



Evaluation of Chamomile (*Matricaria chamomilla* L.) as an Alternative Growth Promoter in Broiler Chicks

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ABSTRACT

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This study was conducted to evaluate *Matricaria chamomilla* (MC), commonly known as chamomile, as a new growth promoter in broiler chicks and its effects on feed intake, weight gain, feed conversion ratio, carcass characteristics, clinical biochemistry, immunity and digestive bacterial count. A total of 200 day-old male broiler chicks were randomly assigned into five experimental treatments each with four replicates of 10 chicks. The treatments were feeding of commercial diet as a control (CD) and the same supplemented with *M. chamomilla* at 0.3 (MC-0.3), 0.6 (MC-0.6), 0.9 (MC-0.9) and 1.2 (MC-1.2) per cent levels. The addition of MC did not affect feed consumption. Although there were no differences ($P > 0.05$) among the groups at any of the periods in the feed intake, BW gain and feed conversion ratio. There were, however, differences ($P < 0.05$) in the European production index (EPI) with the index for chickens fed MC-0.6 being higher as compared with all the other treatments. The immune response to Newcastle disease was higher in MC-0.9 and MC-1.2 groups. The counts of lactobacilli in the intestinal digesta were higher ($P < 0.05$) in broilers receiving MC-0.6 and MC-0.9 diets than those fed on the CD diet. In respect of plasma clinical biochemistry, differences ($P < 0.05$) were found in glucose, HDL and uric acid for the different treatments when compared with the control, but the values were within the normal range for poultry. Overall, the present study indicates that the positive effects, if any, of dietary supplementation of chamomile in broilers apparently failed to present a definite trend. Thus, further evaluation is needed in order to establish the proper use, the level of supplementation and the potential beneficial effects that *M. chamomilla* would impart in broilers.

Keywords: Broiler chicks, Clinical biochemistry, Growth promoter, *Matricaria chamomilla*.

INTRODUCTION

After the prohibition of antibiotics as growth promoters in animal nutrition by the European Union, many research lines have been developed to investigate alternative growth promoting compounds. These new candidates must have, at least, the same

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beneficial activity of the antibiotics, when used at sub-therapeutic doses as a preventive measure against proliferation of pathogenic bacteria (Feighner and Dashkevicz, 1987).

In this regard *Matricaria chamomilla* L. (MC), commonly known as chamomile has been suggested to possess health-promoting effects (McKay and Blumberg, 2006). Chamomile belongs to the Asteraceae family and is one of the important medicinal herb native to southern and eastern Europe. It is also grown in Germany, Hungary, France, Russia and Yugoslavia, and introduced to other countries such as North Africa, Asia, North and South America, Australia, and New Zealand (Singh *et al.*, 2011).

The analysis of chamomile flower extract done by Hamady *et al.* (2017), has showed the presence of several bioactive phytochemical components including pentadecanoic acid, which is a palmitic acid methyl ester reported to have a potential antioxidant and growth promoter activities in broiler chickens (Vijisarl and Arumugam, 2014); α -bisabolol (a monocyclic sesquiterpene alcohol), which was found to have an anti-inflammatory, antibacterial and antifungal properties (Kazemi, 2014) and 4,5,9,10-dehydro-isolongifolene, a sesquiterpene with potent antioxidant properties (Rangasamy and Namasivayam, 2014). Other compounds that were identified in the analysis done by Hamady *et al.* (2017) were, 4,7-dihydroxy-coumarin and 7-methoxy-coumarin. Coumarins have a variety of important biological activities such as anti-inflammatory, antioxidant, antiviral and antimicrobial (Al-Majedy *et al.*, 2016). Farnesol was also identified in the chamomile flower extract (Kazemi, 2014). The tested extract was also found to contain the terpene compounds as farnesyl- β -D-mannofuranoside, phytol and lavandulol that show antimicrobial activity (Gunasekaran *et al.*, 2012). Others activities reported have also been digestive beneficial effects (antispasmodic effect), inhibition of gastric ulcers, or diarrhoea, as well as the modulation of the activity of hepatic cytochrome P450 (Kazemi, 2014). Furthermore, powdered flowers and extracts of chamomile have demonstrated inhibitory effects on the growth of several pathogenic bacteria such as *Salmonella* spp., *Escherichia coli*, *Enterobacter* spp., *Enterococcus* spp. and others (Al-Kaisse and Khalel, 2011; Dada *et al.*, 2015; Hamady *et al.*, 2017).

