

HEMATOLOGICAL INDICES AND MINERAL CONTENT OF SERUM IN CAPTIVE AND WILD AMAZONIAN MANATEES, *Trichechus inunguis*

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ROSAS¹, F.C.W.; LEHTI², K.K.; MARMONTEL³, M. Hematological indices and mineral content of serum in captive and wild amazonian manatees, *Trichechus inunguis*. *Arq. ciên. vet. zool., UNIPAR*, 2(1): p. 37-42, 1999.

ABSTRACT: Blood was sampled from nine captive and five wild Amazonian manatees, *Trichechus inunguis*. Approximately 10 ml of blood were removed from the palmar side of the pectoral flippers. Samples were analysed for hematocrit, hemoglobin, zinc, copper, magnesium, iron, sodium, potassium and calcium. The hematological parameters determined in the wild manatees were assumed to represent normal standards of the species. Copper and sodium levels were similar between captive and wild manatees, but zinc, magnesium, iron, potassium and calcium in wild manatees were significantly greater than those of captives. The results suggest that the diet of the captive animals needed improvement with regard to the latter minerals. The mineral levels of zinc, magnesium, iron and calcium were also significantly different between male and female wild manatees. This could indicate a possible natural difference between the sexes and deserves further research.

KEY WORDS: Amazonian manatee, hematology, nutrition, serum mineral content, *Trichechus inunguis*.

ÍNDICES HEMATOLÓGICOS E CONTEÚDO MINERAL DO SORO DE PEIXES-BOIS DA AMAZÔNIA (*Trichechus inunguis*) CATIVOS E SELVAGENS

ROSAS, F.C.W.; LEHTI, K.K.; MARMONTEL, M. Índices hematológicos e conteúdo mineral do soro de peixes-bois da Amazônia (*Trichechus inunguis*) cativos e selvagens. *Arq. ciên. vet. zool., UNIPAR*, 2(1): p. 37-42, 1999.

RESUMO: Aproximadamente 10 ml de sangue de 9 peixes-bois (*Trichechus inunguis*) cativos e de 5 peixes-bois selvagens foram retirados da região ventral da nadadeira peitoral. Foram analisados os valores de hematócrito, hemoglobina, zinco, cobre, magnésio, ferro, sódio, potássio e cálcio. Os valores dos parâmetros hematológicos determinados nos peixes-bois selvagens foram considerados como padrões normais para a espécie. Os níveis de cobre e sódio foram similares entre os peixes-bois cativos e selvagens. No entanto, os valores de zinco, magnésio, ferro, potássio e cálcio nos peixes-bois selvagens foram significativamente maiores que nos cativos. Os resultados sugerem que a dieta dos animais em cativeiro necessita suplementação dos cinco últimos minerais citados. Os níveis de zinco, magnésio, ferro e cálcio foram também significativamente diferentes entre machos e fêmeas, o que pode ser um indicativo de uma possível diferença natural entre os sexos, e necessita estudos adicionais.

PALAVRAS-CHAVE: Peixe-boi da Amazônia, hematologia, nutrição, conteúdo mineral do soro, *Trichechus inunguis*.

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ÍNDICES HEMATOLÓGICOS Y CONTENIDO MINERAL DE SORO DE MANATIES DE LA AMAZONIA (*Trichechus inunguis*) EN CAUTIVERIO Y SILVESTRES

ROSAS, F.C.W.; LEHTI, K.K.; MARMONTEL, M. Indices hematologicos y contenido mineral de soro de manaties de la Amazonia, (*Trichechus inunguis*) em cautiverio y silvestres. *Arq. ciên. vet. zool., UNIPAR*, 2(1) : p. 37-42, 1999.

RESUMEN: Cerca de 10 ml de sangre de 9 manaties de la Amazonia (*Trichechus inunguis*) en cautiverio y 5 manaties silvestres fueran colectados de la face ventral de la aleta pectoral. Los valores de hematocrito, hemoglobina, zinc, cobre, magnesio, hierro, sodio, potasio y calcio fueran analizados. Los valores de los índices hematológicos determinados para manaties silvestres fueran considerados como los padrones normales para la especie. Los niveles de cobre y sodio fueran similares entre manaties de cautiverio y silvestres. Sin embargo, los valores de zinc, magnesio, hierro, potasio y calcio en los manaties silvestres fueran significativamente más altos que en animales de cautiverio. Los resultados sugieren que la dieta de esos últimos necesita suplementación de zinc, magnesio, hierro, potasio y calcio. Los niveles de zinc, magnesio, hierro y calcio tambien fueran significativamente diferentes entre machos y hembras, lo que puede indicar una diferencia natural entre los sexos, y que necesita estudios adicionales.

