

SHORT COMMUNICATION

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## Effects of purslane extract on performance, immunity responses and cecal microbial population of broiler chickens

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### Abstract

The aim of present study was to evaluate the effect of purslane extract on performance, cecal microflora composition and immune responses of broiler chickens. One hundred and ninety two 1-day old broiler chicks (Ross 308) were allocated randomly in 4 groups with 4 replicates to receive diets supplemented with 0 (control), 100, 200 and 300 ppm of purslane extract for 42 days. Body weight gain, feed intake (FI) and feed conversion ratio were measured weekly and calculated for starter (1-21 d), grower (22-42 d) and overall periods (1-42 d). All diets were isocaloric, isonitrogenous and provided *ad libitum*. Antibody response against sheep red blood cell (SRBC) was measured on d-28 and d-42. At the age of 42 d, eight chicks per treatment killed aseptically for enumeration of cecal bacteria. The results of this experiment indicated that FI increased significantly with inclusion of purslane extract in grower and overall period ( $p \leq 0.05$ ). Purslane extract did not affect coliform and *Escherichia coli* populations but increased *Lactobacillus* population of cecal content significantly ( $p \leq 0.05$ ). There were no significant differences in primary and secondary antibody titer against SRBC and no differences among the treatments for relative weight of thymus and spleen ( $p > 0.05$ ). Relative weight of bursa was affected with inclusion of purslane extract in the diet. Therefore, it was concluded that purslane inclusion had a positive significant effect on cecal microflora composition, but had no effect on immune response of broiler chickens.

**Additional key words:** broiler performance; cecal microflora; immune response; antibody titer.

*Portulaca oleracea* L. (commonly called purslane) is an herbaceous weed widely distributed throughout the world and cultivated in some countries as Iran. The plant has been used as a vegetable and for medical purpose for hundreds of years. Purslane is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term 'Global Panacea' (Lim & Quah, 2007). Its nutritional potential was first reported by Simopoulos & Salem (1986) who indicated that purslane was the richest vegetable sources of omega-3 fatty acids. In addition, similar studies have revealed that purslane is a rich source of nutrients like flavonoids (kaempferol, quercetin and apigenin),

vitamins A, C and E, beta-carotene, and minerals (Cai *et al.*, 2004; Lim & Quah, 2007; Dkhil *et al.*, 2011).

The use of purslane as a feed supplement in poultry has been reported by several workers. Aydin & Dogan (2010) working with laying hens suggested that supplementing diets with dried purslane increased egg production and egg weight. Zhao *et al.* (2013) reported that supplementing 0.2% purslane extract to broiler feed improved weight gain and feed conversion ratio (FCR), increased the population of *Lactobacillus* sp. and *Bifidobacterium* sp. and decreased the population of *Escherichia coli* in the gastrointestinal tract. The objective of the present study was to confirm previous

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Abbreviations used: BW (body weight); BWG (body weight gain); CFU (colony forming units); FCR (feed conversion ratio); FI (feed intake); HA (haemagglutination); ME (metabolizable energy); SRBC (sheep red blood cell).

work on dietary supplementation of purslane on growth performance, cecal microbial population and immune response in broiler chickens.

The experiment was conducted at the poultry station of Ramin Agricultural and Natural Resources University in Iran. One hundred and ninety two 1-d old broiler chicks (Ross 308) of mixed sex were purchased from a local hatchery (Sahrae Jonoub, Khuzestan Province). On the first day, 12 chicks were placed randomly into each of 16 pens (100 × 150 cm). The trial was planned as randomized design during a period of 42 days. A lighting program of 23L:1D was used for the entire 3-42 d growing period. The chicks (4 replicate pens of 12 birds each) were fed a corn-soybean meal-based diet supplemented with 0 (control), 100 (Pur1), 200 (Pur2) and 300 (Pur3) ppm of purslane extract. Each replicate pen was considered as an experimental unit. All diets were isocaloric and isonitrogenous (2983.1 kcal kg<sup>-1</sup> metabolizable energy (ME), 21.30% crude protein in starter period and 3012.6 kcal kg<sup>-1</sup> ME, 18.90% crude protein in grower period). Feed and water were provided *ad libitum*. The ingredient composition of the experimental diets is shown in Table 1.

