Pak. j. life soc. Sci. (2016), 14(1): 18-23 E-ISSN: 2221-7630;P-ISSN: 1727-4915

Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

RESEARCH ARTICLE

Effect of Vitamins, Probiotics and Low Protein Diet on Lipid Profile, Hormonal Status and Serum Proteins Level of Molted White Leghorn Male Layer Breeders

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ARTICLE INFO
Received: Jan 13, 2015
Accepted: Jan 25, 2016
Online: Mar 08, 2016

Keywords
Layer Breeder
Molting
Probiotics
Vitamins
White Leghorn

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ABSTRACT

In the present research, we evaluated the supportive effects of different dietary supplements including vitamin E, vitamin C, probiotics, 12% crude protein diet and combination of all these treatments on lipid profile, hormonal status and serum proteins level of post-molt White Leghorn male breeders. For this purpose 270 commercially available male layer breeders at the age of 59 weeks were procured and introduced to Zn-induced molting after which supplemented feed was given. Serum samples were collected to assess the lipid profile including triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol; hormonal status including triiodothyronine (T_3), thyroxin (T_4), thyroid stimulating hormone (TSH), cortisol and serum proteins level including albumin, globulin and total protein to evaluate the effect of supplements on general health status of post molt birds. Results showed that as a whole, vitamin C and probiotic treatments helped in improvement of lipid profile and vitamin E helped in improving thyroid hormones level. Vitamin C helped in a better way in declining corticosterone level as compared to other groups. Probiotics and combination treatments helped in improving serum proteins level. To the best of our knowledge, this is the first time that effect of these treatments is evaluated in molted male layer breeders.

INTRODUCTION

According to Ministry of National Food Security & Research of Pakistan, about 14,556 million eggs were produced in the country during the year 2013-14. The dynamics of commercial layer farming largely depends upon the status of breeding stock. As a result, currently about 10.19 million breeding stock birds are being raised in different parts of the country (Anonymous, 2014). In general, health status of female layer breeders is considered crucial for a prosperous egg industry however for economic reasons health status of male birds is also equally important.

Economic crisis may occur by culling the birds and time spent in rearing new chicks (20-22 weeks) after the completion of production age of one flock, which can be avoided by the induction of molting in male breeder birds at the end of their production cycle for rejuvenation of reproductive system (Berry, 2003; Iftikhar et al., 2015). Different poultry feed additives like vitamin E (Giraudeau et al., 2013), vitamin C (Khan et al., 2014) and probiotics (Li et al., 2014) are believed to be very beneficial in upgrading the overall health status of different species of birds.

Thyroid hormones are known to have important role in regulating energy metabolism, increasing the basal and oxidative metabolism rate by increasing the respiratory rate, mitochondrial mass and mitochondrial cytochrome contents of the cell (Yen, 2001). Factors like age, temperature and feeding affects the blood levels of thyroid hormones (Decuypere and Kuhn, 1988; Stojevic et al., 2000). Similarly stress response in poultry birds is considered to be associated with high serum level of cortisol (Onbasilar et al., 2007). Hypothalamic-pituitary-

adrenal system is triggered when body undergoes stress. This results in stimulation of hypothalamus to cause production of ACTH hormone from anterior pituitary. Thus raised ACTH level causes adrenal gland to release corticosterone (Virden et al., 2007).

Merging the phenomenon of molting with feed supplementation of beneficial feed additive in post molt male layer birds is believed to have better results than molting alone. Though studies have been conducted on assessment of useful effects of different supplements on overall health status of post molt layer (Anwar et al., 2012), broiler and broiler breeders (Khan et al., 2014), yet very few research work is available from the literature archives on layer breeders (Iftikhar et al., 2015). Hence, the following research was planned with an aim to inquire the beneficial effects of vitamin C and E, probiotics and low CP feed on lipid, hormonal and protein profile of molted male layer breeders.

MATERIALS AND METHODS

Experimental design

A total of 270 commercially available White Leghorn male layer breeders (Bovans®) of 59 weeks age were procured from the market and kept at the Animal House, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan. Birds were acclimatized for one week. During this period, birds were given normal recommended diet with 16% crude protein, *ad libitum* water and recommended light only. All the birds were immunized against Newcastle Disease and Infectious Bronchitis. After that the birds were allotted randomly to six different groups. At the beginning of the second week birds of all six groups were introduced to ZnO induced molting along with recommended decrease in daily light to12 hours. It took birds about 4 weeks to reduce 20-25% of body weight. After achieving targeted reduction in body weight, group I was nominated as control (16% CP feed), group II was started given vitamin E (16% CP feed + 100 mg vitamin E), group III was given vitamin C (16% CP feed + 500 mg vitamin C), group IV was fed probiotics (16% CP feed + 50 mg probiotics), group V was given feed with 12% crude protein (Table 1) while group VI was given combination of all above treatments. Vitamin E was purchased from BDH®, Germany while vitamin C was procured from RDH®, England. Probiotics used was of Protexin®, Hilton Pharma, Holland brand which contains *Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Streptococcus thermophilus, Streptococcus faecium* and *Bifidobacterium bifidum* species.

