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Artigo

Commercial feed acceptance of diets for Amazonian manatees *(Trichechus inunguis)* in captivity

Aceitabilidade de rações comerciais na dieta do peixe-boi da Amazônia (Trichechus inunguis) em cativeiro

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Resumo: A manutenção de peixes-bois amazônicos (*Trichechus inunguis*) em cativeiro é cara e complexa. A obtenção de alimentos naturais é sazonal e os custos de alimentá-los com plantas cultivadas são bastante onerosos, requerendo uma logística e planejamento de alto custo. Dessa forma, esse estudo teve como objetivo obter uma alternativa alimentar de boa qualidade visando reduzir os custos com a alimentação e diminuir os riscos de doenças infecto-contagiosas por alimentos contaminados. O experimento foi desenvolvido em duas fases: teste de aceitabilidade e teste de palatabilidade. Foram utilizados quatro animais adultos e em bom estado de saúde, sendo dois machos e duas fêmeas. Testou-se dois tipos de ração comercial extrusada, uma específica para equinos e outra para roedores de laboratório. Durante o experimento o estado corpóreo e de saúde dos animais foram monitorados por biometria e hemograma completo. Os animais estudados comportaram-se favoravelmente a introdução do novo alimento, e mantiveram um estado de saúde e massa corporal satisfatória, considerando às condições de estresse em que foram submetidos. As duas fêmeas mostraram-se menos receptivas ao novo alimento e o tamanho da ração interferiu diretamente no seu consumo, uma vez que pellets de maior diâmetro são mais facilmente capturados pelos lábios preênsil de *T.inunguis*. O peixe-boi da Amazônia mantido em cativeiro aceita a inclusão de alimento concentrado na dieta desde que de tamanho favorável a sua captura.

Palavras chave: Aceitabilidade, alternativa alimentar, ração.

Abstract: Keeping Amazonian manatees (*Trichechus inunguis*) in captivity is expensive and complex. Access to natural food is seasonal, and feeding manatees farmed plants is quite costly, requiring expensive logistics and planning. This study aimed to obtain alternative, good-quality feed to reduce feeding costs and decrease the risk of infectious and contagious diseases transmitted by contaminated food. The experiment was conducted in two stages: an acceptance test and a palatability test. Four adult animals in good health were used, two males and two females. Two types of extruded commercial feed were tested, one specific for horses and the other for laboratory rodents. During the experiment, the body and health status of the animals were monitored by biometrics and complete blood counts. The animals studied behaved favorably toward the introduction of the new feed and maintained satisfactory health status and body mass, considering the stressful conditions to which they were subjected. The two females were less receptive to the new feed, and the ration size directly affected intake because larger diameter pellets are more easily captured by the prehensile lips of *T. inunguis*. Captive Amazonian manatees accept the addition of concentrate to their diet as long as the size is conducive to capturing the pellets.

Keywords: Acceptability, dietary alternative, feed.

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Introduction

The Amazonian manatee (*Trichechus inunguis*) (Natterer, 1883) is classified as "vulnerable" by the Endangered Species Red List of the International Union for Conservation of Nature (IUCN, 2017). In Amazonas State (AM), Brazil, manatees still experience pressure from hunting and accidental capture of calves (Rosas, 1994; da Silva *et al*., 2008). When rescued, they are sent to authorized research centers such as the Robin C. Best Water Park (Parque Aquático Robin C. Best) at the Brazilian National Institute of Amazon Research (Instituto Nacional de Pesquisas da Amazônia - INPA) in Manaus, AM.

Captivity studies have found that adult and young Amazonian manatees consume approximately 9% to 14% of their live weight daily, respectively (Best, 1981). This animal is a monogastric herbivore, exhibiting post-gastric fermentation of food, similar to horses (Burn, 1986). In nature, manatees feed on over 50 plant species, especially aquatic and semi-aquatic macrophytes (Best, 1981). As opportunistic herbivores, they most likely also consume leaves, fruit, and organic matter decomposing at the bottom of lakes during low water season (Guterres et al., 2008).

The diet of the INPA manatees consists of a wide variety of farmed plants, such as tomato, cucumber, green beans, lettuce, cabbage, kale, gherkins, and pumpkin, among others, acquired at market price from the markets in Manaus (Chacon, 2001). The annual consumption of the INPA manatees is estimated to be 100 tons. In addition to these plants, grass forage of the genus *Brachiaria sp.* harvested from the city's surroundings is provided to nutritionally complement the captivity diet and reduce maintenance costs. Keeping manatees in captivity is quite costly and requires intensive

management, and feeding is mainly responsible for these costs. Providing the animals with food that meets their nutritional needs and that is contaminant-free is expensive and limits keeping these animals in captivity. Thus, it has been recommended that low-cost and high nutritional value diets should be formulated for young and adult animals in captivity. This study aimed to test the acceptance of two commercial feeds for Amazonian manatees (*Trichechus inunguis*) in aptivity to reduce feeding costs and decrease the risk of infectious-contagious diseases transmitted by contaminated food acquired from city markets.

