Effect of supplementation of lutein and DL-methionine on certain haematobiochemical parameters and serum concentration of corticosterone in captive Golden pheasants (*Chrysolophus pictus*)


Centre for Wildlife Conservation, Management and Disease Surveillance, ICAR-Indian Veterinary Research Institute, Izatnagar-243 122, Uttar Pradesh, India.

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**ABSTRACT**

Twenty four male golden pheasants were divided into 4 groups of 6 each. First group basal diet contained no supplementary lutein or DL-methionine (M₀). The diet of the birds in group M₁ were supplemented with lutein (40 mg/kg DM) without supplementary DL methionine, whereas those in group M₂ were supplemented only with DL-methionine (1.5g/kg DM). Birds in group M₃ were fed both the supplements. A feeding trial of 60 days duration was conducted. Heterophil counts was significantly lower (P<0.001) in groups M₂ and M₃ as compared to group M₀. It was concluded that supplementation of lutein (40mg/kg DM) but not DL-methionine (0.15% DM) improved the capability of the Golden pheasant to combat stress.

**Key words:** Blood parameters, *Chrysolophus pictus*, Corticosterone, Lutein.

**INTRODUCTION**

Golden Pheasant (*Chrysolophus pictus*) is a very colourful bird belonging to the family Phasianidae in the order Galliformes. Concerned with the declined population of pheasants in the wild, zoo community is coming forward to make their contribution in ex-situ/in-situ conservation. However, to make such programme successful, it is mandatory that proper nutrition and diet be provided to the birds, which will improve reproductive performance, longevity and reduce health related problems. Information available on feeding and nutrition of Golden pheasants are meager which mostly pertains to feeding behaviour (Wu et al., 2010) and energy budget (Luqiang et al., 2005).

Molting is a natural phenomenon in life cycle of birds during which they lose their feather to regenerate newer ones. As feathers are rich in sulphur containing amino acid (20-22%), any deficiency of these nutrients would impair feather growth (Leeson and Walsh, 2004). Carotenoids are required for bright colouration of plumages and must be supplied through diet because Golden pheasants cannot synthesize carotenoids de novo (Goodwin, 1984). This carotenoid based ornamentation signals individual’s condition and is used by females while selecting mates (Hill, 1992). Considering the higher demand of carotenoids and sulphur containing amino acids for feather growth and pigmentation, it would be logical to assume that any deficiency of these nutrients will induce stress in captive Golden pheasants. Carotenoids can also acts as an anti-stressor in birds (Sahin et al., 2008) however, Costantini et al. (2008) observed no association between circulatory carotenoids and corticosterone in kestrel. Such variation in response could be due to type of carotenoids and interspecies differences with respect to carotenoids metabolism and utilisation (Parker et al., 1996; Rodriguez et al., 2013). Similarly, there are studies, which indicate that methionine plays crucial role in the defense against oxidative stress (Stadtman et al., 2005; Métyer et al., 2008). However, Willemesen et al. (2011) 2-hydroxyl 4-methyl-thio-butanoic acid, but not DL-methionine reduced stress in broiler chicken. Thus it seems that the role of methionine in alleviation of stress is dependent on source of supplementary methionine.

Hematological and blood biochemical parameters are known to be influenced by various factors like life stage, nutritional status and disease conditions (Fudge, 2000). There are some studies that indicate that supplementary methionine (Yodseranee and Bunchasak, 2012; Francis et al., 2012) and carotenoids (Selim et al., 2013) affect the hematological and serum biochemical parameters in chicken. To the best of our knowledge there is no information available regarding normal blood profile of Golden pheasant. Considering the paucity of information on effect of supplementary carotenoids and methionine in pheasants it would be desirable to study the...
effect of these nutrients on hematopoietic profile and their ability to reduce stress in captive Golden pheasants. Primary objective of this experiment was to study the effects of supplementation of lutein and DL-methionine on hematopoietic profile and serum concentration of corticosterone of captive Golden pheasants. Additionally the study intended to generate baseline data on hematological and serum biochemical profile of the species.

