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Journal of Applied Animal Research

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/taar20

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To cite this article: Maria Imtiaz, Naila Chand, Shabana Naz, Ibrahim A. Alhidary, Sina Gul & Rifat Ullah Khan (2023) Effects of dietary inclusion of *Moringa oleifera* methanolic extract on productive performance, humoral immunity and nutrient digestibility in Japanese quails, Journal of Applied Animal Research, 51:1, 743-748, DOI: 10.1080/09712119.2023.2278878

To link to this article: https://doi.org/10.1080/09712119.2023.2278878

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Published online: 20 Nov 2023.

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JOURNAL OF APPLIED ANIMAL RESEARCH
2023, VOL. 51, NO. 1, 743–748
https://doi.org/10.1080/09712119.2023.2278878

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Effects of dietary inclusion of *Moringa oleifera* methanolic extract on productive performance, humoral immunity and nutrient digestibility in Japanese quails

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ABSTRACT

A total of 180 unsexed, 1-day-old quail chicks were divided into four treatments (three replicates). The treatment groups were designated as MOLE-0, MOLE-1, MOLE-2 and MOLE-3. In the MOLE-0 group, quails received a basal diet without supplementation. For MOLE-1, quails were provided with 100 mg/L of MOLE in their water, while MOLE-2 received 200 mg/L of water with MOLE supplementation. The fourth treatment, MOLE-3, received a concentration of 300 mg/L of MOLE in their drinking water. The findings revealed that MOLE-3 exhibited significantly higher weight gain and dressing percentage, along with a lower feed conversion ratio ($P < 0.05$), while the control group (MOLE-0) exhibited the least growth performance. MOLE-3 showed statistically higher antibody titres and immune organ weights, followed by MOLE-2 and MOLE-1. Conversely, the control group (MOLE-0) had a lower antibody titre against ND. Additionally, nutrient digestibility analysis indicated that MOLE-3 had higher digestibility of dry matter and crude protein ($P < 0.05$), while group MOLE-0 exhibited lower digestibility ($P < 0.05$). We concluded that supplementing *Moringa oleifera* leaf extract at a rate of 300 mg/L resulted in improved growth performance, immunity and nutrient digestibility in Japanese quails.

ARTICLE HISTORY
Received 18 September 2023
Accepted 30 October 2023

KEYWORDS
Growth; Japanese quails; moringa; immunity; mortality; nutrient digestibility

Introduction

Antibiotics are usually added into chicken diet for several purposes such as the treatment of infectious diseases or used sub-therapeutically to prevent infections and promote growth performance in broilers (Khan et al. 2022a). However, the widespread use of antibiotics has led to the emergence of antimicrobial resistance. This resistance is linked to humans consuming poultry products containing traces of antibiotics (Khan et al. 2022c). As a reaction, the European Union has prohibited the utilization of antibiotics in feed to reduce their harmful effects (Fatima et al. 2022). Consequently, there is a need to find alternative growth stimulants to replace antibiotics in chicken production.

The prohibition of antibiotics as growth enhancers has spurred comprehensive research into alternatives for poultry production, including probiotics (Khan and Naz 2013), prebiotics (Haq et al. 2020), enzymes (Jabbar et al. 2021), organic acids (Khan et al. 2021), herbs (Kairalla et al. 2022a, 2022b) and other compounds (Rahman et al. 2017; Kareem et al. 2018; Chand et al. 2020). Among these alternatives, researchers are exploring phytogenic compounds for use in chickens due to their numerous benefits, such as improved feed conversion ratio, better immunological status and augmented gastrointestinal health (Zamani et al. 2017; Kairalla et al. 2023). *M. oleifera*, a compact drought-resistant deciduous tree standing 5–12 cm tall (Khan et al. 2021), holds exceptional value for humans and animals alike, providing nutrition, medicinal properties and industrial applications (Mbikay 2012). This plant is rich in beta-carotene, amino acids and vitamins, contributing to its therapeutic potential in treating various ailments (Fuglie 1999). Moreover, it serves as an antioxidant, antimicrobial agent and growth stimulant (Ullah et al. 2022), with antioxidant benefits derived from caffeic acid and cinnamic acid (Khan et al. 2021). With over 92 beneficial components, *M. oleifera* proves to be a valuable natural resource (Nnam 2009).

