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Combination of synthetic carotenoids enhanced the haematological attributes in broiler breeders

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Abstract
A biological trial was conducted to assess the effect of feeding carotenoid combinations on haematological constituents in broiler breeders. Sixty adult healthy birds were used and these were randomly divided into three groups. The treatments were control (basal diet), T_1 (6 mg each of canthaxanthin and apo- carotenoid) and T_2 (12 mg each of canthaxanthin and apo- carotenoid). The blood sample was collected and haematological profiles were analysed. In female broilers, higher dose of carotenoids (T_2) fed group showed significantly ($P<0.05$) higher in lymphocyte, heterophils, haematocrit, red blood cell and its related attributes. No significant ($P>0.05$) difference in white blood cell, monocyte and red blood cells diameter (RDWc) was observed. In case of male breeders, feeding carotenoids combination showed significantly ($P<0.05$) higher count of lymphocyte, heterophils, haematocrit, red blood cell and its attributes. There was no significant difference was noticed in monocyte and RDWc. In conclusion, supplementation of combination of carotenoids to broiler breeder chickens improved the haematology profile, as well as the health and welfare status.

Keywords: Broiler breeders, carotenoids, haematology profile, canthaxanthin, apo-carotenoid

Introduction
Carotenoids are lipid soluble pigments. They are red, yellow or orange pigments and widely distributed in nature. Carotenoids have been identified in photosynthetic centres in plants, bird feathers, crustaceans and marigold petals, they are especially abundant in yellow-orange fruits and vegetables and dark green, leafy vegetables. More than 700 naturally occurring carotenoids colours red (lycopene), orange (α-carotene, ß-carotene and γ-carotene) yellow (luetin, α-cryptoxanthine or violaxanthine) is identified. Carotenoids are important to avian species due to its pigmentation properties, which are being used as a magnitude to assess the bird's health status (Olson and Owens, 1998) [9]. Additionally carotenoids improves the meat flavour, pigmentation bodies and delaying oxidation and reducing oxidative stress (Von Schantz *et al.,* 1999; Blount *et al*., 2000) [12, 4] and increase birds immune status. However, the effect of these carotenoids on haematological attributes in broiler breeders is not available as they serves as an indicator of health status of the birds. Hence, the present study was carried out to assess influence of carotenoids combination on health status of broiler breeder birds with respect to the changes in haematological attributes in both male and female breeders.

Materials and methods
Selection and feeding management of birds
Sixty adult healthy male broiler birds (CARIBRO VISHAL) were taken randomly and were divided equally into three treatments, in each treatment have ten male and ten female broiler breeder birds. The birds were maintained under uniform standard managemental conditions in individual cages. The experimental study was carried out during summer season. Sex separate experimental diets were formulated and prepared as per the recommendation of BIS 2007. Combination of two synthetic carotenoids (Canthaxanthine and Apo - carotenoid) were used. The pigments were obtained from *Guangzhou Juyuan Bio-Chem, China*. Control birds did not received any carotenoids supplementation and two different dose levels are (combination of canthaxanthin and apo- carotenoid each 6 mg/kg and 12 mg/kg diet). The dose were calculated as per the recommendation of European food safety authority, 2014.

Sample collection and analysis

Blood samples were collected in both male and female birds randomly with heparin coated containers. The blood samples were analysed for the following parameters such as white blood cell, monocyte, lymphocyte, heterophils, red blood cells and haemoglobin, HCT, MCV, MCH and MCHC using automatic blood analyser (Abacus Junior Vet5, Datron, UK).

Statistical analysis

The data obtained for various parameters were subjected to statistical analysis using statistical package for social sciences (SPSS) version 16.0. The means were compared for significant using Tukey's multiple range test.

Result and Discussion

Mean (±S.E.) haematological parameters of female broiler breeders (12 and 18th week of age) as influenced by dietary supplementation of carotenoids were furnished in Table 1 and 2. Data pertaining to white blood cell in female broiler breeder (12th and 18th week), there was no significant difference between control and dietary treatment groups was observed. The lymphocyte count showed significant difference ($P<0.05$) was noticed between control and Carotenoid supplemented group during 18th week. The T_2 group showed higher lymphocyte value (20.15%) and lowest (13.67%) in control group. However, the monocytes did not show significant result was observed difference between the groups at both 12 and 18th week. The heterophils count (homologous to neutrophils in mammalians), revealed significant difference ($P<0.05$) between treatment groups. The higher heterophils value (3.05%) was observed in T_2 group and lower least by control group. The analysis of variance of data on red blood cell did not show any significant difference between groups. This result was in accordance with the findings of Ali *et al*. (2016) [2]. The haemoglobin concentration showed significant difference ($P<0.05$) between treatment groups at both 12 and 18th week. The T_2 treatment showed higher haemoglobin concentration (13.74 and 13.78 g/dl) in 12 and 18th week, respectively and lower concentration was observed in control group (10.47 and 11.87g/dl). This result was in harmony with the findings of Ali *et al*. (2016) [2] in broiler chicken. They reported that supplementation canthaxanthin improved haemoglobin concentration in broilers. Data on haematocrit value revealed significant difference between treatment groups. Highest haematocrit value in T_2 group (28.98 and 22.41%) and lower value observed in without carotenoid supplement group. Similar result was also observed by Ali *et al.* (2016) [2]. This increase in haematocrit value might be due to the anti-oxidants activity of carotenoids, which could enhance bird's immune performance.

