Journal of Animal and Feed Sciences, 24, 2015, 341–347
The Kielanowski Institute of Animal Physiology and Nutrition PAS, Jabłonna

The effect of rosemary herb as a dietary supplement on performance, egg quality, serum biochemical parameters, and oxidative status in laying hens

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KEY WORDS: rosemary powder, performance, blood profile, antioxidant status, laying hens

Received: 23 June 2015
Revised: 1 November 2015
Accepted: 25 November 2015

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ABSTRACT. The aim of this study was to evaluate the effect of rosemary herb on performance, egg quality, blood profile, immune function and antioxidant status in Hi-sex Brown laying hens. A total of 96 thirty-six-week-old laying hens were assigned to 4 dietary treatments (6 replications of 4 hens per group) and were fed the control diet or diets supplemented with 3, 6 or 9 $g \cdot kg^{-1}$ of rosemary powder until 52 weeks of age. There were no differences in live body weight, feed consumption, feed conversion ratio or egg weight due to adding rosemary. Egg numbers and egg mass linearly increased with rosemary supplementation. Adding rosemary to laying hen diets resulted in a linear increase in yolk percent ($P < 0.05$) and yolk-to-albumen ratio ($P = 0.01$), and a decrease in albumen percent ($P < 0.05$) compared with the non-supplemented group. Serum constituents were not significantly influenced by rosemary, except urea, total cholesterol, immunoglobulins M (IgM) and A (IgA) concentrations. In comparison with the control group, the diet enriched with rosemary numerically reduced serum triglycerides, cholesterol and LDL-cholesterol concentrations, but HDL-cholesterol level was elevated with the same addition. Superoxide dismutase activity was linearly and quadratically ($P = 0.007$ and 0.002, respectively) increased in rosemary groups, and was maximized (290 $U \cdot ml^{-1}$) at 6 $g \cdot kg^{-1}$ diet. In conclusion, rosemary supplemented up to 6 $g \cdot kg^{-1}$ diet can be used as effective feed additive to improve performance, immunity and antioxidant status in laying hens.

Introduction

Medicinal plants and their bioactive components are presently attaining importance in animal and poultry production as well as health care systems because of their broad beneficial effects promoting growth and production, immune enhancement, and safeguarding health (Farag et al., 2014; Alagawany et al., 2015a,b). Studies have described various biological impacts and protective effects of rosemary (*Rosmarinus officinalis* L.), including antioxidant, antibacterial, anti-inflammatory, anticancer, immunomodulatory and health-promoting activities (Florou-Paneri et al., 2006; Chun et al., 2014).

Phytogenic feed additives (PFA), including rosemary, exert their antioxidant mechanism *via* eradication of free radicals, constitution of chelates with metal ions, and prevention or reduction of oxidation (Rice-Evans et al., 1995). Certain reports stated that dietary herbal plants or their essential oils improved growth and productive performance (Alçiçek et al., 2003; Basmacioğlu Malayoğlu et al., 2004), while others observed no such effects (Lee et al., 2003; Papageorgiou et al., 2003). Rosemary powder, a spice produced by drying the leaves of the rosemary herb, contains a large number of different phenolic compounds with biological activities, such as carnosol, carnosic acid, rosmanol and epirosmanol. Carnosic acid is the most active antioxidant present in rosemary powder or extract (Angioni et al., 2004) with an antioxidant activity approximately three times higher than carnosol and seven times higher than synthetic antioxidants.

The main objectives of the present study were to evaluate the ability of different levels of rosemary to improve productive performance, egg quality criteria and blood metabolites, as well as oxidative status parameters of laying hens during the experimental period (from 36 to 52 week of age).

Material and methods

This study was conducted at Poultry Research Farm, Department of Poultry, Faculty of Agriculture, Zagazig University (Egypt). All of the experimental procedures were carried out according to the Local Experimental Animals Care Committee, and approved by the institutional ethics committee. The birds were cared for using husbandry guidelines derived from Zagazig University standard operating procedures.

