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Influence of dietary *Lactobacillus*-based probiotic or essential oil blend on laying performance, blood parameters and humoral immunity of laying hens reared in summer

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ABSTRACT

This study was conducted to investigate the effects of dietary supplementation with *Lactobacillus*-based probiotic (PRO) and/or essential oil blend (EOB) in laying hens reared under heat stress on laying performance and egg quality, on some blood parameters, and on immunity. Laying hens, exposed to high ambient temperatures during the summer, were allocated in 4 experimental treatments for 16 weeks. (192), 52-week-old, Birds were fed a basal diet (control) or the same diet supplemented with PRO (0.5 g/kg) or EOB (36 mg/kg) or PRO+ EOB (with the same dosages). The egg weight were markedly enhanced in eggs from hens treated with EOB, and the dietary supplementation with PRO and EOB alone or combined with each other has significantly increased Haugh unit and albumen height ($P<0.05$). The intestinal pH was dramatically depressed in PRO and in PRO+EOB supplemented hens, whereas the intestinal viscosity was significantly reduced only in birds treated with both additives ($P<0.01$). Supplementing PRO and EOB in diet tend to improve (not significantly) antibody responses against Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV). It was concluded from this study that PRO and/or EOB supplementation in laying hens exposed to high temperatures improves egg weight and egg qualities and partly promotes humoral immunity.

Key words: Essential oils, Heat stress, Immunity, Laying hens, Probiotic

Avian species exposed to high ambient temperature exhibit various behavioural and physiological changes — in laying hens, heat stress characterised by decreased egg production, egg weight, eggshell thickness and strength, feed intake as well as immunosuppression (May *et al.* 1986, Mashaly *et al.* 2004, Franco-Jimenez *et al.* 2007). Suzuki *et al.* (1983) demonstrated that heat stress caused marked changes in the intestinal bacterial composition. Shoail *et al.* (2010) suggested that probiotic plays an important role in stimulating the immune system of poultry during heat stress.

Herbs, parts of herb plants or herbal plant extracts can beneficially affect feed intake, secretion of digestive juices and the immune system of animals (Mikulski *et al.* 2008) due to the presence of essential oils. These have some antimicrobial, antioxidant, enzymatic, digestion stimulating biological properties (Hernandez *et al.* 2004), anti-heat stress effects (Cabuk *et al.* 2006) as well as activate immune system (Hu 1997). The present study aimed at investigating the potential role of *Lactobacillus*-based PRO and EOB supplementation diets alone or in combination with each other on laying performance, egg quality, some blood parameters and humoral immunity in laying hens reared in hot summer season.

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MATERIALS AND METHODS

Experimental treatments and diets: Laying hens (192) of 52 weeks of age were randomly divided into 4 treatment groups of 48 birds each, and birds were housed individually. Each treatment consisted of 6 replicates of 8 birds / replicate. The dietary treatments were as follow: (i) basal diet (negative control), (ii) basal diet supplemented with 0.5 g/kg commercial PRO, (iii) basal diet supplemented with 36 mg/kg commercial EOB, and (iv) basal diet supplemented with 0.5 g/kg PRO plus 36 mg/kg EOB.

The ingredients and chemical composition of the basal diet are presented in Table 1. EOB is a blend of 6 different essential oils derived from selected herbs (Oregano oil (*Origanum* sp.), laurel leaf oil (*Laurus nobilis* L.), sage leaf oil (*Salvia triloba* L.), myrtle leaf oil (*Myrtus communis*), fennel seeds oil (*Foeniculum vulgare*), and citrus peel oil (*Citrus* sp.)) and included carvacrol, thymol, 1:8-cineole, p-

Table 1. Ingredients and chemical composition of basal layer stage 2 diet (52–68 week)

	%
Ingredients	
Corn	57.65
Soybean meal (44% CP)	17.40
Sunflower meal (28% CP)	7.95
Full-fat soybean (32% CP)	4.00
Soybean oil	1.00
Dicalcium phosphate	1.30
Vitamin premix[1]	0.10
Trace mineral premix[2]	0.10
Limestone	9.80
Salt	0.35
D-L methionine	0.20
Sawdust	0.15
Chemical composition %	
Dry matter	90.00
Crude protein	16.00
Crude oil	3.70
Crude fiber	4.20
Crude ash	13.00
Starch	41.80
Calculated composition	
ME, Kcal/kg	2884
Ca, %	4.20
Available P, %	0.35
Methionine, %	0.44
Lysine, %	0.80

[1]Vitamin premix, per kg diet: 15 000 IU vitamin A; 5 000 IU vitamin D_3; 50 mg vitamin E; 10 mg vitamin K_3; 4 mg vitamin. B_1; 8 mg vitamin B_2; 5 mg vitamin B_6; 0.025 mg vitamin B_{12}; 50 mg niacin; 20 mg panthothenic acid; 2 mg folic acid 0.25 mg biotin; 75 mg ascorbic acid; 175 mg choline.

