Short communication

Effects of rosemary essential oil and zinc on performance, egg quality traits, and some serum metabolites in laying hens

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Abstract This study was conducted to evaluate the effects of dietary supplementation of rosemary essential oil (REO) and zinc (as zinc sulfate) on performance, egg quality traits, and some serum metabolites in laying hens. Lohmann LSL-Lite laying hens (44-weeks-old) were randomly allocated into four treatments, each comprising of six replicates of six birds. One group served as the control and was fed a corn-soybean meal based diet without REO or zinc supplement. The other groups received the control diet supplemented with 200 mg/kg REO, 40 mg/kg zinc, or both REO and zinc, respectively. Supplementation of REO and zinc to the diet had no significant (P > 0.05) effects on egg production, egg weight, egg mass, feed intake, feed conversion ratio, body weight and production of abnormal eggs. Compared with the control, egg shape index was increased (P < 0.05) in laying hens fed with REO. Birds fed REO had lower (P < 0.05) serum total cholesterol level compared with the control. However, this effect was not observed by feeding both zinc and REO. These results indicated that the dietary inclusion of REO had a potential to improve the egg quality traits and serum cholesterol level in laying hens.

Keywords: blood metabolites, egg quality, laying hen, performance, rosemary, zinc

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Introduction

Rosemary (*Rosmarinus officinalis* L.) is known as an herbal remedy for the prevention and treatment of different diseases (Sedighi et al., 2015). Alagawany and Abd El-Hack (2015) demonstrated that productive performance and egg quality traits were improved by supplementation of 3000 mg/kg rosemary powder to laying hen diets. However, Bölükbaşi et al. (2008) reported that egg yolk percent and Haugh unit were decreased by supplementation of 200 mg/kg REO to laying hen diets.

Studies have shown that rosemary compounds have a potential to decrease the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in the liver (Valenzuela et al., 2004), and to reduce blood levels of glucose, triglyceride (Al Sheyab et al., 2012), and cholesterol (Alagawany and Abd El-Hack, 2015) in poultry and in other animals. On the other hand, Polat et al. (2011) observed that serum levels of triglyceride and cholesterol were increased by supplementation of REO to broiler diets.

Zinc is an important element deficiency of which caused loss of appetite and reduced efficiency of feed utilization leading to growth retardation, and decreased egg production and reproductive performance (Sahin et al., 2009). It is also a component of the enzyme carbonic anhydrase, which has a crucial role in supplying the carbonate ions during eggshell formation. Mabe et al. (2003) suggested that zinc can affect mechanical properties of eggshell by affecting calcite crystal formation and modifying crystallographic structure of eggshell.

Moreover, zinc has been suggested to reduce blood cholesterol levels by regulating the activity of HMG-CoA reductase in the liver and by reducing the secretion of cholesterol and triglyceride from the liver into the blood (Eder et al., 1999). Sahin et al. (2002) reported that serum total cholesterol and triglyceride levels were lower in cold-stressed laying hens fed dietary supplementation of 30 mg/kg zinc compared with those fed a basal diet. Other study observed no such differences in serum total cholesterol and triglyceride by supplementation of 40 mg/kg zinc to laying hen diet (Torki et al., 2015).

In view of these conflicting results, further studies should be conducted to determine the effects of dietary REO and zinc on laying hens. Also, to the best of our

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knowledge, no information is available on the combined effect of REO and zinc on laying hens. Therefore, this study was carried out to evaluate the effects of supplementation of REO and zinc alone or together to laying hen diet on performance, egg quality traits, and serum levels of glucose, triglyceride, and cholesterol.

Materials and methods

Zinc sulfate (ZnSO₄. 7H₂O, 98% purity) was used as the source of zinc. Dried aerial parts of rosemary were purchased from a commercial herbs market. They were ground to a powder and steam-distilled using a clevenger-type apparatus for 5 h. The REO samples, which were light buttery yellow in color, were dried over anhydrous sodium sulfate and stored in sealed vials at 4°C.

