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Influence of Season on Biochemical Attributes of Bhadawari Buffalo Bull Semen: Effect of Temperature and Humidity

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ABSTRACT

The present study was undertaken to establish the seasonal variations in semen biochemical indices in Bhadawari bull. Four fertile, healthy adult Bhadawari bulls aged between 2 to 4 years were used as semen donors. The study was conducted over a period of six months and divided into three seasons as winter season (February- March), dry summer season (April-May) and wet summer season (June-July). Sixteen semen samples were collected in each season (four ejaculates from each bull) by using Artificial Vagina and seminal plasma were harvested by centrifugation. The results of the study showed gradual and significant rise in values of ALT, AST, ALP, cholesterol and triglycerides and decline in values of amylase with increase in ambient temperature and THI. Amylase showed negative correlation and other indices showed positive correlation with ambient temperature and THI. The ambient temperature and THI showed positive correlation with biochemical attributes except amylase while humidity showed negative correlation. It could be concluded from the study that temperature and THI of season significantly affects the semen biochemical attributes of Bhadawari bulls.

Keywords: Biochemical, Season, Temperature, Humidity, Semen, Bull, Buffalo

The reproductive performance of bulls directly depends on the genetic potential, management and environment. The interrelationship of these factors determines the adaptation of the animals and their reproductive efficiency (Robertshaw, 1982). In temperate regions, reproductive characteristics of animals are influenced by a combination of photoperiod and temperature, while in tropical regions the environmental effect seems to be related to rain and its indirect effect on the amount and quality of forage (Rege *et al*., 2000). However, changes in other climate factors such as humidity and temperature can cause thermal discomfort, resulting in a decrease in food intake and interference with spermatogenesis and semen quality (Kunavongkrit *et al*., 2005). In tropical and sub-tropical countries, climatic heat is the major factor restricting animal productivity. Growth, milk production and reproduction are impaired as a result of drastic changes in biological functions caused by heat stress (Marai *et al.,* 1995).

Besides elevation of ambient temperature affects puberty deleteriously that leads to testicular degeneration and reduces percentages of normal and fertile spermatozoa in the ejaculate of males. The ability of male to mate and fertilize are also affected by ambient temperature. Biological backgrounds of such phenomenon include disturbances in each of sexual activity, endocrine and testis functions; spermatogenesis and physical and chemical characteristics of the semen (Abdel-Samee *et al.,* 1997).

Since physical characteristics of semen alone are not completely satisfactory for semen appraisal in the current practice, hence determinations of biochemical constituents of seminal plasma are also needed for semen evaluation (Mann and Lutwak- Mann, 1981). Amongst domesticated animals, sufficient literature is available on seminal enzymes and biochemistry of bulls of different breeds (Dhami and Sahni, 1994), but the information on Bhadawari buffalo bull and impact of stressful season on semen biochemistry is meagre. Bhadawari breed of buffalo is water buffalo (*Bubalus bubalis*) reared in Mathura and adjoining areas and well known for highest fat percentage in milk. There is relatively little information available in the literature on various seminal attributes of this breed. In our recently published studies we have published preliminary data on biochemical attributes (Pandey *et al.,* 2012) and influence of season on semen characteristics of this breed (Sharma *et al.,* 2014). The present study was aimed at studying biochemical constituents of seminal plasma and their relationship with ambient attributes (temperature, humidity and THI) during winter, dry and wet summer seasons.

MATERIALS AND METHODS

Animals and location

Four fertile, healthy adult Bhadawari buffalo bulls aged between 2 to 4 years and with live weight of 300-400 kg stationed at the ILFC, College of Veterinary Science and Animal Husbandry, Mathura were used as semen donors. All these bulls were in good health and were maintained in nearly identical nutritional and managerial condition throughout the study period.

Collection of semen and preparation of seminal plasma

The whole study was conducted over a period of six months and divided into three seasons as winter season when temperature is low with high humidity due to fog (February- March), dry summer season when temperature is slightly high with low THI (April-May) and wet summer season when temperature is high with high THI (June-July). Four semen samples were collected from each bull in each season by using Artificial Vagina in the morning hours and average ambient temperature and humidity of the day was recorded. The daily THI was determined based on average daily temperature and relative air humidity on a given day. (Vitali *et al.,* 2009). Semen samples were collected fortnightly (1st and 15th day of every month) from each bull and immediately after collection, seminal plasma was harvested by centrifugation at 5000 rpm for 10 minutes at 4^{0}C. The supernatants were transferred into 1.5 mL eppenddorf tubes and re-centrifuged to eliminate the remaining sperms and separated seminal plasma was stored at -20°C until used. Out of these collected samples, sixteen semen samples in each season (four ejaculates from four bulls per season) were selected for further biochemical analysis.

