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Effect of some phytogenic additives as dietary supplements on performance, egg quality, serum biochemical parameters and oxidative status in laying hens

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ABSTRACT

The aim of the present study was to investigate the impact of some phytogenic feed additives on performance and quality of eggs as well as blood constituents and antioxidant indices in laying hens. Hi-sex Brown laying hens (72) were assigned to 3 dietary treatments (6 replications of 4 hens in a group) and were fed the basal diet or diets supplemented with 0.9% of rosemary and thyme herbs till 52 wk-old. There were no differences in feed intake and egg weight due to herbs addition. Feed conversion ratio, egg production and egg output improved with herbal plants supplementation compared to control. Egg quality traits were significantly affected by herbs supplementation.There were significant differences among the different experimental groups and control on all serum constituents studied except total protein, albumin and HDL-cholesterol. In comparison to control, the diet enriched with rosemary herb significantly lowered serum triglyceride, cholesterol and LDL-cholesterol concentrations. Super oxide dismutase (SOD) activity was statistically improved in phytogenic additive groups, and was maximized (277 U/ml) with dietary thyme. While, malonaldehyde (MDA) level decreased compared to control. It can be concluded that thyme and rosemary supplemented up to 0.9% can be used as effective natural feed additives in layer diets to improve performance, immune response and antioxidant status.

Key words: Antioxidant status, Blood profile, Layer, Performance, Phytogenic additives

Herbs or their derivatives are attaining importance now-a-days in livestock production and health care systems, these plants or their bioactive components have beneficial uses as growth enhancer, immunomodulator, natural antioxidant etc. (Farag *et al.* 2014, Alagawany *et al.* 2015a,b). Certain studies and researches described various protective and biological impacts of rosemary (*Rosmarinus officinalis* L.), including antibacterial, antioxidant, antiinflammatory, immunomodulatory, anticancer and health-promoting activities (Florou-Paneri *et al.* 2006, Chun *et al.* 2014).

Natural feed additives including rosemary and thyme herbs exert their antioxidant mechanism via elimination of free radicals, constitution of chelates with metal ions and reduction of oxygen formation (Rice-Evans *et al.* 1995, Khan *et al.* 2012). Some reports pointed out that dietary herbs or their products (cold pressed oil, essential oil and phytochemicals) improved growth and production of poultry (Alcicek *et al.* 2003, Basmacioglou-Malayoðlu *et al.* 2004), while others observed no such impacts (Lee *et al.* 2003, Papageorgiou *et al.* 2003). Rosemary powder is produced by drying rosemary leaves, and contains a large number of phenolic compounds like carnosic acid, carnosol, rosmanol, rosmarinic acid and epirosmanol. Carnosic acid is the largest antioxidant compound present in rosemary or its extract, with an antioxidant effect approximately seven times higher than the synthetic antioxidants and three times higher than carnosol (Angioni *et al.* 2004).

Thyme (*Thymus vulgaris* L.) and its effective compounds improve the activity of digestive enzymes such as amylase, lipase and protease which results in improving the digestibility and absorption of nutrients attributed to increase of intestine length, width and depth of digestive tract villi which increases the opportunity for absorption of nutrients (Alcicek *et al.* 2003, Abd El-Wareth 2012). The main objectives of this study were to investigate the ability of rosemary and thyme herbs to improve performance, blood metabolites and oxidative status of laying hens during the experimental period (36 to 52 wk of age).

MATERIALS AND METHODS

Animals and diets: All protocols in this experiment were approved by the guidelines of the Animal Ethics Committee of Zagazig University (Zagazig, Egypt). Hi-sex Brown laying hens (72), 36 wk-old, were randomly divided into 3

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treatment groups with 6 cage replicates of 4 hens each. The cages were equipped with a trough feeder and a nipple drinker. The laying hen house was provided with adequate ventilation and programmable lighting (17 h light and 7 h dark). The water and rations were provided *ad lib.* during the experiment. The basal diet (2800 Kcal of ME/kg, 18% CP) was formulated to meet nutrients recommendation of Hi-sex Brown management guide which meet or exceeded the NRC (1994) recommendations (Table 1). Dietary treatments were control (basal diet with no additive), control diet with 0.9% of thyme powder, and control diet with 0.9% of rosemary powder, and the diets were fed as a mash. The duration of the experiment was 16 wk (36 to 52 wk-old). Rosemary and thyme herbs as commercial products were obtained from Free Trade Egypt Company, Behira, Egypt.

