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# Enhancement Effect of *Moringa olifera* leaves Alcoholic Extract on Broiler Chicks' Performance

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## ABSTRACT

The current study was conducted to assess the consequences of *Moringa oleifera* leaves alcoholic extract *(*MOLE*)* based on growth performance and immunological response of broiler chicks. There was a total of a hundred and Eighty-one-day-old chicks were assigned at random into three dietary groups undergoing treatment with three replicates of 20 each bird. Control group was given drinking water without additives. The second and third groups were supplemented with MOLE at doses of 125 and 250 mg/L of water, respectively. Supplementation of broiler chicks with MOLE enhanced their growth performance represented by increasing final body weight, body weight gain, phagocytic activity and phagocytic index while significantly decreased feed intake and food conversion ratio. Moreover, it increased serum levels of insulin like growth factor-1 (IGF-1), interferon-gamma (IF-γ) and Immunoglobulin G (IgG).

This study indicated that *Moringa oleifera* extract in drinking water enhanced the growth performance and immunological response of broiler chicks through increasing protein expression of IGF-1, IF-γ .

*Keywords*: *Moringa oleifera* leaves alcoholic extract, Insulin-like growth factor-1, interferon-gamma.

## INTRODUCTION

Poultry contribute significantly to human well-being by producing food and improving income and employment opportunities. For most Egyptians, poultry meat is the most important source of animal protein, accounting for about 45 percent of total protein consumption (Andeyhun, 2014). Poultry processing compares to conventional production of animal's systems in various ways, such as broilers' fast Feed consistency and growth rates (Duclos *et al.*, 2007).

Poultry production plays a vital socioeconomic role in emerging nations since chickens are a valuable and inexpensive animal protein source (Olwande *et al.*, 2010*;* Melesse *et al.*, 2013). Modern poultry production techniques aim for maximum profit at the lowest possible production cost. The price of feed accounts for 60-70 percent of the whole production cost, according to reports of Tesfaye *et al.*, 2013. In most emerging nations, poultry production sectors encounter issues such as rising feed prices, since the primary sources of protein used to manufacture rations in this sector (soybean meal, fish meal, etc.) are sometimes insufficient and expensive (Abbas, 2013; *Moreki* and Gabanakgosi, 2014).

This has resulted in the search for less expensive, locally accessible, and options that are less competitive for some chicken components for animal feed and, to a lesser extent, protein sources *(Gadzirayi et al., 2012). Moringa oleifera* leaves,

for example, are a low-cost alternative protein source that can be utilised in chicken diets in this situation (Melesse *et al.,* 2013*;* Tesfaye *et al.,* 2013)

Under the order Brassicales, under the genus Moringa (family Moringaceae), *Moringa oleifera* is a well-known cultivated plant. *Moringa oleifera* is also known as the horseradish tree or the drumstick tree, and the ben oil or benzoil tree or miracle tree (Gupta *et al.,* 2018; Kalibbala *et al.,* 2009). *Moringa oleifera* is a kind of tree in South Asian in origin, specifically Sri Lanka, India, Bangladesh, Afghanistan, Arabia, and Northeastern and Southwest Africa (Fahey, 2005; Moyo *et al.,* 2016). The tree is also known as "Never die" or "wonder tree" in Africa and has been selected Botanical of the Year 2007 by the National Institute of Health (USA) (Gupta *et al.,* 2018).

*Moringa* seeds and leaves are commonly used in the food industry as well as in treatment of some diseases (Fahey, 2005). It is commonly used in human diet and as herbal medicine for its seeds, flowers, and leaves (Oyeyinka and Oyeyinka, 2018). Various parts of *Moringa oleifera* trees are utilized in traditional diets and as an excellent source of human nutrition in various areas across the world (Olugbemi *et al*., 2010*;* Onunkwo and George, 2015).

Leaves of *Moringa* are a feedstock for both mammals and birds due to their high level of protein, rich profile of minerals, and availability of vitamins (particularly A, B, and C). It contains between 30 and 40% edible oil (ben oil) (Pandey, 2012). Ben oil is high in oleic acid, sterols, and tocopherols, all of which help to avoid rancidity (FAO, 2018). It contains antiviral, antioxidative, and anti-inflammatory properties, cardioprotective, anticancer, and anti-asthmatic anticancer effects. Antibiotic and antifungal properties of *pterygospermin*, a component found in *Moringa* seeds, against *Pseudomonas aeruginosa, Fusarium solani, Bacillus subtilis, Staphylococcus aureus, and Fusarium solani* (Sultana *et al*., 2014*;* Jabeen *et al.,* 2008)

