Global Veterinaria 7 (3): 270-275, 2011
ISSN 1992-6197

Egg Quality Characteristics and Productive Performance of Laying Hens Fed Diets Supplemented by *Echinacea purpurea* Extract, Immunofin and Vitamin E

M. Nasiroleslami and M. Torki

Department of Animal Science, Agriculture Faculty,
Razi University, Imam Avenue, Kermanshah, Iran, Postal Code: 6715685418

Abstract: This investigation was conducted to assess effects of adding *Echinacea purpurea*, vitamin E (Vit E) and Immunofin® (Imm) to laying hens' diets on egg quality characteristics and productive performance. A total number of 240 Lohmann LSL-Lite hens (71-wk-old) were divided in 40 cages (n=6). Eight iso-caloric and iso-nitrogenous diets (ME=2720 Kcal/Kg and CP=154.2 g/Kg), including the basal diet (control) or the basal diet supplemented with either 300 (mg/kg of diet) of ethanolic extracts of Ech, or 60 (mg/kg of diet) of α-tocopherol acetate (Vit E), or 200 (mg/kg of diet) of Imm and four other dietary groups included the basal diet with mixed form of the mentioned additives, were formulated. Each experimental diet was assigned to hens in 5 randomly selected cages (replication). To determine egg quality parameters, all eggs during three frequent days were collected on week 4 of trial. Collected data of feed intake (FI), egg production (EP), egg mass (EM) and calculated feed conversion ratio (FCR) were analyzed based on completely randomized design using GLM procedure of SAS. Although FI in the hens was not significantly affected by treatment, FCR, EP, EM and EW were affected by diets ($P \leq 0.05$). Feed efficiency in hens fed diets included Imm and Ech (Imm+Ech) was the best compared to other dietary groups. Adding Imm to diet increased EP, EM. By considering the data obtained from the present trial, diet supplementation by Imm and vit E could have beneficial effect on performance of laying hens during the period of 71-81 week of age.

Key words: Ehinacea Purpurea · Immunofin® · Feed Intake (FI) · Egg Production (EP) · Egg Weight (EW) · Egg Mass (EM) Feed Conversion Ratio (FCR) · Significant Effect · Experimental Diets

INTRODUCTION

A number of experiments conducted to find alternatives to antibiotics revealed that no single alternative exists with the effects comparable to antibiotics. There is need to find more efficient feed additives or combinations of them for maintaining health and improving performance of poultry and other livestock species [1]. Supplementation of a diet with a mixture powder of garlic and thyme assisted in improving performance of laying hens and egg quality traits [2]. Supplementing corn-soybean or corn-soybean-guar meal diets by β-mannanase had beneficial effects on performance of hens especially in terms of FCR and EP [3]. Zarei *et al.* [4] reported that probiotic Yesture® and A-Max® in laying hens' diets improved egg mass (EM). In addition, these researchers suggested that commercial feed additives (Yeasturer, A-Max, Thepax®, Fermacto® and Biomin®) could have beneficial effects on egg shell quality characteristics in terms of shell weight and thickness.

During recent years, phytigenic feed additives have attracted increasing interest as an alternative growth promoter to replace the use of antibiotic feed additives. In principal, the primary mode of action of growth promoting feed additives arises from beneficially affecting the ecosystem of gastrointestinal microbiota through controlling potential pathogens. Based on the producing company, Immunofin® (Pars Imen Daro Co. Tehran, Iran) contains active substances of Echinacea and some other medicinal plants and supposed to increase immune system of poultry especially in viral and bacterial disease. It has been also believed to increase immunoglobulin G (IgG) in poultry. *Echinacea purpurea* (Ech) belongs to

Corresponds Author: M. Torki, Animal Science Department, Agriculture Faculty, Razi University Imam Avenue, Kermanshah, Iran, Postal Code: 6715685418.