Materials and methods

First step

Animals

This study was performed with Amazonian manatees in captivity at the Robin C. Best Water Park, which is part of the INPA. Four healthy animals were used, two males (Tupy-28 years old and Anamã-8 years old) and two females (Tukano-22 years old and Cunhataí-11 year old), between 8 and 28 years old, and weighing 150 to 220 kg.

Manatees that fed regularly, had no injuries, and were free of infections or signs of nutritional deficiency were considered healthy.

The selected animals were kept in intermediate tanks (two meters deep, 2.5 meters long, and 1.5 meters wide) and constantly monitored throughout the entire experiment, which lasted approximately 300 hours during November and December 2006 and January 2007.

Feed

Two commercial feeds were tested: Feed A - PURINA LABINA® and Feed B - PURINA HIPPUS®. The selection of Feed A was based on the mean pellet size, considering that studies that have examined the pellet size in relation to feed intake capacity are scarce, and Feed B was selected due to its nutritional composition (Table 1).

Table 1. Commercial information for the feeds used in the experiment

Feed		A	B
Physical characteristics		Extruded	Pelletized
Size (cm)	Length	2.5	5.0
	Diameter	1.5	0.4
Nutritional value (%)	Moisture content	12	13
	Crude protein	23	14
	Ether extract	4	3
	Crude fiber	5	22
	Minerals	10	15
	Calcium	1.5	1.5
	Phosphorus	0.85	0.45

Basic composition:

Feed A contained calcium carbonate, soybean meal, wheat bran, dehydrated alfalfa, dicalcium phosphate, ground whole corn, degummed soybean oil, sodium chloride (salt), vitamin-mineral premix, amino acids, fish meal, and an antioxidant additive.

Feed B contained ground whole corn, calcium carbonate, soybean meal, alfalfa hay, corn gluten meal-60, sodium chloride (salt), coast cross hay, an antioxidant additive, a fungistatic antifungal additive, vitamin-mineral premix, wheat bran, molasses, and ground whole soybeans (pressure treated).

Feed B was ground to 2-mm pellets, and 5% (2 kg) wheat bran was mixed into each 40 kg of ground feed.

Then, this mixture underwent the extrusion process, i.e., an instantaneous vaporization process in which the feed reaches high temperatures (145-150 °C) for a short period of time, gelatizing the carbohydrate contained in the feed.

Acceptance test and feed palatability

The acceptance test consisted of four steps, each lasting one week.

First week (adaptation)

The amount of feed usually provided to the animals was reduced by 50% (half consisting of legumes and vegetables and the other half consisting of *Brachiaria mutica* grass). Each animal received 100 g of feed daily, along with the forage. The feed was placed in floating circular feeders (approximately 1 meter diameter) made of black polyethylene tubes 2.5 cm in diameter and attached at the ends.

Second week (introduction)

During this phase, only concentrate (500 g of feed/animal/day) was provided in the feeder (no forage).

Third week (maintenance)

During this step, both forage (50% of the daily feed) and concentrate (500 g of feed/animal/day) were provided.

Concentrate acceptance was tested by supplying only feed (250 g every 30 minutes) in the feeder. The forage was provided only after supplying the concentrate.

Fourth week (palatability test)

The animals were fed forage at the total amount usually provided with 500 g of concentrate added per animal.

After completing the test for **Feed A**, **Feed B** was tested using the same group of animals. Considering that these animals were already adapted to the new feed, only the introduction, management, and palatability tests were performed for **Feed B**.

Second step

Animals

For this step, four male animals were selected, two sub-adults and two adults,

aged between 4 and 14 years old, in good health, and weighing between 95 and 135 kg. Two of these manatees were born in captivity (I-Kinjá - 4 years old and II-Erê – 10 years old), and two arrived as orphans and were raised in captivity. (III-Tapajós – 7 years old and IV-Guarany – 14 years old). The animals were isolated in the same tank used in the first step of the study. The experiment lasted two months, consisting of approximately 300 hours between MARCH & MAY (2008).

Feed

Pelletized Corcelina® Purina commercial feed, which was formulated for horses performing average physical activity, was used. Because this feed has high fiber content, which does not allow starch to expand during the extrusion process, it was necessary to reformulate the feed without altering its nutritional value so that it could then be extruded. This process was necessary to increase the feed use efficiency because manatees in captivity are accustomed to eat floating food.

First, the size of the commercial feed was reduced to approximately 0.8 mm in a hammer mill (specific for cereals). Once it was transformed into powder, the feed was placed in a kneading machine together with 10 kg of wheat flour, 1 kg of dextrin (natural gum obtained by hydrolyzing corn and used to help cluster the feed ingredients), and 1.8 kg of Protenose® (corn protein ingredient obtained by separating and concentrating the gluten extracted from corn through the wet milling process, to complement the protein losses caused by temperature increase, which causes protein denaturation) for each 40 kg of feed and was mixed for 20 minutes.