Moringa oleifera is commonly used in broiler rations in the form of powder or aqueous extract. However, there have been limited studies investigating the effects of its aqueous extract on broiler performance and health. One such study by Alabi et al. (2020) reported that administering 90 mL of *M. oleifera* leaf extract (MOLE) reduced feed intake while improving feed to gain ratio in broilers. Similarly, in another study conducted by Paul et al.(2018) the aqueous extract of *M. oleifera* leaves was found to decrease feed intake and improve feed conversion ratio compared to antibiotics in broilers. In a recent study, Khan et al. (2022b) demonstrated that supplementing broiler chicks with MOLE (120 mL/L) of drinking water led to enhanced carcass yield, improved immunity, blood

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metabolites, and a reduction in melanodialdehyde. Few studies have documented the methanolic extract of moringa on growth, digestibility of nutrients and immune response of quails. Therefore, the aim of the present study was to find different levels of methanolic extract of growth performance, carcass quality, nutrient digestibility and antibody titre against New Castle disease (ND) virus in Japanese quails.

Materials and methods

Preparation of Moringa oleifera *MOLE*

The leaves of *M. oleifera* were obtained from the Medicinal Plants Section of Pakistan Forest Institute, Peshawar. The green and fresh leaves of moringa were air dried in a shaded area till the constant weight was achieved. To inhibit fungal growth continuous turning of leaves were done. The dried leaves were properly grinded and were sieved through 0.15 mm sieve. In Soxhlet, 200 grams of leaf powder was soaked overnight in 100 mL of methanol (100 percent methanol). About 50 mL extract were dissolved in dimethyl sulfoxide using clean vessel for 72 h, yielding a compact mass having 10 mg/mL concentration known as *Moringa oleifera* methanolic extract.

Experimental house and management

The poultry house and equipment's were cleaned using water and disinfected with formalin. Before chick's arrival the house was left empty for 14 days to get microbial free environment. Temperature and humidity were maintained according to age and condition of chicks.

Birds management and diet composition

A total of 180 unsexed, 1-day-old quail chicks were divided into four treatment groups. Each treatment consisted of three replicates, with 15 quails per pen. Throughout the study, all quails had free access to commercial feed (Table 1) and water. The birds were housed in metallic cages measuring 80 × 45 × 45 cm, equipped with feeders and drinkers. The light program initially provided 14 h of light daily, which was later increased to 16 h. The corn-soybean diets were in mash form and were formulated following the National Research Council's guidelines (1994) as shown in Table 1. The birds received vaccinations by a veterinarian at the appropriate age using distilled water. Ambient temperatures were recorded daily with the highest and lowest temperatures ranging from 14 to 23°C. The relative humidity averaged around 60–70%. All quails were raised in wire batteries under consistent management, hygiene, and environmental conditions. The four groups were labelled as MOLE-0, MOLE-1, MOLE-2, and MOLE-3. In the MOLE-0 group, quails received a basal diet without any supplementation. For MOLE-1, quails were provided with MOLE at a rate of 100 mg/L of water, while MOLE-2 received 200 mg/L of water with MOLE supplementation. The fourth treatment, MOLE-3, received MOLE at a concentration of 300 mg/L in their drinking water.

Table 1. Dietary composition of broiler chickens during finisher diet.

Ingredient (%)	Percent availability
Yellow corn	59.12
Soybean meal	23.45
Palm oil	2.96
Corn gluten meal	5.5
Wheat bran	3.1
Dicalcium phosphate	2.10
Ground limestone	0.64
Choline chloride	0.04
DL-methionine	0.080
L-lysine	0.37
Salt	0.1
Threonine	0.16
Vitamins- minerals premix (standard level)	0.50
Analyses	
ME, kcal/kg	3995
Crude protein, %	22.5
Non phytate *P*, %	0.46
Calcium, %	0.95
D. Lysine, %	1.25
D. Methionine, %	0.65
Sulphur amino acids, %	0.91
Threonine, %	0.84

Productive traits

To assess productive response, the following indicators were used: feed intake (FI), weight gain (WG), and feed conversion rate (FCR). At the end of the experiment, 96 male Japanese quails (3 animals in each subgroup, totalling 12 animals in each group) were randomly selected. The cervical dislocation method was used to euthanize the quails, and their skin and internal organs were collected to assess the dressing percentage.

