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Effect of Heat Stress on Buck's Adaptability and Semen Characteristics

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Abstract

The experiment was conducted to investigate the effect of heat stress on adaptability and semen quality of buck. Almost similar 10 Black Bengal bucks were selected for the study at Artificial Insemination Centre, Bangladesh Agricultural University, Bangladesh. Prerequisite biological data were collected from each animal before keeping them sun heat and after 1 hour sun exposure. Different semen characteristics of each animal were measured. Temperature humidity index (THI) was 19.95 in the morning which indicate all the animals were in absence of heat stress and THI was 25.54 in the noon which indicate all the animals were in severe heat stress at noon. Buck 1 and buck 5 were more heat resistant ($p<0.01$), because their thermolysis capacity (TC) values were less than the others. There were no differences in tunica dartos index and semen characteristics among different bucks ($p>0.05$). Positive correlation between sperm motility and testis length ($p<0.05$), and negative correlation between sperm concentration and scrotal skin temperature ($p<0.05$) were found. From the study it can be concluded that all the bucks were in severe heat stress at noon time and TC can be used as an indicator for selection of buck for heat tolerance.

Key words: Black bengal buck, Sun exposure, Temperature humidity index, Thermolysis capacity, Tunica dartos index

Introduction

There are many factors which affect the heat tolerance and adaptability of small ruminants. Rectal temperature is the major physiological parameter to measure the degree of tolerance to heat (Johnson, 1980). Heat loss occurs by conduction, convection, radiation and evaporation. The test used to evaluate the heat tolerance is a measure of the capacity of heat loss by recovery of the rectal temperature. The thermolysis capacity (TC) can be used to evaluate the heat tolerance in ruminants (Aguilar *et al*., 2010; Titto *et al*., 2011). To find out the TC, animals are exposed to a heat challenge by sun exposure. Animal with low TC value are more heat-tolerant than higher TC valued animals (Titto *et al*., 2011). The TC found during a heat tolerance test is a valuable tool that should be used on a routine basis to guide decisions about animals to be selected for selective breeding or crossbreeding programs.

The tunica dartos (TD) muscle controls the distance between the anterior abdominal wall and the upper tip of the testes. A parameter for the tolerance of the rams to adverse hot climatic conditions, designated as the tunica dartos index (TDI), was estimated as the multiplication of the percentage change in scrotal length and that of the percentage difference between rectal temperature and scrotal skin temperature (Marai *et al*., 2006). Semen parameters are correlated with TDI value and can be a reliable parameter to indicate the tolerance of rams to hot subtropical conditions (Marai *et al*., 2000). Black Bengal goats are numerically and economically very important and promising animal genetic resources in Bangladesh, but there is no data dealing with the effect of heat stress challenge as TC and TDI as adaptability indicators on Black Bengal buck. Thus, the present study aimed to assess the degree of TC as a characteristic of heat tolerance and examine the TDI as a parameter for adaptability of Black Bengal buck to adverse hot climatic conditions.

Materials and Methods

Experimental site, animal and design

The experiment was conducted at the Artificial Insemination Laboratory under the Department of Animal Breeding and Genetics, Faculty of Animal Husbandry, Bangladesh Agricultural University, Mymensingh, Bangladesh from July 15 to October 15, 2012. A total of 10 Black Bengle bucks having similar age known to be healthy and clinically free of external and internal parasiteswere selected for the experiment. The same animal was being used at two periods, during the cooler period (before keeping them sun exposure) and after heat stress condition (after 1 hour sun exposure). Feed and water were offered as adlibitum. The bucks were kept in individual pens at the Artificial Insemination Centre. Cleaning and hygienic management was maintained regularly. The floor, stall, water trough, and feeder were cleaned every day. Two months were given to animal for their psychological and physiological adjustment.The data about ambient temperature and relative humidity of these experimental days were collected from Meteorological Centre, Bangladesh Agricultural University, Mymensingh. The experimental animals were selected, maintained and treated in adherence to accepted standards for the humane treatment of animals.