Performance and egg quality parameters: Feed intake (FI) and feed conversion ratio (FCR) (g of feed consumed/ g of egg produced) were recorded and calculated weekly. Egg number (EN) and egg weight (EW) were recorded daily to calculate the egg production (EP) and egg output (EO) (EN × EW). Eighteen eggs from each treatment were randomly selected to measure egg quality traits in terms of albumin (%), yolk (%), shell (%), shell thickness, egg shape index, yolk index and Haugh unit score. Haugh unit was calculated according to formula proposed by Card and Nesheim (1972). The thickness of egg shell was a mean value of measurement at 3 locations on the shell (air cell, equator, and sharp end) by using a dial and pipe gage.

Blood sampling and laboratory analyses: Blood samples were randomly collected from two hens per replicate treatment from wing vein in non-heparinized tubes at the end of experimental period. The samples were let to coagulate and centrifuged at 3,500 rpm for 15 min to obtain serum. Serum samples were analyzed for total protein (g/ dl), albumin (g/dl), triglycerides (mg/dl), total cholesterol (mg/dl), low-density lipoprotein (LDL, mg/dl), high density lipoprotein (HDL, mg/dl), and immunoglobulin M (IgM) and (IgG) by spectrophotometer (Shimandzu UV-1700) using commercial diagnostic kits provided from Biodiagnostic Co. Giza, Egypt according to Akiba *et al.* (1982). For antioxidant parameters, activity of superoxide dismutase (SOD) was measured by the xanthine oxidase method, which monitors the inhibition of reduction of nitro blue tetrazolium by the sample (Winterbourn *et al.* 1975). Reduced glutathione (GSH) was analyzed according to Beutler *et al.* (1963). The malondialdehyde (MDA) concentration was determined with 2-TBA, monitoring the change of absorbance at 532 nm with the spectrophotometer (Hitachi, Japan) (Jensen *et al.* 1997).

Statistical analysis: All the data (performance, quality of eggs, blood parameters and oxidative staus) were subjected to ANOVA method using a completely randomized design using the GLM procedures of SPSS (version, 17.0, 2008). The differences among treatments were computed using the post-hoc Newman-Keuls test ($P<0.05$).

Table1. Composition and nutrient content of the control diet fed to laying hens

	The basal diet
Ingredient	
Corn	56.71
Soybean meal (44% CP)	28.62
Soybean oil	3.13
Limestone	9.33
Di-calcium phosphate	1.45
NaCl	0.30
Vitamin-mineral premix*	0.30
Dl-Methionine	0.16
Calculated composition (%) **	
ME, kcal/kg	2800
Crude protein	17.51
Calcium	4.00
Available phosphorus	0.38
Lysine	0.92
Methionine + cystine	0.73

*Layer vitamin-minerals premix: Each 1 kg consists of vit. A, 8,000 IU; vit. D_3, 1,300 ICU, vit. E, 5 mg; vit. K, 2 mg; vit. B_1, 0.7 mg; vit. B_2, 3 mg; vit. B_6, 1.5 mg, vit B_{12}, 7 mg; biotin, 0.1 mg; pantothenic acid, 6 g; niacin, 20 g; folic acid, 1 mg; manganese, 60 mg; zinc, 50 mg; copper, 6 mg; iodine, 1 mg; selenium, 0.5 mg; cobalt, 1 mg. **Calculated according to NRC (1994).

RESULTS AND DISCUSSION

Hen productive performance: The impact of dietary thyme and rosemary supplement on laying hen performance is shown in Table 2. There were no significant differences ($P<0.05$) in egg weight and feed intake due to phytogenic additives treatments. While, FCR, EP and EO significantly ($P<0.05$) improved with herbal plant supplementation compared to control. The diets enriched with herbs were consumed without any palatability risks. Our results are in line with the results of Cho *et al.* (2014) who stated that supplemental phytogenic additives to control diet had no impact on FI compared to unsupplemented diet. Similarly, Radwan *et al.* (2008) noted that dietary rosemary and thyme herbs increased egg number and egg mass as well as improved FCR, while the FI was not statistically affected.

