# EFFECT OF DIETARY SUPPLEMENTATION OF ASTXANTHIN, PRILL FAT AND THEIR COMBINATION ON ANTIOXIDANTS AND IMMUNITY STATUS OF LACTATING BUFFALOES DURING HEAT STRESS

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## ABSTRACT

To evaluate the role of dietary supplementation of astaxanthin, prill fat and their combination on antioxidants and immune status of lactating buffaloes during heat stress. Twenty four lactating buffaloes (first to fourth parity) were selected and divided equally into four groups i.e. Group I (control), Group II (astaxanthin 0.25 mg/kg body wt/day), Group III (prill fat 100 g/animal/day) and Group IV (astaxanthin + prill fat). Plasma was separated from the blood collected at fortnightly interval for the analysis of antioxidant enzymes and interleukins levels. The levels of superoxide dismutase and catalase were significantly lower in astaxanthin supplemented groups (Group II and Group IV) compared to Group I and Group III. The levels of pro-inflammatory cytokine (Interleukin -2) was significantly ($P<0.05$) lower in astaxanthin supplemented groups (Group II and Group IV) compared to Group I whereas levels of anti-inflammatory cytokines (Interleukin -10) was significantly ($P<0.05$) higher in all supplemented groups (Group II, Group III and Group IV) than Group I. Results showed that, dietary supplementation of astaxanthin (potent antioxidant) was able to alleviate the heat stress induced changes by lowering the levels of antioxidant enzymes (SOD and CAT) and pro-inflammatory cytokine (IL-2) and improving the levels of anti-inflammatory (IL-10) cytokines in lactating buffaloes during summer season.

**Keywords**: *Bubalus bubalis*, buffaloes, antioxidant, astaxanthin, heat stress, interleukins, prill fat

## INTRODUCTION

Heat stress is one of the factor which cause reactive oxygen species mediated oxidative stress in farm animals. Production performance of buffaloes are mostly affected during summer stress due to change in physiological responses, feed intake, antioxidant system and decreased immunity. Antioxidants are substances that can prevent or slow the damage to cells caused by free radicals, unstable molecules that the body produces as a response to environmental and other stresses. Superoxide dismutase (SOD) and

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catalase protect the cell against damage from superoxide ion to $H_2O_2$ due to the secondary generation of highly reactive hydroxyl group (Miyazaki *et al*., 1991). Summer stress is one of major factor contributing to lower immunity in the buffaloes. Inflammatory cytokine (pro and anti-inflammatory) levels were significantly differed in heat stress and cooling conditions at different time intervals (Do Amaral *et al*., 2009). Buffaloes are mainly affected by oxidative stress, is one of major cause for inflammatory and immune dysfunction. Astaxanthin (AX) is a lipophilic, pinkish orange carotenoid antioxidant found in algae, seafood (crustacean shells, crabs, shrimps, fish) and various plants that give them their unique colour appearance (Higuera-Ciapara *et al*., 2006). Several studies revealed AX's beneficial health effect as antioxidant, photo protective, anti-inflammatory, immunity improvement, and other important applications in the nutraceutical, cosmetic, food and feed industries (Higuera-Ciapara *et al*., 2006; Guerin *et al*., 2003). Astaxanthin found naturally in different living organisms such as microalgae (*Haematococcus pluvialis*, *Chlorella zofingiensis*, and *Chlorococcum* sp), fungi, red yeast (*Phaffia rhodozyma*), seafood and some birds such as flamingos and quail. Astaxanthin is extracted from *Haematococcus pluvialis* for dietary supplements in humans and animals (Guerin *et al*., 2003). Antioxidant strength of astaxanthin is 10 times greater than other carotenoid and 100 times more than α-tocopherol. AX is termed as "super vitamin E" (Pan *et al*., 2003). Inflammatory cytokine expression like nuclear factor (NF) kB, tumor necrosis factor (TNF)-α and interleukin (IL)-1β were significantly reduced in astaxanthin fed mice (Yasui *et al*., 2011). When microalgal biomass *(H. pluvialis, S. platensis* or *B. braunii)* was fed to rats, showed significantly high antioxidase catalase, superoxide dismutase and peroxidase activity Rao *et al.* (2010). Surai *et al*., (2016) reported substantially lower levels of malondialdehyde and antioxidant enzyme systems in astaxanthin-fed fish compared with basal dietary feed. Dietary absorption of astaxanthin is same as that of fat or lipid soluble vitamins. Presence of dietary fat influences the absorption of astaxanthin in small intestine. Astaxanthin absorption is higher when emulsified with olive oil and significantly improves the use of astaxanthin supplements with additional fat (Odeberg *et al*., 2003). Astaxanthin supplemented along with lipid based diets resulted in increased bioavailability of astaxnthin in humans (Hussein, 2015). Due to its inertness in rumen, fat can be incorporated up to 9% of the dietary DM (Sharma *et al*., 2016). Bypass fat supplementation increases dietary energy density, improves body condition score and animal productivity (Ganjkhanlou *et al*., 2009).

