Performance, physiological and microbiological responses of broiler chicks to Moringa oleifera leaf powder, garlic powder or their combination

by Endang Widiastuti

Performance, physiological and microbiological responses of broiler chicks to *Moringa oleifera* leaf powder, garlic powder or their combination

S Sugiharto, I Isroli, T Yudiarti, E Widiastuti, H I Wahyuni and T A Sartono

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia (50275)
sgh_undip@yahoo.co.id

Abstract

The study aimed to investigate the effect of *Moringa oleifera* leaf powder, garlic powder or their combination on growth performance, blood parameters and ileal pathogenic bacteria population of broiler chickens. A hundred of day-old broiler chicks were raised for 28 days in four dietary groups including CONT (corn-soybean meal-based basal diet, without any additive, antibiotics or coccidiostats), MOLP (the same basal diet added with 1% *Moringa oleifera* leaf powder), GARLIC (basal diet added with 1% garlic powder) and MOLIC (basal diet added with 1% *Moringa oleifera* leaf powder and 1% garlic powder). Compared to other birds, CONT birds consumed more feed. There was no effect of treatments on body weight (BW) gain and feed conversion ratio (FCR) of broilers. MOLP and MOLIC had lower abdominal fat content compared to CONT birds. MOLIC chicks had lower values of mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) compared to other chicks. Coliform were lower in ileum of GARLIC than other birds. MOLIC had lower ileal *Enterobacteriaceae* counts in the ileal content, when compared with that in other chicks. In conclusion, combination of *Moringa oleifera* leaf powder and garlic powder was beneficial in reducing the abdominal fat deposition and ileal *Enterobacteriaceae* content in broiler chickens. However, the use of such combined phytobiotic products may adversely affect the erythrocyte indices of broilers.

Keywords: *antibiotics, growth promoters, immune system, phytobiotics*

Introduction

Antibiotic growth promoters (AGP) has traditionally been included in broiler rations to control the outbreak of bacterial infections and promote the growth rate of broiler chickens. However, the emergence of antibiotic-resistant pathogenic bacteria due to the application of AGP may impose to the ban of such additive for broiler production. Considering that the AGP withdrawal in feed may increase the health and production problems, any alternative to AGP is therefore necessities. There are a number of AGP alternatives, one of which is phytobiotic. This plant-based product has been included in the rations to serve as natural antibiotics, immunomodulatory agents as well as growth promoter for broiler chickens (Sugiharto 2016). Among the phytobiotics, *Moringa oleifera* has long been known to have high nutritional contents (Mune et al 2016) and functional properties (e.g., antimicrobial activity) (Al_husnan and Alkahtani 2016). In poultry, *Moringa oleifera* leaf powder has been included in broiler rations as a cheap alternative to AGP. Nkukwana et al (2014) and Mousa et al (2017) documented that feeding *Moringa oleifera* leaf meal improved the growth performance of broilers. Also, Khan et al (2017) noticed that feeding *Moringa oleifera* leaf meal resulted in positive effects on intestinal morphology and health in broiler chickens. In term of immune system, dietary inclusion of *Moringa oleifera* leaf powder improved immune response of broilers to Newcastle disease and infectious bursal disease vaccination (Liaqat et al 2016).

