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Physiological responses, energy metabolites and prolactin levels of buffaloes supplemented with dietary astaxanthin, prill fat and their combination during heat stress

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ABSTRACT

Study was conducted to assess the effect of dietary supplementation of astaxanthin, prill fat and their combination on physiological responses, energy metabolites and prolactin levels in buffaloes during summer season. Twenty four lactating buffaloes were equally divided into four groups, viz. Gr I (control), Gr II (astaxanthin supplementation @ 0.25 mg/kg body wt/day), Gr III (prill fat @ 100 g/animal/day) and Gr IV (combination of both). The respiration rate was lower in treatment groups than control. Plasma glucose in combination group was higher than control. Plasma NEFA was lower in treatment groups compared to control. Prolactin level was lower in supplemented groups compared to control. Based on the results it can be concluded that astaxanthin supplementation helped in ameliorating effects of heat stress whereas prill fat worked as source of energy to maintain the energy balance in lactating buffaloes. The combinations of both the products are more effective as far as stress markers and increase in milk production is concerned but due to higher price of astaxanthin the cost benefit ratio is lower than prill fat supplementation alone. Therefore, farmers prefer to feed prill fat to their lactating buffaloes during summer conditions.

Key words: Astaxanthin, Buffaloes, Energy metabolites, Heat stress, Prill fat

Livestock production is likely to be adversely affected by climate change. Climate change poses a serious threat to livestock production due to the impact on feed and forage quality, water availability, milk production, diseases, breeding and biodiversity. All animals have a thermal comfort zone, which is a range of ambient temperatures that are beneficial to physiological functions (Clarke *et al.* 1986). Altan *et al.* (2003) reported that the variation in climate variables such as temperature, humidity and radiation was recognized as the potential hazards in all domestic livestock species for their growth and production. High ambient temperature accompanied with high air humidity caused an additional discomfort and enhanced the stress levels which in turn resulted in depression of the physiological and metabolic activities viz. behavioural, endocrine, cardio-respiratory and immune system (Ghavi *et al.* 2013). Exposure of buffaloes to heat stress conditions causes a series of drastic changes in biological functions that include deviation from normal physiological functions, decreased feed intake and utilization, disturbances in blood metabolites (Ganaie *et al.* 2013). These changes finally result in impairment of growth and production performance. In order to sustain production performance of buffaloes in hot environment some of the measures are to be taken, which includes physical modifications of micro-environment, dietary modification (minimize diet-induced thermogenesis), increasing energy density (compensate for lower feed intake) and use of dietary antioxidant (vitamin E, Zn, CrPic, betaine) to overcome the adverse effects of heat stress.

Astaxanthin is a potent antioxidant with unique oxygen containing groups of hydroxyl (OH) and ketone (C = O), responsible for its high antioxidant activity and ability to span and stabilize cell membranes. Antioxidant activity of astaxanthin is 10 times higher than other carotenoids and 100 times higher than á-tocopherol. Therefore, Astaxanthin was named as "super vitamin E" (Pan *et al.* 2003). Whereas, Prill fat is a form of bypass fat that resist lipolysis and bio hydrogenation by rumen microbes, but gets digested in lower digestive tract and increases caloric density without reducing the content and digestion of dietary fibres (Schauff and Clark 1989). Astaxanthin absorption is similar to that of dietary lipids or vitamins that are fat soluble. The presence of dietary fat in the small intestine is known to influence the degree of astaxanthin absorption (Odeberg *et al.* 2003).

Considering the above fact, the current study was

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designed to examine individual as well as combined effect of astaxanthin and prill fat supplementation on physiological responses, energy metabolites and prolactin levels of buffaloes during summer season.