the family of Asteraceae. Preparations from *E. purpurea* are among the most widely used herbal medicines. Most uses of *E. purpurea* are based on its reported immunological properties. Echinacea and its different preparations contain a variety of active substances like alkamides, glycoproteins, polysaccharides, phenolic compounds, cinnamic acids, essential oils and flavonoids [5-6]. It is widely used around the world to treat common cold and other infectious disorders with the claim to have non-specific immune responses stimulating effects. Health-promoting properties of extracts derived from plants of the genus Echinacea has been already reported [7]. Vitamin E (Vit E), a fat-soluble vitamin of plant origin, is essential for the integrity and optimal function of the reproductive, muscular, circulatory, nervous and immune systems. Chickens, however, cannot synthesize vit E; therefore, vit E requirements must be met from dietary sources [8]. The immunomodulatory effects of vit E have been demonstrated in humans and a variety of animal species and were most evident in very young, very old and immunocompromised individuals [9]. The aim of the present experiment was to determine effects of dietary supplementation by Imm, Ech, vit E and mixed forms of the mentioned additives on productive performance of laying hens and egg quality characteristics.

MATERIALS AND METHODS

All procedures used in this ten-week experiment were approved by the Animal Ethics Committee of Razi University and complied with the "Guidelines for the Care and Use of Animals in Research". A total number of 240 white laying hens (Lohman, LSL-lite) at late production phase (with average body weight: 1470±23) were distributed between 40 cages and hens in 5 cages (replication) were assigned to each of 8 experimental diets (n=6). Eight iso-caloric and iso-nitrogenous diets (ME=2720 Kcal/Kg and CP=154.2 g/Kg), including the basal diet (control) or the basal diet supplemented with either 300 (mg/kg of diet) of ethanolic extracts of *E. purpurea* (Ech), or 60 (mg/kg of diet) of á-tocopherol acetate (Vit E), or 200 (mg/kg of diet) of Immunofin (Imm) and four other dietary groups included the basal diet with mixed form of the mentioned additives (as Imm+Ech, Imm+Vit E, Ech+Vit E and Imm+Ech+Vit E), were formulated (Table 1). The dosage of the *E. purpurea* juice for hens was adjusted on the basis of human medical recommendations to 0.25 ml/kg $BW^{0.75}$ due to a lack of data for poultry. The hens were housed in laying cages made from galvanized metal wire which provided approximately 430 cm2/hen. The cages were located in a

Table 1: The ingredients of experimental diets

Ingredients	1	2	3	4	5	6	7	8
Corn	68.08	68.08	68.08	68.08	68.08	68.08	68.08	68.08
Fish meal	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Soybean meal	18.45	18.45	18.45	18.45	18.45	18.45	18.45	18.45
Limestone	9.02	9.02	9.02	9.02	9.02	9.02	9.02	9.02
DCP	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Salt	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Vitamine E	-	-	-	0.06	-	0.06	0.06	0.06
Echinacea	-	-	0.3	-	0.3	-	0.3	0.3
Immunofin	-	0.2	-	-	0.2	0.2	-	0.2
Sand	1.88	1.68	1.58	1.82	1.38	1.62	1.52	1.32
Mineral Premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Calculated analysis								
ME (Kcal/Kg)	2720	2720	2720	2720	2720	2720	2720	2720
CP %	14.58	14.58	14.58	14.58	14.58	14.58	14.58	14.58
Ca	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
Available P	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
Na	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Arginine	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Isolucine	0.59	0.59	0.59	0.59	0.59	0.59	0.59	0.59
Lucine	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36
Lysine	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72
Methionine	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Met + Cys	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56

[1]The mineral premix provide the following quantities,per kilogram of diet: manganese, 110 mg; zinc, 100 mg; iron, 60 mg; copper, 10 mg; iodine, 100 mg and selenium, 0.2 mg

windowless and environmentally controlled room with the room temperature kept at 21-23 °C and the photoperiod set at 16 h of light (incandescent lighting, 10 lux) and 8 h dark. Water was available *ad libitum* throughout the experiment. Feed consumption was measured on a weekly basis. Collected data of feed intake (FI), egg production (EP), egg mass (EM) and calculated feed conversion ratio (FCR) were submitted to analysis of variance and means were tested ($P \leq 0.05$), according to their distributions, using the GLM procedure of SAS. Significant effects of dietary treatments were compared by using Duncan's multiple range tests. Values of different parameters were expressed as the mean ± standard deviation (Mean±SD).