Several studies have revealed the potentials of garlic (*Allium sativum*) as an alternative to AGP in broiler diets. Toghyani et al (2011) reported that garlic reduced the intestinal pathogenic bacteria loads and improved digestion and absorption in broiler chickens. Concomitant with this, garlic was able to decrease and increase the intestinal populations of *Escherichia coli* and lactic acid bacteria (LAB), respectively, and improved the immune competences of broilers (Rahimi et al 2011). Likewise, Karangiya et al (2016) showed that dietary supplementation of garlic improved the final body weight of broilers as compared to control. To improve their efficacy as AGP substitute, phytogenic products may be used in combination with other products. It was apparent in the study of Mousa et al (2017) that combination of *Moringa oleifera* and *Cichorium intybus* in the diets resulted in better growth performance in broilers, when compared with *Moringa oleifera* or *Cichorium intybus* alone. Such combination was also associated with better immunological responses in broilers as compared to each one alone (Mousa et al 2017). On the basis of the above study and considering the functional traits of each phytogenic product, the combination of *Moringa oleifera* leaf powder and garlic powder was expected to exert synergistic and complementary effects, which may produce particular benefits for the growth performance and health of broiler chickens. The present study aimed to investigate the effect of *Moringa oleifera* leaf powder, garlic powder or their combination on growth performance, blood parameters and ileal pathogenic bacteria population of broiler chickens.

Materials and methods

Green and mature *Moringa oleifera* leaves were harvested from the plants around the campus. The leaves were dried under the shade for 5 days and then milled to a fine powder. The latter product was subsequently regarded as *Moringa oleifera* leaf powder. Garlic powder (100% purity) was bought from PT. Gunacipta Multirasa, Tangerang (Indonesia). The proximate compositions of *Moringa oleifera* leaf powder and garlic powder are shown in Table 1.

Table 1. Proximate compositions of *Moringa oleifera* leaf meal and garlic powder

Compositions (%)	*Moringa oleifera* leaf meal	Garlic powder
Moisture	15.8	9.44
Crude protein	27.7	1.22
Crude fat	2.59	1.17
Crude fiber	17.8	1.30
Ash	12.1	10.2

A hundred of day-old chicks (Lohmann broiler meat; body weight of 37.4 ± 2.68 g, means ± standard deviation) were randomly distributed to one of four dietary groups with five replicates (five birds in each). The dietary groups included CONT (corn-soybean meal-based basal diet, without any additive, antibiotics or coccidiostats), MOLP (the same basal diet added with 1% *Moringa oleifera* leaf powder), GARLIC (basal diet added with 1% garlic powder) and MOLIC (basal diet added with 1% *Moringa oleifera* leaf powder and 1% garlic powder). For the entire trial (28 days), the birds were kept on rice husk litter in an open-sided broiler house. The chicks were also given free access (*ad libitum*) to fresh water and feed throughout the study period. The basal diet was prepared as a single feed and formulated (Table 2) to meet the nutrient requirements of broiler chickens (SNI 2006). Feed intake, body weight and feed conversion ratio (FCR) were recorded on weekly basis.

Table 2. Ingredients and nutrient compositions of basal diet

Ingredients	**Compositions (%, unless otherwise noted)**
Maize	56.0
Soybean meal	31.0
Meat bone meal	4.00
Molasses	4.00
Vegetable oil	2.50
Mineral and vitamin mix[1]	2.50
Calculated nutrients:	
Metabolizable energy (kcal/kg)[2]	3,000
Crude protein	22.0
Crude fat	5.00
Crude fiber	5.00
Ash	7.00
Ca	0.90
P (available)	0.60

[1] *Provided minerals per kg of the feed: 1,100 mg Zn, 1,000 mg Mn, 75 mg Cu, 850 mg Fe, 4 mg Se, 19 mg I, 6 mg Co, 1,225 mg K, 1,225 mg Mg, 1,250,000 IU vitamin A, 250,000 IU vitamin* D_3*, 1,350 g pantothenic acid, 1,875 g vitamin E, 250 g vitamin* K_3*, 250 g vitamin B1, 750 g vitamin B2, 500 g vitamin B6, 2,500 mg vitamin B12, 5,000 g niacin, 125 g folic acid, 2,500 mg biotin* [2] *Value was estimated based on formula (Bolton, 1967) as follow: 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen free extract] + 2.5}*

The birds were vaccinated with Newcastle disease virus (NDV) vaccine at days 4 and 18 through eye drops and drinking water, respectively. At day 28, 10 chicks from each group (two chicks per replicate) were randomly selected, and from which the blood was collected from their wing veins. The collected blood was placed in ethylene diamine tetra acetic acid (EDTA)-vacutainers for the determination of blood cells counts. The rest of the blood was placed in anticoagulant-free vacutainers, allowed to clot at room temperature, and centrifuged at 3,000 rpm for 10 min to obtain serum. Immediately after blood sampling, the birds were slaughtered, and internal organs and abdominal fat pad were obtained and weighed. For the enumeration of the selected bacteria population, digesta were expelled from the ileum into sample bottles.