[2]Mineral premix, per kg diet: 56 mg Mn; 140 mg Zn; 56 mg Fe; 10.5 mg Cu; 1.05 mg I; 0.28 mg Co; 0.28 mg Se; 0.7 mg Mo.

cymene, and limonene as active components. PRO is a *Lactobacillus*-based probiotic, and the microbial composition of it is as following (colony forming unit-cfu/kg): *Lactobacillus acidophilus* 3.09×10^{10}, *Lactobacillus plantarum* 1.89×10^{10}, *Lactobacillus del.* subsp. *bulgaricus* 3.09×10^{10}, *Lactobacillus rhamnosus* 3.09×10^{10}, *Bifidobacterium bifidum* 3.00×10^{10}, *Streptococcus sal.* subsp. *thermophiles* 6.15×1010, *Enterecoccus faecium* 8.85×10^{10}, *Aspergillus oryzae* 7.98×10^{10}, *Candida pintolopesii* 7.98×10^{10}.

The diets were formulated to meet or exceed NRC (1994) specifications. The basal diet (layer stage 2) was analysed for dry matter, crude ash, crude protein, crude oil, crude fibre, sugar, starch, according to chemical analytical methods recommended by the Association of German Agricultural Analysis and Research Institutes (VDLUFA) (Naumann and Bassler 1993). A conventional vaccine programme was used for the experimental animals.

Housing and management: The trial was conducted in a layer pen during the summer, between June and September 2010, in Ankara, located in central Turkey. The hens were housed in individual layer cages. The experimental diets and drinking water were offered *ad lib*. A photoperiod of 17 h/day was maintained. Hen house temperature was recorded all hours of the day during the experiment. Mean hen house temperatures for June, July, August and September were 26, 29, 30 and 28 °C, respectively.

Productivity and egg quality parameters recording and analyses: Individual body weights were measured at the beginning and at the end of the experiment. The mean body weights (mean ± standard error) of birds in control, PRO, EOB and PRO+EOB treatments at the beginning of the study were 1963±12, 1979±34, 2018±31 and 2000±44, respectively. The final body weights (g) were measured as 2023±26, 2122±42, 2152±23 and 2152±32 for the same treatments, respectively. During the experiment, 30 randomly sampled eggs from each treatment were collected on 2 consecutive days biweekly. Hence, 960 eggs were weighed to determine the average egg weight. Food intake was recorded on a weekly basis. Egg mass and food conversion ratio were calculated as per (Mizrak *et al*. 2010).

Eggs (768), 24 from each treatment were randomly collected to evaluate egg quality once every 4 week. Egg-shell thickness, egg-shell strength, yolk colour score, Haugh unit (HU), and albumen height were examined. When determining egg quality characteristics, the sample of eggs were individually weighed at initiation, and then egg shell breaking strength was measured using egg shell tester equipment. Eggs were cracked and the shell was weighed. Egg shell thickness (with membrane) was measured at 3 different points (i.e., the top, middle, and bottom) using a micrometer. Albumen height was measured electronically. Hangh unit was calculated as sper Haugh (1937).

Digestive function analyses: At the end of the experiment, 6 hens from each treatment were slaughtered severing the bronchial vein to determine intestinal viscosity and pH. The pH value of the intestinal (duodenum and jejunum) content was determined with a pH meter. Several aliquots were filled with each sample, and centrifuged (4000 *g* for 10 min at 4°C). The supernatant was withdrawn and the viscosity of a 0.5 mL aliquot was measured using a digital viscometer maintained at 40°C and expressed in cP.