Antibody titre

On day 35 of age, blood samples were collected randomly from five birds per replicate pen. The samples were used to analyse the antibody titre against New Castle disease virus. After collection, the blood samples were centrifuged at room temperature for 10 min at 3000 rpm to obtain serum.

Apparent nutrient digestibility

Upon reaching 35 days of age, three birds from each replicate pen were selected at random and placed into meticulously cleaned cages for the purpose of measuring nutrient digestibility. The cages were equipped with feed troughs and water nipples. The same treatments given during the growth study were administered to the birds. After a 3-day acclimatization period, measurements were taken over 5 days. Excreta collected were meticulously screened to remove any spilled feed and feathers. The collected excreta were then air-dried under ambient room temperature, finely ground, and subsequently employed for proximate analysis. The samples were analysed for crude protein (CP), ether extract (EE), crude fat (CF) and nitrogen free extract (NFE) using the methods of the Association of Official Analytical Chemists (2005). The apparent digestibility values for CP, EE, CF, and NFE were then calculated as follows:

$$\text{Apparent nutrient digestibility (\%)} = \frac{\text{nutrient intake}}{\text{excreta nutrient nutrient intake}} \times 100.$$

Statistical analysis

The data was subjected to statistical analysis using a completely randomized design (CRD) to compare means. The least significant difference (LSD) test was applied at a significance level of 5% to determine significant differences between the groups.

Results

The feed intake was non-significant ($P < 0.05$) during experimental trail from day 0 to day 42 as shown in Table 2. Numerically high feed consumption was recorded during finisher period in MOLE-0 (Control) which were not treated with methanolic extract of *M. oleifera* as compared to the treated groups (MOLE 1, 2, 3) which were supplemented with *Moringa oleifera* at the rate of 100, 200 and 300 mg per litre in drinking water. Similarly, on overall basis, the treated groups showed low feed intake as compare to MOLE-0.

The data of weight gain in Japanese quails fed with different levels of methanolic extract of moringa is given in Table 3. Weight gain was substantially ($P > 0.05$) increased with increasing rate of methanolic extract of *M. oleifera* in all treated groups. During starter phase, high ($P < 0.05$) weight gain was recorded in MOLE-3 linearly followed by MOLE-2 and MOLE-1, while low ($P < 0.05$) weight gain was recorded in MOLE-0. During finisher phase, weight gain was increased ($P < 0.05$) with increasing level of moringa. Significantly ($P < 0.05$) high weight gain was recorded in MOLE-3 while the control group experienced the lowest weight gain. Similar pattern of weight gain was recorded for overall.

With increasing levels of methanolic extract of *M. oleifera* during the course of the trial, FCR was significantly ($P < 0.05$) enhanced (Table 4). MOLE-0 recorded the greatest (poor) feed FCR during the starter phase. Similarly, higher (poor) FCR was noted in MOLE-0 (control) during the finisher phase, while lower (good) and the same feed conversion ratio were recorded in all treated groups. Overall FCR of MOLE-3 was significantly ($P < 0.05$) lower and was followed by MOLE-2 and MOLE-1. Overall FCR for the control group was recorded poor.

Different concentrations of *Moringa oleifera* methanolic extract considerably ($P < 0.05$) impacted the dressing percentage (Table 5). While MOLE-0 (control) had the lowest dressing %, MOLE-3 had the highest ($P < 0.05$) dressing percentage, followed by MOLE-2 and MOLE-1. Mortality was not differed significantly ($P = 0.0770$) among the treatment groups. However, MOLE-0 (no inclusion of MOLE) had numerically higher mortality than *Moringa oleifera* treated groups. Statistically higher ($P < 0.05$) antibody titre was recorded in MOLE-3 followed by MOLE-2 and MOLE-1. Lower antibody titre against ND was recorded in MOLE-0 (control group).

Immune organs weight was significantly ($P < 0.05$) improved with increasing level of MOLE (Table 6). Statistically higher

Table 2. Mean feed intake (g) in broilers fed with different levels of methanolic extract of *Moringa oleifera* leaves (MOLE).

