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Effect of Neem (Azadirachta indica) and Tulsi (Ocimum sanctum) leaves powder on growth performance, hemato-biochemical profile and carcass traits of Japanese quail (Coturnix japonica)

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Abstract: The current research investigated the growth performance, hematology, serum biochemistry of Japanese quails fed Neem (Azadirachta indica) and Tulsi (Ocimum sanctum) leaves powder with basic diet. A total number of 300 one-week old Japanese quail chicks were divided randomly into four dietary treatments with five replicates each (15 birds per replicate). Dietary treatments were: T0 or control or basal diet, T1 (Neem 0.5%), T2 (Tulsi 1%) and T3 (Neem 0.25% + Tulsi 0.5%) in basal diet. Growth performance parameters (feed intake, body weight, weight gain and feed conversion rate) were determined weekly. Hematology, serum biochemistry, carcass traits were evaluated on 35 days of the age. Neem and Tulsi alone and their combination as a dietary supplement, significantly $(p \le 0.05)$ increased the body weight and body weight gain. The combination of Neem and Tulsi also resulted in significantly lower FCR. Carcass traits were significantly ($p \le 0.05$) better in T3 except for the liver weight that was nonsignificantly (p > 0.05) different among the treatment groups. Hemato-biochemical parameters (glucose, hemoglobin, WBCs, MCH, total cholesterol, HDL and LDL cholesterol, total protein) were different significantly different ($p \le 0.05$) among the treatment groups. However, RBCs, HCT, MCV, MCHC, platelets, triglycerides and VLDL were not affected by herbal treatments (p > 0.05). It was concluded that a combination of leaves 'powder of Neem and Tulsi can be used for improving the growth performance, carcass traits and health of the Japanese quails.

Key words: Body weight, blood profile, meat yield, herbal diet, health

1. Introduction

Poultry production is a well-known and one of the fastgrowing food producing industry around the globe [1]. In Pakistan, this sector is contributing 35.19% in total meat production, 60.6% in the agriculture sector and 11.7% overall gross domestic product. Over the most recent years, this industry has shown brilliant development and is employing more than 1.5 million peoples [2].

Quail farming is a subsector of the poultry industry and is famous for rapid meat production enterprise [3,4]. There are several genotypes of quails around the globe but Japanese quails are mostly used for farming purpose. Japanese quails are a newly introduced species in the poultry industry of Pakistan [3,5]. Japanese quail is very famous as "Batair" in Pakistan. Quail farming has some particular benefits. Quails can be utilized for the production of meat in a short period (4-5 weeks) and it becomes mature at the age of six weeks [6]. Quail farming

is becoming a popular business because of better overall revenues, among different segments of poultry. Japanese quails are the best choice for meat production due to better egg production, rapid growth rate, high disease resistance and shorter production time [7].

For all types of poultry businesses, feed is an essential and important element for poultry business as it constitutes 60% to 70% of the production costs [3]. Various feed supplements or growth promoters have been designed to improve feed efficiency, growth and product quality and to reduce production costs [3,8]. The feed additives substance is applied from an expansive perspective, which could be added with basic feed to get some specific effects. The basic purpose of giving feed additives is to increase the performance of animals by enhancing their growth, improving feed conversion ratio, sustainability and to reduce mortality of poultry animals. Such feed additives are often called nonnutrient feed additives and known

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as growth promoters [9]. Most of the growth stimulants and synthetic drugs are given to poultry animals for better and faster growth, but many disadvantages such as high costs, side effects on the health of birds and longer residual properties shown by the use of these stimulants and drugs [10]. On the other hand, medicinal herbs are believed to have valuable properties such as antioxidant, antifungal and antimicrobial properties as supplements to animal and poultry feeds [11]. Herbal feed additive also improves nutrient utilization, absorption and the stimulation of the immune system [12]. Utilization of herbs and therapeutic plants in poultry feeding could be more beneficial as growth promotors and prevent many common poultry diseases. Moreover, these herbs would be easily available and can be used effectively in poultry diets [13].

