

Original Research Article

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Effect of Astaxanthin Supplementation on Blood Plasma Leptin and IgG Profiles in Pre and Postpartum Murrah (*Bubalus bubalis*) Buffaloes during Different Seasons

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ABSTRACT

The study was conducted to evaluate the effect of astaxanthin supplementation on blood plasma leptin and IgG profiles in pre and postpartum Murrah buffaloes during different seasons. For this, thirty two pregnant dry Murrah buffaloes were selected from Livestock Research Centre at National Dairy Research Institute, Karnal. The buffaloes were divided into four groups consisting of eight Murrah buffaloes in each control and supplemented groups of buffaloes during summer (THI=83.2; RH=72.78%) and winter season (THI=58.0; RH=55.20%), respectively. All groups were fed according to nutrient requirement of buffaloes (ICAR, 2013). The treatment group was supplemented with astaxanthin @ 0.25 mg/kg body weight/animal/day during the period 30 days prior to expected date of calving and upto 30 days postpartum. Blood samples were collected on -30, -15, -7, 0, +7, +15, +30,+45 and +60 days with respect to calving where day '0' represents the day of calving. The plasma leptin and IgG levels of treatment group differed significantly ($P<0.05$) as compared to control group during both seasons. Plasma leptin and IgG was higher ($P<0.05$) in winter as compared to summer season. The increase of heat load during summer season presented as THI on buffalo decreases leptin and total IgG level in plasma.

Keywords

Astaxanthin, IgG, Leptin, Summer, Winter

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Introduction

Transition period in dairy buffaloes presents a risk interval for metabolic disorders. An increased capability of milk production is associated with the changes of metabolic and energy homeostasis (Butler, 2000; Lucy, 2003). An increase in energy requirements during late pregnancy and early lactation makes dairy cows highly susceptible to negative energy balance (NEB) which

commonly occurs in the transition period. The metabolic adaptation to NEB requires interactions of metabolic fuels and its failure may occur in various tissues like the liver, adipose tissue and others (Herdt, 2000). Intensified processes of NEFA oxidation proceeded in the liver, result in the increased production of reactive oxygen species (ROS) and oxidative stress development. Thus, metabolic profiles as well as antioxidative/prooxidative status is a useful tool for

monitoring health and reproduction status in buffaloes during the transition period. Astaxanthin is a lipophilic, pinkish-orange carotenoid (3,3'-dihydroxy- β,β -carotene-4,4'-dione) found in algae, seafood (crustacean shells, crab, shrimps, fish) by Qin *et al.*, (2008) and various plants, giving them their exclusive colored aspect (Zhao *et al.*, 2011).

The main source of astaxanthin is the microalga *Haematococcus pluvialis*, which contains maximum concentrations (Boussiba *et al.*, 2000). Astaxanthin is also used as a dietary additive in the USA, Japan, South Korea and Sweden (Choi *et al.*, 2011). Like other carotenoid, astaxanthin manifests high protective antioxidant (Aoi *et al.*, 2008; Derosa *et al.*, 2014) and anticancer (Kavitha *et al.*, 2013; Kowshik *et al.*, 2014; Palazza *et al.*, 2015) properties which decreases oxidative stress and inflammation (Choi *et al.*, 2011; Wolf *et al.*, 2010; Sahebkar *et al.*, 2015), reduces rethrombosis after thrombolysis (Lauver, 2008) and is efficient in ischemia-reperfusion (Gross and Lockwood, 2004), arterial hypertension (Hussein *et al.*, 2005; Hussein *et al.*, 2006) and dyslipidemia (Hussein *et al.*, 2005; Banach *et al.*, 2014).

Leptin is a protein hormone secreted by adipose tissues and acts on hypothalamus to regulate feed intake (Hossner, 1998) and energy balance (Soliman *et al.*, 2002). Leptin is also associated with other biological processes such as reproduction, hematopoiesis, immune response and bone formation (Olusi *et al.*, 2003). It has been reviewed that an increase in the circulating leptin concentration is involved in regulation of metabolic rate, macrophage function and induction of immune cell proliferation or differentiation. Moreover, leptin concentration in plasma has been a direct reflection of the amount of body fat and reproductive function through its effect on nutritional status (Agrawal *et al.*, 2008).

IgG includes circulating antibodies which act against diseases antigens. It is formed by plasma cells found within lymphatic system of animal body (Choudhary *et al.*, 2006). Shearer and Beede, (1990) reported that serum IgG concentrations were altered in peripheral serum of Holstein calves in subtropical climate.