The conditions and standards of laying hens rearing used in this study were approved by the Department of Animal Science of Razi University and complied with the guidelines for the care and use of animals in research. A total of 144 laying hens (44 weeks of age; Lohmann LSL, white egg) were randomly allocated to 24 cages (40×40 cm; six hens per unit). Feed was offered in a trough feeder in the morning and in the evening, whereas water was offered *ad libitum* in trough drinkers. Light was provided for 16 h daily and temperature was maintained at 23 ± 3 °C throughout the experiment.

The birds were randomly divided into four treatment groups. Each group had six replicates. The first group was served as control and fed a corn-soybean meal based diet without zinc or REO. The other three groups received control diet supplemented with 200 mg/kg REO, 40 mg/kg zinc, or both feed additives together. Basal diet (Table 1) was formulated to meet or exceed the nutrients requirements listed in the National Research Council (NRC, 1994). The actual analyzed zinc contents of the experimental diets were determined by graphite furnace atomic absorption, using a PerkinElmer AAnalyst 600 graphite furnace system (PerkinElmer, Waltham, MA). A representative sample of each diet was ashed (550°C for 14 h) and then solubilized in 10 mL HCl (6 N) and 30 mL of demineralized water at a high temperature by using a sand heater (300°C for 15 min). After filtration (Whatman No. 41) the volume was increased to 50 mL with demineralized water. Zinc was measured at a wavelength of 324.8 nm. The analyzed levels of zinc in diets were 111.4, 145.5, 105.5, and 138.0 mg/kg, respectively.

Production performance was measured from 44 to 56 weeks of age. Egg production and egg weight were recorded daily for each replicate, and the cumulative aver-

age egg production and egg weight per bird were calculated for the total period. The generated data were used to calculate egg mass per bird. Abnormal eggs, including the soft-shelled, cracked, and broken eggs, were also recorded daily. Feed intake was measured on a weekly basis, and mortality was recorded daily. Data on feed intake and egg mass were used to calculate feed conversion ratio. All the hens were weighed by replicate at the beginning and at the end of the experiment and body weight changes were determined. The eggs laid during the last 3 days of the study were collected for determination of quality traits. Egg shape index, egg specific gravity, eggshell thickness, eggshell percentage, Haugh units, yolk index, and yolk color were determined as described elsewhere (Torki et al., 2014).

At 56 wk of age, blood samples were collected from the wing vein of six randomly selected birds per treatment (one hen per replicate). Blood was collected into sample bottles containing no anticoagulant and centrifuged at $3000 \times g$ for 15 min. Sera were stored at -20° C until further analysis. Serum glucose, triglyceride, and total cholesterol levels were analyzed using the diagnos-

Table 1. Ingredients and nutrient composition of the experimental basal diet

Ingredients	% or as noted			
Corn	67.64			
Soybean meal	21.01			
Wheat bran	0.15			
Soybean oil	0.07			
Lime stone	3.00			
Oyster shell	5.47			
Dicalcium phosphate	1.64			
Sodium bicarbonate	0.18			
Common salt	0.19			
Vitamin-mineral premix ¹	0.50			
DL-methionine	0.15			
Calculated analysis	% or as noted			

Calculated analysis	% or as noted
Metabolizable energy (kcal/kg)	2750
Crude protein	14.69
Calcium	3.64
Available phosphorus	0.37
Sodium	0.15
Crude fiber	2.32
Lysine	0.71
Methionine	0.37
Methionine + Cysteine	0.63
Threonine	0.54
Tryptophan	0.16

¹Mineral-vitamin premix provided following per kg of diet: 20 mg Cu; 100 mg Fe; 100 mg Mn; 0.4 mg Se; 110 mg Zn; 1 800 IU vitamin A; 4 000 IU vitamin D₃; 36 mg vitamin E; 4 mg vitamin K; 0.03 mg vitamin B₁₂; 1.8 mg thiamine; 13.2 mg riboflavin; 6 mg pyridoxine; 60 mg niacin; 20 mg calcium pantothenate; 2 mg folic acid; 0.2 mg biotin; 500 mg choline chloride.

tic kits (Pars Azmun, Tehran, Iran), and enzymatic methods, whereas serum zinc level was measured using an atomic absorption spectrophotometer (PerkinElmer, Waltham, MA) with a graphite furnace atomizer in deuterium background correction method. All measurements were run in triplicate.

