EFFECT OF DIFFERENT PROTEIN LEVELS ON THE PERFORMANCE AND APPARENT PROTEIN DIGESTIBILITY OF ORPHAN CALVES OF AMAZONIAN MANATEES (*Trichechus inunguis*)

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Abstract

The objective of this study was to evaluate the effect of four dietary crude protein (CP) levels, provided by mixing two commercial milk replacers, on the performance and protein apparent digestibility of captive orphan calves of Amazonian manatees (*Trichechus inunguis*). Four individuals (two males and two females), with an average weight of 28.95 kg and 8.75 months-old in average were used. The dietary treatments were: Diet 1 (7.22 g CP), Diet 2 (9.77 g CP), Diet 3 (12.25 g CP) and Diet 4 (14.88 g CP). Each animal received one of each diet during 14 d period with a 7 d adaptation period between diets. Feed intake, weight gain and feed conversion were recorded. Chromium oxide was used to determine apparent protein digestibility; additionally, the protein content of manate's milk was determined. Registered data was analyzed under 4x4 Latin Square Design and mean comparisons were performed using the statistical software IBM SPSS 24.0. The results showed that feed intake and weight gain were not influenced ($P>0.05$) by the dietary treatments; however, the lowest feed conversion corresponded to Diet 3, which was different ($P<0.05$) from Diet 1 but it was not ($P>0.05$) from D2 or Diet 4. The highest apparent protein digestibility corresponded to Diet 4 and the protein content of manatee's milk was 9.70%. In conclusion, orphan calves of manatees can be fed with different proportions of commercial milk replacers without affecting their performances.

Key words: Amazonian manatee, performance, protein, digestibility.

Introduction

The Amazonian manatee is an aquatic herbivorous mammal belonging to the Sirenia Order, of high ecological value as it contributes to the fertilization of water to produce plankton. Endemic species of the Amazon watershed (Brazil, Colombia, Ecuador and Peru) *(Best, 1984; Rosas, 1994; Domming, 1981; Timm et al., 1986; Rosas, 1991)* of sweet water as exclusive habitat *(Best, 1984; Husar, 1977; Rosas, 1994).*

There are several risk factors that threaten its current population by placing it in Appendix I of CITES (The Convention on International Trade in Endangered Species of Wild Fauna and Flora) *(Soini et al., 1996; Silva & Montes, 2014; MINAM, 2014)* and in a vulnerable situation by IUCN (International Union for Conservation of Nature) *(IUCN, 2016)*. Currently, in Peru, it is considered as a "protected Amazonian species" under the Peruvian Amazonian Regulation of Fishing Regulations *(Ministerial Resolution Nº 147-2001-PE, 2001)* and cataloged "in danger of extinction at national level" *(Supreme Decret Nº 034-2004-AG, 2007).*

Hunting has led to an increase in the number of orphan infant offspring, establishing rescue and conservation strategies *(Soto, 2007)*; being necessary the investigation at nutritional level and to establish requirements of the animal for the appropriate alimentary management in captivity in rescue centers. The few reports of this species in the nutritional field have been evaluations of diets adjusting to the nutritional content of breastmilk *(Best et al., 1982; Rodriguez Chacon et al., 1999; Barbosa, 2011, Maduro, 2014*); all with the aim of approaching the nutritional requirements of the species, which are still unknown.

Among the different nutrients, the protein is a macronutrient that stands out for its great importance in tissue regeneration, muscle mass development, enzyme production and hormones; know the protein requirement of a species would not only allow an adequate development of the animal but also make an adequate contribution of this nutrient in the diet, avoiding nutritional deficiencies, anemia and excess protein supplied which would increase the nitrogen removed in urine and feces favoring the eutrophication of the rivers where the animal lives.

The objective of the present study was to evaluate the nutritional profile of the Amazonian manatee (*Trichechus inunguis*) lactating in captivity, fed with four different levels of protein in the diet, considering the productive performance, digestibility and nutritional composition of milk; as a reference of the protein requirement of the species.

Materials & Methods

Experimental animals and location

A total of 4 lactating manatees (2 males and 2 females), with an average weight of 28.95±1.36 kg and an age of about 8.75 months; previously identified, were randomly distributed in individual pools provided with shade and a water filtration system. The trial was carried out at the facilities of the Amazon Rescue Center (CREA) in the city of Iquitos-Peru **(Figure 1 - 2)**.
The daily management consisted of monitoring the ambient temperature and water, where it was maintained at a temperature of not less than 23 °C.

Treatments

Four diets were evaluated, being a mixture in different proportions of two lactose-free dairy substitutes, to obtain the proposed nutritional contribution. The milk replacers used were a specialized formula (ZMM) and another commercial formula (NLF). The proportions of the milk replacer and the calculated crude protein content for each diet are presented in the **Table 1**.

