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Influence of dietary *Moringa oleifera* on broilers performance, intestinal microbial population and humoral immune competence

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ABSTRACT: This study was designed to evaluate the effect of using different levels of phytobiotic containing *Moringa oleifera* (*M. oleifera*) leaf powder (MOLP) on broiler chickens body growth performance parameters, intestinal microbial population, and the humoral immune response. Day-old Hubbard broiler chicks (*n* = 200) were randomly allocated into 4 treatment diet. Basal diet was supplied with treatments T0, T1, T2 and T3 representing (0, 1%, 5% and 7.5% MOLP); respectively. Chickens were kept under observation for 5 weeks. Body performance parameters, total viable bacterial and coliform counts and humoral immune response to Newcastle disease (ND) virus vaccine were detected using the haemagglutination inhibition (HI) test. The results revealed significant ($P < 0.05$) improvement in performance parameters in groups supplemented with dietary MOLP. However, the best significant ($P < 0.05$) performance was observed in the group fed 1% MOLP. There was no significant ($P < 0.05$) difference among treatments considering the total viable intestinal bacterial and coliform counts. Significant ($P < 0.05$) increase in the means of HI in dietary MOLP supplemented groups was observed, where the highest means were seen in 1% MOLP treated birds. In conclusion, dietary supplementation with MOLP could improve the performance parameters and the immune response while reducing the total viable intestinal bacterial and coliform counts of broiler chickens. The study recommended using of dietary level of 1% MOLP to improve performance, intestinal health, and immune competence.

Keywords: *M. oleifera*, chickens, growth, bacterial count, HI

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INTRODUCTION

Antibiotic growth promoters have been widely used for the prevention of poultry infection and improvement of meat and egg production. Using antibiotics as growth promoters have been banned in several countries due to the development of bacterial drug resistance, presence of drug tissue residues and destruction of normal gut microflora (Hong et al., 2012; Kostadinović et al., 2019). Nowadays, supplementation of alternatives phytogenic feed additives in poultry ration is commonly anticipated as growth promoters to enhance the performance of broiler chickens (Hashemipour et al., 2016; Popović et al., 2018).

Leaf powder of *M. oleifera* is considered as chep source of poultry ration protein (Tesfaye et al., 2013), in addition to vitamins, acids, minerals, and various phenolics compounds (Moyo et al., 2012). Dietary supplementation of poultry with *M. oleifera* leaf powder (MOLP) improved the body performance traits (Okafor et al., 2014; Mousa et al., 2017), as well as enhanced both the intestinal health (Gomashe et al., 2014) and the humoral immune response (Eze et al., 2013 and 2014).

Therefore, this work was planned to detect the influence of using different levels of MOLP on body growth performance parameters, intestinal microbial population and humoral immune response of broiler chickens.

MATERIALS AND METHODS

Experimental chickens and design

Day-old Hubbard broiler chicks were obtained from a commercial hatchery. Birds were fed on starter, grower and finisher diets at ages 2, 2-4 and 4-5 weeks, respectively. No antibiotics or coccidiostats was added to the ration. The ration was formulated to meet the nutrient requirements of broiler chicks according to NRC (1994) which were formulated from the local feed ingredients commonly used for poultry feed in Egypt. The main ingredients composition of diets is represented in Table 1. Feed and water were given *ad libitum*. Vaccination was done against Newcastle disease (ND) and infectious bronchitis viruses using living Hitchner HB1 and H120 strains, respectively at 5 days old, against avian influenza (AI) virus using inactivated H5N1 strain at 7 days old, against Gumboro disease virus using intermediate strain at 12 days old and against ND virus using La Sota strain at 19 days old. Vaccines were administrated using eye drop method except AI vaccine using a subcutaneous inoculation method. A total of two hundred, one day old mixed broiler chicks were randomly allocated into 4 equal groups of 50 chicks. Each group was further subdivided into 2 replicates with 25 chicks per each. The chicks of each replicate were housed in thoroughly cleaned and disinfected deep litter houses. Basal diet was supplied with different levels of MOLP. Treatments were T0, T1, T2, and T3 representing 0, 1%, 5% and 7.5% MOLP, respectively they were fed for 5 weeks of experimental study. The study was done in accordance with the National regulations on animal welfare and Institutional Animal Ethical Committee recommendations and approval.

