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Effect of *Astaxanthin* supplementation on liver profile in growing Vrindavani calves exposed to thermal stress

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Abstract

Thirty healthy growing Vrindavani cattle, with an average body weight 170 ± 6.50 were selected for the experiment. The calves were randomly divided into five groups (6 animals each) viz. Group I (control), Group II heat stress (HS), Group III (HS+ *Astaxanthin* supplementation), Group IV (shed +*Astaxanthin* supplementation) and Group V ambient temperature exposure (En+ *Astaxanthin* supplementation) for monitoring the effect of *Astaxanthin* supplementation on various group of animal. Dietary supplementation of *Astaxanthin* was given at 150mg/day/animal. To assess the effect of heat stress on liver profile the Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) level in the serum were analyzed. Blood serum sample was collected at weekly interval for the estimation of ALT and AST. ALT and AST level was significantly ($P < 0.05$) higher in treatment groups compared to control. However, heat stressed *Astaxanthin* supplemented group showed lower ALT and AST level as compare to Heat stress (HS) group. Present study indicated that the *Astaxanthin* supplementation helps to reduce ALT and AST level to its normal value during heat stress condition. The dietary supplementation of *Astaxanthin* may be very helpful in mitigating the effect of heat stress in growing Vrindavani cattle.

Keywords: Vrindavani, mitigating, assess

Introduction

The country economy is mainly depends upon agriculture and livestock sector. Livestock sector plays a vital and significant role in increasing the GDP of India. In 2050, the demand for animal products worldwide is predicted to nearly treble. In the meantime, current climate models predicted that temperatures may rise by 0.2°C every ten years, bringing the global average surface temperature closer to 4.0°C by 2100 (IPCC 2007). India now has 192.52 million cattle (20th livestock census). Cattles are mostly raised in subtropical climates. Temperature, humidity, precipitation, air movement, radiation, barometric pressure, and ionisation are some of the components that make up climate (Maurya *et al.*, 2015) [15]. Globally, different regions have different climates that are influenced by several elements such as latitude, dominant winds, evaporative conditions, water supply, elevation, and proximity to mountains (West, 2003) [21]. It is believed that the temperature ranges for lactating dairy cows is between -0.5 and 20°C in temperature region of the world (West, 2003) [21]. Livestock production is expected to be negatively impacted by climate change. Climate change poses a serious threat to livestock production because of its impact on diseases, breeding, biodiversity, water availability, feed and forage quality and milk Production (Godde *et al.*, 2021) [9]. The harsh climate badly affects the liver function which depicts in the change in various enzymes. The ALT and AST are two of the most reliable markers of liver condition (An-Qiang *et al.*, 2009) [4]. Blood ALT and AST values are greater in cross-bred dairy calves exposed to heat stress (Berian *et al.*, 2019) [5]. In growing and adult cattle, summer and the hot, humid season showed considerably greater plasma enzyme activity (ALT, AST) than winter and spring (Bhan *et al.*, 2013) [6]. *Astaxanthin*, an oxygenated carotenoid, is included in the class of carotenoids known as xanthophylls, which is mostly composed of lutein, zeaxanthin, β -cryptoxanthin, and canthaxanthin

(Majumder *et al.*, 2024) [14]. The primary colourful pigment found in seafood, such as salmon, trout, red sea bream, prawns, lobster, etc., is called astaxanthin. Astaxanthin contains keto groups, hydroxyl groups, and conjugated double bonds. It has characteristics that are both lipophilic and hydrophilic (Majumder *et al.*, 2024) [14]. Astaxanthin forms an internal and exterior link with the cell membrane; it exhibits higher biological activity than other antioxidants (Ambati *et al.*, 2014) [3]. Astaxanthin molecules include both hydroxyl and ketone groups, which make them more polar and improve membrane function, the ionone rings at both ends of the molecule have a functional role in scavenging the free radicals (Majumder *et al.*, 2024) [14]. The major source of natural Astaxanthin is *Haematococcus pluvialis* and most of studies it has been extensively used (Higuera-Ciupara *et al.*, 2006) [10]. Numerous research demonstrated the beneficial effects of astaxanthin on immunity, anti-inflammatory, photoprotective, and ocular health, among other critical applications in the nutraceuticals, cosmetics, and food and feed sectors (Soren *et al.*, 2017) [20]. The antioxidant activity of astaxanthin is 100 times more than that of α -tocopherol and up to 10 times higher than that of other carotenoids such as zeaxanthin, lutein, canthaxanthin, and β -carotene. As a result, astaxanthin is referred regarded as "super vitamin E." (Somagond *et al.*, 2022) [19]. In this study dietary Astaxanthin has been used as an antioxidant to ameliorate heat stress.

