In vitro efficacy and ameliorating effect of *Moringa oleifera* on growth, carcass, stress and digestibility of nutrients in *Escherichia coli*-infected broilers

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**ABSTRACT**

The present study was conducted to evaluate the *in vitro* efficacy of *Moringa oleifera* against *E. coli* infection and *in vivo* effect of *M. oleifera* on the growth, carcass quality, heterophil to lymphocyte ratio (H:L) and digestibility of nutrients in broilers infected with *E. coli* (1.5 × 10\(^9\) CFU/ml). A total of 350 old broiler chicks (Hubbard) were assigned to different groups as follows: (1) negative control (uninfected, untreated), (2) positive control (infected, untreated), (3) infected + treated with *M. oleifera* leaf extract at the rate of 50 ml/l (MOLE\(_{50}\)), (4) infected + treated with *M. oleifera* leaf extract at the rate of 100 ml/l (MOLE\(_{100}\)), (5) infected + treated with *M. oleifera* leaf extract at the rate of 150 ml/l (MOLE\(_{150}\)). The results revealed that 12% *M. oleifera* extract produced significantly (*P* < 0.05) higher bacterial inhibition in the *in vitro* study. Body weight, feed conversion ratio (FCR), carcass quality, H:L ratio and digestibility of proteins and energy were significantly (*P* < 0.05) higher in MOLE\(_{100}\) supplemented broilers. From the results of the present study, it was concluded that MOLE\(_{100}\) improved the growth performance, carcass quality, welfare issue and nutrient digestibility in broilers infected with *E. coli*.

**Introduction**

Avian colibacillosis, which is caused by the bacteria *Escherichia coli*, is a major source of concern for poultry breeders since it results in significant financial losses (Haq et al. 2020). Septicemia is present in the acute stage, while airsacculitis and fibrinous polyserositis are present in the subacute stage (e.g. pericarditis, perihepatitis and peritonitis). As a result, mortality is raised, resulting in economic losses due to increasing vaccine and treatment expenditures (Kumari et al. 2020).

Antibiotics are often used in chickens to treat illness, as a preventive measure, or as a growth booster. However, it has the potential to increase medication toxicity and exacerbate residual effects (Hafeez et al. 2021; Israr et al. 2021; Shuaib et al. 2021a; Shuaib et al. 2021b). Antibiotic overuse renders many antimicrobials useless in the treatment of certain microbiological infections (Abudabos et al. 2018; Chand et al. 2021). Resistance to antimicrobial agents has become a worldwide problem. Efforts are made via research in discovering novel, powerful and creative antibiotics as well as useful chemotherapeutic medicines to cope with various diseases; however the usage of antibiotics should be minimal to reduce the incidence of resistant bacteria (Abudabos et al. 2017). Regardless of the many methods to medication development, plants continue to be the primary source of natural herbal medicines (Abudabos et al. 2016; Alhidary et al. 2017; Rahman et al. 2017; Chand et al. 2018; Wahab et al. 2019; Ahmad et al. 2020). Now from the last couple of decades the trend has been changed. Plants have been used to maintain the health of animals as an important part of natural products. Plants contain a large number of chemical substances with significant preventive effects (Khan et al. 2012a; Khan et al. 2012b; Khan et al. 2012c; Khan et al. 2012d; Alzawqari et al. 2016; Tehseen et al. 2016).

*Moringa oleifera* is a great source of nutritional, medicinal and industrial compounds that may be used to treat human and animal ailments (Khan et al. 2021a). It is a tiny, rapidly growing, drought-resistant ‘deciduous’ tree that grows to a height of between 5 and 12 m. Moringa seeds and leaves are widely used in food and medicine. The leaves are an excellent source of amino acids, carotene, ascorbic acid and vitamins, and owing to their exceptional medical and therapeutic properties, they may be used as a medication to prevent a variety of illnesses (Makkar and Becker 1997; Anwar et al. 2005; Simbaya et al. 2020; Khan et al. 2021b). Additionally, the leaves may be utilized as an antimicrobial, antioxidant and growth stimulant (Mahfuz and Piao 2019).