Biochemical and serological analyses: At the end of the study, blood samples were drawn from the jugular vein of 6 birds from each treatment in microtubes without anticoagulant. After clotting for 2 h at room temperature, serum was separated by centrifugation at 3000 g for 10 min at room temperature, and stored at –20 °C until biochemical analyses –determination of the serum concentrations of glucose- by the glucose oxidase method (Trinder 1969) of triglycerides by the glycerol-3-phosphate-oxidase para-aminophenazone GPO-PAP colorimetric enzymatic method

(Trinder 1969), and total cholesterol by the cholesterol-oxidase para-aminophenazone (CHOD-PAP) colorimetric enzymatic method (Roeschlau *et al.* 1974).

Serum samples were collected from 6 hens from each treatment at the beginning and at the end of the experiment to investigate the antibody titres against the Newcastle disease virus (NDV), infectious bronchitis virus (IBD) and infectious bursal disease virus (IBDV) by ELISA technique using commercial kits. Plates were read at 650nm on an ELISA reader.

Statistical analysis: All data were subjected to analysis of variance in the general linear model using the SPSS 15.0 (SPSS 2006) statistical package. The experimental unit for the analyses of egg production and LWG was a cage containing 1 hen but the experimental unit for the other parameters were treatment means. *Post-hoc* analyses were carried out to separate the significant differences between treatment means using the Duncan's test.

RESULTS AND DISCUSSION

The egg weight increased significantly in hens supplemented with EOB alone (Table 2). Moreover, LWG tended to be higher in supplemented birds than in the controls (P>0.05). Considering dietary probiotic supplementation in agreement with the results of our study, Nahashon *et al.* (1994) have reported that *Lactobacillus* added diets increased body weight in laying hens. Similarly, Yörük *et al.* (2004) showed that probiotic dietary supplementation in laying hen diets improves egg production and egg weight, without decreasing feed efficiency. In the present study, the egg production tended to be lower in supplemented hens than that in the control (not significantly). Contrarily, Çabuk *et al.* (2006) reported that hen-day egg production of brown layers, 54- to 74- weeks-old, receiving diets supplemented with an EOB was significantly higher compared to the control (P<0.01) in summer. Ma *et al.* (2005) reported beneficial effects of herbal additives on egg production of laying hens during heat stress; but, Bozkurt *et al.* (2009) showed that dietary EOB supplementation did not affect egg production and egg weight from broiler breeders. Botsoglou *et al.* (2005) investigating the effects of dietary aromatic plant extracts on the laying performance of 32- to 40-week old hens, reported no significant differences in egg production and egg weight among the treatments. These discrepancies would be related to the amounts of PRO and plant extracts added to the diets and to the nature and source of such additives.

Our results showed no significant differences in eggshell weight, eggshell thickness, eggshell strength, and egg yolk colour among treatments. Haugh unit and albumen height increased significantly in EOB supplemented birds compared to the controls (P<0.05). Latter parameter was also higher in PRO+EOB supplemented birds than that of the control birds. Improvement in Haugh unit indicated that essential oil can improve egg quality by increasing its shelf life. However, these results were in disagreement with those of Botsoglou *et al.* (2005) and Florou-Paneri *et al.* (2005) who reported that Haugh unit and albumen height were not affected by oregano essential oil supplementation to diet.

Significant differences were observed in intestinal pH and viscosity among treatments (Table 3). PRO supplementation combined with EOB to diet significantly decreased intestinal pH. Low pH in stomach and intestine suppresses growth of

Table 2. Effects of probiotic, essential oil blend and probiotic + essential oil blend included in the basal diet of laying hens on productivity and egg quality parameters

Parameters	Treatments				SEM	P
	Control	PRO	EOB	PRO + EOB		
Productivity parameters						
Egg production, hen-day, %	78.25	76.73	77.44	75.44	0.79	NS
Egg weight, g	66.70^{b}	66.51^{b}	68.79^{b}	67.00^{b}	0.31	P<0.05
Egg mass, g/hen/day	52.16	51.04	53.23	50.51	0.56	NS
Feed intake, g/hen/day	108.18	110.44	109.90	107.31	1.03	NS
Feed conversion, g feed/g egg	2.08	2.16	2.06	2.14	0.03	NS
Body weight gain, g	60	134	143	152	15.70	NS
Livability, %	99	99	98	98	0.32	NS
Egg quality parameters						
Cracked/broken/ egg ratio, %	3.92	4.10	4.12	5.08	0.31	NS
Egg yolk color score[1]	5.30	5.37	5.21	5.19	0.04	NS
Haugh unit, HU	76.80^{b}	79.20^{ab}	80.80^{a}	79.70^{ab}	0.50	P<0.05
Albumen height, mm	6.39^{b}	6.72^{ab}	7.05^{a}	6.81^{a}	0.07	P<0.05
Egg shell weight, g	5.82	5.82	5.96	5.81	0.04	NS
Egg shell thickness, µm	324.61	329.92	320.67	319.75	1.86	NS
Egg shell strength, kg/cm^2	3.61	3.55	3.67	3.51	0.05	NS