Seminal plasma compositions

The seminal plasma samples were analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), amylase, total cholesterol and triglycerides using commercially available kits (Span Diagnostic Limited).

Statistical analysis

The statistical significance was determined by ANOVA and Tukey's post-hoc multiple comparison tests followed by Pearson correlation among indices using SPSS software for Windows (version 16.0). The data are presented as the mean ± SE. A p value < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

The results of the semen biochemical parameters of buffalo bulls in winter, dry summer and wet summer seasons are summarized as mean ± SE in Table 1. The results of present study showed significant effect of season on all the biochemical parameters, corroborated with the findings of Khawaskar *et al.* (2012) and Pandey *et al.* (2014). All biochemical parameters showed gradual increase in values with increasing ambient temperature however, amylase activity showed reducing trend with increase in ambient temperature. The highest values were observed in wet summer and lowest values in winter season (except for amylase).

Table 1. The ambient attributes and semen biochemical parameters of Bhadawari buffalo bulls in winter, dry summer and wet summer seasons

Biochemical Attributes	Winter (Feb-Mar)	Dry Summer (Apr-May)	Wet summer (Jun-Jul)
Average Temperature (^{0}C)	15.37±1.25^a	29.12±0.50^b	34.25±0.85^c
Relative Humidity (%)	79.50±1.80^a	60.75±0.76^b	49.25±3.43 ^{c}
Temperature-Humidity Index (THI)	59.15±1.97^a	78.77±0.81^b	83.26±0.58^c
Alanine Transaminase (IU/L)	15.83±0.63^a	25.89±1.39^b	41.48±1.58^c
Aspartate Transaminase (IU/L)	44.02±1.75^a	50.96±2.85^a	61.39±2.33^b
Alkaline Phosphatase (IU/L)	2033.13±106.51^a	2236.23±82.82^{ab}	2486.53±84.27^b
Amylase (IU/L)	13.26±0.50^c	8.90±0.27^b	3.16±0.63^a
Cholesterol (mg/dl)	24.70±3.74^a	101.01±5.61^b	192.98±11.22^c
Triglycerides (mg/dl)	164.69±5.73^a	217.81±11.00^b	414.20±20.16^c

The values of AST and ALT observed in present study is comparable with the values reported by Khawaskar *et al.* (2012) in Surti buffaloes but lower than the value (AST) reported by Shukla *et al.* (2009). The activity of ALT, AST and ALP were found significantly elevated ($p<0.05$) during wet summer season compared to other seasons. Similar kind of seasonal effects were noticed by Dhami and Kodagali (1988), Juma (2000) and Pandey *et al.* (2014) in cattle and Khawaskar *et al.* (2012) in buffaloes. However higher values of ALT and AST in winter season were noticed by Taha *et al.* (2000) and Juma and Kassab (2009) in rams.

The concentration of transaminase enzymes (AST and ALT) in semen is a good indicator of semen quality because it measures sperm membrane stability (Corteel, 1980) and acrosomal damage (Sharma *et al.,* 2001). Daader *et al.* (1993) reported negative correlation of percentage of live spermatozoa in semen with AST level and AST/ALT ratio. Thus, high concentration of transaminase enzyme in the extra cellular fluid (seminal plasma) indicate increasing percentage of abnormal

spermatozoa in ejaculate occurring due to sperm membrane damage leading to leakage of enzymes from spermatozoa (Gundogan, 2006).

The present study showed increasing trend in levels of alkaline phosphatase from winter to wet summer season. The higher levels of ALP in summer season were also reported by Dhami and Kodagali (1988) and Pandey *et al.* (2014) in cattle, Chanad *et al.* (1985) and Juma and Kassab (2009) in rams and Khawaskar *et al.* (2012) in buffalo. The majority of ALP enzyme in semen of bulls originates from the seminal vesicles and, to a lesser extent, from the testes and epididymis (Dogan *et al.,* 2009). Seminal phosphatases play an important dephosphorylating role in sperm metabolism (Khawaskar *et al.,* 2012), and also reported to be index of sperm cell integrity and acrosomal damage (Chauban *et al.,* 1993; Okab, 2007). Thus estimation of ALP activities in seminal plasma reflects the functional state of accessory sex glands, metabolic activity of spermatozoa, and sperm membrane integrity that are helpful in differentiating the reproductive biology of bulls of different breeds/ species (Ibrahim *et al.,* 1985). The increase in ALP activity in wet summer may be due to increase secretion of adrenocorticotrophic hormone (ACTH) due to environmental stress (Litwack, 1972).