Materials and Methods

The study was carried out at the ICAR-Indian Veterinary Research Institute in Bareilly (UP) at the Nuclear Research Laboratory and the Physiology and Climatology Division. The proposed experiment was conducted in a psychrometric chamber by the Physiology and Climatology Division of the Indian Veterinary Research Institute, Izatnagar Bareilly (UP). A total of thirty crossbred calves bearing good health, aged between one and one and a half years, were used in the experiment. Animals were divided into 5 groups. Group I was non heat stress control group (NHSC). Group II was Heat stress group, where animals were exposed to heat stress (HS). Group III was heat stress with Astaxanthin supplemented group (HS+A). Group IV was Astaxanthin supplemented group and animals were kept in normal shed without any heat stress. Group V where animal kept under direct sunlight with *Astaxanthin supplementation*. Dietary supplementation of Astaxanthin was given at 150mg/day/animal. The duration of all experimental groups of animal was 28 days for each group. Blood samples were collected from animal on weekly basis. Blood samples were collected on pre exposure (7 day before exposure) and 1st, 7th, 14th, 21st, 28th day of exposure at respective schedule of experiment. In addition to above, all the animals were

given 35th day recovery period. After blood collection serum sample were separated for the estimation of ALT and AST.

- **ALT (Alanine Amino Transferase):** Serum ALT activity was measured using a commercially available ALT test kit (Coral Clinical System).
- **AST (Aspartate Amino Transferase):** Serum AST activity was measured using a commercially available AST test kit (Coral Clinical System).
- **Statistical analysis:** The data collected throughout the experimental period was analyzed by using two way ANOVA.

Results and Discussion

The results of different parameters of Liver profile test i.e ALT and AST are being presented in the Table no.1 and Table no.2 and Figure no.1 and Figure no.2 respectively.

Alanine aminotransferase (ALT)

The Mean $\pm$ SE value of control and treatment (HS, HS+A, Sh+A, and En+A) group have been presented in the Table 1 and Figure 1. Prior to exposure, there was no significant difference in the ALT levels between the control, HS, HS+A, En+A, and Sh+A, respectively. On the first day of the trial, there was a significant ($p < 0.05$) rise in the ALT values in the HS, HS+A, and En+A groups of animals as compared to the NHSC and Sh+A groups. The 7th, 14th, 21st, and 28th days of the experiment showed the same pattern in the variation of ALT levels for the various treatments (HS, HS+A, and En+A) respectively. After 35 days of recovery period, there was no significant trend in the variation in ALT for any of the treatments. There was no significant difference on Pre exposure, 1st, 7th, 14th, 21st, 28th and 35th day in control and Sh+A group of animal. As study progress there was significant increase ($P < 0.05$) in ALT level on 7th, 14th, 21st day of study period compare to pre exposure and recovery period.

Yadav *et al.* (2016) [22] reported that the cattle kept in a climate chamber showed greater serum ALT activity. As temperature rises, serum ALT activity rises as observed by Sharma *et al.* (2011) [18]. According to Cerutti *et al.* (2018) [8], calves' serum ALT activity was higher in the summer than in the winter. Kittur *et al.* (2024) [13] found that goats experiencing heat stress have elevated serum ALT levels but AST levels did not increased. *Astaxanthin supplementation* reduces the impact of heat stress in goats (Kittur *et al.*, 2024) [13]. All Previous study results support our finding, where we found that heat stress increase in serum ALT level and supplementation Astaxanthin causes an ameliorative effect in growing Vrindavani cattle by reduction in ALT level.

Table 1: Mean $\pm$ SEM of ALT (U/ml) of different groups over the study period in growing Vrindavani cattle

	ALT				
	Control	HS	HS+A	Sh+A	En+A
Pre expo	18.37 $\pm$ 0.07aA	18.36 $\pm$ 0.10aE	18.37 $\pm$ 0.04aE	18.37 $\pm$ 0.02aA	18.35 $\pm$ 0.02aD
1	18.39 $\pm$ 0.10dA	24.02 $\pm$ 0.26aD	22.16 $\pm$ 0.78bD	18.38 $\pm$ 0.05dA	19.73 $\pm$ 0.40cD
7	18.38 $\pm$ 0.09dA	31.64 $\pm$ 0.68aC	28.43 $\pm$ 1.30bB	18.24 $\pm$ 0.36dA	23.37 $\pm$ 1.04cC
14	18.37 $\pm$ 0.07dA	35.45 $\pm$ 0.35aA	32.58 $\pm$ 0.91bA	18.28 $\pm$ 0.17dA	29.65 $\pm$ 1.17cA
21	18.38 $\pm$ 0.04dA	33.40 $\pm$ 0.40aB	30.51 $\pm$ 1.38bAB	18.29 $\pm$ 0.16dA	26.52 $\pm$ 0.55cB
28	18.38 $\pm$ 0.02dA	32.01 $\pm$ 0.40aC	25.00 $\pm$ 1.04bC	18.29 $\pm$ 0.37dA	23.11 $\pm$ 0.82cC
35	18.38 $\pm$ 0.04dA	23.36 $\pm$ 0.43aD	21.67 $\pm$ 0.59bD	18.24 $\pm$ 0.07dA	20.05 $\pm$ 0.33cD