*Moringa oleifera* has been mostly used in broiler rations as a powder. Fewer research reports have been conducted to determine the impact of aqueous extract on broiler performance and health (Alabi et al. 2017; Hashem et al. 2019; Paul et al. 2019). The majority of previously performed research studies on the effects of aqueous extract of *M. oleifera* on broiler performance and health have shown ambiguous results. Additionally, the majority of research tested *M. oleifera* as crushed powder in broiler diets. Few studies have evaluated the aqueous extracts...
of seeds of *M. oleifera* against *E. coli*, *V. cholerae* and *S. typhii* (Sharma et al. 2006; Yang et al. 2006; Walter et al. 2011). The objective of the present study was to evaluate the effect of aqueous extract of *M. oleifera* on the *in vitro* antibacterial activity, growth performance, digestibility of protein and apparent metabolizable energy and heterophils to lymphocyte ratio in broiler infected with *E. coli*.

Materials and methods

All the experimental procedures were conducted according to the Rights and Welfare of Animals, The University of Agriculture, Peshawar, Pakistan (Letter No. 432/FAHVS).

**In vitro antibacterial activity of *M. oleifera* leaf extract against *E. coli***

Antimicrobial activity of *M. oleifera* leaf extract (MOLE) against *E. coli O78* was tested by agar well diffusion method. Briefly, the test organism was inoculated into nutrient broth and incubated overnight at 37°C to achieve a turbidity of 0.5 McFarland standards, yielding a final inoculum of $1.5 \times 10^9$ CFU/ml. A uniform microbial culture broth was used to cultivate the plate. Various concentrations 3%, 6%, 9%, 12% and 15% of *M. oleifera* leaf extract were prepared. Five millimetre wells were drilled into the infected medium using a sterilized cork-borer. Wells were filled with the given concentration of the extracts. The plates sealed properly with para film, was labelled and incubated for 24–48 h at 37°C after loading the extracts. After 48 h the plates were observed with clear zone of inhibition around the wells against *E. coli* and the antimicrobial activities were expressed in millimetre (mm).

### Table 1. Composition of basal feed and chemical composition as fed basis.

| Ingredients (%) | Starter phase (1–21 days) | Finisher phase (22–35 days) | Moringa oleifera |
|-----------------|----------------------------|-----------------------------|------------------|
| Corn            | 55.5                       | 56.00                       |                  |
| Soybean meal (44%) | 28.4                     | 27.5                        |                  |
| Canola meal     | 6.06                       | 5.51                        |                  |
| Sunflower meal  | 3.3                        | 4.2                         |                  |
| Vegetable oil   | 2.1                        | 2.1                         |                  |
| Molasses        | 1.00                       | 1.00                        |                  |
| Dicalcium phosphate | 1.9                    | 1.9                         |                  |
| Limestone       | 1.00                       | 1.00                        |                  |
| NaCl            | 0.01                       | 0.01                        |                  |
| NaHCO$_3$       | 0.01                       | 0.01                        |                  |
| DL-Methionine   | 0.20                       | 0.1                         |                  |
| Lysine-HCl      | 0.22                       | 0.37                        |                  |
| Vitamins minerals premix | 0.3            | 0.3                         |                  |
| Chemical composition |              |                             |                  |
| ME, kcal/kg    | 3000                       | 3150                        |                  |
| Crude protein, % | 23.5                      | 21.30                       |                  |
| Methionine, %   | 0.55                       | 0.44                        |                  |
| Lysine, %       | 1.42                       | 1.23                        |                  |
| Sulfur amino acids, % | 0.96               | 0.80                        |                  |
| Threonine, %    | 0.95                       | 0.85                        |                  |
| Calcium, %      | 1.05                       | 0.90                        |                  |
| Available       | 0.50                       | 0.45                        |                  |
| phosphorus, %   | 14.21                      | 14.21                       |                  |
| Crude protein, % | 23.45                      | 23.45                       |                  |
| Crude fats, %   | 2.52                       | 2.52                        |                  |

1Vitamins–minerals premix contains in the following per kg: vitamin A, 2400,000 IU; vitamin D, 1000,000 IU; vitamin E, 16,000 IU; vitamin K, 800 mg; vitamin B1, 600 mg; vitamin B2, 1600 mg; vitamin B6, 1000 mg; vitamin B12, 6 mg; niacin, 8000 mg; folic acid, 400 mg; panthenic acid, 3000 mg; biotin 40 mg; antioxidant, 3000 mg; cobalt, 80 mg; copper, 2000mg; iodine, 400; iron, 1200 mg; manganese, 18000 mg; selenium, 60 mg, and zinc, 14,000 mg.

