



SHORT COMMUNICATION

Efficacy of Vitamins, Probiotics and Protein Supplementation on Serum Health Biomarkers of Molted Male Layer Breeders

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ABSTRACT

The effects of vitamin C, vitamin E, probiotics, low crude protein diet and their combination were evaluated on health biomarkers including total antioxidant capacity, total oxidant status, homocysteine, paraoxonase, arylesterase, ceruloplasmin and liver enzymes of post molt male layer breeders. For this purpose male layer breeders (n=270) at the age of 59 weeks were procured and undergone induced molting. Results obtained after biochemical and statistical analysis of serum samples showed that both vitamin C and vitamin E are very helpful in regaining a better health status and recovery from oxidative stress induced by forced molting. Probiotics also helped in improving some of the parameters like paraoxonase and ceruloplasmin. Combination treatment also showed positive effects in ameliorating the molting induced oxidative stress. These findings advocate the use of vitamin C, vitamin E and their combination in poultry feed to improve the overall health status of molted White Leghorn male layer breeders.

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INTRODUCTION

The phenomenon of forced molting in poultry industry has become a very common practice to increase the productive and reproductive life span of birds. Different feed supplements like vitamin E (Giraudeau *et al.* 2013), vitamin C (Khan *et al.*, 2014) and probiotics (Li *et al.*, 2014) are also considered very useful in improving health status of different species of birds. Merging the phenomenon of molting with supplementation of useful feed additive in post molt birds is considered a better approach than molting alone. Although researches have been conducted on evaluation of beneficial effects of different supplements on overall health status of post molt layer (Anwar *et al.*, 2012), broiler and broiler breeders (Khan *et al.*, 2014) but very little research work is available from the literature archives on layer breeders. Hence, the current research was conducted to evaluate the efficacy of different feed supplements in improving the health status of molted male layer breeders.

MATERIALS AND METHODS

Two hundred and seventy male layer breeders at the age of 59 weeks were purchased from open market and were maintained at the Animal House, Faculty of Veterinary Science, University of Agriculture, Faisalabad. Birds were acclimatized for one week. During this period, birds were given 16 hours light, normal 16% CP feed and *ad libitum* water. Birds were immunized against Newcastle disease (ND) and infectious bronchitis (IB). At the beginning of the second week, all birds were molted through supplementation of ZnO with a modest decrease in daily light to 12 hours. Molting was continued for four weeks till a desired 20-25% decrease in body weight was obtained. On induction of molting birds stopped producing the semen. After completion of molting birds were allocated randomly into six equal groups and were started given supplanted feed keeping one group as control (CP: 16% feed), second group was fed vitamin E (CP: 16% feed + 100 mg vitamin E; BDH® Germany), third group was given vitamin C (CP: 16% feed + 500 mg

Table 1: Ingredients and composition of 12% crude protein diet fed to layer breeders.

Ingredients	g/kg feed
Corn	356
Wheat	80
Rice Broken	260
Sorghum	50
Rice Polish	120
Guar Meal	20
Sunflower Meal	30
Corn Glutton 60 %	20.5
Soya Bean Meal	20
Molasses	40.5
Lysine	3.0
<i>Calculated Composition:</i>	
Crude Protein (%)	12.01
Metabolizable Energy (ME; kcal/Kg)	2893

vitamin C; RDH[®] England), forth group was given probiotics (CP: 16% feed + 50 mg probiotics; Protexin[®] Hilton Pharma, Holland), fifth group was given feed with 12% CP (Table 1) while last group was given combination of all above treatments till the end of experiment.

After five weeks of supplemented feeding, birds started producing semen again. First blood sampling was collected after one week of first ejaculate and sampling continued for next five consecutive weeks. Serum thus extracted was stored at -20°C till further analysis. Biological health biomarkers including total antioxidant capacity (TAC), total oxidant status (TOS), homocysteine, paraoxonase, arylesterase and ceruloplasmin were determined to assess general health status and oxidative stress of post molt birds. Serum ALT and AST levels were also measured to estimate the oxidative damage of liver cells. TAC and TOS were measured followed method described by Erel (2004) and Erel (2005), respectively. Homocysteine estimation was done through commercially available kit procured from Diazyme[®] Laboratories, USA. The ALT and AST levels were also estimated through commercially available kits by Randox[®] Laboratories, UK. A semi auto-analyzer (Biosystem BTS-330, Costa Brava, Barcelona, Spain) was used for spectrophotometric analysis. All blood samples were centrifuged in Beckman TJ-6[®] Centrifuge, USA to collect the serum samples.

The data was subjected to two-way ANOVA and Duncan Multiple Range test to estimate the significance of difference between groups. The software package GraphPad Prism 5.04[®] was used to analyze the data and significance level was at P<0.05.

RESULTS AND DISCUSSION

Results obtained showed that overall mean serum TAC level was significantly (P<0.05) increased in vitamin

E, vitamin C and combination treatment groups while a significant decrease was found in case of probiotics group. Level of TOS was also decreased (P<0.05) in vitamin E and vitamin C groups indicating retrieval from oxidative stress induced after the practice of molting (Table 2). These results were quite similar to the findings of Khan *et al.* (2014) in post-molt male broiler breeders. Panda and Cherian (2014) also reported that vitamin E is quite important in protection against the development of oxidative stress. It prevents oxidative injury of PUFAs, thiol rich protein constituents of cellular membranes and the cytoskeleton and nucleic acid and thereby maintains the structural and functional integrity of sub cellular organelles. Erel (2004) also found the higher TAC level and considerably lower TOS level in vitamins treated groups of birds as compared to control one. In the current study, a significant increase in oxidative stress and significant decrease in TAC level was observed in probiotics group which is contrary to the results reported by Anwar *et al.* (2012) whereas an increase in TAC and a significant decrease in oxidant status were found in probiotic treated female layer birds.