Because *Moringa oleifera* leaves are said to be free of heavy metals like arsenic, cadmium, and mercury and high in vitamins (B, A, and C), incorporating them into poultry feeds is nutritious and may improve poultry production efficiency (Donkor *et al.,* 2013). Furthermore, the nutrient content that is required for life of *Moringa* leaves/twigs, such as vitamins B and A, iron, calcium, copper, sulfur, and protein, as well as Its capacity to absorb and eliminate toxins in food might be beneficial to make the plant important as a major local feed stuff (Lanaon 2007). Adequate dietary *Moringa* leaf intake could have a significant impact on avian development, production performance, and carcass characteristics. Higher digestibility and antibacterial capabilities against gut infections have been linked to improved feed quality (Ayssiwede *et al.,* 2011; *Olugbemi et al.,* 2010) reported that *Moringa oleifera* has a hypocholesterolemia It has an effect, and it can be used in chicken feed to lower cholesterol levels in eggs. Abou-Elezz *et al. (2011)* confirmed that Moringa leaf meal as a dietary supplement (up to 10 percent) may improve the color of the yolk while having no significant negative effects on the rate of egg laying. As a result, a sustainable feed substitution of 10% *Moringa* leaf meal in laying hen diets has been advocated. Five percent level of *Moringa* leaf meal was found to be beneficial on birds, however the 15 percent and 20 percent levels exhibited negative effects. *(*Abou-Elezz *et al.,* 2011*;* Kakengi *et al.,* 2007). Safa and Tazi (2014) found that, broilers fed a diet containing 5% *Moringa* leaf meal for 7 weeks gained weight, had greater overall feeding intake, and had a superior feed conversion ratio in comparison to the control group. *Moringa oleifera* extracts have been found to contain a variety of biochemical compounds, including quercetin, various glycosides, various isothiocyanates, and kaempferol glucosides, all of which have anti-inflammatory activities (Maheshwari *et al.,* 2014; Stohs and Hartman, 2015). The presence of numerous proteins and peptides (isothiocyanates, glycoside cyanides, etc.) in *Moringa oleifera* leaf extracts was able to positively affect the immunological response (Gupta *et al., 2010;* Rachmawati and Raifi, 2014). The immune system functions of *Moringa oleifera* are also established by several *in vitro* studies (Gupta *et al.,* 2018). It also contains high content of phytonutrients like antioxidants such as carotenoids, tocopherols, and ascorbic acid, which are abundant in the diet (Qwele *et al*., 2015*;* Saini *et al.,* 2014).

Therefore, the present research was conducted to evaluate the effect of *Moringa oleifera* leaves ethanolic extract (MOLE) on basis of growth

performance and immunological response in broilers chicks.

## MATERIALS AND METHODS

### Chemicals and reagent:

Assaying kits for measuring serum levels of cholesterol (CAT. NO.230004), triacylglycerols (TAG) (CAT. NO.314004) were obtained from Spectrum Company. (Cairo, Egypt). Malondialdehyde (MDA) detection kits (CAT. NO. MD 2529) were obtained from Bio Diagnostics Ltd. (Giza, Egypt). Kits for assaying serum levels of immunoglobulins G (CAT. NO. MBS260043) & immunoglobulins M (CAT. NO. MBS706158) from my BioSource Company. (Cairo, Egypt). Assay kits for serum levels of IGF-1 (CAT. NO. In-Ch0104), IF- γ (CAT. NO. In-Ch0031) by ELISA were obtained from Bio nova Co., Ltd (Beijing, China). The other compounds employed in this experiment were analytic grade.

### preparation of *Moringa oleifera* leaves alcoholic extract (MOLE):

A total of 2 kilograms of Moringa oleifera fresh green leaves were obtained from a local source of *Moringa oleifera* farm located in Sadat City, Menoufia, Egypt. Ethanolic extract of *Moringa oleifera* leaves (MOLE) was prepared according to Sinha *et al*. (2012); Mousa *et al.,* (2019). Fresh *Moringa oleifera* leaves were carefully cleaned with distilled water before being shade room temperature drying. Then, by using a simple hammer mill, it was ground into fine particles. The powder was steeped in a 70% ethanol solution and gently shaken for 48 hours at room temperature (22 °C). Filter paper was used to filter the contents (Whatman size No. 1), which was dried in a hot air oven at 50 °C and stored in an airtight container at 4 °C until usage. *Moringa oleifera* leaves alcoholic extract MOLE diluted using distilled water to form (volume/volume) 125 and 250 mL /L water for Treatments of groups 2 to 3, respectively.

### Experimental chicks and housing:

This experiment was carried out in conformity with the ethical norms of the university of al Sadat's animal care and use committee.