RESULTS

Effects of dietary treatment on FI, FCR, EP, EM and EW of laying hens are presented in Tables 2 to 6, respectively. Dietary treatment did not significantly affect FI, but FCR was significantly affected by diet inclusion of feed additives. In hens fed diets included Imm and Ech, FCR was the best compared to other dietary groups. EP and EM of hens fed diets supplemented by Imm were higher compared to hens fed other experimental diets. Hens fed diet supplemented by Vit E had the highest EW compared to hens within other dietary groups. Hens fed the diet supplemented with Vit E showed significantly

Table 2: Feed intake (FI, g/hen/day) of laying hens fed iso-caloric and iso-nitrogenous experimental diets included Immunofin (Imm), Echinacea (Ech), Vitamin E (Vit E), Imm+Ech, Imm+Vit E, Ech+Vit E, Ech+Vit E and Imm+Ech+Vit E

	Feed intake (FI, g/hen/day)		
Week	1-5	5-10	1-10
Treatments			
Control	111.92± 5.79	117.52± 2.84	114.72± 3.87
Immunofin (Imm)	113.81± 4.27	117.94± 3.45	115.87± 3.19
Echinacea (Ech)	115.71± 3.16	117.67± 1.59	116.69± 2.26
Vitamin E (Vit E)	114.72± 4.26	118.22± 2.24	116.47± 3.02
Imm + Ech	114.31± 4.63	115.67± 3.49	114.95± 4.04
Imm + Vit E	110± 10.17	117.55± 1.20	113.78± 5.35
Ech + Vit E	115.51± 3.59	117± 2.54	116.25± 2.51
Imm + Ech + Vit E	114.56± 2.68	117.05± 1.83	115.80± 1.80
SEM	0.813	0.380	0.513
P-Value	0.699	0.839	0.880
CV	4.51	2.05	2.80

SEM = Standard Error of Means

Table 3: Feed conversion ratio (FCR, g egg/ g feed) of laying hens fed iso-caloric and iso-nitrogenous experimental diets included Immunofin (Imm), Echinacea (Ech), Vitamin E (Vit E), Imm+Ech, Imm+Vit E, Ech+Vit E, Ech+Vit E and Imm+Ech+Vit E

	Feed conversion ratio (FCR, g egg/ g feed)		
Week	1-5	5-10	1-10
Treatments			
Control	2.97± 0.43^{bc}	2.86± 0.49	2.91± 0.40^{b}
Immunofin (Imm)	3.13± 0.43^{abc}	2.60± 0.34	2.86± 0.37^{ab}
Echinacea (Ech)	3.10± 0.54^{abc}	2.73± 0.26	2.92± 0.38^{ab}
Vitamin E (Vit E)	3.22± 0.40^{abc}	2.72± 0.25	2.97± 0.26^{ab}
Imm+Ech	2.66± 0.02^{c}	2.81± 0.29	2.73± 0.15^{b}
Imm+Vit E	3.30± 0.45^{abc}	2.88± 0.17	3.09± 0.18^{ab}
Ech+Vit E	3.72± 0.68^{a}	3.04± 0.35	3.38± 0.50^{a}
Imm+Ech+Vit E	3.53± 0.66^{ab}	3.04± 0.47	3.28± 0.49^{a}
SEM	0.086	0.055	0.062
P-Value	0.060	0.449	0.126
CV	16.96	12.26	12.98

^{ab}Means within column with different superscripts are significantly different ($P < 0.05$). SEM = Standard Error of Means

Table 4: Egg production (EP, %) of laying hens fed iso-caloric and iso-nitrogenous experimental diets included Immunofin (Imm), Echinacea (Ech), Vitamin E (Vit E), Imm+Ech, Imm+Vit E, Ech+Vit E, Ech+Vit E and Imm+Ech+Vit E

	Egg production (EP, %)		
Week	1-5	5-10	1-10
Treatments			
Control	65.72± 11.25^{ab}	68.91± 10.89^{ab}	67.32± 9.61^{abc}
Immunofin (Imm)	67.06± 5.39^{ab}	77.07± 6.03^{a}	72.07± 5.16^{a}
Echinacea (Ech)	66.28± 9.34^{ab}	70.66± 5.81^{ab}	68.47± 7.22^{abc}
Vitamin E (Vit E)	63.17± 4.25^{b}	67.95± 5.29^{ab}	65.56± 4.31^{abc}
Imm+Ech	73.14± 2.25^{a}	69.38± 8.47^{ab}	71.26± 4.86^{ab}
Imm+Vit E	59.26± 4.89^{b}	66.78± 3.83^{ab}	63.20± 3.76^{abc}
Ech+Vit E	58.84± 6.94^{b}	63.53± 8.41^{b}	61.19± 7.64^{c}
Imm+Ech+Vit E	60.48± 6.60^{b}	64.50± 7.39^{b}	62.49± 6.51^{bc}
SEM	1.225	1.222	1.101
P-Value	0.040	0.160	0.085
CV	12.06	11.26	10.48