Full blood counts were measured with a hematology analyzer according to manufacturer's instruction (Prima Fully-auto Hematology Analyzer, PT. Prima Alkesindo Nusantara, Jakarta, Indonesia). The hemagglutination inhibition (HI) test (Villegas 1987) was carried out to determine the serum NDV antibody titers. The measurements of total triglyceride, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were conducted according to enzymatic colorimetric/color methods. Total protein and albumin in serum were measured based on the spectrophotometric/photometric tests. The difference between total protein and albumin was considered as globulin value. The serum biochemical analyses were performed using kits (DiaSys Diagnostic System GmbH, Holzheim, Germany) following the producer's protocols. The numbers of coliform and lactose-negative enterobacteria were determined on MacConkey agar (Merck KGaA, Darmstadt, Germany) following overnight aerobic incubation at 38°C as red and colourless colonies, respectively. The sum of coliform and lactose-negative enterobacteria was regarded as *Enterobacteriaceae*.

The results obtained in this study were analyzed based on a completely randomized design by ANOVA. Duncan's multiple-range test was subsequently conducted when there was a significant difference among dietary groups. Data are presented as least squares means and standard error.

Results

Data on the performance of broilers are presented in Table 3. Compared to the treated birds, birds in CONT group consumed more feed. Yet, there was no significant effect of dietary supplementation of phytobiotics on BW gain and FCR of broilers. In general, no substantial effect of dietary treatments was observed with regard to the relative weight of internal organs of broilers (Table 4). However, it was apparent that MOLP and MOLIC had lower abdominal fat content as compared to CONT birds.

Table 3. Performances of broiler chicks at 28 days of age

Items	**Treatment groups**				**SE**	***p* value**
	CONT	**MOLP**	**GARLIC**	**MOLIC**		
BW gain (g/bird)	683	629	654	713	27.0	0.20
Feed intake (g/bird)	1,229^{a}	1,029^{d}	1,135^{c}	1,187^{b}	4.78	<0.01
FCR	1.83	1.64	1.75	1.67	0.08	0.38

^{a,b,c,d} Means in the same row with different letters show significant differences ($p<0.05$) among dietary treatments CONT: corn-soybean meal-based basal diet, MOLP: basal diet added with 1% Moringa oleifera leaf powder, GARLIC: basal diet added with 1% garlic powder, MOLIC: basal diet added with 1% Moringa oleifera leaf powder and 1% garlic powder, BW: body weight, FCR: feed conversion ratio, SE: standard error

Table 4. Internal organs relative weight of broiler chicks

Items (% live BW)	**Treatment groups**				**SE**	***p* value**
	CONT	**MOLP**	**GARLIC**	**MOLIC**		
Heart	1.00	0.92	0.93	0.97	0.07	0.83
Liver	2.89	2.91	2.94	2.96	0.13	0.98
Proventriculus	0.53	0.51	0.52	0.54	0.02	0.76
Gizzard	2.04	2.09	1.93	1.98	0.10	0.69
Duodenum	0.74	0.75	0.76	0.76	0.06	0.99
Jejunum	1.22	1.24	1.13	1.28	0.08	0.56
Ileum	0.85	1.03	0.86	1.02	0.07	0.16
Pancreas	0.54	0.52	0.48	0.49	0.03	0.58
Caeca	0.38	0.38	0.34	0.39	0.02	0.43
Spleen	0.13	0.16	0.12	0.10	0.02	0.35
Thymus	0.30	0.29	0.27	0.25	0.03	0.67
Bursa of Fabricius	0.24	0.27	0.22	0.24	0.02	0.50
Abdominal fat	1.31^{a}	0.60^{c}	1.05^{ab}	0.80^{bc}	0.13	<0.01