Table 2. Effects of phytogenic additives on performance of laying hens from 36 to 52 wk of age (n=6)

Performance	Control	Thyme (0.9%)	Rosemary (0.9%)	SEM[1]	*P*-value[2]
Feed intake (g)	87.13	87.35	95.45	2.21	0.108
Feed conversion ratio	1.98[a]	1.92[b]	1.83[c]	0.07	0.048
Egg weight (g)	63.22	62.02	63.30	0.52	0.541
Egg production (%)	72.30[c]	75.76[b]	[a]86.86	0.68	0.025
Egg output (g)	1368[c]	[b]1408	1648[a]	44.83	0.017

Different superscripts within rows are significantly different ($P<0.05$); [1]SEM, standard error of mean; [2]overall treatment P-value.

Medicinal plant supplementation to poultry diet can improve FCR (Hong *et al.* 2012). On the other hand, thyme herb supplementation to layer diet increased egg weight during the period from 60 to 70 wk-old (Mansoub 2011). Significant impacts on egg output and egg weight could be due the positive impact of thyme herb in reducing the count of pathogens in the digestive tract, enhancing immune response and productive performance of the birds (Shahryar *et al.* 2011). Lee *et al.* (2003) suggested that the presence of harmful bacteria in the digestive tract causes an increase in lysis and deamination of protein and amino acids of nutrients may be due to secretary substances from these bacteria such as urease.

The improvement in EP and EO with supplementation of natural feed additives may be attributed to the provision of some bioactive components that improve digestion and absorption of nutrients in the digestive tract (Alagawany *et al.* 2015a). Moreover, it could be due to the bioactive role of phenolic molecules like carnosol, carnosic acid, rosmarinic acid, and thymol which are found in rosemary and thyme causing greater feed efficiency, resulting in improved performance indices (Bozin*et al.* 2006, Abd El-Hack *et al.* 2016a). Furthermore, Moreno *et al.* (2006) reported that carnosic and rosmarinic acids may be the key bioactive antimicrobial components present in rosemary herb. Dietary feeding of cold pressed or essential oil extracted from herbal plants enhanced the secretion of intestinal enzymes, and improved the performance and digestibility of nutrients in birds (Jang *et al.* 2004).

Egg quality parameters: The impacts of supplemental herbal plants on egg quality traits of laying hens are illustrated in Table 3. All parameters of egg quality were significantly affected by different treatments (P<0.05). The highest values of yolk (%), shell (%), shell thickness, yolk index, and yolk to albumin ratio were achieved by hens fed diet enriched with thyme in comparison with the control or rosemary groups. Conversely, compared to the basal diet, supplementation of herbal plants to layer diets decreased albumin (%) and egg shape index. Haugh unit score was statistically (P=0.027) decreased (78.07) and increased (88.77) with the dietary thyme and rosemary, respectively. These findings were in partial agreement with Radwan *et al.* (2008) who found that yolk index and yolk weight were increased by rosemary treatment in laying hens diets (P<0.05), where the highest value of yolk index was recorded in the 0.5% rosemary group. On the other hand, Mansoub (2011) noted that egg quality traits were significantly (P<0.05) influenced by thyme addition to layer diets, the highest value of yolk index was seen in the 2% thyme group. On the contrary, thyme supplementation to layer diets had no significant impacts on quality of eggs among treatments (Mohebbifar and Torki 2010).

Table 3. Effects of phytogenic additives on egg quality criteria of laying hens from 36 to 52 wk of age (n =6)

Egg quality criteria	Control	Thyme (0.9%)	Rosemary (0.9%)	SEM[1]	*P*-value[2]
Albumen (%)	66.48^{a}	60.06^{c}	64.72^{b}	0.36	0.040
Yolk (%)	24.36^{c}	28.54^{a}	26.39^{b}	0.36	0.013
Shell (%)	11.84^{b}	14.08^{a}	11.57^{b}	0.20	0.047
Egg shape index	79.30^{a}	76.71^{b}	78.96^{ab}	0.64	0.043
Shell thickness (µm)	0.35^{c}	0.42^{a}	0.38^{b}	0.01	0.049
Yolk index	36.67^{b}	40.62^{a}	40.50^{a}	0.58	0.040
Yolk:albumen ratio	0.38^{c}	0.49^{a}	0.42^{b}	0.01	0.010
Haugh unit score	84.30^{b}	78.07^{c}	88.77^{a}	1.25	0.027

Different superscripts within rows are significantly different (P<0.05); [1]SEM, standard error of mean; [2]overall treatment P-value.

