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Effect of Probiotics, Yeast, Vitamin E and Vitamin C Supplements on Performance and Immune Response of Laying Hen During High Environmental Temperature

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Abstract: In order to evaluate the effects of dietary probiotics, yeast, vitamin E and vitamin C supplementation on performance, serum and yolk cholesterol and immune response of heat stressed laying hens, a trial was conducted with sixty white layer hens of Hy-Line variety. Experiment was conducted by using completely randomized design with 5 treatments, 3 replicates and 4 hens in each replicate. The treatments involved: control, basal diet plus 50 mg multi strains probiotic, basal diet plus 1 g yeast of *Saccharomyces cerevisiae*, basal diet plus 200 mg vitamin C and basal diet plus 200 mg vitamin E per Kg of diet. Results indicated no significant difference in hen performance, egg quality (shell thickness, shell resistance, shell percent and haugh unit) and serum and yolk cholesterol concentrations. Yolk percent was increased significantly and the highest yolk percent was observed in vitamin E treatment. Immune response of laying hens with multi strains probiotic and yeast supplementation was greater than others. However, dietary vitamin E and C supplementation increased immune response, but differences were not significant compare with other groups.

Key words: Vitamin E, vitamin C, probiotics, yeast, laying hen, high environmental temperature

Introduction

High temperature results in reduced feed intake, egg production, egg weight, Haugh units and yolk index (Smith and Oliver, 1972). Heat stress stimulates the release of corticosterone and catecholamines and initiates lipid peroxidation in cell membranes (Freeman and Crapo, 1982), including membranes of T and B lymphocytes.

Use of vitamins C and E, selenium (Sahin and Kucuko, 2001; Sahin *et al*., 2002), antibiotics and probiotics (Manner and Wang, 1991; Zulkifli *et al*., 2000) as additives in feeds was aimed at reducing the heat stress in birds. Researches showed that using vitamin E can reduce the negative effects of corticosterone (Tengerdy, 1989), improve egg production, feed intake and yolk and albumen solids (Kirunda *et al*., 2001), improve egg quality (Puthpongsiriporn, 1998), release of vitellogenine that is necessary for yolk formation (Bollengier-Lee *et al*., 1998) and develop immune response by antioxidation property (Franchini *et al*., 1991; Meydani and Blumberg, 1993) in hens exposed to heat stress. In the same way, under hot conditions, birds are not able to synthesize sufficient amounts of ascorbic acid (Kutlu and Forbes, 1993) and supplemental ascorbic acid could significantly reduce the body temperature (Orban *et al*., 1993; Pardue *et al*., 1985).

Regarding antioxidant property, there is a positive synergistic effect of vitamins E and C on the immune response. In addition to antioxidation, vitamin C has been reported to enhance immune response by modifying corticosteroid synthesis in adrenal glands (Pardue *et al*., 1985).

The addition of probiotics to diets benefit the host animal by stimulating appetite (Nahashon *et al*., 1992), improve intestinal microbial balance (Fuller, 1989), stimulate the immune system (Toms and Powrie, 2001), decrease pH and release bacteriocins (Rolfe, 2000) that compete with other microbes for adhesive site, improve egg mass, egg weight, egg size in layers (Nahashon *et al*., 1992; Jin *et al*., 1997) and feed consumption in layers and also depress serum and egg yolk cholesterol concentrations in hens (Mohan *et al*., 1995; Kurtoglu *et al*., 2004). However, there are scarce reports on the effects of probiotic supplementation on immune response in chickens under heat stress conditions, although it has been suggested that the effectiveness of probiotics may be more obvious in stressed chickens (Jin *et al*., 1997). According to mention summery, we were observed that the different kind of supplements can improved hen performance with different mechanism during heat stress. There was no information about comparing of using different dietary supplementation that improves hen performance during high environmental temperature. This study was conducted to investigate the effects of dietary supplementation of probiotics, yeast, vitamin E and vitamin C on performance, egg quality, immune response and serum and egg yolk cholesterol levels of laying hens exposed to high environmental temperature in the same condition.

