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SERUM BIOCHEMICAL CHANGES DURING DIFFERENT TEMPERATURE HUMIDITY INDICES IN PANDHARPURI BUFFALO

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Abstract: *The present study was conducted on ten healthy Pandharpuri buffaloes more than 2 years of age to study the levels of stress enzyme during different seasons. All animals were maintained under standard feeding and management practices. Five ml blood was collected from each buffalo aseptically from jugular vein during morning hours. Serum samples were separated out by cenfrigugation. All these serum samples were used for estimation of stress enzymes activity viz, aspartate aminotransferase (AST), Alanine aminotransaminase (ALT), Alkaline phosphatase (ALP), Gama - Glutamyl transferase (GGP), Total bilirubin, Total Protein, Albumin and Globulin. On the basis of mean temperature and relative humidity, Temperature Humidity Index (THI) was calculated for the peak summer season (Month of May), peak rainy season (Month of August) and peak winter season (Month of December). THI calculated for summer, rainy and winter seasons were 76.97 ± 0.38, 73.32 ± 0.38 and 68.81 ± 0.49 respectively. The serum concentration of AST ±S.E.(U/L) in Pandharpuri buffaloes during summer, rainy and winter seasons were 82.09 ± 0.68, 73.89 ± 0.87 and 64.06 ± 0.70 respectively. The serum concentration of ALT ± S.E. (U/ L) during summer, rainy and winter seasons were 71.54 ± 0.83, 42.23 ± 0.85 and 34.68 ± 0.79 respectively where as serum concentration of ALP ± S.E. (IU/ L) during summer, rainy and winter seasons were 137.52 ± 1.78, 122.18 ± 2.715 and 109.73 ± 2.0 respectively.*

Significantly ((P<0.05) increased levels of GGT and total bilirubin during summer season indicated that hot dry or summer season is more stressful for these buffaloes as compared to rainy and winter season and values of protein profiling in these animals differs non – significantly in different seasons.

Key Words: *Serum, AST, ALT, ALP, GGT, Total bilirubin, protein profile, Seasons, Pandharpuri buffalo.*

Introduction:

Heat stress is one of the major influencing factors to reduce animal reproduction and productivity. Excessive heat load caused by a combination of air temperature, relative humidity, low wind velocity and solar radiation increases body physiological characters and can reduce feed intake and milk production. The variation in climatic variables like temperature, humidity and radiations were recognized as the potential hazards in the growth and production of all domestic livestock species (West, 2003). Under thermoneutral environmental conditions, most of the large domestic animals are able to maintain equilibrium between the heat production and heat loss. But, in stressful conditions, the physiological and behavioural responses will vary in relation to genetic make-up and environmental factors. Environmental conditions and in particular seasonal variations in air temperature and relative humidity are considered physiological stressors which affect the animal's biological system (Mazzullo et al, 2014).

Excessive heat load affects the animal's thermoregulatory mechanism, creates a stressful condition and negatively affects the breeding performance in dairy cattle, by reducing fertility and negatively affects the breeding performance in dairy cattle and in extreme cases results in death. Therefore, the evaluation of the biochemical profile of Pandharpuri buffaloes is an essential prerequisite for the selection of animals in dry and hot environmental conditions for the selection of animals and estimation of production and reproduction efficiency of animals. Hence, the present study was undertaken to study the biochemical constituents of Pandharpuri buffaloes in hot and dry conditions.

Materials and Methods:

The present study was conducted at Department of Veterinary Biochemistry, Bombay Veterinary College, Parel, Mumbai-400012. The experiment was conducted on 10 apparently healthy Pandharpuri buffaloes above two years of age maintained under loose housing ondition at Livestock Instructional and Research Demonstration Farm, K.N.P. College of Veterinary Science, Shirwal. Dist. Satara. On the basis of mean temperature and relative humidity, THI was calculated using the formula of Mader et al. (2006). Five ml of blood was collected from each of the buffaloes from jugular vein at morning hours during peak summer season (Month of May), peak rainy season (Month of August) and peak winter season (Month of December) and kept into the centrifuge machine for centrifugation at 3000 rpm for 20 min. to separate out the clear transparent serum samples.