Collection of samples

After induction of molting, birds took about five weeks to start semen production again. The birds were given supplemented feed after completion of molting. Sampling was started after one week of first ejaculate and continued for five consecutive weeks onwards. Blood was collected in sterilized, chilled test tubes lacking anticoagulant to separate the serum. All blood samples were centrifuged in Beckman TJ-6® Centrifuge, USA to collect the serum samples. The sera thus separated were collected in eppendorf tubes and kept at -20ºC till further analysis. Guidelines regarding ethical use of animals for research, obtained from the Institutional Committee, University of Agriculture Faisalabad, Pakistan, was strictly followed.

Table 1: Ingredient with parts used, CP share and ME share of 12% crude protein diet fed to treated birds.

Ingredients	Parts used (%)	Crude protein share	ME share
Corn	30	2.4	1032
Wheat	8	0.96	251.2
Rice Broken	26	1.898	780
Sorghum	5	0.5	163.15
Rice polish	12	1.32	330
Guar meal	2	0.8	44
Sunflower meal	3	0.96	48
Corn gluten 60 %	2.5	1.5	94.25
Soya bean meal	2	0.94	50.8
Molasses	4.5	0.135	88.29
Lysine	0.3	0.2895	11.97
Total	100	12.0145	2893.66

Biochemical analysis

After proper thawing, samples were used to estimate lipid profile including triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol; hormonal status including triiodothyronine (T_3), thyroxin (T_4), thyroid stimulating hormone (TSH), cortisol and serum proteins level including total protein, albumin and globulin to evaluate general health status of post molt birds. Levels of triglycerides, total cholesterol and HDL-cholesterol were measured by using commercially available Fluitest kit by Biocon® Diagnostic, Germany. Level of LDL-cholesterol was measured by using subtraction method. Triiodothyronine and thyroxin level were determined by using the ELISA kits supplied by Biocheck®, USA while ELISA kits for TSH and cortisol were procured from Diametra®, Italy and Accubind®, USA, respectively. Commercially available kits by ELITech®, France were used for the assessment of total protein and albumin levels while level of globulin was assessed again through subtraction method. A semi auto-analyzer (Biosystem BTS-330, Costa Brava, Barcelona, Spain) was used for spectrophotometric analysis.

Statistical analysis

The results were expressed as the mean ± SE. All data were analyzed using two way analysis of variances (ANOVA) followed by Duncan Multiple Range test, using software package CoStat 6.4® and GraphPad Prism 5.04®.