Materials and Methods

A total of 60 laying hens, 62 week old, Single Comb White Leghorn (Hyline-W36 strain) were divided into five groups. Experiment was conducted by using completely

Table 1: Composition of the basal diet

Ingredients	% in diet
Yellow corn	45.38
Wheat	21.00
Soybean meal	20.25
Soybean oil	1.80
Oyster shell	9.60
Sodium bicarbonate	0.15
Potassium bicarbonate	0.10
Salt	0.15
Vitamin premix[1]	0.20
Mineral premix[2]	0.20
DL-Methionine	0.12
L-Lysine	0.03
Dicalcium phosphate	1.02
Nutrient analysis	
Metabolizable energy (kcal/kg)	2806.4870
Crude protein (%)	15.0170
Lysine (%)	0.7518
Methionine (%)	0.3576
TSAA[3] (%)	0.6191
Calcium (%)	3.9510
Available phosphorous (%)	0.3013

[1]Vitamin premix provided per kilogram of diet: vitamin A, 8800 IU; vitamin D_3, 2500 IU; vitamin E, 11 IU; vitamin K_3, 2.2 mg; vitamin B_1, 1.5 mg; vitamin B_2, 4 mg; pantothenic acid, 8 mg; vitamin B_6, 2.46 mg; niacin, 35 mg; vitamin B_{12}, 0.01 mg; folic acid, 0.48 mg; biotin, 0.15 mg; cholin chloride, 200 mg., [2]Mineral premix provided per kilogram of diet: manganese, 75 mg; iron, 75 mg; copper, 6 mg; iodine, 0.87 mg; selenium, 0.2 mg; zinc, 64.68 mg., [3]TSAA: Total Sulfur containing Amino Acids

randomized design and five dietary treatments were utilized. The treatment involved: control, basal diet plus 50 mg/Kg multi strains probiotic (a product containing nine strains of variable organisms namely *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Aspergillus oryzae* and *Candida pintolopessi*), basal diet plus 1 g/Kg yeast of *Saccharomyces cerevisiae*, basal diet plus 200 mg/Kg L-ascorbic acid (vitamin C) and basal diet plus 200 mg/Kg α-tocopheryl acetate (vitamin E). These levels of supplementation selected base on optimum recommended level in some researches. The composition of basal diet is shown in Table 1.

This study was conducted in the northern research farm of Animal Science Research Institute. Hens were randomly assigned to cages so that there were three replications. Each replicate consisted of 2 adjoining cages with 2 hens per individual cage for a total of 4 hens per replicate. Before the start of the experiment, all hens fed basal diet for 2 weeks and were similar in body size and production. Layers were fed with experimental diets for 42 days. Feed (in mash form) and water were provided *ad-libitum* throughout the experiment. The experiment was conducted in the summer and the temperature and lighting schedules (16L: 8D) were similar to guidelines set in the Hy-Line W-36 Commercial Management Guide (Hy-Line International, 2003). During the experiment, hens exposed to cycling short-time heat stress. The constant temperature and relative humidity of hen house was 24±2°C and 50±10%, respectively. During experimental period in summer we had 4-h/day high environmental temperature. In addition to this we increased temperature and relative humidity of house for 5-h/day upped to 33±2°C and 45±11%, respectively.

Egg production was monitored daily and feed consumption was recorded at the end of each six weeks of the experimental period. Egg weight was measured two times in a week. Shell thickness, shell hardness, shell weight, albumen quality (Haugh unit score), yolk weight and were measured every two weeks. Internal egg quality, Shell thickness and shell hardness were measured by Egg Multi Tester EMT-5200, Ultrasonic Thickness Gauge (Echometer 1062) and Digital Egg Shell Force Gauge (model-II), respectively. Yolk cholesterol and plasma cholesterol were determined during the last week of the trial. These measurements were made by spectrophotometer (UV-visible S2100, Scinco, Korea) using commercial kits by method of Pasin *et al.* (1998).

For experimental immunization, antibody against Sheep Red Blood Cells (SRBC) was measured using the method designed by Trout *et al.* (1996). Briefly, birds were injected intravenously (brachial vein) with 0.2 mL of 9% SRBC and after 5 days of inoculation, birds were bled. Then at the same day SRBC was injected again. Serum samples were obtained 5 days after the second injection to determine anti-SRBC secondary antibody titers. The sera were inactivated at 56°C for 30 min. Antibody production was measured by an agglutination test using the microtiter technique.

Data were analyzed by ANOVA using General Linear Models procedure of SAS software (SAS Institute, 1999). Means were compared using Duncan's multiple range test. Level of significance used in all results was 0.05.

