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Concentrations of Fructose, Citric Acid and Total Protein in the Seminal Plasma of Goats Raised under Tropical Climates in Northeastern Brazil

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Abstract: Concentrations of fructose, citric acid and total protein in the seminal plasma of goats were measured between the different seasons of the year. The seasons affected all biochemical components investigated in the seminal plasma of goats as there was variation between the different months as well. The highest fructose concentrations in goats were recorded during the rainy and dry/rainy seasons. Citric acid concentrations were highest in the rainy period only whereas total protein concentrations were lower in the rainy/dry period compared to the other seasons of the year. Thus, climate conditions in the Brazilian Northeast are probably associated with these changes in the biochemical composition of bucks.

Key words: Seminal plasma, buck, tropical climate, biochemical components, season, Brazil

INTRODUCTION

It is well known that the semen quality of small ruminants is influenced by season in temperate climates (Eaton and Simmons, 1952; Colas, 1983; Roca *et al*., 1992; Tuli and Holtz, 1995). In those regions these differences have been related to the photoperiod (Chemineau *et al*., 1991). However in tropical regions, changes in day length are less pronounced and goats are considered continuous polyestrous animals.

Seminal plasma is a complex mixture of secretions originated from the testis, the epididymis and the male accessory sex glands (Manjunath *et al*., 1994; Rossi *et al*., 1997). SP contains a variety of biochemical components some of which are relatively specific in the modulation of sperm function and potentiating its fertilizing capacity (Thomas *et al*., 2003; Marti *et al*., 2007). Fructose is one of the main constituents of seminal plasma and its role as a nutrient consists in supplying energy to sperm in the form of an easily glycolyzable material through the glycolytic pathway (Mann, 1946; Flipse and Anderson, 1969; Harrison, 1971) as well as the most abundant saccharide in the ejaculate.

This saccharide has been described as a molecular marker of the seminal vesicle function (Gonzales and Villena, 2001). Other research studies have demonstrated the existence of a positive correlation between serum testosterone levels and fructose concentration in the seminal plasma of goats (Singh and Penbey, 1995). Therefore, fructose is presented as an important seminal component for sperm metabolism and its concentration has been associated with sperm quality, metabolic activity and normal functioning of the vesicular glands (Lewis-Jones *et al*., 1996).

Another important constituent is citric acid which has been known to act as a buffering system in seminal plasma (Mendoza *et al*., 1989) moreover, studies have demonstrated that its citrate also takes part in the regulation of glycolytic activity of sperm metabolism (Nelson and Cox, 2005; Kamp *et al*., 2007). The seminal plasma protein is an important category of molecule present in SP with important effects on sperm physiology (Ollero *et al*., 1997). Some SP proteins participate in the regulatory mechanisms of sperm function which include protection of the spermatozoa, maintenance of motility, sperm capacitation, acrosome reaction and interaction with the oocyte (Calvete *et al*., 1994; Jonakova *et al*., 2000; Barrios *et al*., 2000) and also prevent and reverse sperm membrane-induced damage by heat shock (Barrios *et al*., 2000; Perez-Pe *et al*., 2001). In a earlier study, Cardozo *et al*. (2006) demonstrated the existence of the seasonal changes in the protein profile of rams under

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temperate climatic conditions with significant differences between the breeding and non-breeding seasons. However, reports in the literature about variations in total protein concentration under tropical climatic conditions are scarce and few are conclusive. Therefore, the present study was conducted to evaluate the existence of variation in the concentrations of fructose, citric acid and total protein in the seminal plasma of goats raised under the tropical climatic conditions prevailing in the Brazilian Northeast in different seasons and months of the year.

MATERIALS AND METHODS

Research was conducted in the Animal Science Department of the Federal University of Ceara (Fortaleza, Ceara, Brazil), at 3°45'02"S, 38°32'35"W and 15.5 m above sea level. The experiment was performed from September, 2005 to August 2006. The climate, according to the Koppen classification is AW, hot and humid. During this study, average temperature was 27.3+0.5°C and air relative humidity was 75.8+4.9%. The FUNCEME meteorology station of FUC provided the weather data. Twenty crossbred male goats were used with an average of 22.4+5.9 months of age, weighing 36.2+6.9 kg and featuring scrotal circumference of 24.7+1.5 cm. This study was aware that crossbreed is any animal with no defined standard breed. These individuals are formed by the crosses involving several native and exotic breeds therefore, it is not possible to precisely determine their genetic composition. They are very well adapted to tropical climates and very common in Northeastern Brazil. The animals were raised in an intensive system individually penned in a ventilated building and fed uniformly according to the National Research Council (1981) protocol for goats. Feeding was a mix of Tifton 85 hay (*Cynodon dactilum*) and concentrate mixture (9.01% CP, 55.41% TDN, 64.41% NDF, 0.31% Ca, 0.24% P and 3.03% EE).