The study was separated into 4 experimental periods of 14 days, with intervals of 7 days of adaptation between each experimental period. During each experimental period the four diets were evaluated in a different animal **(Figure 3).** Feeding was performed daily using the following feed protocol:

-During the evaluation periods 1 and 2, 1 liter per animal (4 ration of 250 ml/ration/day).

-During periods 3 and 4 increased supply to 1.5 liters per animal (6 ration of 250 ml/ration/day) in response to the increased requirement of consumption for the growth of animals. The amount of feed offered and residual was measured with a graduated cylinder ± 1 ml and of 250 and 50 ml of capacity **(Figure 4).**

Response variables

Zootechnical performance

At the beginning of the experiment, the individual weight of the 4 infants was recorded before the consumption of the diet to be evaluated. Throughout the experiment the weight at the beginning and end of each experimental period was recorded with an electronic balance of 200 kg of capacity and ±1 g of sensibility. The variables obtained from each diet per period (14 days) were: body weight gain (BWG), total feed intake and feed conversion rate (FC) **(Figure 5)**.

Apparent protein digestibility

The digestibility observational evaluation was performed using an inert marker of chromium oxide (Cr_2O_3), added in each diet. Between the fourth and fifth evaluation days of the first and third periods, 1.19% of chromic oxide (0.5 g) in dry matter was added for one day in each nipple **(Figures 6 - 8).**

For a significant sample collection, animals should be continuously monitored to avoid feces samples being lost as they disintegrate in the water or that the animals limit an optimal collection by habit of coprophagy. The difference between the consumed and the excreted determined the coefficient of apparent digestibility.

The dry matter digestibility was calculated through the following equations (Harshaw, 2012):

$$\text{DMD diets} = 100 - (100 * \frac{\text{\% Cr2O3 in feed}}{\text{\% Cr2 O3 in feces}})$$

$$\text{DMD nutrients} = 100 - (100 * \frac{\text{\%nutrient in feces x \% Cr2O3 in feed}}{\text{\%nutrient in feed x \% Cr2 O3 in feces}})$$

The representative samples of the diets offered were collected at the end of the experiment, taking total samples of 100 g for each diet evaluated. Samples from the diets and feces collected from each diet were stored in airtight bags and transported for determination of moisture content, dry matter and protein (Kjeldhal, 1883), In addition the content of chromium in feces was determined **(Figure 9).**

Milk sample collection

In the semi-upland area of the CREA, fishing net was used to carry out the physical containment of the lactating mother **(Figure 10 - 14).** Once near the shore, the animal was placed on a care stretcher. The animal under observation was a first time it was found in its second semester of lactation. After disinfecting the mammary gland, the animal was milked manually, placing the sample in a recent sterile autoclavable 100 ml capacity **(Figure 15 - 16).** After the milk collection, the sample vessel was placed in a cooler to be transported and stored in refrigeration at 4° C until its analysis of protein content.

Statistical analysis

A Latin Square Design (LSD) was used with four treatments and four replicates per treatment (4x4). The data were subjected to a normality analysis and subsequently to a one-way ANOVA by the general linear model (GLM) procedure and the comparison of means of the diets for the final weight, weight gain, feed intake and feed conversion rate by the Duncan test. A p-value less than 0.05 were considered as a level of significance. The statistical software IBM SPSS 24.0 was used.

Results

Zootechnical performance

La **Table 2** describes the results obtained by the Duncan test, in which no significant differences were observed between the four diets regarding the final weight, weight gain and food consumption. In contrast, a significant difference was found in cumulative feed conversion ($p<0.05$). Being the diet 3 of minor feed conversion (6.22) and diet 1 the highest feed conversion (9.16).

Diet 3 presented higher food efficiency in reference than the others, reducing the consumption of food and increasing the weight of the animal.

Apparent protein digestibility

In **Table 3** it can be see the reported of observational results in the total protein and dry matter digestibility of the four diets evaluated.

High digestibility of dry matter (63.64-84.27%) and total protein (77.08-94.62%), nutrient of greater utilization of the lactating animal were obtained. An observational comparison of the different diets suggests that Diet 4 presented the highest digestibility of dry matter and crude protein.

Analysis of protein content of milk.

It was observed that the Amazonian manatee milk has a liquid appearance, slightly creamy, homogeneous and quite viscous with an opaque white coloration. When the collected milk sample was left standing, the formation of a layer of fat on the surface could be observed. An average of 9.70% protein content of the Amazonian manatee milk sample was obtained in the second semester of lactation.

