



Short Communication

Evaluation of *Calendula officinalis* L. (marigold) flower as a natural growth promoter in comparison with an antibiotic growth promoter on growth performance, carcass traits and humoral immune responses of broilers

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ABSTRACT

The objective of this experiment was to investigate the effects of dried powder of *Calendula officinalis* L. (marigold) flower as an antibiotic growth promoter substitute on growth performance, organ weights, and immunological parameters in broilers. A total of 240 mixed sex broilers (Ross 308) at 1 d of age were individually weighed and randomly assigned to 4 treatments with 4 replicates of 15 birds for 6 wk. The dietary treatments were: 1) a basal diet (control); 2) control plus 4.5 mg flavophospholipol/kg of diet; 3) control plus 5 g marigold/kg of diet; 4) control plus 10 g marigold/kg of diet. Antibody responses against Newcastle (NDV), influenza (AI) viruses, and sheep red blood cells (SRBC) were measured. Supplementing 4.5 mg flavophospholipol/kg of diet enhanced body weight (BW) of broilers at 14 and 28 d of age ($P < 0.05$), but final BW at 42 d was not markedly affected. At 14 and 28 d of age, broilers fed diets supplemented with 10 g marigold/kg of diet had significantly lower BW compared with broilers fed the basal diet supplemented with antibiotic or 5 g marigold/kg of diet. During the starter period, broilers fed diets supplemented with antibiotic had significantly ($P < 0.05$) better FCR compared with broilers supplemented with 10 g marigold/kg of diet, but did not differ from broilers fed the basal diet or basal diet supplemented with 5 g marigold/kg of diet. There were no significant differences in FCR between treatments, during grower, finisher phases as well as for the whole experiment. Carcass yield was significantly ($P < 0.05$) higher in broilers supplemented with 5 g marigold/kg of diet compared with broilers in other groups. The treatments failed to induce any marked effect on immune parameters. In conclusion, the results of this experiment showed that supplementation of 5 and 10 g dried powder of marigold/kg of diet has no affirmative influence on growth performance of broilers.

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1. Introduction

Antibiotic growth promoters (AGP) have been used in animal diets for more than 50 yr to promote growth and protect health of

the birds in the Persia and other countries (Feanti et al., 1971; Ghalamkari et al., 2012; Fekri Yazdi et al., 2014a; b; Goodarzi et al., 2014). The AGP were supposed to improve growth performance by control growth and proliferation of pathogen microorganisms in gastrointestinal tract, resulting in better digestion, absorption and metabolism of nutrients (Kheiri et al., 2018; Gheisari et al., 2017). Although application of AGP shows some affirmative effect on poultry production, the continuous consumption resulted in problems such as development of cross-resistance and multiple antibiotic resistances in pathogenic bacteria (Sorun and Sunde, 2001), drug residues in the final product, and dysbacteriosis (Andremont, 2000). Because of the immense need for suitable AGP alternatives, probiotics (Landy N, Kavayani, 2014;

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Toghyani et al., 2015), prebiotics, and aromatic plants and herbal products (Landy et al., 2012) have received increased attention as possible AGP substitutions.

Marigold (*Calendula officinalis* L.) is a medicinal plant in the genus *Calendula* of the family Asteraceae and that is widely distributed in Germany, Persia, Australia, Czech, Austria, Switzerland, Hungary, Egypt and Syria. Marigold flower possess many active substances such as saponins, flavonoids (Vahed et al., 2016) and carotenoids which have antioxidant effect and are essential for the immune system (Breithaupt, 2007; Jung et al., 2012). Furthermore, Wang et al. (2017) reported that dietary supplementation of marigold extract enhances the antioxidant capacity and meat quality in broilers. Rajput et al. (2012) reported that dietary supplementation with marigold flower extract increases carcass trait, antibody titers against Newcastle Disease virus (NDV) and Avian Influenza virus (AIV), and growth performance of broilers. Vahed et al. (2016) observed the ability of marigold oil extract to decrease the negative effects of CCl₄-induced hepatic cell injuries and reported that it can be used as a suitable natural antioxidant in poultry nutrition. Keeping in view the mentioned pharmaceutical advantages of marigold derivatives, an investigation was carried out to examine the impact of 2 levels of dried powder of marigold flower on growth performance, carcass characteristics and immune responses in broilers.

2. Material and methods

2.1. Ethical matters

The broilers were raised in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. Handling, sampling and killing of broilers complied with the ethical guidelines of the Isfahan Azad University's Ethical Committee (approval ref no. 2018-032).