Keeping in mind the above fact, the present study was planned to examine the individual as well as combined effect of astaxanthin and prill fat supplementation on levels of antioxidant enzymes and interleukins during heat stress condition in lactating buffaloes.

## MATERIALS AND METHOD

### Selection and management of animals

A total of 24 lactating Murrah buffaloes were selected from Kathwad Village of district Kaithal, Haryana (India). Experiment was carried out during summer season (July to October - 2018). The buffaloes were divided into four groups i.e. six animals in each group based on milk yield. All groups were treated uniformly in terms of feeding (NRC, 2001) and other management practices.

Group II was additionally supplemented with 0.25 mg/kg body wt/day of dietary astaxanthin, Group III with 100 g/animal/day of prill fat, Group IV with both astaxanthin 0.25 mg/kg body wt/day and prill fat 100 g/animal/day and Group I as control. Astaxanthin (Herbal antioxidant) was purchased from Herbo Nutra, New Delhi and Prill fat (Bergafat T-300) from Nutriprof Company for the supplementation to the experimental animals during summer season. Blood samples (10 ml/animal) were collected at fortnightly intervals in sterile heparinised vacutainer (BD Vacutainer TM, UK) tubes by puncturing the jugular vein, posing minimum disturbance to the animal, on days 0, 15, 30, 45, 60, 75 and 90$^{th}$ day of experiment period. Immediately after blood collection, blood samples were kept in ice box and transported to the laboratory for further processing. The plasma was separated by centrifugation at 3000 rpm for 30 minutes and stored at -20$^{o}$C in different aliquots for the estimation of plasma antioxidant enzymes and interleukins. Temperature humidity index (THI) was calculated using the formula of NRC (1971). The THI during experiment period was ranged from 76.73 to 83.10. Ethical approval for experiment was taken from Institutional Animal Ethics Committee (IAEC), as per rules laid down by the Government of India (IAEC Approval No. 42-IAEC-18-7).

**Estimation of antioxidant enzymes and plasma interleukins (IL)**

Superoxide dismutase (SOD), Catalase, Interleukin 2 and Interleukin 10 was estimated in plasma samples using "Bovine ELISA Kit" supplied by Bioassay Technology, 1713 Junjiang International Building, 218 Ningguo Rd. Yangpu Dist. SH. China.

**Statistical analysis**

Statistical analysis of the data was carried out to find mean ± S.E using software version (22) of the SPSS system and Prism 5 for window. 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 $P<0.05$). Models used for analysis:

$$Y_{ij} = \mu + S_i \times B_j + S \times B + S \times B ..... + £_{ij}$$

Where, $\mu$ = overall mean

$Y_{ij}$ = K$^{th}$ observation on i$^{th}$ treatment at j$^{th}$ interval

$S_i$ = effect of i$^{th}$ tratment

$B_j$ = effect of j$^{th}$ interval

$£_{ij}$ = random error

## RESULTS AND DISCUSSION

Temperature Humidity Index (THI) during experiment period (August to October - 2018) was used as a scale for measuring heat stress on animals (Armstrong, 1994). THI during study period was ranged from 76.73 to 83.10 (Figure 1) indicating stressful situation for the buffaloes.

**Antioxidant enzymes**

**Plasma Superoxide Dismutase (SOD)**

The results of SOD (ng/ml) levels in Murrah buffaloes during summer season for control and treatment groups (astaxanthin, prill fat and their combination) have been presented in Table 1 and

statistical analysis in Table 3. The mean value of SOD for control group was higher than the values of all treatment groups. The overall mean values of SOD of Group II (14.45±0.35 ng/ml) and Group IV (14.84±0.43 ng/ml) was significantly (P<0.05) lower than Group I (16.61±0.39 ng/ml) and Group III (16.07±0.46 ng/ml) of buffaloes (Table 1). No statistical difference in SOD value was observed during different intervals of experiment. Further, within the supplemented group astaxanthin (14.45±0.35 ng/ml) and combination (14.84±0.43) groups have lower SOD values than the prill fat (16.07±0.46 ng/ml) supplemented group (Table 1). Analysis of variance of data showed significant (P<0.01) variation between groups (Table 3).

As observed in present study several of researchers reported significant higher serum SOD activity during both moderate and high THI compared to low THI (Zhang *et al*., 2014; Yatoo *et al*., 2014). Lallawmkimi (2009) examined the effect of winter and summer seasons on the antioxidant status of lactating Murrah buffaloes and reported significantly higher SOD levels during summer compared to winter in Murrah buffaloes. Similaly, significantly higher SOD levels in seminal plasma of Karan Fries bulls during hot dry season followed by spring and winter was observed (Soren *et al*., 2016). Kumar *et al.* (2011) reported similar higher trend in erythrocyte SOD activity in buffaloes during hot dry and hot humid season. The levels of SOD was positively correlated with THI in present study and are in accordance with Chaudhary *et al*. (2015) who also showed a positive association between SOD and THI in Surti buffaloes. The results of present study showed that astaxanthin (potent antioxidant) supplementation neutralise, the higher ROS production and therefore SOD levels were low in supplemented group by breakdown of superoxide ($O_2^-$) to $H_2O_2$ and oxygen. Pan *et al.* (2003) stated that in ammonia stressed shrimp there was significantly lower SOD in astaxanthin treated group indicating improvement of antioxidation capacity by dietary astaxanthin and accordingly, the improvement of survival rate against ammonia stress. Astaxanthin contains a long conjugated double bond system with a relatively unstable orbital electron, it can scavenge oxygen radicals in cells and reduces cellular damage (Chien *et al*., 2003). Soren *et al*. (2017) reported significantly (P<0.01) lower levels of SOD in seminal plasma of Karan Fries bulls supplemented with astaxanthin compared to control group of animals during hot humid season.

