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 overall period (p ≤ 0.05). Purslane extract did not affect coliform and Escherichia coli populations but increased Lactobacillus population of cecal content significantly (p ≤ 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.
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−1 metabolizable energy (ME), 21.30% crude protein in starter period and 3012.6 kcal kg –1 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 conversation 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 xg 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 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 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                     | 5.00                     |
| 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 | 0.50                    | 0.50                     |

### Calculated analysis

|                        | Starter diet | Grower diet |
|------------------------|-------------|-------------|
| ME (kcal kg⁻¹)         | 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        |

1 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. 2 ME: metabolizable energy.
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 \textit{Lactobacillus} spp. count in the caecum when compared to other groups. Diet, however, had no significant effect on coliform and \textit{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⁻¹ 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, phytogenic 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 phytogenic material with a strong antimicrobial action against gut pathogens while not harming beneficial bacteria such as bifidobacteria and lactobacilli.

The present findings showed that purslane 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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