Materials and Methods

Location of the farm

The present study was conducted at the Cattle Yard of National Dairy Research Institute (NDRI), Karnal (Haryana) which is situated on an altitude of 250 meter above mean sea level at latitude of 29^o42'N and longitude of 79^o54'E.

The maximum ambient temperature during summer goes up to 45^oC and minimum temperature in winter comes down to 0^oC with a diurnal variation of 15-20^oC.

Experimental animals

Thirty two pregnant Murrah buffaloes (zero to third parity) were selected 16 in summer (April-June) and 16 in winter season (December-February) from the livestock herd of National Dairy Research Institute, Karnal. From these 16 animals were further randomly divided equally (8 each) into two groups i.e. control and supplemented group of buffaloes. The duration of experiment was from 30 days before expected date of calving to 60 days after parturition.

Ethics Approval

Experiment was approved and conducted under the established standard of the institutional Animal Ethics Committee (IAEC), constituted as per the article number 13 of the Committee for the Purpose of

Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India.

Feeding of experimental animals

All the animals were fed as per ICAR feeding standard. The rations consisted of green fodder and concentrate mixture. Control groups of buffaloes remained as without supplementation of astaxanthin during summer and winter seasons, respectively. Treatment groups of buffaloes were supplemented with astaxanthin during summer and winter seasons, respectively. The astaxanthin as powder form was fed @ 0.25 mg/kg body weight/day mixing with concentrate mixture, from 30 days before parturition till 30 days after parturition.

Collection of blood samples

Blood samples (7-9 ml) were collected in sterile Potassium-EDTA coated vacutainer tubes (BD-PlymouthPL6 7BP, UK) from jugular vein puncture, posing minimum disturbance to animal, on the days -30, -15, -7, 0, 7, 15, 30, 45 and 60 with respect to day of parturition. Day '0' represented the day of calving. Samples were brought to the laboratory in chilled ice boxes soon after collection and centrifuged at 1200xg at 4°C for 20 minutes to separate the plasma from packed erythrocytes. The plasma was decanted in another numbered, clean, dried plastic eppendorf vials. The plasma samples were analyzed for leptin and IgG.

Leptin was determined in plasma using "Bovine Leptin Enzyme-Linked Immunosorbent Assay Kit" (Catalog No. E0014Bo) supplied by Bioassay Technology Laboratory. Immunoglobulin G was determined in plasma using "Bovine Immunoglobulin G Enzyme-Linked Immunosorbent Assay Kit" (Catalog No.

E0010Bo) supplied by Bioassay Technology Laboratory.

Statistical analysis

The data analysis was carried out by SAS software, Version (9.3) of the SAS system for Window, Copyright© (2011) SAS Institute Inc., Cary, NC, USA and Prism 5 software. Three way ANOVA and Tukey's multiple comparison tests were used.

Results and Discussion

The plasma levels of leptin in periparturient Murrah buffaloes during summer and winter seasons are presented in Fig. 1 and Fig. 2. The mean plasma level of leptin during summer season were 5.94±0.29 vs. 5.98±0.30, 2.25±0.19 vs. 3.88±0.17 and 3.07±0.16 vs. 4.44±0.17, respectively of control and treatment groups of buffaloes on -30, 0, +60 days. The levels significantly decreased by 62.12% and 35.11% in control and treatment groups of buffaloes on 0 day. The plasma leptin level of treatment group differed significantly (P<0.05) as compared to control group on day 7 before calving upto day 60 after calving. The mean plasma level of leptin during winter season were 3.93±0.16 vs. 3.94±0.17, 2.36±0.23 vs. 3.01±0.22 and 2.99±0.19 vs. 3.52±0.12 ng/ml, respectively of control and treatment groups of buffaloes on -30, 0, +60 days. The levels significantly decreased by 40% and 23.60% in control and treatment groups of buffaloes on 0 day. The plasma leptin level of treatment group differed significantly as compared to control group on day 15th before calving and from day 7 to 45 after calving. Plasma leptin was higher (P<0.05) in winter as compared to winter season. The analysis of variance of data has been provided in Table 1. leptin revealed a significant difference between season (p<0.01), day (p<0.01), animal (p<0.01), season*day (p<0.01).