All serum samples were used for estimation serum biochemical activities. The serum biochemical activities were determined at 37 ^{0}C with the help of AGD semi- automated biochemical analyzer using commercially available kits.

1. Serum aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Serum alkaline phosphatase (ALP), Serum Gamma - Glutamyl transferase (GGT) and serum total bilirubin activities was determined by Robonik Prietest Kit developed as per Tietz (1986) and Young, (1995).
2. Serum total protein was estimated by Robonik Prietest Kit developed as per the procedure described by Tietz (1986) and Christensen (1983).
3. Serum albumin was estimated by Robonik prietest Kit developed as per the procedure described by Thomas (1998) and Young, (1995).
4. The serum globulin concentration was determined indirectly as a difference of the total protein and the albumin concentration. The data for all the parameters were analyzed using Completely Randomized Design as per standard statistical procedure cited by Snedecor and Cochran (1994).

Result and Discussion

1) Temperature humidity index (THI):

Mean values of temperature humidity index (THI) during different seasons of the study period are presented in the **Table 1** along with its analysis of variance in the **Table 1.2** The THI recorded significantly ($P<0.05$) higher in summer as compared to the winter season and THI of summer and rainy season differ significantly. Dikmen and Hansen, (2008) reported that the THI value of 75 to 80 indicated moderate to high intensity of thermal stress and THI of 72 and below was considered as no heat stress, 73 to 77 as mild heat stress, 78 to 89 as moderate and above 90 as severe heat stress. Moreover, Heat stress has adverse effects on reproductive performances of cattle and buffaloes. The Higher ambient temperature during the summer has been associated with reduced fertility in dairy cattle through its deleterious impact on oocyte maturation and early embryo development (Das, et al, 2013; Chandrabhan, et al., 2012)

2) Serum aspartate aminotransferase (AST) activity (U/L);

Mean values of serum AST activities during different seasons of the study period are presented in the **Table 2** along with its analysis of variance in the **Table 2.1** The serum concentration of AST recorded significantly ($P<0.05$) higher in summer as compared to the rainy and winter seasons and serum concentration of AST of summer, rainy and winter seasons differ significantly. The activity of AST is high in the liver of all domestic animals and serum is used routinely in all species for the evaluation of the liver cell injury (Kaneko et al., 1997). Significantly higher ($P<0.05$) serum AST activity during summer season in Pandharpuri buffalo observed in the present study (Table 2, Fig. 2) could be due to free radicals induced damage to the hepatic tissue and possibly to the other tissues also leading to leakage of this intracellular enzyme into the blood stream resulting in its elevated level in the serum. Our findings corroborates with the findings reported by Kalmath (2015) in Hallikar cattle, Rasooli et al.

(2004) in Holstein heifers and Mazzullo et al, (2014) in cows.

3) Serum alanine aminotransferase (ALT) activity (IU/L)

Mean values of serum AST activities during different seasons of the study period are presented in the **Table 2 and fig. 3** along with its analysis of variance in the **Table 2.1** The serum concentration of ALT recorded significantly (P<0.05) higher in summer as compared to the rainy and winter seasons and serum concentration of ALT of summer, rainy and winter seasons differ significantly. Increase in ALT in summer season is in agreement with the findings of Koubkova et al. (2002), Chandrabhan et al. (2012) and Kalmath (2015). Serum ALT could be used as one of the most universal markers for hepatic injury across species and its elevated activities in serum indicate hepatic injury (Amacher, 1998, Marai et al., 1997). In buffaloes, higher concentration of this enzyme is found in liver and muscles (Kaneko et al., 1997).

4) Alkaline Phosphatase (ALP):

Mean values of serum ALP activities during different seasons of the study period are presented in the **Table 2** along with its analysis of variance in the **Table 2.1** The serum concentration of ALT recorded significantly (P<0.05) higher in summer as compared to the rainy and winter seasons and serum concentration of ALP of summer, rainy and winter seasons differ significantly. ALP activities in IU/L are found in many tissues including bone, liver, intestine, kidney, placenta and germ cells (Sarkar, 2012). Significantly higher (P<0.05) serum ALP activities in Pandharpuri buffalo during summer season observed in the present study (Table 2 and Fig. 4) could be due to oxidative damage to the liver cell during the summer season and therefore ALP level in the serum could be used as an indicator of heat stress in buffalo.