**Plasma catalase**

The results of Catalase (ng/ml) in Murrah buffaloes during summer season for control and treatment group (astaxanthin, prill fat and their combination) have been presented in Table 2 and statistical analysis in Table 3. The mean values for control group was higher than the all treatment groups. The overall mean values of catalase in buffaloes of Group II (46.64±0.96 ng/ml) and Group IV (46.32±0.72 ng/ml) was significantly (P<0.05) lower than the control (49.37±0.61 ng/ml) and prill fat (47.28±0.86 ng/ml) supplemented group (Table 2). No significant difference was observed in Catalase values at different intervals in plasma of Murrah buffaloes under field condition. Further, with in the supplemented groups, no significant variation was observed in Catalase levels. Astaxanthin (46.64±0.96 ng/ml) and combination of astaxanthin and prill fat supplemented groups (46.32±0.72) have numerically lower Catalase values than the prill fat (47.28±0.86 ng/ml) group (Table 2). Analysis of variance of data showed significant (P<0.01) variation between groups as well as intervals (Table 3). Plasma catalase during

summer showed significant (P<0.01) positive correlation with THI (Table 4).

The results of present study are in agreement with those of Shibu *et al.* (2008) who reported higher catalase levels in Murrah buffaloes during heat stress conditions. Increased catalase activity is in connection to increase tolerance from thermal stress in order to prevent protein denaturation during heat stress condition (Kundu *et al*., 2014). Several research findings demonstrated the beneficial effect of dietary inclusion of various antioxidants *viz*, sodium bicarbonate, potassium carbonate, ascorbic acid etc during heat stress in buffaloes. Kumar *et al*. (2011) observed significant higher erythrocyte catalase activity in control group of buffaloes than supplemented group. Lallawmkimi *et al*. (2013) studied the influence of vitamin E supplementation in Murrah buffaloes and reported a significantly higher catalase in control group of animals compared to the vitamin E supplemented animals. Zhang *et al*. (2014) in his study on white shrimp (*litopenaeus vannamei*) showed a significant (P<0.01) lower catalase level in astaxanthin (125 mg/kg and 50 mg/kg in diet) supplemented group as compared to control. There was significant lower levels of plasma catalase in astaxanthin supplemented group of post-partum Murrah buffaloes than control group (Priyadarshini, 2017). Kumar (2018) reported significant (P<0.01) lower levels of catalase in Karan Fries and Tharparker heifers supplemented with astaxanthin compared to control group during summer season.

**Immunity parameters**

**Plasma interleukin - 2 (IL-2)**

The mean ± S.E values of IL-2 for control and treatment groups have been presented in Figure 2 and statistical analysis in Table 3. The overall mean values of IL-2 for control and treatment groups (astaxanthin, prill fat and their combination) of Murrah buffaloes were 65.17±1.10, 57.46±1.14, 64.21±1.08 and 56.91±1.46 ng/L respectively (Figure 2). Significant (P<0.05) lower mean values of IL-2 was observed in Group II (astaxanthin) and Group IV (combination of astaxanthin + prill fat) of buffaloes compared to Group III (prill fat) and Group I (control). Statistical analysis of data showed significant (P<0.01) variation between groups, intervals and interaction between groups and intervals (Table 3). Plasma IL-2 showed significant (P<0.01) positive correlation with THI, SOD and catalase, whereas significant (P<0.01) negative correlation with IL-10 during summer (Table 4).

Many of earlier findings showed significant changes in the levels of inflammatory cytokines (IL-1β, IL-2, IL-6, IL-10, and IL-12) during cold and heat stress conditions. Compromised immune status in thermally stressed buffalo heifers was associated with reduced lymphocyte proliferation and IL-2 production (Patir and Upadhyay, 2007). Do Amaral *et al.* (2011) reported higher levels of plasma prolactin levels in cows during heat stress was associated with reduced lymphocyte proliferation and increased pro inflammatory cytokine production. Sordillo and Aitken (2009) reported that inflammatory and immune dysfunction are mainly because of oxidative stress during summer season. Wang *et al.* (2015) reported significantly higher values of IL-2 in rats exposed to heat stress. There was reduction in levels of pro-inflammatory cytokines, such as tumor necrosis factor-α and interleukin-6 in astaxanthin supplemented group (Macedo *et al*., 2010). Priyadarshini and Aggarwal (2018) found lower (P< 0.05) expression of IL-6 and TNF-α in Murrah buffaloes supplemented with astaxanthin during summer seasons. Kumar