Birds, experimental design and diets

Ninety-six Hi-sex Brown laying hens were used in the experiment. A completely randomized design was used, with six replications of 4 hens each; four birds were housed per wire cage (50×50×45 cm). The cages were equipped with a nipple drinker and trough feeders. The bird house was provided with programmable lighting and adequate ventilation. The lighting programme at the start of the trial was 14 h of light and was increased by 15 min each week to 17 h of light. The diets and water were provided *ad libitum* throughout the experiment. The control diet (2800 kcal of ME$\cdot$kg^{-1}, about 18% CP) was formulated to meet the nutrient recommendations of the Hi-sex Brown management guide, which meet or exceed the NRC (1994) recommendations, as shown in Table 1. The four experimental dietary treatments were: 1. control (basal diet with no additive), 2. control diet with 3 g$\cdot$kg^{-1} of rosemary powder, 3. control diet with 6 g$\cdot$kg^{-1} of rosemary powder, and 4. control diet with 9 g$\cdot$kg^{-1} of rosemary powder; the diets were fed as a mash. The experiment lasted 16 weeks (between 36 and 52 week of age). Rosemary powder as a commercial product was purchased from Free Trade Egypt Company, Behira (Egypt).

Table 1. Composition of control diet

Ingredient	g · kg^{-1}
Yellow maize	567.1
Soyabean meal (44% CP)	286.2
Soyabean oil	31.3
Limestone	93.3
Dicalcium phosphate	14.5
NaCl	3.00
Vitamin-mineral premix[1]	3.00
DL-methionine	1.60
Calculated analysis[2]	
ME, kcal · kg^{-1}	2800
crude protein	175.1

[1] vitamin-mineral premix provided per kg diet: IU: vit. A 8000, vit. D_3 1300; mg: vit. E 5, vit. K 2, vit. B_1 0.7, vit. B_2 3, vit. B_6 1.5, vit. B_{12} 7, biotin 0.1, folic acid 1, pantothenic acid 6, niacin 20, Mn 60, Zn 50, Cu 6, I 1, Se 0.5, Co 1; [2] calculated according to NRC (1994)

Data collection and egg parameters

Laying hens were weighed at the beginning and the end of the experiment. Feed consumption (FC) was recorded weekly and adjusted for mortality; the feed conversion ratio (FCR) (grams of feed consumed per gram of egg produced) was calculated. Egg weight (EW) and egg number (EN) were recorded daily to calculate the egg mass (EM) as EN × EW.

Egg quality criteria

Eggs were examined for interior and exterior quality. Egg components were measured monthly using three eggs from each treatment replicate. Eggs were weighed, then their length and width were determined before breaking. The egg was carefully broken on a glass plate (35 × 25 cm) to measure both internal and external egg quality characteristics. Yolks were separated from albumen. Egg shells were cleaned of any adhering albumen. Albumen weight was calculated by subtracting the weight of yolk and shell from the whole egg weight. The egg shape index was computed as the ratio of egg width to length, the yolk index as the average yolk height divided by yolk diameter (mm) following removal of the yolk from the albumen. Yolk height was measured by means of tripod micrometer reading to the nearest 0.01 mm, while yolk diameter was measured by a vernier caliper to the nearest 0.05 mm. The Haugh unit was calculated as: Haugh units (%) = $100 \times \log (H + 7.57 - 1.7W^{0.37})$, where H is the height of the albumen and W is the weight of the egg. The eggs were examined for shell quality on the basis of shell thickness (with shell membrane) that was measured by a micrometer. Shell thickness (μm) is given as the mean value of measurements at three locations on the eggs (air cell, equator, sharp end).