5) γ-glutamyltransferase (GGT) :

Mean values of serum GGT activities during different seasons of the study period are presented in the **Table 2** along with its analysis of variance in the **Table 2.1** The serum concentration of GGT recorded significantly (P<0.05) higher in summer as compared to the rainy and winter seasons and serum concentration of GGT of summer, rainy and winter seasons differ significantly. Significantly higher (P<0.05) serum GGT activities in Pandharpuri buffalo during summer season observed in the present study (Table 2 and Fig. 5) could be due to oxidative damage to the liver cell during the summer season and therefore GGT level in the serum could be used as an indicator of heat stress in buffalo. Our findings are in accordance with the study of Mazzullo et al, (2014) in cows. Furthermore, increased serum values of GGT (U/L) usually reflect liver damage in dairy cattle (Ronchi et al., 1999). Our results showed higher level of GGT during summer season is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream, which reflects liver damage and disruption of normal liver function.

6) Total bilirubin (mg/dL):

Mean values of serum total protein activities during different seasons of the study period are presented in the **Table 2** along with its analysis of variance in the **Table 2.1** The serum concentration of total bilirubin recorded significantly (P<0.05) higher in summer as compared to the rainy and winter seasons and serum concentration of total bilirubin of summer, rainy and winter seasons differ significantly.

Significantly higher (P<0.05) serum total bilirubin activities in Pandharpuri buffalo during summer season observed in the present study (Table 2 and Fig. 7) could be due to oxidative damage to the liver cell during the summer season and therefore total protein level in the serum could be used as an indicator of heat stress in buffalo. Our findings corroborates with the study of Mazzullo et al, (2014) in cows.

7) Serum total protein (g/dL)

Mean values of serum total protein activities during different seasons of the study period are presented in the **Table 2** along with its analysis of variance in the **Table 2.1** The serum concentration of total protein recorded significantly (P<0.05)

higher in summer as compared to the rainy and winter seasons and serum concentration of total protein of summer, rainy and winter seasons differ significantly.

Significantly lower serum total protein concentration observed during the summer season compared to winter season (Table 3 and Fig. 7) in control group could be due to impaired protein synthesizing capacity of the liver induced by oxidative damage to the hepatic cells. Reduction in the protein levels could also be due to higher levels of plasma cortisol observed in the present study as the catabolism of the proteins (Guyton and Hall, 2006) and reduced total protein levels during summer could also be attributed to the corresponding decline in the secretion of insulin during summer observed in the present study. Our study corroborates with the study of Nazafi et al. (2003) in sheep, Gudev et al. (2007) in buffaloes and Omran et al. (2011) in Egyptian buffalo calves.

8) Serum albumin (g/dL)

Mean values of serum albumin activities during different seasons of the study period are presented in the **Table 2** along with its analysis of variance in the **Table 2.1** The serum concentration of albumin recorded significantly ($P<0.05$) higher in summer as compared to the rainy and winter seasons and serum concentration of albumin of summer, rainy and winter seasons differ significantly. Non-significant variation ($P>0.05$) in the serum albumin levels between the seasons observed in the control group (Table 2 and Fig. 8) could be to enable the animal to maintain normal plasma osmotic pressure to maintain blood volume and body fluid distribution during heat stress. Das et al. (2013) also reported non significant difference in the albumin concentration between seasons in Nili-Ravi buffaloes.

9) Serum globulin (g/dL)

Mean values of serum globulin activities during different seasons of the study period are presented in the **Table 2** along with its analysis of variance in the **Table 2.1** The serum concentration of globulin recorded significantly ($P<0.05$) higher in summer as compared to the rainy and winter seasons and serum concentration of globulin of summer, rainy and winter seasons in Pandharpuri buffalo differ significantly. The non-significant variation ($P>0.05$) in the serum globulin levels during different seasons in the control group (Table 2 and Fig. 9) observed in the present study is in agreement with the finding of Das et al. (2013) who also reported non significant variation in plasma globulin concentration during different seasons in Nili Ravi buffaloes. However, Abdelatif et al. (2009) in female Nubian goats and Al-Haidary et al. (2012) in Najdi rams have reported higher levels of globulins during hot summer compared to winter.