Neem and Tulsi are some of the important native herbs that can be used in poultry diets. These herbs are not only cheaper sources of feed but also have broad medicinal properties like antiprotozoal, hepato-protective, antimicrobial (antiviral, antibacterial, antifungal) and many other properties having not any serious adverse effects and has attracted worldwide eminence [14]. However, there is no concrete information about using Neem and Tulsi in quail diet and their possible effects on health and performance of the quails. This study is an effort to explore the possibility of using Neem and Tulsi leaves' powder in the diet of Japanese quail.

2. Materials and methods

2.1. Study site

The study was conducted at Avian Research and Training Centre (ARTC), University of Veterinary and Animal Science (UVAS), Lahore, Pakistan. Experimental birds were procured from a running project (HEC-NRPU-8352) and reared until the birds reached the age of 35 days from the end of October to end of November 2019.

2.2. Treatments and experimental design

A total number of 300 Japanese quails (7-days old, mixedsex) were obtained from ARTC and distributed into 20 replicates of four treatment groups (five replicates per treatment group). Each replicate was populated with 15 quails. The replicates were managed according to the completely randomized design. T0 was the control group where the only basal diet was offered to the birds whereas in T1, T2 and T3 groups, quails were offered 0.5% Neem, 1% Tulsi, 0.25% Neem + 0.5% Tulsi in basal diet, respectively. The dose rates of Neem and Tulsi were determined based on earlier literature [18,19]. The composition and feed formulation of the experimental diets are shown in Table 1. The trial was continued for four weeks up to 35 days of the age of Japanese quails.

2.3. Management of the experimental birds

The birds were kept in battery cages at ARTC having five tiers from top to bottom. The experimental birds were placed randomly into quail farming shed measuring 914.4 \times 396.24 \times 365.76 cm in length, width and height. The *ad-libitum* feed was provided to birds and ensure the free supply of water through automatic nipples lines installed inside cages. Feed was provided through trough feeders which installed in front of cages.

3. Parameters evaluated

3.1. Growth performance

A measured amount of feed was offered on daily basis and unconsumed feed was recorded to calculate the daily feed intake by subtraction. Daily feed intake data was further converted into weekly data to analyze the final results.

All the birds in each replicate were weighed weekly by using a weighing scale having the least count of 0.01 g (Gromy Industry Co., Ltd, Hangzhou, China). Body weight gain was calculated on weekly basis by subtracting initial weight from the final body weight.

3.2. Carcass traits and internal organs

At the age of 35 days, three quails from each replicate were selected and weighted before slaughter. The slaughtering was performed by using a sharp knife following the Islamic Halal method of slaughtering (PS 3733). After complete bleeding, quails were again weighed and subsequently defeathered and dissected. The carcass without viscera and internal organs (liver, gizzard and heart) were weighed on a weighing balance.

3.3. Blood chemistry/hematology

For blood biochemistry and hematological assessment, three quails were randomly selected from each replicate and about 3 mL blood was taken from the jugular vein of birds into 2 sets of vacutainers (with anticoagulant for hematology and without anticoagulant for serum biochemical examination). Then different biochemical tests performed for getting results. For biochemical examination, blood samples were subjected to centrifugation to obtain serum. These serum samples were collected in Eppendorf tubes and stored at -4 °C. Later, hematological and biochemical examinations were carried out at Quality Lab, Specialized Laboratory Services, Jail Road Lahore. For serum biochemistry, glucose, total cholesterol, VLDL-cholesterol, HDL-cholesterol and LDL cholesterol, triglycerides, total protein were analyzed. For hematological assessment, complete blood count (hemoglobin, WBCs, total RBCs, hematocrit value, mean corpuscular volume, mean corpuscular hemoglobin concentration, and platelets) assessment was performed on Sysmex hematology analyzer (Model XP100; Sysmex Suisse AG, Yverdon-les-Bains, Switzerland).