Groups	Starter phase	Finisher phase	Overall
MOLE-0	152.75 ± 1.05	376.38 ± 9.41	529.12 ± 9.12
MOLE-1	150.74 ± 1.35	363.72 ± 13.98	514.46 ± 14.84
MOLE-2	151.89 ± 1.36	360.00 ± 19.99	511.89 ± 20.61
MOLE-3	152.08 ± 1.25	363.97 ± 9.94	516.05 ± 11.07
P value	0.3355	0.5368	0.5120

Means in the same column with different superscripts are statistically different ($P < 0.05$).
MOLE represents methanolic extract of *Moringa oleifera*; 100–300 mg per litre of drinking water.

Table 3. Mean weight gain (g) in broilers fed with different levels of methanolic extract of *Moringa oleifera* leaves (MOLE).

Groups	Starter phase	Finisher phase	Overall
MOLE-0	$69.87^{c} \pm 1.33$	$120.47^{d} \pm 0.53$	$190.35^{d} \pm 0.91$
MOLE-1	$70.95^{bc} \pm 0.99$	$125.17^{c} \pm 2.00$	$196.13^{c} \pm 2.59$
MOLE-2	$72.29^{bc} \pm 1.32$	$129.80^{b} \pm 0.90$	$202.09^{b} \pm 1.69$
MOLE-3	$76.43^{a} \pm 1.03$	$133.80^{a} \pm 1.03$	$210.24^{a} \pm 1.88$
P value	0.0007	0.0000	0.0000

Means in the same column with different superscripts are statistically different ($P < 0.05$).
MOLE represents methanolic extract of *Moringa oleifera*; 100–300 mg per litre of drinking water.

Table 4. Mean feed conversion ratio in broilers fed with different levels of methanolic extract of *Moringa oleifera* leaves (MOLE).

Groups	Starter phase	Finisher phase	Overall
MOLE-0	$2.19^{a} \pm 0.03$	$3.12^{a} \pm 0.07$	$2.78^{a} \pm 0.05$
MOLE-1	$2.13^{ab} \pm 0.04$	$2.91^{b} \pm 0.07$	$2.62^{b} \pm 0.05$
MOLE-2	$2.10^{b} \pm 0.0558$	$2.77^{b} \pm 0.14$	$2.53^{bc} \pm 0.09$
MOLE-3	$2.00^{c} \pm 0.0215$	$2.79^{b} \pm 0.04$	$2.45^{c} \pm 0.04$
P value	0.0021	0.0035	0.0009

Means in the same column with different superscripts are statistically different ($P < 0.05$).
MOLE represents methanolic extract of *Moringa oleifera*; 100–300 mg per litre of drinking water.

Table 5. Mean dressing percentage and mortality in broilers fed with different levels of *Moringa oleifera* leaves (MOLE).

Groups	Dressing Percentage	Mortality	Antibody titre (Log_{10})
MOLE-0	$67.80^{d} \pm 0.34$	1.00 ± 0.00	$3.33^{c} \pm 0.58$
MOLE-1	$69.06^{c} \pm 0.74$	0.67 ± 0.58	$4.33^{b} \pm 0.58$
MOLE-2	$70.19^{b} \pm 0.18$	0.33 ± 0.58	$4.67^{b} \pm 0.58$
MOLE-3	$71.62^{a} \pm 0.55$	0.00 ± 0.00	$6.00^{a} \pm 0.00$
P value	0.0001	0.0770	0.0013

Means in the same column with different superscripts are statistically different ($P < 0.05$).
MOLE represents methanolic extract of *Moringa oleifera*; 100–300 mg per litre of drinking water.

Table 6. Mean immune organ index in quails fed with different levels of methanolic extract of *Moringa oleifera* leaves (MOLE).

Groups	Thymus	Spleen	Bursa
MOLE-0	0.28c ± 0.01	$0.08^{d} \pm 0.01$	$0.10^{c} \pm 0.02$
MOLE-1	$0.32^{b} \pm 0.03$	$0.11^{c} \pm 0.02$	$0.12^{b} \pm 0.05$
MOLE-2	$0.35^{a} \pm 0.01$	$0.12^{b} \pm 0.01$	$0.14^{a} \pm 0.01$
MOLE-3	$0.36^{a} \pm 0.00$	$0.14^{a} \pm 0.03$	$0.15^{a} \pm 0.01$
P value	0.0000	0.0001	0.0003

$\alpha = 0.05$, means in the same column with different superscripts are substantially different.
MOLE represents methanolic extract of *Moringa oleifera*; 100–300 mg per litre of drinking water.