A total of 180 unsexed avian broiler chicks were allocated into three groups of 60 chicks each at random; the first group is control, which received plain water; second, which received MOLE at dosages of 125 mg/L of drinking water; and third, which received MOLE at doses of 250 mg/L of drinking water.

Each group was separated into three sub-groups and kept in three separate cages, each with 20 chicks.

### Ration and additives:

Broilers were fed broiler starter for 12 days, broiler grower from 12 to 22 days, and broiler finisher from 22 to the end of the trial (37 days). Feed and water were freely available (Table1).

The experiment was carried out at room temperature with a 14-hour light/ten-hour dark cycle. Throughout the experiment, the room temperature was kept at 23-25°C with a relative humidity of 50:70%. The birds were kept under consistent climatic and nutritional settings throughout the duration of the experiment.

| Ingredient and composition % | Starter | Grower | Finisher |
|---|---|---|---|
| Corn | 57.89 | 61.26 | 62.4 |
| Soybean meal 48% | 35.00 | 31.00 | 29.00 |
| Soybean oil | 2.40 | 3.20 | 4.50 |
| Monocalcium phosphate | 1.30 | 1.15 | 1.00 |
| Limestone | 1.50 | 1.50 | 1.45 |
| DL-Methionine | 0.35 | 0.33 | 0.30 |
| L-Lysine (%) | 0.44 | 0.37 | 0.28 |
| Threonine | 0.18 | 0.23 | 0.23 |
| Tryptophan | 0.00 | 0.01 | 0.17 |
| L-Valine | 0.10 | 0.11 | 0.08 |
| NaCl | 0.23 | 0.24 | 0.23 |
| Choline Chloride (%) | 0.10 | 0.10 | 0.10 |
| Trace mineral permix[1] | 0.10 | 0.10 | 0.10 |
| Vitamin permix[2] | 0.10 | 0.10 | 0.10 |

| | | | |
|---|---|---|---|
| Sodium sulphate | 0.26 | 0.25 | 0.23 |
| Ronozyme NP[3] | 0.02 | 0.02 | 0.02 |
| Rovabio Excel AP[4] | 0.01 | 0.01 | 0.01 |
| Ronozyme ProAct[5] | 0.02 | 0.02 | 0.02 |
| Antioxidant | 0.01 | 0.01 | 0.01 |

[1]Trace mineral premix per kilogram of diet: Iron carbonate 50 mg, Manganese oxide 100 mg, Copper sulphate 12 mg, Zinc 100 mg, Calcium iodide 1.6 mg, Sodium selenite 3 mg, Cobalt sulphate 0.4 mg; Calcium iodide 1.6 mg; Sodium selenite 3 mg; Cobalt sulphate 0.4 mg Vitamin A, 13000 IU; Vitamin D3, 4000 IU; Vitamin E, 100 mg; Vitamin B1, 3 mg; Vitamin B2, 9 mg; B6, 6 mg; B12, 0.4 mg; Folic acid, 2 mg; Biotin, 0.25 mg. [2] Vitamin premix per kilogram of diet: Vitamin A, 13000 IU; Vitamin D3, 4000 IU; Vitamin E, 100 mg; Vitamin B1, 3 mg; Vitamin B2, 9 mg; B6, 6 mg; B12, , 0.4 mg; Folic acid, 2 mg; Biotin, 0.25 mg.[3] Ronozyme NP contains a minimum of 10000 units of Phytase per gramme. [4] Rovabio Excel AP contains at least 22000 units of B-Xylanase and 2000 units of B-gluconase per gramme. [5] Ronozyme ProAct has a minimum of 75000 units of protease per gramme. commercial mixture (Lumance™, Innovad NV, Belgium) **(Taha *et al.*, 2014).**

Chicks were vaccinated by IBD and ND vaccines during its suitable schedule.

## Methods:

### Performance parameter:

### Feed intake (g):

The difference between the left over and the initial quantity of feed delivered was used to compute the daily feeds eaten. The feed given to the birds in each treatment was weighed on a regular basis (weekly).

### Body weight gain (g):

By subtracting the starting body weight at the start of the experiment from the final body weight on day 37, The weight of individual birds in each replicate was added together and then divided by the number of birds in each replicate to get the average body weight per replicate.

### Feed conversion ratio:

The average weight gained, and average feed consumed by the birds in each treatment were used to calculate the feed conversion ratios (FCR).

$$\text{Feed conversion ratio} = \frac{\text{Average feed intake (g)}}{\text{Average body weight gain (g)}}$$

### Sampling:

At the end of the experimental period, blood samples were taken from all groups. Blood samples, they were incubated for 1 hour at room temperature for coagulation. Blood samples were centrifugated for 15 minutes at 3500 rpm then clear sera were separated for analysis.