M. oleifera plant

The leaf of *M. oleifera* was obtained from the Agricultural Research Center, Giza, Egypt. They are dried and ground as a powder. The powder of MOLP was subjected to proximate analysis according to the Association of Official Analytical Chemists (AOAC, 2000) methods. The composition of plant leaves extract was the followings; 26.2% crude protein, 18.0% crude fiber, 3.0% crude fat, 6.0% ash and 10.0% moisture content. It was added to the ration in different concentrations (0, 1%, 5% and 7.5%).

Studied parameters

Performance variables

At day old arrival, all birds were weighed and then weekly weighed till the end of the experiment (5 weeks old). The feed consumption was also recorded weekly (g feed/g body weight). The FCR was estimated by dividing the total feed consumption/Kg by the total body weights/Kg. The performance parameters variables were estimated according to Bell and Weaver (2002). The birds were observed daily for signs or mortalities. The European production efficiency factor (EPEF) for each group was calculated at the end of the study as the following; [Average live body weight (Kg) × Liveability (%)] × 100 / [Marketing age (day) × FCR].

Intestinal bacterial count

At the end of the study (5 weeks old), ten birds from each treatment were sacrificed and the intestinal contents were collected for determination of total viable bacterial count and coliform count (ISO, 1995). The results of the total bacterial count were expressed as the number of the organism of colony-forming units per gram (CFU/g) contents of the intestine.

Humoral immune response

Blood samples were collected weekly from the wing vein of ten birds in each treatment. The serum was separated by blood centrifugation. The blood was kept in refrigerator for several hours, and then centrifuged for serum. The titers of humoral immune response were investigated using the haemagglutination inhibition (HI) test with 8 haemagglutinating units (Swayne et al., 1998).

Statistical analysis

The results were statistically analyzed using the General Linear Model procedure of the Statistical Analysis System software (SAS®, 2000). Overall data were analyzed using one way ANOVA test.

Significant differences between treatment means were considered significant at level P <0.05.

Table 1. Main ingredients composition of diets given for broiler chickens for 5 weeks

Elements (g/kg feed)	Type of diets		
	Starter	Grower	Finisher
Metabolized energy (kcal/kg)	3000	3150	3200
Crude protein %	23.0	22.0	19.0
Soybean meal (45%)	330.5	302.7	250.9
Yellow maize (9%)	57.94	57.94	57.94
Maize gluten meal (60%)	70.2	70.1	65.7
Fat	32	45	41
Lysine	2.9	2.7	3.5
Methionine	2.2	2.0	2.2
Dicalcium phosphate	18	18	18
Sodium chloride	4	4	4

Each g of the mineral mixture of the diet contained: IU: vit. A 9000, vit. D3 2500, vit. E 17; mg, vit. K3 2.5, vit. B1 1.7, vit. B2 6.6, vit. B6 2.4, vit. B12 0.015; mg choline chloride 400, Mn 80, Fe 40, Zn 70, Cu 8, Se 0.3.

RESULTS AND DISCUSSION

There was no clinical signs or mortalities was recorded during the experimental period.

The results of Table 2, showed significant (P < 0.05) improvement in broilers body weights with different levels of MOLP (1, 5 and 7.5%). Similarly, Nkukwana (2012) found that birds supplemented with MOLP had higher body weight than the birds fed the control diets throughout the production period. Treatment fed on a diet with 1% *M. oleifrea* leaf meal gained significantly (P < 0.05) higher body weights than the control group and other treatments with higher levels (5 and 7.5%). Lower concentrations of MOLP were found to induce a positive significant effect on broilers performance than a higher one. This observation is supported by the study of Olugbemi et al. (2010) found that increasing the inclusion level of *M. oleifera* leaf meals in broiler diets results in depressed growth performance. Khan et al. (2017) observed that among the levels of MOLP included in the basal diets (i.e., 0.6, 0.9, 1.2 and 1.5%), only MOLP at the level of 1.2% that increased the body weight gain, whereas the other levels did not exert any effect. Kout et al. (2015) and Agashe et al. (2017) observed significantly higher body weights on diets containing different levels (0.2, 0.4 and 0.6%) of *M. oleifera* leaf meal. Divya et al. (2014) demonstrated that addition of MOLP at 0.5, 1.0, 1.5 and 2.0% levels or antibiotic slightly decreased body weight. Ochi et al. (2015) also found significant reduction in weight gain and body weight at inclusion level of 2% *M. oleifera*, while supplementation with 0.5% resulted in significant increase in feed consumption. Authors have speculated that this may be due to the presence of phytate which acted as an anti-nutritional factor. Bitter test of *M. oleifera* leaves (tannin at 1-23 g/kg) inducing reduced palatability and feed intake of birds when consumed in higher levels (Onunkwo and George, 2015).