Blood sampling and laboratory analyses

Blood samples were randomly collected from six birds per treatment from the wing vein into sterilized tubes that were closed with rubber stoppers. Samples were left to coagulate and centrifuged at 3500 rpm for 15 min to obtain serum, and the serum samples were kept in Eppendorf tubes at –20 °C until analysed. The following serum biochemical parameters: total protein ($g \cdot dl^{-1}$), albumin ($g \cdot dl^{-1}$), urea ($g \cdot dl^{-1}$), triglycerides (TG; $mg \cdot dl^{-1}$), total cholesterol ($mg \cdot dl^{-1}$), high-density lipoprotein (HDL) cholesterol ($mg \cdot dl^{-1}$), low-density lipoprotein (LDL) cholesterol ($mg \cdot dl^{-1}$), and immunoglobulin G (IgG), M (IgM) and A (IgA) levels were determined spectrophotometrically using commercial diagnostic kits from Biodiagnostic Co. Giza (Egypt), according to Akiba et al. (1982).

For antioxidant assays, serum samples were subjected to measurement of superoxide dismutase (SOD) activity as well as reduced glutathione (GSH) and malondialdehyde (MDA) concentrations by spectrophotometric methods using a spectrophotometer (Hitachi, Japan). SOD activity was measured by the xanthine oxidase method, which monitors the inhibition of reduction of nitroblue tetrazolium by the sample (Winterbourn et al., 1975). The GSH concentration was analysed according to Beutler et al. (1963). The MDA level was analysed with 2-thiobarbituric acid (TBA), monitoring the change of absorbance at 532 nm with a spectrophotometer (Jensen et al., 1997).

Statistical analysis

Data were subjected to the ANOVA procedure for a completely randomized design using the GLM procedures of SPSS (version, 17.0; 2008). Orthogonal polynomial contrasts were used to test the linear and quadratic effects of the increasing levels of supplementation of rosemary herb.

Results and discussion

Hen productive performance

The effect of the dietary rosemary supplement on the performance of laying hens during the experimental period is shown in Table 2. There were no linear or quadratic differences ($P < 0.05$) in final body weight (FBW), FC, FCR and EW due to rosemary treatments in different phases. But EN and EM were significantly (linearly; $P < 0.05$) increased with rosemary supplementation compared with the basal diet. The mortality rate was nil in all treatments and all diet formulations. The rosemary diets were consumed without any palatability problems. The results of the current study are in line with the findings of Cho et al. (2014), who found that phytogenic feed additives (PFA) supplementation to the basal diet had no effect on feed intake (FI) or FCR compared with an unsupplemented diet. Similarly, Radwan et al. (2008) noted that dietary rosemary increased egg production, EM and improved FCR, while the FI was not statistically affected.

Table 2. Effects of rosemary powder on performance of laying hens from 36 to 52 week of age (n = 6)

Performance	Rosemary powder, $g \cdot kg^{-1}$				SEM[1]	P^2	
	0	3	6	9		linear	quadratic
Body weight, g							
initial	1604	1667	1645	1634	21.84	0.726	0.409
final	1822	1842	1780	1773	20.85	0.270	0.753
Daily feed consumption, g	87.13	86.39	94.45	95.45	2.21	0.108	0.843
Feed consumed per g egg produced	1.98	1.62	1.71	1.83	0.07	0.584	0.134
Egg weight, g	63.22	61.92	64.62	63.30	0.52	0.541	0.993
Egg number	21.69	26.15	26.04	26.06	0.68	0.025	0.080
Total egg mass, g of egg/hen	1368	1615	1687	1648	44.83	0.017	0.083

[1] SEM – standard error of means; [2] linear and quadratic effect of rosemary supplementation

Medicinal plant supplementation to poultry diets can improve FCR (Hong et al., 2012). Some studies observed improvements in body weight, body weight gain and FCR of chickens when birds fed 0.5% rosemary powder (Radwan et al., 2008). In the present study supplementing rosemary up to 0.6% of layer diets led to numerical improvement in EN and EM at all ages. The improvement in egg production parameters with phytogenic additive supplementation may be due to the provision of certain compounds that improve digestion and absorption of nutrients in the digestive tract. Also, it could be attributed to the biological activity of phenolic compounds such as carnosol and carnosic acids that are found in rosemary and are able to cause greater feed efficiency and utilization, resulting in improved productive performance (Bozin et al., 2006). Also, Moreno et al. (2006) stated that rosmarinic and carnosic acids may be the key bioactive antimicrobial compounds present in rosemary. Dietary feeding of essential oil extracted from medicinal plants increased the secretion of digestive enzymes, so enhanced nutrient digestibility and improved the performance of broilers (Jang et al., 2004). In the present study, the findings indicate that the supplementation of 0.6% rosemary to laying hen diets can give the best productive performance in terms of EN and EM.