2.2. Animals and dietary treatments

A total of 240 one-day-old newly hatched broiler chicks (Ross 308) were purchased from a local hatchery, individually weighed (35 ± 1 g) and distributed randomly to 4 treatment groups with 4 replicates of 15 birds. The dietary treatments were: 1) a basal diet (control), 2) control plus 4.5 mg flavophospholipol/kg of diet, 3) control plus 5 g marigold/kg of diet, 4) control plus 10 g marigold/kg of diet. Nutrient concentrations of the basal diet met or exceeded minimum requirements of the 2014 Ross 308 broiler strain (Aviagen, 2014).

Chicks were fed starter diets from d 1 to 14, grower diets from d 15 to 28, and finisher diets from d 29 to 42 (Table 1). Chicks were raised on floor pens (120 cm × 120 cm × 80 cm), provided supplemental heat and ventilation, and given free access to feed and water. The lighting program was provided continuously for the first week. The duration was reduced gradually (1 h per d) to 18 h by d 13, which was maintained throughout the study. The ambient temperature was initially maintained at 32 °C. It was gradually lowered by 2 °C/wk, and finally fixed at 22 °C till the end of the experiment.

2.3. Performance and carcass components

Chicks were weighed at 1, 14, 28, and 42 d of age. Daily feed intake (DFI) was measured during starter, grower, finisher and entire the experimental period. Mortality was recorded as it occurred to correct DFI. Feed conversion ratio (feed:gain) was calculated.

At 42 d of age, following weighing, 8 broilers/treatment were selected, based on the average weight of the group and sacrificed by

Table 1

Ingredients and calculated composition of basal starter, grower, and finisher diets.

Item	Starter	Grower	Finisher
Ingredients, g/kg (as-fed)			
Corn (8% CP)	549.6	555.1	612.5
Soybean meal (44% CP)	387.0	366.0	310.0
Soybean oil	13.6	34.0	36.0
Mono calcium phosphate (15% Ca, 22.7% P)	15.6	13.7	12.0
Calcium carbonate	16.0	14.8	13.7
Sodium chloride	1.6	1.9	1.9
Sodium bicarbonate	2.7	2.3	2.3
Trace mineral premix ¹	2.5	2.5	2.5
Vitamin premix ²	2.5	2.5	2.5
DL-methionine	3.7	3.3	3.0
L-lysine	3.0	2.1	2.1
L-threonine	1.0	0.7	0.5
Choline chloride	1.2	1.1	1.0
Calculated composition, g/kg			
Metabolizable energy, kcal/kg	2,870	3,027	3,110
Crude protein	220	210	190
Calcium	9.5	8.7	7.9
Available P	4.8	4.3	3.9
Methionine + Cysteine	10.3	9.6	8.8
Lysine	13.8	12.6	11.3
Threonine	9.3	8.6	7.6

¹ Provided the following per kilogram of diets: Mg, 120 mg; Fe, 20 mg; Cu, 16 mg; Zn, 110 mg; Se, 0.3 mg; I, 0.25 mg.

² Provided the following per kilogram of diets: vitamin A, 12,000 IU; vitamin D₃, 5,000 IU; vitamin E, 80 IU; vitamin K, 3.2 mg; riboflavin, 8.6 mg; vitamin B₁₂, 0.017 mg; pantothenic acid, 20 mg; nicotinic acid, 65 mg; folic acid, 2.2 mg.

cervical dislocation. Internal organs including abdominal fat, liver, gizzard, heart, proventriculus, and small intestine were excised, weighed and calculated as a percentage of live weight. Carcass yield was measured accordingly and ceca and small intestine length were assayed.

2.4. Immunity

At 9 d of age, each bird was subcutaneously vaccinated with 0.2 mL an inactivated oil emulsion against NDV and AIV. At 21 d of age, chicks were also orally revaccinated with live LaSota Newcastle disease (ND) vaccine. At 42 d of age, birds' spleen and bursa of Fabricius were removed, weighed and expressed as a percentage of live weight. At 25 d of age, 8 birds per treatment were injected intravenously with 1 mL of 1% sheep red blood cell (SRBC) suspension. At 6 d post-SRBC, blood samples were collected to measure antibody titers against SRBC by the microtiter procedure described by Wegmann and Smithies (1966). At 28 d of age, 8 birds per treatment were selected based in the mean of cages, and blood samples were taken to determine antibody titers against NDV and AIV. Serum antibody titers for NDV and AIV were measured using a hemagglutination inhibition test (HI) and HI antibodies were then converted to log₂.

2.5. Statistical analysis

Data were analyzed using the General Linear Model procedures of SAS (SAS, 2012). Means were separated using Duncan's multiple range test at significance level of $P < 0.05$.

3. Results and discussion

3.1. Performance and carcass traits

Data on performance indices are summarized in Table 2. Final BW of broilers did not statistically differ between treatments, although it tended ($P > 0.05$) to increase in broilers fed the basal diet supplemented with antibiotic. At 14 d of age, broilers

Table 2
Effect of experimental diets on the performance of broilers at different ages.