MATERIALS AND METHODS

This experiment was conducted at Pandit G. B. Pant High Altitude Zoo, Nainital (Uttarakhand), located at the latitude of 29° 23' N, longitude of 79° 30' E, at an altitude of 2,084 meters above mean sea level.

Twenty four male Golden pheasants (18-22 months of age, 620-640 g body weight) were randomly distributed into four groups of 6 each in an experiment based on 2x2 factorial design. All the birds were fed a conventional zoo diet comprising of wheat, 28%; bajra, 10%; soyabean meal, 10%; commercial poultry feed, 44.35%; green, 1.5%; onion, 1.5%; garlic, 2.0%; tomato, 0.7%; bottle gourd, 1.1%; hardboiled egg with shell, 3.1%; limestone powder 0.85%; dicalcium phosphate 0.7% and premix 0.2% on dry matter basis to meet their nutrient requirements (NRC, 1994). Basal diet M\(_0\)-C\(_0\) contained no supplementary lutein or DL-methionine (M\(_0\)C\(_0\)). The diet of the birds in group M\(_1\)C\(_0\) were supplemented with lutein (40 mg/kg DM) without supplementary DL-methionine, whereas, those in group M\(_0\)C\(_1\) were supplemented only with DL-methionine (1.5g/kg DM). Birds in group M\(_1\)C\(_1\) were fed both the supplements.

During the entire experimental period, the birds were housed in pairs in their designated enclosures (3x3x6 m), which were designed and built as per Central Zoo Authority of India (CZA, 2012) standards. Proper management and sanitary guidelines as suggested by CZA (2012) were followed during the course of experimentation. All the birds were dewormed with Albandazole at 5 mg/kg BW, 1 m prior to the feeding trial. They were kept under natural lighting system. Institute Animal Ethics Committee of Indian Veterinary Research Institute, Izatnagar, India, approved all procedures followed in this experiment.

Blood sample (1 ml) was collected from right wing vein of each bird in EDTA vial on day 56 of the trial period. Serum was harvested by centrifugation at 3000 rpm for 10 minute and stored in sterilized vials at -20°C for laboratory analysis.

Hematological parameters including total erythrocyte count (TEC) and total leucocyte count (TLC), Haemoglobin (Hb) estimation of total erythrocyte count (TEC) and total leucocyte count (TLC), were estimated in Neubauer chamber. Haemoglobin (Hb) concentration of blood samples were estimated by using Sahli’s haemoglobinometer. Blood smears were prepared at the time of blood collection and preserved in absolute methanol to estimate the relative microscopic differential leucocyte count (DLC), stained with May-Grunwald-Giemsa stain.

Serum biochemical parameters were estimated by using Span Diagnostics kits (Span Diagnostics Limited, Surat, India) Serum concentration of corticosterone was estimated by using Corticosterone ELISA Kit. Total carotenoids in feed samples were estimated by spectrophotometric method given by (Luterotti et al., 2011)

Statistical analysis: Data obtained were subjected to analysis of variance (ANOVA) for 2x2 factorial design (Snedecor and Cochran, 1994). Treatment means were tested by applying Tukey’s test. All analysis were performed by using SPSS software package, version 16 (SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

