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The Effects of Different Levels of *Portulaca oleracea*, Medicinal Plant, on Performance, Egg Quality, Blood Biochemical and Immunity Parameters of Mature Laying Hens

Research Article

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ABSTRACT

This experiment was conducted to evaluate the effects of different levels of *Portulaca oleracea* (PO), medicinal plant, on performance, egg traits, blood biochemical and immunity parameters of laying hens. One hundred eighty Hi-Line (W-36 strain) mature laying hens 65 up to 76 weeks of age were allotted in a completely randomized design consisting of 5 treatments and 3 replicates (12 birds per replicate). Treatment groups consisted of a control group (1) with no PO supplementation and experimental groups 2, 3, 4 and 5 which contained 0.5%, 1.0%, 1.5% and 2.0% of PO powder, respectively. Using different levels of PO significantly affected the performance, egg traits, blood biochemical and immunity parameters of laying hens ($P<0.05$). The highest values for egg weight, egg production percentage, egg mass, feed intake, feed conversion ratio, egg yolk color index, albumin percentage and blood high density lipoprotein (HDL) were obtained by feeding 2.0% of PO. The highest level of blood hemoglobin was observed by feeding 1.0% of PO. The overall results showed that feeding *Portulaca oleracea* powder up to 2.0% in the diet has a positive effect on mature laying hens performance and egg traits.

KEY WORDS blood metabolite, egg production, egg traits, layer hens, medicinal plants.

INTRODUCTION

In poultry, herbs and spices are not only appetite and digestion stimulants, but also impact other physiological functions, help to sustain good health and welfare and improve their performance (Frankic *et al.* 2009). World Health Organization encourages use of medicinal herbs and plants to minimize the use of chemicals through the global trend to go back to nature. Attempts to use natural materials such as medicinal plants could be widely accepted as feed additives to enhance efficiency of feed utilization and animal productive performance (Levic *et al*. 2008). Francois (2006) found that medicinal herbs are desirable for stimulating digestion, particularly affecting bile secretion and activity of pancreatic enzymes. *Portulaca oleracea* (PO) is an herbaceous weed that contains many biologically active compounds and is a source of many nutrients. PO is one of the richest green plant sources of omega-3 fatty acids, such as alpha-linolenic acid (18:3 n-3), but also contains high levels of linoleic acid (18:2 n-6), both essential for normal human growth, health promotion and disease prevention (Zeinali *et al.* 2012). PO leaves also have high contents of antioxidants, such as α-tocopherol, ascorbic acid and glutathione (Simopoulos *et al.* 1992). Some recent studies investigated the effects of the dietary inclusion of PO supplement in poultry diets. It was reported that the use of PO powder up to 3.0% in quails diets has a positive effect on their performance (Zeinali *et al.* 2012). Also, it was reported that

PO supplementation significantly altered the fecal bacterial community without affecting the intestinal pH (Zhao *et al.* 2012) and that broilers` diets using PO grains up to 7.5% improved their performance and health status (Kaveh, 2012). With broilers` diets using PO polysaccharides, it was found an increase in growth performance and feed conversion and a decrease in the serum lipid level (Yung *et al.* 2013). Using 0.5 g/L of PO in ISA brown laying hens' water improved their egg production performance (Dougnon *et al.* 2012). Our study is focused on evaluating the effects of different dietary levels of *Portulaca oleracea* powder on performance, egg traits and blood biochemical and immunity parameters of laying hens.

MATERIALS AND METHODS

Animals and dietary treatments

One hundred eighty Hi-Line (W-36 strain) laying hens from 65 up to 76 weeks of age were allotted in a completely randomized design consisting of 5 treatments and 3 replicate (12 birds per replicate). The treatment groups consisted of a control group (1) with no PO supplementation, and experimental groups 2, 3, 4 and 5 containing 0.5%, 1.0%, 1.5% and 2.0% of PO powder, respectively. Powder aerial parts of PO were supplied from a local market and had the following compositions according to AOAC. (2002): crude protein (17.75%), crude fiber (31.22%), calcium (0.41%) and phosphorous (0.26%). The diets were formulated to meet the requirements of laying hens as established by the NRC (1994) (Table 1).

The diets and water were provided *ad libitum*. The lighting program during the experimental period consisted of 16 hours of light and 8 hours of darkness and mean environmental temperature was 15 ˚C.