^{a,b,c} Means in the same row with different letters show significant differences ($p<0.05$) among dietary treatments CONT: corn-soybean meal-based basal diet, MOLP: basal diet added with 1% Moringa oleifera leaf powder, GARLIC: basal diet added with 1% garlic powder, MOLIC: basal diet added with 1% Moringa oleifera leaf powder and 1% garlic powder, BW: body weight, SE: standard error

Data on complete blood counts and serum antibody titer and biochemical parameters of broiler chicks are presented in Table 5 and Table 6, respectively. Compared to other birds, birds in MOLIC group had lower values of MCH and MCHC. There was no significant effect of treatments with regard to the other blood profiles and serum biochemical parameters of broilers.

Table 5. Complete blood counts of broiler chicks

Items	**Treatment groups**				**SE**	***p* value**
	CONT	**MOLP**	**GARLIC**	**MOLIC**		
Hemoglobin (g/dL)	10.7	11.3	11.8	9.44	0.70	0.13
Erythrocytes (10^6/µL)	2.53	2.62	2.83	2.33	0.17	0.23
Hematocrit (%)	27.5	28.5	30.5	25.4	1.81	0.27
MCV (fl)	110	110	109	110	1.45	0.98
MCH (pg)	42.2^{a}	43.0^{a}	41.8^{a}	40.2^{b}	0.49	<0.01
MCHC (g/dL)	38.9^{a}	39.6^{a}	38.7^{a}	36.9^{b}	0.56	0.02
Leukocytes (10^3/µL)	69.9	75.9	77.2	76.4	5.99	0.81
Heterophils (10^3/µL)	6.38	7.31	8.63	5.63	1.12	0.28
Eosinophils (10^3/µL)	3.94	4.75	4.69	4.25	0.33	0.28
Lymphocytes (10^3/µL)	59.6	63.8	63.9	66.2	5.34	0.85

Thrombocytes ($10^3/\mu L$)	7.88	8.25	8.88	9.38	0.65	0.39

a,b Means in the same row with different letters show significant differences (p<0.05) among dietary treatments
CONT: corn-soybean meal-based basal diet, MOLP: basal diet added with 1% Moringa oleifera leaf powder, GARLIC: basal diet added with 1% garlic powder, MOLIC: basal diet added with 1% Moringa oleifera leaf powder and 1% garlic powder, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, SE: standard error

Table 6. Serum antibody titer and biochemical parameters of broiler chicks

Items	Treatment groups				SE	*p* value
	CONT	MOLP	GARLIC	MOLIC		
Antibody titer against NDV (Log_2 GMT)	1.63	1.88	1.75	1.63	0.21	0.81
Total triglyceride (g/dL)	105	125	123	135	12.9	0.45
Total cholesterol (g/dL)	168	181	173	158	10.5	0.48
LDL (g/dL)	43.0	41.1	38.2	38.0	7.34	0.95
HDL (g/dL)	104	112	110	102	6.90	0.70
Total protein (g/dL)	2.24	2.47	2.70	2.42	0.15	0.21
Albumin (g/dL)	1.01	1.10	1.14	1.09	0.05	0.31
Globulin (g/dL)	1.24	1.38	1.56	1.34	0.10	0.18
A/G ratio	0.82	0.81	0.76	0.81	0.03	0.35

CONT: corn-soybean meal-based basal diet, MOLP: basal diet added with 1% Moringa oleifera leaf powder, GARLIC: basal diet added with 1% garlic powder, MOLIC: basal diet added with 1% Moringa oleifera leaf powder and 1% garlic powder, NDV: Newcastle disease virus, GMT: geometric mean titers, LDL: low-density lipoprotein, HDL: high-density lipoprotein, A/G ratio: albumin to globulin ratio, SE: standard error

Table 7 shows the population of selected bacterial populations in the ileal digesta of broiler chickens. Coliform bacteria counts were lowerin GARLIC co mpared to other birds. The birds in MOLIC group had lower ($p<0.05$) numbers of *Enterobacteriaceae* in the ileal content, when compared with that in other chicks.