Data pertaining to effect of supplementation of lutein and DL methionine on haematology and serum metabolic profile of Golden pheasant are presented in Table 1. Heterophil counts were significantly lower (P<0.001) in groups M\(_1\)C\(_0\) and M\(_1\)C\(_1\) as compared to groups M\(_0\)C\(_0\) and M\(_0\)C\(_1\) and lymphocyte count was higher (P<0.05) in lutein supplemented group as compared to non-lutein supplemented group. An index comprising the relative abundance of both lymphocytes and heterophils is the heterophil/lymphocyte (H/L) ratio, which is widely used to estimate stress in poultry (Gross and Siegel, 1983; Maxwell, 1993) and wild birds (Birkhead et al., 1998). In this experiment, lutein supplemented group showed lower (P<0.05) H/L ratio. During initial responses to infection some white blood cells produce free radicals to kill and inactive foreign organisms in the body. While this is a combat mechanism, over-production of free radicals may cause damage to white blood cells and surrounding tissues. Luteins are large lipophilic molecules that due to their double-bond structure are able to mop up these excessive free radicals and protect cells from oxidative stress (Bendich and Shapiro, 1986). Meriwether et al. (2010) reported that lutein supplementation at 40 mg/ kg DM decreased IL-6 and several other inflammatory parameters in the liver following an LPS injection in chickens. Interluekin-10, an anti-inflammatory cytokine, has a very little basal expression, but supplementation of lutein enhanced its expression (Rothwell et al., 2006). Further, Shanmugasundaram and Selvaraj (2011) reported that dietary lutein supplementation at 50 mg/kg DM decreased oxidative damage and inflammatory responses post-LPS injection by decreasing IL-18 production and increasing IL-10 production in turkeys. From the results and the review it is evident that supplementation of lutein at 40 mg/kg DM would decrease H/L and improve capability of captive Golden pheasants to combat stress.

There was no significant difference in concentration of Haemoglobin, TEC, WBC, basophils, eosinophils and...
monocyte count among the groups (Table 1). As there is no report concerning haematological parameters of Golden pheasant, we compared the values obtained in this study with those of other species of pheasants. In this experiment, RBC counts ranged from 3.6-3.8x10^6/µl, which is similar with range of RBC, count (2.6-3.9x10^6/µl) in Ring necked pheasant (Lloyd and Gibson, 2006). In this study, haemoglobin concentrations ranged from 13.0-13.4 mg/dl, which is lower than the range of 14.4-18.9 mg/dl reported by Lloyd and Gibson (2006) for Ring necked pheasants. However, Hauptmanova et al. (2006) reported that haemoglobin concentrations ranged from 11.4-14.1 mg/dl in Ring-necked pheasants, which was similar to the range observed in this study. In present study, average WBC counts ranged 23-25x10^3/µl. Kececi and Col (2011) reported that WBC counts of Ring-necked pheasant were 28.8±8.0 x 10^3/µl, which was similar to that observed in this study. The differential leukocyte counts differ among different avian species (Perelman et al., 1999; Puerta et al., 1989). Differential leukocyte count also differ within the same species (Hauptmanova et al., 2006) due to factors like season, age, individual properties of birds, hormones, stress, immune status, as well as the time of blood sampling during the day (Bounous, 2000). In spite of these variations lymphocytes were the major leukocyte in Ring-necked pheasants (Hauptmanova et al., 2006). Similarly, lymphocytes are the most abundant leukocytes in Golden pheasant. Results of this experiment demonstrate that haematological parameters of Golden pheasants are similar to those of Ring-necked pheasants.

Serum concentration of glucose, total protein, albumin, globulin, triglycerides and uric acid was not significantly different among the groups (Table 1). Serum concentrations of glucose, triglycerides, total protein (TP), albumin and globulin and uric acid were not affected by supplementation of lutein and DL methionine. Lloyd and Gibson (2006) reported that serum concentration of glucose and triglycerides ranged from 284-426 mg/dl and 35.4 to 123 mg/dl respectively in Ring-necked pheasants which is in accordance with results of present study. Serum concentration of total protein ranged from 3 to 5 g/dl in wide range of avian species (Campbell and Coles, 1986; Kaneko et al., 1997 and Khazaainia et al., 2006). In this study, concentration of total protein ranged from 3.8-4.31 g/dl, which is similar to the range of 3.5 g/dl to 4.9 g/dl reported earlier in Ring-necked pheasants (Lloyd and Gibson, 2006; Suchy et al., 2010). In Ring-necked pheasant, concentration of serum albumin ranged from 2.64-2.96 g/dl and that of globulin ranged from 1.9-2.3 g/dl (Schmidt et al., 2007), which is similar to the range (2.13 -2.33 g/dl of albumin and 1.76 -2.14 g/dl of globulin) observed in this study. Uric acid is the major nitrogenous waste product of birds (Harr, 2002). In the present study, concentration of serum uric acid ranged from 5.61-6.12 mg/dl, which is similar to the range of 6.01-6.5 mg/dl reported for common pheasants (Schmidt et al., 2007). From the results of this experiment and from the review of literature it seems that serum biochemical parameters (glucose, triglycerides, total protein, albumin, globulin and uric acid) of Golden pheasants are similar to those of Ring-necked pheasants. In this experiment we did