Egg quality parameters

The effects of rosemary supplementation on egg quality parameters are illustrated in Table 3. Addition of rosemary to laying hen diets resulted in a significant linear increase in yolk percent ($P < 0.05$) and yolk-to-albumen ratio ($P = 0.01$), and decrease in albumen percent ($P < 0.05$) compared with the non-supplemented group. These results partially agree with Radwan et al. (2008), who showed that yolk weight and yolk index were significantly ($P < 0.05$) increased by rosemary supplementation to layer diets; the highest yolk index value was seen in the 0.5% rosemary group. In the present study, compared with the control diet, shell thickness was significantly (quadratically; $P = 0.006$) increased with the dietary rosemary level, obtaining the highest values of shell thickness at 3 g·kg^{-1} diet. The Haugh unit score was significantly (quadratically; $P < 0.05$) decreased with increasing rosemary supplementation up to 6 g·kg^{-1} diet. Increased shell thickness by addition of rosemary may be attributed to carnosic and rosmarinic acids; the main components in rosemary having antioxidant and antimicrobial activities (Moreno et al., 2006). A study by Bölükbaşi et al. (2008) revealed that Haugh units and yolk percent were decreased by rosemary oil supplementation to layer diets, but the albumen percent was not affected. The Haugh unit score is a key indicator of interior egg quality, and it was decreased by diets supplemented with rosemary oil (Botsoglou et al., 2005). Pertinent reports on the use of rosemary herb in hen feeding have not yet been presented in the literature.

Blood constituents

Biochemical blood parameters usually reflect the health of an animal. These parameters are vital indicators of the nutritional and physiological status of birds and animals (Abd El-Hack and Alagawany, 2015). The effect of supplemental rosemary on blood metabolites of laying hens are shown in Table 4. Serum constituents were not significantly ($P > 0.05$) influenced by rosemary supplementation, except urea, total cholesterol, IgM and IgA. Dietary supplementation of rosemary exhibited a positive impact on IgM and total cholesterol, which is in accordance with Hashemipour et al. (2013). Compared with the control group, supplementation of diets with rosemary linearly and quadratically ($P < 0.001$ and 0.062, respectively) elevated the IgM concentration and depressed (linearly and quadratically; $P < 0.001$) IgA and total cholesterol levels in the serum of hens.

In the present study, the diet enriched in rosemary (3, 6 and 9 g·kg^{-1} diet) numerically reduced serum triglycerides and total cholesterol, as well as LDL-cholesterol concentrations, but HDL-cholesterol concentrations were elevated with the same addition. In partial accordance with the present findings, Bölükbaşi et al. (2008) reported that rosemary supplementation to laying hen diets significantly depressed serum triglyceride and total cholesterol levels. In the same context, Rahimi et al. (2011) pointed out that blood triglyceride, total and LDL-cholesterol concentrations were significantly reduced by addition of PFA to chicken diets, but HDL-cholesterol concentrations increased. Radwan et al. (2008) observed that layers fed diets sup-

Table 3. Effects of rosemary powder on egg quality criteria of laying hens from 36 to 52 week of age (n = 6)