Homocysteine is also thought to increase during oxidative stress (Anwar *et al.*, 2012). In the current research, serum homocysteine concentration was significantly decreased in vitamin C, vitamin E and 12% CP groups as compared to that of control (Table 2). In current study, level of paraoxonase (PON1) was also higher (P<0.05) in vitamin E and vitamin C groups as compared to control one while the level of arylesterase (PON3) was higher (P<0.05) in vitamin C treated groups as compared to control group (Table 2). Gursu *et al.* (2004) reported a significant increase in paraoxonase and arylesterase activity in rats given vitamin C supplements. Anwar *et al.* (2012) found a significant increase in paraoxonase and arylesterase level of molted layer birds when treated with probiotics. These findings were similar to our findings where a significant increase in paraoxonase level was found in probiotics supplemented group. In another study by Sohail *et al.* (2011) levels of paraoxonase and arylesterase were significantly decreased in group given probiotics supplemented feed. Ceruloplasmin is an α_2 -globulin present in the blood and is mainly associated with the copper reserves of the body. The zinc supplementation in diet is considered responsible for a decrease in ceruloplasmin level. Ceruloplasmin level was found significantly decreased (P<0.05) in vitamin C treated group and significantly decreased in probiotics group (Table 2) as has also been reported by Anwar *et al.* (2012).

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), important health status indicators, also explain the oxidative damage at liver level. Increased

Table 2: Serum level of biological health biomarkers in different trial groups of post molt male layer breeders

Groups	TAC	TOS	OSI	Homocysteine (μmolL^{-1})	Paraoxonase ($\text{Umin}^{-1}\text{ml}^{-1}$)	Arylesterase ($\text{Umin}^{-1}\text{ml}^{-1}$)	Ceruloplasmin (UL^{-1})	AST (UL^{-1})	ALT (UL^{-1})
	mmol Trolox equivalent L^{-1}	($\mu\text{mol H}_2\text{O}_2$ equivalent L^{-1})							
Control	1.00±0.09 ^C	1.46±0.12 ^{AB}	152.3±15.1 ^B	31.86±1.17 ^A	416.7±43.5 ^D	147.3±7.5 ^C	36.77±4.24 ^B	49.59±1.75 ^A	15.51±1.43 ^A
Vitamin E	1.38±0.14 ^A	1.02±0.08 ^D	75.13±6.82 ^D	25.29±2.53 ^C	536.4±39.8 ^A	166.7±6.2 ^B	36.89±2.97 ^B	43.06±3.38 ^B	12.01±0.90 ^C
Vitamin C	1.19±0.08 ^B	0.94±0.08 ^D	82.03±12.32 ^D	25.42±2.62 ^C	535.7±25.5 ^A	179.7± 6.1 ^A	22.84±3.96 ^D	44.22±3.61 ^B	11.53±0.84 ^C
12% CP	1.01±0.08 ^C	1.37±0.08 ^{BC}	140.5±9.8 ^{BC}	25.55±2.63 ^C	492.0±41.3 ^B	174.4±15.2 ^{AB}	31.04±5.40 ^C	51.21±1.00 ^A	14.22±0.45 ^B
Probiotics	0.84±0.11 ^D	1.51±0.09 ^A	201.3±29.8 ^A	30.69±3.42 ^A	469.1±40.6 ^C	144.6±9.7 ^C	40.73±3.75 ^A	50.34±2.75 ^A	15.13±1.70 ^{AB}
Combination*	1.12±0.16 ^B	1.30±0.16 ^C	133.4±28.0 ^C	28.25±4.18 ^B	417.9±79.7 ^D	175.6±4.7 ^{AB}	23.67±3.15 ^D	49.42±1.75 ^A	15.43±1.16 ^A

Values (mean±SE) within a column having different alphabets differ significantly (P<0.05); *12% CP feed + 100 mg vitamin E + 500 mg vitamin C + 50 mg probiotics (Protexin[®]) per kg of feed.

activities of AST and ALT indicate liver damage (Peric *et al.*, 2009). AST and ALT levels were found significantly lower ($P < 0.05$) in treated groups (Table 2). Variation in liver transaminases is also directly related to the changes in the rate of protein metabolism, so increase in the demand of unessential amino acids and enhanced protein metabolism after probiotic supplementation could be the reason for increased AST concentrations in this group. Similar to our findings, Khan *et al.* (2005) also found a significant decrease in ALT and AST enzymes in White Leghorn breed when given vitamin C supplemented feed. Khan *et al.* (2014) also found very significant decrease in serum AST and ALT levels in vitamin C and vitamin E treated groups of male broiler breeders which are again quite similar to our findings.

Conclusion: Both vitamin C and vitamin E are very much helpful in ameliorating the molting induced oxidative stress by improving the oxidative status and other health biomarkers of male layer breeders. Combination treatment also showed positive effects in improving overall health status and retrieval from oxidative stress. Likewise, both vitamins were also found supportive in recovery from oxidative liver damage provoked by molting. Hence these vitamins are highly recommended to be added in post molt poultry birds feeding regime.

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Author's contribution: This work is a product of the intellectual environment of the whole team; and all the members have contributed in various degrees in designing

the study, developing the methodology, performing the analysis and writing the manuscript.

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