and Singh (2019) reported astaxanthin as useful in reducing levels of pro-inflammatory cytokines in $H_2O_2$-stimulated U937 mononuclear cells by hindering the ROS-induced production of NF-κB transcription factor. Kumar (2018) reported NF-κB signaling pathway was inhibited in astaxanthin supplemented group, hence a significant reduction in pro-inflammatory mediators like IL-2 and IL-12 was observed. Oral astaxanthin administration alone induced the production of IL-2 and IFN-γ in mice's lymphocytes (Lin *et al*., 2016). The results of the present study are in general agreement with those of earlier worker's studies on different species of animals, which showed a significant ($P<0.05$) lower values of IL-2 in astaxanthin and combination supplemented group of buffaloes compared to prill fat and control group during summer season.

**Plasma interleukin - 10**

The mean ± S.E values of IL-10 for control and treatment groups have been presented in Figure 3 and statistical analysis in Table 3. The overall mean values of IL-10 for Group I, II, III and IV were 175.5±1.89, 212.5±2.14, 182.5±1.97 and 208.6±2.29 (ng/L) respectively (Figure 3). Overall mean values for control group was significantly lower than the all supplemented groups. Significantly higher values of IL-10 were found in combination group followed by astaxanthin group, prill fat group and control group. There was also significant difference in IL-10 among supplemented groups (Figure 3) being significantly lower in prill fat supplemented group compared to other two supplemented groups. No significant difference was observed in IL-10 values among astaxanthin and combination supplemented groups during summer season. Statistical analysis of data showed significant variation between groups, interaction of groups and intervals, but no significant ($P<0.01$) variation was observed between intervals. Plasma IL-10 showed positive correlation with THI and negative significant ($P<0.01$) correlation with SOD, catalase and IL-2 level during summer season in buffaloes (Table 4).

The higher values of IL-10 in astaxanthin supplemented group indicated lower inflammatory response in these buffaloes during heat stress conditions. Sheikh *et al.* (2017) showed higher anti - inflammatory action in heifer's supplemented with zinc, which helped in reduction ($P<0.05$) of IL-12 concentration due to rise in IL-10 levels, which is a potent inhibitor of IL-12. Similarly Kumar *et al.* (2015) reported that supplementation of inorganic chromium to heat stressed buffalo calves improved T and B cell proliferation, neutrophil phagocytic activity and immunoglobulin and improved their immune status during heat stress. Pearce *et al.* (2015) also reported that dietary supplementation of zinc to heat stressed pigs subsided the changes caused by heat stress that were noticeable from changes in TNF-α levels.

## CONCLUSION

Astaxnthin used as a potent antioxidant helped in ameliorating the adverse effect of oxidative stress by scavenging oxygen free radicals in cells and reduces cellular damage during summer season. Supplementation of astaxanthin alone and with combination with prill fat reduced the levels of pro-inflammatory cytokines (interleukin-2) and improved levels of anti-inflammatory cytokines (interleukin-10) indicated lower inflammatory response in treated buffaloes during heat stress conditions. The results confirm that dietary supplementation of astaxanthin along

Table 1. Effect of dietary supplementation of astaxanthin, prill fat and combination (astaxanthin + prill fat) on superoxide dismutase (SOD) (ng/ml) of buffaloes during summer season.

| Fortnight interval | Control | Supplementation | | |
|---|---|---|---|---|
| | | Astaxanthin | Prill fat | Combination |
| 0 | 16.24±1.07 | 14.53±1.05 | 15.29±1.22 | 14.76±1.19 |
| I | 16.23±1.45 | 15.34±1.23 | 15.83±1.35 | 14.7±0.96 |
| II | 17.35±1.02 | 13.8±0.70 | 15.65±1.12 | 14.48±1.05 |
| III | 16.81±1.24 | 15.57±0.97 | 16.00±1.57 | 15.18±1.29 |
| IV | 15.53±1.05 | 14.2±0.79 | 15.17±1.49 | 14.63±1.33 |
| V | 17.47±0.79 | 14.66±1.15 | 17.49±1.05 | 14.49±1.09 |
| VI | 16.68±0.86 | 13.07±0.70 | 17.08±0.89 | 15.64±1.53 |
| Mean ± S.E. | $16.61^{Y}$±0.39 | $14.45^{X}$±0.35 | $16.07^{Y}$±0.46 | $14.84^{X}$±0.43 |

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

Table 2. Effect of dietary supplementation of astaxanthin, prill fat and combination (astaxanthin + prill fat) on catalase (ng/ml) of buffaloes during summer season.