Mineral mixture was added to the concentrate and water was freely available. Health management (deworming and vitamin supplementation) was conducted according to the guidelines of Embrapa/CNPC (Brazilian Goat Research Center). The different seasons were defined based on the average environmental data for precipitation, humidity and temperature. The months of the year were divided in: dry season from September to December with precipitation of 0 mm, relative humidity at 71.5+1.5% and temperature at 27.7+0.4°C dry/rainy transition season from January to February with precipitation at 65.1+9.9 mm, relative humidity at 75.0+2.0% and temperature at 27.8+0.1°C rainy season from March to June with average precipitation at 298.2+101.4 mm, relative humidity at 81.2+2.9% and temperature at 26.9+0.3°C and the rainy/dry transition season July and August with average precipitation at 35.1+21.8 mm, relative humidity at 74.7+4.6% and temperature at 26.8+0.2°C. The Temperature-Humidity Index (THI) was calculated for each season according to the formula: $THI = T_A+0.36\ TP_O+41.5$ described by Silva (2005).

The ejaculates were collected weekly using an artificial vagina from September 2005 to August 2006. At the end of experimental period, 48 samples of semen were collected. Upon collection on the semen, the ejaculated volume was individually recorded and a small aliquot taken for assessment of sperm counts. After collection of the semen each semen sample was immediately centrifuged in a Sigma refrigerated centrifuge (Model 4K15) at 3,417 g/20 min/+4°C and the supernatant was frozen at -18°C for subsequent analysis of biochemical composition. At the end of the collection period due of the low volume of seminal plasma of some animals, a mix (pool) was made from four samples of each animal totaling 240 observations resulting in one sample from each animal per month.

The concentrations of fructose, citric acid and total proteins in seminal plasma in the different seasons of the year were assayed in triplicate using colorimetric commercial kits of the *in vitro*® brand. The influence of the seasons of the year on the concentrations of fructose, citric acid and total proteins in the seminal plasma of goats was analyzed using a randomized blocks design in which each animal represented a block to remove the effects of age, weight and scrotal circumference. Analysis of variation was used to evaluate possible variations in biochemical components of seminal plasma throughout the year.

Data were analyzed using a randomized blocks design to evaluate possible variations in the concentrations of fructose, citric acid and total proteins in the seminal plasma of goats during different seasons and between the months of the year. Comparisons among means of these variables were carried out using Turkey's statistical test with a 5% probability of error. Associations between mean temperature, mean relative air humidity, rainfall and the temperature-humidity index with biochemical components of seminal plasma were calculated using Pearson's correlation coefficient.

RESULTS AND DISCUSSION

In this study, researchers describe the seasonal and monthly changes in the concentrations of fructose, citric acid and total protein in the seminal plasma of goats raised under the tropical climatic conditions prevailing in Northeastern Brazil (Table 1 and 2).

Table 1: Concentrations of fructose, citric acid and total protein in the seminal plasma of goats raised in Northeastern Brazil during different seasons of the year

	Season				
Parameters	Rainy	Rainy/Dry	Dry	Dry/Rainy	Annual average
Fructose (mg dL^{-1})	613.0±176.9^{a}	399.9±182.6^{c}	540.8±168.4^{b}	652.9±166.2^{a}	559.4±127.2
Citric acid (mg dL^{-1})	498.7±109.5^{a}	429.9±109.4^{b}	458.9±104.9^{b}	453.5±96.0^{b}	466.0±82.4
Total proteins (g dL^{-1})	5.8±1.3^{a}	4.6±1.9^{b}	5.6±1.2^{a}	5.75±0.8^{a}	5.46±0.9
THI	75.4^{c}	76.5^{b}	76.5^{b}	77.0^{a}	76.4

Table 2: Monthly variation of the biochemical composition of goats raised in tropical environment, state of Ceara, Brazil