Dietary treatments with *M. oleifera* showed better FCR when compared with control treatment. The best FCR was obtained by using 1% MOLP in all periods compared to other higher treatment levels and control (Table 3). These results are in agreement with Banjo (2012) who found that inclusion of *M. oleifera* leaf meal at 1, 2 and 3% in the diet did not significantly enhance FCR. Onunkwo and George (2015) reported a significant decrease in FCR of the birds fed MOLP levels 0.0, 5.0, 7.5 and 10%. Inoculation of low levels of MOLP was investigated by Aderinola et al. (2013) who recorded better FCR after feeding of broilers with *M. oleifera* leaves meal-based diets (0, 0.5, 1.0, 1.5 and 2.0%) than control. Also, Kout et al. (2015) recorded the best FCR in birds fed on 0.2% MOLP.

The results showed no significant (P < 0.05) difference among treated groups and the control one (Table 4). The improvement in the performance of broilers observed due to the supplementation of MOLP may also be attributed to the significant quantities of vitamins (A, B and C), calcium, iron, and protein. The leaves of *M. oleifera* have been reported to have an antioxidant activity due to the higher amount of polyphenols, tannins, anthocyanin, glycosides, and thiocarbamates, which remove free radicals, activate antioxidant enzymes and inhibit oxidases (Moyo et al., 2012).

Table 5 reveals that there was no significant (P > 0.05) difference in the means of mean total viable intestinal bacterial and coliform count among different treatments. Nevertheless, 1% MOLP revealed the lowest total vial intestinal bacterial count as compared with control. Parallel results were seen by Moez et al. (2014) who detected enhancement of the antimicrobial activity of dry *M. oleifera* leaves. Divya et al. (2014) detected that supplementation of MOLP (0.5, 1.0, 1.5 and 2.0%) significantly reduced the gut microflora and coliform population. Moreover, Onsare et al. (2013), Gomashe et al. (2014) and Hossam et al. (2016) observed that *M. oleifera* leaf extract inhibited the growth of *Escherichia coli* and *Salmonella* spp. and act as antibacterial. Yang et al. (2006) and Younis and Elbestawy (2017) proved the positive effect of *M. oleifera* (3% dried leaves) on the enhancement of duodenum traits, reduced *E. coli* and increased *Lactobacillus* spp. counts which helped in improving the intestinal health of broilers and increasing the production of digestive secretions and nutrient absorption. Al-Husnan and Alkahtani (2016) demonstrated that MOLP possesses antibacterial properties that may be beneficial in lowering the populations of potentially pathogenic bacteria in broilers intestine. Extracts of *M. oleifera* seeds may contain antibiotic metabolites, such as carboxylic acid, 2,4-diacetyl phloroglucinol, and cell wall-degrading enzymes and chitinases. It also possesses antimicrobial activity due to its principle component pterygospermin which kills bacteria by blocking the enzymes cysteine proteinase and alcohol dehydrogenase and thus blocks bacteria metabolism and survival (Yang et al., 2006).