Discussion

Zootechnical Performance

In the weight gain, a range of 0.9-1.21 kg / week was obtained. This agrees with the average weekly weight gain reported in previous studies performed in similar conditions developed by Best et al. (1982), Rodriguez Chacon et al. (1999), and Maduro (2014). Although there was no significant statistical difference in body weight gain between the diets, it was observed that diet 3 (2.42 kg per 14 days) showed some improvement in the body weight gain, being more than half a kilo higher than the weight gain obtained by diet 1 (1.80 kg/period), which was the diet that presented the lowest weight gain.

The resulting daily feed intake was 1.05-1.11 kg of milk per animal, which was much lower than reported by Best et al. (1982). However, authors such as Rodriguez Chacon et al. (1999a) and

Maduro (2014), also obtained daily food consumption lower than that reported by Best et al. (1982), being slightly closer to those presented in the present study. Although the average food consumption was lower than previously reported, weight gains were similar to the studies mentioned above. It is suggested that this would be because inputs from the diets supplied were possibly more usable than the inputs used in the dairy formulas of previous studies.

Water temperature is an important factor that can affect food consumption, temperatures lower than 20 °C and higher than 31 °C results in depression and/or loss of appetite in infants, which are more susceptible that adults (Vanoye, 2002). In the present study, the water temperature and pH were recorded, in which no variations were observed that could affect the test, obtaining an average ambient temperature of 28.8 °C and average water temperature of 27.4 ± 0.5 °C, which was Within the temperature range of comfort for the young.

The feed conversion obtained in the present work from 6.22 to 9.16 was lower than that reported in previous studies by Rodriguez Chacon et al. (1999a) and Maduro (2014). In this aspect, significant statistical differences were found, being the Diet 3 the one of better feed conversion ($p<0.05$) suggesting a better dietary efficiency of the diet. With the provision of a more nutritious food, the frequency of feeding during the day can be reduced, reducing human contact with the animals, which could have an influence on the reintroduction programs of these animals in the wild.

Apparent protein digestibility

Manatees appear to be more efficient than other wild mammals in their ability to digest protein, with the cecum and colon being the primary sites where manatees digest the protein and lipid components of the feed (Burn, 1986). This was evidenced by the fact that the average protein content in feces (13.74%) was lower by approximately 47.68% with respect to the average crude protein content of the diet (26.26%). The crude protein content in the feces of the four diets evaluated presented independent variations of the crude protein content of the diet. Crude protein content in feces was expected to increase as crude protein intake from diets increased, however, Diets 2 and 4 had lower crude protein values in feces (10.57% and 12.11%, respectively) compared with Diet 3, which showed a higher value (17.06%) despite having a lower contribution than diet 4. This could be due to a different to other factors such as digestibility and amino acid profile of different diets, but not the amount of crude protein; which was evidenced when determining the protein digestibility coefficient of each of the diets, obtaining for the Diets 2 and 4 the highest coefficients of protein digestibility (85.59% and 94.62%), respectively.

In a direct comparison, Diet 4 showed the highest protein digestibility coefficient (94.62%), however, it did not present the best productive performance suggesting that it has highly digestible protein sources, but does not take into account the bioavailability of the amino acid profile of diets. Among the four diets evaluated, diet 4 had the highest dry matter digestibility coefficient (84.27%), which is similar to the range (68-82%) reported by Harshaw (2012) in adult *T. manatus latirostris*. However, a high digestibility is not necessarily an indicator of a suitable diet for the species, since its nutritional content could be highly fermentative causing changes in the gastro-fecal pH, alteration of the intestinal biota, accelerated growth rhythm with alterations such as obesity if frequent monitoring is not (Harshaw, 2012).

In another context, the present preliminary digestibility assessment remains a matter of study, in response to the scarce bibliographic information available. The high values of apparent digestibility reported by Harshaw (2012) and the present study is not conclusive evidence of the physiological condition of the animal or of the feed offered, since the species practices the coprophagy habit as a strategy to maximize nutrient utilization (Rodriguez Chacon et al., 1999b), a factor that estimates the digestibility, so it is suggested to make adjustments in the method of evaluation of digestibility.