Conclusion:

From the present study, our results showed variations in biochemical parameters related to temperature-humidity index in Pandharpuri buffaloes. It can be concluded that THI is a sensitive indicator of heat stress and is impacted by ambient temperature more than the relative humidity in Pandharpuri buffaloes. Higher THI is associated with significantly increased AST, ALT, ALP, GGT and Total bilirubin. Higher levels of serum enzymes such as AST, ALT and ALP indicated the oxidative stress induced hepato cellular injury where as lowered serum protein levels during summer season indicated impaired protein synthesis due to heat stress in the Pandharpuri buffalo.

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Table 1: Mean ± S.E. Temperature Humidity Index (THI) during different seasons

Seasons(Month /)	Tdb(^{0}C)	Twb (^{0}C)	Relative humidity (%)	(THI)
Winter (December)	20.88± 0.30	14.48± 0.42	82.19 ± 0.78	68.81 ^{c} ± 0.49
Summer (May)	27.87± 0.26	21.94 ±0.28	59.74 ± 0.77	76.97 ^{a} ± 0.38
Rainy (August)	23.46± 0.23	22.35± 0.08	87.55 ± 1.10	73.32 ^{b} ± 0.38

Mean THI value with different superscripts differ significantly.

Table 1.1: Analysis of Variance Table for Temperature Humidity Index (THI) during different seasons

ANOVA TABLE					
Source of Variation	**Degree of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**C.D. Values**
Treatments	2	1035.965	517.983	85.298	(0.01)= 1.65*
Error	90	546.536	6.073		(0.05) =1.24**
Total	92	-	-	-	

Table 2: Mean ± S.E. serum biochemical parameters during different seasons

Parameters	Summer season	Rainy season	Winter season
AST (U/L)	82.09 ^{a} ± 0.68	73.89 ^{b} ± 0.87	64.06 ^{c} ± 0.70
ALT (U/L)	71.54 ^{a} ± 0.83	42.23 ^{b} ± 0.85	34.68 ^{c} ± 0.79
ALP (IU/L)	137.52 ^{a} ± 1.78	122.18 ^{b} ± 2.715	109.73 ^{c} ± 2.20
GGT (U/L)	41.55 ^{a} ± 0.58	30.28 ^{b} ± 0.81	17. 55 ^{c} ± 1.80
Total Bilirubin(mg/dl)	0.43 ^{a} ± 0.01	0.34 ^{b} ± 0.01	0.27 ^{c} ± 0.01
Total Protein (gm/dl)	6.45 ^{c} ± 0.09	6.87 ^{b} ± 0.05	7.15 ^{a} ± 0.08
Albumin (gm/dl)	3.36 ^{b} ± 0.04	3.96 ^{a} ± 0.05	3.82 ^{a} ± 0.12
Globulin (gm/dl)	3.08 ^{a} ± 0.09	2.91 ^{b} ± 0.06	3.33 ^{b} ± 0.07

Table 2.1: Analysis of variance of AST, ALT, ALP, GGT, Total Bilirubin, Total Protein, Albumin and Globulin during different seasons

Source of Variation	Degree of freedom	Sum of squares	Mean sum of squares	F cal	C. D.
AST					
Treatments	2	1629.833	814.916	143.632	
Error	27	153.189	5.674	-	(0.01) = 2.952
Total	29	-	-	-	(0.05) = 2.186
ALT					
Treatments	2	7584.830	3792.415	559.163	(0.01) = 3.227
Error	27	183.122	6.782	-	(0.05) = 2.390
Total	29	-	-	-	