Data were subjected to ANOVA in a completely randomized design using the GLM procedure (SAS Institute, 2003). Means were compared by the Duncan's multiple-range tests at P < 0.05 and P < 0.1 was considered tendency for an effect.

Results

Supplementation of REO and zinc to the diet had no significant (P > 0.05) effect on productive performance in laying hens. Similarly, dietary treatments did not affect (P > 0.05) eggshell weight, eggshell thickness, egg specific gravity, Haugh unit, yolk color, and yolk index. However, compared with the control, egg shape index was increased (P < 0.05) in laying hens fed with REO (Data not shown).

The effects of dietary REO and zinc on serum metabolite levels are shown in Table 2. Serum zinc level was increased (P < 0.05) by supplementation of zinc to the diet. Dietary treatments had no significant (P > 0.05) effect on serum triglyceride level. Birds fed diet supplemented with REO had lower (P < 0.05) serum total cholesterol level compared with the control. However, this effect was not observed by feeding both REO and zinc. Serum glucose level tended to be (P = 0.072) lower in birds fed combination of zinc and REO than for those fed the other diets.

Discussion

In this study, supplementation of REO to the basal diet had no significant effect on productive performance in laying hens. These results do agree with those of other researchers (Florou-Paneri et al., 2006), which showed that feed intake, egg weight, egg mass, feed conversion ratio, and average body weight were not affected by supplementation of 5000 or 10000 mg/kg rosemary herb to laying hen diets. However, Bölükbaşi et al. (2008) reported that dietary supplementation of 200 mg/kg REO increased egg weight, reduced feed intake, and improved feed conversion ratio in laying hens. In other report (Alagawany and Abd El-Hack, 2015), a linear increase in egg weight and egg mass was observed as the level of rosemary powder increased from 3000 to 9000 mg/kg diet. The reason for this discrepancy between the results is unknown, but it likely relates to the level of supplementation, preparation methods, the stability of chemical components, and the duration of study (Polat et al., 2011).

Zinc supplementation did not affect productive performance in laying hens. This result is not unexpected based on inconsistencies reported on zinc supplementation and productive performance in laying hens. Similar to our results, Swiatkiewicz and Koreleski (2008) reported that supplementation of 30 mg/kg zinc to the laying hen diet did not influence egg production, egg weight, egg mass, feed intake, and feed conversion ratio. In contrast, in other studies (Zamani et al., 2005; Idowu et al., 2011), an improvement in feed intake, egg production, egg weight, body weight, and feed conversion ratio was observed by supplementation of zinc to laying hens diets. The inconsistent effect of dietary zinc on productive performance in laying hens may be due to the mineral concentration in the basal diet. A level of 35 mg/kg dietary zinc has been recommended by NRC (1994) for laying hens. However, the basal diet used in the present study contained 111.4 mg/kg zinc, and this level of zinc seems to be adequate to provide maximum productive performance in laying hens.

Our results showed that the production of abnormal eggs and the values of eggshell weight, eggshell thick-

Table 2. Effects of dietary treatments on serum zinc and biochemical parameters of laying hens at 56 weeks of age (week 12 of the experiment)

Items -	Diet			SEM	P values	
	С	ZN	REO	ZN+REO	SEM	r values
Zinc (µg/mL)	11.22 ^{bc}	12.62a	10.77°	12.21 ^{ab}	0.265	0.028
Glucose (mg/dL)	155.6a	152.8a	140.5^{ab}	122.6 ^b	5.041	0.072
Triglyceride (mg/dL)	788.4	717.7	712.7	770.8	19.59	0.445
Total cholesterol (mg/dL)	252.7a	245.1ab	215.8 ^b	263.5a	6.35	0.038

Means within row with different superscripts are significantly different (P < 0.05), Duncan's multiple-range test were applied to compare means.

