

Seasonal Variations in Blood Parameters of the Amazonian Manatee, *Trichechus inunguis*

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ABSTRACT

Seasonal variations in body weight, food consumption and blood glucose, total lipids, urea, total proteins, albumin and globulins of captive Amazonian manatees, *Trichechus inunguis*, were determined. Body weight changed significantly along the year, increasing from autumn to spring and decreasing in summer. The mean daily food intake of paragrass remained almost unchanged along the year. Paragrass administered to the manatees showed important variations in crude protein and lipid content along the year. No significant differences in blood parameters were registered between males and females in all seasons. Further, there were no significant differences in blood total proteins, albumin and globulins along the year. On the other hand, significant differences in the mean blood glucose, lipids and urea were registered. An increase in the blood glucose in the spring and summer was observed. Blood urea and lipids levels were positively related to paragrass protein and lipids content. These two correlations suggested that these blood parameters are good indicators of the animal nutritional status in the Amazonian manatee.

Key words: Amazonian manatee, *Trichechus*, blood biochemistry, nutrition, Amazon

INTRODUCTION

Comparative studies on blood biochemistry can provide important information on the physiology and health of aquatic mammals (Ridgway, 1972; Kastelein *et al.*, 1990; Fothergill *et al.*, 1991; Colares *et al.*, 1992; Koopman *et al.*, 1995) and season is one factor, among others, interfering on the normal mammal physiology. In the brown bear, Halloran & Pearson (1972) observed variations in blood concentrations of calcium, potassium, urea, and creatinine along the year. Brown & White (1980) observed variations in the total protein in the blood of elephants which were related to the habitat they occupied and the period of the year considered. Knick *et al.* (1993) studying the hematology and blood chemistry of bobcats, *Felis rufus*, observed variations in cholesterol levels between animals captured in spring and autumn. Several authors have suggested that seasonal variations in the blood parameters could be related to the seasonal changes in the food composition and availability (Brown *et al.*, 1978; Warren *et al.*, 1982; Colares *et al.*, 1992; Hellgren *et al.*, 1993; Schweigert, 1993).

The Amazonian manatee, *Trichechus inunguis*, is endemic to the basin of the Amazon River. It is a

non-ruminant herbivore that feeds on a large variety of aquatic and semi-aquatic plants (Lemire, 1968). However, it has preference for semi-aquatic and aquatic grasses (Best, 1984; Colares, 1991b). The environmental availability of these plants presents seasonal variations related to the rainy and dry seasons occurring in the Amazon rivers and lakes (Junk, 1970). Studies developed during the dry seasons between 1979 and 1981 in the lakes from Central Amazon showed that no food source for the Amazonian manatee was available until the rain season was arrived (Best, 1983).

Application of blood biochemistry analysis to study Amazonian manatee has not been described for captive and natural populations. Taking into account that (1) little scientific information on manatee nutrition is available, (2) animal health has been determined employing biomedical methodologies, and (3) the necessity of careful handling of manatees, this investigation was developed to study some aspects of nutrition and blood biochemistry in the Amazonian manatee, *Trichechus inunguis*. The possible seasonal and sexual variations in these parameters have also been considered.

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MATERIALS AND METHODS

Ten adult Amazonian manatees (five males and five females) were kept in captivity for at least ten years at the National Institute of Amazon Research (INPA) at Manaus City (Brazil). Experiments were developed from May, 1989 to April, 1990. The manatees were maintained in a round concrete tank of 78.5 m² and 60 cm depth. Every day, they were fed paragrass, *Brachiaria mutica*. Daily food intake was calculated based on the difference of food administered (corresponded to 7% of the animal body weight per day) and the one that remained in the tank after 12 h. For this, the remaining food was collected, dried at ambient air temperature for 24 h, and weighed (Colares, 1991b). Every month, the mean daily food intake was calculated. A seasonal mean was then estimated.

To detect possible seasonal variations in paragrass composition, weekly samples of the food stock were randomly collected during one year. These samples were then dried at 38°C until steady weight in order to determine paragrass water content. The dried material was then pooled and stored until the chemical analysis, which was performed at the end of the season in triplicate. In each pool, crude proteins, total lipids, carbohydrates, and ashes were determined in triplicate, following the analytical procedure described by Silva (1981). Results were expressed in percentage of dry weight.

