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Individual and Combined Effects of Moringa and Neem Leaves on Immune Response and Gut Microflora in Japanese Quails

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ABSTRACT

The current study investigated the individual and combined immunomodulatory and gut microflora modulatory effects of moringa and neem leaf powder in Japanese quails. A total of eighty (80) unsex day-old Japanese quail chicks were distributed into the four groups. The T0 offered only a basal diet, while T1, T2 and T3 were supplemented with moringa (5%), neem (1%) and moringa (5%) + neem (1%) leaf powder respectively in the basal diet. Results showed that live body weight and carcass weight were better ($p<0.05$) in T3 than in control and other supplemented groups. The relative weight of the spleen was found higher ($p<0.05$) in T2 and T3 groups as compared to T0 and T1 groups. The relative weight of the thymus was recorded as higher ($p<0.05$) in T3 as compared to control and other treated groups. The relative weight of the bursa was higher ($p<0.05$) in the T1 and T3 groups as compared to T0 and T2 groups. All treated groups exhibited significantly high ($p < 0.01$) total bacterial count, *Lactobacillus* and *Bifidobacterium* count as compared to the control group (T0). Whereas, *E. coli* and *Salmonella* count was found significantly lower ($p < 0.01$) in treated groups as compared to the control group. Hemoglobin and erythrocyte count was higher ($p < 0.05$) in T1 and T3 groups as compared to T2 and T0. MCHC (mean corpuscular haemoglobin concentration) was found higher ($p < 0.05$) in T3 as compared to T0 groups. White blood cell count was recorded higher ($p < 0.05$) in T3 as compared to T1 group. Lymphocytes (L) percentage was recorded highest ($p < 0.05$) in T3 followed by T2, T1 and T0 groups. While, heterophil (H) percentage and H/L ratio were found lowest ($p < 0.05$) in T3 followed by T2, T1, and T0 groups. It is concluded that moringa and neem leaf meal in combined form (5%+1%) have a significant potential to modulate immunity, haematological profile and gut microflora in Japanese quails.

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AAK conceived the study, RAC carried out the research work, while MB helped in manuscript writing and analysis.

Key words
Quails, Immunity, Microflora, Hematology, Moringa, Neem

INTRODUCTION

In an attempt, to augment the animal protein for human use quails were introduced. They are very prolific, have a short generation interval, and are disease resistant. Quails are a popular protein source because of their fast growth rates, early sexual maturity, short generation intervals, and resistance to a number of bird diseases (Mulaudzi *et al*., 2019). Quail farming is a relatively new industry that complements the production of low-cost animal proteins such as chicken, turkey, and ostrich. As a result, they helped to bridge the gap between the availability of other animal products on the table for human use. Traditionally used synthetic feed additives like antimicrobial growth stimulants have recently banned in commercial poultry farming (including quails' farmin), prompting further research into alternative growth promoters for animal production (Kamboh *et al*., 2015; Shahin *et al*., 2020). Many alternatives to these growth boosters, such as organic acids and medicinal herbs for chicken feed, have been proposed to enhance the performance of the birds (El-Saadany *et al*., 2022; Saki *et al*., 2012). Several plants like moringa, neem, ginger etc., were rported in recent literature for their significant potential to replace antibiotics in the poultry industry (Ashour *et al*., 2020; Untari *et al*., 2022).

Moringa oleifera has been shown to contain natural antioxidants like flavonoids, flavones and other phenolic compounds (Siddhuraju and Becker, 2003). Moringa leaves are rich in protein, vitamins (A, B, and C), iron, phosphorus, and calcium (Murro *et al*., 2003). Furthermore,

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heavy metals that might be harmful, such as mercury, arsenic, and cadmium, are not present in *M. oleifera* leaves, making its use in poultry diets safe (Donkor *et al*., 2013). Furthermore, polyphenols, anthocyanin, tannins, thiocarbamates, and glycosides, which activate antioxidant enzymes, remove free radicals, and inhibit oxidases, contributed to the antioxidant activity of moringa leaves (Luqmans *et al*., 2012) that ultimately effects immunity and modulate it towards the positive direction (Eladia and Ampode, 2021). According to a recent study conducted by our research group, moringa supplementation showed significant immunomodulatory effects in broilers (Afzal *et al*., 2020).