### Biochemical analysis:

Serum total cholesterol and triacylglycerol levels were assayed according to Ellefson and Caraway (1976); Bucolo and David (1973) respectively. Malonaldehyde (MDA) total antioxidant capacity were determined in serum according to Ohkawa *et al.,* (1979) and Koracevic *et al*., (2001) respectively. Serum IgG & IgM concentration using ELISA was determined according to Mallery et al., (2010).

### ELISA assay for determination of serum insulin like growth factor 1 (IGF-1) and Interferon γ (IFN-γ):

Serum levels of IGF-1 and IFN-γ were measured using commercially available ELISA kits, following instruction protocol of the manufacturer (Bio nova Co., Ltd, Beijing, China). Both kits based on Sandwich-ELISA technique. Briefly, 50 µl of each standard and/or sample diluted with dilution buffer were added to the appropriate well of the microtiter plate precoated with the corresponding specific antibody (against IGF-1 or IFN-γ respectively). After 30 min incubation at 37℃, the solution in the wells was aspirated and discarded and wells were washed 5 times with wash buffer. Then, 50 µl of a Horseradish Peroxidase (HRP)-conjugated antibody specific for IGF-1 or IFN-γ respectively was added and incubated for 30 min at 37°C. Then, the plate was washed 5 times in wash buffer, the color was developed by adding 100 µl substrate reagent and incubation for 15 min at 37°C. Finally, reaction was terminated by adding 50 µl of stop solution which turns the color from blue to yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of IGF-1/or IFN-γ. You can calculate the concentration of IGF-1/or IFN-γ was calculated in the samples by comparing the OD of the samples to the standard curve (Jansen *et al.,* 1983; Digby and Lowenthal, 1995).

### Statistical analysis:

Statistical Analysis System was used to evaluate the data acquired using one-way analysis of variance (ANOVA) based on the Completely Randomized Design model (SAS, 2012). Duncan's Multiple Range Test (SAS, 2012) was

used to establish the significance of the differences between groups, with differences of 5% (p0.05) being significant.

## RESULTS

**Effect of MOLE on growth performance parameters**:

To look at the impact of MOLE on growth performance parameters, the changes in body weight gain, feed intake and food conversion ratio in response to MOLE treatment at a dose of 125 mg/L (M1) and 250 mg/L (M2) from day 0 till the end of experiment were estimated. The results showed that, supplementation of broiler chicks with MOLE increased body weight gain and decreased both feed intake and food conversion ratio compared to those of control group ($p \leq 0.01$). On the other hand, there was no significant differences concerning the above-mentioned parameters between the second and third groups (Table 2).

**Table 2: The effect of MOLE on body weight gain, feed intake and food conversion ratio of broiler chicks.**

| Parameters | Control group | MOLE (125 mg./L) | MOLE (250 mg./L) |
|---|---|---|---|
| Initial weight (g) | 39.3±.2 | 40.8±.3 | 40.5±.4 |
| Final body weight (g) | 1993.3±58.2[b] | 2325±36.3[a] | 2448±48.2[a] |
| Body weight gain (g) | 1952.5± 62.83[b] | 2284.167±40.01[a] | 2407.5±53.02[a] |
| Daily feed intake (g) | 4316 ± 60.75[a] | 4124± 55.41[b] | 4074 ±72.07[b] |
| Food conversion ratio | 2.219± 0.08[a] | 1.801667± 0.03[b] | 1.69± 0.04[b] |

- Data are presented as means ± SE (standard errors of means).
- The mean difference is significant at $p$ <0.05. The values carrying different letters in the same ROW were statistically different.
- MOLE, *Moringa oleifera* leaves alcoholic extract

**serum lipid profile and total antioxidant capacity**

As shown in Table 3, supplementation of broiler chicks with MOLE at either used doses (M1 or M2) from day zero till the end of experiment on caused no significant changes in serum level of total cholesterol, triacylglycerols, total antioxidant capacity or malonaldehyde among different groups.