Analysis of protein content of milk

The difficulty in obtaining sufficient milk volume for analysis occurred in previous studies performed with *T. manatus* by Bachman & Irvine (1979) and Vergara et al. (2000). Likewise, the observed physical characteristics are in agreement with previous studies carried out with manatee milk by Barbosa (2011), for *T. inunguis*; and Pervaiz & Brew, (1986), for *T. manatus latirostris.*

The protein content of breast milk obtained in the present study (9.7%) was found within the range of 4.24-10.47% previously reported by the only previous report in this species, carried out by Barbosa (2011). When comparing with similar studies performed on the two subspecies of the marine manatee, one can observe slight superiority to the protein content of Florida manatee milk (*Trichechus manatus latrirostris*) (6.9-9.0%; 9.65% y 9.7%) reported by Bachman & Irvine, 1978; Pervaiz & Brew, 1986 and Worthy, 1990, respectively; also presents great superiority with the protein content of the milk described for the manatee of the Antilles (*Trichechus manatus manatus*) who indicates a value of 5.25% (Vergara et al., 2000). The difference in protein content in addition to being influenced by the species may be related to different factors such as: type of food offered to the animals in captivity, conditions of the animal from which the sample was extracted (live or dead animal), conditions In which the animal lives (in captivity or in free life) and stage of lactation since previous studies have shown decreasing levels of total lipids and proteins with the increase of the lactation phase (Pervaiz & Brew, 1986; Eichelberger et al., 1940).

Previous studies of the protein content of milk from various aquatic mammals have reported the following data: baleen whale (*Suborder Mysticeti*) with 9-15% and toothed whale (*Suborder Odontoceti*) with 8-11% (Oftedal, 1997); pink river-dolphin (*Inia geoffrensis*) with 9.6% (Rosas & Lehti, 1996), bottlenose dolphins (*Tursiops truncatus*) with 12.2% (Pervaiz & Brew, 1986), spotted dolphin (*Prodelphinus plagiodon*) with 9.4% (Eichelberger et al., 1940), humpback dolphin (*Sousa plumbea*) with 11.30% and common dolphin (*Delphinus delphis*) with 10.30% (Peddemors et al., 1989); respectively. The range of protein content in breast milk in other cetaceans varies from 7.1 to 12.8%. When comparing the milk protein content of the Amazonian manatee (*Trichechus inunguis*) with the aquatic mammals mentioned above a high approximation between values can be observed, evidencing one of the most common characteristics of the milk of this group of animals: the high protein content in milk (Jenness and Sloan, 1970).

The transfer of protein for breeding is much greater than that given in other terrestrial herbivorous mammals such as the Brazilian tapir (*Tapirus terrestris*) with a content of 4.4% (Jensen, 1995), mountain zebra (*Equus zebra*) with 1.6% protein (Jensen, 1995), and domestic animals with 3.5 and 3.3% protein in cattle (FAO, 2017) and domestic goats (Boza & Sanz, 1997), respectively. For which, Barbosa (2011) explains that the higher concentration of protein in aquatic mammals could be due to the increased need for oxygen retention for diving and proteins are essential nutrients for the formation of structures and even blood cells responsible for the transport of oxygen.

When comparing the four diets evaluated with breast milk in relation to protein content, productive performance and digestibility, it was observed that Diet 3 (12.25 g CP) presented the best feed consumption and feed conversion, being caused by the slightly higher protein intake, it was also observed that Diet 4 (14.88 g CP), despite presenting a high digestibility of the protein, was not accompanied by the better productive performance, probably due to the profile of amino acids and to the excess of protein comparing with the one of breast milk.

Conclusions

1. With the exception of feed conversion, the zootechnical performance of the experimental animals was not significantly influenced by the dietary treatments.
2. The apparent protein digestibility varied from 77.08 to 94.62 %, and the highest value corresponded to Diet 4 (14.88 g CP).
3. The crude protein content of Amazonian manatee´s milk was 9.70%

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Literature Cited

Bachman KC, and Irvine AB (1979) Composition of milk from the florida manatee, *Trichechus latirostris*. *Comparative Biochemistry and Physiology* 62: 873–878.

Barbosa, PDS (2011) Composição Quimica do Leite de Peixe-boi da Amazônia (*Trichechus inunguis*, NATTERER 1883) em cativeiro nos diferentes estágios de lactação. Master Thesis. Instituto Nacional de Pesquisas da Amazônia-INPA. 19-26.

Best RC, Ribeiro GA, Yamakoshi M, and Da Silva VMF (1982) Artificial feeding for unweaned Amazonian manatees *(Trichechus inunguis). International Zoological Yearbook* 22:263-277.

Best RC (1984). The aquatic mammals and reptiles on the Amazon. In H. Sioli (Ed.), The Amazon: Limnology and landscape ecology of a mighty tropical river and its basin. Dordrecht, Netherlands.

Boza J, and Sanz (1997) Aspectos Nutricionales De La Leche De Cabra. *Agronomía Mesoamericana* 16: 239–252.

Burn DM (1986) The digestive strategy and efficiency of the west indian manatee, *Trichechus manatus*. *Comparative Biochemistry and Physiology -- Part A: Physiology* 85: 139–142.

Decreto Supremo Nº 034-2004-AG (2007). Aprueban categorización de especies amenazadas de fauna silvestre y prohíben su caza, captura, tenencia, transporte o exportación con fines comerciales.