Every season the body weight of each manatee was determined with a Salter balance (500 x 0.5 kg). At the same time blood samples were collected. The manatees were immobilized using a restraining apparatus described by Colares (1991a), and blood was withdrawn from the branches of the brachial artery on the ventral side of the pectoral flippers. A disposable 20 ml syringe with scalp needle (19G) was used. Blood sampling was always performed in the morning before feeding.

Blood samples from each animal were placed in assay tubes containing anticoagulant (heparin or GLISTAB[®] from LABTEST, Belo Horizonte-MG - Brazil). Glucose, total lipids, urea, albumin, and total proteins were determined by the following methods, respectively: glucose-oxidase, sulphophosphovanilin, modified diacetyl, bromocresol green, and biuret. All determinations were performed using diagnostic kits from Labtest (Belo Horizonte, MG, Brazil). Blood globulin concentrations were calculated from the difference

between the total protein and albumin concentrations.

To verify possible seasonal and sexual differences in the blood parameters, data were submitted to MANOVA. *A posteriori* comparisons were made employing the Tukey's test. These statistical analysis were performed using the software Statgraphics (Statistical Graphics System; version 5.1). Correlation between blood parameters and paragrass chemical composition were tested with Kendall tau analysis, employing the software "Statistica for Windows" (StatSoft Inc., 1995). Significance level adopted for all statistical analyses was 95% ($\alpha = 0,05$).

RESULTS

The mean body weight of the Amazonian manatees changed significantly along the year (Figure 1). It increased approximately 7% from autumn to spring. In summer, the body weight decreased attaining a mean value similar to that registered in autumn. The mean daily consumption of paragrass by all manatees remained almost unchanged (approximately 3% of the total biomass) along the year, except in winter, where it almost attained 4% of total biomass (Figure 1).

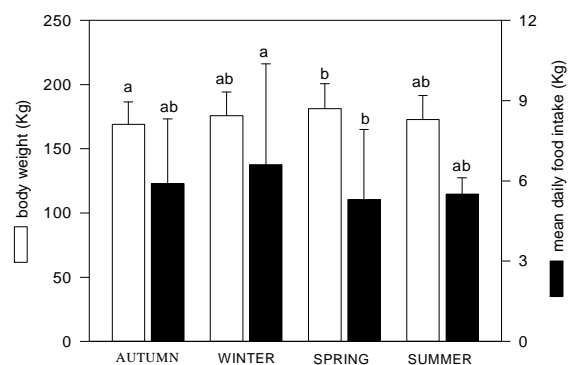


Figure 1 - Seasonal variations in body weight and daily food intake of the Amazonian manatee, *Trichechus inunguis*. Data are means (\pm 1SE; n= 10). Different letters indicate significantly different means ($P < 0.05$; Tukey's test).

Results from chemical analysis carried out on the paragrass administered to the manatees are presented in the Table I. The carbohydrates, ash and water contents did not show important variations

along the year. However, lipid content decreased from autumn to summer, while the crude protein content increased during the same period (see also Figures 2 and 3). No correlation between the mean body weight and daily consumption of paragrass or the seasonal variations in paragrass composition was observed.

Table 1 - Seasonal variations in the chemical composition of the paragrass, *Brachiaria mutica*. Data for nutrients are expressed in percentage of dry weight and represents mean of three determinations of pooled samples.

Parameter	Season			
	Autumn	Winter	Spring	Summer
Crude Protein (%)	11.5	11.8	14.7	17.5
Lipids (%)	6.0	5.1	2.1	2.7
Carbohydrates(%)	74.2	75.6	76.1	71.3
Ashes (%)	8.3	7.5	7.1	8.5
Water content (%)	65.3	65.1	64.6	68.4