[1]Roche yolk colour score:1, light yellow,; 15, orange. $^{a-b}$ values in the same line not sharing a common superscript differ significantly. EOB, Essential oil blend; NS, not significant; PRO, probiotic; SEM, pooled standard error mean; P, probability.

Table 3. Effects of probiotic, essential oil blend and probiotic + essential oil blend included in the basal diet of laying hens on intestinal viscosity and pH, blood and immune response parameters

Parameters	Treatments				SEM	P
	Control	PRO	EOB	PRO + EOB		
Intestinal viscosity and pH						
viscosity, cP	1.89[a]	1.70[ab]	1.89[a]	1.45[b]	0.06	P<0.05
pH	6.22[a]	5.88[b]	6.30[a]	5.66[b]	0.08	P<0.01
Some blood and immune parameters						
Glucose, mg/dl	228	237	233	231	2.56	NS
Total cholesterol, mg/dl	157	139	193	183	9.41	NS
Triglyceride, g/l	2.21	1.88	2.72	2.67	0.19	NS
NDV antibody titers (ELISA titers)						
At 52 week old	13756	15417	12132	13174	771	NS
At 68 week old	4291	5050	6519	5876	672	NS
IBV antibody titers (ELISA titers)						
At 52 week old	7716	9474	10904	6934	729	NS
At 68 week old	11611	10337	9374	12018	1202	NS
IBDV antibody titers (ELISA titers)						
At 52 week old	9214	8392	9245	8936	321	NS
At 68 week old	8427	9425	8684	9293	448	NS

[a-c]Values in the same line not sharing a common superscript differ significantly. EOB,essential oil blend; IBV, infectious bronchitis virus; IBDV, infectious bursal disease virus; NDV, Newcastle disease virus; NS, not significant; PRO, probiotic; P, probability; SEM, pooled standard error mean.

acid-intolerant bacteria. Low gastric pH accelerates the conversion of pepsinogen to pepsin, which enhances the absorption rate of proteins and minerals (Park *et al.* 2009). Combined with PRO and EOB supplementation in layer diets synergistically depressed the intestinal viscosity.

Neither PRO nor EOB supplementation to the diet affected serum glucose, triglyceride and total cholesterol concentrations, but total cholesterol and triglyceride concentrations tended to increase in birds treated with EOB alone or in combination with PRO compared to the control. Likewise, Sohail *et al.* (2010) reported that dietary probiotic supplementation decreased cholesterol concentration in broiler. Supplementing EOB or PRO or EOB+PRO in diets had no statistically significant advantageous effect on antibody titers against NDV, IBV and IBDV in summer, however, as numerical, there were positive effects of EOB and PRO on antibody titers against NDV and IBDV, respectively. The antibody titer levels of NDV for all treatments declined from 52 through 68 weeks. Although the changes of antibody titers for NDV (from 52 to 68 weeks of age) over time had similar trends in the all treatments ($P > 0.05$), the NDV antibody titer levels of EMO and PRO+EOB treatments were higher compared to the other treatments. These results showed that EOB and PRO supplementation in diet tend to increase immunity. Kong *et al.* (2006) reported that appropriate doses of Chinese herbal ingredients had a positive effect on immune enhancement for chicken. Likewise, it was reported that dietary supplementation of *Lactobacillus*–based probiotic can improve humoral immunity (Sohail *et al.* 2010).

Dietary supplementation of EOB alone showed positive effects on egg weight and Haugh Unit, and dietary supplementation of EOB alone or in combination with PRO increased albumen height. Combined supplementation of PRO with EOB decreased intestinal viscosity. Additionally, PRO or PRO+EOB supplementation decreased intestinal pH. Collectively, these findings suggested that PRO and EOB supplementation in commercial layer reared under heat stress can be beneficial to egg weight, Haugh unit, albumen height, digestive function and humoral immunity.

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