Table 7. Nutrient digestibility in quails fed with different levels of methanolic extract of *Moringa oleifera* leaves (MOLE).

Groups	DM %	Ash %	CP %	EE %	CF %	NFE %
MOLE-0	$66.70^{c} \pm 0.94$	18.54 ± 1.63	$52.63^{b} \pm 1.15$	80.36 ± 0.42	24.15 ± 2.04	74.48 ± 1.14
MOLE-1	$66.85^{c} \pm 0.67$	20.86 ± 1.51	$55.68^{b} \pm 0.95$	81.15 ± 1.45	24.77 ± 2.51	74.57 ± 1.20
MOLE-2	$69.46^{b} \pm 0.93$	20.86 ± 1.65	$57.71^{b} \pm 5.12$	81.46 ± 0.61	24.87 ± 2.09	75.86 ± 1.33
MOLE-3	$72.36^{a} \pm 1.36$	21.15 ± 1.18	$64.08^{a} \pm 3.48$	81.76 ± 1.20	25.86 ± 5.09	75.92 ± 2.30
P value	0.0004	0.1926	0.0130	0.4187	0.9282	0.5435

Means in the same column with different superscripts are statistically different ($P < 0.05$).
MOLE represents methanolic extract of *Moringa oleifera*; 100–300 mg per litre of drinking water.

relative weight of thymus was found in MOLE-3 followed by MOLE-2, MOLE-1 and MOLE-0 (control). Similarly, high ($P < 0.05$) and the same relative weight of spleen was found in MOLE-3 and MOLE-2, while lowest ($P < 0.05$) spleen weight was recorded in the control group. Relative weight of bursa was found to be lowest ($P < 0.05$) in the control group (MOLE-0) and MOLE-1, while highest ($P < 0.05$) value was recorded for MOLE-3.

The data in Table 7 showed the effect of addition of different MOLE on nutrients digestibility of quails. There was no effect ($P > 0.05$) in digestibility of ash, EE, CF, and NFE. The digestibility of dry matter and CP was significantly affected by supplementation of MOLE. The digestibility of DM and CP was higher ($P < 0.05$) in MOLE-3 and lower ($P < 0.05$) in group MOLE-0 (control group).