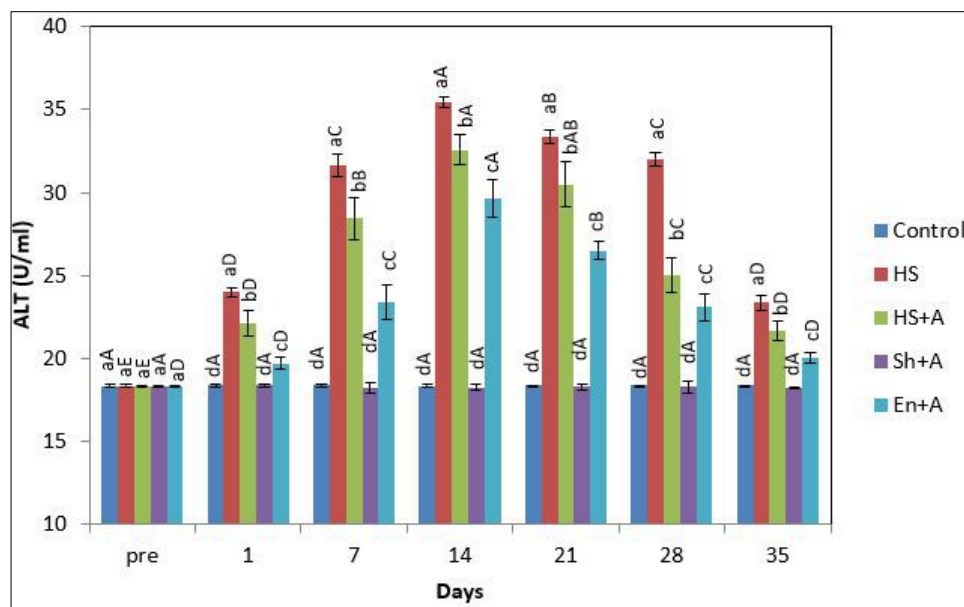


Fig 1: ALT (U/ml) of different groups over the study period. All values are shown as Mean $\pm$ SEM. abc... values with different superscripts represents significant difference (<0.05) between the groups ABC... values with different superscripts represents significant difference (<0.05) within the treatment

Aspartate aminotransferase (AST)

Table No. 2 and Figure No. 2 present the Mean $\pm$ SE of AST (U/ml) in serum of growing Vrindavani calves recorded after heat exposure in all groups during the research period. Before the exposure, there was no significant ($p < 0.05$) difference in the AST values amongst control, HS, HS+A, En+A, and Sh+A, respectively. On the first day of the trial, there was a notable ($p < 0.05$) rise in the AST values in the HS, HS+A, and En+A groups of animals as compared to the NHSC and Sh+A groups. The 7th, 14th, 21st, and 28th days of study for HS, HS+A, and En+A showed the similar pattern in the change of AST levels. After 35 days of the recovery period, there was no discernible pattern in the variation in AST level for any of the treatments; it was essentially unchanged from the pre-exposure phase.

Following a statistical analysis of the data, variations in AST were found between the pre-exposure, 1, 7, 14, 21, 28 and 35 days. Over the course of the investigation, it was observed that there was no significant difference ($P > 0.05$) in the animals in the control group and Sh+A group. AST was found to be significantly ($p < 0.05$) greater in the HS group of animal throughout the duration of study. As the investigation went on, the AST increased significantly

($P < 0.05$) on days 7th, 14th, 21st as compared to pre-exposure and 35 days of recovery period. The highest AST value was seen on the 7th, 14th, and 21st days of the trial. On the 28th day of the study, the AST value was lower but remained significantly ($P < 0.05$) greater than the pre-exposure and recovery period. The AST concentration for the two treatments i.e. HS+A and En+A followed same trend like HS group of animals.

Bueno *et al.* (2017) [7] found elevated serum AST activity in broiler after their heat exposure. High summertime ambient temperatures may raise transaminase enzyme activity and have negative effects on thyroid, liver, and kidney function as reported by Ramadan *et al.* (2015) [16]. Rathwa *et al.* (2017) [17], also observed higher levels of AST and ALT in the summer than in the winter. Aleena *et al.* (2020) [2] worked on heat-stressed Osmanabadi, Malabari, and Salem Black goats and reported that the Osmanabadi had significantly higher levels of AST than the Malabari, and Salem Black goat respectively. In accordance to our research finding on *Astaxanthin supplementation* in heat cattle Islam *et al.* (2017) [12] also reported a reduced in AST and ALT level in rat treated with Astaxanthin.