Other studies described a wide range of different properties of *Matricaria chamomilla* in animals (McKay and Blumberg, 2006). There are reports that chamomile flower powder supplementation improved feed intake, weight gain and growth rate of broiler chicks compared with control one (Al-Kaisse and Khalel 2011). However, Dada *et al.* (2015) reported no beneficial effects in broiler chicks receiving MC. Furthermore, Hamada *et al.* (2015) reported that chamomile supplementation at different levels reduced final body weight and weight gain in poultry. The aim of this study, therefore, was to evaluate the effects of supplementation of *Matricaria chamomilla* L. on the growth performance, carcass characteristics, clinical biochemistry, immunity and digestive bacterial count of broiler chicken.

MATERIALS AND METHODS

Housing and animals

The experiment was performed in a commercial poultry farm located in Astaneh-Ashrafiyeh. Prior to the experiment, the facility was cleaned and disinfected, including drinkers and feeders. Continuous controlled temperature (22-25°C), air humidity (55 to 65%) and ventilation were implemented according to the instructions for Ross 308 broilers (Aviagen, Newbridge, Scotland, UK 35805).

Chicks were one-day-old male, Ross 308 strain (Aviagen, Newbridge, Scotland, UK 35805), with similar body weights in all groups. The experiment complied with the guidelines for the use of experimental animals by the Ethics Committee of the Islamic Azad University. Environmental conditions and management were identical for all groups during the experiment.

Experimental design, feedstuffs and treatments

The experimental design was a randomized complete block, with five treatments, four replicates per treatments with ten animals per replicate (a total of 200 male broiler chicks). The experiment lasted 42 d. All chicks were fed a commercial diet according to the producer's recommendation (Table 1). The ingredients of the commercial diet were corn, soybean meal, fish meal, soybean oil, all acquired from the local market (Rasht, Iran). The nutritional contents of the commercial diet in the different growth phases are summarized in Table 1. The treatments were feeding a commercial diet as a control (CD) and the same supplemented with *M. chamomilla* flower powder at 0.3 (MC-0.3), 0.6 (MC-0.6), 0.9 (MC-0.9) and 1.2 (MC-1.2) per cent levels. The MC flower powder was purchased locally (Rasht, Iran). Feed and water were supplied *ad libitum*.

Growth performance and feed intake

Body weight (BW) and feed intake (FI) were recorded weekly for 6 weeks and calculated at d 21 and 42. Feed conversion ratio (FCR) was calculated as the ratio

Table 1. Nutritional composition of the basal diet

Attributes	Pre-starter (0-6 d)	Starter (7-20 d)	Grower (21-30 d)	Finisher 1 (30-38 d)	Finisher 2 (39-42 d)
Crude protein, %	23.5	20.5	18.6	17.0	16.2
Crude fiber, %	NA	3.5	3.2	3.6	3.0
Crude fat, %	NA	4.0	3.6	3.6	3.5
Arg, %	1.33	1.45	1.25	1.15	0.99
Met+Cys, %	1.01	0.91	0.82	0.75	0.72
Lys, %	1.29	1.20	1.13	0.99	0.93
Met, %	0.63	0.59	0.50	0.44	0.42
Ca, %	1.05	0.95	0.91	0.85	0.81
Available P, %	0.50	0.47	0.45	0.42	0.38
ME, kcal/kg	3000	2980	3000	3020	3050

(g/g) of average feed intake and average BW gain. The European production index (EPI) was calculated according to the formula proposed by Nollet *et al.* (2008): $[(\text{Live weight (kg)} \times \text{livability (\%)}) / (\text{FCR} \times \text{age (days)})] \times 100$.