Purslane was cultured in the herbal station of Ramin Agricultural and Natural Resources University and harvested during their flowering period. The parts of the plant suitable for consumption, which consisted of the soft upper stems of the plant and leaves, were used. Purslane samples were dried in an oven at 40°C. For preparation of hydroalcoholic extract, 2 kg of purslane powder was weighted and macerated with 10 L ethanol (80%) for 72 hours at room temperature. The extract was then shacked, filtered and evaporated in rotating evaporator under reduced pressure until solvent was disappeared (Movahedian *et al.*, 2007).

Broiler performance parameters such as body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured weekly and calculated for starter (1-21 d), grower (22-42 d) and whole (1-42 d) periods.

For determination of immune response, at 21 and 35 days of age, 1 mL of 5% SRBC was injected into brachial vein of 2 chicks per cage and blood samples were taken in non-heparinized tubes by puncturing the brachial vein 7 days after each injection. Serum was obtained by centrifuging at 1,500 ×g for 15 min at 25°C, and stored at -20°C until assayed. Individual serum samples were analyzed for antibody responses against SRBC by haemagglutination (HA) method as

**Table 1.** Composition and calculated analyses of the basal diets

Item (%)	Starter diet (days 1-21)	Grower diet (days 22-42)
Maize	50.30	53.55
Soybean meal	28.30	29.00
Fish meal	5.00	0.00
Wheat	7.00	7.00
Wheat middling	3.00	3.00
Vegetable oil	3.00	3.50
Dicalciumphosphate	1.10	1.50
L-Lys-HCl	0.15	0.10
DL-Met	0.25	0.25
Sodium chloride	0.20	0.28
Oyster shell	1.00	1.12
Sodium bicarbonate	0.15	0.15
Vit. and min. premix <sup>1</sup>	0.50	0.50
<b>Calculated analysis</b>		
ME <sup>2</sup> (kcal kg <sup>-1</sup> )	2983	3013
Crude protein	21.30	18.90
Calcium	0.97	0.85
Available phosphorus	0.48	0.42
Methionine + Cystine	1.01	0.86
L-Lysine	1.33	1.09
Na	0.16	0.17

<sup>1</sup> Vitamin and mineral premix supplied the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 1,800 IU; vitamin E, 11 mg; vitamin K3, 2 mg; vitamin B2, 5.7 mg; vitamin B6, 2 mg; vitamin B12, 0.024 mg; nicotinic acid, 28 mg; folic acid, 0.5 mg; pantothenic acid, 12 mg; choline chloride, 250 mg; Mn, 100 mg; Zn, 65 mg; Cu, 5 mg; Se, 0.22 mg; I, 0.5 mg; and Co, 0.5 mg. <sup>2</sup> ME: metabolizable energy.

described previously (Khatibjoo *et al.*, 2011). At the end of the experiment, two birds per pen were selected randomly and lymphoid organs including spleen, bursa of fabricius and thymus were weighed and their relative weights were calculated (percentage of live body weight).

At the age of 42 d, two birds per cage that had not been injected with SRBC were selected randomly and killed aseptically and one gram of cecal content of each bird was pooled for serial dilution. Microbial population were determined by serial dilution of cecal samples.

*Lactobacillus* sp. was grown on Rogosa SL agar; *E. coli* and coliforms were grown on eosin methylene blue agar. Plates for *Lactobacillus* sp. were incubated anaerobically at 37°C. *E. coli* and coliforms were incubated aerobically at 37°C. Plates were counted between 24 and 48 h after inoculation. Colony forming units (CFU) were defined as distinct colonies measuring at least 1 mm in diameter.