**Table 3: The effect of *MOLE* on Total cholesterol, triacylglycerols and antioxidant capacity of broiler chicks.**

| Parameters | Control group | MOLE (125 mg./L) | MOLE (250 mg./L) |
|---|---|---|---|
| Total cholesterol (mg/dl) | 75.44 ± 2.12 | 78.9 ± 2.23 | 77.28 ± 1.84 |
| Triacylglycerols (mg./dl) | 50 ± 16.95 | 59.25 ± 10.61 | 66 ± 10.68 |
| Total antioxidant capacity (mM/L) | 0.94 ±0.03 | 0.97 ± 0.01 | 0.93 ±0.02 |
| Malonaldehyde (nmol/ml) | 10.04 ±0.1 | 10.346 ± 0.08 | 9.97 ± 0.37 |

- Data are presented as means ± SE (standard errors of means).
- The mean difference is significant at $p$ <0.05. The values carrying different letters in the same ROW were statistically different.
- MOLE, *Moringa oleifera* leaves alcoholic extract

**Effect of MOLE on Phagocytic activity % and Phagocytic index:**

Effect of administrating the broiler chicks with *MOLE* at a dose of 125 mg/L (M1) and 250 mg/L (M2) from day zero till the end of experiment on phagocytic activity % and phagocytic index was tested. As presented in Table 4, both used doses of MOLE increased phagocytic activity % and phagocytic index as compared to that of control group ($p \leq 0.01$). Moreover, this stimulatory effect of MOLE on either phagocytic activity % or phagocytic index was significantly higher in second group treated with lower dose (M1) than in third group treated with higher dose (M2) ($p \leq 0.01$).

**Table 4: Effect of MOLE on Phagocytic activity % and phagocytic index of broiler chicks**.

| Parameters | Control group | MOLE (125 mg./L) | MOLE (250 mg./L) |
|---|---|---|---|
| Phagocytic activity % | 55.69 ± 1.50[c] | 67.51 ±0.5[a] | 65.47 ± 0.65[b] |
| Phagocytic index | 2.003 ± 0.076274[c] | 2.75±0.05[a] | 2.38 ± 0.06[b] |

- Data are presented as means ± SE (standard errors of means).
- The mean difference is significant at $p$ <0.05. The values carrying different letters in the same ROW were statistically different.
- MOLE, *Moringa oleifera* leaves alcoholic extract.

**Effect of MOLE on immunoglobulin IgG and Immunoglobulin IgM:**

Treatment of broiler chicks with MOLE at a dose of 125 mg/L (M1) and 250 mg/L (M2) resulted in different changes in serum level of IgG and IgM. There was a significant increase in IgG in M2-treated group (third group) compared to either control group or M1-treated group (second group) ($p \leq 0.01$). However, there was no significant differences between second group and control group. while Ig-M showed that there was no significant change in M2 and M1 as compared to that of control group ($p \leq 0.01$). On the other hand, there was no significant differences between second and third groups (Table 5).

**Table (5): The effect of Moringa oleifera leaves alcoholic extract on Ig-G and Ig-M of broiler chicks.**

| Parameters | Control group | MOLE (125 mg./L) | MOLE (250 mg./L) |
|---|---|---|---|
| IgG (mg/dl) | $114.33 \pm 0.33^{b}$ | $116 \pm 2.17^{b}$ | $133 \pm 4.15^{a}$ |
| IgM (mg/dl) | $7 \pm 0.91$ | $8 \pm 0.58$ | $8.75 \pm 0.48$ |

- Data are presented as means ± SE (standard errors of means).
- The mean difference is significant at $p$ <0.05. The values carrying different letters in the same ROW were statistically different.
- MOLE, *Moringa oleifera* leaves alcoholic extract.

**Effect of MOLE on IFN-γ and IGF-1:**

Serum level of IFN-γ and IGF-1 in response to supplementation of broiler chicks with MOLE at a dose of 125 mg/L (M1) and 250 mg/L (M2) was investigated. As shown in Table 6, there was significant increase in in serum IF-γ level in M2-treated group (third group) as compared to of control group and M1-treated group (second group) ($p \leq 0.05$). However, there was no significant change between second group and control group. On other hand, IGF-1 serum level showed that, there was significant increase in M1-treated group (second group) compared to that of control group and M2-treated group (third group) ($p \leq 0.05$). On the other hand, there was no significant change in serum IGF level between third group and control group.