| Ingredients | T0 (control) | T1 (Neem powder) | T2 (Tulsi powder) | T3 (Neem + Tulsi powders) |
|------------------------------|--------------|------------------|-------------------|---------------------------|
| Broken rice | 10.00 | 9.50 | 9.00 | 8.50 |
| Neem powder | 0.00 | 0.50 | 0.00 | 0.50 |
| Tulsi powder | 0.00 | 0.00 | 1.00 | 1.00 |
| Corn | 58.79 | 58.79 | 58.79 | 58.79 |
| Soybean meal (44%) | 19.83 | 19.83 | 19.83 | 19.83 |
| Sunflower meal | 4.90 | 4.90 | 4.90 | 4.90 |
| Rape seed meal | 4.00 | 4.00 | 4.00 | 4.00 |
| DL-methionine | 0.23 | 0.23 | 0.23 | 0.23 |
| L-threonine | 0.13 | 0.13 | 0.13 | 0.13 |
| Calcium carbonate | 0.45 | 0.45 | 0.45 | 0.45 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 |
| Bone ash | 0.56 | 0.56 | 0.56 | 0.56 |
| Lysine suphate | 0.45 | 0.45 | 0.45 | 0.45 |
| *Premix | 0.30 | 0.30 | 0.30 | 0.30 |
| Bicarb | 0.09 | 0.09 | 0.09 | 0.09 |
| Quantum Blue 1000FTU | 0.02 | 0.02 | 0.02 | 0.02 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| Crude protein | 22.01% | 22.2% | 22.04% | 22.10% |
| Metabolizable energy kcal/kg | 2834 | 2837 | 2830 | 2832 |
| Lysine | 1.29 | 1.30 | 1.29 | 1.30 |
| Calcium | 0.91 | 0.90 | 0.90 | 0.88 |
| Available phosphorus | 0.46 | 0.45 | 0.43 | 0.44 |

Table 1. Composition of experimental diets offered to Japanese quail

*Premix contained Vit-A 9000 I.U, Vit-D 3250 I.U, Vit-E 30 I.U, Vit-K3 4mg, Thiamine 3.5 mg, Riboflavin 8 mg, Vit-B6 4.4 mg, Vit-B12 1.5 mg, Folic Acid 1 mg, Vit-B5 12 mg, Niacin 55 mg, Biotin 5 mg, Choline Chloride 700 mg, Selenium 50 mg, Zinc 110 mg, Copper 67.2 mg, Iron 394 mg, Manganese 172 mg, Potassium Iodide 0.8 mg, Furazolidon 100 mg, Maduramycin 50 mg.

3.4. Statistical analysis

All results data were recorded and collected and analyzed through a one-way analysis of variance (ANOVA) technique [15] by using SPSS v.16 (SPSS Inc., Chicago, IL, USA). Significant means were compared through Duncan's multiple range test [16].

4. Results and discussion

4.1. Growth performance

Growth performance parameters of Japanese quail supplemented Neem and Tulsi are shown in Table 2. Statistical analysis showed a significant difference ($p \le 0.05$) in feed intake of the experimental groups. The feed intake of T0, T1, T2, and T3 was 609.98 g, 642.58 g, 667.18 g, 635.57 g, respectively. When T0 and T2 were compared, the results were significantly different ($p \le 0.05$). T2 group consumed more feed than T0 group. However, when T1 and T3 were compared, there was no significant difference

(p > 0.05). T0 control group consumed less feed than all the treatment groups supplemented with Neem and Tusli. The increased feed intake might be due to hunger increasing and digestion stimulating, antibacterial and hepato-protective properties of Neem and Tulsi, which might have helped the quails to improve their gut health [13]. Similar findings were observed in previous studies [10,17,18,19] where higher feed intake was observed in Neem fed birds. However, the findings of the present study are contradictory to the results of [20,21] who observed nonsignificant feed consumption in the birds fed with Neem and Tulsi supplemented diet.

