title of "Miracle Tree" and received viable attention. Phytochemicals, minerals, and vitamins, are present in large amounts in leaves and can be employed in cosmetic and dietary supplements [3]. Every component, including flowers, fruit, pods, and leaves, is used for both dietary and commercial purposes. *Moringa oleifera* aids in milk production. *Moringa oleifera leaves* powder can be used to protect the liver heart, kidney, and lungs; reduce pain, and belly fat [4].

Moringa oleifera Lam is used for pharmaceutical, dietary, and antibacterial purposes. Moringa oleifera has many nutrients, proteins, vitamins, and amino acids present in various parts of the plants. Leaf meal shows considerable beneficial remuneration, no adverse effects, and improved development and meat quality at various nutritional incorporation levels (5%-10% in chick ration and 10% in broiler ration) [5]. Moringa oleifera leaves supplement increase meat weight, high density lipoprotein (HDL) and decreased the level of LDL, VLDL, cholesterol, blood glucose intensity in egg laying hens and broiler hens Moringa oleifera leaves can improve blood chemistry, increase RBC and WBC counts as well as an increased in haemoglobin in broiler chickens [5].

2. MATERIALS AND METHODS

The experiment was performed at Minhaj University Lahore to examine the effect of powder Drumstick Tree *Moringa oleifera* leaves on lipid profile and haematological indices after consumption of high fat diet in chickens. Disinfectants were sprayed to disinfect the chicken wooden coops.

2.1. *Moringa oleifera* Leaves Supplement Preparation

Moringa oleifera leaves were used for experiment, these leaves were collected from the village Sehjowal in the District Kasur; the leaves were dark green in colour. After collecting from trees, leaves were carefully picked off from the branches, washed carefully and dried for seven days in direct sunlight to get rid of moisture completely and easy for grinding to make powder. To make leaf powder dry leaves were grinded by hammer mill to attain the leave residue. For further use the plastic bags were employed to store the powdered leaves as shown in Figure 1. The Feed-13 was bought from Islamabad Feeds (Pvt) Ltd. Rawat Rawalpindi. Feed-13 contains (soyabean oil, canola meal, fish meal, mineral mixture, wheat, maize, rice polish). Nutrient composition: (Crude Protein 15-17%, Crude Fiber 3.0-6.5%, Crude Fat 2% Minimum, Total Ash 13%).

2.2. Collection of Animals

Ethical approval was taken prior to study from ethical approval committee of Minhaj University Lahore. Twenty chickens were bought from the poultry research facility Lahore when they were 7.5 months (around 30 weeks) old. The Golden Misri breed of chicken was chosen for the experiment. Before the experiment, they underwent two weeks of acclimatization. These chickens were kept in the animal house of Minhaj University Lahore at room temperature (22 °C \pm 2 °C). The animals were separated into 3 Experimental and one Control group, after six days of acclimation. 5 animals in each group (N = 5) were weighted before the experiment.

Group 1: Control Group (CG-1) was given Feed-13 (1.96 g only. Group 2: Experimental Group (EG-2) was given 5 ml of mustard oil which was mixed with simple diet Feed-13 (1.96 g). Group 3: Experimental Group (EG-3) was given 5 ml of



Fig. 1. Powdered Moringa oleifera leaves as a dietary supplementation.

mustard oil + 1.08 g of powdered *Moringa oleifera* leaves which were combined with the Feed-13 (1.96 g). **Group 4:** Experimental Group (EG-4) received treatment with 1.08 g of *Moringa oleifera* leaves powder + Feed-13 (1.96 g) to monitor weight utilizing blood chemistry, and lipid profile.

2.3. Blood Sample Collection

At the time of dissection after the experiment of 30 days the mean body weight of each group of chickens was recorded. Blood samples (about 5 ml each) were obtained from the jugular vein of each hen with the help of disposable syringe. Blood was collected in EDTA test tube for measuring haematological parameters, Hb, RBCs, WBCs, Lymphocytes, MCV, MCH, HCT, platelets count, and lipid profile TG, TC, HDL, LDL, and VLDL. After collection, blood was centrifuged for 15 minutes at 3000 revolutions per minutes, carefully collecting the serum require the use of pasture pipette. Serum was stored in Eppendorf tube at 20 °C in refrigerator. Serum was used for lipid profile and Haematological assessments.

2.4. Lipid Profile Tests

These tests were performed at University of Veterinary and Animal Sciences, Lahore, state of the art Roche Cobas c 111 Chemistry Analyzer was used for the determination of lipid profile by enzymatic calorimetric method (Electro Chemiluminescence Technology).