Carotenoid supplementation increased humoral immune response against Newcastle disease (ND) virus in broiler breeders (Prabakar *et al.,* 2017) [5]. Dietary ß-carotenoid stimulated the neutrophils against phagocytic and bacterial killing ability under stress condition in cows (Tjoelker *et al*., 1988) [11]. The mean corpuscular haemoglobin (MCH) exhibited significant difference between treatment and control groups. The highest MCH value (62.94 pg) observed in T_1 followed by T_2 (60.64 pg) and control (55.40 pg) group in 18th week. Mean corpuscular volume (MCV), revealed significant ($p>0.05$) difference between carotenoid supplemented and un-supplemented (control) group. The higher dose of carotenoid fed group showed significantly higher MCV value (11.00) than control group. The analysis of variance of data on RDWc and MCHC showed no significant difference between treatment and control group.

The mean (± S.E.) haematological parameters of male broiler breeders as influenced by dietary supplementation of carotenoids were furnished in Table 3 and 4. Data on white blood cell showed significant difference ($P<0.05$) among the treatment and control group. The T_2 group showed significantly higher white blood cell value (27.78µl) than control groupat18th week, but there was no significant difference noticed during 12th week. Lymphocytes showed significantly ($P<0.05$) higher value in T_1 (24.56) during 18th week. The carotenoid fed group showed significantly ($P<0.05$) higher heterophils value (4.31 and 4.38%) in 12 and 18th week respectively. Red blood cells showed significant difference between treatment and control group as higher red blood cells value (2.87 10^6 / mm^3 and 2.14 ($10^6/mm^3$)was observed in carotenoid fed group. Data on haematocrit value significantly higher (28.90%) in T_2 followed by T_1 (21.55%) and control (20.54%) group at 12th week however, no significant difference was observed at 18th week of age. Higher MCV value (112.00 μm^3) observed in higher dose of carotenoid supplemented group than control group at 12th week. The analysis of variance of data on MCH showed significantly higher (62.94pg) in low dose carotenoid supplemented group followed by high dose supplemented group (60.64pg) and control group (55.40 pg). Statistical analysis of data on lymphocyte, red blood cells width (RDW_C) and MCHC in male broilers revealed no significant difference between treatment groups. Carotenoids have important antioxidant and immune properties. Lutein-zeaxanthin mixture in breeder diets, an important increase of enzymes favouring the elimination of free radicals occurred (Gao *et al*., 2013). Carotenoids can directly stimulate the immune response by inducing lymphocyte proliferation, immunoglobulin and cytokine production as well as gene regulation (Bendich, 1991) [3] and by favouring intercellular communication (Chew and Park, 2004). Feeding carotenoids, lutein and astaxanthin increased cell mediated andhumoral immune response in zebra finch (Mcgrwa *et al.*, 2003) and male green finch (Aguilera and Amat, 2007) [1]. Lopez-rull *et al*. (2015) [7] reported that plasma carotenoid levels plays an essential role in the development and activation of immune system in nestling vulture birds compared to adult birds.

Table 1: Effect of feeding Carotenoid on blood parameters at 12th week in female broiler breeders

Treatment	WBC /µl	LYM (%)	MON (%)	HET (%)	RBC (10^6 / mm^3)	Hb (g/dl)	HCT (%)	MCV (μm^3)	RDWC (µm)	MCH (pg)	MCHC (g/dl)
Control	28.25±2.84	23.75±1.56	1.08±0.27	2.21±0.69	1.27±0.12	10.47^b±0.96	18.96^b±1.84	78.20^b±1.39	13.23±0.56	47.32±4.20	46.66±0.69
T1	24.17±5.35	24.71±6.87	1.06±0.29	2.06±0.77	0.96±0.35	11.36^{ab}±0.48	20.21^{ab}±4.31	108.10^a±1.27	12.36±0.48	49.54±5.91	49.37±4.15
T2	23.75±1.16	20.32±1.56	0.76±0.39	2.67±0.59	1.91±0.13	13.74^a±0.39	28.98^a±1.24	110.00^a±1.34	13.62±0.42	62.56±4.53	56.72±3.65
P- Value	0.625	0.741	0.811	0.380	0.019	0.010	0.019	0.022	0.402	0.114	0.124

WBC: white blood cells; LYM- Lymphocyte; MON – Monocyte; HET- Heterophils; RBC- Red blood cell; Hb – Haemoglobin; HCT – Haematocrit; MCV - Mean Cell Volume; RDWC – Red blood cell width; MCH - Mean Corpuscular Haemoglobin; MCHC - Mean Corpuscular Haemoglobin Concentration

Table 2: Effect of feeding Carotenoid on blood parameters at 18th week in female broiler breeders