Neem (*Azadirachta indica*) is a fast-growing evergreen tree that has the potential to provide medical and nutritional benefits to broilers. Extracts of neem leaves have been demonstrated in the literature to have antibacterial activity against bacteria like *Streptococcus* spp., *Pseudomonas* spp., *Staphylococcus* spp., and *Escherichia coli*, as well as against certain fungal species (Valarmathy *et al*., 2010). In addition, neem supplementation has been found to have immunomodulatory effects in farm animals (Landy *et al*., 2011) and aquaculture (Kaur *et al*., 2019).

Keeping in view the biological activities of moringa and neem, current study planned to investigate the individual and combined immunomodulatory and gut microflora modulatory effects of *Moringa oleifera* and *Azadirachta indica* leaf powder in Japanese quails.

MATERIALS AND METHODS

Experimental plan and housing

The experimental design and procedures were approved by the Directorate of Advanced Studies, Sindh Agriculture University Tandojam (No. DAS/1425/of 2021) and were carried out according to prescribed ethical standard. Total eighty unsex day-old Japanese quail chicks were purchased from a commercial hatchery in Karachi and were raised in cage system available in the Department of Animal Nutrition, SAU, Tandojam. After arrival, chicks were first weighed and equally divided into four groups i.e., T0, T1, T2, and T3. Each treatment was consists of four replicates with 5 chicks in each replicate. T0 was fed control diet (basal diet), whereas T1 was supplemented 5% moringa leaf meal (MLM) in basal diet, T2 was supplemented 1% neem leaf meal (NLM) and T3 was supplemented with mixtures of 5% MLM and 1% NLM in basal diet respectively. Both the doses of neem and moringa (1 and 5%, respectively) were chosen based on a pilot study conducted on broilers (unpublished data). The experiment was lasted until five weeks of age.

Chicks were kept in cages during whole experimental period. Using disinfectant and fresh water, the cages were fully cleaned before transfer chicks to them. Rice husk was utilised as litter up to a depth of 2-4 inches for each treatment of birds. To minimise gas generation, litter was turned on a regular basis. Paper sheets were utilised to shield the litter during the first week of brooding. The temperature was kept at 90-95°F for the first week, then reduced by 5°F each week until the temperature was kept at 70°F. It was customary to feed the birds twice a day. The birds have unlimited access to fresh and clean water. The feed was prepared according to the instructions of National Research Council (NRC, 1994) and its ingredients and chemical composition is shown in Table I. While the nutritive value of MLM and NLM is presented in Table II.

Determination of relative weight of lymphoid organs

Eight birds from each group were weighed and killed via islamic slaughtering method of neck cutting at 35 days of age, and the carcass weight as well as the weight of lymphoid organs (thymus, spleen, and bursa) was determined using a digital balance. The relative weight of lymphoid organs was calculated using organ and live bird weight (Memon *et al*., 2019).

Complete blood count

At 35 day, eight birds from each group were slaughtered and a two milliliter of blood sample was collected in tubes from the both treated and control group. The sample were used for hematological analysis using an automatic hematology analyser (SINNOWA Medical Science and Technology Co., Ltd., Nanjing, China) in order to investigate the supplemental effects of moringa and neem on hematological indices including RBC (red blood cell) count, MCH (mean corpuscular haemoglobin), MCV (mean corpuscular volume), PCV (packed cell volume), MCHC (mean corpuscular haemoglobin concentration), PDW (platelet distribution width), PCT (plateletcrit), MPV (mean platelet volume), and WBC (white blood cell) count. The hematology profile also illuminated the heterophil to lymphocytes ratio (H/L index), which is a vital indicator of immunity.