PALABRAS-CLAVE: Manaties, hematología, nutrición, contenido mineral de soro, *Trichechus inunguis*

Introduction

The Laboratory of Aquatic Mammals at the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil, has carried out rehabilitation programs with Amazonian manatees (*Trichechus inunguis*), on both orphaned calves, raised on artificial milk (BEST *et al.*, 1982; ROSAS, 1992; ROSAS, 1994) and adult animals, which quickly become adapted to captivity (ROSAS, 1994). The presence of these animals in captivity has allowed a series of studies to be carried out, which would be impossible to conduct in the wild, such as determination of metabolic rate (GALLIVAN & BEST, 1980), thermoregulation (GALLIVAN *et al.*, 1983), hearing (BULLOCK *et al.*, 1980), biochemical blood parameters (COLARES, 1992) and growth (ROSAS, 1992).

Hematocrit, hemoglobin and some mineral content analyses were conducted by MEDWAY *et al.* (1982), BOSSART & DIERAUF (1990) and CONVERSE *et al.* (1994) with the West Indian manatee (*Trichechus manatus*). However, virtually nothing is known about blood parameters of wild Amazonian manatees (*Trichechus inunguis*).

The ease with which blood is removed from manatees and the absence of information about normal mineral concentrations in the serum of the

Amazonian manatee, led to the development of this study. We believe that the information obtained can be used to improve the management of Amazonian manatees in captivity.

Materials And Methods

Part of this study used up to 9 Amazonian manatees kept in captivity at INPA, which, at the start of the study, were fed a diet consisting of 90% grass (*Brachiaria mutica*) and 10% cabbage and lettuce. Towards the end of the study, these proportions were changed to 60% *B. mutica* and 40% cabbage and lettuce. As part of the Mamirauá Project, five wild animals were also sampled during capture-release procedures for the attachment of radio-transmitters. Blood samples were removed from the internal region (palmar side) of the pectoral fin, with the animals restrained on a stretcher. Butterfly needles (Scalp 19 G) attached to a syringe were inserted between the radius and the ulna, according to GERACI & LOUNSBURY (1993). Approximately 10ml of blood were collected from each animal, and then transferred to a test tube containing 6 drops of sodium heparin anticoagulant. In order to separate the plasma, the blood samples were centrifuged at 3000 rpm for 10-15 minutes.

The blood samples obtained from the captive manatees were collected on four different occasions

(Table 1). On the first one, only the hematocrit (Ht) and hemoglobin (Hb) concentrations were determined. The hematocrit levels were obtained by centrifuging the samples in micro-capillary tubes containing heparin. The hemoglobin concentration was determined by the cyanmethemoglobin method, using Drabkin's solution. On the second collection, the mineral contents were also determined in addition to hematocrit and hemoglobin. For this, 0.3 ml of serum was treated with 0.9 ml of 6.7% trichloroacetic acid (TCA), and following centrifugation, the mineral contents were analysed using an atomic absorption spectrophotometer (AAS). On the third blood collection of captive manatees, the mineral serum concentrations were determined by simple dilution (1 : 4) of the serum with de-ionized water and the mineral content directly analysed in the AAS. Finally, on the fourth collection of the captive animals, only the hematocrit concentrations were determined. For the wild manatees, the serum mineral levels were determined by simple dilution with de-ionized water as described above for the captive manatees (Table 1).

The serum samples of the captive and wild manatees were analysed for zinc, copper, magnesium, iron, sodium, potassium and calcium. The mean concentrations (and standard deviations) were calculated for male and female manatees according to the following groups: treatment with TCA (Group 1), simple dilution (Group 2), and wild manatees (Group 3) (Table 2). The results were submitted to 2-sample t-test analysis to determine any significant differences between the means of different sexes in the same groups and between the same sexes of different groups.

For purposes of comparison, the hematocrit and hemoglobin levels obtained from the blood samples collected on the first occasion were grouped with those obtained in the second collection, and the comparisons of mineral concentrations between wild and captive manatees were carried out between Group 2 and Group 3, which underwent the same preparations for mineral analyses. In order to determine the effect of an improved diet on the hematocrit values, the data obtained for the captive Amazonian manatees in 1992-93 were compared with the values obtained for the same animals in samples collected in 1995 (Table 3).