^{ab}Means within column with different superscripts are significantly different (P < 0.05). SEM = Standard Error of Means

Table 5: Egg mass (EM, g/hen/day) of laying hens fed iso-caloric and iso-nitrogenous experimental diets included Immunofin (Imm), Echinacea (Ech), Vitamin E (Vit E), Imm+Ech, Imm+Vit E, Ech+Vit E, Ech+Vit E and Imm+Ech+Vit E

	Egg mass (EM, g/hen/day)		
Week	1-5	5-10	1-10
Treatments			
Control	39.87± 6.64^{ab}	43.21± 6.69^{ab}	41.54± 5.82^{ab}
Immunofin (Imm)	41.85± 4.15^{ab}	48.71± 5.38^{a}	45.28± 4.57^{a}
Echinacea (Ech)	40.78± 6.48^{ab}	44.42± 3.68^{ab}	42.60± 4.84^{ab}
Vitamin E (Vit E)	40.13± 3.47^{ab}	45.25± 3.79^{ab}	42.69± 3.23^{ab}
Imm+Ech	43.88± 1.74^{a}	42.52± 4.53^{ab}	43.20± 2.43^{ab}
Imm+Vit E	37.24± 3.33^{ab}	43.64± 2.18^{ab}	40.44± 2.03^{ab}
Ech+Vit E	36.85± 4.61^{b}	40.53± 4.25^{b}	38.69± 4.40^{b}
Imm+Ech+Vit E	37.57± 4.52^{ab}	41.72± 5.10^{b}	39.65± 4.57^{ab}
SEM	0.757	0.761	0.676
P-Value	0.239	0.218	0.282
CV	12.05	11	10.25

^{ab}Means within column with different superscripts are significantly different (P < 0.05). SEM = Standard Error of Means

Table 6: Egg weight (EW, g) of laying hens fed iso-caloric and iso-nitrogenous experimental diets included Immunofin (Imm), Echinacea (Ech), Vitamin E (Vit E), Imm+Ech, Imm+Vit E, Ech+Vit E, Ech+Vit E and Imm+Ech+Vit E

	Egg weight (EW, g)		
Week	1-5	5-10	1-10
Treatments			
Control	60.68± 1.09	62.63± 1.48^{bc}	61.65± 1.22^{bc}
Immunofin (Imm)	61.73± 2.60	62.76± 2.86^{bc}	62.25± 2.67^{abc}
Echinacea (Ech)	61.27± 1.69	62.73± 0.90^{bc}	62± 1.24^{abc}
Vitamin E (Vit E)	63.09± 3.10	66.50± 2.12^{a}	64.79± 2.55^{a}
Imm+Ech	60.14± 3.39	61.27± 2.31^{c}	60.71± 2.77^{c}
Imm+Vit E	62.34± 0.83	65.35± 1.56^{ab}	63.85± 0.54^{ab}
Ech+Vit E	62.14± 3.09	63.85± 2.95^{abc}	62.99± 2.75^{abc}
Imm+Ech+Vit E	61.75± 1.95	64.49± 1.58^{ab}	63.12± 1.67^{abc}
SEM	0.371	0.391	0.357
P-Value	0.619	0.009	0.101
CV	3.81	3.88	3.60

^{ab}Means within column with different superscripts are significantly different (P < 0.05). SEM = Standard Error of Means

Table 7: Egg quality characteristics of laying hens fed iso-caloric and iso-nitrogenous experimental diets included Immunofin (Imm), Echinacea (Ech), Vitamin E (Vit E), Imm+Ech, Imm+Vit E, Ech+Vit E, Ech+Vit E and Imm+Ech+Vit E