Final body weight Japanese quail differed significantly $(p \le 0.05)$ among treatments groups. T3 and T1 groups presented higher body weight than T2 and T0 control groups. Similar findings were reported in broilers fed Neem and Tulsi [10,13]. In contrast, it had been reported that body weight was not affected by the dietary

supplementation of Neem and Tulsi leaf powder in broiler chicken [21,22,23,24]. Weight gain of Japanese quails was significantly ($p \le 0.05$) affected by supplementation of Neem and Tusli. Weekly weight gain of the experimental birds is shown in Figure. On cumulative basis, the birds from the T3 group gained significantly more weight than T1 and T2. Accordingly, dietary supplementation of Neem in broiler chickens was found to enhance the weight gain in broilers [13,25]. In another study, Tulsi had been reported to increase the weight gain in broilers [19,26]. The combination of Neem and Tulsi was also found to improve the overall weight gain in poultry birds [10]. This shows the potential of using Neem and Tulsi herbs in the diet of Japanese quail. However, some previous reports showed no difference in the weight gain of the birds when broiler chickens were fed with Neem and Tulsi leaves in diet [12,24,27].

Regarding, feed conversion ratio (FCR), statistical analysis showed a significant difference in the FCR of the experimental groups where better FCR (3.31) was noted in T3 and poor FCR was recorded in T0 or control group. It was observed that FCR of the entire period of the experiment was best in the T3 group followed by T1, T2 and T0 control group. Better FCR in T3 might be due to better feed efficiency and higher weight gain. It has been earlier described that Neem and Tulsi have antibacterial

Table 2. Growth performance of Japanese quail fed diets supplemented with Neem and Tulsi powders.

| | Initial body weight $(n = 5)$ | Final body weight $(n = 5)$ | Weight gain $(n = 5)$ | Feed intake (n = 5) | FCR (n = 5) |
|---------|-------------------------------|-----------------------------|------------------------------|------------------------------|---------------------|
| | g/bird | | | | |
| T0 | 21.64 ± 0.22 | 189.22°±1.97 | 167.57°± 1.95 | $609.98^{b} \pm 14.59$ | $3.66^{a} \pm 0.06$ |
| T1 | 22.11±0.33 | $206.27^{ab} \pm 1.96$ | $184.15^{b} \pm 1.91$ | 642.56 ^{ab} ± 6.99 | $3.45^{ab}\pm0.05$ |
| T2 | 22.05 ± 0.18 | $205.54^{b} \pm 2.57$ | $183.49^{\text{b}} \pm 2.49$ | 667.18 ^a ± 9.05 | $3.60^{a} \pm 0.11$ |
| T3 | 22.02 ± 0.25 | 213.20 ^a ± 2.69 | $191.18^{a} \pm 2.84$ | 635.57 ^{ab} ± 10.69 | $3.32^{b} \pm 0.07$ |
| p-value | 0.5514 | <0.0001 | <0.0001 | 0.0139 | 0.0227 |

The values in the table are mean \pm standard error.

Different letters ^{a-c} within a column show significant differences when $p \le 0.05$ whereas common letters show nonsignificant differences at p > 0.05.

FCR is feed conversion ratio.

T0 = control diet; T1 = Neem powder in diet; T2 = Tulsi powder in diet; T3 = Neem + Tulsi powders in diet.

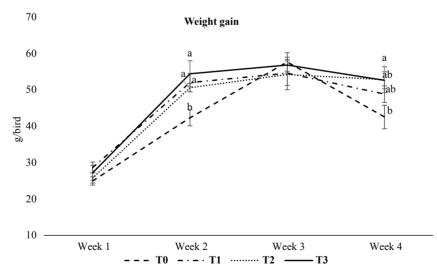


Figure. Weekly weight gain of Japanese quails fed diet supplemented with Neem and Tulsi powders. Different letters a-b show significant differences among the treatment groups. T0 = control diet; T1 = Neem powder in diet; T2 = Tulsi powder in diet; T3 = Neem + Tulsi powders in diet.

properties that might have led to better absorption and utilization of nutrients present in the gut and improved feed conversion ratio [10]. Previously, similar observations were reported in poultry where improved FCR was noted due to Neem and Tulsi leaves in basic diet and [19,28]. In another study, FCR was found better in Neem and Tulsi group compared with the control group [10]. However, contrary to present findings, no effect of Neem and Tulsi supplementation on overall FCR was reported in broiler chickens [12,29]. On an overall basis, Neem and Tulsi leave powder with basic diet had improved the growth performance of the birds.