**Table 2.** Influence of supplementation of purslane extract on growth performance of broiler chickens

Treatments <sup>1</sup>	d 1-21			d 22-42			d 1-42		
	FI	BWG	FCR	FI	BWG	FCR	FI	BWG	FCR
Control	926.1	691.4	1.34 <sup>b</sup>	2492.4 <sup>c</sup>	1366.7	1.83	3418.5 <sup>c</sup>	2058.2	1.66
Pur1	926.1	679.1	1.37 <sup>ab</sup>	2635.0 <sup>ab</sup>	1412.3	1.87	3561.1 <sup>ab</sup>	2091.4	1.70
Pur2	926.0	673.2	1.38 <sup>a</sup>	2591.5 <sup>b</sup>	1412.5	1.84	3517.4 <sup>bc</sup>	2085.7	1.69
Pur3	930.8	671.7	1.39 <sup>a</sup>	2703.2 <sup>a</sup>	1451.7	1.87	3634.0 <sup>a</sup>	2123.4	1.71
SEM <sup>2</sup>	3.98	4.25	0.01	23.83	15.85	0.01	25.22	16.67	0.01
<b>p values</b>									
Linear effect	0.728	0.107	0.006	0.001	0.089	0.483	0.002	0.246	0.128
Quadratic effect	0.789	0.530	0.439	0.614	0.920	0.792	0.708	0.950	0.726
Cubic effect	0.899	0.956	0.802	0.025	0.553	0.317	0.041	0.607	0.258

<sup>1</sup> Control = corn-soybean basal diet. Pur1, Pur 2, Pur 3 = 100, 200, 300 ppm purslane extract, respectively. <sup>2</sup> SEM: n = 4 (4 replicates and 12 birds/replicate). <sup>a, b, c</sup> Means in the same column with different superscripts differ significantly ( $p < 0.05$ ). FI: feed intake. BWG: body weight gain. FCR: feed conversion ratio.

**Table 3.** Effects of different levels of purslane extract on cecal microbial population and immunity response of broilers at 42 days

Treatments <sup>1</sup>	Microbial population (log CFU g <sup>-1</sup> )			SRBC (log2)		Immunity organ (%)		
	<i>Lactobacillus</i>	Coliforms	<i>E. coli</i>	d 28	d 42	Spleen	Bursa	Thymus
Control	7.04 <sup>b</sup>	7.47	6.68	4.25	9.37	0.15	0.19 <sup>ab</sup>	0.43
Pur1	8.06 <sup>a</sup>	7.26	6.32	4.12	9.00	0.14	0.17 <sup>ab</sup>	0.43
Pur2	7.25 <sup>b</sup>	7.41	6.27	3.88	8.88	0.13	0.23 <sup>a</sup>	0.56
Pur3	7.89 <sup>a</sup>	7.41	6.52	4.62	8.50	0.18	0.16 <sup>b</sup>	0.43
SEM <sup>2</sup>	0.14	0.05	0.12	0.13	0.17	0.02	0.01	0.03
<b>p value</b>								
Linear effect	0.066	0.952	0.641	0.458	0.086	0.670	0.759	0.578
Quadratic effect	0.333	0.309	0.227	0.103	1.000	0.311	0.249	0.306
Cubic effect	0.002	0.274	0.996	0.341	0.748	0.749	0.048	0.177

<sup>1</sup> Control= corn-soybean basal diet. Pur1, Pur 2, Pur 3= 100, 200, 300 ppm purslane extract, respectively. <sup>2</sup>SEM: n=8 (8 replicates and 1 bird/replicate). <sup>a, b, c</sup> Means in the same column with different superscripts differ significantly ( $p < 0.05$ ). CFU: colony forming units. SRBC: sheep red blood cell

For analysis of variance all data were analysed using the GLM procedure of SAS software with an average of 12 birds in a cage as the experimental unit for performance characteristics and an average of 2 birds as the experimental unit for microbiological characteristics and immune responses. Differences between treatment means were tested using Duncan's multiple comparison test. Statistical significance was declared at  $p \leq 0.05$ . Microbiological concentrations were subject to log10 transformation before analysis.