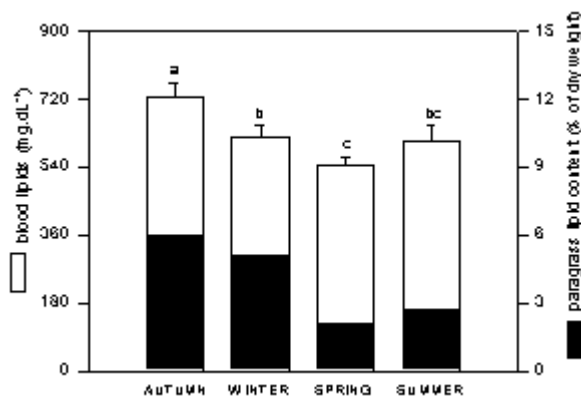


Figure 2 - Seasonal variations in blood lipids of the Amazonian manatee, *Trichechus inunguis*, and lipid content of the paragrass, *Brachiaria mutica*. Results for blood lipids are means of ten individual measures (\pm 1SE), while those for paragrass lipid content are means of three determinations of pooled samples. Different letters indicate significantly different means ($P < 0.05$; Tukey's test).

Because no significant differences ($P > 0.05$) between males and females were found for all blood parameters analyzed (Table 2), data were pooled and a seasonal mean was then calculated (Table 3).

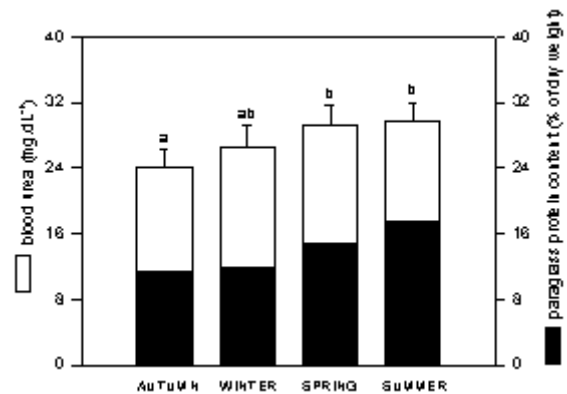


Figure 3 - Seasonal variations in blood urea of the Amazonian manatee, *Trichechus inunguis*, and the protein content of the paragrass, *Brachiaria mutica*. Results for blood urea are means of ten individual measures (\pm 1 SE), while those for paragrass protein content are means of three determinations of pooled samples. Different letters indicate significantly different means ($P < 0.05$; Tukey's test).

No significant seasonal variation in the blood total proteins, albumin, and globulins could be detected ($P > 0.05$). However, significant differences in the mean blood glucose, lipids and urea were found. A higher blood glucose concentration was observed in spring, when compared to that registered in winter. Blood total lipid concentrations decreased from autumn to spring remaining, in the summer, lower than in autumn. Blood urea level increased from autumn to spring and was also higher in summer than in autumn (Table 3). Significant ($P < 0.05$) correlation between blood urea and paragrass protein content and between blood lipids and paragrass lipid content were detected (Figures. 2 and 3, respectively).

DISCUSSION

Despite the lack of correlation between mean body weight and daily consumption of paragrass, or seasonal variations in paragrass composition, we must consider that during winter manatees increase their daily food intake. This fact could explain the higher body weight attained in spring, despite the lower consumption of paragrass in this season. It is also important to note that during this season paragrass protein content is increasing.

Table 2 - Blood parameters of captive male and female Amazonian manatees, *Trichechus inunguis*, through a year. Results are means (\pm 1SE; n=5).

Sex	Season	Blood parameter					
		Glucose (mg.dL ⁻¹)	Total lipids (mg.dL ⁻¹)	Urea (mg.dL ⁻¹)	Proteins (g.dL ⁻¹)	Albumin (g.dL ⁻¹)	Globulins (g.dL ⁻¹)
Male	Autumn	36.8 \pm 6.2	711.6 \pm 50.0	24.9 \pm 3.7	6.3 \pm 0.1	3.6 \pm 0.1	2.7 \pm 0.3
	Winter	35.9 \pm 2.7	624.6 \pm 46.5	27.9 \pm 5.4	6.4 \pm 0.1	3.5 \pm 0.2	2.9 \pm 0.2
	Spring	44.3 \pm 5.3	558.8 \pm 29.8	28.7 \pm 3.7	6.2 \pm 0.1	3.6 \pm 0.1	2.6 \pm 0.2
	Summer	39.3 \pm 5.8	607.3 \pm 71.1	29.6 \pm 5.7	6.1 \pm 0.2	3.5 \pm 0.1	2.6 \pm 0.1
Female	Autumn	39.7 \pm 1.0	731.8 \pm 64.7	23.3 \pm 2.8	6.5 \pm 0.1	3.4 \pm 0.1	3.1 \pm 0.1
	Winter	32.0 \pm 3.6	606.6 \pm 57.1	25.3 \pm 1.8	6.4 \pm 0.2	3.3 \pm 0.1	3.1 \pm 0.1
	Spring	42.2 \pm 1.7	528.8 \pm 30.8	30.0 \pm 3.3	6.3 \pm 0.2	3.4 \pm 0.1	3.0 \pm 0.2
	Summer	39.1 \pm 3.4	611.4 \pm 53.4	29.7 \pm 2.9	6.3 \pm 0.1	3.5 \pm 0.1	2.9 \pm 0.1