### Plant extract preparation

*M. oleifera* leaves were harvested fresh from trees near The University of Agriculture Peshawar. The leaves were washed thoroughly and stored for 5–8 days on a floor covered with plastic sheet to provide shade and anaerobic conditions, before being ground into fine powder using a miller machine (grinder) at the Department of Animal Nutrition, The University of Agriculture Peshawar. Different percentages of *M. oleifera* leaves fine powder were dissolved in absolute distilled water, namely, 3%, 6%, 9%, 12% and 15%. The antibacterial activity against *E. coli* was significantly increased when 12% *M. oleifera* leaf aqueous extract was used. After that, 12.3% (40 g) *M. oleifera* leaf powder was dissolved in 333.33 ml of absolute freshwater. The solution was stored for 24 h and shocked on a regular basis. The water extract of *M. oleifera* leaves was then filtered using muslin cloth or a sieve. Following filtering, the aqueous leaves extract was kept at 4°C for future investigation. The aqueous extract of *M. oleifera* leaves was divided into three concentration levels of 50, 100 and 150 ml.

### Housing and management of birds

We bought 350 day-old broiler chicks (Hubbard; average starting weight 44 g) from a hatchery in Lahore. All birds were assisted via the provision of optimal management and environmental circumstances, as well as ad libitum food and regular immunization. The experiment lasted 35 days and was divided into two phases: a starting phase (0–21 days) and a finisher phase (21–35 days). Table 1 contains the ration formulation. Birds were assigned to different groups as follows: (1) negative control (uninfected, untreated), (2) positive control (infected, untreated), (3) infected + treated with *M. oleifera* leaf extract at the rate of 50 ml/l (MOLE$_{50}$), (4) Infected + treated with *M. oleifera* leaf extract at the rate of 100 ml/l (MOLE$_{100}$), (5) Infected + treated with *M. oleifera* leaf extract at the rate of 150 ml/l (MOLE$_{150}$).

### E. coli challenge method

Except negative control, all birds in each group were infected with pathogenic strain of *E. coli* (O1:157: H7). After 24 h of culturing on a nutrient broth medium, the strain was diluted in sterile water. Prior to inoculation, birds were denied water for two hours before being treated with *E. coli*-contaminated water. Birds were inoculated per os with a cell culture containing $1.0 \times 10^8$ CFU/ml.

### Performance traits

A known quantity of feed was offered twice a day. The rejected feed was collected and then weekly feed intake was calculated.
The starting body weight was recorded. Then, every week, the body weight was measured using a digital balance. Feed conversion ratio was calculated on the basis of feed consumption and weight gain. On day 35, two birds were killed for carcass characteristics such as dressing percentage/carcass yield. The skull, feet and viscera were removed from the bird. The dressing % was calculated using the dressed and living body weights.

**Blood collection and Heterophil to lymphocyte ratio**

A 3 ml blood sample was taken with EDTA from two birds/replicate at 35 days. Heterophil to lymphocyte ratio was determined under light microscope (89,404-886, VWR International, Radnor, PA) at 40× magnification.

**Total tract digestibility**

On day 35, total tract N retention was evaluated using acid-insoluble ash as a digestibility mark. The marker was added into the experimental diets on day 32 of the trial and given to the birds (five birds per replicate), separated in steel cages, until the study ended. Excreta samples were collected twice a day in plastic bags and kept at 20°C on day 28 of the experiment. The ground feed and excreta samples were sieved and kept at 20°C after passing through a 0.5 mm sieve. The total tract nitrogen retention was calculated using the Kjeldahl method and multiplied by 6.25. A bomb calorimeter was used to calculate gross energy (GE).