ALP					
Treatments	2	3875.8	1937.9	37.963	(0.01) = 8.854 (0.05) = 6.557
Error	27	1378.261	51.047	-	
Total	29	-	-	-	
GGT					
Treatments	2	2883.984	1441.992	102.117	(0.01) = 4.66 (0.05) = 3.45
Error	27	381.266	14.121	-	
Total	29	-	-	-	
Total Bilirubin					
Treatments	2	0.125	0.063	73.07	(0.01) = 0.04 (0.05) = 0.03
Error	27	0.023	0.001	-	
Total	29	-	-	-	
Total Protein					
Treatments	2	2.734	1.367	23.99	(0.01) = 0.30 (0.05) = 0.22
Error	27	1.539	0.057	-	
Total	29	-	-	-	
Albumin					
Treatments	2	1.915	0.958	16.106	(0.01) = 0.30 (0.05) = 0.22
Error	27	1.605	0.059	-	
Total	29	-	-	-	
Globulin					
Treatments	2	0.861	0.43	7.113	(0.01) = 0.31 (0.05) = 0.23
Error	27	1.634	0.061	-	
Total	29	-	-	-	

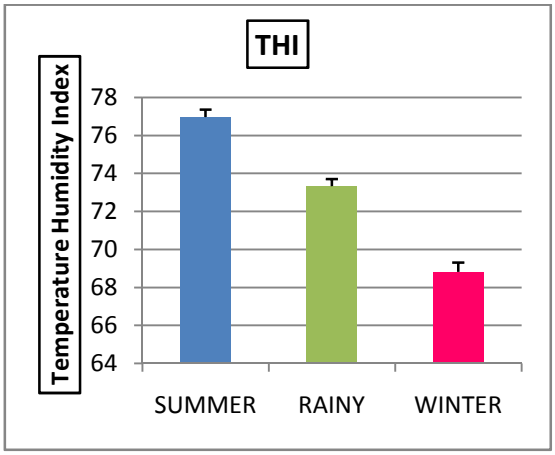

Figure 1: Temperatute Humidity Index during Pandharpuri buffalo

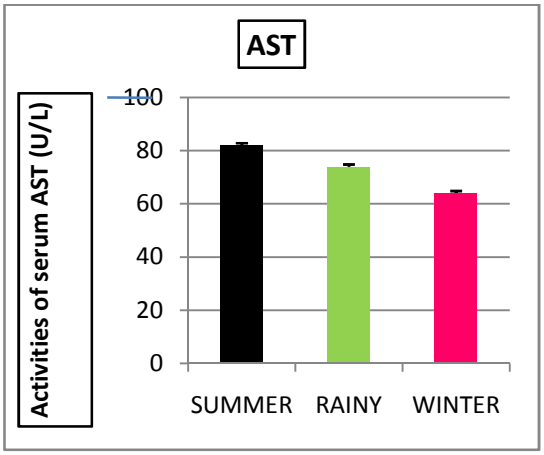