Carcass traits

At d 42, and after 4 h of fasting, one bird randomly chosen from each replicate (n=20) was slaughtered and used to measure organ weights of the broilers. Carcass components were dissected and separately weighed and recorded.

Clinical biochemistry, immunity response and bacterial count

At 42 d of age a 5-ml of blood sample was collected from the ulnaris vein of one randomly chosen bird from each of the replicates (4 birds per treatments), and placed into sodium EDTA. Total plasma cholesterol and triglyceride levels were determined using enzymatic methods according to Schmid and Forstner (1986), while the cholesterol fractions (HDL and LDL) were measured directly with diagnostic kits. Plasma glucose was determined using a glucose oxidase kit according to Trinder (1969) and Barham and Trinder (1972). Plasma uric acid was measured using a uric acid-uricase enzyme kit based on Kato *et al.* (2000). All the assays were carried out using commercial kits (Teif Azmoon Pars, Co., Tehran, Iran). Birds were vaccinated against different diseases such as infectious bronchitis (IB) at d 1 and d 18, against Newcastle disease at d 10 and 18, against avian influenza at the d 1, and against Gumboro's disease at the d 14 of age. Blood samples were collected from each bird at the d 42 of age to quantify anti-Newcastle Disease antibody titers measured through hemagglutination inhibition test according to Cunningham (1971).

At d 21 of age, one bird per replicate (n=20) was injected intravenously in the breast muscle with 0.3 ml of a sheep red blood cells (SRBC) suspension (1 mL of PBS with 10 mL of SRBC). Blood samples were collected from each bird at d 42 of age to quantify anti-SRBC antibody titers. Blood samples were collected by puncturing the vena cava into 6-ml vacuutainers with heparin as an anticoagulant (Sigma, Germany) and centrifuged at 10,000 rpm for 15 min. The obtained serum samples were pipetted and stored in 2-ml eppendorf tubes at 20°C. The total immunoglobulin M (IgM), and G (IgG) anti-SRBC antibody titers were determined using a micro-hemagglutination technique (Pourhossein *et al.*, 2015). The antibody data were expressed as the \log_{10} of the reciprocal of the highest dilution giving visible agglutination. Bacterial count in the digesta samples was carried out according to Dibaji *et al.* (2014) using MRS agar for Lactobacilli, EMB for *E. coli* and MacConkey agar for coliforms. A suspension of bacteria isolated from gastrointestinal digesta from two birds from each replicate was prepared. Lactobacilli bacteria were counted after incubation at 37°C in anaerobic conditions for 72 h, and *E. coli* and coliforms counted after incubation at 35°C in aerobic conditions for 45 h. Bacteria were counted by colony counter as colony forming units (CFU) and reported as \log_{10} number of bacteria/gram sample.

Statistical analysis

Data were subjected to statistical analysis using the General Linear Model procedures (SPSS Statistical package v.20). Differences among means were assessed via Duncan's multiple range tests. Values were considered as statistically significant when $P < 0.05$.

RESULTS

Growth performance

The broilers showed good health and normal behavior during the entire experiment. During the starter period (d 1-21), the finisher period (d 22-42) and in the total period (d 1-42), there were no differences ($P > 0.05$) in feed intake and BW gain among the dietary groups (Table 2). The FCR (g feed intake/g gain) and EPI values over the 42-d period showed differences ($P < 0.05$) among the groups, but were similar when period-wise (d 1-21 or d 22-41) comparison was made. The highest FCR was achieved by the MC-0.6 group, which showed significant differences when compared to control, MC-0.3, MC-0.9 and MC-1.2 groups, and EPI was lower in MC-0.6 group as compared with the remaining treatments.