**Statistical analysis**

Data were analyzed under completely randomized design using statistical package SAS (1998). Tukey test was applied to separate the means. A probability value of less or equal to 0.05 was considered statistically significant.

**Results**

The impact of M. oleifera on the inhibition of E. coli is given in Table 2. The impact of M. oleifera leaf extract on inhibition against E. coli was significant (p<0.01). The inhibition of MOLE against E. coli increased gradually when the concentration of MOLE was increased from 3% to 12%. When the MOLE concentration was increased from 3% to 12%, it rose from 7.9 to 18.1 mm. When the concentration of MOLE was raised from 3% to 9%, the inhibition against E. coli was increased by 5.0 mm (7.9 vs 13.1 mm). When supplemented with 12% MOLE, the inhibition was increased even further, reaching 10.2 mm greater than on 3% MOLE (18.1 vs 7.9 mm). However, increasing the MOLE concentration beyond 12% did not result in greater inhibition, and when the MOLE concentration was raised from 12% to 15%, the inhibition was reduced by 5.2 mm (18.1 vs 12.9 mm).

**Average weight gain, feed intake and FCR from 7 to 14 days of age**

Table 3 shows that treatment with MOLE had no impact on weight increase from 7 to 14 days (p>0.05). The groups given OLE gained the same amount of weight as the NC. The treatment with MOLE had no impact on feed intake from 7 to 14 days of age (P>0.05). FCR was significantly (P<0.05) better in MOLE150 and MOLE100 compared with PC.

**Average weight gain, feed intake and FCR from 15 to 21 days of age**

The supply of MOLE was shown to be significant (P<0.05) in terms of weight growth from 15 to 21 days of age as shown in Table 3. MOLE100 had significantly higher (P<0.05) weight gain and PC had the lowest (397.10). Feed consumption was significantly (P<0.05) higher in MOLE100 and the lowest feed intake was found in MOLE150.

**Average weight gain, feed intake and FCR from 22 to 28 days of age**

Table 3 shows that weight gain was significantly (P<0.05) higher in MOLE150 while the highest (P<0.05) feed intake was found with MOLE100. The FCR was found better with MOLE100 and MOLE150 compared with PC.

**Average weight gain, feed intake and FCR from 29 to 35 days of age**

Table 3 indicates that weight gain was significantly (P<0.05) higher in NC, MOLE100 and MOLE150 compared with the PC. The feed intake means in the table showed that effect was found not significantly different during the age of the 29–35

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### Table 2. In vitro antibacterial activity ‘Zone of Inhibition’ of MOLE against E. coli using the agar well diffusion method.

| Concentration of MOLE | MOLE zone of inhibition against E. coli (mm) |
|-----------------------|------------------------------------------|
| 3%                    | 7.9a                                     |
| 6%                    | 8.1a                                     |
| 9 %                   | 13.1a                                    |
| 12%                   | 18.1a                                    |
| 15%                   | 12.9a                                    |
| Pooled SEM            | 0.12                                     |
| P-value               | <0.01                                    |

Notes: Means given in the table which having change superscripts are different from each other significantly at P=5% (p = 0.05). MOLE is the abbreviation of Moringa oleifera leaf extract, used in the water source @ of 50, 100 and 150 ml/litre, respectively. NC stands for negative control which was untreated and uninfected. PC stands for positive control which was infected with E. coli virulent strain @ 1.0 × 10⁹ CFU and untreated.
Table 3. Effect of MOLE supplementation on the average weight gain (g), feed intake (g) and FCR of broiler chicks at different intervals during the experiment.