Our findings showed that there were significant (P < 0.05) differences in the means of HI titers between MOLP and the control where the highest means were seen in 1% MOLP treated birds (Table 6). Several investigations are indicative of beneficial effects of *M. oleifera* in boosting the immune systems (Olugbemi et al., 2010;Al-Majali et al., 2017). The study of Yang et al. (2006) showed that dietary dehydrated leaves of Moringa induce a significant increase in the concentrations of total globulin, γ-globulin and lymphocyte ratio, antibody titer levels with comparison to the standard (sheep erythrocytes), and delayed-type hypersensitivity of broiler chickens. Comparable results also were obtained by Chollom et al. (2012), Eze et al. (2013), Hossam et al. (2016) and Younis and Elbestawy (2017) who demonstrated that *M. oleifera* extracts have nutritional value as well as strong antiviral activity against ND virus.

Table 2. The effect of different treatments level of MOLP on body weights of broiler chickens

Treatments	Age in weeks					
	0	1	2	3	4	5
T0	46.61±0.7	86.10±1.6^{b}	253.31±3.0^{c}	675.01 ±6.9^{c}	970.80±12.1^{c}	1296.19±16.9^{c}
T1	45.82±0.6	109.40±1.4^{a}	310.65±5.0^{a}	786.76±5.7^{a}	1290.72±11.7^{a}	1907.45±14.3^{a}
T2	44.91±0.3	102.75±1.1^{a}	296.93±4.6^{b}	754.84±5.9^{b}	1198.52±11.9^{a}	1710.21±14.6^{b}
T3	45.35±0.5	107.98±1.5^{a}	291.87±4.0^{b}	715.93±5.1^{ab}	1065.43±12.0^{b}	1800.76±14.9^{b}

Means with different superscripts, within age, are significantly different (P < 0.05)

Table 3. The effect of different treatments level of MOLP on FCR of broiler chickens

Treatments	Age in weeks				
	1	2	3	4	5
T0	1.50±0.20	3.10±0.41^{a}	2.97± 0.31^{a}	2.76 ± 0.72^{a}	3.09 ± 0.21^{a}
T1	1.21±0.03	1.56±0.05^{c}	1.42±0.02^{c}	1.63±0.04^{c}	1.27±0.03^{c}
T2	1.35±0.02	1.90±0.03^{b}	1.69±0.01^{ab}	1.87±0.03^{b}	1.63±0.02^{b}
T3	1.38±0.01	2.09±0.02^{b}	1.90±0.01^{b}	1.94±0.02^{b}	1.67±0.02^{b}

Means with different superscripts are significantly different (P < 0.05)

Table 4. The effect of different treatments level of MOLP on EPEF of broiler chickens

Treatments	European Production Efficiency Factor (EPEF)
T0	275.5±7.91
T1	298.3±6.84
T2	283.7±8.0
T3	279.9±9.76

Table 5. The effect of different treatments level of MOLP on total viable count and Coliform count in broiler chickens

Treatment	Total viable count log10 (CFU/g)	Coliform count log10 (CFU/g)
T0	6.2	5.8
T1	5.7	4.9
T2	5.9	5.2
T3	6.1	5.6

Table 6. The effect of different treatments level of MOLP on HI antibody titers against ND virus vaccine in broiler chickens

	Age in weeks				
Treatments	1	2	3	4	5
T0	4.7 ±3.0[a]	3.9±3.5[a]	4.8±3.0[a]	5.1±3.2[a]	5.03±2.2[a]
T1	5.9±2.5[b]	4.7±2.9[b]	6.5±2.1[b]	6.3±2.8[b]	6.8±1.7[b]
T2	5.6±2.3[b]	4.7±2.9[b]	6.3±3.4[b]	6.0±2.8[b]	6.5±1.7[b]
T3	5.4±2.3[b]	4.2±3.7[b]	6.1±3.4[b]	6.1±3.0[b]	6.2±1.8[b]

Means with different superscripts are significantly different ($P < 0.05$)

CONCLUSIONS

In conclusion, dietary supplementation of broiler chickens with levels 0, 1%, 5% and 7.5% MOLP could improve the body weight, FCR and EPEF, enhance the gut health by reducing the total and coliform bacterial count as well as boost the titers of HI humeral immune response to ND virus vaccine. However, 1% of MOLP induced the best results among the other levels. So, it is recommended to include this level in broiler ration.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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