Performance parameters and egg traits

Feed intake, feed conversion, egg production percent, egg mass and egg weight were determined weekly on bird bases. Mortality was recorded as it occurred. The collected eggs were classified as normal or damaged; the latter included the following: fully cracked eggs (an egg with broken shell and destroyed membrane), hairline cracked eggs (an egg with broken shell but intact membrane) and the eggs without shell (an egg without shell but with intact membrane).

For measurement of egg traits at the end of the experiment period, 3 eggs were sampled from each replicate. Determination of egg specific gravity was done by floating eggs in brine at different levels of salt. Content of the egg shells were cleaned and shells were maintained at environmental temperature for 48 h until dried, then weighed with a digital scale with an accuracy of 0.01 (g). The thickness of egg shell was measured by micrometer with an accuracy of 0.001 mm in the middle of the egg and in three spots on each of three eggs. Then their average was considered as final thickness of egg shell for each experimental unit. Color index of the yolk (Roche color index), yolk index, albumen index, Haugh units were determined according to Card and Nesheim (1972).

Table 1 Composition of experimental diets

Feeds ingredients	Portulaca oleracea (%)				
	0	0.5	1.0	1.5	2.0
Corn	50.00	50.00	50.00	50.00	50.00
Wheat	23.73	23.18	22.63	22.10	21.49
Soybean meal (44%)	16.31	16.25	16.21	16.15	16.15
Vegetable oil	0.24	0.36	0.48	0.61	0.73
Portulaca oleracea	0	0.50	1.00	1.50	2.00
Oyster shell	7.83	7.83	7.81	7.79	7.78
Dicalcium phosphate	1.11	1.10	1.10	1.09	1.09
Salt	0.28	0.28	0.27	0.26	0.26
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25
Calculated composition					
ME (kcal/kg)	2800	2800	2800	2800	2800
CP (%)	14.00	14.00	14.00	14.00	14.00
Ca (%)	3.28	3.28	3.28	3.28	3.28
Available phosphor (%)	0.31	0.31	0.31	0.31	0.31
CF (%)	2.86	3.28	3.98	4.54	4.54
Sodium (%)	0.15	0.15	0.15	0.15	0.15
Lysine (%)	0.63	0.63	0.63	0.63	0.63
Met + Cys (%)	0.55	0.55	0.55	0.55	0.55
Tryptophan (%)	0.18	0.18	0.18	0.18	0.18

[1] Vitamin premix per kg of diet: vitamin A (retinol): 8500000 IU; vitamin D_3 (cholecalciferol): 2500000 IU; vitamin E (tocopheryl acetate): 11000 IU; vitamin k_3: 2200 mg; Thiamine: 1477 mg; Riboflavin: 4000 mg; Panthothenic acid: 7840 mg; Pyridoxine: 7840 mg; Cyanocobalamin: 10 mg; Folic acid: 110 mg and Choline chloride: 400000 mg.
[2] Mineral premix per kg of diet: Fe ($FeSO_4$ $7H_2O$ 20.09% Fe): 75000 mg; Mn ($MnSO_4$ H_2O, 32.49% Mn): 74.4 mg; Zn (ZnO, 80.35% Zn): 64.675 mg; Cu ($CuSO_4$ $5H_2O$): 6000 mg; I (KI, 58% I): 867 mg and Se ($NaSeO_3$, 45.56% Se): 200 mg.
ME: metabolisable energy; CP: crude protein and CF: crude fiber.

Biochemical and hematological parameters and immunity cells

At the end of this experiment, two hens from each replicate were randomly chosen for blood collection from the brachial vein (approx. 5 mL). One mL of blood was transferred to tubes with ethylenediaminetetraacetic acid (EDTA) for determining heterophil, lymphocyte, hematocrit, hemoglobin, red blood cells and leukocyte blood cell counts. One hundred leukocytes per sample were counted by heterophil to lymphocyte separation under an optical microscope the heterophile to lymphocyte ratio was calculated and recorded (Gross and Siegel, 1983). The remaining 4 mL of blood was centrifuged to obtain serum for the determination of the biochemical parameters using the Anision-300 autoanalyzer system: glucose, cholesterol, triglyceride, albumin, total protein and uric acid that were accomplished with appropriate commercial kits (Pars Azmoon Company; Tehran, Iran).

Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS (2005). Means were compared using the Duncan multiple range test. Statements of statistical significance were based on P<0.05.

RESULTS AND DISCUSSION

Performance parameters

The effects of different levels of powdered aerial parts of PO in feeds on the performance of laying hens are summarized in Table 2. Using different levels of PO had significant positive effects on the performance of laying hens (P<0.05).

The highest values of egg weight, egg production percentage, egg mass, feed intake and feed conversion ratio resulted from using 2.0% of PO in hens diets. It is known that most spices stimulate the function of pancreatic enzymes (lipases, amylases and proteases), and that some also increase the activity of digestive enzymes of gastric mucosa (Srinivasan, 2005).

Besides the effect on bile synthesis and enzyme activity, extracts from herbs and spices accelerate the digestion and shorten the time of feed/food passage through the digestive tract (Suresh and Srinivasan, 2007). These effects could be seen in PO treatments. In addition, PO contained some special compounds such as antioxidants, α-tocopherol, ascorbic acid and glutathione (Simopoulos *et al.* 1992). The highest feed intake of group 5 provided sufficient levels of nutrients to support the high performance of laying hens.

α-tocopherol and other antioxidants can improve the laying hens performance by preventing nutrient oxidation (Nobakht and Mehmannavaz, 2010). The beneficial effects of PO in laying hens diets is in agreement with previous reports in quails, broilers (Kaveh, 2012) and ISA laying hens (Dougnon *et al.* 2012).

Egg traits

The effects of different levels of PO on egg traits of laying hens are shown in Table 3. Using different levels of PO had significant effects on specific gravity, yolk color index, shell, albumin and yolk percentages and shell thickness (P<0.05). Using 2.0% of PO compared with control group, improved the egg specific gravity, yolk color index and albumin weight, while reduced the values of eggshell and egg yolk percentages and egg shell thickness. Specific gravity is an index of calcium deposition in shell. Significant increase in egg specific gravity, egg yolk color and egg albumin by using PO may be associated with the amount of feed intake, since by increasing the amount of PO, the amount of feed intake increased and, so, a higher amount of calcium deposited in shell and improved the egg specific gravity.

Green plants are rich source of carotenoids such as beta carotene, so, by using 2.0% of PO in laying hens diets, may be the highest amounts of these carotenoids were transferred into egg yolk and elevated its color. Improve in yolk color density by using PO medicinal plant is in agreement with previous observations (Nobakht and Mehmannavaz, 2010; Sayedpiran *et al.* 2011; Nobakht and Mehmannavaz, 2013).

Table 2 Effects of using different levels of *Portulaca oleracea* powder on the performance of laying hens

Portulaca oleracea levels	Egg weight (g)	Egg production (%)	Egg mass (g)	Feed intake (g)	Feed conversion ratio
0	65.31^{a}	58.12^{a}	38.18^{a}	113.86^{a}	3.06^{a}
0.5	65.72^{a}	59.92^{a}	39.39^{b}	113.81^{a}	2.96^{b}
1.0	65.81^{a}	63.08^{b}	41.64^{c}	113.86^{a}	2.78^{c}
1.5	67.38^{b}	67.22^{c}	47.95^{d}	114.16^{a}	2.57^{d}
2.0	67.55^{b}	71.10^{d}	48.03^{e}	115.95^{b}	2.48^{e}
SEM	0.326	0.66	0.350	0.029	0.029
P-value	0.002	0.0001	0.0001	0.0001	0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of using different levels levels of *Portulaca oleracea* powder on egg traits of laying hens

Portulaca oleracea levels	Specific gravity (mg/mL^{3})	Yolk color index	Eggshell (%)	Albumin (%)	Egg yolk (%)	Haugh unit	Shell thickness (mm)
0	1.071^{a}	2.44^{a}	9.24^{a}	61.04^{a}	29.71^{a}	85.34	0.370^{a}
0.5	1.075^{ab}	3.00^{ab}	8.76^{ab}	62.65^{ab}	28.55^{a}	85.34	0.342^{b}
1.0	1.083^{c}	3.33^{ab}	8.78^{bc}	64.64^{b}	26.56^{a}	86.67	0.335^{bc}
1.5	1.085^{c}	3.78^{bc}	8.42^{ab}	64.88^{b}	26.68^{a}	81.00	0.319^{cd}
2.0	1.080^{ac}	4.33^{c}	7.82^{a}	70.07^{c}	22.34^{b}	85.34	0.312^{d}
SEM	0.002	0.281	0.194	1.066	0.965	2.087	0.006
P-value	0.003	0.007	0.005	.002	0.003	0.418	0.006