MATERIALS AND METHODS

Geographical location of the study area: The present study was carried out at farmer's door in Kathwad village of district Kaithal (Haryana), India. Kathwad is at an altitude of 248 metres above sea level and at latitude of 29º82′N and longitude of 76º49′E. In summer, the highest ambient temperature rise to 45ºC and the minimum winter temperature is around 2ºC with a diurnal variation of 15–20ºC. The region's average rainfall is about 700 mm. The THI during experiment period (July to October 2018) ranged from 76.73 to 83.10.

Selection of experimental animals: The study was conducted during summer season (July to October, 2018) on 24 healthy lactating Murrah buffaloes (first to fourth parity). These animals were equally divided into four groups, i.e. Gr I (control), Gr II (astaxanthin supplementation @ 0.25 mg/kg body wt/day), Gr III (prill fat @ 100 g/animal/day) and Gr IV (combination of both) based on the milk yield. Mass deworming was done before start of experiment.

Management of experimental animals: All experimental animals were fed (wheat and rice straw, maize, Sorghum, Bajra etc.) as per the availability with the farmers (NRC 2001). The different ingredients of the concentrate mixture were—maize 33%, groundnut cake 21%, mustard oil cake 12%, wheat bran 20%, deoiled rice bran 11%, mineral mixture 2% and common salt 1%, while animals of group II additionally supplemented with astaxanthin @ 0.25 mg/kg body wt/day, group III additionally supplemented with prill fat @ 100 g/animal/day and group IV additionally supplemented with combination of astaxanthin @ 0.25 mg/kg body wt/day and prill fat @ 100 g/animal/day. Fresh and clean water was provided free choice to each buffalo four times a day. Buffaloes were hand milked twice a day.

Ethical approval: The 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 (IAEC Approval No. 42-IAEC-18-7).

Measurement of respiration rate (RR): Respiration rate was recorded by flank method. One complete outward and inward movement of flank was counted as one respiration. Respiration rate was counted for 2–3 minutes and expressed as breaths per minute.

Measurement of pulse rate (PR): Pulse rate (PR) was counted by palpating the pulsation of middle coccygeal artery at the base of the tail. The pulse rate was counted for 2–3 minutes and expressed as pulse rate per minute.

Measurement of skin temperature (ST): The skin temperature at fore head region were recorded using non-contact body infrared thermometer by keeping it 2–3 inches away from the desired surface site.

Estimation of plasma glucose: Glucose was estimated in plasma samples using GOD-POD method kits supplied by Span Diagnostics Ltd by using method given by Trinder (1969).

Estimation of Plasma NEFA (Non-esterified fatty acids): Non-esterified fatty acids were estimated in plasma samples using 'Bovine NEFA ELISA Kit' (Cat No.E0021Bo) supplied by Bioassay Technology, 1713 Junjiang International Building, 218 Ningguo Rd. Yangpu Dist. SH. China.

Estimation of plasma prolactin: Prolactin was estimated in plasma samples using 'Bovine Prolactin ELISA Kit' (Cat No.E0237Bo) supplied by Bioassay Technology, 1713 Junjiang International Building, 218 Ningguo Rd. Yangpu Dist. SH. China.

Statistical analysis: Statistical analysis of the obtained data was performed using software version (22) of the SPSS system and Prism 5. Statistical analysis of the data was carried out to find mean ± S.E. Two way ANOVA and one way ANOVA was done to find out the significant difference between treatments and fortnight intervals and their interaction. The pair wise comparison of means was carried out using post-hoc Duncan multiple comparison test. Evaluation of the correlation between all the factors in respective set of animals was made using a correlation coefficient at the level of probability ($P<0.01$ and $P<0.05$).

RESULTS AND DISCUSSION

The THI during experiment period (July to October, 2018) was ranged from 76.73 to 83.10. The results of physiological responses (RR, PR and ST) and prolactin levels are presented in Tables 1 and 2, whereas results of blood glucose and NEFA are presented in Figs 1 and 2 respectively.