| Fortnight interval | Control | Supplementation | | |
|---|---|---|---|---|
| | | Astaxanthin | Prill fat | Combination |
| 0 | 48.94±1.71 | 49.63±2.25 | 45.65±1.81 | 46.43±1.90 |
| I | 50.58±1.80 | 50.44±2.31 | 51.13±2.12 | 50.25±1.49 |
| II | 49.21±1.85 | 46.53±1.77 | 49.58±1.89 | 48.21±1.64 |
| III | 49.99±2.09 | 46.07±2.56 | 47.78±1.24 | 46.56±2.17 |
| IV | 49.18±1.31 | 46.14±2.91 | 46.90±3.87 | 45.80±2.50 |
| V | 49.20±1.26 | 44.01±3.75 | 44.75±1.70 | 45.17±1.14 |
| VI | 48.47±1.98 | 43.68±1.54 | 45.17±2.22 | 41.80±1.07 |
| Mean ± S.E. | $49.37^{Y}$±0.61 | $46.64^{X}$±0.96 | $47.28^{XY}$±0.86 | $46.32^{X}$±0.72 |

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

Table 3. Analysis of variance (ANOVA) of antioxidant enzymes in buffaloes during study period.

| Source of variation | df | Mean sum of square | | | |
|---|---|---|---|---|---|
| | | SOD | Catalse | IL-2 | IL-10 |
| Group | 3 | 43.482** | 78.811** | 796.391** | 14380.722** |
| Interval | 6 | 3.786 | 83.713** | 1061.402** | 163.996 |
| Group* interval | 18 | 3.079 | 11.302 | 49.330* | 873.353** |
| Error | 140 | 7.842 | 26.641 | 20.328 | 94.259 |

**(P<0.01); *(P<0.05).

Table 4. Correlation of Temperature Humidity Index (THI) with antioxidant enzymes and interleukins in buffaloes during study period.

| Parameters | THI | SOD | Catalase | IL - 2 | IL - 10 |
|---|---|---|---|---|---|
| THI | 1 | | | | |
| SOD | -.146 | 1 | | | |
| Catalase | .228** | -.020 | 1 | | |
| IL - 2 | .340** | .607** | .145 | 1 | |
| IL - 10 | .082 | -.089 | -.204** | -.221** | 1 |

**(P<0.01); *(P<0.05)

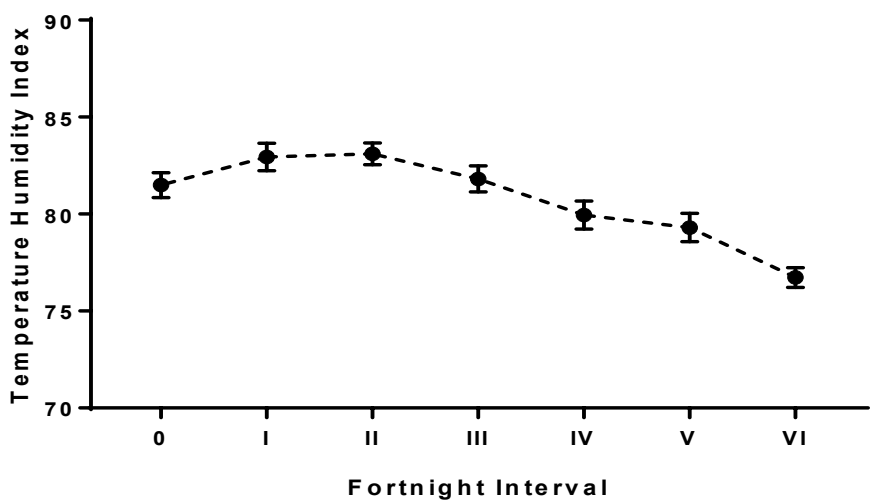


Figure 1. Temperature Humidity Index (THI) during summer season (July to October - 2018).

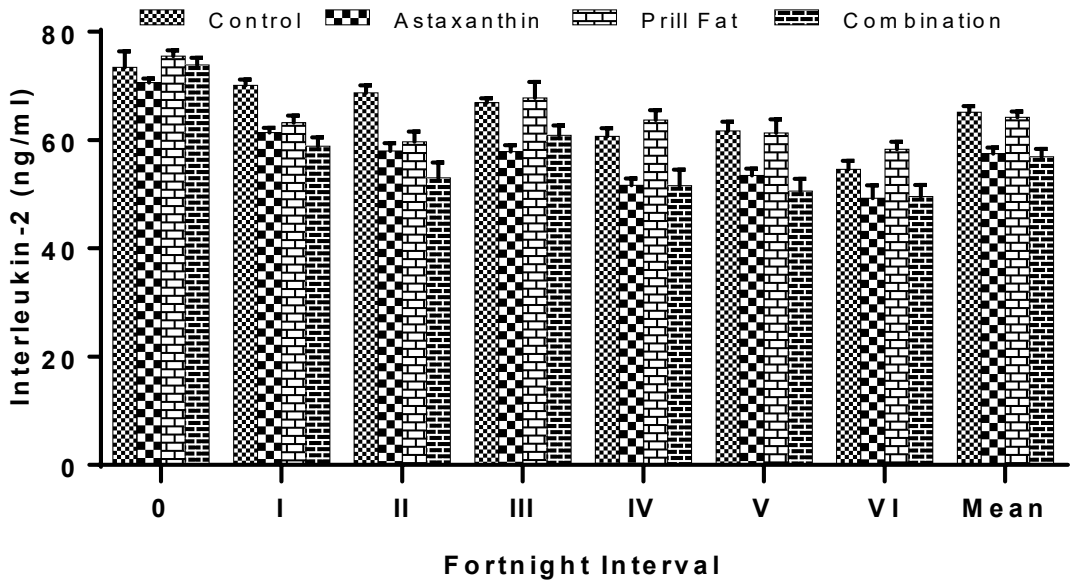


Figure 2. Effect of dietary supplementation of astaxanthin, prill fat and combination (astaxanthin + prill fat) on interleukin - 2 (IL-2) (ng/L) of buffaloes during summer season.