- **Table 6: The effect of MOLE on serum levels of INF- γ and IGF-1 in broiler chicks.**

| Parameters | Control group | MOLE (125 mg./L) | MOLE (250 mg./L) |
|---|---|---|---|
| INF- γ (ng/ml) | $27.40 \pm 2.88^{b}$ | $31.47 \pm 2.59^{b}$ | $50.67 \pm 10.06^{a}$ |
| IGF-1 (ng/ml) | $60.15 \pm 6.99^{b}$ | $191.36 \pm 33.97^{a}$ | $131.36 \pm 39.10^{ab}$ |

- Data are presented as means ± SE (standard errors of means).
- The mean difference is significant at $p$ <0.05. The values carrying different letters in the same ROW were statistically different.
- MOLE, *Moringa oleifera* leaves alcoholic extract

## DISCUSSION

The ability of the broiler chicken business in emerging nations to compete on a global scale, which is strongly based on the efficacy of its manufacturing method, is critical to its survival and expansion. In poultry management, Antibiotics in the water supply are routinely utilized as growth promoters and as a means of preventing or controlling harmful bacterial infections. The advantage of such an approach is that it promotes excellent health, minimizes avian mortality, and is environmentally friendly encourages maximum growth through increased nutrient use, and, as a result, enhances profit (Zeweil *et al*., 2006). However, it was shortly realized that the usage of synthetically manufactured chemicals, particularly antibiotics as growth promoters, had adverse side effects (Makanjuola, 2014*)*. Antibiotic growth promoters are no longer allowed because of not just cross-resistance but also multiple resistances. Researchers changed the usage by concentrating on organic or natural ingredients for feed and water additions rather than synthetic pharmaceuticals to improve the economic condition of chicken production (Zeweil *et al*., 2006). Medicinal plants used as prospective

therapeutic treatments, whether alone or in combination, has become a hot topic in science (Oyewole, 2012). Because of the inclusion of bioactive phytochemicals or phytonutrients, there are a few therapeutic plant items that are known to improve the host's natural resistance to infection (Soetan and Oyewole, 2009).

Carotene, protein, vitamin C, calcium, and potassium, as well as natural antioxidant components such flavonoids, phenolics, and carotenoids, have been detected in *Moringa oleifera* leaves (Anwar and Bhanger, 2003; Mousa et al., 2019). Because of its key therapeutic features such as antibacterial and antifungal activity, it is one of the herbs having bioceutical compounds that might be used to substitute some medications as synthetic growth enhancers and supplements in broiler and other animal production (Nickon et al., 2008).

In this research, we investigated inclusion of *MOLE* in broiler chicks' water enhanced the features of growth performance through increasing the final body weight and body weight gain and increasing serum levels of insulin-like growth factor-1 (IGF-1) and resulted in decreasing food conversion ratio and feed intake. Our results were parallel with that of Sultana *et al.,* (2014) who reported that supplementing soybean meal (SBM) with *Moringa* leaves had a significant impact on poultry growth performance (body weight and body weight gain). In addition, the birds were healthier and had a greater feed conversion ratio (FCR). Furthermore, nutritional supplementation with *Moringa oleifera* leaves at concentrations ranging from 5% to 20% of feed resulted in better growth performance in broilers (Moreki and Gabanakgosi, 2014). Moreover, Abdulsalam *et al.* (2015) mentioned that *Moringa oleifera* leaves supplemented diets have been shown to boost growth performance during the finisher stage. In addition, Alabi *et al.* (2017) showed that the extract-supplemented groups, average daily body weight growth and ultimate body weight were higher than in the control groups. Similarly, Khan *et al.* (2017) found that using *Moringa* leaf powder as a dietary supplement at a dosage of 1.2 percent of feed resulted in increased body weight in broilers. Commonly, body weight is used to keep track of the animals' dietary needs and growth (Ndlovu *et al.,* 2009). It could also be linked to the accessible certain growth-stimulating chemicals and dietary components in *Moringa oleifera,* which likely led in an increase in the chickens' live body weight (Kakengi *et al.*, 2007). In addition, it's also been suggested that *Moringa oleifera's* crude extract, like other herbal medicines, may have digestion-enhancing characteristics that encourage the growth of helpful bacteria while suppressing the growth of dangerous microbes (Hernandez *et al.,* 2004). However, Anti-nutritional variables that impact feed palatability were shown to be insignificant in compromising nutrients and growth-stimulating chemicals bioavailability found in *Moringa oleifera* leaves (Foidl *et al.,* 2001). In addition, Ambali and Furo (2012) reported that the presence of pharmacological chemical substances (carbohydrates, saponins, cardiac glycosides, terpenes, steroids, flavonoids, and alkaloids) in the *Moringa oleifera* extract could explain the rise in final body weight and body weight gain.

The decrease in feed intake in broilers supplemented by MOLE in the current research is consistent according to the findings of Portugaliza and Fernandez (2012), who reported that broiler feed intake was dramatically lowered as the concentration of Moringa oleifera aqueous leaf extracts in drinking water rose. This could be related to the increased functions of digestion and metabolism of Moringa oleifera (Ghazalah and Ali, 2008), which meet nutritional needs while feeding at a reduced rate. *Moringa oleifera* leaves are also high in carotenoids, vitamins, minerals, amino acids, flavonoids, and alkaloids flavonoids (Siddhuraju and Becker, 2003). In addition, it contains a unique blend of phenolic chemicals (zeatin, quercetin, kaempferol, and apigenin) that promotes growth and reduces the spread of illness in the gastrointestinal tract (Teixeira et al., 2014) As a result, food use improved, and less feed was required to meet the maintenance and production needs of the birds.