Temperature humidity index

Ambient air temperature and relative humidity (RH, %) were recorded with a mercury thermometer inside the sheds at the times of semen collection and when carrying out physiological and scrotal measurements. Maximum and minimum temperatures were also recorded. Relative humidity was recorded using a hairhygrometer. The average ambient temperatures and relative humidity values estimated in the present study were, 20.5ºC and 70.5% during morning and

27.2ºC and 60.5% during noontime respectively. The average duration of daylight during experimental period was 12.25 h. To gauge the extent of heat stress induced by the environmental conditions at various times, THI was estimated according to the following equation: THI = dbºC-[(0.31-0.31 RH) (dbºC-14.4)], where dbºC= average dry bulb temperature in degrees Celsius, and RH= average relative humidity percentage/100. THI values were classified as follows: <22.2= absence of heat stress, 22.2ï 23.2= moderate heat stress, 23.3ï 25.5= severe heat stress and 25.6= very severe heat stress.

Tunica dartos index

TDI, a parameter that correlates well with bucksô reproductive ability, were developed to gauge the ability of bucks to tolerate elevations in ambient temperature or, in other words, to indicate the tolerance of bucks to hot subtropical conditions in Bangladesh, was estimated. The TDI was estimated using three methods according to Marai *et al*. (2001): TDIa, TDIb and TDIc. TDIa expressed the percentage change in scrotal length relative to maximum scrotal length minus the testis length, TDIb expressed the difference between the rectal temperature (RT) and the scrotal skin temperature as a percentage of RT and TDIc expressed the combination of the above formula. The formula of these indices is as follows:

$$TDIa = ((Max.\ SCL - Min.\ SCL)/(Max.\ SCL - TL)) \times 100,$$

$$TDIb = ((RT - SST)/RT) \times 100$$

TDIc is a combination of the other two formulae, ((TDIb × TDIa)/100), that is,

$$TDI = (((RT - SST)/RT) \times ((Max.\ SCL - Min.\ SCL)/(Max.\ SCL - TL))) \times 100.$$

where,

RT= average rectal temperature
SST= average scrotal skin temperature
Max. SCL= average maximum scrotal length
Min. SCL= average minimum scrotal length and
TL= average testis length. Other factors affecting scrotal characteristics and testis size were considered to be constant. Pearson correlation coefficients were calculated for associations between the three TDIc values (<4.5, 4.5ï 7.4 and Ó7.5) and both libido and semen characteristics.

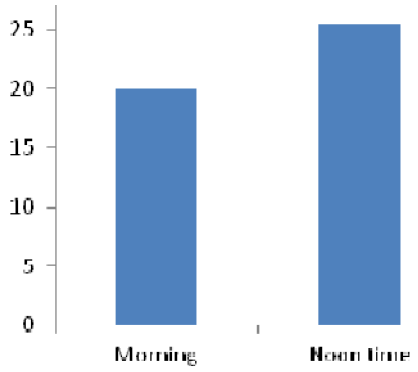

Fig. 1. Heat stress measuring by THI

Evaluation of thermolysis capacity

The TC as a characteristic of heat tolerance was measured in accordance with modified methods of Titto *et al*. (1998). The rectal temperatures were collected by restraining and holding the animals. A digital thermometer was used to collect rectal temperature. No feed bunks or water troughs were available to the animals during the test. The test consists of a heat stress challenge, in which the animals were stayed inside the house from 11.00 to 12.00 hours. At 12.00 hourthe first rectal temperature were taken (RT°1). From 13.00 to 14.00 hours, all animals were staying 1 h in a sunny place. After this hour in the sun, the animals were bringing back to the house, where they were staying for one 1 h more (14.00ï 15.00 hours). At 15.00 hour the second rectal temperature were taken (RT°2). The individual animalôs rectal temperatures were applied to a formula where the inverse of the difference among the two rectal temperatures represents an index of TC =10-(RT° 2- RT° 1) where RT°1 is rectal temperature at 12.00 hour and RT°2 is rectal temperature at 15.00 hour. The individual TC was calculated based on three TC measures of each animal. All animals were tested at the same time on five typical summer days with clear skies and hardly any wind observe.