C: diet without zinc and rosemary oil supplements, ZN: diet supplemented with 40 mg zinc/kg, REO: diet supplemented with 200 mg rosemary essential oil/kg, ZN+REO: diet supplemented with 40 mg zinc/kg + 200 mg rosemary essential oil/kg, Zinc was provided as zinc sulfate (ZnSO₄, 7H₂O).

SEM: standard error of the mean.

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ness, egg specific gravity Haugh unit, yolk color, and yolk index were not affected by supplementation of REO to laying hen diets. However, compared with the control, egg shape index was increased by dietary supplementation of REO. Egg shape index is a good indicator of external egg quality, and the higher the index the better the external quality (Kgwatalala et al., 2013). Results from the literature are mixed regarding the effect of rosemary on egg quality traits in laying hens. Florou-Paneri et al. (2006) showed that supplementation of 5000 or 10000 mg/kg rosemary powder to laying hen diets had no effect on egg shape index, volk diameter, yolk height, yolk color, Haugh unit, and eggshell thickness. In contrast, Bölükbaşi et al. (2008) reported that eggshell thickness was increased but Haugh unit was decreased by supplementation of 200 mg/kg REO to laying hen diets. Similar results have been reported by Alagawany and Abd El-Hack (2015), who added different levels (3000 to 9000 mg/kg) of rosemary powder to laying hen diets. The reasons for these differences are not clear; however, several factors may affect the efficacy of an herbal product, as indicated previously.

In this study, supplementation of zinc to laying hen diets had no effect on egg quality traits. Similar to our results, Idowu et al. (2011) reported that supplementation of 105 mg/kg zinc (zinc sulfate or oxide) to laying hen diet did not affect eggshell thickness, eggshell weight, eggshell index, egg specific gravity, and Haugh unit. They showed that a level of 30 mg/kg dietary zinc was sufficient for high eggshell quality. Therefore, in the present study, the control diet alone may supply sufficient zinc for normal egg quality. However, a study by Zamani et al. (2005) showed that supplementation 100 or 150 mg/kg zinc to a basal diet containing 50 mg/kg zinc improved eggshell thickness and eggshell mechanical properties such as stiffness, elastic modulus, breaking strength, and fracture toughness. In other study (Swiatkiewicz and Koreleski, 2008), it was observed that supplementation of 30 mg/kg zinc to laying hen diet improved eggshell breaking strength while had no effect on eggshell weight, eggshell thickness, and eggshell density. These results suggest that in order to determine the effect of dietary zinc on egg quality traits factors other than those recorded in this study must also be considered as criterion measures.

In this study, supplementation of REO to laying hen diets did not affect serum zinc and triglyceride levels. However, compared with the control, serum total cholesterol level was decreased by supplementation of REO to the diet. This may be caused by the inhibition of HMG-CoA reductase activity as reported by Valenzuela et al. (2004). In accordance with the present results, Al-

agawany and Abd El-Hack (2015) indicated that serum total cholesterol level was decreased by supplementation of different levels of rosemary herb to laying hen diets, but the triglyceride level was not affected. Also, in partial accordance with the present results, Bölükbaşi et al. (2008) reported that REO supplementation to laying hen diets depressed serum total cholesterol and triglyceride levels. However, Polat et al. (2011) reported that serum triglyceride, total cholesterol, LDL-cholesterol, and HDL-cholesterol levels were increased by supplementation of REO to broiler diet. This discrepancy among the studies may be due to the differences in the product type (powder, essential oil, extract, etc.), level and type of administration, as well as experimental conditions.