2.5. Haematology Tests

These tests Hb, RBCs, WBCs, lymphocytes, MCV, MCH, HCT, PLT, were performed at University of Veterinary and Animal Sciences, Lahore, using fully automated Haematological analyzer (Sysmex, Japan) by using immunofluorescence techniques.

2.6. Statistical Analysis

Data analysis was done by using Graph pad prism version 5. Student t-test was used to analyze the numerical data. The data was considered significant when the P-value was less than 0.05, ($p \le 0.05$). Mean \pm Standard error mean was used to express the values.

3. RESULTS

In this study 20 Golden Misri chickens were used for experiment and examined the effect of *Moringa Oleifera* on lipid profile and Haematological indices in chickens. The following parameters were compared to examine the statistically identifying similarities and differences among them.

3.1. Body Weight

Statistically, significant differences were observed in the initial, final and mean body weight gain of control, and *Moringa oleifera* treated groups. There is a significant p < 0.01 increase in the final weight and weight gain p < 0.05 of EG-2 compared to control group vs dosage group (Figure 2 and Table 1).

3.2. Lipid Profile Analysis

The mean of total Cholesterol and high-density lipoprotein concentration was increased. High density lipoprotein is good cholesterol. There was a significance (p < 0.05) and (p < 0.001) distinction occur increase in Mean value of *Moring*a treated groups as compared to control group (Figure 3). Total protein and low-density lipoprotein show significance (p < 0.05) distinction decreased in mean value of *Moringa oleifera* treated groups as compared to control group (Figure 4 and Table 2).

Table 1. Effect of *Moringa oleifera* and combination of oil, at the final body weight and weight gain of 9 months old

 Golden Misri chickens.

Groups	Initial Weight (kg)	Final Weight (kg)	Weight Gain (kg)
CG-1	1.85 ± 0.1	2.15 ± 0.13	0.3 ± 0.05
EG-2	1.6 ± 0.20	$1.5 \pm 0.15a^*$	0.1 ± 0.06
EG-3	1.6 ± 0.15	$2.15 \pm 0.12b^{**}$	$0.55\pm0.18b\texttt{*}$
EG-4	1.75 ± 0.07	$2.15 \pm 0.18a^*$	0.35 ± 0.06

Mean \pm SEM is expressed values (a) = Control vs Experimental group (b) = diet + Oil vs diet + Oil + *Moringa oleifera* powder, (c) = diet + Oil + *M. oleifera* powder vs. diet + *M. oleifera* powder, P < 0.05*, P < 0.01**.



Fig. 2. *Moringa oleifera* and combination of oil on the final weight and weight gain of 9 months old Golden Misri chickens Mean \pm Standard Error Mean are used to express data. There is a significant p < 0.05 increase in the final weight and weight gain of EG-2 compared to control group vs. dosage group.

Table 2. Moringa oleifera and combination of oil, effect on Lipid profile of 9 months old Golden Misri chickens.

Groups	TG	ТР	TC	HDL	LDL
Units	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)
CG-1	64.4 ± 6.34	205 ± 2.12	100.8 ± 7.67	48.8 ± 3.39	22.8 ± 2.63
EG-2	67.6 ± 9.91	$200\pm13.15a^{\boldsymbol{*}}$	82.2 ± 10.73	58.2 ± 1.31	13. ± 1.39*
EG-3	54.2 ± 5.89	191.4 ± 7.29	$112.2 \pm 6.98a^{***}$	$76.6 \pm 5.66a^{**}b^{*}$	18.4 ± 1.96
EG-4	68.4 ± 8.58	199.8 ± 4.28	$166 \pm 4.13a^{***}b^{***}$	$69.8\pm5.54a^{\boldsymbol{*}}$	$11.8 \pm 2.03b^{**}$



Fig. 3. *Moringa oleifera* and combination of oil effect on total Cholesterol and high-density lipoprotein concentration was increased. Mean \pm Standard Error Mean are used to express data. There is significance (p < 0.05) and (p < 0.001) increased in cholesterol and high-density lipoprotein in Mean value of Moringa treated groups as compared to control groups in 9 months old Misri chickens.