Results and Discussion

As shown in Table 2, dietary supplementation of probiotics, yeast, vitamin E and vitamin C during heat stress caused higher egg production than control, but these differences were not significant statistically ($p>0.05$). Also, egg weight, egg mass, feed consumption and feed conversion ratio was not effected by treatments. Probiotic inclusion did not influence the egg weight significantly, which has already been reported by Mohan *et al.* (1995), Haddadin *et al.* (1996) and Chen and Chen (2003). But there are also some reports which disagree with our findings (Nahashon *et al.*, 1992; Tortuero and Fernandez, 1995), which might be related to the strain of bacteriae, concentration and the form of bacteria used (viability, dryness or their products).

Table 2: Effect of dietary supplementation of probiotics, vitamin E and vitamin C during heat stress on performance of laying hens

Parameters	Control	Multi strain probiotic	Yeast	Vitamin C	Vitamin E	SEM
Hen-day Egg Production (%)	70.29	70.46	71.35	72.08	71.28	0.67
Egg Weight (gr)	61.06	61.17	60.63	61.93	59.50	0.41
Egg Mass (gr/hen/day)	42.90	43.11	43.95	44.63	42.39	0.61
Feed Consumption (gr/hen/day)	103.39	106.10	104.90	107.23	106.50	0.99
Feed Conversion Ratio (gr feed/gr egg)	2.411	2.463	2.398	2.403	2.513	0.24

Table 3: Effect of dietary supplementation of probiotics, vitamin E and vitamin C during heat stress on egg quality treats

	Control	Multi strain probiotic	Yeast	Vitamin C	Vitamin E	SEM
Shell thickness (mm×10^2)	29.58	30.20	30.73	29.98	30.21	0.17
Shell Resistance (kg/cm^2)	2.76	2.54	2.91	2.79	2.70	0.05
Egg shell (%)	9.07	9.29	9.27	8.89	9.34	0.07
Egg yolk (%)	26.60^{b}	27.62^{ab}	27.24^{ab}	27.77^{ab}	28.33^{a}	0.21
Haugh unit	82.70	85.63	82.76	85.27	84.25	0.49

^{a,b}Row means with common superscripts do not differ significantly ($p>0.05$)

Table 4: Effect of dietary supplementation of probiotics, vitamin E and vitamin C during heat stress on serum and egg yolk cholesterol and immune response

	Control	Multi strain probiotic	Yeast	Vitamin C	Vitamin E	SEM
Serum cholesterol (mg/dl)	148.85	137.95	147.34	149.04	133.24	3.72
Egg cholesterol (mg/gr yolk)	11.86	11.33	11.39	10.56	11.38	0.19
Antibody titer (log_2) against SRBC[1]	6.33^{b}	8.67^{a}	8.83^{a}	7.83^{ab}	8.03^{ab}	0.31

^{a,b}Row means with common superscripts do not differ significantly ($p>0.05$), [1]SRBC: Sheep Red Blood Cells

Balevi *et al.* (2001) were fed commercial multi strain probiotic to 40-week-old layers and showed no statistically significant differences in egg production and egg weight compared with the control. They were stated that the difference between their results and previous works may be related to differences in the ages of the hens. Furthermore, in the present study, short period of experiment was an additional factor that inhibits appearing the effect of probiotic on performance. Kurtoglu *et al.* (2004) showed that probiotic effect on egg production was not specific until day 60, but significant increase in egg production by probiotic supplementation were seen on days 60-90 of their experiment.

Egg quality (shell thickness, shell resistance, shell percent and haugh unit) didn't affected ($p>0.05$) by probiotics, vitamin E and vitamin C supplementation (Table 3). Egg shell thickness in all treatment was higher than control and this showed the positive effects of probiotics, vitamin E and vitamin C during heat stress. Yolk percent was increased in all of the treatments compared with control but the highest yolk percent (28.33%) was observed in vitamin E treatment ($p<0.05$). Hosseini *et al.* (2006) reported that addition of yeast in commercial layer hen diet had not any positive effect on egg shell thickness, haugh unit, egg breaking strength and egg shell quality. Mahdavi *et al.* (2005) realized that using the different levels of probiotic caused significant decrease in plasma cholesterol, plasma triglyceride and egg cholesterol, but it had no significant effects on egg production, egg weight, egg mass, feed consumption, feed conversion ratio, shell thickness, shell hardness and Haugh unit. Haugh unit is major indicator determining egg quality and does not change by dietary regimen (Silversides and Scott, 2001).