Physiological measurements

Physiological measurements of buckôs rectal and scrotal skin temperatures, scrotal length (the vertical distance between the anterior abdominal wall and the bottom of the scrotum) and testes length were estimated at 12.00 hours on the day of semen collection, once every week, during the experimental periods. The scrotal skin temperature was measured using a thermometer between the skin folds. The scrotal length and testes length were measured with a flexible metal tape (Marai *et al*., 2000).

Semen collection and evaluation

Collection of semen was done by artificial vagina method. Each time, two ejaculates were collected in two sterilized inner liner and graduated collecting tubes and evaluated, and the average result of the two ejaculates was included in the data to be analyzed. Semen was collected thrice in a week. After collection, semen sample was kept in warm water immediately at about 37.78 to 40.56°C. Just after collection of semen was evaluated for its quality such as color, volume, pH, consistency, motility, concentration, morphology and percentage of live spermatozoa. The color of semen may be milky, creamy cloudy, watery, greenish, yellowish and light yellowish. But the required color is creamy white or yellowish white. The volume of semen was measured directly with the help of a graduated collecting vial. The hydrogen ion concentration was tested with pH paper. The mass

motility percentage was determined in a drop of fresh semen examined under a microscope (.20), using a warm stage adjusted to 37ºC (Melrose and Laing, 1970). The total number of spermatozoa per ml of raw semen was enumerated by haemocytometer method according to Herman and Madden (1963). The sperm concentration (109/mL) was determined using a spectrophotometer (Spectronic 20 machine, USA) with set wave-length 550 nm. Semen (0.1 ml) was added to the tube containing 7.9 ml of 2.9% sodium citrate. Live and dead sperm percentages and the percentage of abnormal spermatozoa were estimated immediately after semen collection in a smear made from a drop of fresh semen stained by eosin (5%) according to the method of Hancock (1951). To evaluate the mass activity, one small drop (20 ul) of semen was placed on a clean pre-warmed (37°C) slide and examined under microscope at low magnification (4x) without cover slip. Mass motility was scored into 0 to 5 scales according to Herman and Madden (1963) are present in Table 1.

Statistical analysis

Data were statistically analyzedby using SAS statistical computer package programmed in accordance with the principle of Completely Randomized Design (CRD). Duncanôs Multiplerange test was done to compare variations between treatments where ANOVA showed significant differences. Pearson co-relation co-efficient were done with rectal temperature, scrotal skin temperature, scrotal length, testis length and with various semen characteristics.

Table 1. Mass motilityscore ofsemen (Hermanand Madden,1963)

Score	Grade	Criteria
5	Excellent quality	80% or more of the spermatozoa are in very vigorous motion
4	Verygoodmotility	Approximately 70-80% of the spermatozoa are in vigorous rapid motion. Waves and eddies form and droprapidly, but notsorapidasinexcellentmotility.
3	Good motility	About 50 to 70% of the spermatozoa are in motion. Motion is vigorous but waves and eddies formed and movedslowlyacrossthefield.
2	Fairmotility	From 20 to 50% of the sperm are in motion. The movements arelargely vigorous butnowaves or eddiesareformed.
1	Poor motility	Less than 30% of the sperm are in motion. The motionis mostlyweakand oscillatory, not progressive.
0	Dead	Nomotilitydiscernable.

Results and Discussion

Temperature humidity index

In the present study during experimental period average temperature was 20.5^{0}C and average relative humidity was 70.5% in the morning and the average temperature was 28.2^{0}C and average relative humidity was 60.5% in the noon time. Hence, THI value was 19.95 in the morning which indicate all the experimental animals were in absence of heat stress and THI value was 25.54 in the noon which indicate all the experimental animals were in severe heat stress (Fig. 1).