4.2. Carcass characteristics

Carcass characteristics of Japanese quail supplemented Neem and Tulsi are presented in Table 3. Statistical analysis showed significant ($p \le 0.05$) differences in all carcass traits except Liver weight (p > 0.05) among the treatment groups. Highest values of preslaughtered weight slaughtered weight and dressed weight were observed in T3 group followed by T2 and T1 while the lowest value was noted in the control group. Heart weight was significantly ($p \le 0.05$) higher in T3 compared to the control group. Gizzard weight was observed comparable among the birds from T0 and T1 groups (p > 0.05) that differed significantly from T2 and T3 groups ($p \le 0.05$). Similarly, in a previous study, improvements in the carcass traits such as dressing percentage and heart weight with no change in gizzard weight were observed when Neem leaves extracts were supplemented to the broiler chickens [30]. In contrast to present findings, some earlier reports showed that the supplementation of Neem and Tulsi in basic diet had no impact on the carcass characteristics [17,28]. Varying results of the current study may be due to the concentrations of Neem or other herbal extracts supplied to the experimental birds of current and past trials. Moreover, the length of the experimental period and slaughtering age also may contribute toward carcass characteristics [30].

4.3. Blood parameters

Serum chemicals are the indicators of individuals' health and physical conditions [23]. The role of red blood cells (RBCs) and hemoglobin (Hb) is well understood in terms of oxygen supply throughout the body systems. White blood cells (WBCs) and platelets are involved in producing and maintaining the cellular immune response [23,34].

Blood parameters in Japanese quails fed the diet supplemented with Neem and Tulsi are shown in Table 4. Statistical analysis showed nonsignificant differences (p > 0.05) among the treatment groups for red blood cells, haematocrit (HCT), Mean corpuscular volume (MCV), mean corpuscular hameoglobin concentration (MCHC), platelets, triglycerides and very low density lipoproteins (VLDL) values. Earlier to this, nonsignificant differences of RBCs and HCT in Neem and Tulsi supplemented and control groups were reported [10,31]. Similarly, in another experiment, no effects of treatment on RBCs, MCV, MCH and MCHC was recorded [32,33].

Regarding glucose, hemoglobin, cholesterol, MCH, WBCs, HDL, LDL and total protein, dietary treatments had a significant impact on these health parameters in Japanese quails ($p \le 0.05$).

Glucose concentration was significantly higher in T0 control group than in all other treatment groups. Simply, the Neem and Tulsi powder alone and in combination resulted in lowering the glucose level in Japanese quails. Tulsi (O. sanctum) contains some active insulinogenic ingredient which helped in lowering of hyperglycemia [34]. The lower glucose contents in Neem and Tulsi supplemented groups could be attributed to hypoglycemic effects of these herbs [35, 36].

Hemoglobin was significantly higher in the T2 group than in the T1 group ($p \le 0.05$) but there was no difference

| | Preslaughter weight | Slaughtered weight | Dressed weight | Liver | Gizzard | Heart |
|-------------|---------------------------|------------------------|---------------------------|-----------------|-------------------------|-------------------------|
| | (g) | | | | | |
| T0 (n = 15) | $191.40^{\rm b} \pm 2.41$ | $178.20^{b} \pm 1.98$ | $112.80^{\rm b} \pm 2.11$ | 4.86 ± 0.29 | $5.52^{a} \pm 0.26$ | $1.77^{\rm b} \pm 0.09$ |
| T1 (n = 15) | $202.80^{a} \pm 4.34$ | $185.47^{ab} \pm 5.58$ | $115.82^{b} \pm 2.13$ | 4.72 ± 0.29 | $6.09^{a} \pm 0.41$ | $2.13^{b} \pm 0.14$ |
| T2 (n = 15) | $203.96^{a} \pm 2.43$ | $193.20^{a} \pm 2.71$ | $118.29^{ab} \pm 2.02$ | 5.24 ± 0.32 | $5.38^{ab} \pm 0.17$ | $2.00^{b} \pm 0.12$ |
| T3 (n = 15) | $213.00^{a} \pm 4.80$ | $195.47^{a} \pm 4.31$ | $123.40^{a} \pm 3.19$ | 4.14 ± 0.22 | $4.55^{\rm b} \pm 0.34$ | $2.96^{a} \pm 0.42$ |
| p- value | 0.0015 | 0.0113 | 0.0223 | 0.0587 | 0.0091 | 0.0044 |