Domning DP (1981) Distribution and Status of Manatees *Trichechus* spp. Near the mouth of the Amazon River, Brazil. Biol. Conserv 19: 85-97.

Eichelberger L, Fletcher ES, Geiling EMK, and Vos BJ (1940) The composition of dolphin milk. *J. Biol. Chem* 134: 171-176.

Harshaw, LT (2012) Evaluation of the Nutrition of Florida Manatees (*Trichechus manatus latirostris*). Doctoral Thesis, University of Florida. 57-67.

Husar SL (1977) *Trichechus inunguis. The American Society of Mammalogists* 72: 1-4

[IUCN] The International Union for Conservation of Nature (2016). Red List of Threatened Animals. http://www.iucnredlist.org/ Accessed January 3, 2017.

Jensen RG (1995) Handbook of Milk Composition. San Diego, California: Academic Press.

Maduro, AHP (2014) Perfil Metabólico e Desempenho Zootécnico de Filhotes de Peixe-boi da Amazônia (*Trichechus inunguis*) mantidos em cativeiro. Alimentados com diferentes Sucedâneos do Leite materno. Master Thesis, Instituto Nacional de Pesquisas da Amazônia-INPA. 54-71.

[MINAM] Ministerio del Ambiente (2014) Especies de Fauna Silvestre Peruana en los apéndices de la CITES, (CoP 16).

Oftedal OT (1997) Lactation in Whales and Dolphins: Evidence of Divergence between Baleen and Toothed Species. *Mammary Gland Biology and Neoplasia* 2: 205-230.

Peddemors VM, De Muelenaere HJH, and Devchand K (1989) Comparative milk composition of the bottlenosed dolphin (*Tursiops truncatus*), humpback dolphin (*Sousa plumbea*) and common dolphin (*Delphinus delphis*) from southern African waters. *Comparative Biochemistry and Physiology* 94: 639–641.

Pervaiz S, and Brew K (1986) Composition of the milks of the bottlenose dolphin (*Tursiops truncatus*) and the florida manatee (*Trichechus manatus latirostris*). *Comparative Biochemistry and Physiology* 84: 357–360.

Resolución Ministerial Nº 147-2001-PE (2001). Aprueban el Reglamento de Ordenamiento Pesquero de la Amazonía Peruana.

Rodriguez Chacon ZM, Da Silva VM, and Neto JA (1999) Teste de Fórmula Láctea na Alimentação de filhotes órfãos de Peixe-boi da Amazônia (*Trichechus inunguis*). In: Instituto Nacional de Pesquisas da Amazônia. Conservação e manejo do peixe-boi da Amazônia (*Trichechus inunguis*) em cativerio. pp 214-218.

Rosas FCW (1991) Peixe-boi da Amazonia *Trichechus inunguis* (Natterer, 1883). In: *Estado de Conservación de los Mamíferos Marinos del Atlántico Sudoccidental*. Ed. Capozzo, H.I., & Junin, M. Informe y estudios del Programa de Mares Regionales del PNUMA, No 138. pp 178-181.

Rosas FCW (1994) Biology, conservation and status of the Amazonian manatee *Trichechus inunguis*. *Mammal Review* 24: 49-59.

Rosas FCW, and Lehti KK (1996) Nutritional and mercury content of milk of the Amazon River dolpin, *Inia geoffrensis*. *Comparative Biochemistry and Physiology* 115: 117–119.

Silva J, Montes D, and Elías R (2014) Conocimientos , conservación y avistamiento del manatí amazónico (*Trichechus inunguis*), según los pobladores de la cuenca del río Ucayali (Loreto, Perú). *Salud tecnol. Vet* 2: 32–38.

Soini P, Sicchar LA, Gil GG, Fachín AT, Pezo R, Chumbe MA (1996) Una evaluación de la fauna silvestre y su aprovechamiento de la Reserva Nacional Pacaya Samiria, Perú. Documento Técnico *Instituto de Investigaciones de la Amazonía Peruana - Iquitos* 24: 64.

Soto A (2007) Caza del manatí amazónico en la Reserva Nacional Pacaya Samiria, Perú. Centro de Datos para la Conservación, Universidad Nacional Agraria La Molina. pp: 23.

Timm RM, Albuja LV, and Clauson BL (1986) Ecology, Distribution, Harvest, and Conservation of the Amazonian Manatee *Trichechus inunguis* in Ecuador. *Biotropica* 18: 150-156.

Vanoye, FFL (2002) Constantes Hematicas para crías de manatí (*Trichechus manatus manatus*) de hasta dos años de edad en cautiverio. Undergrade Thesis, Universidad Veracruzana. 4-11.