|                | NC     | PC     | MOLE100 | MOLE150 | Pooled SEM | P-value |
|----------------|--------|--------|---------|---------|------------|---------|
| Weight gain    | 293.63a | 262.43b | 280.07ab | 284.17a | 292.23a    | 12.31   | 0.0371 |
| Feed intake    | 421.29  | 407.41  | 426.71  | 398.58  | 399.22     | 6.54    | 0.2630 |
| FCR            | 1.43b   | 1.55a   | 1.52a   | 1.40bc  | 1.37c      | 0.11    | <0.01 |
| 7–21 days Weight gain | 474.93ab | 397.10c | 436.87bc | 511.65a | 430.90bc   | 7.76    | 0.0225 |
| Feed intake    | 746.67ab| 724.33bc| 754.40b | 830.16a | 698.67b    | 5.64    | 0.0035 |
| FCR            | 1.58    | 1.83    | 1.73    | 1.63    | 1.63       | 0.11    | 0.2514 |
| 7–21 days Weight gain | 768.57ab | 659.53c | 716.93bc | 795.81a | 723.13a    | 9.12    | 0.0054 |
| Feed intake    | 1168.43b| 1131.74d | 1181.13b | 1228.71a | 1097.99c   | 11.45   | 0.0002 |
| FCR            | 1.52ab  | 1.72a   | 1.65ab  | 1.55b   | 1.52b      | 0.13    | 0.0333 |
| 22–28 days Weight gain | 535.23b | 471.33f | 501.72bc | 558.75bc | 580.20b    | 7.54    | 0.0113 |
| Feed intake    | 872.07  | 833.23  | 874.97  | 878.33  | 874.20     | 8.51    | 0.9029 |
| FCR            | 1.63ab  | 1.77a   | 1.74a   | 1.57b   | 1.51b      | 0.12    | 0.0214 |
| 29–35 days Weight gain | 818.96a | 589.26b | 644.13b | 784.32a | 786.03a    | 7.59    | 0.0002 |
| Feed intake    | 1326.94a| 1141.99c| 1227.66ab| 1263.22a| 1211.26ab  | 7.11    | 0.1499 |
| FCR            | 1.62ab  | 1.94a   | 1.90a   | 1.61b   | 1.54b      | 0.12    | 0.0002 |
| 7–35 days Weight gain | 2122.8a | 1720.1c | 1862.8b | 2138.9a | 2089.4a    | 27.54   | <0.01 |
| Feed intake    | 3366.9a | 3106.9b | 3283.7ab| 3370.3ab| 3183.3ab   | 33.45   | 0.0459 |
| FCR            | 1.59b   | 1.81a   | 1.76a   | 1.59b   | 1.5b       | 0.17    | <0.01 |

Notes: Means given in the table which having change superscripts are different from each other significantly (P < 0.05). MOLE is the abbreviation of Moringa oleifera leaf extract, used in the water source at the rate of 50, 100 and 150 ml/l, respectively. NC stands for negative control which was untreated and uninfected. PC stands for positive control which was infected with E. coli virulent strain at the rate of 1.0 × 109 CFU and untreated.

The effect of MOLE on carcass yield, heterophils to lymphocytes ratio, crude protein and gross energy is given in Table 4. Carcass yield significantly (P < 0.05) changed with MOLE100 and MOLE150 compared with PC. MOLE100 and MOLE150 compared with PC. MOLE100 and MOLE150 compared with PC. MOLE100 and MOLE150 compared with PC. MOLE100 and MOLE150 compared with PC.

Average weight gain, feed intake and FCR from 7 to 35 days of age

Table 3 shows that the weight gain is achieved with MOLE100, MOLE150 and NC. The feed intake means with MOLE100 (3370.3) was found lower (P < 0.05) in PC compared to MOLE and NC.

Carcass yield, heterophils to lymphocytes ratio, crude protein and gross energy

The effect of MOLE on carcass yield, heterophils to lymphocytes ratio, crude protein and gross energy is given in Table 4. Carcass yield significantly (P < 0.05) changed with Moringa oleifera leaf extract (MOLE). High carcass yield was obtained at the mean 100 and MOLE150 compared with NC, PC and MOLE50. Heterophils to lymphocytes ratio was significantly (P < 0.05) lower in MOLE treatment and NC. Crude protein digestibility was significantly (P < 0.05) higher in MOLE100 and MOLE150. Similarly, gross energy was also significantly (P < 0.05) higher in NC, MOLE100 and MOLE150 compared with PC.