Item	Dietary treatments				SEM	P-value
	Control	antibiotic	5 g marigold/kg	10 g marigold/kg		
Body weight, g						
14 d	266.9 ^{bc}	280.2 ^a	273.0 ^{ab}	257.3 ^c	2.09	0.04
28 d	1,000.0 ^{abc}	1,031.2 ^a	1,022.0 ^{ab}	961.2 ^c	7.76	0.03
42 d	2,023.4	2,084.0	2,009.6	2,028.4	16.16	0.08
Daily feed intake, g/d						
1 to 14 d	22.6	23.3	23.2	22.3	0.70	0.09
15 to 28 d	91.8	91.3	87.9	87.3	6.25	0.07
29 to 42 d	131.3	136.8	131.9	136.1	3.45	0.06
1 to 42 d	81.0	82.9	81.1	81.0	1.48	0.08
Feed:gain, g/g						
1 to 14 d	1.36 ^{ab}	1.33 ^b	1.36 ^{ab}	1.41 ^a	0.02	0.04
15 to 28 d	1.75	1.70	1.64	1.73	0.07	0.09
29 to 42 d	1.79	1.81	1.87	1.78	0.09	0.07
1 to 42 d	1.71	1.70	1.72	1.70	0.08	0.07

^{a–c} Within a row, values not sharing a common superscript differ at $P < 0.05$.

supplemented with the antibiotic had higher ($P < 0.05$) BW compared with broilers in control group, and broilers supplemented with 10 g marigold/kg of diet did not differ ($P > 0.05$) from broilers supplemented with 5 g marigold/kg. Similarly, at 28 d of age, the highest ($P < 0.05$) BW is obtained in broilers fed diets containing antibiotic compared with broilers supplemented with 10 g marigold/kg of diet, and BW did not differ ($P > 0.05$) from broilers fed the basal diet or basal diet supplemented with 5 g marigold/kg of diet. Treatments failed to induce any marked effect on feed consumption during all of the experimental growth phases as well as for the whole experiment, although during the grower phase (14 to 28 d), it tended ($P > 0.05$) to decrease in broilers supplemented with 5 or 10 g marigold/kg of diet. Significant differences between treatments were noted in FCR during the starter phase, and broilers fed diets containing antibiotic had significantly ($P < 0.05$) better FCR compared with broilers supplemented with 10 g marigold/kg of diet, but did not differ ($P > 0.05$) from broilers fed the basal diet or basal diet supplemented with 5 g marigold/kg of diet. There were no significant differences in FCR between treatments, during grower, finisher phases as well as for the whole experiment. As AGP could control and reduce the growth of opportunistic pathogens and subclinical infection in chicks' gut, thus it may conduct to a better feed utilization, resulting in increased growth performance (Bedford, 2000). Similar to our results, Kavyani et al. (2012) reported a greater efficiency in the feed utilization in broilers fed diets containing flavophospholipol, resulting in a better FCR compare with the control group. Landy et al. (2011a) reported the affirmative impact of flavophospholipol on the final BW of broilers. In the current trial, the positive impact of antibiotic on BW of broilers is more pronounced at 14 and 28 d of age, and it did not reflect at 42 d of age probably due to a better developed digestive tract in older birds. In the present trial, significant differences between treatments was noted in FCR during the starter phase, although there were no significant differences in FCR between treatments, during grower, finisher phases as well as for the whole experiment. Similarly, Wang et al. (2017) reported that dietary marigold extract supplementation had not any marked effect on growth performance of broilers. Rajput et al. (2012) reported that dietary supplementation of marigold extract flower improved live BW of broilers, however, DFI and FCR of broilers were not affected by the dietary treatments. Mirzah and Djulardi (2017) reported that dietary supplementation of marigold flower extract improved production performance of laying quails. Probably the dosage applied in the present trial has not been such a level that would cause a positive effect on growth performance, since there are reports of beneficial influences of

marigold flower extract on performance criteria of broilers. In the current trial, BW of broilers at 14 and 28 d of age were significantly higher in broilers supplemented with 5 g marigold/kg of diet than in broilers supplemented with 10 g marigold/kg of diet. Landy et al. (2011b) reported that addition of 5 g dried aerial part of *Echinacea purpurea* L. per kilogram of diet had beneficial effects on performance parameter when compared to the 10 g/kg of diet. Our findings on performance parameter are in accord to those of Toghiani et al. (2010) who reported that supplementation of high dosage of medicinal plants in the diet may have adverse effects on some beneficial microbial populations, resulting in lower growth rate. Furthermore, as reported, marigold possesses polyphenols (Gong et al., 2012), acylated flavonoid-O-glycosides and methoxylated flavonoids, amino acids (Abasova et al., 1995), alkaloids, carotenoids, saponins, tannins (Duke, 2000). Tannins can bind proteins in gastrointestinal tract and decrease digestion and absorption of proteins resulting in decreased growth performance.