The plasma levels of IgG in control and treatment groups of periparturient Murrah buffaloes during summer and winter conditions are presented in Fig. 3 and Fig. 4. The mean plasma IgG level in control and treatment groups of Murrah buffaloes were 57.21 ± 0.38 vs. 57.08 ± 0.28 , 40.68 ± 0.27 vs. 42.93 ± 0.29 and 50.23 ± 0.18 vs. 54.58 ± 0.36 $\mu\text{g/ml}$, on -30, 0 and +60 days, respectively during summer season and 58.34 ± 0.46 vs. 58.87 ± 0.36 , 48.01 ± 0.40 vs. 53.59 ± 0.22 and 54.11 ± 0.41 vs. 57.02 ± 0.23 $\mu\text{g/ml}$ during winter season, respectively.

The plasma IgG level of treatment group was significantly ($P < 0.05$) higher as compared to control group on day 15 before calving, on day of calving and postpartum period. The plasma IgG level of treatment group was significantly ($P < 0.05$) higher as compared to control group on day 7 before calving, on day of calving and on days 15, 30 and 60 after calving. Plasma IgG level was higher ($P < 0.05$) in winter than in summer season.

The low concentration of leptin during the month of August when THI was more is in agreement with the results of Mann *et al.*, (2000) who reported that leptin reached a nadir in late winter (August to September), while being at its peak in late winter (January to March).

This is because of the critical role of leptin in regulating energy metabolism (Block *et al.*, 2003). Astaxanthin treated groups had higher leptin level. It may be due to the fact that astaxanthin is an antioxidant which reduces the oxidative damage of source of leptin i.e. white adipose tissues. During parturition, there was reduction in the level of circulating leptin due to onset of energy deficit. Leptin concentration during pregnancy was higher which declined rapidly towards calving (Maurya, 2011). In the present study also we found the plasma leptin concentration was

significantly lower on the day of parturition in both the seasons. The leptin concentration depressed during postpartum period despite a gradual increase in energy balance (Block *et al.*, 2001). Energy conservation increased by hypoleptinemia in early postpartum period and by promoting enhanced metabolic efficiency (Leury *et al.*, 2003). Leptin has role in feed intake and energy disposition as well as help in the co-ordination of metabolism during the pre-partum to postpartum period. Transition from pregnancy to lactation in dairy cows was associated with a decreased in the plasma leptin level (Thorn *et al.*, 2008; Maurya, 2011). In our result, plasma leptin level was higher ($p < 0.05$) in winter season as compared to summer season.

Decline in plasma leptin around parturition might be due to decrease in adiposities and insulin concentration. Vaidya *et al.*, (2012) found leptin level in high and low yielding Sahiwal cow (5.81 ± 0.14 vs. 5.54 ± 0.10) in summer and (6.12 ± 0.10 vs. 5.90 ± 0.21 ng/ml) in winter season on 45th day of prepartum, however, (5.98 ± 0.07 vs. 5.98 ± 0.31) in summer and (6.26 ± 0.20 vs. 5.79 ± 0.13) in winter in Karan Fries cows, respectively. Hafez (2013) reported that the increase of heat load during August to September presented as THI on lactating buffalo decreases their leptin hormone level in blood plasma.

Aarif (2014) showed that plasma leptin (ng/ml) level were significantly ($P < 0.05$) different (4.53 ± 0.09 vs. 3.13 ± 0.06) in Murrah buffaloes without provision of cooling and managed under cooling, respectively, on day of calving during summer season.

The mean plasma level of leptin (ng/ml) in Sahiwal cows during thermoneutral to summer season on -15, 0, +15 days of parturition were 6.35 ± 0.21 vs. 6.60 ± 0.12 , 5.40 ± 0.11 vs. 6.46 ± 0.18 and 5.56 ± 0.08 vs. 6.78 ± 0.06 ng/ml, respectively (Somal and Aggarwal, 2014).

Table.1 ANOVA Table for leptin and IgG

Source of Variation	df	Mean sum of square	
		Leptin	IgG
Season	1	48.81**	1220.14**
Day	8	13.39**	437.51**
Animal	7	7.62**	1.63
Season x Day	8	4.61**	40.60**
Season x Animal	7	0.46	1.42
Day x Animal	56	0.12	1.08
Season x Day x Animal	56	0.10	0.83
Residual	144	0.67	4.68
Total	287	1.25	20.37

Where ** p<0.01

Fig.1 Changes in plasma leptin (ng/ml) of control and treatment groups of buffaloes during pre and post-partum period in summer season