Insulin-like growth factor 1 (IGF-1) is a polypeptide hormone of 70 amino acids generated primarily by the liver (which accounts for about 75% of circulating IGF-1) because the liver's endocrine system is stimulated by GH and insulin. IGF-1, on the other hand, stimulates somatostatin production in the pituitary, which acts as an inhibitory feedback signal on GH release in the hypothalamus (Ohlsson *et al.*, 2009). The increasing of serum insulin-like

growth factor-1 (IGF-1) level in our study is considered a good marker on enhancement growth performance parameters and is in accordance with those of Guobin *et al.,* (2011), who reported that in mammals and chickens, IGFs were major positive body and muscle growth modulators. Abdel Reheem and Hassan, (2021) mentioned that in beef cattle, IGF-1 and GH have been used as indications of possibility for growth, changes in average daily growth (ADG)and body composition. The considerable increase in IGF-1 in calves given 15 percent Moringa leaf meal in diet was discovered, along with a strong link between high levels of nutrition, a high average daily increase, and body weight with plasma IGF-1 concentration in growing steers are all factors to be consider (Torrentera *et al.,* 2009). Growth hormones and IGF-1 are required to support normal growth (Scanes, 2009).

In our study, addition of *MOLE* in water caused no significant changes in serum lipid profile and total antioxidant capacity. moreover, according to *Olugbemi et al. (2010)*, *Moringa oleifera* has a hypocholesterolemic effect and can be used in chicken feed to lower cholesterol levels in eggs. *Abou-Elezz et al. (2011)* reported that the use of dietary Moringa leaf meal (up to 10%) can increase yolk colour while having no effect on egg laying rate. Lipid peroxidation is commonly employed as a measure of harm caused by reactive oxygen species (ROS) *(Kuun and Borchert, 2002),* and Melondialdehyde (MDA) levels in blood and tissues are routinely employed as a lipid peroxidation indicator *(Sehirliet al., 2008; Yousef et al., 2009).*So, according to, *Ojo and Adetoyi (2017)* and *El-Kholy et al. (2018b)* mentioned that *Moringa oleifera* leaves treatment increased total anti-oxidant capacity (TAC) while decreasing lipid peroxidation in growing rabbits. The presence of flavonoids and polyphenols in Moringa oleifera leaves, which might lower oxidative stress, may explain these findings. *(El-Kholy et al., 2018b).* Furthermore, Caffeic and chlorogenic acids are antioxidant phytochemicals found in *Moringa oleifera* leaves *(Siddhuraju and Becker, 2003),* which increase enzymatic antioxidants *(Oseni and Idowu, 2014).* Moringa oleifera possesses antioxidant activity due to its phytochemical composition, which might affect the stability, palatability, processing qualities, and shelf life of chicken products *(Abbas et al., 2012; Jung et al., 2010).* The most important antioxidants in Moringa are flavonoids, particularly flavanols *(Pandey al., 2012).* They had higher antioxidant activity than vitamin C and could be used to extend the shelf life of poultry products. In addition, moringa had a higher reducing power and fewer free radicals remained when compared to these vegetables. Moringa leaves also contain important flavonoids such as kaempferol and quercetin, which have higher antioxidant activity than ascorbic acid (*Al-Asmari et al., 2015; Anwar et al., 2003).* These antioxidants protect animals against degenerative illnesses and infections, which might be linked to the direct trapping of free radicals to prevent DNA damage caused by excessive oxidation *(Sreelatha et al., 2009).*