Discussion

In the current investigation, using a MOLE concentration of 12% resulted in substantial changes in E. coli inhibition. Because of their different potential/efficacy levels, it was assumed that using different concentrations of MOLE would produce different results. However, no significant differences were discovered between the effects of MOLE concentrations of 3% and 6% and 9% and 15% on E. coli. It was suggested that the growth suppression could be caused by peptides acting directly on microorganisms, affecting cell membrane production or the creation of key enzymes (Suárez et al. 2003; Bukar et al. 2010). The increased zone of inhibition against E. coli could be due to the antibacterial action of flavonoids and alkaloids in MOLE (Bukar et al. 2010). The inclusion of phytochemicals (alkaloids, flavonoids and saponins) in an aqueous extract of Moringa oleifera leaf improved antibacterial activity against E. coli (Abalaka et al. 2012). The presence of bioactive substances such as flavonoids, phenolic acid and carotenoids in the M. oleifera plant may explain its antibacterial efficacy against both microorganisms. Our findings were supported by Siddhuraju and Becker (2003), Vaghasiya and Chanda (2007) and Peter et al. (2011), who found that using MOLE improved antibacterial activity (zone of inhibition) in vitro against E. coli. Peter et al. (2011) backed up our findings by stating that MOLE improved antibacterial action against E. coli. According to Shekhar et al. (2000), using high MOLE concentrations resulted in a decline in antibacterial effectiveness and E. coli resistance.

In the current study, the provision of MOLE100. MOLE150 increased the bird’s final body weight gain in the infected...
birds. Ambali and Furo (2012) found that supplementing with 100 ml *Moringa oleifera* leaf increased final body weight gain. There were flavonoids, alkaloids, carbohydrates, steroids, saponins, terpenes and cardiac glycosides in *Moringa oleifera* leaf extract. Jamroz et al. (2005) agreed with our findings, stating that the mechanism of action of plant extract on bird performance was unknown. The active site was present in the GIT, which was changed by gut microorganisms, increasing growth rate. Zanu et al. (2012) found a high final body weight gain with up to 10% *Moringa oleifera* leaf provision. Studies by Melesse et al. (2011) found similar results to ours, with substantial ultimate body weight gain in Senegalese chicks and RIR. Weight increase was due to GIT nutrition digestion with substantial ultimate body weight gain in Senegal chicks pounds in the MOLE would a (2010) agreed with our growth, hence a small dosage of MOLE supplementation may could have an anti-nutritive effect on chick growth, hence a small dosage of MOLE supplementation may be more useful and safe. Mbikay (2012) and Wallace et al. (2010) agreed with our findings and postulated that some compounds in the MOLE would affect the birds’ immunity, food intake, processing, and red-ox status. MOLE containing anti-oxidative substances such as tannins, polyphenols, glycosides and anthocyanin, antibacterial lipophilic components, connected to the cytoplasm membrane and excluded free radicals, anti-oxidative enzymes became active and effect oxidase, which may have increased nutrient availability to birds, according to Luqman et al. (2012).

The overall feed intake showed a significant response when supplemented with MOLE. It declined in chicks fed on the positive control, however, the feed intake restored when enriched with MOLE. There was tendency towards decrease when the level of MOLE was raised beyond 100 ml. Ogbe and Affiku (2012) indicated similar results that birds reduced feed intake from seven to eight weeks of age when fed on a high level of *M. oleifera* leaf extract. The reduced feed intake with a high level of *M. oleifera* leaf in the feed might be due to the detrimental effects of MOLE that had deteriorated the taste of the feed. Similar findings were reported by Lannao (2007) in broilers fed with *M. oleifera* aqueous leaf extract. Mbikay (2012) stated that quercetin and kaempferol (glycosides gives flavour) are abundantly present in the *M. oleifera* leaf and Moringingine (alkaloid moringingine) might have stimulated the feed intake voluntarily by controlling haemostasis of glucose. Wach et al. (2007) also supported our study and stated that quercetin glycosides having sugar group on three positions, from which glucose was the commonest, with rhamnose and galactose were found abundantly. Portugaliza and Fernandez Jr (2012) also favoured our results by providing *Moringa oleifera* leaf extract in the water and stated that feed intake lowers while providing high *Moringa oleifera* leaf extract. Ghazalah and Ali (2008) stated that high provision of *M. oleifera* improve digestion and metabolism with low feed intake. Teixeira et al. (2014) stated that quercetin, zeatin, apigenin, kaempferol (phenolic components) in *Moringa oleifera* leaf were fundamental for less GIT problems, growth of the birds and thus maximized the use of feed and lowered the feed intake for maintenance and production traits.