Months	Total proteins (g dL^{-1})	Citric acid (mg dL^{-1})	Fructose (mg dL^{-1})
January	5.67±0.55^{ab}	507.48±61.980^{ab}	607.45±128.11^{ab}
February	5.66±0.66^{ab}	398.21±64.120^{c}	695.23±104.46^{a}
March	5.33±0.92^{b}	493.13±81.130^{abc}	684.18±92.130^{a}
April	6.72±1.03^{a}	558.61±110.50^{a}	698.89±109.92^{a}
May	5.74±0.84^{ab}	462.83±74.100^{abc}	584.80±111.28^{abc}
June	5.08±1.27^{bc}	480.26±86.600^{abc}	489.27±132.16^{bcd}
July	5.53±0.54^{bc}	435.06±82.810^{bc}	404.48±191.07^{cd}
August	4.01±1.25^{c}	423.62±104.49^{bc}	392.06±173.90^{d}
September	5.76±1.44^{ab}	392.01±83.280^{c}	480.53±134.26^{bcd}
October	5.03±0.75^{bc}	474.96±69.130^{abc}	578.98±123.48^{abc}
November	5.40±1.07^{b}	454.61±85.990^{abc}	567.33±130.21^{abcd}
December	5.65±0.74^{ab}	511.56±84.210^{ab}	529.86±95.700^{abcd}
Annual average	5.46±0.92	466.02±82.360	559.42±127.22

Different letters in different columns are statistically different ($p<0.05$)

An increase in fructose concentration was observed from the rainy/dry transition to dry/rainy season ($p<0.05$) followed by a significant ($p<0.05$) decrease during the rainy/dry transition season (Table 1).

However, low ($p<0.05$) fructose concentration was found during the rainy/dry transition and dry seasons. The highest fructose concentrations were observed in February (695.2±104.5), March (684.2±92.1) and April (698.9±109.9) whereas July (404.5±191.1) and August (392.1±172.9) were the months with the lowest concentration. These results were similar to those reported by Pinheiro *et al.* (1996) with goats of the Alpine and Moxoto breeds and their crosses raised under extensive conditions however, those researchers found low values for fructose concentration during the dry season (348.8±21.8 mg dL^{-1}) for all breeds. A probable explanation for this difference between the studies is that the feeding factor in the present experiment was uniform throughout the year. Thus, diet factors do not exert influence on seasonal changes in the biochemical composition in the seminal plasma of goats.

Therefore, it is believed that the change in fructose concentration observed during this study was probably due to an environmental effect including temperature and humidity acting together on the males. Adequate knowledge of seasonal variation on fructose concentration is an important tool to determine the proper season to preserve and use goat semen. Such conditions seem to have been achieved in the rainy season of the research but not in the dry season.

Citric Acid (CA) was significantly higher only in the rainy season (Table 1). In April, the highest monthly concentration of citric acid was found (558.6±110.5), whereas September (392.0±83.3) had the lowest. Citric acid concentrations during the dry and rainy seasons (458.9±104.9 and 498.7±109.5, respectively) were similar to those reported by Pinheiro *et al.* (1996), (rainy: 461.4±10.6; dry: 338.4±12.3 mg dL^{-1}, respectively). However, they were higher than those recorded by Rodrigues (1997) with crossbred male goats (314.7±96.6 mg dL^{-1}) both in tropical regions.

In temperate climate conditions, Roca *et al.* (1993) found high fructose and citric acid concentrations in the summer (867.57 and 311.98 mg dL^{-1}, respectively) and fall season (931.42 and 327.66 mg dL^{-1}, respectively) and low concentration in the spring (567.95 and 249.11 mg dL^{-1}, respectively) whereas winter (765.57 and 285.11, respectively) was considered a transitional season whose seasonality was related to plasma testosterone concentration changes induced by photoperiod. The process of citricolysis although, rather slow may continue in semen for some time after the spermatozoa have exhausted the entire reserve of seminal fructose, i.e., after fructolysis has come to an end. Moreover, the rate of citricolysis in the SP is only observed in the presence of sperm cells in semen, the seminal plasma itself being unable to metabolize citric acid because only the spermatozoa have the necessary enzymes to use that metabolite (Humphrey and Mann, 1949). The same authors found daily individual fluctuations in citric acid and fructose concentration as well as a variable ratio between fructose and citric acid which seems to be specific semen characteristic.