3.3. Haematological Analysis

The mean of haematological parameters Haemoglobin and total red blood cells count show significance (p < 0.05) distinction in the Mean value increase red blood cells and haemoglobin of *Moringa* treated group 3 as compared to control group 1 (Figure 5). Lymphocytes count and monocytes level observed and shows significantly (p < 0.05) distinction in Mean value increase monocytes level of *Moringa oleifera* treated group 3 as compared to control group. In Lymphocytes count show significance (p < 0.05) increase in EG-3 and EG-4 *Moringa oleifera* treated group (Figure 6 and Table 3).



Fig. 4. *Moringa oleifera* and combination of oil effects on total protein and low-density lipoprotein in 9 months older Golden Misri chickens Mean \pm Standard Error Mean is used to express the data. There is significance (p < 0.05) decrease in mean value of *Moringa oleifera* treated groups as compared to control groups.

 Table 3. Moringa oleifera and combination of oil, effect on Haematological indices of 9 months old Golden Misri chickens.

chickens.					
Parameters	Units	CG1	EG 2	EG 3	EG 4
Haemoglobin (HB)	(g/dl)	6.82 ± 0.87	$3.86 \pm 0.53a^*$	$3.2 \pm 0.33a^{**}$	2.9 ± 0. 51a**
Hematocrit (HCT)	(%)	8.18 ± 2.61	19.14 ± 3.29	$16.06 \pm 3.03a^*$	18.4 ± 1.86
TOTAL RBCs	(×10 ¹² /L)	2.24 ± 0.23	1.84 ± 2.20	$1.62 \pm 0.19a^*$	1.08 ± 0.25
MCV	(fl)	119 ± 7.82	110.2 ± 10.26	105 ± 7.53	109.6 ± 8.95
МСН	(pg)	28.2 ± 1.019	24.4 ± 2.42	21.4 ± 1.91	22.6 ± 2.89
МСНС	(g/dl)	30.2 ± 1.62	24.4 ± 2.20	$22.4 \pm 1.86a *$	26 ± 2.34
Platelets Count	(×10 ⁹ /L)	7.6 ± 1.16	4.94 ± 1.36	4.94 ± 0.75	4.6 ± 1.04
TLC (WBCs)	(×10 ⁹ /L)	7.56 ± 0.95	7.32 ± 0.76	5.04 ± 1.34	5.62 ± 0.65
Neutrophil	(%)	64.2 ± 5.48	39.8 ± 6.09	47.6 ± 9.98	38.6 ± 6.56
Lymphocytes	(%)	58.2 ± 3.99	43.8 ± 6.18	$34\pm3.18a^{\boldsymbol{**}}$	$33.2 \pm 4.21a^{**}$
Monocytes	(%)	3.76 ± 0.28	$1.92 \pm 0.32a^*$	2.14 ± 0.56	$1.92 \pm 0.41a^*$



Fig. 5. Moringa oleifera and combination of oil on Haemoglobin and total red blood cells count of 9 months old chickens. Mean \pm SEM are used to express data. There is significance (p < 0.05) distinction in the Mean value increase red blood cells and haemoglobin of Moringa oleifera treated EG-3 as compared to CG-1.



Fig. 6. *Moringa oleifera* and combination of oil on Lymphocytes count and monocytes level of 9 months old Golden Misri chickens. Mean \pm SEM is used to express data. There is significantly (p < 0.05) distinction in Mean value increase monocytes level of *Moringa oleifera* treated group1 and treated group 3 as compared to control group. In Lymphocytes count show significance in EG-3 and EG-4 *Moringa oleifera* treated group.

4. **DISCUSSION**

The present study was conducted to inspect the effect of Drumstick tree (Moringa oleifera) leaves powder on lipid profile and hematological indices after consumption of high fat diet in chickens at the age of 30 weeks. Healthy and vaccinated chickens were used for the experiment. Lipid profile parameters included triglyceride, TC, high density lipoprotein, low density lipoprotein, and very low-density lipoprotein. The blood parameters included hematocrit, erythrocyte, neutrophil leukocyte, hetrophil, lymphocyte, and monocytes. They also include HB, MCV, PCV, MCH, MCHC, WBCs, RBCs, and PLT. Mean ± standard error mean was used to express the data. Initial and final weights were measured for all chickens in control and experimental groups before and after the experiment. The equal amount of diet was given to all the chickens. The experimental Moringa oleifera treated group has additional weight $(2.15 \pm 0.13 \text{ kg})$ as compared to CG-1 (1.6 ± 0.15 kg). Villarruel-López et al. [6] concluded that the Moringa olifera leaves play a significant role in enhancing the weight ratio in rats. Moringa oleifera helps with weight gain in treated groups but not in control groups.