Egg quality criteria	Rosemary powder, g · kg^{-1} diet				SEM[1]	P^2	
	0	3	6	9		linear	quadratic
Albumen, %	66.48	66.76	65.28	64.72	0.36	0.040	0.536
Yolk, %	24.36	23.78	25.35	26.39	0.36	0.013	0.196
Shell, %	11.84	12.14	12.05	11.57	0.20	0.657	0.391
Egg shape index	79.30	81.72	78.41	78.96	0.64	0.453	0.467
Shell thickness, µm	0.35	0.43	0.39	0.38	0.01	0.587	0.006
Yolk index	36.67	40.06	41.14	40.50	0.58	0.240	0.410
Yolk albumen ratio	0.38	0.36	0.40	0.42	0.01	0.010	0.174
Haugh unit score	84.30	81.68	78.77	88.77	1.25	0.277	0.005

[1] SEM – standard error of means; [2] linear and quadratic effect of rosemary supplementation

Table 4. Effects of rosemary powder on blood components in laying hens at 52 week of age (n = 6)

Blood components	Rosemary powder, g · kg^{-1} diet				SEM[1]	P^2	
	0	3	6	9		linear	quadratic
g · dl^{-1}							
total protein	4.47	4.83	5.20	5.09	0.13	0.074	0.370
albumin	2.45	2.35	2.56	2.55	0.04	0.222	0.615
urea	2.47	1.46	2.17	2.87	0.18	0.010	0.060
Lipid parameters, mg · dl^{-1}							
triglycerides	183	141	124	167	15.98	0.417	0.064
total cholesterol	171	129	127	119	5.92	<0.001	<0.001
HDL-cholesterol	93.28	134.00	95.00	93.01	9.05	0.616	0.247
LDL-cholesterol	56.61	43.00	42.46	37.30	3.73	0.105	0.571
Immunoglobulin, mg · dl^{-1}							
IgG	1.27	2.09	2.00	1.78	0.14	0.854	0.130
IgM	12.54	14.75	17.95	17.74	0.71	<0.001	0.062
IgA	85.55	53.00	57.34	65.02	8.68	<0.001	<0.001

[1] SEM – standard error of means; [2] linear and quadratic effect of rosemary supplementation

plemented with rosemary leaves at 0.5% or 1% had lower ($P < 0.05$) total lipids level, while the concentrations of total cholesterol, HDL- and LDL-cholesterol in serum were not affected in the same way with the various rosemary supplementations compared with the control group. In contrast, Abd El-Latif et al. (2013) found that addition of rosemary to chicken diets led to increased serum concentrations of TG, total cholesterol and LDL-cholesterol, while concentrations of total protein, albumin and uric acid did not show significant changes. Also, Osman et al. (2010) noted that rosemary supplementation at levels of 0.5 and 1 $g \cdot kg^{-1}$ diet did not significantly affect the serum concentration of protein, albumin, creatinine or cholesterol.

Medicinal plants or their products often influence blood lipid parameters in opposing ways (Alagawany et al., 2015a,b). Hyperlipidaemic effects were reported with certain herbal plants (Bölükbaşi et al., 2006; Alagawany et al., 2015a), whereas hyperlipidaemia was seen with others (Farag et al., 2014). The discrepancies among these studies might be due to the differences in the PFA used, product type (powder, essential oil, phenolic compounds, etc.), doses and style of administration, as well as experimental conditions.

There are a large number of herbal plants having immunomodulatory properties that have been used to provide alternative potential to traditional chemotherapy for several of diseases, especially diseases related to immunodeficiency (Kumar et al., 2011; Farag et al., 2014). In the poultry industry, it is important to elevate the immune system to reduce or prevent infectious diseases. A variety of factors such as failure in vaccination and abuse of antibiotics can induce immunodeficiency and infection by immune-suppressive diseases. Using immune enhancers is a key solution to the problem of improving immunity and reducing susceptibility to infectious disease in poultry farms. Those PFA that are rich in flavonoids extend the biological activity of ascorbic acid, act as antioxidants and may enhance immune function (Acamovic and Brooker, 2005). These findings can partially explain the nutritional and biological effects of treatments on immune parameters presented in Table 4.