### Table 1: Effect of supplementation of lutein and DL-methionine on haematological profile of captive Golden pheasants.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>M1C1</th>
<th>M1C2</th>
<th>M1C3</th>
<th>M3C</th>
<th>C</th>
<th>M</th>
<th>MxC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (mg/dl)</td>
<td>13.2±0.20</td>
<td>13.2±0.14</td>
<td>13.0±0.24</td>
<td>13.2±0.18</td>
<td>0.560</td>
<td>0.452</td>
<td>0.887</td>
</tr>
<tr>
<td>Total erythrocyte count (10^12/µl)</td>
<td>3.79±0.054</td>
<td>3.81±0.059</td>
<td>3.80±0.055</td>
<td>3.82±0.059</td>
<td>0.975</td>
<td>0.553</td>
<td>0.921</td>
</tr>
<tr>
<td>Total leucocyte count (10^3/µl)</td>
<td>24.5±0.20</td>
<td>24.7±0.14</td>
<td>24.7±0.21</td>
<td>24.7±0.25</td>
<td>0.638</td>
<td>0.754</td>
<td>0.456</td>
</tr>
<tr>
<td>Heterophils(10^3/µl)</td>
<td>27.7±0.34</td>
<td>28.1±0.15</td>
<td>26.3±0.18</td>
<td>26.0±0.20</td>
<td>0.1</td>
<td>0.432</td>
<td>0.678</td>
</tr>
<tr>
<td>Lymphocytes (10^3/µl)</td>
<td>64.8±0.234</td>
<td>64.2±0.139</td>
<td>65.8±0.082</td>
<td>66.4±0.119</td>
<td>0.001</td>
<td>0.653</td>
<td>0.774</td>
</tr>
<tr>
<td>Monocytes (10^3/µl)</td>
<td>2.44±0.329</td>
<td>2.38±0.359</td>
<td>2.37±0.263</td>
<td>2.21±0.346</td>
<td>0.964</td>
<td>0.568</td>
<td>0.784</td>
</tr>
<tr>
<td>Eosinophils (10^3/µl)</td>
<td>1.67±0.432</td>
<td>1.80±0.366</td>
<td>1.70±0.280</td>
<td>1.77±0.474</td>
<td>0.521</td>
<td>0.987</td>
<td>0.834</td>
</tr>
<tr>
<td>Basophils(10^3/µl)</td>
<td>3.42±0.503</td>
<td>3.31±0.658</td>
<td>3.86±0.319</td>
<td>3.67±0.454</td>
<td>0.488</td>
<td>0.376</td>
<td>0.456</td>
</tr>
<tr>
<td>Heterophils’ lymphocytes</td>
<td>0.43±0.059</td>
<td>0.44±0.029</td>
<td>0.40±0.027</td>
<td>0.39±0.049</td>
<td>0.001</td>
<td>0.865</td>
<td>0.6567</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>224.6±2.68</td>
<td>229.5±1.90</td>
<td>226.0±2.33</td>
<td>224.0±2.81</td>
<td>0.432</td>
<td>0.822</td>
<td>0.762</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>123.6±3.05</td>
<td>122.6±4.04</td>
<td>123.8±3.14</td>
<td>126.9±3.11</td>
<td>0.443</td>
<td>0.657</td>
<td>0.453</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>5.96±0.511</td>
<td>5.90±0.434</td>
<td>5.83±0.484</td>
<td>6.08±0.460</td>
<td>0.585</td>
<td>0.546</td>
<td>0.546</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>4.15±0.096</td>
<td>4.13±0.077</td>
<td>4.22±0.073</td>
<td>4.19±0.076</td>
<td>0.685</td>
<td>0.558</td>
<td>0.445</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>2.19±0.122</td>
<td>2.27±0.065</td>
<td>2.30±0.084</td>
<td>2.32±0.085</td>
<td>0.663</td>
<td>0.782</td>
<td>0.567</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.01±0.060</td>
<td>1.94±0.060</td>
<td>1.86±0.109</td>
<td>1.88±0.063</td>
<td>0.662</td>
<td>0.824</td>
<td>0.275</td>
</tr>
<tr>
<td>Corticosterone (ng/ml)</td>
<td>41.3±0.301</td>
<td>38.5±0.18</td>
<td>34.7±0.303</td>
<td>33.1±0.35</td>
<td>0.001</td>
<td>0.845</td>
<td>0.277</td>
</tr>
</tbody>
</table>