The chickens were intaking high fat diet oil after a 30-days experiment. Comparing the initial body weight of the chicken to the weight rise in experimental group EG-2 is shown in Figure 3. The chickens were intaking mustard oil diet shows a significant change in the body weight as compared to the control group, and the body showed a larger size appearance because of the high consumption of fatty diet weight is increased Kilany et al. [7]. In the present study, lipid profile parameter measurements demonstrated the impact of mustard oil and high fat diet on Moringa oleifera. The mean value of Moringa oleifera treated groups in comparison to Control groups does not differ significantly (p > p)0.05). The triglyceride level of 9-month-old Golden Misri chickens was measured using a mixture of mustard oil and Moringa oleifera on lipid profile. There was no significance in any of the groups compared to CG-1 vs EG-2 giving (Moringa oleifera + Feed-13) compared to EG-3 (Feed-13 + Moringa oleifera + oil). Elbashier and Ahmed [8] observed that triglyceride levels increased by decreasing the treatment of supplementation of Moringa oleifera diets by 20% compared with the normal diet percentage of 10%. The high percentage of Moringa oleifera increased the level of triglycerides and showed significance in p-value. In current study a low dose of Moringa oleifera was used.

In the present study, high density lipoprotein (HDL) dose dependence was shown to be extremely significant (p < 0.05) and (p < 0.01) enhanced high density lipoprotein and dose dependent *Moringa oleifera* combination of high fat diet group compared to CG-1 in 9-month-old Golden Misri chickens. In the previous studies, Basmacioglu and Ergul [9] observed the *Moringa oleifera* leaf meal ratio showed significant difference as compare to

CG-1. Elkloub et al. [10] observed an increase in HDL level in broiler chickens treated 4 weeks with three different doses of Moringa oleifera (0.2%, 0.4%, and 0.6%). In current study an increase in high density lipoprotein was observed in Moringa oleifera treated groups. In the present study, low density lipoprotein (LDL) showed dose dependent increase among Moringa oleifera treated groups. In current study in Moringa oleifera treated group 9-month-old Golden Misri chickens were given high fat diet in combination with Moringa oleifera. Low density lipoprotein is called bad cholesterol Mobolaji et al. [11] observed a decrease in low density lipoprotein levels in broiler chickens treated with three different Moringa oleifera doses (0.40%, 0.60%, or 0.80%) over three weeks. Zanu et al. [12]. found a decrease in LDL concentration after treatment with 0.20% Moringa oleifera for 4 weeks.

In this study, an increase in the total protein (TP) was found in *Moringa oleifera* and a combination of mustard oil treated groups. The difference between EG-2 and CG-1 is statistically significant (P < 0.05). This increase in total protein concentration might be due to mustard oil increasing in the activity of 9 months old Golden Misri chickens. Stanley [13] found that increasing total protein concentration by giving a *Moringa oleifera* leaf meal diet for 32 days, showed a significant difference in p-value.

In the present study, highly significantly (p < 0.001) reduced cholesterol levels (p < 0.001) in *Moringa oleifera* and mustard oil treated groups compared to the control groups in 9-month-old Golden Misri chickens. Alnidawi *et al.* [14] found that total cholesterol was decreased with an increase in the level of *Moringa oleifera* diet of 20%. The decreased level of *Moringa oleifera* was 5%, 10%, and 15% compared with the control group. Ghasi *et al.* [15] found that different levels of extract from *Moringa oleifera* leaves used in a fat diet reduced blood cholesterol levels significantly in Wister rats. In our study, a low dose of *Moringa oleifera* was used.

In a previous study, Gasmalbari *et al.* [16] found that Moringa treated groups caused a reduction in RBCs as compared to control groups, when *Moringa oleifera* leaf meal, 0.25%, was given to 24-day old albino rats for four weeks. In the present study, there was no significant (p > 0.05)

difference in WBCs. In the current study, there was a considerable rise in Hb level (p < 0.05). Moringa oleifera and mustard oil treated groups were compared to control groups. EG-3 and EG-4 have revealed an optimistic outcome in haemoglobin level as compared to the Control Group. Verma et al. [17] observed an increase in haemoglobin (Hb) level of 9 weeks old chickens fed by Moringa oleifera and high fat diet mustard oil for three weeks. In the current study, the hematocrit (HCT) demonstrated that the mean values of Moringa treated group EG-2 showed a significant (p <0.05) difference increase (HCT) level compared to the control groups in 9 months old Golden Misri chickens. Similarly, Zanu et al. [12] observed an increase in Hematocrit concentration in Moringa oleifera treated group at 5% compared to the control group. At 9 weeks old the Moringa oleifera treated group caused highly significant change in p-value. In previous studies, a high level of Moringa oleifera was used and showed a high significance, Ahmed et al. [18]. However, in the current study a low dose of Moringa oleifera was used. Onu and Aniebo [19] discovered no significant difference in Hematocrit level after treatment of Moringa oleifera leaf meal 0.75% given for three weeks.