Thermolysis capacity

The values of TC of different bucks are shown in Table 2. It showed that buck 1 and buck 5 were more heat resistant than other bucks ($p<0.01$), because their TC values were less than the others. Buck 2, buck 3, buck 4, buck 6, buck 7, buck 8, and buck 10 were similar in heat tolerant because their TC values were statistically similar. Titto *et al*. (1998) found in bucks to a heat challenge by sun exposure that, less TC value is more heat tolerant. The TC was found to evaluate the heat tolerance. Mean rectal temperature after sun exposition was higher than the first measure ($P < 0.001$) showing that bucks were submitted to a hot environment that can be confirmed by the rise of respiratory frequency. Initial mean respiratory frequency was 54/min indicating no heat stress, and increased in all animals after 1 h in the sun to 80-90/min. One hour later, respiratory frequency decreased to 65/min. The TC data show that heat tolerance varies with each individual animal, with a minimum TC of 8.25, a maximum of 9.58 and a mean value of 8.98 for all studied animals; 10 could be the higher possible TC when rectal temperature measured after sun exposition returned to the initial (basal temperature. Thus bucks with low TC values are more heat-tolerant than higher TC valued animals. The present findings in agreement with the findings of animal with low TC value are more heat-tolerant than higher TC valued animals (Aguilar *et al*., 2010; Titto *et al*., 2011). The thermolysis capacity found during a heat tolerance test is a valuable tool that should be used on a routine basis to guide decisions about animals to be selected for selective breeding or crossbreeding programs

Table 2. Thermolysis capacity of different buck (mean±SD)

Treatment	TC values	Level of significance (p<0.01)
Buck 1	$8.25^{d} \pm 0.33$	**
Buck 2	$8.88^{bc} \pm 0.01$	
Buck 3	$9.26^{ab} \pm 0.18$	
Buck 4	$9.14^{ab} \pm 0.22$	
Buck 5	$8.56^{dc} \pm 0.17$	
Buck 6	$9.36^{ab} \pm 0.13$	
Buck 7	$8.90^{bc} \pm 0.06$	
Buck 8	$8.98^{bc} \pm 0.14$	
Buck 9	$9.58^{a} \pm 0.13$	
Buck 10	$8.88^{bc} \pm 0.08$	

Within a column showing different superscripts are significantly different (p <0.01);
** indicates 1% level of significance; TC= Thermolysis capacity

Adaptability of different bucks based on TDI values

The effect of heat stress on TDI values of bucks are shown in Table 3; and Table 4 show the semen characteristics (motility, total sperm count, concentration, dead sperm, and pH) of bucks. There were no significant differences in TDI and semen characteristics among different bucks (p>0.05). All the bucks are similar in adaptability and semen characteristics.

Table 5 shows the correlation co-efficients between rectal temperature, scrotal skin temperature, scrotal length, testis length and with various semen characteristics. There were significant and positive correlation between sperm motility and testis length (p<0.05). Significant and negative correlation were found between sperm concentration and scrotal skin temperature (p<0.05).

Most of the traits studied were adversely affected during summer. pH, incidence of dead spermatozoa, and the decreases in sperm motility and sperm concentrations in buck semen during the hot season were in agreement with the results obtained in buck by Curtis (1983) and El-Darawany (1999). Ortavant *et al*. (1985) also reported that sperm output and semen characteristics were adversely affected with exposure to long daylight (long daylight is associated with an increase of ambient temperature). The significant increase in rectal, body skin and scrotal skin temperatures in rams during summer compared to winter agreed with the results obtained by Marai *et al.* (1997). Similar results were also found by El-Darawany

Table 3. Effect of heat stress on TDI values of buck (mean±SD)

Treatment	TDIa	TDIb	TDIc	level of significance
Buck 1	17.32±4.15	8.30±0.37	1.42±0.32	NS
Buck 2	13.36±1.82	8.44±0.34	1.12±0.13	
Buck 3	20.70±4.99	8.16±0.41	1.70±0.39	
Buck 4	29.26±7.52	8.10±0.93	2.32±0.61	
Buck 5	15.16±1.46	8.94±0.30	1.34±0.13	
Buck 6	13.58±1.85	8.70±0.72	1.14±0.10	
Buck 7	16.98±7.65	8.56±0.26	1.46±0.68	
Buck 8	17.34±5.62	8.54±0.26	1.54±0.55	
Buck 9	21.30±1.99	7.9±0.62	1.70±0.24	
Buck 10	10.24±2.18	8.38±0.41	0.86±0.20	

NS=Non-significant, p>0.05; TDI=Tunica dartos index.