Table 7. Selected population of bacteria in the ileal digesta of broiler chicks

Items	Treatment groups				SE	*p* value
	CONT	MOLP	GARLIC	MOLIC		
Coliform	8.79^a	9.09^a	7.41^b	8.54^a	0.36	0.03
Lactose-negative enterobacteria	8.98	8.96	9.43	8.47	0.29	0.19
Enterobacteriaceae	9.54^a	9.41^a	9.47^a	8.91^b	0.15	0.04

a,b Means in the same row with different letters show significant differences (p<0.05) among dietary treatments CONT: corn-soybean meal-based basal diet, MOLP: basal diet added with 1% Moringa oleifera leaf powder, GARLIC: basal diet added with 1% garlic powder, MOLIC: basal diet added with 1% Moringa oleifera leaf powder and 1% garlic powder, SE: standard error

Discussion

In response to the ban of antibiotics as growth promoters, broiler industries are now searching for any alternatives to AGP. Of the alternatives, phytobiotics such as *Moringa oleifera* leaf (Nkukwana et al 2014; Mousa et al 2017) and garlic (Karangiya et al 2016) have been suggested to improve the growth performance of broilers. Data in our present study show that dietary additions of *Moringa oleifera* leaf powder, garlic powder or their combination resulted in lower feed intake as compared to control. This reduced feed intake did, however, not significantly affect the BW gain and FCR of broilers. In line with our data, Divya et al (2014) showed no change in BW gain and FCR in broilers with feeding *Moringa oleifera* leaf powder (at the levels of 0.5, 1.0, 1.5 and 2.0% in basal diet) as compared to control. Likewise, Khan et al (2017) noted that although dietary supplementation of *Moringa oleifera* leaf powder increased BW gain of broilers, the treatment did not change feed consumption and FCR of broilers at 35 days of age. From the aforementioned studies, it may be suggested that the growth promoting effect of *Moringa oleifera* leaf powder seems to be less consistent. Our inference was also supported by the fact in the study of Khan et al (2017) that among the levels of *Moringa oleifera* leaf powder included in the basal diets (i.e., 0.6, 0.9, 1.2 and 1.5%), only *Moringa oleifera* leaf powder at the level of 1.2% that increased the BW gain, whereas the other levels did not exert any effect. With regard to garlic, although significantly reducing feed intake, this phytobiotic treatment did not change BW gain and FCR of broilers in the present study. In accordance with our finding, Toghyani et al (2011) did not observe any effect of garlic powder (included at the levels of 2 and 4% in the diets) on final BW, feed intake and FCR of broilers. Likewise, feeding 1.5% and 3% garlic powder had no effect on final live weight, feed consumption and FCR of broiler chickens as reported by Milošević et al (2013). Unlike the above studies, Karangiya et al (2016) showed that feeding 1% garlic powder increased final BW of broilers. Yet, the treatment did not improve FCR of broilers. In this study, the combination of *Moringa oleifera* leaf powder and garlic did not substantially affect the BW gain and FCR, though decreased feed intake of broilers. In this respect, the synergistic effect of both phytobiotic compounds did not appear in the present study in term of improving the growth performance of broilers.