Treatment	WBC /µl	LYM (%)	MON (%)	HET (%)	RBC (10^6 / mm^3)	Hb (g/dl)	HCT (%)	MCV (µm3)	RDWC (µm)	MCH (Pg)	MCHC (g/dl)
Control	24.31±1.75	13.67^{b}±1.26	1.33±0.16	2.47^{b}±0.45	1.81±0.11	11.87^{b}±0.41	17.77^{b}±0.38	109.60±1.63	12.44±0.18	55.40^{b}±2.85	60.63±0.85
T1	21.95±1.72	19.67^{a}±1.05	1.70±0.24	2.78^{ab}±0.78	1.59±0.15	12.00^{b}±0.32	19.71^{ab}±1.22	107.80±1.93	12.74±0.24	62.94^{a}±1.07	60.11±1.53
T2	24.17±1.37	20.15^{a}±2.07	1.11±0.22	3.05^{a}±0.22	1.93±0.11	13.78^{a}±0.19	22.41^{a}±0.75	107.80±1.02	12.68±0.25	60.64^{ab}±0.48	62.46±1.08
P- Value	0.532	0.019	0.173	0.746	0.188	0.322	0.008	0.657	0.628	0.031	0.369

WBC: white blood cells; LYM- Lymphocyte; MON – Monocyte; HET- Heterophils; RBC- Red blood cell; Hb – Haemoglobin; HCT – Haematocrit; MCV - Mean Cell Volume; RDWC – Red blood cell width; MCH - Mean Corpuscular Haemoglobin; MCHC - Mean Corpuscular Haemoglobin Concentration

Table 3: Effect of feeding Carotenoid on blood parameters at 12th week in male broiler breeders

Treatment	WBC /µl	LYM (%)	MON (%)	HET (%)	RBC (10^6 / mm^3)	Hb (g/dl)	HCT (%)	MCV (μm^3)	RDWC (µm)	MCH (pg)	MCHC (g/dl)
Control	24.85±0.60	17.81±0.69	1.49±0.20	2.47^{b}±0.53	1.58±0.13^{b}	10.26±0.81^{b}	20.54^{b}±1.55	110.40^{ab}±1.34	12.96±0.35	51.18^{b}±0.98	46.30^{b}±0.37
T1	23.54±0.39	19.11±0.92	1.23±0.32	3.20^{ab}±0.47	1.97±0.07^{b}	10.96±0.17^{b}	21.55^{b}±0.73	109.40^{b}±0.34	12.66±0.15	55.88^{ab}±1.23	51.08^{ab}±1.19
T2	23.55±0.28	20.58±0.48	1.65±0.28	4.31^{a}±0.39	2.87±0.04^{a}	13.26±0.17^{a}	28.90^{a}±0.41	112.00^{a}±0.47	12.44±0.25	58.72^{a}±1.48	52.52^{a}±1.44
P- Value	0.173	0.214	0.550	0.018	0.026	0.019	0.031	0.002	0.318	0.021	0.013

WBC: white blood cells; LYM- Lymphocyte; MON – Monocyte; HET- Heterophils; RBC- Red blood cell; Hb – Haemoglobin; HCT – Haematocrit; MCV - Mean Cell Volume; RDWC – Red blood cell width; MCH - Mean Corpuscular Haemoglobin; MCHC - Mean Corpuscular Haemoglobin Concentration

Table 4: Effect of feeding Carotenoid on blood parameters at 18th week in male broiler breeder

Treatment	WBC /µl	LYM (%)	MON (%)	HET (%)	RBC (10^6 / mm^3)	HB (g/dl)	HCT (%)	MCV (µm3)	RDWC (µm)	MCH (pg)	MCHC (g/dl)
Control	22.97^{b}±0.79	18.12^{b}±0.95	1.40±0.34	2.40^{b}±0.28	1.90^{b}±0.09	12.81±0.38	21.13±1.12	111.20±1.27	13.38±0.27	68.10±2.44	61.30±1.94
T_1	25.72^{ab}±1.04	24.65^{a}±1.37	0.85±0.30	4.38^{a}±0.53	2.14^{a}±0.06	13.28±0.25	23.38±0.73	109.20±1.02	13.44±0.08	62.32±0.94	58.08±0.90
T_2	27.78^{a}±1.70	23.21^{a}±0.83	1.25±0.48	3.99^{ab}±0.96	2.00^{ab}±0.08	13.24±0.28	22.20±0.91	111.20±1.02	13.52±0.13	66.88±2.17	60.32±2.31
P- Value	0.048	0.034	0.294	0.001	0.003	0.436	0.514	0.629	0.714	0.539	0.629

WBC: white blood cells; LYM- Lymphocyte; MON – Monocyte; HET- Heterophils; RBC- Red blood cell; Hb – Haemoglobin; HCT – Haematocrit; MCV - Mean Cell Volume; RDWC – Red blood cell width; MCH - Mean Corpuscular Haemoglobin; MCHC - Mean Corpuscular Haemoglobin Concentration

Conclusion

Supplementation of combination of carotenoids (canthaxanthin and apo- carotenoid) each at 6mg/kg to basal diet enhanced the haematological attributes and thereby increases the health and welfare of broiler breeders during heat stress.

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