Discussion

Our findings showed that supplementing MOLE at a rate of 300 mg/L improved body weight gain and feed conversion ratio (FCR). The leaves of *M. oleifera* are remarkably rich in protein, containing approximately 25–27%, making them highly suitable for animal consumption. The amino acid profile of *M. oleifera* leaves and soybean meal exhibited similarity. Moreover, these leaves contain all the essential amino acids needed by animals. Studies have reported that *M. oleifera* possesses higher iron content four glasses of milk and twice the protein content of milk. The nutritional analysis of *M. oleifera* leaf meal revealed it contains 22.5% crude fibre, 29.7% crude protein, 4.38% ether extract, 0.26% phosphorus, 27.9% calcium and minimal tannin content. According to a study by Lu et al. (2016) while *M. oleifera* did not significantly affect feed intake, however, it positively impacted FCR. In comparison to the control group, Riry et al. (2016) observed a reduction in feed consumption among Japanese quails that were fed *M. oleifera* seed meal. Likewise, Elkloub et al. (2015) discovered that Japanese quails provided *M. oleifera* leaf meal recorded the lowest feed intake and the most favourable FCR. Kulkarni and Durge (2019) observed an improved FCR in Japanese quails provided with *M. oleifera* leaf meal. On the other hand, Ahmed and El-Rayes (2019) noted an increased FCR in Japanese quails fed *M. oleifera* leaf meal in their diet. Nevertheless, there exist inconsistencies in the research outcomes concerning feed intake in birds supplemented with *M. oleifera*. Castillo et al. (2018) documented a noteworthy rise in feed conversion ratio (FCR) among Japanese quails administered *M. oleifera* leaf flour, while observing no substantial alteration in feed consumption. Conversely, Alabi et al. (2020) noted that the Moringa meal supplement led to reduced feed consumption, possibly due to increased nutritional satisfaction. These discrepancies may be attributed to factors such as the dosage, duration, preparation of Moringa, the quail strains used, and variations in experimental design. Adding Moringa leaf meal to broilers' feed has shown to improve weight gain in other studies (Nouman et al. 2014). Likewise, Elkloub et al. (2015) observed that Japanese quails receiving 0.2% Moringa meal exhibited greater weight gain compared to those in the control diet. Talukdar et al. (2020) also identified a notable rise in growth among Japanese quails that were supplemented *M. oleifera* leaf meal in comparison to the control group that received no supplementation. This indicates that *M. oleifera* leaf meal holds a promising profile as a viable natural feed additive for boosting the overall performance of Japanese quails. Moreover, Kulkarni and Durge (2019) noted a rise in weight gain among Japanese quails that were supplemented *M. oleifera* leaf meal, surpassing the weight gain observed in the control group. Similarly, Ahmed and El-Rayes (2019) reported noteworthy increases in weight gain among Japanese quails that were fed *M. oleifera* leaf meal as part of their diet, in comparison to the control group. In the current study, Mole supplementation at the level of 300 mg/L resulted in significantly higher dressing percentage or carcass weight. *M. oleifera* leaves are rich in alpha-linoleic acid and various essential amino acids (Khan et al. 2021). The occurrence of dietary antioxidants within *M. oleifera* is likely accountable for the observed rise in carcass weight (Hekmat et al. 2015). These antioxidants play a role in mitigating stress among birds and enhancing the processes of protein absorption and digestion (Qwele et al. 2013; Saini et al. 2014). Ahmed and El-Rayes (2019) recorded significant rises in dressing weight within the group of Japanese quails that incorporated *M. oleifera* leaf meal into their diet. The abundance of carbohydrates, protein, and fibre in *M. oleifera*, along with its low fat content, may offer an explanation for the noticeable increase in carcass weight. Moreover, the substantial presence of pepsin and total soluble protein in *M. oleifera* leaf meal affirms its appropriateness as a dietary protein source for non-ruminant animals. This, in turn, contributes to the enhancement in carcass weight, as highlighted by Nouman et al. (2014). In the present study, the antibody titre and the weight of immune organs were significantly higher in Japanese quails fed MOLE at the level of 300 mg/L. Similar to our findings, Hassan et al. (2016) also reported that supplementation of *Moringa oleifera* leaf meal (MOLM) at a rate of 15% resulted in an enhanced antibody titre against New Castle Disease Virus (NDV) in birds. Previous studies, like the one conducted by Du et al. (2007), have also shown that MOLM supplementation in broiler diets can increase antibody titre against diseases. The increased antibody titre observed with MOLM may be attributed to the presence of lectins in

Moringa leaves, which are known to enhance the body's defence system (Hassan et al. 2016). As a result, *Moringa oleifera* is considered an immune-boosting plant that helps the body strengthen its defence against diseases. According to Katanbaf et al. (1989), an increase in relative organ weight is considered an indicator of immunological improvement. The enhanced immunity observed may be attributed to the unique combination of phenolic compounds present in the leaves of *M. oleifera*, including zeatin, quercetin, kaempferol, and apigenin.

In the current study, the digestibility of dry matter and crude protein was significantly higher in quails supplemented with MOLE 300 mg/L. Nutrient digestibility refers to the proportion of feedstuff absorbed by an animal's body upon consumption. Egbu et al. (2022) conducted a study on Cobb 500 broilers and found that the administration of *Moringa oleifera* seed extract (MSE) in drinking water had digestion-enhancing qualities similar to other herbal medications. MSE appeared to promote the growth of beneficial bacteria while inhibiting harmful microbes, potentially influencing poultry growth and intestinal microbiota (Akhouri et al. 2013). According to Siti et al. (2019), the active ingredient in MSE works by inhibiting microbial pathogens and endotoxins in the intestine, as well as increasing pancreatic activity, leading to improved nutrient metabolism and utilization. Ossebi (2002) conveyed that the incorporation of Moringa leaves up to a 24% level in feed exhibited no detrimental impacts on nutrient absorption. Conversely, it led to a substantial enhancement in protein digestibility, energy utilization and mineral utilization.

Conclusion

From the results of the present study, it was concluded that supplementation of *Moringa oleifera* leaves extract at the rate of 300 mg/L resulted in improved growth performance, immunity and nutrient digestibility in Japanese quails.

Acknowledgement

We extend our appreciation to the Researchers Supporting Project (No. RSPD2023R833), King Saud University, Riyadh, Saudi Arabia

Disclosure statement

No potential conflict of interest was reported by the author(s).

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