RESULTS AND DISCUSSION

Results obtained after the biochemical and statistical analysis are shown in Table 2 and Table 3. In present study, a significant decrease in serum triglycerides was found in vitamin E, vitamin C, 12% CP and combination treatment groups when compared with the control one. No significant change was found in probiotics group. Total cholesterol level was significantly decreased in probiotics and 12% CP groups. Level of HDL was found increased in vitamin C and probiotics groups while LDL was decreased significantly in probiotics supplemented group. The increase in HDL- cholesterol and decrease in LDL-cholesterol are healthy effects. HDL has the property of reverse cholesterol transport, thus it has the ability to protect against the development of arteriosclerosis and coronary artery diseases (Barter and Rye, 1996; Stein and Stein, 1999). The transportation of free cholesterol from endothelial macrophages to the liver and excretion in the bile is the main mechanism of how HDL protects against the formation of atherosclerotic plaques (Davidson and Toth, 2007). Tawfeek et al. (2014) found a decrease in serum cholesterol level in vitamin E treated group of broiler birds. Similar to our findings, Aluwong et al. (2013) also observed a significant decrease in serum cholesterol level in the group given yeast supplemented feed. In same study, significant differences were also observed in HDL concentrations in all experimental groups supplemented with the probiotic when compared with the control one. Similarly, Sohail et al. (2010) also found a significant decrease in cholesterol levels in *Lactobacillus*-based probiotic supplemented group of broiler birds. However in another study on rabbits by Cavallini et al. (2009) it was found that the addition of *Enterococcus faecium* as a probiotic did not influence the serum total and HDL cholesterol; however triglyceride concentration were significantly increased. Similarly, Shareef and Al-Dabbagh (2009) reported that the addition of 2% yeast as a probiotic in the diet of broiler birds significantly reduced the triglyceride and cholesterol concentration in the serum. In another study by Anwar et al. (2012) on layer birds, the triglyceride concentration was increased after probiotic supplementation; however, the LDL concentration was decreased in the symbiotic supplemented birds. All these findings could be attributed to its healthy impact in the molted laying hens. In the current study a significant decrease in T_3 level was observed in probiotics and 12% CP groups an increase in T_3 level was observed in vitamin E treated group, while in case of T_4 a significant increase was found both in vitamin C and vitamin E groups while level of T_4 was declined in 12% CP group. As expected, level of TSH was significantly high in probiotics and CP 12% groups while its level was significantly lower in vitamin E and combination groups. Serum quantity of T_3 and T_4 hormones are considered to be changed with age, temperature, fasting, feeding and pathophysiology (Decuypere and Kuhn, 1988; Stojevic et al., 2000). Kaya et al. (2001) supplemented 100-200 ppm zinc oxide to 56 week old Hisex brown laying hens and noticed a decrease in plasma T_3 and T_4 hormone as compared to the group fed with less zinc. Broiler chickens were supplemented with zinc at a level of 5280 ppm of feed and a decreased concentration of T_3 and T_4 was observed (Dean et al., 1991). Spent layers were supplemented with ZnO at a rate of 3000 ppm and an increase in the level of thyroxin was observed (Sandhu, 2007). Williamson et al. (1985) proposed that, the reduction in T_3 is a consequence of the reduction in food intake as well as the increased adrenocortical activity. Dean et al. (1991) observed that excessive dietary zinc (5289 ppm) in day old chicks reduced the size of thyroidal follicles and changed the secretion of thyroid hormone. In a research by Aluwong et al. (2013) observed significant decrease in thyroxin (T_4) level in broiler given feed supplemented with yeast. Mahmoud et al. (2013) observed that vitamin C results into a significant increase in triiodothyronine and thyroxin level in heat stressed broilers and help the body in attenuating heat stress induced oxidative damage. In the same research, an increase in level of T_3 was found in vitamin E treated group. Similarly Sohail et al. (2010) found a significant increase in T_3 and T_4 level in *Lactobacillus*-based probiotic group of broiler birds. Khan et al. (2014) also observed significant increase in T_3 and T_4 levels both in vitamin E and

Table 2: Mean concentration of serum triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol (mean±SE) in different trial groups of post molt male layer breeders.

	Triglycerides mg/dl ± SE	Total cholesterol mg/dl ± SE	Cholesterol-HDL mg/dl ± SE	Cholesterol-LDL mg/dl ± SE
Control	192.72±6.28^{A}	169.208±8.842^{B}	68.527±1.451^{B}	100.680±8.785^{B}
Vitamin E	173.04±2.94B^{C}	166.477±3.358^{BC}	67.761±2.353^{BC}	98.716±4.943^{B}
Vitamin C	175.40±4.56^{B}	172.572±6.242^{AB}	72.418±1.645^{A}	100.154±5.725^{B}
12% CP	161.64±4.73^{C}	157.105±8.632^{D}	64.439±2.426^{C}	92.665±9.219^{BC}
Probiotics	203.72±7.88^{A}	159.738±4.425^{CD}	73.651±0.688^{A}	86.087±4.435^{C}
Combination*	161.88±4.40^{C}	178.888±4.304^{A}	67.494±2.529^{BC}	111.394±6.692^{A}

$^{A-D}$ Mean values 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.

Table 3: Mean concentration of different serum hormones and proteins (mean±SE) in different trial groups of post molt male layer breeders

	Triiodothyronine ng/dl ± SE	Thyroxine µg/dl ± SE	TSH mlU/L ± SE	Corticosteronen g/mL ± SE	Total Proteins g/dl ± SE	Albumin g/dl ± SE	Globulin g/dl ± SE
Control	3.085±0.034^{AB}	8.320±0.19^{D}	0.504±0.011^{B}	30.486±2.367^{B}	2.874±0.185^{C}	1.224±0.027^{BC}	1.651±0.163^{B}
Vitamin E	3.190±0.051^{A}	9.354±0.401^{B}	0.410±0.039^{C}	31.257±1.163^{B}	2.953±0.249^{BC}	1.268±0.078^{B}	1.686±0.261^{B}
Vitamin C	3.086±0.066^{AB}	10.960±0.350^{A}	0.523±0.008^{B}	28.158±2.740^{C}	3.014±0.477^{B}	1.191±0.048^{C}	1.823±0.453^{A}
12% CP	2.699±0.121^{D}	7.195±0.374^{E}	0.614±0.033^{A}	31.250±2.337^{B}	2.923±0.281^{BC}	1.124±0.036^{D}	1.799±0.250^{A}
Probiotics	2.940±0.107^{C}	8.530±0.371^{C}	0.606±0.057^{A}	36.433±2.839^{A}	3.014±0.216^{B}	1.331±0.036^{A}	1.683±0.207^{B}
Combination*	2.999±0.097^{BC}	9.437±0.549^{B}	0.418±0.026^{C}	30.548±2.034^{B}	3.158±0.215^{A}	1.347±0.040^{A}	1.811±0.205^{A}