Table 3 - Seasonal variations in blood parameters of the Amazonian manatee, *Trichechus inunguis*. Pooled data from 5 males and 5 females. Data are means (\pm 1SE). Different letters indicate significantly different means (P<0.05; Tukey's test).

Season	Blood parameter					
	Glucose (mg.dL ⁻¹)	Total lipids (mg.dL ⁻¹)	Urea (mg.dL ⁻¹)	Proteins (g.dL ⁻¹)	Albumin (g.dL ⁻¹)	Globulins (g.dL ⁻¹)
Autumn	38.2 \pm 2.8	721.7 \pm 36.5	24.1 \pm 2.1	6.4 \pm 0.1	3.5 \pm 0.1	2.9 \pm 0.2
Winter	34.4 \pm 2.1	615.6 \pm 31.2	26.6 \pm 2.6	6.4 \pm 0.1	3.4 \pm 0.1	3.0 \pm 0.1
Spring	43.2 \pm 2.5	543.6 \pm 19.8	29.3 \pm 2.2	6.2 \pm 0.1	3.5 \pm 0.1	2.7 \pm 0.1
Summer	39.2 \pm 2.8	609.4 \pm 39.6	29.6 \pm 2.2	6.2 \pm 0.1	3.5 \pm 0.1	2.7 \pm 0.1

When comparing males and females, results from blood parameter analysis are in accordance with those reported for the African elephant, *Loxodonta africana* (Moore & Sikes, 1967); for the deer, *Odocoileus virginianus* (Seal & Erickson, 1969; LeResche *et al.*, 1974); for the seal, *Phoca hispida* (Geraci *et al.*, 1979); and for the West Indian manatee, *Trichechus manatus* (Medway *et al.*, 1982), among others.

In wild animals, blood glucose concentration can change as a function of diet, age, physical activity, reproduction, environmental conditions, animal handling, time of the day and metabolism (Halloran & Pearsson, 1972; LeResche *et al.*, 1974; Seal *et al.*, 1975). Lee *et al.* (1977) described variations in blood glucose levels of the polar bear along the year, and suggested that this particular variation could be due to an increase in activity as a response to the lower temperature and ice formation. According to Marques *et al.* (1994), climatic changes induced stress in cattle, *Bos taurus indicus*,

which was verified by changes in the plasmatic cortisol. The blood glucose level we found for the Amazonian manatee were higher in spring than in winter, but we cannot relate this increase with a lower environmental temperature. *Trichechus inunguis* lives in warm places with little temperature variations. The annual mean water temperature of the Amazon river is 29 \pm 1° C (Sioli, 1984).

On the other hand, several authors have suggested that changes in blood glucose could be due to the handling stress during blood sampling, even though such procedure is standardized (Seal *et al.*, 1972; LeResche *et al.*, 1974; Hyvarinen *et al.*, 1975; Koopman *et al.*, 1995). However, O'Shea *et al.* (1985) showed that the West Indian manatee was tolerant to capture and that wild animals have blood glucose levels higher than captive ones. The blood glucose levels registered in the present study were similar to those registered for other Amazonian manatees kept captive (Colares *et al.*, 1992).