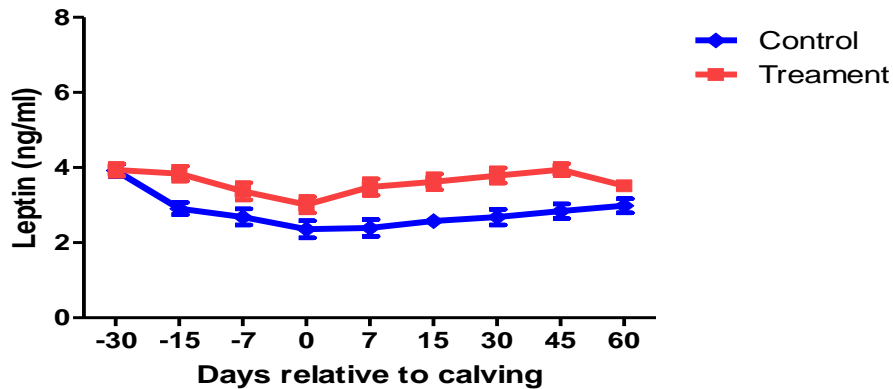


Fig.2 Changes in plasma leptin (ng/ml) of control and treatment groups of buffaloes during pre and post-partum period in winter season

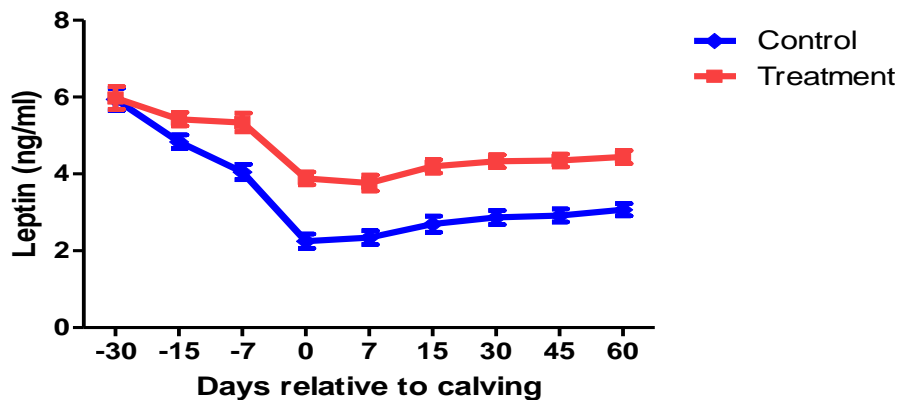


Fig.3 Changes in plasma IgG ($\mu\text{g/ml}$) of control and treatment groups of buffaloes during pre and post-partum period in summer season

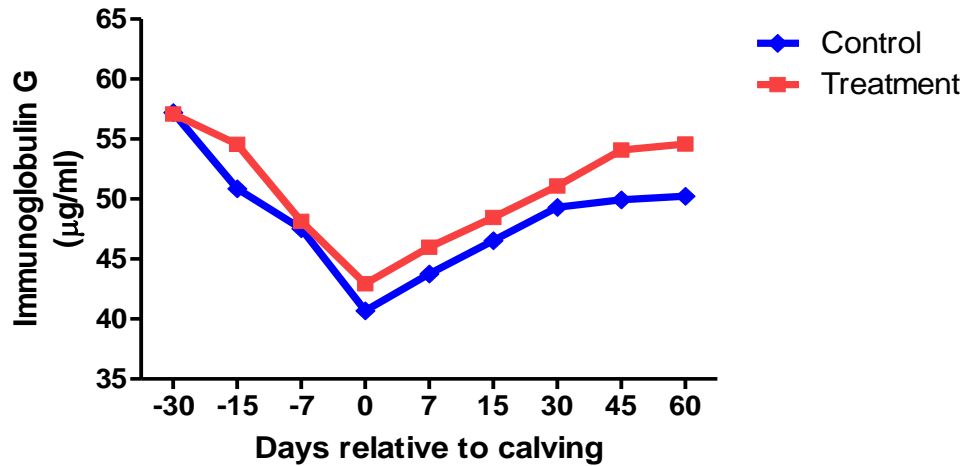
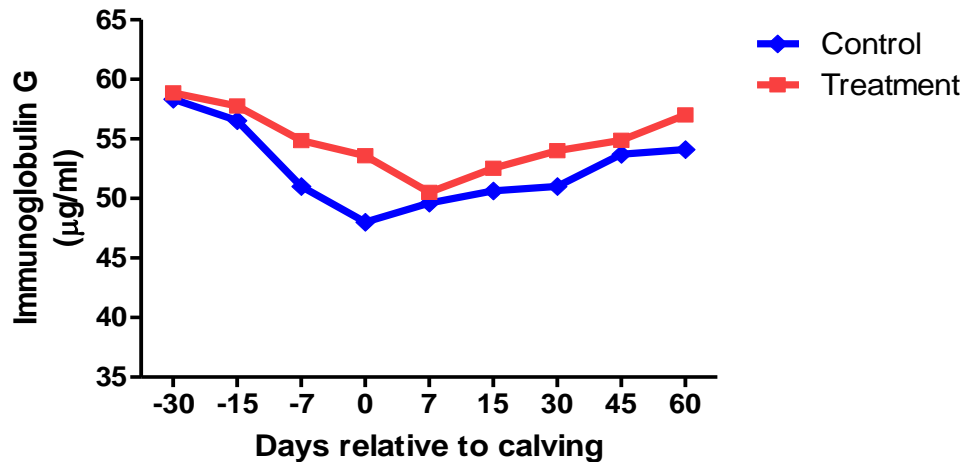