Table 3. Carcass characteristics of Japanese quail fed diets supplemented with Neem and Tulsi powders.

The values in the table are mean \pm standard error.

Different letters ^{a-b} within the column show significant differences.

T0 = control diet; T1 = Neem powder in diet; T2 = Tulsi powder in diet; T3 = Neem + Tulsi powders in diet

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| | T0 (n = 15) | T1 (n = 15) | T2 (n = 15) | T3 (n = 15) | p - value |
|----------------------------------|---------------------------|-----------------------------|--------------------------|----------------------------|-----------|
| Glucose (mg/dL) | $97.20^{a} \pm 1.33$ | $69.80^{\mathrm{b}}\pm9.84$ | $64.20^{b} \pm 3.59$ | $66.00^{b} \pm 6.50$ | 0.0010 |
| Hemoglobin (g/dL) | $12.63^{ab} \pm 0.25$ | $11.98^{b} \pm 0.25$ | $13.40^{a} \pm 0.32$ | $12.97^{a} \pm 0.33$ | 0.0081 |
| WBCs (×10 ³ /µL) | $2.56^{a} \pm 2.27$ | $2.51^{a} \pm 0.96$ | $2.23^{b} \pm 0.88$ | $2.53^{a} \pm 1.23$ | < 0.0001 |
| RBCs (×10 ⁶ /µL) | 2.68 ±0.06 | 2.54 ± 0.10 | 2.74 ± 0.06 | 2.72 ± 0.09 | 0.2960 |
| HCT (%) | 45.78 ± 0.86 | 44.00 ± 1.41 | 47.60 ± 0.89 | 47.40 ± 1.19 | 0.0897 |
| MCV (fL) | 168.07 ± 0.77 | 174.00 ± 1.03 | 172.40 ± 1.39 | 167.80 ± 3.41 | 0.0649 |
| MCH (pg) | $42.40^{\rm b} \pm 2.16$ | $67.20^{a} \pm 10.29$ | $48.40^{\rm b} \pm 1.03$ | $47.20^{b} \pm 0.74$ | 0.0090 |
| MCHC (g/dL) | 31.40 ± 2.20 | 27.40 ± 0.70 | 28.00 ± 0.54 | 27.20 ± 0.78 | 0.0715 |
| Platelets (×10 ³ /µL) | 9.80 ± 0.77 | 6.80 ± 0.61 | 6.27 ± 0.57 | 9.27 ± 2.09 | 0.0992 |
| Cholesterol (mg/dL) | $202.40^{b} \pm 8.08$ | $196.40^{b} \pm 14.95$ | $252.80^{a} \pm 23.61$ | $240.60^{ab} \pm 11.75$ | 0.0317 |
| Triglycerides (mg/dL) | 221.60 ± 34.32 | 178.40 ± 17.09 | 167.40 ± 10.28 | 143.20 ± 13.32 | 0.0728 |
| HDL (mg/dL) | $110.20^{\circ} \pm 1.84$ | $105.40^{\circ} \pm 9.02$ | $172.67^{a} \pm 10.67$ | 137.87 ^b ± 9.28 | < 0.0001 |
| LDL (mg/dL) | $96.00^{b} \pm 2.17$ | $94.80^{\rm b} \pm 7.92$ | $151.00^{a} \pm 11.54$ | $128.47^{a} \pm 8.71$ | < 0.0001 |
| VLDL (mg/dL) | 43.00 ± 6.33 | 35.40 ± 3.39 | 32.60 ± 2.21 | 28.67 ± 2.64 | 0.0861 |
| Total Protein (g/dL) | $6.44^{a} \pm 0.10$ | $6.08^{ab} \pm 0.13$ | $6.20^{ab} \pm 0.13$ | $5.90^{b} \pm 0.12$ | 0.0236 |

Table 4. Blood profile of Japanese quail fed diet supplemented with Neem and Tulsi powders .