Carcass traits

There were no significant differences in the weight of the different organs except for the neck and spleen (Table 3). In case of the neck, the MC-0.3 group

Table 2. Effects of graded levels of chamomile supplementation on growth performance of the broilers

Attribute	Dietary groups [†]					SEM
	CD	MC-0.3	MC-0.6	MC-0.9	MC-1.2	
<i>Body weight (g)</i>						
Starter	661	662	665.6	666.8	664.5	16.3
Finisher	1240.6	1242.6	1112.2	1168.5	1290.8	123.1
Overall	1901.7	1904.6	1777.8	1835.3	2010.3	123.8
<i>Feed intake (g)</i>						
Starter	1022.2	1026.5	1026.3	1026.5	1025.7	16.6
Finisher	3440.2	3440.2	3258.5	3321.2	3531.7	42.5
Overall	4462.5	4462.5	4285	4347.5	4557.5	97.5
<i>Feed conversion ratio (g feed/g BW gain)</i>						
Starter	1.54	1.55	1.54	1.53	1.54	0.03
Finisher	2.78	2.77	2.93	2.85	2.74	0.10
Overall	2.34 ^a	2.34 ^a	2.41 ^b	2.37 ^{ab}	2.33 ^a	0.07
EPI [‡]	200.1 ^a	206.2 ^a	187.0 ^b	198.4 ^a	208.7 ^a	11.31

[†]The treatments included feeding of commercial diet as a control (CD) and the same supplemented with *M. chamomilla* at 0.3 (MC-0.3), 0.6 (MC-0.6), 0.9 (MC-0.9) and 1.2 (MC-1.2) per cent levels.

[‡]European production index.

^{ab}Means with different letters within treatments differ significantly ($P < 0.05$).

showed the highest values, while MC-0.9 and MC-1.2 groups showed the lowest ($P < 0.05$). On the other hand, the weight of spleen was significantly ($P < 0.05$) higher in MC-0.3 group as compared with the other treatments.

Clinical biochemistry, immunity response and bacterial count

The data on effects of MC on clinical biochemistry are shown in Table 4. The plasma levels of glucose, HDL and uric acid varied significantly ($P < 0.05$) among the MC-supplemented groups when compared with the control. The plasma levels of glucose showed a significant reduction in MC-0.9 group and higher plasma levels in MC-0.6

Table 3. Effects of graded levels of chamomile supplementation on the organ weights of the broilers

Attributes	Dietary groups [†]					SEM
	CD	MC-0.3	MC-0.6	MC-0.9	MC-1.2	
<i>Organ weight (g)</i>						
Liver and bile	54.0	61.0	48.0	54.5	54.6	4.41
Neck	56.0 ^{ab}	60.5 ^a	52.0 ^{ab}	50.0 ^b	50.0 ^b	1.0
Heart	10.5	12.5	11.5	11.0	11.0	0.69
Spleen	2.5 ^b	4.0 ^a	2.0 ^b	2.0 ^b	2.0 ^b	1.80
Thymus	6.5	6.0	6.0	5.0	5.0	0.74
Bursa	2.0	2.0	2.0	2.0	2.0	0.39
Breast	539.0	489.5	468.0	493.5	493.6	0.64
Gizzard	61.0	61.5	58.5	59.5	59.4	3.24
Wings	110.0	115.5	118.5	111.0	111.3	5.93
Thigh	508.0	501.5	466.0	479.0	479.5	5.90
Abdominal fat	22.5	29.5	23.5	23.0	23.0	2.85

[†]The treatments included feeding of commercial diet as a control (CD) and the same supplemented with *M. chamomilla* at 0.3 (MC-0.3), 0.6 (MC-0.6), 0.9 (MC-0.9) and 1.2 (MC-1.2) per cent levels

^{ab}Means with different letters within treatments differ significantly ($P < 0.05$).