Vergara JE, Parente CL, Sommerfeld PA, and Lima RP (2000). Estudo da composição do leite do peixe-boi marinho (*Trichechus manatus manatus* LINNEAUS 1856) no Nordeste do Brasil com inferências para uma dieta artificial. *Ciências Veterinária Nos Trópicos* 3: 159–166.

Worthy GAJ (1990) Nutritional Energetics for Marina Mammals; Addendums, in: CRC Handbook of marine mammal medicine: health, disease, and rehabilitation. CRC Press, Boston.

Jenness R, and Sloan RE (1970) The composition of milks of various species: a review. *Dairy Sci.* Abstr. 32: 599-612.

Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO) (2017). Producción y productos lácteos: Composición de la leche. http://www.fao.org. Accessed April 16, 2017.

Table 1. Composition and crude protein content of the experimental diets.

Item	Diet			
	1	2	3	4
ZMM %	25	50	75	100
NLF %	75	50	25	0
Mix of ZMM + NLF fed/animal/day, g	42	42	42	42
Crude protein of ZMM + NLF Mix, %	17.19	23.25	29.16	35.43
Amount of crude protein fed/animal/ day, g	**7.22**	**9.77**	**12.25**	**14.88**

Table 2. Mean initial body weight, final weight, body weight gain, feed intake and cumulative feed conversion rate of the four experimental diets in 14 days of evaluation.

MEASUREMENTS[1]	DIET[2]			
	1	2	3	4
Final body weight, kg	34.17 [a]	34.16 [a]	34.54 [a]	34.68 [a]
Body weight gain 14 d-period, kg	1.80 [a]	2.28 [a]	2.42 [a]	2.16 [a]
Total feed intake in 14 d-period, kg	15.34 [a]	15.57 [a]	14.73 [a]	14.69 [a]
Feed conversion (FC)	9.16 [a]	6.95 [ab]	6.22 [b]	7.38 [ab]

[a,b] Different letters in the same row are statistically different values (p <0,05).
[1] Values for each diet represent the average of the 4 replicates.
[2] Diets: D1 (7.22 g CP), D2 (9.77 g CP), D3 (12.25 g CP) and D4 (14.88 g CP).

Table 3: Dry matter and crude protein digestibility of the four diets evaluated.

Diet[1]	Digestibility, %	
	Dry matter	Crude Protein
1	74.13	77.08
2	63.90	85.59
3	63.64	78.73
4	84.27	94.62

[1] Diet: D1 (7.22 g CP), D2 (9.77 g CP), D3 (12.25 g CP) and D4 (14.88 g CP).

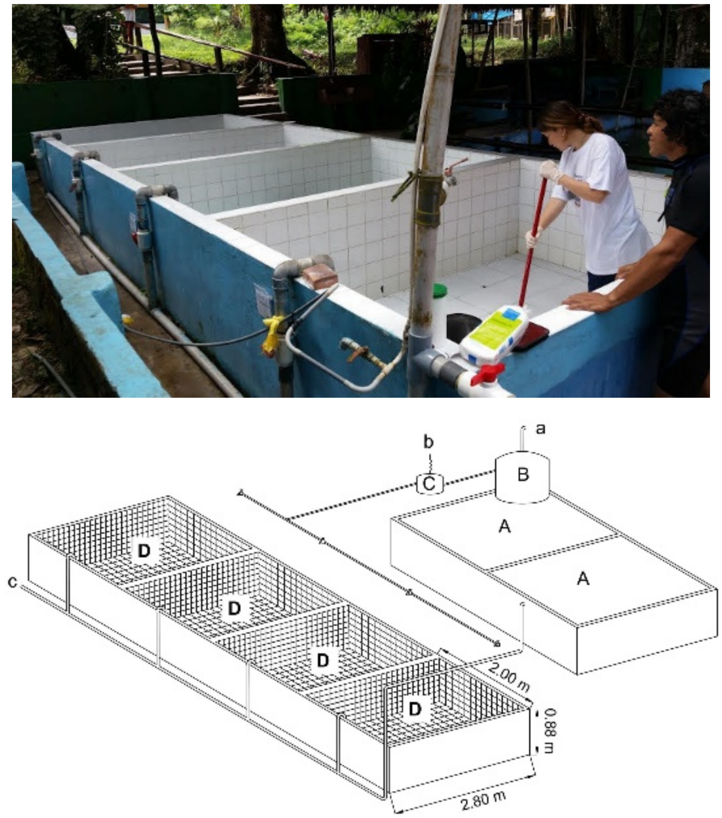

Figure 1: Facilities of the Quarantine Zone of the Amazon Rescue Center (4 pools of 2.79 m long x 2.01 m wide x 0.88 m deep). And schema of distribution in the area (A: water reserve pools; B: treated tank for the water filtration system; C: water thermoregulation thermos; D: individual pools; a: water supply; b: electrical supply: c: lead of water.