Respiration rate (RR): The overall mean values of respiration rate for group I, II, III and IV of Murrah buffaloes were 32.52±0.30, 29.54±0.31, 29.47±0.25 and 29.40±0.23 breaths per minute respectively (Table 1). The overall mean values of respiration rate was significantly ($P<0.05$) higher in control compared to the different treatment groups. However, mean values of respiration rate did not differ

Table 1. Overall mean values of physiological responses of buffaloes during summer season

Parameter	Control	Supplementation (breaths/min)		
		Astaxanthin	Prill fat	Combination
Respiration rate	32.52^{Y}± 0.30	29.54^{X}± 0.31	29.47^{X}± 0.25	29.40^{X}± 0.23
Pulse rate (pulse/min)	73.73^{X}± 0.29	72.80^{X}± 0.34	73.02^{X}± 0.35	72.66^{X}± 0.41
Skin temperature (°C)	35.33^{X}± 0.22	34.81^{X}± 0.20	35.09^{X}± 0.17	34.94^{X}± 0.17

Values are mean±SE of seven observations on six animals. X, Y and Z values with different superscripts within a row differed signiûcantly ($P<0.05$).

Table 2. Overall mean values of prolactin (ng/ml) of buffaloes during summer season

Fortnight interval	Control	Supplementation		
		Astaxanthin	Prill fat	Combination
I	$161.73^{Xbcd}\pm$ 1.75	$162.09^{Xd}\pm$ 1.55	$168.13^{Xd}\pm$ 4.39	$165.11^{Xc}\pm$ 2.14
II	$167.78^{Yd}\pm$ 4.27	$148.26^{Xc}\pm$ 3.20	$163.06^{Ycd}\pm$ 1.54	$141.05^{Xb}\pm$ 7.68
III	$162.33^{Ycd}\pm$ 1.62	$142.03^{Xbc}\pm$ 4.01	$146^{XYbc}\pm$ 4.76	$145.58^{XYb}\pm$ 9.42
IV	$145.86^{Yab}\pm13$ 8.84	$2.3^{Yb}\pm$ 4.19	$148.66^{Ybc}\pm$ 6.31	$111.51^{Xa}\pm$ 5.33
V	$147.29^{Xabc}\pm$ 6.62	$140.76^{Xbc}\pm$ 2.68	$134.25^{Xab}\pm$ 5.32	$145.2^{Xb}\pm$ 7.00
VI	$144.73^{XYa}\pm x$ 7.00	$141.83^{XYbc}\pm$ 2.37	$147.0^{Ybc}\pm$ 8.75	$128.61^{Xab}\pm$ 4.13
VII	$138.70^{Ya}\pm$ 4.54	$114.06^{Xa}\pm$ 4.58	$121.5^{Xa}\pm x$ 4.58	$110.28^{Xa}\pm$ 4.07
Mean±S.E	$152.63^{Z}\pm$ 2.44	$140.19^{X}\pm$ 2.43	$146.94^{Y}\pm$ 3.09	$135.33^{X}\pm$ 3.58

Values are mean ± S.E of seven observations on six animals. a, b, c, X, Y and Z values with different superscripts within a column and within a row differed significantly (P<0.05) within treatment and among treatment groups respectively.

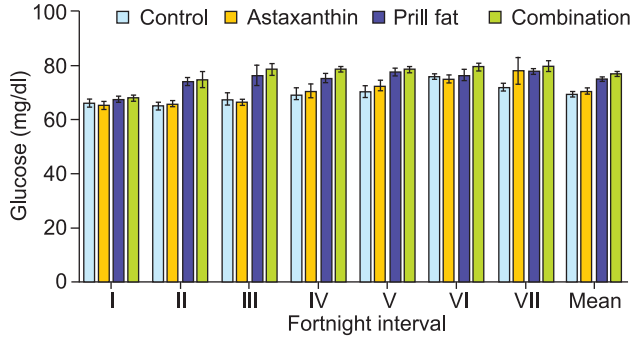


Fig. 1. Effect of dietary supplementation of astaxanthin, prill fat and combination (astaxanthin + prill fat) on plasma glucose (mg/dl) levels of buffaloes during summer season.