Table 4. Effects of graded levels of chamomile supplementation on clinical biochemistry of the broilers

Attributes	Dietary groups [†]					SEM
	CD	MC-0.3	MC-0.6	MC-0.9	MC-1.2	
Glucose, mg/dL	174.2 ^{ab}	176.4 ^{ab}	187.5 ^a	154.4 ^b	183.6 ^{ab}	11.1
Cholesterol, mg/dL	135.4	211.8	141.0	139.7	216.8	7.1
Triglycerides, mg/dL	67.2	86.4	64.4	30.5	61.5	13.7
HDL, mg/dL	78.0 ^b	81.2 ^{ab}	83.0 ^{ab}	89.5 ^{ab}	95.5 ^a	7.1
LDL, mg/dL	74.2	106.4	69.1	44.1	109.0	13.8
Uric acid, mg/dL	2.4 ^a	3.8 ^{ab}	4.9 ^b	3.8 ^{ab}	5.0 ^b	0.79

[†]The treatments includee feeding of commercial diet as a control (CD) and the same supplemented with *M. chamomilla* at 0.3 (MC-0.3), 0.6 (MC-0.6), 0.9 (MC-0.9) and 1.2 (MC-1.2) per cent levels

HDL: high density lipoprotein; LDL: high density lipoprotein.

^{ab}Means with different letters within treatments differ significantly ($P < 0.05$).

as compared with all other groups. While there were no effects ($P > 0.05$) of the dietary treatments on the plasma levels of cholesterol, triglyceride and LDL, the HDL values were found to be significantly ($P < 0.05$) higher in the MC-1.2 group compared to control (CD) group. The serum levels of uric acid was significantly ($P < 0.05$) higher in MC-0.6 and MC-1.2 groups compared with the CD group which showed the lowest value.

The immune response data are shown in Table 5. No significant differences were found in SRBC and the IgG titres when compared to control and with other treatments. But differences ($P < 0.05$) were found in IgM values where MC-0.3, MC-0.6 and MC-0.9 groups showed significant increase. Furthermore, differences ($P < 0.05$) were found in ND titres where MC-0.3, MC-0.9 and MC-1.2 groups showed the highest levels.

The viable counts of bacteria (lactobacilli, *E. coli* and coliforms) are summarized in Table 6. While there was no variations ($P > 0.05$) apparent in the *E. coli* counts in the digesta, a higher ($P < 0.05$) lactobacilli count was found in the MC-0.6 and MC-0.9 groups in comparison to the control CD group. However, the coliform count was significantly ($P < 0.05$) higher in MC-0.6 group in comparison to all other dietary groups.

Table 5. Effects of graded levels of chamomile supplementation on immune parameters of broilers

Attributes	Dietary groups [†]					SEM
	CD	MC-0.3	MC-0.6	MC-0.9	MC-1.2	
<i>Immune response for total SRBC, IgG and IgM (log₁₀) values</i>						
SRBC	3.7	4.5	4.2	3.5	4.5	0.63
IgG	1.7	1.5	2.0	1.7	2.5	0.49
IgM	2.0 ^b	3.0 ^a	2.2 ^{ab}	2.7 ^{ab}	2.0 ^b	0.42
<i>Immune response to Newcastle Disease</i>						
HI titre (log ₁₀)	2.5 ^b	3.7 ^a	2.2 ^b	4.5 ^a	4.0 ^a	0.51

[†]The treatments included feeding of commercial diet as a control (CD) and the same supplemented with *M. chamomilla* at 0.3 (MC-0.3), 0.6 (MC-0.6), 0.9 (MC-0.9) and 1.2 (MC-1.2) per cent levels

^{ab}Means with different letters within treatments differ significantly ($P < 0.05$).

IgG: immunoglobulin G; IgM, immunoglobulin M; SRBC: sheep red blood cells; ND: Newcastle disease.

Table 6. Effects of graded levels of chamomile supplementation on viable counts bacteria in the intestinal digesta of broilers

Attributes	Dietary groups [†]					SEM
	CD	MC-0.3	MC-0.6	MC-0.9	MC-1.2	
Lactobacilli, log ₁₀ CFU/g	5.0 ^b	5.6 ^{ab}	6.7 ^a	6.5 ^a	6.1 ^{ab}	0.27
Coliforms, log ₁₀ CFU/g	5.7 ^b	5.7 ^b	7.0 ^a	6.0 ^b	5.9 ^b	0.44
<i>E. coli</i> , log ₁₀ CFU/g	5.4	5.2	6.0	5.2	4.5	0.45

[†]The treatments included feeding of commercial diet as a control (CD) and the same supplemented with *M. chamomilla* at 0.3 (MC-0.3), 0.6 (MC-0.6), 0.9 (MC-0.9) and 1.2 (MC-1.2) per cent levels.