In our research, addition of *MOLE* in water also resulted in an improvement of immunity indices in broilers such as phagocytic activity, phagocytic index, IgM, IgG and IFN-γ. This finding agrees with the findings of Madubuike and Ekenyen (2006); Olugbemi *et al.* (2010); Oyewo *et al.* (2012) who found that supplementation of *Moringa oleifera* aqueous extract improves the health of broiler chicks through boosting immunological response. According to Oyewo *et al*., (2012), the immune-boosting properties of *Moringa oleifera* may be attributable to phytochemical elements found in aqueous preparations of the plant, such as alkaloids and saponins. Mineral content, such as selenium, manganese, iron, zinc, and magnesium, has also been connected to the immunomodulatory impact of aqueous leaf extracts (Prasad, 2000; Ravaglia *et al.* 2000; Madubuike and Ekenyen, 2006; Oyewo *et al.*, 2012). As well as *Moringa* is rich in ginseng; ginseng saponins which has been shown to have anti-inflammatory, antioxidant, anti-apoptotic, and immune-stimulant effects, leading to the hypothesis that *Moringa* and its extracts could help with immunomodulation (Rausch et al., 2006) and could explain immune-boosting effect of MOLE in the current result. Furthermore, Anti-inflammatory biochemical components such as quercetin, different isothiocyanates, different glycosides, and kaempferol glucosides have been found in *Moringa oleifera* extracts from diverse regions (Azra *et al.*, 2012; Rehman *et al.*, 2014). The presence of different proteins and peptides (isothiocyanates, glycoside

cyanides, etc.) in *Moringa oleifera* leaves extracts was able to positively alter immunological response (*Rady et al., 2013; Salem, 2016).* Furthermore, different *Moringa oleifera* doses resulted in a considerable rise in white blood cell counts and immunoglobulin levels (Adedapo *et al.,* 2005). Because of their critical roles in immune function, serum immunoglobulin and complement components are commonly utilized to assess the immunological state of hens. *Moringa oleifera* leaf extracts have been discovered to have immunosuppressive as well as immunostimulatory properties (Rachmawati *et al.,* 2014). Leaves have an immunomodulatory effect, decreasing the immunosuppression caused by cyclophosphamide and enhancing both cellular and humoral immunity. (Gupta *et al.,* 2010), which is attributed to the presence of compounds like glycoside cyanides and isothiocyanates (Sudha *et al.,* 2010*).* Furthermore, Yang *et al.,* (2020) reported during the starter period (0–4 wk.), ducks were fed 2% *Moringa oleifera* stem (MOS) diet and 4% *Moringa oleifera* stem (MOS) diet had higher total protein and albumin, as well as slightly higher IgM, IgG, and IgA values. *Moringa oleifera* aqueous leaf extract increased lymphocyte counts and total white blood cell in experimental broiler chickens. This emphasizes its immunomodulatory ability, as white blood cells are involved in fighting infection and clearing away injured or dead cells and tissues in the body (Oyewo *et al.,* 2012). In the same context, the current study's improved phagocytic activity could be attributed to the contents of MOLM derived from minerals and vitamins, which play important roles in the secretion of various cytokines required for phagocytic activities (Fakurazi *et al.,* 2008).

In this regard, in the present study, the increased in serum levels of INF- γ was considered good evidence on immunomodulation effect of MOLE. INF- γ is created by lymphocytes after they have been activated by antigens or mitogens (Halminen *et al.,* 2001). The major Macrophage Activating Cytokine (MAC) that activates macrophages and stimulates phagocytic activity is interferon-gamma (IFN- γ). (Sulistiani and Hesti, 2015). Increased hydrolytic enzymes in the cytoplasm accompany macrophage activation. Flavonoids (components of MOLE) have a mode of action that stimulates IFN production by activating NK cells (Vongsak *et al*., 2013). IFN- plays a critical function in detecting and eliminating infections. Because it is the primary effector of cell mediated immunity, IFN- may coordinate a range of antimicrobial effects. Antiviral reactions are induced by the production of reactive oxygen species and reactive nitrogen intermediates, which can enhance antigen presentation via antigen presenting cells by increasing antigen recognition via cognate T-cell contact, increasing the Antiviral reactions are induced by the production of reactive oxygen species and reactive nitrogen intermediates, which can enhance antigen presentation via antigen presenting cells (Abbas *et al.,* 2014*).* Our findings are in line with Khan *et al.,* (2012) who reported that the ability of natural feed additives to promote phagocytosis of prospective microphages, generation of interleukins, IFN- γ, and the immunomodulatory effects of tumor necrosis factor, macrophage secretory metabolism, antigen-presenting cells, and antioxidant capacities are all related. Furthermore, our findings go in agreement with those of Kim *et al*., (2010) and Lillehoj *et al*., (2011*)* who noticed that in comparison to chickens fed a regular diet, supplementing one-day-old chickens' diets with medicinal plants resulted in higher rates of IFN- γ, encoding gene transcripts.

## CONCLUSION

Administration of MOLE in drinking water of broiler chicks for 35 days improved broiler performance by improving BW, BWG, serum levels of IGF-1 and decreasing FCR in addition increasing immunological response through increasing serum levels of IgM, IgG, IF- γ, phagocytic activity and phagocytic index. Therefore, MOLE could be a promising growth promotor and immunostimulant candidate in broiler chicken.

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