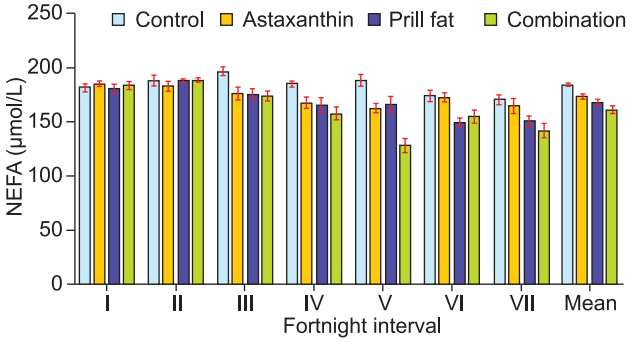


Fig. 2. Effect of dietary supplementation of astaxanthin, prill fat and combination (astaxanthin + prill fat) on plasma NEFA (µmol/L) levels of buffaloes during summer season.

among treatment groups. Respiration rate of Murrah buffaloes showed significant (P<0.01) positive correlation with THI, and other physiological parameters. Analysis of variance (ANOVA) showed significant (P<0.01) variation between groups, intervals and interaction between group and interval.

Respiration rate is considered as an indicator of heat stress. Higher respiration rate was found during hot humid season. Respiration rate showed significant positive correlation with circulating levels of corticoids and it increases with increase in environmental temperature (Vijayakumar, 2005). Higher respiration rate with increase in Tmax could enables animals to dissipate excess of heat from the body, which accounts for 30% of total heat loss by expiration.

The lower values of respiration rate in astaxanthin treated groups is in agreement with Kumar (2018) who reported lower respiration rate may be due to lower production of free radicals in astaxanthin treated Karan Fries and Tharparker heifers during heat stress conditions. Astaxanthin helped in scavenging free radicals that are produced during summer stress and ameliorates effect of heat stress in Murrah buffaloes.

Pulse rate (PR): The overall mean values of pulse rate of group I, II, III and IV of Murrah buffaloes were 73.73±0.29, 72.80±0.34, 73.02±0.35 and 72.66±0.41 beats/min respectively (Table 1). No significant difference was found between the control and all the treatment groups. However, pulse rate in group II and group IV was numerically lower than control group. Pulse rate of Murrah buffaloes showed positive correlation with THI, prolactin levels and ST and negative correlation with glucose.

The results of present study indicated higher average values of pulse rate during study period. A positive correlation of pulse rate with an increase in ambient temperature and relative humidity was noticed in present study, these results are in accordance to those in Surti buffaloes (Chaudhary *et al.* 2015) and in Murrah buffaloes (Singh *et al.* 2014b). Dietary supplementing of astaxanthin to Sahiwal and KF heifers (Kumar, 2018) and implantation of melatonin to growing Murrah buffaloes (Kumar, 2017) helped to maintain the pulse rate in normal range during summer season.

Skin temperature (ST): The overall mean values of skin temperature of group I, II, III and IV of Murrah buffaloes were 35.33±0.22, 34.81±0.20, 35.09±0.17 and 34.94±0.17°C respectively (Table 1). No significant difference was found in skin temperature of the control and treatment groups but skin temperature was lower in treatment groups than control group. The ST was decreased with decrease in ambient temperature and THI. ST of Murrah buffaloes showed significant (P<0.01) positive correlation with THI, Tmax and other physiological parameters. Analysis of variance (ANOVA) of skin temperature data showed significant (P<0.01) variation among intervals.