In the current trial carcass yield, gizzard and abdominal fat pad were significantly ($P < 0.05$) influenced by the dietary treatments (Table 3). Relative gizzard weight was affected by the dietary treatments, so that the relative weight of gizzard was greater in broilers supplemented with 10 g marigold/kg of diet compared with broilers fed the basal diet, but did not differ ($P > 0.05$) from broilers fed the basal diet supplemented with antibiotic or 5 g marigold/kg of diet. Relative weight of abdominal fat was greater in broilers supplemented with 10 g marigold/kg of diet compared with broilers fed the basal diet or basal diet supplemented with 5 g marigold/kg of diet, but did not differ ($P > 0.05$) from broilers fed the basal diet supplemented with antibiotic. As reported, marigold possesses tannins (Duke, 2000); tannins can bind proteins, reduce the absorption of proteins resulting in higher deposition of fat. Carcass yield was significantly ($P < 0.05$) higher in broilers supplemented with 5 g marigold/kg of diet compared with broilers fed the basal diet, the basal diet supplemented with the antibiotic or the basal diet supplemented with 10 g marigold/kg of diet. The relative weights of other internal organs were not markedly affected by the dietary treatments. These results are consistent with results reported by Hernandez and Madrid (2004) and Landy et al. (2011a) who did not find any differences among the control group and those supplemented with neem (*Azadirachta indica*) or mixtures of plant extracts on relative weight of organs.

3.2. Immune responses

The effects of experimental diets on immune parameter are presented in Tables 3 and 4. Relative weights of bursa of Fabricius

Table 3
Effects of marigold flower and antibiotic on carcass yield and internal relative organ weight of broilers at 42 d of age (%).

Item	Dietary treatments				SEM	P-value
	Control	antibiotic	5 g marigold/kg	10 g marigold/kg		
Carcass	65.6 ^b	65.7 ^b	66.6 ^a	65.3 ^b	0.27	0.03
Abdominal fat	0.54 ^b	0.57 ^{ab}	0.42 ^b	0.79 ^a	0.03	0.04
Liver	2.88	2.69	2.61	2.67	0.04	0.07
Gizzard	1.72 ^b	1.83 ^{ab}	1.94 ^{ab}	2.04 ^a	0.04	0.03
Heart	0.59	0.65	0.68	0.64	0.01	0.07
Proventriculus	0.41	0.41	0.40	0.41	0.01	0.06
Small intestine	3.66	3.73	3.61	3.64	0.09	0.07
Bursa	0.115	0.119	0.132	0.131	0.005	0.08
Spleen	0.15	0.16	0.12	0.13	0.007	0.08
Small intestine, cm	194	189	185	197	2.0	0.09
Cecum, cm	37	37	38	38	0.6	0.07

^{a, b} Within a row, values not sharing a common superscript differ at $P < 0.05$.

Table 4
Effects of marigold flower and antibiotic on antibody titers against Newcastle and Influenza viruses at 28 d and sheep red blood cells (SRBC) at 31 d of age (\log_2).

Item	Dietary treatments				SEM	P-value
	Control	antibiotic	5 g marigold/kg	10 g marigold/kg		
New castle	7.0	7.1	6.9	6.8	0.13	0.07
Influenza	3.4	3.3	3.9	3.7	0.17	0.08
SRBC	7.0	7.0	6.1	7.4	0.57	0.06

and spleen were not affected by the dietary treatments. The differences in antibody titers against NDV and AI viruses at 28 d of age and SRBC at 31 d of age were not significant among treatments. Rajput et al. (2012) reported that marigold flower extract supplementation enhanced relative thymus weight, although spleen and bursa weights were not affected by the dietary treatments. The researchers reported that marigold flower extract supplementation enhanced antibody titers against NDV and AI viruses. Wang et al. (2017) reported that marigold extract supplementation enhanced the total antioxidant activity, and it also improved the activities of superoxide dismutase in thigh muscle and liver. As marigold flower has been reported to have many active substances such as saponins, flavonoids (Vahed et al., 2016) and carotenoids which have antioxidant effects, an enhance in immune responses of chicks was anticipated. However, treatments failed to induce any significant effect on immune related parameters, which is probably due to the dosage applied in the present trial.

4. Conclusion

In conclusion, the overall results of the current study showed that supplementation of 5 and 10 g dried powder of marigold/kg of diet has no affirmative influence on growth performance of broilers. The results indicated that more experiments are needed to investigate the probable effects of lower levels of marigold in broilers.

Conflicts of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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