Addition of rosemary to layer diets may improve their serum IgM concentration versus the control diet. In previous studies it was reported that medicinal plants and their components could activate such immune functions as lymphocyte proliferation, phagocytosis, and red blood cell, haemoglobin and white blood cell counts (Hashemipour et al., 2013; Alagawany et al., 2015b).

Antioxidant indices

The antioxidant properties of aromatic herbs deserve special attention because undesirable oxidation produces unacceptable changes in flavour, colour, odour, and other quality factors of the eggshell. The effect of dietary rosemary on antioxidant parameters, including serum activity of superoxide dismutase (SOD) and concentrations of reduced glutathione (GSH) and malondialdehyde (MDA) of hens, is illustrated in Table 5. Serum SOD activity was linearly and quadratically ($P < 0.01$) increased in the rosemary groups, and reached a maximum (290 $U \cdot ml^{-1}$) at 6 $g \cdot kg^{-1}$ diet. The GSH and MDA concentrations were not linearly and quadratically influenced in comparison with the control group. Hashemipour et al. (2013) noted that the intake of herbs or their contents resulted in an increase in serum antioxidant enzyme activities such as SOD and glutathione peroxidase (GSH-Px) and a decrease in the MDA concentration. Elevated levels of antioxidant enzymes may improve the steady state of the antioxidant system of poultry. SOD is metalloprotein enzyme whose main activity is in the antioxidant defense system. Lopez-Bote et al. (1998) demonstrated that the MDA concentrations in meat from chickens fed diets supplemented with rosemary and sage extracts ranged from 0.30 to 0.35 mg $MDA \cdot kg^{-1}$ meat, and were significantly lower than those from chickens fed on the control diet not enriched with antioxidants.

It seems that rosemary supplementation to layer diets was effective in enhancing the antioxidant ability of birds. Rosemary is a rich source of beneficial phenolic compounds, carnosol, carnosic and rosmarinic acids, and related effective compounds having strong antioxidant, anti-cancer and anti-inflammatory activities (Chun et al., 2014; Rocío-Teruel et al., 2015). Herbal additives may act by more than one mechanism, affecting feed intake and conversion, stimulating the secretion of digestive enzymes and gastrointestinal motility, as well as immune and endocrine functions in addition to their antioxidant, antimicrobial, antiviral, anti-

Table 5. Effects of rosemary powder on superoxide dismutase (SOD) activity reduced glutathione (GSH) and malondialdehyde (MDA) concentrations in serum of laying hens at 52 week of age (n = 6)

Oxidative status	Rosemary powder, $g \cdot kg^{-1}$ diet				SEM[1]	P^2	
	0	3	6	9		linear	quadratic
SOD, $U \cdot ml^{-1}$	207	269	290	257	10.20	0.007	0.002
GSH, $ng \cdot ml^{-1}$	9.02	9.24	15.46	9.19	1.13	0.438	0.117
MDA, µmol	4.47	4.51	4.41	3.89	0.31	0.411	0.151

[1] SEM – standard error of means; [2] linear and quadratic effect of rosemary supplementation

inflammatory, anthelminthic and coccidiostatic activities (Hernández-Hernández et al., 2009; Basmacioğlu Malayoğlu et al., 2010).

Conclusions

The results obtained in this experiment showed that rosemary supplementation to layer diets tended to improve egg number, egg mass, serum total cholesterol and immunoglobulin M concentrations, and superoxide dismutase activity, while reducing immunoglobulin A serum content without a positive or negative effects on final body weight, feed consumption, feed conversion ratio, egg weight, shell percent, yolk index and some blood constituents including total protein, albumin, high-density and low-density lipoprotein cholesterol, immunoglobulin G and reduced glutathione concentrations as well as lipid peroxidation. On the other hand, shell thickness increased with the dietary rosemary level, obtaining the highest values of shell thickness at 3 $g \cdot kg^{-1}$ diet, while the Haugh unit score decreased with increasing rosemary supplementation up to 6 $g \cdot kg^{-1}$ diet. In summary, rosemary may be used in laying hens, but further studies are required before arriving at definite conclusions.

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