Blood parameters: Blood measurements are crucial indicators of physiological, immunological and nutritional status of livestock. The impact of supplemental herbs on blood parameters of layers are illustrated in Table 4. Serum total protein, albumin and HDL-cholesterol concentrations were not statistically affected by supplemental herbs (P>0.05). The results from herbal plant supplementation were in agreement with Osman *et al.* (2010) who found that rosemary addition at levels of 0.5 and 1g/kg diet did not induce any significant impact on the concentration of protein and albumin in serum.

Triglyceride and total cholesterol were significantly (P=0.041 and P<0.001; respectively) decreased by rosemary herb supplementation in comparison to control or thyme groups, while LDL-cholesterol was lowered with herbal groups compared to control. In partially accordance with the present results, Bolukbasi *et al.* (2008) found that inclusion of rosemary herb in layer diets significantly depressed triglyceride and total cholesterol concentration in serum. On the same line, Rahimi *et al.* (2011) showed that the concentrations of blood triglyceride, total cholesterol and LDL-cholesterol were statistically reduced by phytogenic feed additives to chicken diets. Radwan *et al.* (2008) noted that the birds fed diets containing rosemary leaves at 0.5, or 1% had lower serum total lipids, but the concentrations of total cholesterol and its fractions (HDL and LDL cholesterol) were not affected in the same way with the various rosemary additions compared to control. Contrarily, supplementations of rosemary herb to chicken diets led to increased serum levels of triglycerides, total cholesterol and LDL, but the serum content of total protein and albumin did not show significant changes (Abd El-Latif *et al.* 2013).The observable improvements of lipid constituents may be returned to bioactive agents like thymol and rosmarinic acid that stimulate lipase and bile production and hence lipid digestion (Hernandez *et al.* 2004, Abd El-Hack 2016a). Thymol essential oil which is found in thyme plant has been described to improve the gut secretions such as salivary amylase, bile salt and pancreatic enzymes like lipase, trypsin and chymotrypsin (Platel and Sriinivasan 2004). The effect of herbal plants or their extracts on blood lipid constituents has been shown to be dialectical

(Alagawany *et al.* 2015a,b). Hyperlipidemic impacts were reported with some medicinal plants (Bolukbasi *et al.* 2006, Alagawany *et al.* 2015a); on the contrary, hyperlipidemia was seen with other herbs (Farag *et al*. 2014). The variations among these studies may be returned to the differences in phytogenic feed additives used, product kind (powder, cold pressed oil, essential oil, phenolic compounds, etc.), levels and method of administration as well as experimental conditions (Abd El-Hack *et al.* 2015).

Dietary supplementation of phytogenic additives exhibited a positive effect on immunological parameters which is in accordance with Hashemipour *et al.* (2013). In comparison to control group, diets supplemented with thyme or rosemary herb significantly (P=0.049 and P<0.001, respectively) increased IgM level, but IgG value was significantly elevated with rosemary only. In the current study, the diet enriched in rosemary herb (0.9%) numerically increased the concentration of IgG and IgM, in comparison with control or thyme herb groups.

There are a large number of medicinal plants and their products which possess immunomodulatory properties and were used to provide alternative potential to traditional chemotherapy for many diseases, such as immuno deficiency diseases (Kumar *et al.* 2011, Farag *et al.* 2014). In poultry industry, it is important to improve the immune functions to lower and prevent infectious diseases. There are some factors which can induce immunodeficiency such as vaccination failure and inhibition of antibiotics. Use of synthetic or natural immune enhancers is a key solution to enhance immune system and to reduce susceptibility to many diseases in animal house. Phytogenic feed additives which are rich in phenolic compounds and flavonoids extend the biological activity of vitamin C, act as antioxidants and may improve immune response (Acamovic and Brooker 2005). These results can partially explain the biological and nutritional impacts of treatments on immune parameters (Table 4).