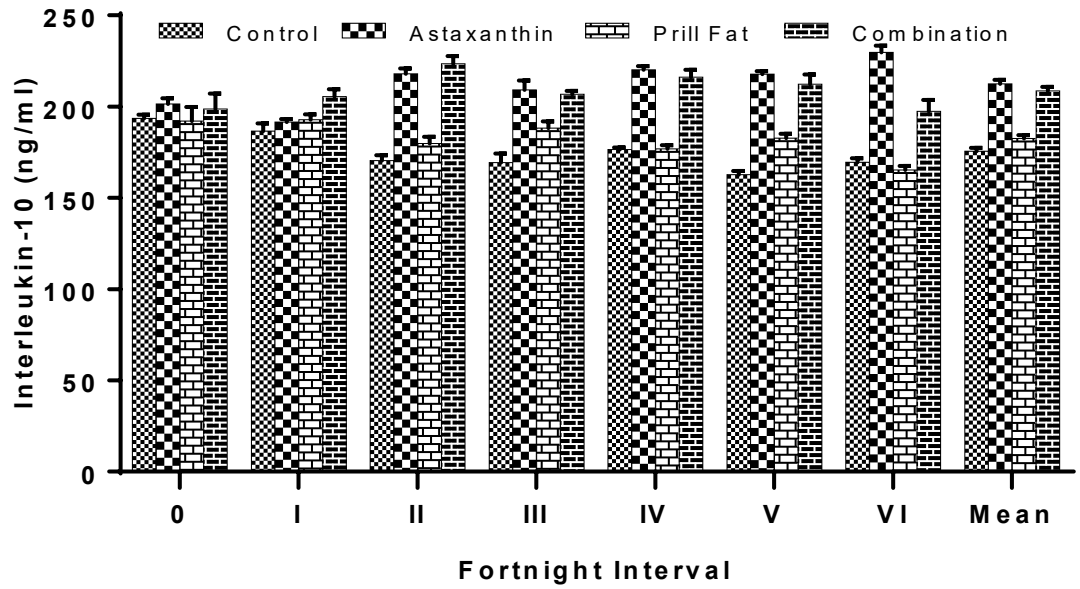


Figure 3. Effect of dietary supplementation of astaxanthin, prill fat and combination (astaxanthin + prill fat) on of interleukin - 10 (IL-10) (ng/L) of buffaloes during summer season.

with prill fat was more effective than individual supplementation in alleviating the heat stress induced changes in buffaloes during summer season, which was evident from significant lower levels of antioxidant enzymes (SOD and Catalase) and pro-inflammatory cytokine (IL-2) and higher levels of anti-inflammatory (IL-10) cytokines in combination supplemented group. Based on the results of the study it can be stated that for amelioration of adverse effect of heat stress, the combination of astaxanthin and prill fat can be supplemented for normal physiology and welfare for buffaloes.

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## REFERENCES

Armstrong, D. 1994. Heat stress interaction with shade and cooling. *J. Dairy Sci*., **77**(7): 2044-2050. DOI: 10.3168/jds.S0022-0302(94)77149-6

Chaudhary, S.S., V.K. Singh, R.C. Upadhyay, G. Puri, A.B. Odedara and P.A. Patel. 2015. Evaluation of physiological and biochemical responses in different seasons in Surti buffaloes. *Vet. World*, **8**(6): 727. DOI: 10.14202/vetworld.2015.727-731

Chien, Y.H., C.H. Pan and B. Hunter. 2003. The resistance to physical stresses by *Penaeus monodon* juveniles fed diets supplemented with astaxanthin. *Aquaculture*, **216**(1-4): 177-191. DOI: 10.1016/S0044-8486(02)00056-X

Do Amaral, B.C., E.E. Connor, S. Tao, J. Hayen, J. Bubolz and G.E. Dahl. 2009. Heat-stress abatement during the dry period: Does cooling improve transition into lactation?. *J. Dairy. Sci*., **92**(12): 5988-5999. DOI: 10.3168/jds.2009-2343

Do Amaral, B.C., E.E. Connor, S. Tao, M.J. Hayen, J.W. Bubolz and G.E. Dahl. 2011. Heat stress abatement during the dry period influences metabolic gene expression and improves immune status in the transition period of dairy cows. *J. Dairy. Sci*., **94**(1): 86-96. DOI: 10.3168/jds.2009-3004