Banerjee and Ashutosh (2011) also reported a similar positive correlation among physiological parameters (PR, ST, RR) in climatic chamber studies. The higher ST in rural buffaloes in present study are in agreement with Thankachan

(2007) who also reported similar increase in ST of Murrah buffaloes during hot humid environment. The increase in ST may be due to higher environmental temperature during summer season causes vasodilation of skin capillaries which increase the blood flow to the skin to enhance the heat loss (McManus *et al.* 2009). The results of present study are in accordance with Kumar (2018). Schutz *et al.* (2011) who reported that the solar radiation during heat stress condition causes elevated ST and are directly related to each other.

Plasma glucose: The mean ±S.E. values of plasma glucose for control and treatment groups have been presented in Fig. 1. The overall mean values of plasma glucose (mg/dl) for control and treatment groups II, III and IV of Murrah buffaloes were 57.47±0.94, 58.14±0.78, 58.94±0.79 and 59.67±0.83 mg/dl respectively. Significant (P<0.05) higher plasma glucose levels was found in group III and IV compared to group I and II. However, plasma glucose (mg/dl) in astaxanthin supplemented group was numerically higher than control group, but statistically was not significant. Further, the plasma glucose levels for combination group (59.67±0.83) was higher than the prill fat (58.94±0.79) supplemented group. Analysis of variance of data showed significant (P<0.01) variation between groups and intervals but interaction between group and intervals was not significant. Plasma glucose was negatively (P<0.01) correlated with THI, Tmax and NEFA levels.

The results of the present study are in agreement with those of previous researchers Shabab *et al.* (2016); Chaudhary *et al.* (2015) who reported significant decrease in plasma glucose with increase in THI level during hot humid conditions. The lower plasma levels during higher THI may be attributed to lower feed intake during heat stress.

Elevated glucose level in supplemented buffaloes has contributed to enhance milk yield as the glucose is the precursor for milk lactose synthesis (Wattiaux, 2005). The higher glucose levels in prill fat cows and buffaloes leads to increased milk production (Singh *et al.* 2016; Shabab *et al.* 2016). Dietary supplementation of astaxanthin and prill fat enhanced the milk production and fat % in buffaloes during summer season (Somagond *et al.* 2019). The results of present study are in accordance to those of earlier studies carried out on cattle and buffaloes, whereas Shelke *et al.* (2012) did not find any effect of bypass fat on plasma glucose of Murrah buffaloes. Priyadarshini (2017) reported higher plasma glucose in astaxanthin supplemented group during summer season. In vitamin C supplemented groups, the plasma glucose concentration was significantly higher than the control group indicating the beneficial effects of vitamin C supplementation in ameliorating the heat stress (Kumar *et al.* 2012). Similarly supplementation of vitamin E (1000 IU/day/cow) along with zinc (60 ppm/day/cow) to summer stressed Sahiwal cows showed significantly higher plasma glucose compared to control (Chandra *et al.* 2013).

Plasma NEFA (Non-esterified fatty acid): The mean ±S.E. values of plasma NEFA for control and treatment groups have been presented in Fig. 2. The overall mean values of plasma NEFA (µmol/L) for groups I, II, III and IV of Murrah buffaloes were 183.35±1.99, 172.69±2.18, 167.64±2.75 and 160.99±3.69 µmol/L respectively. The plasma NEFA levels were higher in control group than supplemented groups of lactating buffaloes. Further, with in the supplemented groups, combination group showed significantly lower NEFA levels compared to astaxanthin and prill fat supplemented group. However, no significant difference in plasma NEFA levels of group II and III was observed. The mean values of plasma NEFA for supplemented groups (astaxanthin, prill fat and their combination) started showing decreasing trend from 2nd fortnight and was continued till the end of experiment. Statistical analysis of variance of data showed significant (P<0.01) variation between groups, intervals and interaction between group and interval.