Table:2 Mean $\pm$ SEM of AST (U/ml) of different groups over the study period in growing Vrindavani cattle

	AST				
	Control	HS	HS+A	Sh+A	En+A
Pre expo	21.86 $\pm$ 0.46aA	21.86 $\pm$ 0.29aE	21.84 $\pm$ 0.30aD	21.85 $\pm$ 0.29aA	21.81 $\pm$ 0.26aD
1	21.88 $\pm$ 0.30aA	23.22 $\pm$ 0.37aDE	22.36 $\pm$ 0.63aD	21.82 $\pm$ 0.50aA	22.02 $\pm$ 0.39aD
7	21.88 $\pm$ 0.27dA	33.44 $\pm$ 0.72aBC	26.96 $\pm$ 0.49bB	21.64 $\pm$ 0.16dA	24.27 $\pm$ 0.52cBC
14	21.86 $\pm$ 0.46dA	36.65 $\pm$ 0.90aA	31.08 $\pm$ 0.54bA	21.68 $\pm$ 0.52dA	28.40 $\pm$ 0.58cA
21	21.86 $\pm$ 0.31dA	34.11 $\pm$ 0.54aB	27.25 $\pm$ 0.62bB	21.67 $\pm$ 0.38dA	25.61 $\pm$ 0.79cB
28	21.87 $\pm$ 0.32cA	31.75 $\pm$ 0.74aC	25.37 $\pm$ 0.42bC	21.60 $\pm$ 0.45cA	23.85 $\pm$ 0.75bC
35	21.86 $\pm$ 0.31bA	24.38 $\pm$ 0.36aD	22.61 $\pm$ 0.27bD	21.77 $\pm$ 0.29bA	21.98 $\pm$ 0.29bD

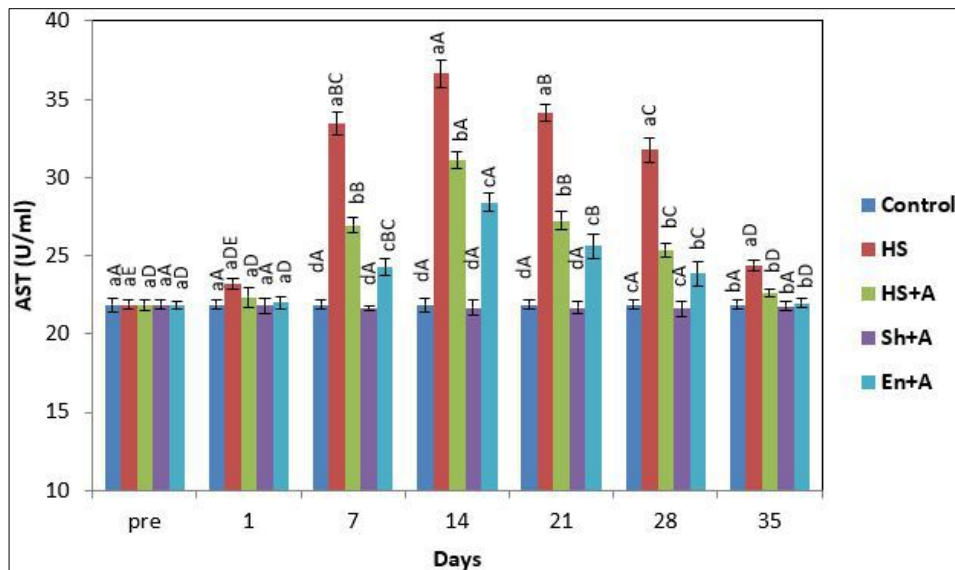


Fig 2: AST (U/ml) of different groups over the study period. All values are shown as Mean± SEM.

abc...values with different superscripts represents significant difference (<0.05) between the groups

ABC...values with different superscripts represents significant difference (<0.05) within the treatment.

Conclusion

The HS group had the highest ALT and AST values. The result showed that there is significant difference between NHSC group and heat exposed group HS, HS+A and En+A group. There was no significant ($P>0.05$) difference between NHSC and Sh+A group. On the fourteenth day of the research period, highest value of AST and ALT value was observed in all heat exposed and direct sunlight exposed group. The ALT and AST levels were significantly lower in the Sh+A group (as compared to HS+A and En+A) among the supplemented groups. The finding of our study showed a negative effect of heat stress on liver profile of animal by increasing the ALT and AST level in the blood serum. *Astaxanthin supplementation* normalize the ALT and AST level in the serum which is an indicative of ameliorative effect of Astaxanthin in heat stressed growing Vrindavani Cattle.

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