The values in the table are mean \pm standard error.

Different letters ^{a-c} within a row show significant differences when $p \le 0.05$ whereas common letters show nonsignificant differences at p > 0.05.

T0 = control diet; T1 = Neem powder in diet; T2 = Tulsi powder in diet; T3 = Neem + Tulsi powders in diet.

mg/dL is milligrams per deciliter, g/dL is grams per deciliter, µL is microliters, fL is femtoliters, pg is picograms.

WBCs is white blood cells, RBCs is red blood cells, HCT is hematocrit, MCV is mean corpuscular volume, MCH is mean corpuscular hemoglobin, MCHC is mean corpuscular hemoglobin concentration, HDL is high density lipoproteins, LDL is low density lipoproteins, VLDL is very low density lipoproteins.

from the control group. Similar findings were reported in earlier literature where supplementation of Tulsi and Neem showed significantly (p < 0.05) different hemoglobin contents in control and supplemented groups [37,38,39]. Hemoglobin contents are correlated with the endogenous sex hormone levels especially the testosterone in serum where a higher testosterone level may result in higher hemoglobin contents in quails [40]. According to Sethi et al. [38], Tulsi can enhance the serum testosterone level in the rabbit. Thus, the higher level of hemoglobin in the current study could be attributed to enhanced endogenous serum hormones.

MCH was lower in T0 group while a higher value of MCH was observed in the T1 group. Similarly, Unigwe et al. [32] reported a higher MCH value in Neem fed birds than the control group. MCH and MCHC reflect the hemoglobin contents in RBCs [41], thus showing the enhanced oxygen carrying capacity and better health. Higher MCH in T1 groups in current experiment favor the Neem supplementation in quail diet.

In the case of cholesterol, there was no difference between T0 and T1 but when T0 compared with T2

and T3 there was a significant difference. Similar results were reported by Elangovan et al. [37] who found that there was no effect of Neem on cholesterol of Japanese quail. In contrast to the present findings of cholesterol, in another study a significant decrease in the concentration of cholesterol [42]. In the case of LDL, the quails in T2 had significantly higher LDL contents than T0 and T1. Additionally, the beneficial fraction of the cholesterol i.e HDL was also significantly higher in the T2 group followed by T3 and the least HDL were observed in T1 and T0. In past, herbs used in poultry diets had been evaluated for any impact on HDL and LDL contents of the birds and positive results were shown with higher HDL values in the birds fed with herbal treatments [43]. Faghani et al. [44] described higher HDL in broilers fed with turmeric extract. Herve et al. [45] reported a similar finding in Japanese quail fed with ginger (Zingiber officinale, Roscoe). Thus, it can be concluded that the use of Tulsi of powder can be great value for using in the diet of Japanese quail

Total protein contents of Japanese quail were significantly different ($p \le 0.05$) among treatment groups.

Highest contents of total protein in T0 followed by T1 and T3, however, T2 was nonsignificantly different from all treatment groups. Various factors can change the status of total proteins in the blood of the avian species. The normal range of total protein in Japanese quails was earlier reported to be 0.62 to 5.7 g/dL in male and 2.9 to 4.3 g/dL depending upon the age, sex and health condition [46]. Possibly, the quails in T3 had more stable body metabolism and converted most of the blood proteins into their body parts. Higher blood proteins indicate several metabolic issues such as dehydration and less protein accretion into muscle mass. The findings of the current

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study suggest that the inclusion of Neem and Tulsi had no harmful impact on the health status of the Japanese quails.

From the above discussion, it can be concluded that supplementation of Neem and Tulsi leaves in diet can improve the health, overall growth and carcass traits of Japanese quail.

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