Figure 2: System for filtering and renewing water from a treated tank (B).

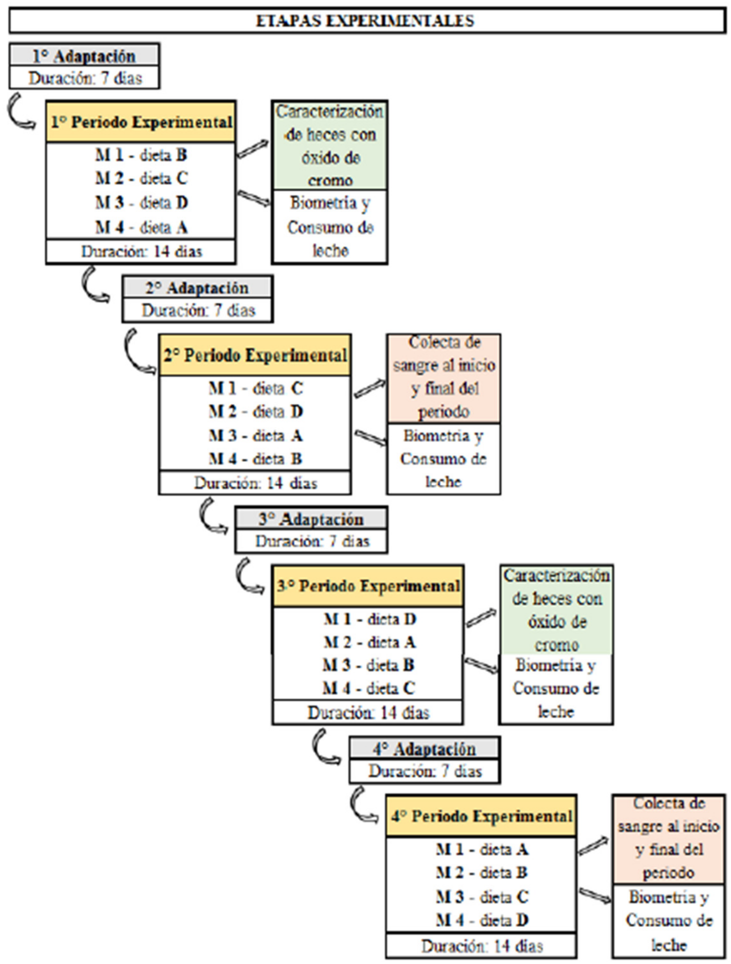

Figure 3: Scheme of the stages of the experiment, duration of the periods and activities.

Figure 4: Nipples and feeding bottles used for the daily feeding of animals.

Figure 5: Periodical and individual weighing of animals by physical condition.

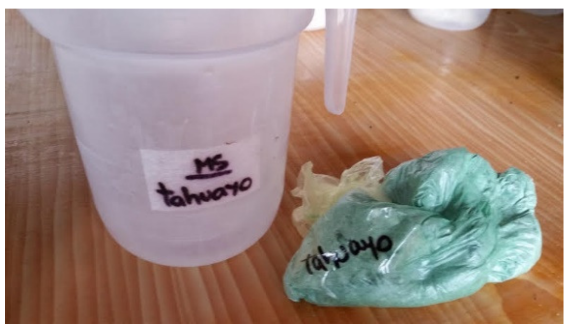

Figure 6: Use of chromic oxide as an external marker and mixed "in dry" with dietary treatments.

Figure 7: Chromic oxide mixed and diluted in the respective nipples.

Figure 8: Milk replacers offered with the external marker.

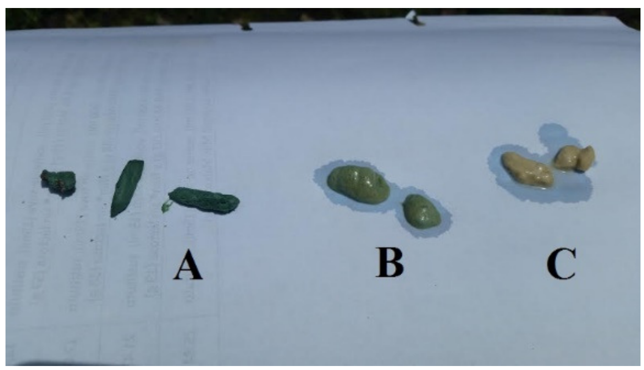

Figure 9: Difference in coloration of marked (A), partially marked (B) and unmarked feces (C).

Figure 10: Artificial body water in the Zone of Semi-captive.

Figure 11: Displacement of the manatee mother towards the riverside for the its subjection.