Quantification of cecal microflora

To count the cecal microflora in each group, duplicate cecal contents were collected aseptically from the intestines of slaughtered Japanese quail birds at 35 days into sterile Eppendorf tubes. These were well combined and kept in the refrigerator at 4°C for future examination. The method of Memon *et al*. (2019) with slight modifications was used for overall bacterial counts and enumeration of particular species such as *E. coli* bacteria and lactobacilli.

Table I. Feed formulation for quails diet (g/100g).

Ingredients	T0 (Control) basal diet	T1 (Basal diet + 5% moringa leaf meal)	T2 (Basal diet + 1% neem leaf meal)	T3 (Basal diet + 5% moringa leaf meal and 1% neem leaf meal)
Corn	56.90	56.90	56.90	56.90
Soybean meal	27.90	27.90	27.90	27.90
Canola meal	5.05	5.05	5.05	5.05
Fish meal	4.00	4.00	4.00	4.00
Guar meal	2.00	2.00	2.00	2.00
Lime stone	1.29	1.29	1.29	1.29
Calcium carbonate	1.09	1.09	1.09	1.09
Vitamin-mineral mixture[1]	0.47	0.47	0.47	0.47
L-Lysine	0.57	0.57	0.57	0.57
DL-methionine	0.32	0.32	0.32	0.32
NaCl	0.23	0.23	0.23	0.23
L-Threonine	0.18	0.18	0.18	0.18

[1] vitamin A 12000mg, vitamin K 2mg, vitamin E 10mg, vitamin B6 1.5mg, vitamin B2 5mg, vitamin B1 1mg, vitamin B12 10mg, folic acid 1mg, nicotinic acid 30mg, biotin 50mg, pantothenic acid 10mg, choline chloride 500mg, Fe 30mg, Cu 10mg, Zn 50mg, Mg 60mg, I 1mg, Co 0.1mg, Se 0.1mg. Composition: Crude Protein = 23.00%; Ether Extract = 6.00%; Metabolizable Energy = 2900Kcal/kg.

Table II. Nutritive value of *Moringa oleifera* leaf meal (MLM) and Neem (*Azadirachta indica*) leaf meal (NLM) on dry matter percentage (%) basis.

Item	MLM composition on DM% basis	NLM composition on DM% basis
Moisture	7.29	7.67
Dry matter	92.71	92.33
Crude protein	29.54	21.61
Ether extract	9.61	4.17
Crude fiber	10.70	17.82
Nitrogen free extract	38.90	48.95
Ash	11.25	7.45
Gross energy (kcal/g)	4.94	4.18

In brief, a total of five dilution tubes holding 9 mL of sterile normal saline solution were used. One mL of the sample was used to prepare a 10-fold dilution. A 0.1 ml of every tube were then cultured on nutrient agar (Oxoid, UK) plates and stored for 24 h at 37°C. After incubation, colonies were counted using a colony counter and CFU/g results were calculated using following formula:

CFU/g = (colony no. x dilution factor)/ volume plated

All bacterial isolates were recognized based on the standardized cultural, staining and biochemical properties following the Bergey's Manual of Systematic Bacteriology (Whitman *et al*., 2012).

Statistical analysis

The results obtained in the work were analyzed using one-way analysis of variance (ANOVA). The JMP statistical package software (version 5.0.1a; SAS Institute, 2000) was used for calculating the differences between different supplementation levels and $p < 0.05$ was considered as limit for significance.