- Mean ± standard error values with different superscripts within a row for a particular parameter differ significantly.
- g, gram; ml, milliliter; µg/ml, microgram per milliliter; ng/ml, nanogram/ml.

Basal diet contained no supplementary lutein or DL-methionine (M1C3). The diet of the birds in group M1C1 were supplemented with lutein (40 mg/kg DM) without supplementary DL methionine, whereas those in group M1C2 were supplemented only with DL-methionine (1.5g/kg DM). Birds in group M1C2 were fed both the supplements.
not find any effect of supplementation of DL-methionine on haematobiochemical parameters studied. Studies conducted earlier have indicated that response of methionine supplementation varied according to the species, type and dosage of supplement (Oda et al., 1991). Akpet et al. (2009) reported that feeding of graded level of methionine (0.3-1.5% DM) did not have any effect on haematological parameters in broiler chick. In this experiment, level of methionine was 0.25 and 0.40% in the un-supplemented and DL-methionine supplemented group. Both these level were probably within a narrow range that may upset the haematobiochemical parameters of captive Golden pheasants.

Concentration of serum corticosterone was higher (P<0.001) in groups M\textsubscript{0}C\textsubscript{1} and M\textsubscript{1}C\textsubscript{1} as compared to groups M\textsubscript{0}C\textsubscript{0} and M\textsubscript{1}C\textsubscript{0} (Figure 1). Further there was no difference (P>0.001) in serum concentration of corticosterone between M\textsubscript{0}C\textsubscript{1} and M\textsubscript{1}C\textsubscript{0} group and M\textsubscript{0}C\textsubscript{0} and M\textsubscript{1}C\textsubscript{1} groups (Table 1). Elevation of serum corticosterone, a major glucocorticoids in birds is used as an indicator of stress (Wingfield, 1994). Corticosterone act as modulator of oxidative stress and it may affect the proxidant and antioxidant balance (Costantini et al., 2008). As carotenoids are potent antioxidants (Bertram and Bortkiewicz, 1995) any short supply of carotenoids may adversely upset this balance inducing stress. In this experiment, serum concentration of corticosterone was lower in lutein supplemented groups. This finding is consistent with decreased H/L in lutein supplemented groups observed in this study and decreased lipid peroxidation due to lycopenes supplementation observed in a previous study (Sahin et al., 2008). Serum concentration of corticosterone was negatively correlated (P<0.01) with dietary concentration of total carotenoid. (Figure 2.) In an earlier study it was reported that stress induced by high temperature was reversed by supplementation of lycopene in Japanese quail (Sahin et al., 2006). Thus, it can be deduced that birds in non-lutein-supplemented groups were under more oxidative stress in comparison to those of lutein supplemented groups.

Supplementation of lutein decreased H/L ratio and serum concentration of corticosterone. Supplementation of DL-methionine did not affect these parameters. Other haematological and serum and serum biochemical parameters were similar among the groups. It was concluded that supplementation of lutein (40mg/kg DM) but not DL-methionine (0.15% DM) improved the capability of the Golden pheasant to combat stress.

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