It was apparent in the present study that dietary supplementation with*Moringa oleifera* leaf powder or combination of *Moringa oleifera* leaf powder and garlic powder resulted in lower abdominal fat content relative weight in broilers. This finding was in line with Cui et al (2018) showing a linearly reduced abdominal fat content in broilers with the increased *Moringa oleifera* leaf meal supplementation. The latter authors suggested that feeding *Moringa oleifera* leaf decreased lipid biosynthesis leading to reduced abdominal fat deposition in broilers. The mechanism by which *Moringa oleifera* reduced lipid biosynthesis in broiler was definitely unknown, but it seems that *Moringa oleifera* suppressed the expressions of HMG-CoAR, PPARα1, and PPARγ genes (Sangkitikomol et al 2014). Previous study showed that garlic treatment was associated with the reduced abdominal fat content in broiler chicks (Milošević et al 2013). Different from the above literature, our present study did not show any significant effect of garlic powder on the abdominal fat content of broilers. The differences in the nature of garlic, strains and age of broiler chicks and the conditions of experiment may be responsible for the divergent results. Interestingly, dietary addition of the combination of *Moringa oleifera* leaf meal and garlic powder resulted in lower abdominal fat deposition in broiler as compared to control. Considering the above data, *Moringa oleifera* leaf meal seemed to have major contribution in lowering the abdominal fat content in the respective birds.

It was shown in the present study that dietary addition of *Moringa oleifera* leaf meal combined with garlic powder resulted in lower values of MCH and MCHC of broilers. It was not clear regarding the reason on how the combination of *Moringa oleifera* leaf meal and garlic powder negatively affected the erythrocyte indices of broilers in the present study. With regard to *Moringa oleifera*, feeding such leaf meal did not affect the values of MCH and MCHC of broiler chickens in the studies of Akpodiete et al (2014) and Liaqat et al (2016). Hence, it seems difficult to suggest the contribution of *Moringa oleifera* leaf meal in reducing the MCH and MCHC values of birds in the present study. Study in rats documented the hemolytic anemia-inducing effect of garlic (Oboh 2004). In accordance with this, study in broilers showed that feeding garlic powder reduced the values of MCH and MCHC (Jawad 2007; Hamlaoui-Gasmi et al 2011). Owing to these studies, it was interesting to assume that the reduced values of MCH and MCHC in MOLIC broilers was due to the effect of garlic powder. This inference should, however, be interpreted with caution as the effect of garlic powder alone was not significant on erythrocyte indices in the current study. Concerning the possible combined effects of *Moringa oleifera* leaf meal and garlic powder in lowering the MCH and MCHC values, data supporting this inference are not available in the literature.

Our finding showed that feeding garlic powder reduced the counts of coliform bacteria in the ileal digesta of broilers. Corresponding result was reported by Dieumou et al (2009) in which garlic treatment resulted in reduced numbers of *E. coli* in the ileo-cecum of broiler chicks. It was most likely that thiosulfinate and allicin content in garlic acted as antibacterial agents against coliform bacteria in the intestine of broilers (Peinado et al 2012). It is interesting to report in this present study that combination of *Moringa oleifera* leaf powder and garlic powder resulted in decreased numbers of *Enterobacteriaceae* in the ileal content of broilers. It has been known that both *Moringa oleifera* leaf (Moyo et al 2012; Al_husnan and Alkahtani 2016) and garlic (Peinado et al 2012) possess antibacterial properties that may be beneficial in lowering the populations of potential pathogenic bacteria in the intestine of broilers. It should, however, be noted that the effect of *Moringa oleifera* leaf powder and garlic powder was not substantial when administrated alone in the present study. Owing to these facts, the use of both phytobiotics as a combination seemed to be more effective in inhibiting the growth of *Enterobacteriaceae* in the ileum of broiler chicks.

Conclusion

- The combination of *Moringa oleifera* leaf powder and garlic powder was beneficial in reducing the abdominal fat deposition and ileal *Enterobacteriaceae* content in broiler chickens.

- However, the use of such combined phytobiotic products may adversely affect the erythrocyte indices of broilers.

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