$^{A-D}$ Mean values within a column, having different alphabets do differ significantly (P<0.05); *12% CP feed + 100 mg vitamin E + 500 mg vitamin C + 50 mg probiotics (protexin®) per Kg of feed.

vitamin C groups. In the same research a significant increase in T_4 level was observed in probiotics group. Sahin et al. (2001) found that serum level of T_3 and T_4 increases gradually when given treatment of α-tocopherol (62.5, 125, 250 and 500 mg/kg of diet) in male Cobb broiler birds raised in heat stress. All these findings advocate the use of vitamins in post molt poultry diet which ultimately helps birds to improve its compromised production potential.

During the current study, a significant decrease in corticosterone level was observed in vitamin C group while a significant increase in case of probiotics was seen when compared with control group. Similar to other stresses, molting stress is also considered to increase the level of serum corticosterone. An increase in circulatory cortisol (corticosterone) was reported in fast induced, molted layer birds (Akram et al., 2002). They attributed it to the impact of stress conferred by fasting, used to induce the molting. Corticosterone, a glucocorticoid, serves as an indicator of animal's state of wellbeing as its level increases during times of distress. Its role is to maintain the sympatho-adrenal system, which regulates the homeostasis of the body (Eckert et al., 1988). Hence, the decrease in corticosterone levels in vitamin C groups explains the phenomenon of relieve from molting stress through supplementation. In another research on spent layers, supplemented with ZnO at a rate of 3000 ppm, an increased cortisol level was observed during second production cycle (Sandhu et al., 2010). Onbasilar et al. (2007) also found relationship between elevated circulating levels of corticosterone during stress response in poultry birds. Similar to our results, Khan et al. (2014) also observed significant decrease in level of corticosterone in vitamin C and vitamin E groups. Likewise, Mahmoud et al. (2013) observed that vitamin C results into a significant decrease in corticosterone level in heat stressed broilers and help the body in attenuating heat stress induced oxidative damage. Sohail et al. (2010) found a significant decline in cortisol amount in *Lactobacillus*-based probiotic group of broiler birds. Similar to our findings, Satterlee et al. (1989) also found that vitamin C causes reduction in serum corticosterone level in broiler chickens. Nur-Azlina and Nafeeza (2008) treated male Sprague Dawley rats exposed to restraint stress with 60 mg/kg oral dose of vitamin E and concluded that vitamin E markedly reduced stress induced by elevation of corticosterone level. In another study by Sahin et al. (2004) a significant decrease in blood corticosterone level was found in post molt poultry birds when treated with vitamin E and C.

There has been considerable interest in the possible use of serum protein levels or the albumin-to-globulin ratio as a sensitive biochemical index for appraising protein nutritional status. In present study, a significant increase in serum total proteins level was observed in vitamin C, probiotics and combination treatment groups. Serum albumin level was significantly high in probiotics and combination treatment groups while level of albumin was significantly low in 12% CP group. However in case of globulin a significant increase was observed in vitamin C, 12% CP and combination treatment groups. Feeding low protein diets has been associated with decreased total serum protein and serum albumin levels in rats by Allison (1955), in man by Scrimshaw and Behar (1961) and in chicks by Leveille et al. (1960) and Leveille and Sauberlich (1961). However, Albanese (1959) has cited cases where low protein intakes have not been associated with low plasma protein levels. In another research by Brandt et al. (1951) the level of total protein, α-globulin, and γ-globulin increased with age. Little variations were noted in the albumin or β-globulin fractions. Similarly, in another research Jadhav et al. (2014) also found a significant increase in total protein and serum globulin levels along with a non-significant increase in serum albumin level in vitamin C supplemented groups. Tawfeek et al. (2014) also found an increase in serum level of total protein in vitamin E treated group of broiler birds.

Conclusion

Almost all treatments helped at different level in improving health status of birds after molting. Vitamin C and probiotic treatments helped in improvement of lipid profile while in case of hormonal status vitamin E helped in improving thyroid hormones level. Vitamin C helped in a better way in declining corticosterone level

as compared to other groups. Probiotics and combination treatments helped in improving serum proteins level.

Acknowledgments

This research work was conducted under Research Project PSF/NSLP/P-AU (245). Authors are highly thankful to Pakistan Science Foundation (PSF) for this financial support.

Authors' contribution

This work is a result of the collaborative environment of the whole team; and all the members have contributed intellectually in designing the study, developing the methodology, performing the analysis and inscription of manuscript.

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