Table 2 shows that the diet had no significant effect on FI and BWG of broiler chicks during days 1-21. However, the FCR of birds fed 200 and 300 ppm purslane

extract were significantly poorer than the control birds. In the periods 22-42 d and 1-42 d, birds fed 300 ppm purslane extract consumed significantly more feed than controls and those supplemented with 200 ppm purslane extract. Diet had negligible effect on BWG and FCR during these periods.

Table 3 shows that supplementing broiler chickens with 100 and 300 ppm purslane extract significantly increased *Lactobacillus* spp. count in the caecum when compared to other groups. Diet, however, had no significant effect on coliform and *E. coli* counts. Primary and secondary antibody titres against SRBC were not significantly affected by diet. Except for bursa, the diet

did not significantly influence the mean relative weights of lymphoid organs.

Our findings suggest that supplementing diets with purslane extract did not improve overall weight gain and FCR. This results are in disagreement with others scientist's findings. Zhao *et al.* (2013) supplemented broiler diets with 2 and 4 g kg<sup>-1</sup> of *P. oleracea* extract and showed significant improvement in daily gain of broilers at both dosages. These inconsistencies between the two experiments could be attributed to dosage differences and variations in preparation of extract, rearing condition and composition of diet. The present study suggests that the beneficial effects of purslane were dependent on the age of chickens. Zhao *et al.* (2013) reported that the performance of broiler chickens was better on d-28 and d-42 of rearing periods when *P. oleracea* was added to their diet. Improvement in broiler performance in grower period when feeding purslane has been reported by other scientists. Ghorbani *et al.* (2013b) showed that broiler FI and BWG were increased with inclusion of 1% and 2% purslane powder in broilers diet in grower and overall periods. These scientists reported that adding purslane powder to broiler diet may be led to good palatability and increased FI and consequently weight gain.

From the gut perspective, phytogetic antimicrobial additives can changed gut microbial population and shifted it to positive colony. The antibacterial properties of plant extracts could be attributed mainly to their phenolic components and their mechanisms of action on the microbial cell (Si *et al.*, 2006). Some phenolic compounds such as resveratrol, hydroxytyrosol, kaempferol, quercetin, apigenin, and several phenolic acids have been reported to inhibit various pathogenic microorganisms (Cai *et al.*, 2004). Si *et al.* (2006) reported that it is possible to select phytogetic material with a strong antimicrobial action against gut pathogens while not harming beneficial bacteria such as bifidobacteria and lactobacilli.

The present findings showed that puslane extract at 100 and 300 ppm was beneficial in increasing intestinal *Lactobacillus* spp. count. However, supplemented diets had no significant effect on coliform and *E. coli* counts (Table 3). Zhao *et al.* (2013) reported that the use of purslane extract can increase the quantities of *Lactobacillus* and *Bifidobacterium* in the cecal content of broiler chickens. A possible explanation for the stimulatory effect of polyphenolic compounds on bacterial growth is that some microorganisms are able to

use these compounds as nutritional substrates. In the particular case of lactobacilli, these bacteria possess the ability to metabolize phenolic compounds supplying energy to cells and positively affecting the bacterial metabolism (Garcia-Ruiz *et al.*, 2008).

In the present study, broiler immunity was not affected by inclusion of purslane extract in the diets. Scientists reported that inclusion of purslane in diet has hypolepidemic properties (Movahedian *et al.*, 2007; Ezekwe *et al.*, 2011), can improve antioxidant status of broiler chicken (Ghorbani *et al.*, 2013a), and enhance the population of beneficial bacteria (Zhao *et al.*, 2013). Although, improvement in broiler immunity system were expected, but probably due to normal rearing condition in this study, the plant extract couldn't perform its positive effects. Steiner (2009) and Brenes & Roura (2010) believe that plant secondary metabolite can exert their effects on critical condition.

It can be concluded that purslane had a positive significant effect on cecal microflora composition, but had no effect on immune response of broiler chickens.

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