RESULTS

Live body weight, carcass weight and relative weight of immune organs

Effect of supplementation of MLM and NLM powder in Japanese quail diet on live weight, carcass weight and relative weight of immune organs was shown in Table III. The results exhibited that live body weight, carcass weight and relative immune organs weight (g) of Japanese quail was improved ($p<0.05$) by the supplementation of MLM and NLM powder. T3 (171.28 g) exhibited the highest raise in live body weight which was significantly ($p<0.05$) higher as compared to T2 (136.43 g), while non significantly higher ($p > 0.05$) than T1 (157.67 g) and T0 (156.01 g) groups. Carcass weight was also recorded highest in T3 (94.34 g) which was significantly ($p<0.05$) higher than control (T0, 71.2 g) and other supplemented groups. Relative weight of spleen was found higher ($p<0.05$) in T2 and T3 groups (0.17 g) as compared to T0 (0.14 g) and T1 (0.13 g) groups. Relative weight of thymus was recorded higher ($p<0.05$) in T3 (0.25 g) as compared to control (0.18 g) and other treated groups. Relative weight of bursa was

higher ($p<0.05$) in T1 and T3 groups (0.22 g) as compared to T0 (control, 0.16 g) and T2 (0.14 g) groups.

Cecal bacterial counts

As shown in Table IV total bacterial count was recorded highest in T3 (5.350 log cfu/g) followed by T2 (5.303 log cfu/g), T1 (5.243 log cfu/g) and T0 (5.220 log cfu/g) groups. All treated groups exhibited significantly high ($p < 0.01$) total bacterial count as compared to control group (T0). *Lactobacillus* count and *Bifidobacterium* count was also recorded highest in T3 (4.363 and 4.264 log cfu/g) followed by T2 (4.214 and 4.195 log cfu/g), T1 (4.190and 4.178 log cfu/g) and T0 (4.167 and 4.097 log cfu/g), respectively. All supplemented groups exhibited high ($p < 0.01$) *Lactobacillus* and *Bifidobacterium* count as compared to control group (T0). However, *E. coli* and *Salmonella* count was found highest in T0 (4.380 and 4.365 log cfu/g) followed by T1 (4.264 and 4.243 log cfu/g), T2 (4.130 and 4.195 log cfu/g) and T3 (4.056 and 4.096 log cfu/g), respectively. All treated groups exhibited significantly lower ($p < 0.01$) *E. coli* and *Salmonella* count as compared to control group (T0).

Hematological profile

The results showed that hemoglobin (%) was higher ($p < 0.05$) in T1 (23.75) and T3 (23.29) as compared to T2 (20.60) and T0 (20.30) groups. Similarly, RBC (%) was higher ($p < 0.05$) in T1 (7.65) and T3 (7.45) as compared to T0 (6.75) and T2 (6.70) groups. PCV (%) was found highest in T2 (59.00) followed by T0 (58.50), T3 (55.50) and T1 (48.00) groups. T1 and T3 groups exhibited significantly ($p < 0.05$) lower PCV (%) as compared to control (T0) and T2 groups. MCHC (g/dl) was found statistically higher ($p < 0.05$) in T3 (35.50) as compared to control/T0 group (31.56). Platelet count (10^9/L) was found highest in T3 (203.00) followed by T2 (189.00), T1 (172.50) and T0/ control group (171.00). Both T2 and T3 groups showed significantly higher ($p < 0.05$) platelet count as compared to control and T1 groups. WBC count (10^9/L) was recorded statistically higher ($p < 0.05$) in T3 (24.00) as compared to T1 (19.50), however, it was non significantly higher ($p > 0.05$) than T0 and T2 groups (22.00). Other variables like MCV, MCH, MPV, PDW, PCT, eosinophils, monocytes and basophil count was remained unchanged ($p > 0.05$) in Japanese quails by the dietary supplementation of MLM and NLM powder (Table V).

Heterophil to lymphocyte ratio

As shown in Figure 1, all moringa and neem supplemented groups significantly improved ($p < 0.05$) the lymphocyte %, while decreased ($p < 0.05$) the heterophil % and H/L ratio as compared to control group (T0). Lymphocytes percentage was recorded highest in T3 (67.0) followed by T2 (64.0), T1 (60.5) and T0 (57.5) groups. Heterophil percentage was found lowest in T3 (28.5) followed by T2 (30.5), T1 (33.5), and T0 (38.0) groups. Similarly, H/L ratio was found lowest in T3 (0.661), followed by T2 (0.554), T1 (0.477) and T0 (0.425) group.