In the present study, no significant (p > 0.05) difference in platelet count (PC) and feed intake has been observed in experimental group EG-2 compared to control group CG-1 in 9 months old Golden Misri chickens. When compared to the control group CG-1 experimental group EG-3 and EG-4 showed no significance. Adegbite *et al.* [20] found a significant (p < 0.05) increase in the mean value of *Moringa oleifera* treated groups as compared to control groups. In a previous study by Saleem *et al.* [21] using a high dose of *Moringa oleifera* changed the mean value.

In the current study, the group treated with *Moringa oleifera* and mustard oil had a higher total RBCs count. The increase was statically significant (p < 0.05) in all treated *Moringa oleifera* and oil groups compared to the control group in 9-monthold Golden Misri chickens. Hafsa *et al.* [22] observed highly significant values of WBCs count treated with three doses (1%, 1.5%, 2%) of *Moringa oleifera* compared with Control Group caused change in P-value show increase in significance.

In the current study, Moringa oleifera and a

high fat diet caused no significant rise (p > 0.05)in MCH and MCV levels compared to the control group. In 9-months-old Golden Misri chickens, Verma et al. [17] observed no considerable change (p > 0.05) in MCH and MCV levels, when given Moringa oleifera leaf supplementation diet. In previous studies, Osman et al. [23] found an increase in MCH and MCV due to the large amount of Moringa oleifera. In the present study, there was a significant (p < 0.05) change in MCHC and monocytes count. Moringa oleifera and mustard oil treated groups were compared to control groups CG-1. EG-4 (Feed-13 + oil + *M. oleifera* powder) and EG-2 (Feed-13 + M. oleifera powder) has shown positive results in 9-month-old Golden Misri chickens. Jiwuba et al. [24] found an increase in the mean corpuscular haemoglobin (MCHC) and a decreased concentration of monocytes count at 30% of the inclusion level of Moringa oleifera diet. In the current study, no certain change (p > 0.05) in neutrophil count and feed intake has been observed in experimental group EG-3 (Moringa oleifera + Mustard oil) compared to the control group. In comparison to the control group, Experimental Group-4 (Feed-13 + high fat diet + M. powder) and Experimental Group EG-2 (Feed-13 + M. oleifera) showed no significance. Tijani et al. [25] observed a highly significant (p < 0.05) increase in Neutrophil count in the 15% Moringa oleifera leaf meal treated group compared to control group. In the present study, the mean value was raised significantly (p < 0.05). There was a significant difference in lymphocyte count between all groups treated with Moringa oleifera, and control group show significance in EG-2 and EG-3. In a Wister rat fed a high fat diet, Ghasi et al. [15] discovered that varying doses of leaf extract from Moringa oleifera had no discernible effect on lymphocyte count.

5. CONCLUSIONS

Present study explores the effectiveness of *Moringa* oleifera after consumption of high-fat diet in Golden Misri chickens. The *Moringa oleifera* has increased the total body weight of the Chickens. The weight gain effect was positively correlated with the dose of *Moringa oleifera* in the groups. *Moringa oleifera* showed significant results with an increase in high-density lipoprotein (HDL), triglyceride (TG), RBCs, and Hb causing decrease in TC, MCHC, MCV, monocytes count, total protein count, LDL, Tlc WBCs, neutrophil count,

MCH, and platelet count. *Moringa oleifera* intake is more effective in the treatment after consumption of high fat diet. In Pakistan, the use of plants for beneficial purposes or as an herbal drug is still ignored. Therefore, awareness about the beneficial effect of *Moringa oleifera* should be increased. The cultivation of *Moringa oleifera* should be increased in Pakistan. *Moringa oleifera* supplements can be good with feed for meat gain and egg production in chickens. Fortification of the diet with *Moringa oleifera* extract can be beneficial for the health of animals as well as human.

6. ETHICS APPROVAL

The experimental protocols and procedures used in this study were approved by the Ethical Committee of the School of Zoology, Minhaj University Lahore, Pakistan with reference number: MUL/ZOOL/235.

7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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