As well as shown in Table 4, there was no significant difference in serum and yolk cholesterol concentrations between experimental groups ($p>0.05$). These findings were in agreement with Kurtoglu *et al.* (2004) who showed that probiotic did not affect serum/yolk cholesterol in 30-days period of experiment. But they did not support Mohan *et al.* (1995) or Mahdavi *et al.* (2005) who report that probiotics could depress serum and egg yolk cholesterol concentrations. However, cholesterol depressing effect of probiotics in the serum and egg yolk in layers requires further investigation.

Antibody production against SRBC in laying hens that fed multi strains probiotic and yeast supplementation was greater than control group ($p<0.05$). However, dietary vitamin E and vitamin C supplementation increased immune response, but differences were not significant compared with other groups ($p>0.05$).

Serological data from the present study showed the effectiveness of probiotics supplementation on systemic immunity. The result of this experiment was similar to finding of Panda *et al.* (2000) and Cross (2002). They indicated that some probiotic could stimulate a protective immune response sufficiently to enhance resistance to microbial pathogens. Matsuzaki *et al.* (1998) reported that oral administration of *Lactobacillus casei* enhanced activity of splenic NK cells and stimulated phagocytic activity. The gut and its resident microbiota play a pivotal role in shaping the immune system repertoire (Noverr and Huffnagle, 2004). Germfree animals have less developed gut-associated lymphoid tissue, but gut colonization in these animals by members of commensal gut microbiota results in the

enhancement and diversification of the antibody-mediated immune response (Rhee *et al*., 2004).

Haghighi *et al*. (2005) reported that probiotic-treated birds had significantly more serum antibody (predominantly immunoglobulin M [IgM]) to SRBC than the birds that were not treated with probiotics. Similarly, Inooka and Kimura (1983) have studied the effects of *Bacillus natto* in feed on SRBC antibody response in chickens. They were observed an increase in antibody production in the chickens fed *Bacillus natto* in diet. They were suggested the lymphoid organs in the intestinal tract show a developmentative response to antigenic substance such as bacteria or feed. Therefore, the effect of enhancement of antibody production in present experiment may be associated with the development of these organs.

Portions of the cell wall structure of the yeast organism, *Saccharomyces* contained Mannanoligosaccharid (MOS) which elicit powerful antigenic properties. Ferket *et al*. (2002) suggested that an increase in antibody response to MOS due to the ability of the innate immune system to react to foreign antigenic material of microbial origin. As well as probiotics, vitamin E and C also resulted in higher antibody titer production after SRBC injection than control group. Antioxidant properties of vitamin E have been shown to enhance immunity of laying hens. Vitamin E has been reported to protect cells involved in immune response, such as lymphocytes, macrophages and plasma cells, against oxidative damage and to enhance the function and proliferation of these cells (Franchini *et al*., 1991; Meydani and Blumberg, 1993).

The results of this study did not support data reported by Scheidler and Forning (1996), Bollengier-Lee *et al*. (1998) and Ciftci *et al*. (2005) that vitamin E supplementation at high levels can improve performance of hens exposed to heat stress. It may be related to short time of heat stress. However, percent of egg yolk was significantly increased ($p<0.05$), when hens were fed experimental diet compared with the control diet. The highest yolk percent was observed in Vitamin E group than in other groups. This result confirms the observations of other researchers (Bollengier-Lee *et al*., 1998; Puthpongsiriporn *et al*., 2001; Ciftci *et al*., 2005). Vitamin E-mediated protection of the liver may improve the production or export of egg yolk precursors from the liver and therefore increase egg production during heat stress.

Using vitamin C had a little positive effect on performance, egg quality, serum and yolk cholesterol and immune response which may be due to the short time of heat stress. The weakness of these effects would be resulted from an insufficient dosage of vitamin C, unable to totally recover ascorbate requirement under hot conditions. Njoku and Nwazota (1989) demonstrated that high dietary vitamin C (200, 400, 600 mg/kg) supplementation significantly increased egg production in hens exposed to heat stress. Similarly, Demir *et al*. (1995) reported that vitamin C supplementation in feed (200 mg/kg) during heat stress increased feed intake and egg shell thickness.

In conclusion, evidence from this study suggests that dietary supplementation of laying hens with antioxidant vitamins (vitamin E or vitamin C), probiotics or yeast during heat stress condition can improve the immune response of birds and can leads to improve performance and egg quality.

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