Table 4. Effects of phytogenic additives on blood components in laying hens at 52 wk of age (n=6)

Blood parameter	Control	Thyme (0.9%)	Rosemary (0.9%)	SEM[1]	*P*-value[2]
Total protein (g/dl)	4.47	4.88	5.09	0.12	0.078
Albumin (g/dl)	2.45	2.44	2.55	0.06	0.232
Triglycerides (mg/dl)	183[a]	178[a]	167[b]	7.98	0.041
Total cholesterol (mg/dl)	171[a]	171[a]	119[b]	7.95	<0.001
HDL- cholesterol (mg/dl)	93.28	88.68	93.01	9.05	0.616
LDL- cholesterol (mg/dl)	56.61[a]	36.41[b]	37.30[b]	3.73	0.015
IgG (mg/dl)	1.27[b]	1.31[b]	1.78[a]	0.13	0.049
IgM (mg/dl)	12.54[c]	15.71[b]	17.74[a]	0.74	<0.001

Different superscripts within rows are significantly different (P<0.05); [1]SEM, standard error of mean; [2]overall treatment P-value.

Table 5. Effects of phytogenic additives on superoxide dismutase (SOD) and reduced glutathione (GSH) activities and malondialdehyde (MDA) concentration in serum of laying hens at 52 wk of age (n=6)

Oxidative status	Control	Thyme (0.9%)	Rosemary (0.9%)	SEM[1]	*P*-value[2]
SOD (U/ml)	207[b]	277[a]	257[a]	9.20	0.027
GSH (ng/ml)	9.02	11.98	9.19	1.01	0.084
MDA (µmol)	4.47[a]	3.74[b]	3.89[b]	0.27	0.041

Different superscripts within rows are significantly different (P<0.05); [1]SEM, standard error of mean; [2]overall treatment P-value.

Antioxidant indices: The impact of dietary herbal plants on serum antioxidant indices (SOD, GSH and MDA) is illustrated in Table 5. Serum SOD activity was statistically (P=0.027) increased in thyme or rosemary groups, which was maximized (277 U/ ml) with 0.9% thyme. The concentration of GSH was not significantly influenced by dietary treatments, in comparison to control group. The MDA level was decreased (P=0.041) with thyme or rosemary addition compared to control, which was minimized (3.74) with thyme group. The level of MDA is an indicator for evaluating antioxidant status. The same findings were reported by Hashemipour *et al.* (2013) who showed that the intake of medicinal plants resulted in an increase in the activities of antioxidant parameters like SOD and GSH, and a decrease in MDA value. Elevated levels of antioxidant enzymes may enhance the steady state of antioxidant system. The SOD mainly takes part in the antioxidant defense system. Lopez-Bote *et al.* (1998) found significantly lower MDA level in meat from chickens fed on the diets supplemented with rosemary extract, than those from chickens fed on the diet not enriched with antioxidants (control).

It seems that thyme and rosemary supplementation to laying hen diets were effective in improving the antioxidant ability of birds. Thyme and rosemary are rich sources of phenolic compounds such as thymol, carnosol, carnosic and rosmarinic acids and related effective compounds having strong antioxidant, anti-inflammatory, antimicrobial, and anti-cancer activities as well as growth enhancers (Chun *et al.* 2014, Abd El-Hack *et al.* 2016a). Natural feed additives may have more mechanisms, including affecting feed intake, feed efficiency and feed utilization, improving the secretion of digestive enzymes, and motility of gastro-intestinal tract as well as endocrine and immune functions, besides antioxidant, antiviral, antimicrobial, anti-inflammatory, anthelminthic and coccidiostat activities (Dhama *et al.* 2015, Alagawany and Abd El-Hack 2015, Abd El-Hack *et al.* 2016b).

Generally speaking, thyme or rosemary supplementation (0.9%) did not affect feed intake and egg weight but it improved feed conversion ratio, egg production and egg output as well as immune parameters. In addition, layers

fed diet enriched with thyme or rosemary had lower lipid parameters and lipid peroxidation, and increased activity of antioxidant enzymes in serum.

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