Table III. Live body weight, carcass weight and relative immune organs weight (g) of Japanese quail supplemented with moringa and neem leaf meal powder.

Parameters	T0	T1	T2	T3	P-value	SEM
Live body weight (g)	156.01^{ab}	157.67^{ab}	136.43^{c}	171.28^{a}	0.012	12.231
Carcass weight (g)	71.20^{b}	70.22^{b}	68.48^{bc}	94.34^{a}	0.017	6.311
Relative weight of spleen (g)	0.14^{b}	0.13^{b}	0.17^{a}	0.17^{a}	0.049	0.164
Relative weight of thymus (g)	0.18^{b}	0.20^{b}	0.19^{b}	0.25^{a}	0.028	0.018
Relative weight of bursa (g)	0.16^{b}	0.22^{a}	0.14^{c}	0.22^{a}	0.017	0.035

Different superscript alphabets (a, b, c) in each row showing significant difference between means at $p<0.05$.T0, basal diet (control); T1, 5% Moringa inclusion in basal diet; T2, 1% Neem leaf inclusion in basal diet; and T3, 5% Moringa + 1% Neem leaf inclusion in basal diet; SEM, Standard error mean.

Table IV. Cecal bacterial counts (log cfu/g) of Japanese quail supplemented with moringa and neem leaf meal powder.

Bacterial count	T0	T1	T2	T3	P-value	SEM
Total bacterial count	5.220^{d}	5.243^{c}	5.303^{b}	5.350^{a}	0.009	0.003
Lactobacillus	4.167^{c}	4.190^{b}	4.214^{ab}	4.363^{a}	0.004	0.005
Bifidobacterium	4.079^{c}	4.178^{b}	4.195^{b}	4.264^{a}	0.003	0.001
Escherichia coli	4.380^{a}	4.264^{b}	4.130^{c}	4.056^{cd}	0.002	0.002
Salmonella	4.365^{a}	4.243^{b}	4.195^{bc}	4.096^{d}	0.001	0.004

Different superscript alphabets (a, b, c, d) in each row showing significant difference between means at $p<0.05$. For details of abbreviations see Table III.

Table V. Blood profile of Japanese quails supplemented with moringa and neem leaf meal powder.

Parameters*	T0	T1	T2	T3	P value	SEM
Haemoglobin (%)	20.30^{b}	23.75^{a}	20.60^{b}	23.29^{a}	0.036	0.527
RBC (10^{12}/L)	6.75^{b}	7.65^{a}	6.70^{b}	7.45^{a}	0.045	0.079
WBC (10^{9}/L)	22.00^{ab}	19.50^{b}	22.00^{ab}	24.00^{a}	0.034	1.539
PCV (%)	58.50^{a}	48.00^{c}	59.00^{a}	55.50^{b}	0.037	1.861
MCV (fL)	86.50	84.00	87.50	85.50	0.755	2.150
MCH (pg)	29.00	28.00	29.50	28.00	0.602	1.539
MCHC (g/dl)	31.56^{c}	34.00^{ab}	33.50^{ab}	35.50^{a}	0.042	1.255
Platelets (10^{9}/L)	171.00^{c}	172.50^{c}	189.00^{b}	203.00^{a}	0.028	11.151
MPV (fL)	6.40	6.05	6.35	7.35	0.531	0.384
PDW (fL)	16.00	15.00	16.00	17.00	0.381	1.776
PCT (%)	0.04	0.06	0.07	0.55	0.333	0.000
Eosinophils (%)	2.50	2.00	3.00	2.00	0.584	0.195
Monocytes (%)	1.00	2.00	1.50	1.50	0.256	0.045
Basophils (%)	1.00	1.00	1.00	1.00	1.000	0.002

*RBC, red blood cell; WBC, white blood cell; MCV, mean corpuscular volume; PCV, Packed cell volume; MCHC, mean corpuscular haemoglobin concentration; MCH, mean corpuscular haemoglobin; PDW, platelet distribution width; MPV, mean platelet volume; PCT, plateletcrit. Different superscript alphabets (a, b, c, d) in each row showing significant difference between means at p<0.05. For details of abbreviations see Table III.