Results

The results (Table 2) show that the mean hematocrit value of the captive females in Group 1 was significantly greater ($P < 0.01$) than that of the captive males in the same group. The average hemoglobin, copper and sodium concentrations did not show any significant differences either between the different sexes of the same groups, or between the same sexes of different groups. The mean zinc concentrations of the captive males and females in Group 1 were significantly greater than those of the captive males ($P < 0.02$) and females ($P < 0.01$), respectively, in Group 2. The mean zinc level of the wild male manatees (Group 3) was also significantly greater than that of the captive males in Group 2 ($P < 0.05$) and that of the wild females ($P < 0.05$). In the case of magnesium, iron and calcium, the average levels of the wild male manatees were significantly greater than those of the captive males in Group 2 ($P < 0.01$, $P < 0.001$ and $P < 0.01$, respectively) and those of the wild females ($P < 0.05$, $P < 0.01$ and $P < 0.02$, respectively). The mean potassium level of the wild male manatees was significantly greater ($P < 0.02$) than that of the captive males of Group 2 (Table 2).

The hematocrit values increased in all the 6 manatees which had this blood parameter analysed, after increasing the proportions of lettuce and cabbage in their diet towards the end of the study (Table 3).

Discussion

Reduced levels of hematocrit and hemoglobin reflect a long period of iron deprivation. Therefore, despite a lack of significant difference between the average hematocrit values of the captive and wild male manatees, the greater absolute mean value of the wild males would suggest a slight case of iron deficiency in the captive males.

The significantly greater average zinc value of the wild male manatees, compared with that of the wild females, may be due to a difference between the sexes, since neither of the wild female manatees was knowingly lactating at the time of the sample collection. If it is, this could explain why the captive males were significantly zinc-deficient, while the captive females were not.

With regard to copper, even though there is

not any significant difference between the captive and wild manatees, the mean absolute values of the latter were greater than those of the captive ones, which can probably be solved with a slight diet complementation.

As far as magnesium is concerned, the significant difference between the average values of the captive and wild males reveals a definite deficiency of this mineral in the captive males' diet. Also, the significant difference between the mean values of the wild male and female manatees suggests, once again, a difference between the sexes. There was no significant difference between wild and captive female manatees, although the mean absolute value of the wild females was greater than that of the captive ones.

In relation to iron, the significantly greater mean value of the wild male manatees and the greater average absolute value of the wild females, compared with the captive male and female manatees, respectively, suggests iron deficiency of the captive male animals. However, since the mean hematocrit values were not significantly different between the captive and wild males, this suggests that the captive males were only marginally deficient in iron. This is confirmed by the improved hematocrit values of six captive manatees after being submitted to an improved diet (Table 3). As with zinc and magnesium, the significant differences between the values of the wild male and female manatees may be due to a difference between the sexes.

As to sodium, there was no significant difference between wild and captive manatees. Even the absolute mean values were very close, which suggests that the captive ones had an adequate sodium intake.

With regard to potassium, the significantly greater average value of the wild male Amazonian manatees, compared with the captive males, implies a deficiency of this mineral in the latter's diet. Although there was no significant difference between the captive and wild females, the greater absolute average value of the wild females may also suggest a need for food supplementation for the captive females.

The significantly greater average calcium value of the wild male manatees, compared with the captive males, suggests, once again, that the latter are deficient in this mineral. The significant difference obtained between the wild male and female manatees may, again, be due to a difference between the sexes.

In conclusion, it would appear that on the whole, the captive male manatees were very deficient in zinc, magnesium, iron, potassium and calcium, and marginally deficient in copper. The captive females, on the other hand, appear to be only marginally deficient in copper, magnesium, iron, potassium and calcium. This greater degree of deficiency in the captive males appears to be due to a difference between the sexes, as revealed by the wild manatees in this study.