Ganjkhanlou, M., K. Rezayazdi, G.R. Ghorbani, M.D. Banadaky, H. Morraveg and W.Z. Yang. 2009. Effects of protected fat supplements on production of early lactation Holstein cows. *Anim. Feed Sci. Tech*., **154**(3-4): 276-283. DOI: 10.1016/j.anifeedsci.2009.09.008

Guerin, M., M.E. Huntley and M. Olaizola. 2003. Haematococcus astaxanthin: Applications for human health and nutrition. *Trends Biotechnol*., **21**(5): 210-216. DOI: 10.1016/S0167-7799(03)00078-7

Higuera-Ciapara, I., L. Felix-Valenzuela and F.M. Goycoolea. 2006. Astaxanthin: A review of its chemistry and applications. *Crit. Rev. Food Sci*., **46**(2): 185-196. DOI: 10.1080/10408690590957188

Hussein, M.A. 2015. Cardioprotective effects of astaxanthin against isoproterenol-induced cardiotoxicity in rats. *Journal of Nutrition and Food Sciences*, **5**(1): 1-6. DOI: 10.4172/2155-9600.1000335

Kumar, A., G. Singh, B.S. Kumar and S.K. Meur. 2011. Modulation of antioxidant status and lipid peroxidation in erythrocyte by dietary supplementation during heat stress in buffaloes. *Livest. Sci*., **138**(1-3): 299-303. DOI: 10.1016/j.livsci.2010.12.021

Kumar, M., H. Kaur, R.S. Deka, V. Mani, A.K. Tyagi and G. Chandra. 2015. Dietary inorganic chromium in summer-exposed buffalo calves (*Bubalus bubalis*): Effects on biomarkers of heat stress, immune status, and endocrine variables. *Biol. Trace Elem. Res*., **167**: 18-27. DOI: 10.1007/s12011-015-0272-0

Kumar, S. 2018. *Influence of astaxanthin supplementation on growth performance, immunity and antioxidant status in heifers during summer season* P.hD. Dissertation, National Dairy Research Institute, Karnal, India.

Kumar, S. and S.V. Singh. 2019. Inhibition of NF-κB signaling pathway by astaxanthin supplementation for prevention of heat stress-induced inflammatory changes and apoptosis in Karan Fries heifers. *Trop. Anim. Health Pro.*, **51**(5): 1125-1134. DOI: 10.1007/s11250-018-01793-y

Kundu, M.S., A.K. De, S. Jeyakumar, J. Sunder, A. Kundu and T. Sujatha. 2014. Effect of prill fat supplementation on hormones, milk production and energy metabolites during mid-lactation in crossbred cows. *Vet. World*, **7**(6): 384-388. DOI: 10.14202/vetworld.2014.384-388

Lallawmkimi, M.C., S.V. Singh, R.C. Upadhyay and S. De. 2013. Impact of vitamin E supplementation on heat shock protein 72 and antioxidant enzymes in different stages of Murrah buffaloes during seasonal stress. *Indian J. Anim. Sci*., **83**(9): 909-915.

Lallawmkimi, M.C. 2009. *Impact of Thermal Stress and Vitamine Supplementation on Heat Shock Protein-72 and Antioxidant Enzymes in Murrah buffaloes.* Ph.D. Dissertation, National Dairy Research Institute, Karnal, India.

Lin, K.H., K.C. Lin, W.J. Lu, P.A. Thomas, T. Jayakumar and J.R. Sheu. 2016. Astaxanthin, a carotenoid, stimulates immune responses by enhancing IFN-γ and IL-2 secretion in primary cultured lymphocytes *in vitro* and ex *vivo*. *Int. J. Mol. Sci*., **17**(1): 44. DOI: 10.3390/ijms17010044

Macedo, R.C., A.P. Bolin, D.P. Marin and R. Otton. 2010. Astaxanthin addition improves human neutrophils function: in vitro study. *Eur. J. Nutr*., **49**(8): 447-457. DOI: 10.1007/s00394-010-0103-1

Miyazaki, T., K. Sueoka, A.M. Dharmarajan, S.J. Atlas, G.B. Bulkley and E.E. Wallach. 1991. Effect of inhibition of oxygen free radical on ovulation and progesterone production by the *in-vitro* perfused rabbit ovary. *J. Reprod. Fertil*., **91**(1): 207-212. DOI: 10.1530/jrf.0.0910207

NRC. 1971. *A Guide to Environmental Research on Animals.* National Academies Press, Washington, DC, USA.

NRC. 2001. *Nutrient Requirements of Domestic Animals: Nutrient Requirements of Dairy Cattle*, 7th ed. National Academy of Sciences, Washington, D.C. USA. 333p.