Fig.4 Changes in plasma IgG ($\mu\text{g/ml}$) of control and treatment groups of buffaloes during pre and post-partum period in winter season



Park *et al.*, (2011) found the IgG levels (mg/ml) were 13.4 ± 1.6 , 13.2 ± 1.8 , 15.3 ± 2.5 and 16.3 ± 1.1 in 1st week, 12.3 ± 2.1 , 14.6 ± 2.3 , 13.9 ± 0.9 and 18.7 ± 2.8 in 8th week and 14.5 ± 2.6 , 15.6 ± 2.2 , 23.6 ± 4.5 and 18.9 ± 2.7 in 12th week of experimental period after 0, 1, 5 and 10 mg/d astaxanthin supplementation, respectively, in female domestic shorthair cats. During August to September when temperature and humidity were high total IgG

level in blood plasma in lactating buffalo decreased (Hafez, 2013).

IgG concentration was found to be significant ($P < 0.05$) higher in treatment groups than that of control during both summer and winter seasons. Tengerdy (1980) showed stimulatory effect of supplementation of vitamin E on serum antibody synthesis, mainly IgG. Mili *et al.*, (2015) reported a significant ($P < 0.05$) rise

in plasma IgG level by supplementation of vitamin E to periparturient buffaloes (24.68 ± 1.06 vs 23.45 ± 0.68 mg/ml). The changes of IgG were also significant between different days in both the groups of buffaloes during summer and winter seasons. Minimum value of IgG was observed on day of calving which further increased in post-partum period. This is supported by Herr *et al.*, (2011) and Mili *et al.*, (2015) who showed IgG level 27.73 ± 1.60 mg/ml 56 days before calving to 17.42 ± 1.54 mg/ml on the day of calving in control and 28.07 ± 1.72 mg/ml to 18.96 ± 1.72 mg/ml in vitamin E supplemented group. El-Desouky (2014) reported that the serum IgG level increased from the 30th day before to the 15th day after parturition in the celmanax (an immune-stimulant prebiotic drug) supplemented group than that of control group in dairy cow. Due to presence of high amount of polyunsaturated fatty acids (PUFA) in cell membrane of immune cells, these are sensitive to oxidative damage. IgG level increased by supplementation of astaxanthin; it could neutralize reactive oxygen species and immune cells protected by lipid peroxidation. In our result, plasma IgG level was significantly higher in winter as compared to summer season. In the present study it was found that the plasma level of IgG declined on the day of calving and then again increased post calving. Our findings were similar to previously reported findings that the immunoglobulin levels decrease towards calving (Chandra, 2009). Maurya, (2011) also reported that lymphocyte proliferation index was reduced towards parturition and then again increased after calving. Negative energy, protein, and/or mineral balance associated with the onset of lactation may be partially responsible for the immunosuppression observed in periparturient dairy cattle (Kimura *et al.*, 1999). This has consequences for both the innate and adaptive immune responses. Neutrophil and lymphocyte function is

diminished in the periparturient period, especially in the dairy cow (Kehrli *et al.*, 1989).

The plasma level of leptin was lowest on the day of calving in control and supplemented groups of buffaloes during summer season with respect to 30 days pre and 60 days post calving. The plasma level of IgG decreased on the day of calving in control and supplemented groups of buffaloes during summer season with respect to 30 days pre and 60 days post calving. Plasma leptin and IgG level was significantly higher in winter as compared to summer season. The increase of heat load during summer season presented as THI on buffalo decreases leptin and total IgG level in blood plasma.

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