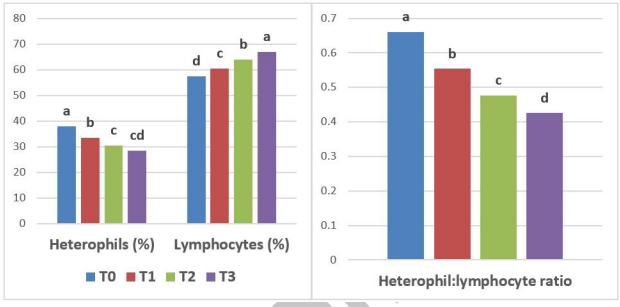

Fig. 1. Heterophil (H) and lymphocyte (L) concentration and H/L ratio of Japanese quail supplemented with moringa and neem leaf meal powder. For statistical details and composition of diet see Table III and IV.

DISCUSSION

There has been a lot of research done on the impact of moringa and neem supplementation in chicken to modulate immunity, digestibility, and gut microbiota in a favourable circumstances (Eladia and Ampode, 2021; Malik *et al*., 2019). However, there is lack of literature on the individual and combined effects of these medicinal plants on immunity and gut microflora in quails. Therefore, MLM and NLM were tested in different doses in broilers in our laboratory (unpublished data) for immune and gut microflora modulatory effects and best doses (1% neem and 5% moringa) were again investigated in current research in Japanese quail to see how they affect growth, immunological responses, and gut microbiota in these birds.

The results obtained from this study exhibited that combined supplementation of MLM and NLM has significantly improved weight gain and carcass weight. This study confirms previous findings indicated that moringa leaf meal promoted growth and productivity in poultry which is attributed to its nutrients and phytochemicals that had antimicrobial properties and significantly may reduce the microbial load of birds and improve the feed utilization that ultimately enhance weight gain (Fahey *et al*., 2001; Kakengi *et al*., 2007). Also, the improvement in final body weight and body weight gain could be credited to best digestibility potential of moringa plant as it has natural enzymes which could enhance nutrients absorption (Backer, 1995). These findings are in agreement with the finding of Elkloub *et al*. (2015) who reported that the supplementation of moringa leaves meal with 0.2, 0.4 and 0.6% levels in the feed of the growing Japanese quails significantly (p<0.05) improved their final body weight and body weight gain compared with control group. Teteh *et al*. (2013) reported that 1 to 2% moringa leaf meal have positive effect (p<0.05) on daily weight gain and FCR, however it has no effect (p>0.05) on daily feed intake of Ross broilers.

The addition of MLM and NLM powder in combined form enhanced the relative immune organ weight (g) of

Japanese quail ($p<0.05$). However, positive effects of individual treatments were also evident as compared to control group, but these effects were little when compared with combined supplementation. In agreement to our findings, the study of Ansari *et al*. (2012) reported a little ($p<0.05$) improvement in the relative weight of immune organs in neem supplemented chickens. In another study, broilers were supplemented with 1 and 2% of moringa leaves in diet and it was reported that moringa leaves dose-dependently improved ($p <0.05$) the weight of bursa, spleen and thymus as compared to control group (Teteh *et al*., 2013).