Figure 12: Cave feeding with breast milk in semi captivity.

Figure 13: Physical containment of the lactating mother.

Figure 14: Manual milking of the animal under evaluation on the care stretcher.

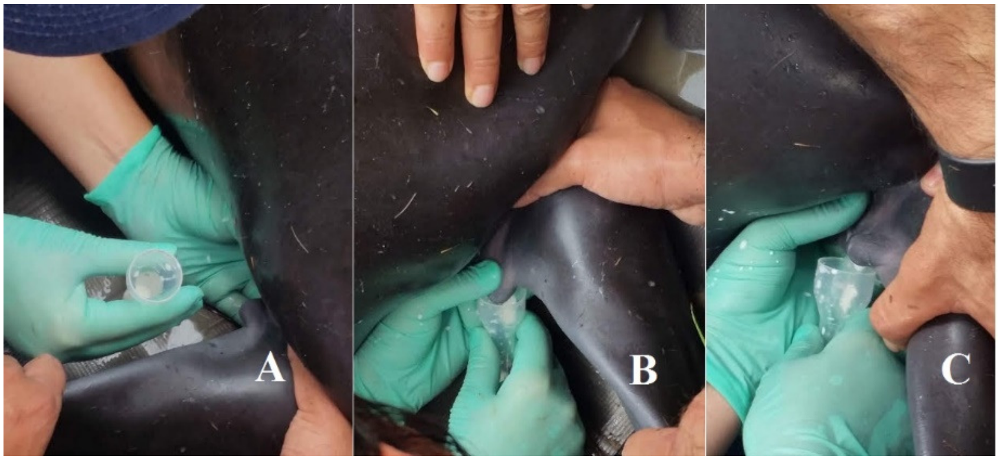

Figure 15: Stimulation of the mammary gland through massages (A), manual milked (B) and prior to milking (C).

Figure 16: Storage of the collected milk sample in a sterile container.

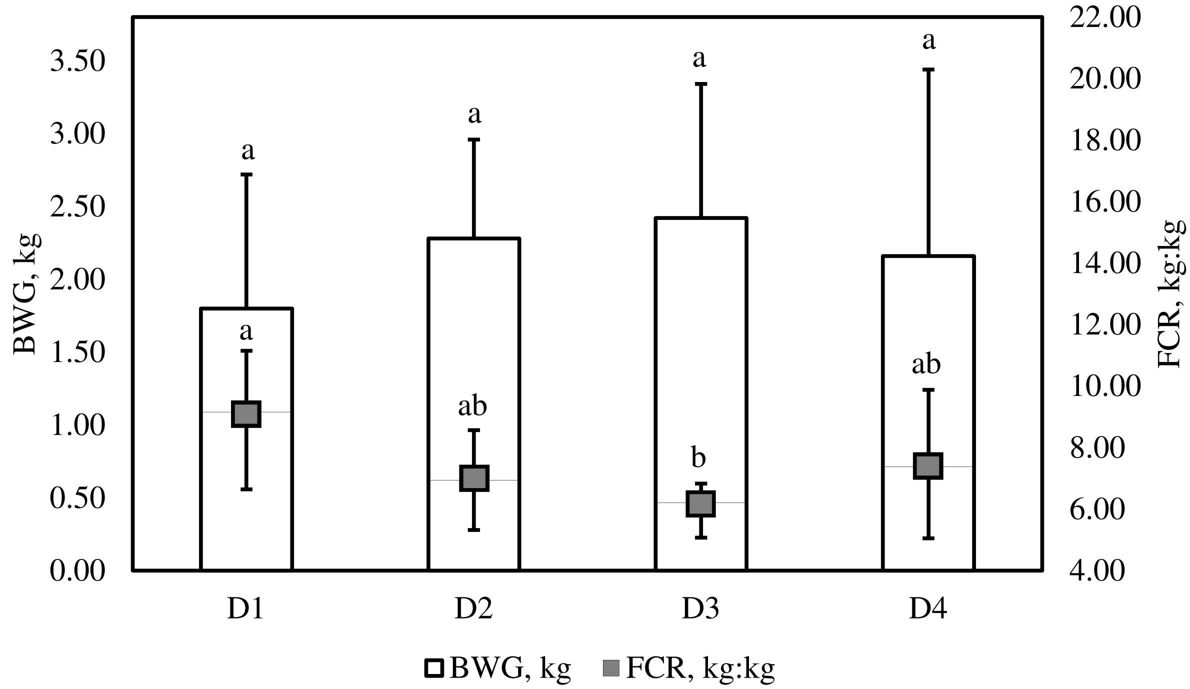

Figure 17: Comparative diagram of the body weight gain (BWG) and feed conversion ratio (FCR) for the dietary treatments.