The blood concentration of NEFA reflects the degree of mobilization of adipose tissue. It has been reported that plasma NEFA levels showed a significant (P<0.01) positive correlation with THI. During summer season the animals will be under negative energy balance, more NEFA will be released from body fat resulting in higher blood NEFA concentration. NEFA levels in prill fat supplemented cow's vs control differed significantly (Singh *et al.* 2014a). The lower NEFA levels in prill fat supplemented group was indicative of lower body fat mobilization and improved energy balance (Sharma *et al.* 2016). The concentration of NEFA in blood reflects the degree of adipose tissue mobilization. The results of the present study showed significant higher NEFA in control group, which indicate state of negative energy balance and increased lipolysis and are associated with decreased milk production in control group of lactating Murrah buffaloes. The significant lower NEFA level in prill fat supplemented group indicated that prill fat was effective in preventing lipolysis from adipose tissue and maintaining the energy balance during heat stress in animals. Plasma NEFA levels showed a significant (P<0.01) positive correlation with physiological parameters, prolactin and THI and showed negative (P<0.01) correlation with glucose.

Plasma prolactin: The mean ± S.E. values of plasma prolactin for control and treatment groups have been presented in Table 2. The overall Mean ± S.E. values of prolactin for control and treatment groups (astaxanthin, prill fat and their combination) of Murrah buffaloes were 152.63±2.44, 140.19±2.43, 146.94±3.09 and 135.33±3.58 ng/ml respectively. The mean values of prolactin of supplemented groups showed decreasing trend from 2nd fortnight and continued till the end of experiment. The higher prolactin value were found during higher THI levels in all the groups. There was significant lower plasma prolactin value in all supplemented groups compared to control. Further, with in the supplemented groups astaxanthin and combination group showed significantly lower prolactin levels compared to prill fat group. However, no significant change in prolactin values was observed in astaxanthin and combination group. Higher values of

plasma prolactin in all group of animals were observed due to higher ambient temperature during study period (July to September). When ambient temperature was increased from 18 to 32°C, prolactin level in Holstein heifers raised by 3 fold (Ronchi *et al.,* 2001). Roy and Prakash (2007) reported that environmental factors such as ambient temperature and photoperiod affect the levels of circulating prolactin in several species. Several other researchers also reported a marked fall in prolactin levels during cold conditions in cattle (Rius *et al.* 2005).

The results of the present study, demonstrated a significant reduction in prolactin levels in astaxanthin treated groups compared to control. Similarly, Kumar (2017) reported a significant decrease in prolactin in melatonin implanted Murrah buffaloes compared to control group. The plasma prolactin was positively correlated with THI, physiological parameters, NEFA, whereas significant ($P<0.05$) negative correlation was found with glucose level. Analysis of variance showed significant ($P<0.01$) variation between groups, intervals and interaction between groups and intervals.

Milk yield: Overall milk yield of control group was significantly lower than the supplemented groups of buffaloes. Significantly higher increase in milk yield (kg/d) was found in group-IV (9.03±0.12) followed by group III (8.89±0.15) and group-II (8.23±0.12) compared to group-I (7.81±0.13). There was also significant change in milk yield among supplemented groups. Milk yield of group II was significantly lower than other two groups, i.e. III and IV. In present study supplementation of astaxanthin, prill fat and their combination enhanced milk yield by 5.12%, 13.8% and 15.6% respectively over control group during summer season.

In conclusion, our results indicated that mean values of physiological responses (ST and PR) were numerically lower in treatment groups compared to control group. RR was significantly ($P<0.05$) lower in all treatment groups than control group. Decreased levels of stress in treatment groups than control, is mainly due to supplementation of potent antioxidant, which facilitated the amelioration of summer stress. Astaxanthin helped in scavenging free radicals that are produced during heat stress and ameliorated its effects. The significant lower NEFA level in prill fat supplemented groups indicated that prill fat was effective in preventing lipolysis from adipose tissue and maintaining the energy balance in lactating buffaloes, which are prone to negative energy balance due to lactation and heat stress conditions. These results indicated the beneficial effects of astaxanthin and prill fat supplementation in ameliorating the heat stress and improving productive performance of buffaloes in rural areas.

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