In Japanese quails, MLM and NLM powder modulated the intestinal microbiota towards a positive direction that results significant increase in total viable bacteria, *Lactobacillus* and *Bifidobacterium* count, while reduced the count of *E. coli* and *Salmonella*. The results support the findings of Djakalia *et al*. (2011) who reported that moringa leaf extracts exhibit antibacterial properties. They stop growth of harmful bacteria (like *Staphylococcus aureus*) from growing in animal intestines and feed. The intestinal microbiota was substantially decreased with MOL powder. The study of Assunção *et al*. (2019) also reported the marvelous decline in the intestinal population of *E. coli* in neem supplemented broilers. The study of Nantapo (2018) indicated that broilers supplemented with 5% moringa leaf powder showed significantly lower *E. coli* and *Clostridium perfringens* and higher *Lactobacillus* count in duodenum and jejunum as compared to those fed 1% moringa leaf powder. These results are also in agreement with the study of Mandal *et al*. (2014), those further reported that 2% moringa in diet of broilers is also beneficial in reducing the microbial load of pathogenic bacteria in the meat. The presence of various photochemical and essential oils in moringa leaves may have role in decreasing the microbial load and coliform count at all levels of MOL feeding compared to control and even antibiotic fed groups (Yang *et al*., 2006).

The results of haematology and serum analysis are often utilised to determine an animal's health condition. Haematological and serum variables have been recognized as an excellent markers of an animal's physiological state, and variations in these parameters are essential in determining the animal's reaction to various physiological circumstances (Khan and Zafar, 2005). Our results demonstrated that all haematological values were within the normal range (Aiello and Mays, 1998). The significant increase in haemoglobin and RBC count in MLM and NLM supplemented groups indicates that these botanicals have potential to prevent animals from anemia (Ansari *et al*., 2012). Moreover, significant raise of haemoglobin in MLM (T1) and MLM+NLM (T3) groups indicates the hepatoprotective effects of both supplements, as liver is known to regulate the synthesis of haemoglobin in the bone morrow via release of erythropoietic factors (Browman *et al*., 1976). Similarly, significant increase in WBC count in MLM and NLM combined supplemented groups reflects that this combinatorial treatment has immune-stimulating potential, because WBCs are the soldiers of body defense system that known to eliminate invading pathogens by several means like neutralization, opsonization and phagocytosis (Lewis Marffy and McCarthy, 2020).

It is well established that raised H/L ratio in birds is a suitable indictor of environmental stress conditions (Landy *et al*., 2011). In our study, all moringa and neem supplemented groups significantly improved ($p < 0.05$) the lymphocyte %, while decreased ($p < 0.05$) the heterophil % and H/L ratio as compared to control group (T0). These results affirmed that like other flavonoid-rich botanicals, MLM and NLM (which are rich in flavonoids) have potential antioxidant effects thus may reduce the stress at cellular level which is evident by decreased H/L ratio (Silas *et al*., 2014; Landy *et al*., 2011) heterophil count which is also recognized as a biomarker of antioxidants against environmental stresses (Oyagbemi and Adejinmi, 2012). These results are parallel with the study of Khan *et al*. (2021) who reported a significant decline in H:L in broilers supplemented moringa extract in drinking water. Similarly, the study of Landy *et al*. (2011) reported a decreased H/L ratio in broilers fed neem fruit powder in diet.

In current investigation, combined effect of MLM and NLM was observed superior as compared to their individual effects, which might be due to tendency of both botanicals to form synergism. In previous research, it is well documented that plant compounds may produce additive, synergistic or antagonistic effects when combined with each other (Kamboh and Zhu, 2014). However, further investigation is recommended to illuminate this phenomenon using specially designed *in vitro* or *in vivo* studies.

CONCLUSIONS

The study concluded that moringa and neem have significant potential to modulate cecal microflora and immunity in quails, particularly when supplemented jointly. Thus, both plant leaves could be used in commercial quail farming (in a dose of 5% moringa + 1% neem) for better production and to overcome immunity-related issues. Meanwhile, further challenge studies are recommended using infections agents and/or their products to estimate the actual immunomodulatory potential of moringa and neem leaf meal powder.

Statement of conflict of interest

The authors have declared no conflict of interest.

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