	Egg quality characteristics			
	Egg index	Shell weight	Shell thickness	HU unit
Treatments				
Control	75.28± 0.85	5.99± 0.61	36.54± 0.60	95.88± 1.92ab
Immunofin (Imm)	73.93± 0.91	6.13± 0.18	36.01± 0.83	94.42± 1.77b
Echinacea (Ech)	75.33± 1.50	5.91± 0.53	36.83± 0.88	96.72± 4.47ab
Vitamin E (Vit E)	74.36± 2.19	6.01± 0.52	36.38± 0.59	95.46± 4.59ab
Imm+Ech	74.81± 1.28	5.94± 0.19	35.84± 0.03	96.39± 1.62ab
Imm+Vit E	75.60± 3.80	6.13± 0.41	36.62± 0.39	96.36± 2.40ab
Ech+Vit E	76.78± 5.58	6.21± 0.43	37.02± 0.59	98.12± 3.02ab
Imm+Ech+Vit E	73.19± 2.88	6.08± 0.56	36.22± 0.23	99.53± 1.54a
SEM	0.438	0.067	0.356	0.480
P-Value	0.628	0.965	0.994	0.210
CV	3.70	7.06	6.17	3.14

[ab]Means within column with different superscripts are significantly different (P < 0.05). SEM = Standard Error of Means

Table 8: Egg quality characteristics of laying hens fed iso-caloric and iso-nitrogenous experimental diets included Immunofin (Imm), Echinacea (Ech), Vitamin E (Vit E), Imm+Ech, Imm+Vit E, Ech+Vit E, Ech+Vit E and Imm+Ech+Vit E (continue)

	Egg quality characteristics		
	Yolk index	Yolk color	Specific gravity
Treatments			
Control	44.03± 1.50[b]	7.60± 0.36[ab]	1.078
Immunofin (Imm)	45.76± 1.95[ab]	7.33± 0.33[b]	1.078
Echinacea (Ech)	45.89± 0.71[ab]	7.86± 0.37[a]	1.072
Vitamin E (Vit E)	45.41± 1.04[ab]	7.26± 0.27[b]	1.078
Imm+Ech	44.16±1.21[b]	7.46± 0.18[ab]	1.080
Imm+Vit E	46.71± 1.34[a]	7.60± 0.28[ab]	1.078
Ech+Vit E	46.08± 2.04[ab]	7.66± 0.33[ab]	1.078
Imm+Ech+Vit E	46.28± 1.32[a]	7.59± 0.43[ab]	1.080
SEM	0.253	0.055	0.001
P-Value	0.057	0.157	0.843
CV	3.52	4.65	0.712

[ab]Means within column with different superscripts are significantly different (P < 0.05). SEM = Standard Error of Means

higher EW compared to hens fed the control diet. Effects of dietary treatment on egg quality characteristics are presented in tables 7 and 8. Among the evaluated egg quality traits, Haugh unit, yolk index and color was significantly affected by dietary treatment. The highest Haugh unit and yolk index was seen in eggs of hens fed the diet included Imm+Ech+Vit E. The highest yolk color was seen in eggs of hens fed the diet included Ech.

DISCUSSION

In the present experiment no beneficial effect of dietary supplementation by Imm and Ech and Vit E on laying hens performance was seen, except for EW which was higher in hens fed diet supplemented by Vit E. Böhmer *et al*. [10] did not find significant effect during a repeated short-time application regime for Echinacea juice as feed additive on performance of layers and fattening pigs. Puthpongsiriporn *et al*. [11] and Lin *et al*. [12] reported that FI, EP were decreased in birds under environmental stress. Studies have shown that antioxidant nutrient supplementation especially vitamins C, E and A, zinc and chromium can be used to attenuate the negative effects of environmental stress [13]. It has been reported that supplementation of dietary vitamin E at 65 IU/kg diet enhanced egg mass and egg quality in white leghorn hens by improving the formation of egg yolk during heat stress [11]. Increased performance in treated birds by Imm and other herbal diets may be due to the various mode of action, including a modified gut microflora [14], stimulated secretion or activity of digestive enzymes [15] (Jang *et al*. 2007), altered immune

functions [16] and histological changes [17]. Actually, a reduction of potentially pathogenic bacteria and a shift in the composition of the gut microflora towards more beneficial bacteria may reduce the competition for nutrients and dietary energy between the host and its microflora.

CONCLUSION

In conclusion, the data indicated that diet supplementation by Imm or Imm+Ech could improve EP in laying hens. In addition, diet supplementation by Imm would have beneficial effect on EM.

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