Our results showed that serum zinc level was increased by supplementation of zinc to laying hen diets, which is consistent with those of other researchers (Idowu et al., 2011). However, zinc supplementation alone caused no change in serum glucose, triglyceride, and total cholesterol levels. Consistent effects of high levels of dietary zinc on serum metabolites have not been reported. Similar to our results, Torki et al. (2015) observed no changes in serum glucose, triglyceride, and total cholesterol levels by supplementation of 40 mg/kg zinc to laying hen diets. In contrast, Sahin et al. (2002) reported that serum total cholesterol and triglyceride levels were lower in cold-stressed laying hens fed dietary supplementation of 30 mg/kg zinc compared with those fed a basal diet. At present, there is no clear explanation for this discrepancy. However, our results showed that, in addition to factors mentioned earlier, the interaction of zinc by other dietary agents can also influence its effect on serum constituents. Interestingly, the lowering effect of REO on serum total cholesterol level was not observed when it was used together with zinc. Conversely, serum glucose level tended to be lower for laying hens fed REO and zinc together than for those fed the other diets. Such interaction between dietary zinc and plant essential oils has also been reported by other researchers (Stensland et al., 2015; Torki et al., 2015).

Conclusions

In the present study, dietary REO and zinc supplementation did not affect laying hen performance. However, egg shape index and serum total cholesterol level were improved by supplementation of REO to laying hen diets. Also, laying hens fed REO and zinc together had the lowest serum glucose level. However, the lowering effect of REO on serum total cholesterol level was not ob-

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served when it was used together with zinc. In summary, this study showed that REO supplementation alone is more effective to improve egg quality traits and serum total cholesterol level in laying hens than supplementation of REO and zinc together.

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اثرات روغن اسانسی رزماری و روی بر عملکرد، ویژگیهای کیفی تخممرغ و برخی متابولیتهای سرم در مرغهای تخمگذار

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چکیده این مطالعه جهت ارزیابی اثرات مکمل نمون جیره با روغن اسانسی رزماری و روی (به شکل سولفات روی) بر عملکرد، ویژگی های کیفی تخم مرغ و برخی متابولیت های سرم در مرغهای تخم گذار انجام شد. مرغهای تخم گذار سویه لوهمن الیاسال – لایت (در سن ۴۴ هفتگی) به صورت تصادفی بین چهار تیمار هریک شامل ۶ تکرار از ۶ پرنده تقسیم شدند. یک گروه به عنوان شاهد انتخاب شد و جیره پایه ذرت – کنجاله سویا بدون مکمل روغن اسانسی رزماری و روی را دریافت نمود. دیگر گروهها به ترتیب جیره شاهد مکمل شده با ۲۰۰ میلی گرم /کیلوگرم روغن اسانسی رزماری و روی گرم /کیلوگرم روغن اسانسی رزماری و روی گرم /کیلوگرم روی یا ترکیب روغن اسانسی رزماری و روی را دریافت کردند. مکمل کردن روغن اسانسی رزماری و روی در جیره اثر معنی داری بر تولید تخم مرغ، وزن تخم مرغ، تولید توده ای تخم مرغ، مصرف خوراک، ضریب تبدیل خوراک، وزن بدن و تولید تخم مرغ های غیر طبیعی نداشت (۹۰ - ۱۰۰۷). در مقایسه با شاهد، شاخص شکل تخم مرغ، در مرغهای تغذیه شده با روغن اسانسی رزماری افزایش یافت (۹۰ - ۱۰۰۷). پرنده های تغذیه شده با روغن اسانسی رزماری در مقایسه با شاهد سطح کلسترول کل سرمی کمتری داشتند (۹۰ - ۱۰۰۷). اما این اثر با ترکیب روی و روغن اسانسی رزماری مشاهده نشد. این اثرات نشان داد که گنجاندن روغن اسانسی رزماری در جیره می تواند باعث بهبود و یژگی های کیفی تخم مرغ و سطح سرمی کلسترول در مرغهای تخم گذار شود.