Figure 2: Level of enzyme AST during different seasons in Pandharpuri buffalo

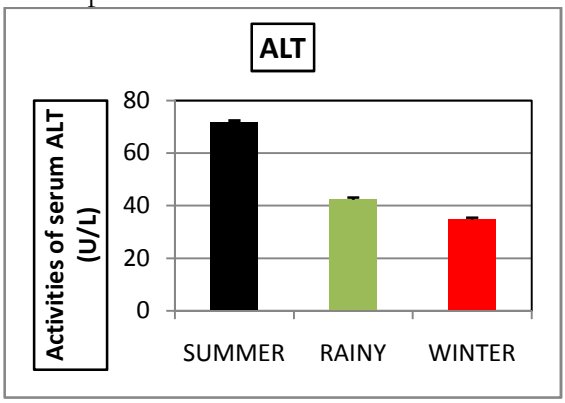

Figure 3: Level of enzyme ALT during different seasons in Pandharpuri buffalo

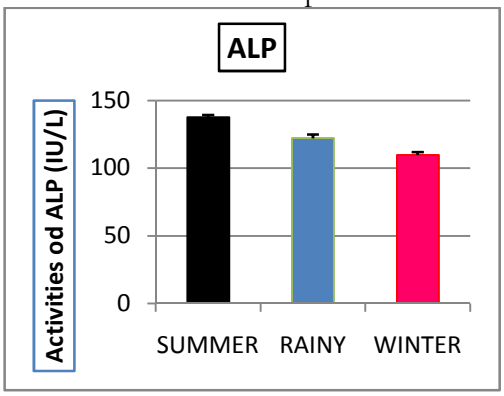

Figure 4: Level of enzyme ALP during different seasons in Pandharpuri buffalo

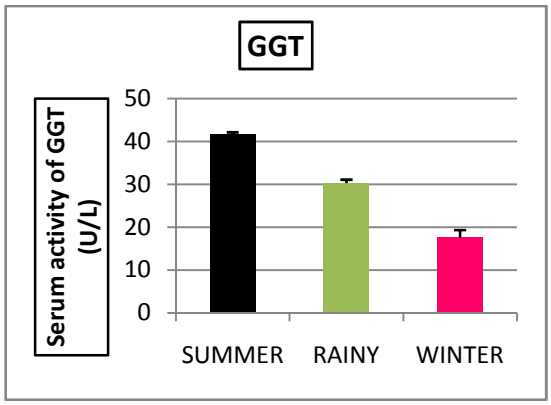

Figure 5: Level of enzyme GGT during different seasons in Pandharpuri buffalo

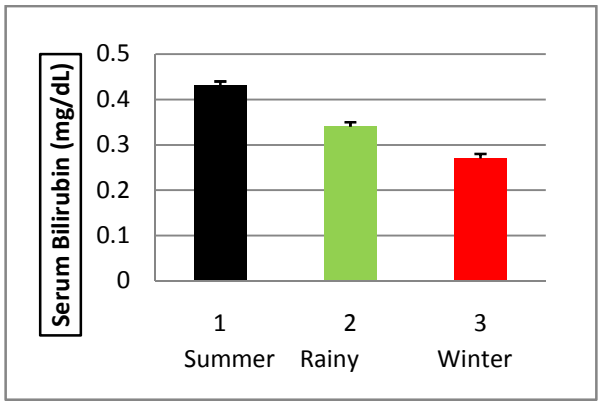

Figure 6: Level of Total Bilirubin during different seasons in Pandharpuri buffalo

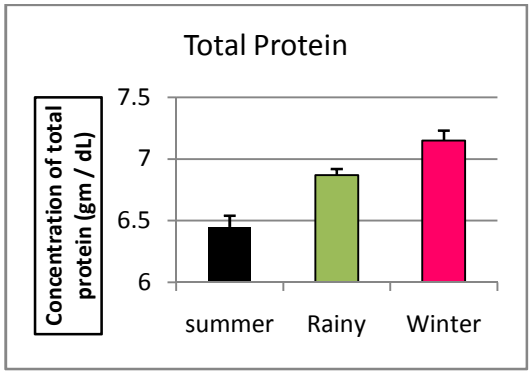

Figure 7: Level of Total Protein during different seasons in Pandharpuri buffalo

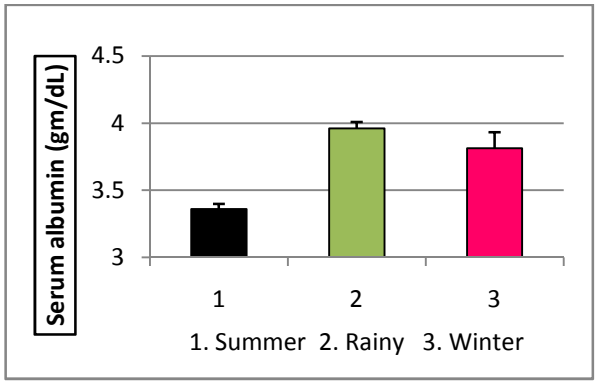

Figure 8: Level of Albumin during different seasons in Pandharpuri buffalo

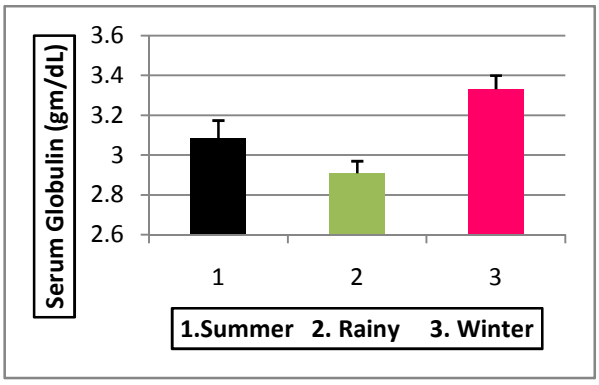

Figure 9: Level of Globulin during different seasons in Pandharpuri buffalo