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Albumin is contained considerable amounts of protein and amino acids. By increasing the amount of feed intake, may be higher the amounts of essential nutrients such as amino acids absorbed and increased the albumin percentages. Also, a decrease in the yolk and eggshell percentage related to an increase in albumin percentage. Increase in the albumin percentage and decrease in egg yolk and egg shell percentages by using PO medicinal plant is not in agree with other reported results (Nobakht and Mehmannavaz, 2010; Sayedpiran *et al.* 2011). On the basis of Nobakht and Mehmannavaz (2013) report, using 1.0% of nettle medicinal plant in laying hens diets not only was not increased the albumin weight, but also it was decreased. Differences in the results could be due to plant species, hens production level and health status of hens. With increasing of egg size, the amount of deposited calcium in per unit of shell surface was reduced and the eggshell thickness was decreased.

Blood biochemical and immunity parameters

The effects of different levels of PO powder in feeds on blood biochemical parameters and immunity cells of laying hens are presented in Tables 4 and 5.

Using PO powder significantly improved the blood high density lipoprotein (HDL) and hemoglobin values ($P<0.05$). The highest amount of high density lipoprotein (HDL) was observed by using 2.0% and other blood parameters did not change by using PO in laying hens diets. PO is a rich source of antioxidants and flavonoids (Zargari, 1990).

It was shown that these compounds have positive effects on increasing the blood level of high density lipoprotein (HDL).

The increase in the amount of high density lipoprotein (HDL) found in the group 5 may be related with these compounds. PO is rich in minerals, vitamins and other chemical compounds.

Table 4 Effects of using different levels of *Portulaca oleracea* powder on biochemical parameters of laying hens

Treatments	Triglyceride (mg/dL)	Cholesterol (mg/dL)	Albumin (g/dL)	Total protein (g/dL)	Uric acid (g/dL)	HDL (g/dL)
0	1191.00	159.86	3.81	5.73	5.61	2.29^{b}
0.5	831.70	111.53	3.19	5.20	4.15	9.07^{a}
1.0	917.20	135.29	2.90	5.05	3.79	6.35^{ab}
1.5	819.50	91.96	3.34	4.12	3.37	5.38^{ab}
2.0	1132.10	121.14	3.22	4.69	5.43	8.47^{a}
SEM	220.66	35.15	0.33	0.56	0.99	1.68
P-value	0.78	0.72	0.45	0.39	0.45	0.1

The means within the same column with at least one common letter, do not have significant difference ($P>0.05$).
HDL: high density lipoprotein.
SEM: standard error of the means.

Table 5 Effects of using different levels of *Portulaca oleracea* powder on immunity cells of laying hens

Treatments	Hematocrit (%)	Hemoglobin (%)	Red blood cells (mm^3)	White blood cells (mm^3)	Hetrophile (%)	Lymphocyte (%)	H / L (%)
0	32.33	10.67^{ab}	3.11	23.20	14.00	84.00	0.18
0.5	35.67	11.77^{bc}	3.32	22.54	16.67	82.67	0.21
1.0	35.65	12.47^{c}	3.33	21.74	13.00	86.34	0.15
1.5	30.00	9.94^{a}	2.98	22.84	14.00	82.34	0.18
2.0	31.32	10.40^{ab}	3.12	22.07	9.34	88.67	0.11
SEM	1.87	0.53	0.13	0.51	4.07	3.63	0.06
P-value	0.19	0.04	0.30	0.33	0.79	0.71	0.80

The means within the same column with at least one common letter, do not have significant difference ($P>0.05$).
H / L: hetrophile / lymphocyte.
SEM: standard error of the means.

Increase in the amount of hemoglobin by using 1.0% of PO may be related with receiving high levels of minerals and vitamins such as iron, copper, vitamin B_{12} and folic acid. These nutrients have positive effects on the level of hemoglobin. The major function of the hemoglobin is to carry oxygen from the lungs to the tissues (Waugh and Grant, 2001).

CONCLUSION

The overall results showed that using *Portulaca oleracea* powder up to 2.0% in laying hens diets has positive effects on their performance and egg traits.

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