These ratios were confirmed in this experiment under tropical conditions and in different seasons: dry/rainy transition (69.44%), rainy (81.35%), rainy/dry transition (93.01%) and dry (84.85%). The concentrations of total proteins in seminal plasma were the most constant metabolites (Table 1) and were lower in the rainy/dry transition period than the other seasons ($p<0.05$). The highest protein concentration was observed in April (6.7±1.1) and the lowest in August (4.1±1.3). Pinheiro *et al.* (1996) found significant differences ($p<0.05$) between the rainy and dry seasons (4.27+0.08; 3.14+0.1 g dL^{-1}, respectively) but those results were not

observed in the current study. A probable explanation for this difference between the studies is the same we outlined above in the discussion. A previous study observed seasonal changes in seminal plasma proteins of Saanen goats under natural conditions in Southern Brazil (subtropical climate) and found an important difference in the standard of proteins between the reproductive and non-breeding seasons suggesting that proteins from goat seminal plasma are under seasonal control and are associated with sperm function during the breeding and nonbreeding seasons (La Falci *et al*., 2002).

Several studies have shown that seminal plasma proteins play an important role in maintaining motility (Elzanaty *et al*., 2002) prevent and reverse sperm membrane-induced damage by heat shock (Barrios *et al*., 2000; Perez-Pe *et al*., 2001) support sperm capacitation, acrosome reaction and interaction with the oocyte (Calvete *et al*., 1994; Jonakova *et al*., 2000; Barrios *et al*., 2000). However, more studies are needed to evaluate and characterize changes in the protein profile throughout the year under tropical climatic conditions as well as possible relationships between semen parameters and fertility. In the research a reduction in the concentrations of fructose and citric acid was detected in the dry season when THI was higher. In addition, we found an interaction between animal and season ($p<0.05$) suggesting that climatic variations may exert an effect on the metabolic patterns of animals and consequently on the biochemical components of seminal plasma as well. In tropical climates such as the conditions prevailing in Northeastern Brazil (>25° latitude) changes in day length are less pronounced therefore, it is believed that these changes may be attributed to the thermal sensation perceived by the animals during the dry period since, feeding was adequate throughout the research. Reports have shown that factors such as temperature may be important in certain environments (Zamiri and Heidari, 2006).

Prior research has shown that exposure of goats in the field to a short season of high ambient temperature for consecutive days could produce seminal degeneration similar to that after continuous exposure in controlled-climate rooms (Smith, 1971). However, more studies are needed to evaluate the correlation between the biochemical composition changes of seminal plasma and semen quality throughout the year. Correlations between biochemical components and climatic parameters are shown in Table 3. The values for correlations between climatic parameters and biochemical components of goat seminal plasma although, low were generally positive. However, moderate correlation was observed only between fructose concentration and THI. In this context it is believed that the seasonal changes in the biochemical components observed in the experiment are under the influence of environmental parameters, especially in the warmer months when temperatures deviate from the thermal comfort zone. The average THI during the experiment time was 78 indicating that the animals were under heat stress.

Table 3: Pearson's simple correlations between biochemical components and climatic parameters in the seminal plasma of goats

Component	CA	Fructose	Temp.	Humid	Precip.	THI
TP	0.49649	0.60386	0.11492	0.19721	-0.14498	0.25679
	(<0.0001)	(<0.0001)	(0.0775)	(0.0023)	(0.0256)	(<0.0001)
CA		0.59719	0.05118	0.19921	-0.04708	0.13960
		(<0.0001)	(0.4329)	(<0.0021)	(0.4706)	(0.0317)
Fructose			0.19474	0.25493	-0.07728	0.35931
			(0.0026)	(<0.0001)	(0.2359)	(<0.0001)

TP = Total Proteins; CA = Citric Acid; THI = Temperature-Humidity Index

Reseachers observed moderate correlations between total proteins, citric acid and fructose. These findings were similar to those reported by Roca *et al*. (1993) who also found a significant correlation ($r = 0.60$) between those components. The correlation between reducible sugars and proteins is probably due to the common origin of these constituents in the male accessory glands (Assumpcao *et al*., 2005). Individual variations were detected for all studied characteristics ($p<0.01$) as well as an interaction between animal and season ($p<0.05$). These findings show that extrinsic factors and interaction between environment factors may have influenced biochemical components.

CONCLUSION

Fructose, citric acid and total protein concentrations in the seminal plasma of goats raised under the tropical climatic conditions prevailing in northeastern Brazil showed seasonal and monthly changes. Thus, climate condition in the Brazilian Northeast is probably associated with these changes in the biochemical composition of goats.

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