The values of total cholesterol obtained in present study were comparable with those reported by Nema (1982) while values were higher than those reported by Shukla *et al.* (2009) and Khawaskar *et al.* (2013) in buffaloes. The present findings showed lowest value of total cholesterol and triglycerides in winter and highest value in wet summer months. The highest cholesterol concentration reported in summer season in present study corroborated with the findings of Okab (2007), Khawaskar *et al.* (2012), Farooq *et al.* (2013) and Pandey *et al.* (2014). However Juma and Kassab (2009) reported highest cholesterol concentrations in seminal plasma in winter months and Zamiri *et al.,*(2010) reported lowest in summer months. Seminal lipids including cholesterol are reported to be associated with sperm membrane structure, sperm metabolism, sperm capacitation and fertilization of the female gamete (Hafez, 1987; Demirci *et al.,* 2002). Higher levels of seminal cholesterol during summer may be due to increased thyroid activity and hepatic mechanisms that remove cholesterol from circulation (Shukla *et al.,* 2009). In addition to this, cholesterol reported to function as potential protective agent against environmental stress (Jacyno *et al.,* 2009). Hence, its increase in summer season in the present study may also be an adaptive mechanism to minimize thermal stress.

Amylase showed significant lower values in wet summer compared to other season. The results of amylase simulated with the findings of Pandey *et al.* (2014).

The results of correlation among semen biochemical attributes are depicted in Table 2. The AST activity showed significant positive correlation with ALT, ALP, cholesterol and triglycerides while negative correlation with amylase. The result of

AST corroborates the findings of Khawaskar *et al.* (2012) and Pandey *et al.* (2014). The ALT activity showed positive association with AST, ALP, cholesterol and triglycerides and negative correlation with amylase activity. Positive correlation was observed among ALP, cholesterol and triglycerides. Amylase activity showed positive correlation with triglycerides and negative correlation with all other parameters. Cholesterol and triglycerides showed positive association with AST, ALP and each other and negative correlation with amylase. The results of correlation simulated with the findings of Pandey *et al. (*2014) and somewhat comparable with the reports of Dogan *et al.* (2009).

The Pearson correlation among ambient attributes and semen biochemical parameter revealed positive correlation of temperature and THI with AST, ALT, ALP, cholesterol and triglycerides except amylase while humidity showed negative correlation with AST, ALT, ALP, cholesterol and triglycerides and positive correlation with amylase activity. The result of correlation of temperature and THI of present study showed resonance with the reports of Pandey et. al. (2014).

Table 2: Correlation among semen biochemical and ambient attributes of Bhadawari bulls.

Biochemical Attributes	AST	ALT	ALP	Amylase	Cholesterol	Triglycerides
Temperature	0.52**	0.75**	0.39**	-0.76**	0.79**	0.67**
Humidity	-0.47**	-0.76**	-0.27	0.83**	-0.62**	-0.52**
THI	0.54**	0.70**	0.41**	-0.68**	0.79**	0.66**
AST	1	0.46**	0.54**	-0.48**	0.66**	0.66**
ALT		1	0.43**	-0.83**	0.77**	0.73**
ALP			1	-0.35*	0.48**	0.51**
Amylase				1	-0.73**	-0.68**
Cholesterol					1	0.93**
Triglycerides						1

CONCLUSION

The biochemical analyses of Bhadawari bull seminal plasma indicated that the highest levels of AST, ALT, ALP, cholesterol and triglycerides were recorded in wet summer months while amylase activity exhibited lowest values. On the other hand the lowest values of these parameters were observed in winter months while amylase showed highest activity in winter months. The ambient temperature and THI showed positive correlation with AST, ALT, ALP, cholesterol and triglycerides and negative correlation with amylase whereas cholesterol and triglycerides showed positive correlation with AST, ALT, ALP and negative correlation with amylase.

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