Odeberg, J.M., A. Lignell, A. Pettersson and P. Hoglund. 2003. Oral bioavailability of the antioxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations. *Eur. J. Pharm. Sci*., **19**(4): 299-304. DOI: 10.1016/s0928-0987(03)00135-0

Pan, C.H., Y.H. Chien and B. Hunter. 2003. The resistance to ammonia stress of *Penaeus monodon* Fabricius juvenile fed diets supplemented with astaxanthin. *J. Exp. Mar. Biol. Ecol*., **297**(1): 107-118. DOI: 10.1016/j.jembe.2003.07.002

Patir, H. and R.C. Upadhyay. 2007. Interrelationship between Heat Shock Protein 70 (HSP70) and Lymphocyte Proliferation in Thermal Exposed Buffalo Heifers. *Ital. J. Anim. Sci*., **6**: 1344-1346. DOI: 10.4081/ijas.2007.s2.1344

Pearce, S.C., M.V. Sanz Fernandez, J. Torrison, M.E. Wilson, L.H. Baumgard and N.K. Gabler. 2015. Dietary organic zinc attenuates heat stress-induced changes in pig intestinal integrity and metabolism. *J. Anim. Sci*., **93**(10): 4702-4713. DOI: 10.2527/jas.2015-9018

Priyadarshini, L. and A. Aggarwal. 2018. Astaxanthin inhibits cytokines production and inflammatory gene expression by suppressing IκB kinase-dependent nuclear factor κB activation in pre and postpartum Murrah buffaloes during different seasons. *Vet. World*, **11**(6): 782. DOI: 10.14202/vetworld.2018.782-788

Priyadarshini, L. 2017. *Immunity Antioxidant Energy Status and Production performance in pre and postpartum Murrah buffaloes supplemented with astaxanthin during summer and winter seasons*. Ph.D. Dissertation, National Dairy Research Institute, Karnal, India.

Rao, A.R., A.H. Reddy and S.M. Aradhya. 2010. Antibacterial properties of Spirulina platensis, Haematococcus pluvialis, Botryococcus braunii micro algal extracts. *Current Trends in Biotechnology and Pharmacy*, **4**(3): 809-819. Available on: https://eds.b.ebscohost.com/eds/pdfviewer/pdfviewer?vid=0&sid=9f5b378d-3674-4eeb-aa32-260fe8b38171%40sessionmgr103

Sharma, S., M. Singh, A.K. Roy and S. Thakur. 2016. Effect of pre-partum prilled fat supplementation on feed intake, energy balance and milk production in Murrah buffaloes. *Vet. World*, **9**(3): 256-259. DOI: 10.14202/vetworld.2016.256-259

Sheikh, A.A., A. Aggarwal, B. Indu and O. Aarif. 2017. Inorganic zinc supplementation modulates heat shock and immune response in heat stressed peripheral blood mononuclear cells of periparturient dairy cows. *Theriogenology*, **95**: 75-82. DOI: 10.1016/j.theriogenology.2017.02.024

Shibu, C.T., S.V. Singh and R.C. Upadhyay. 2008. Impact of temperature rise on pulmonary dynamics, heat dissipation and antioxidant status in KF heifers. *Indian J. Anim. Sci*., **25**: 67-71.

Sordillo, L.M. and S.L. Aitken. 2009. Impact of oxidative stress on the health and immune function of dairy cattle. *Vet. Immunol. Immunop.*, **128**(1-3): 104-109. DOI: 10.1016/j.vetimm.2008.10.305

Soren, S., S.V. Singh and S. Kumar. 2017. Effect of astaxanthin supplementation on semen (Karan fries bulls) storage at 5 C. *International Journal of Current Microbiology and Applied Sciences*, **6**(5): 23-28. DOI: 10.20546/ijcmas.2017.605.003

Soren, S., S.V. Singh and P. Singh. 2016. Influence of season on seminal antioxidant enzymes in Karan Fries bulls under tropical climatic conditions. *Turk. J. Vet. Anim. Sci*., **40**(6): 797-802. DOI: 10.3906/vet-1603-76

Surai, P.F., V.I. Fisinin and F. Karadas. 2016.

Antioxidant systems in chick embryo development. Part 1. Vitamin E, carotenoids and selenium. *Animal Nutrition*, **2**(1): 1-11. DOI: 10.1016/j.aninu.2016.01.001

Wang, L.I., F. Liu, Y. Luo, L. Zhu and G. Li. 2015. Effect of acute heat stress on adrenocorticotropic hormone, cortisol, interleukin-2, interleukin-12 and apoptosis gene expression in rats. *Biomedical Reports*, **3**(3): 425-429.

Yasui, Y., M. Hosokawa, N. Mikami, K. Miyashita and T. Tanaka. 2011. Dietary astaxanthin inhibits colitis and colitis-associated colon carcinogenesis in mice via modulation of the inflammatory cytokines. *Chem. Biol. Interact*., **193**(1): 79-87. DOI: 10.1016/j.cbi.2011.05.006

Yatoo, M.I., D. Umesh and M.C. Sharma. 2014. Seasonal changes in certain blood antioxidants in cattle and buffaloes. *Indian J. Anim. Sci*., **84**(2): 173-176.

Zhang, F.J., X.G. Weng, J.F. Wang, D. Zhou, W. Zhang, C.C. Zhai and Y.H. Zhu. 2014. Effects of temperature-humidity index and chromium supplementation on antioxidant capacity, heat shock protein 72, and cytokine responses of lactating cows. *J. Anim. Sci*., **92**(7): 3026-3034. DOI: 10.2527/jas.2013-6932