The blood glucose increase observed in the Amazonian manatee in spring and summer could be due to an increase in gluconeogenesis. This statement is based on the higher protein availability in the food during these seasons. According to LeResche *et al.* (1974) and Seal *et al.* (1975), an increase in protein assimilation by the animal could lead to a greater gluconeogenic activity. Kirkpatrick *et al.* (1975) have demonstrated that both low energy and high protein contents in diet could lead to an increase in blood urea of elephants. In the present study, both conditions were found, since a decrease in the lipid content and an increase in protein content of the paragrass were registered in spring and summer.

The seasonal changes in blood total lipids could also be a reflex of the annual variation of paragrass composition. In this case, there was a positive correlation between paragrass and blood lipid content. Colares *et al.* (1992) have demonstrated that a decrease in lipid content of the food provided to captive Amazonian manatees reduced blood total lipids. A negative correlation between blood glucose and total lipids was observed in autumn and spring. This fact suggested that a change in the metabolic substrate could be occurring. We must consider that this species lives in areas where the water level can be reduced up to 20 m, especially during the dry season (Sioli, 1984). During this period, the animals remain in deep areas, where the plants which usually serve as food are scarce. As reported by Best (1983), they might even starve. Further, Colares (1991b) demonstrated that besides the reduced plant availability during the dry season, a decrease in the relative amount of protein and an increase in lipid content of several species of plants occurs.

Several authors have indicated that blood urea could be a good indicator of the animal nutritional status, since it rapidly changes with variations in diet protein content (Eggum, 1970; Kumar *et al.*, 1972; Eskeland *et al.*, 1974; Brown *et al.*, 1978; Hellgren *et al.*, 1993). A positive correlation between these parameters was also observed in the Amazonian manatee (Fig. 3). This correlation and that between blood lipid and paragrass lipid content discussed above, could constitute useful tool in nutritional status determination of captive animals. Data on blood lipids and urea from wild specimens of the Amazonian manatee could also provide

important information on nutritional status of this species.

Seasonal variations in blood levels of total proteins, albumin and globulins were also described in several species of mammals. Authors have suggested that such variations could be due to seasonal differences in the percentage of crude proteins in the ingested food (LeResche *et al.*, 1974; Brown *et al.*, 1978; Warren *et al.*, 1982). Further, they have also suggested that such parameters were not good indicators of the nutritional status. Our results support this idea, since, despite the variations in the paragrass composition, no significant changes in these blood parameters were registered along the year.

ACKNOWLEDGMENTS

We thank Francisco Antonio Pinto Colares for the essential help in blood sampling, and the other members of the Laboratory of Aquatic Mammals of INPA for general assistance. We also thank Telma Pasini from the University of São Paulo (Brazil) for technical support and LABTEST Diagnostica Ltda. for donation of analytical reaction kits. A. Bianchini and E.A. Santos are a research fellows from Brazilian CNPq (Proc. nº 300536/90-9 and 300763/87-5 respectively).

RESUMO

Variações sazonais no peso do corpo, consumo de alimento e glicose, lípidos totais, uréia, proteínas totais, albumina e globulinas do sangue de Peixes bois cativos, **Trichechus inunguis**, foram determinadas. Houve diferença significativa no peso dos animais ao longo do ano, sendo este aumento entre o outono e a primavera e decresce no verão. O consumo de capim colônia não variou ao longo do ano. A composição de proteína bruta e lípidos totais de capim colônia dado aos animais mostrou importante variação ao longo do ano. Não foi verificado nenhuma variação nas concentrações dos parâmetros sanguíneos estudados entre os sexos em todas as estações. Também não foi verificado diferenças significativas nas concentrações sanguíneas de proteínas totais, albumina e globulinas ao longo do ano. Por outro lado, diferenças significativas nas concentrações de

glicose, lipídeos e uréia foram verificadas. Um aumento na concentração de glicose foi observado na primavera e verão. As concentrações sagüíneas de uréia e lípidos totais estão correlacionadas, respectivamente, com as quantidades de proteína bruta e gordura no alimento. Estas duas correlações sugerem que estes parâmetros sanguíneos são bons indicadores da condição nutricional do peixe-boi.

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Received: September 24, 1998;
 Revised: March 26, 1999;
 Accepted: July 16, 1999.

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