^{ab}Means with different letters within treatments differ significantly ($P < 0.05$).

DISCUSSION

In the last decade, many studies have reported a wide range of properties of *Matricaria chamomilla* in humans and animals (McKay and Blumberg, 2006). Dada *et al.* (2015) reported no beneficial effects in broiler chicks receiving MC at 0.002 and 0.004% in feed and 0.0018 and 0.0036% in water, which could have been due to the facts that these dosages were very low. Furthermore, Hamada *et al.* (2015) also reported that chamomile supplementation at different levels (0.25, 0.50, 0.75 and 1%) reduced ($P < 0.05$) the final BW and weight gain in broilers. However, the results of this study are in contrast to these reports since chamomile supplementation did not affect feed intake and body weight gain among the dietary groups, although the FCR and EPI values over the 42-d period showed differences ($P < 0.05$) among the groups. However, these were similar when period-wise (d 1-21 or d 22-41) comparisons were made. Interestingly, the lowest performance in terms of FCR and EPI over the 42-d period were recorded with chamomile supplementation at 0.6% level.

Although there were no significant differences in weight gain or feed intake among the groups, the addition of MC flower powder at graded levels in the diet did not affect palatability and acceptability of the feed. In general terms, although our study revealed that the inclusion of MC into the diet at different levels did not cause apparent adverse effects (neither digestive distress nor mortality), the chamomile supplementation seems not to be useful in terms of augmenting the growth performance.

For carcass characteristics, significant differences were found in neck and spleen weights only. In contrast, a previous study assessing MC use in broilers reported significant differences in liver and gizzard weights (Al-Kaisse and Khalel, 2011).

Regarding clinical biochemistry, although significant differences were found in glucose plasma levels, HDL and uric acid for the different MC groups when compared with the control, but all these were within the normal ranges in poultry (Campbell, 2004), and hence may not be of any practical relevance.

Recent studies have reported beneficial effects of MC in human patients with type-2 diabetes and poultry in terms of significantly diminished total cholesterol, triglyceride and low-density lipoprotein cholesterol levels (Khan *et al.*, 2014; Rafraf *et al.*, 2015). We found that glucose plasma levels were lower with 0.9% level of chamomile supplementation and not at the other levels. In contrast, cholesterol, triglyceride and LDL did not show differences ($P > 0.05$) among groups.

Furthermore, broilers fed on MC-0.6 and MC-12 had the highest values of uric acid when compared with the control (CD) group. It is, therefore, apparent that the effects of the bioactives present in MC on the metabolism could not be elucidated in a clear manner in the present study. Regarding immune response, significant differences were found in ND titre values indicative of a higher antibody response

with MC supplementation with the exception of MC-0.6 group. Differences ($P < 0.05$) were also found in IgM values, showing significant increases in MC-0.3 groups. The immunomodulatory activity in MC could be attributed to initiation of immunostimulating properties of heavy erythrocytes (macrocytes), activation of immunoregulation cells of peripheral blood, and increased sensitivity of effector cells to helper signals (Uteshev *et al.*, 1999).

Recent studies have reported the antibacterial activity of MC (Motealleh *et al.*, 2014; Parlinska-Wojtan and Kus-Liskiewicz, 2016; Hamady *et al.*, 2017). In our study, the lactobacilli counts showed an increase in almost all MC groups when compared with the control. However, coliform counts were significantly higher in MC-0.6 group. Although the antimicrobial spectrum against enteric and pathogenic bacteria of lactobacilli strains is well known, the present study would indicate that antibacterial property of MC in broilers is without any clear trend.

CONCLUSION

The present study indicates that the positive effects, if any, of dietary supplementation of chamomile in broilers apparently failed to present a definite trend, further evaluation is needed for establishing the effective level of supplementation and their potential effects that MC would have in broilers.

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