The supplementation of MOLE in the diets changed the FCR significantly. The provision of MOLE up to 100 ml enhanced the FCR significantly and an increase in the level of MOLE beyond 100 ml did not cause further improvement. Thus it is envisaged that *M. oleifera* leaf extract up to 100 ml could be a safe level to use as a growth promoter. Francis et al. (2002) supported our results that high quantity of saponins in Moringa had a positive impact on the growth and utilization of feed in poultry by increasing the feed digestion and absorption. Lannaon (2007), David et al. (2012), Ebenebe et al. (2012) and Safa and Tzai (2012) found enhanced FCR in *Moringa oleifera* fed groups than negative control group.

In the current study, carcass yield was significantly higher in MOLE100 and MOLE150. Many fundamental amino acids were revealed in *M. oleifera* leaf and was found to be a good provider of linoleic acid (Moyo et al. 2011). It was also reported to be a valuable source of vitamins such as E, A and C (Hekmat et al. 2015). Briones et al. (2015) examined that production performance could be enhanced by the provision of *M. oleifera* leaf in broilers diets. Literature review (Qwele et al. 2013; Saini et al. 2014) exhibited that *M. oleifera* leaf contained certain antioxidants such as tocopherol, vitamin C and ascorbic acid that were known as stress reducer, digestibility enhancer and growth promoter. Thus the increased carcass yield could be the result of this phenomenon.

A decrease in H: L was observed with the provision of MOLE100 and MOLE150. A decrease in H:L showed that the birds were healthy and were not under stress because whenever there is stress in the house the ratio of Heterophils to Lymphocytes (H: L) will be higher. Lower H:L ratio resulted in higher immune response thus it revealed about immune status of the birds (Khan et al. 2021b). A decrease in H:L could be because of naturally occurring antioxidants such as tocopherol, vitamin C and ascorbic acid in the *M. oleifera* (Qwele et al. 2013; Saini et al. 2014). Khan et al. (2021(b), Ramadan (2017) and Balami et al. (2018) found similar results who observed decreased H: L in chicks supplemented with *M. oleifera*.

In the current study, MOLE supplementation improved the CP digestibility. Nabizadeh (2012) revealed a significant effect of MOLE supplementation on the nutrient utilization especially CP digestibility in broiler chicks. Awad et al. determined a positive effect of plant extracts on the growth of mucosa, height and width of villi, depth of crpits and height of villi to depth ratio of crpits, thus concluding the improvement in the nutrient stabilization and enhancement in the CP digestion and absorption. Further in the current study, gross energy was significantly higher in MOLE100 and MOLE150. The possible reasons could be the presence of polyphenols or their metabolites in MOL that might have decreased the stress caused by the induction of *E. coli* thus resulting in enhanced AME value (Brenes et al. 2008). Some of the research report found a decrease in energy content when *M. oleifera* was supplemented in birds (Mohammed et al. 2012). The other possible differences could be the use of whole *M. oleifera* leaf in their studies while in the present study aqueous *M. oleifera* leaf extract was included. *M. oleifera* leaf contains fibre that might have decreased the energy value with the increased concentration of MOLE in the diets.

**Conclusion**

The present investigation revealed that *M. oleifera* extract at 12% had the greatest effect on *E. coli* in vitro. In the in vivo
study, MOLE\textsubscript{100} increased growth, carcass yield, H:L ratio, crude protein and energy digestibility.

**Disclosure statement**

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

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