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Literature Cited

- BEST, R.C.; RIBEIRO, G.A., YAMAKOSHI, M. & da SILVA, V.M.F. Artificial feeding for unweaned Amazonian manatees. *International Zoo Yearbook*, v. 22, p. 263-267. 1982.
- BOSSART, G.D. & DIERAUF, L.A. Marine Mammal Clinical Laboratory Medicine. Pages 1-52. In: DIERAUF, L.A. (ed.). *CRC Handbook of Marine Mammal Medicine: Health, Disease, and Rehabilitation*. Boca Raton, Florida: CRC Press, 1990. 735 p.
- BULLOCK, T.H.; DOMNING, D.P. & BEST, R.C. Evoked brain potentials demonstrate hearing in a manatee (*Sirenia:Trichechus inunguis*). *Journal of Mammalogy*, v. 61, p. 130-133. 1980.
- COLARES, E.P. Blood parameters of the Amazonian manatee (*Trichechus inunguis*) - dietary variation. *Comparative Biochemistry and Physiology*, v. 103A, p. 413-415. 1992.
- CONVERSE, L.J.; FERNANDES, B.S.; MACWILLIAMS, P. & BOSSART, G.D. Hematology, serum chemistry, and morphometric reference values for Antillean manatees (*Trichechus manatus manatus*). *Journal of Zoo and Wildlife Medicine*, v. 25, p. 423-431. 1994.
- GALLIVAN, G.J. & BEST, R.C. Metabolism and respiration of the Amazonian manatee (*Trichechus inunguis*). *Physiological Zoology*, v. 53, p. 245-253. 1980.
- GALLIVAN, G.J.; BEST, R.C. & KANWISHER, J.W. Temperature regulation in the Amazonian manatee (*Trichechus inunguis*). *Physiological Zoology*, v. 56, p. 255-262. 1983.
- GERACL, J.R. & LOUNSBURY, V.J. *Marine Mammals Ashore. A field guide for strandings*. Galveston: Texas A&M Sea Grant Publication, 1993. 305 p.
- MEDWAY, W.; BRUSS, M.L.; BENGTON, J.L. & BLACK, D.J. Blood chemistry of the West Indian manatee (*Trichechus manatus*). *Journal of Wildlife Diseases*, v. 18, p. 229-234. 1982.
- ROSAS, F.C.W. Crescimento de filhotes de peixe-boi da Amazônia (*Trichechus inunguis*) criados com leite artificial. *Abstracts V Reunión de Trabajo de Especialistas en Mamíferos Acuáticos de América del Sur*. Buenos Aires, 1992. p. 59.
- ROSAS, F.C.W. Biology, Conservation and Status of the Amazonian Manatee *Trichechus inunguis*. *Mammal Review*, v. 24, p. 49-59. 1994.

Tabela 1. Sampling dates, type of analysis and treatment of blood and serum of captive and wild Amazonian manatees.

Captive	Sampling Dates		Ht	Hb	Serum Treatment	
	Wild				TCA	Simple Dilution
MAR/92			X	X	---	---
JAN/93			X	X	X	---
AUG/93			X	---	---	X
JUL/95			X	---	---	---
		JUN/94	X	X	---	X

Table 2. Average and standard deviation values of hematocrit (Ht), hemoglobin (Hb) and mineral content of the serum of captive (Groups 1 and 2) and wild (Group 3) Amazonian manatees.

	Group 1			Group 2			Group 3			
	(x±sd)	n	(x±sd)	n	(x±sd)	n	(x±sd)	n	(x±sd)	n
Ht(%)	26.7±3.2 ⁺	9	30.2±8.5 ⁺	5	26.0±1.4	2	26.5±0.7	2	36.0±1.4	2
Hb(g%)	12.4±1.8	9	14.1±1.8	6	----	----	----	----	12.2±0.8	3
Zn(ppm)	2.1±0.2 [*]	3	2.5±0.4 ⁺	5	1.3±0.1 ^o	2	1.2±0.1 ⁺	2	3.4±0.7 ^o	3
Cu(ppm)	----	-	----	-	0.24±0.1	2	0.15±0.01	2	0.32±0.2	3
Mg(ppm)	30.2±2.1	3	33.1±4.0	5	16.8±0.6 ⁺	2	15.7±2.4	2	41.8±5.3 ^o	3
Fe(ppm)	0.37±0.1	3	0.41±0.3	5	1.1±0.0 [*]	2	1.3±0.1	2	2.4±0.1 ^{**}	3
Na(ppm)	2411±82.5	3	2411±102.3	5	1890±127.3	2	1830±381.8	2	1869±36.2	3
K (ppm)	261.3±118.8	3	233±19.7	5	145.5±3.5 [*]	2	140.5±33.2	2	287.3±35.5 [*]	3
Ca(ppm)	95.7±20.4	3	95.4±14.2	5	41.8±3.1 ⁺	2	39.9±8.9	2	125.5±11.7 ^{**}	3

Significance values:
^o = p<0.05 * = p<0.02 + = p<0.01 ♦ = p<0.001

Table 3. Hematocrit changes of captive manatees due to dietary improvements.

Animal #	Sex	1992/93	1995
		Ht	Ht
1	F	28%	31%
2	M	28%	33%
3	M	29%	32%
4	M	26%	27%
5	F	28%	29%
6	F	26%	35%