(1999) for rectal and body skin temperatures, Curtis (1983) and El-Darawany (1999) for the scrotal skin temperature of sheep. Mikelsen *et al*. (1981) recorded the greatest scrotal circumference measurements during the autumn season. Yarny *et al*. (1990) claimed that scrotal circumference and testis length in hot summer in sheep decreased to the extent that they became lower than those of the same breeds reared under temperate environmental conditions. Reduction in testicular measurements (testis weight and length) by exposure to heat stress is due to degeneration in the germinal epithelium and partial atrophy in the somniferous tubules (Chou *et al*. 1974). Curtis (1983) indicated that scrotal length denotes the magnitude of heat effect. Scrotal length data from the present study were in agreement with those of El- Darawany (1999).The significant correlations between each of scrotal and testis lengths and each of libidos, semen quality and testosterone concentrations clearly illustrate that the TDI has an important thermoregulatory role in bucks. Its activity can help to control scrotal temperature

through the control of the magnitude of scrotal vascular heat exchange(Curtis,1983).

Table 4. Semen characteristics of different bucks (mean±SD)

Treatment	Motility (%)	Total sperm (million)	Sperm conc. (10^9/ml)	Dead sperm (%)	pH	Level of significance
Buck 1	76.00±4.93	2190±45.09	2.22±0.02	8.50±1.80	6.67±0.17	NS
Buck 2	69.33±1.33	2190±45.09	2.22±0.02	7.50±0.87	6.67±0.17	
Buck 3	79.33±5.67	2190±3.33	2.24±0.01	6.83±0.44	6.66±0.16	
Buck 4	77.00±4.93	2223.3±12.01	2.21±12.01	7.50±0.86	6.83±0.16	
Buck 5	75.00±4.93	2213.3±21.8	2.26±0.02	7.53±0.32	6.67±0.16	
Buck 6	76.00±4.93	2213.3±21.86	2.24±0.01	7.33±0.73	6.67±0.16	
Buck 7	72.00±2.31	2223.3±12.02	2.24±0.01	6.40±0.56	6.83±0.17	
Buck 8	71.66±2.73	2156.6±78.39	2.19±78.38	6.93±0.05	6.83±0.17	
Buck 9	80.66±4.33	2240.0±5.77	2.23±0.01	6.50±0.01	6.67±0.17	
Buck 10	75.67±2.33	2206.67±28.4	2.23±0.01	9.00±0.87	6.50±0.29	

NS= Non-significant, p>0.05.

The mechanism, in summary, is as follows: when the environmental temperature is high, the TDI is relaxed, permitting the testicles to drop away from the body, which increases heat dissipation, and the reverse occurs at low temperature (Maloney and Mitchell, 1996). Curtis (1983) found that the difference between abdominal and testicular temperature is about 4°C in rams. In the present studies, the differences between body skin temperature and scrotal skin temperature were 6.71 and 8.08°C, in noontime and morning, respectively.

Table 5. Correlation coefficients between rectal temperature, scrotal skin temperature, scrotal length, testis length and with various semencharacteristics

Semen traits	Rectal temp.	Scrotal skin temperature	Scrotal length	Testis length
Semen pH	0.2737^{NS}	0.2454^{NS}	-0.0726^{NS}	-0.0839^{NS}
Motility	-0.1970^{NS}	-0.0340^{NS}	0.0615^{NS}	0.4804**
Sperm Concentration	-0.1666^{NS}	-0.4390**	0.0098^{NS}	0.1144^{NS}
Total sperm	-0.1470^{NS}	-0.3450^{NS}	-0.0318^{NS}	0.2831^{NS}
Dead Sperm	0.3242^{NS}	-0.0918^{NS}	0.0640^{NS}	-0.0031^{NS}

NS=Non-significant, p>0.05; **Significant difference, p>0.01.

Conclusions

The results of this study show that all the bucks are similar in adaptability and semen characteristics. There were significant and positive correlation between sperm motility and testis length. Significant and negative correlation was found between sperm concentration and scrotal skin temperature. From the study it can be concluded that all the bucks were in severe heat stress at noon time and TC can be used as an indicator for selection of buck as heat tolerance.

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