Next, the mixture was placed in an extruder at a pressure of 30 ATM and an approximate temperature of 135°C to gelatinize the starch. The mixture was extruded gradually, reaching a maximum diameter of approximately 2.5 cm. The feed was cut into 4-cm long pellets.

The extruded feed was dried in an MA 035 forced-air oven at 55°C for two days and then placed in a plastic bag and stored at 25°C until use.

The experiment was conducted according to the following schedule:

First week (introduction of the experimental diet) For this experiment, the mixed diet based on lettuce, kale, cabbage, and paragrass (*Brachiaria mutica*) proposed by Chacón (2001) was adopted. These ingredients were occasionally replaced with cucumber, gherkin, cowpea, pumpkin, and cabbage, depending on the availability of these foods at the local market.

During this stage of the experiment, the daily supply of the diet was reduced by

50% (based on a daily intake of 14% per animal/day). Each day, 100 g of the concentrate (extruded feed) together with the forage (mixed diet) were offered to the four animals within the floating feeders used in step 1 to familiarize the animals with the presence of the new feed.

Second week (adaptation to the experimental diet) During this phase, the mixed diet was removed, and only the extruded feed (100 g of feed per animal) was provided in the feeder. The feed was offered gradually until the animals started to eat it.

Third to Fourth week (maintaining the experimental diet) During this step of the experiment, 200 g of feed/animal was provided daily to all of the animals gradually, according to their intake. The typical amount (100%) of raw forage was only provided after the manatees ingested all of the concentrate.

Fifth to Eighth week (experimental evaluation) A total of 200 g of feed per animal was provided, gradually offered as the animals ate. The raw forage was only provided after the animals ingested all of the concentrate. During this period, the costs associated with regular feeding were monitored, considering only the purchase of vegetables, fruit, and legumes.

Considering a daily food intake of 14% of an animal's body weight per day, the amount provided was calculated with the following values: total weight of the animals = 318 kg, and estimated intake = 45 kg/day. The diet consisted of 9 kg/day of grass, 32 kg/day of fruit, vegetables, and legumes, and 600 g of feed (10% total dry matter).

Results and Discussion

First step:

The animals were very interested in and curious about the new feed offered during the adaptation phase. It was possible to observe exploratory behaviors, such as touching the feed pellets with the tactile lip hairs and mimicking discrete pinches with the prehensile upper lips, suggestive of ingestion. Burn (1986) reported that manatees have approximately 2,000 sensitive hairs in the rostral region, and 600 are located in a circular region between the nostrils and mouth. These strategically placed hairs are mainly used to feel and explore objects, considering the animal's reduced senses of vision and taste. Additionally, sirenians use facial muscles together with perioral bristles to capture, manipulate, and ingest aquatic vegetation (Marshall et al., 2003).

The rate of acceptance of the new feed by the manatees in this study was 100%, with a gradually increased intake after the adaptation phase. Feed A obtained a higher degree of acceptance than Feed

B, most likely due to its size because Feed B was relatively thinner, which greatly hindered the capture of the feed pellets.

The health status and body mass were maintained throughout the experiments, considering the stressful conditions to which the animals were subjected. It became evident that feed size directly affected acceptance and consequently consumption of pellets because larger pellets (> 1.5 cm diameter) were more easily captured by the manatee's prehensile lips.

However, throughout the experiment, the females showed more resistance to accepting the new feed than the males, eating the feed sporadically when there were no people near the tank and taking longer to consume all of the feed provided. The males in turn ingested the feed much more quickly.

Feed consumption in this species is greatly affected by the nutritional value of the diet, in which factors such as the content of lipoproteins, carbohydrates, fibers, and moisture seem to act as food intake moderators (CAVALLANTE, 1995). In addition to these factors, the metabolic rate, digestive physiology of the species, and feed palatability determine the amount of feed ingested.

Both **Feed A** and **Feed B** exhibited good palatability with satisfactory intake, even in the presence of the animals' regular feed. Chacón (2001) tested three different extruded feeds that were provided by hiding them in the regular food. Compared to that study, the present results were more efficient because the feed was not hidden, facilitating daily management and food supply.

Using feed as a supplement in the diet of herbivores has several advantages, e.g., it provides essential nutrients that fulfill the nutritional requirements of the species, which are typically obtained when a wide variety of food items are provided. This type of diet is quite complicated and can be extremely expensive when keeping wild animals in captivity. In nature, manatees feed on a wide variety of foods with different nutritional levels, fulfilling their daily nutritional demand. However, providing food as pellets makes management more efficient, facilitates feed storage, and reduces transport, infrastructure, and labor costs (ENSMINGER, 1973).

In horses, diet supplementation with feed is very important for working animals living in confinement because it serves as a source of readily available nutrients that meet the dietary needs of the animals and lowers maintenance costs. However, the main disadvantage of feeding horses this type of feed is errors in the administration of the feed. Such errors may cause abdominal discomfort

syndrome, caused by a sudden change in diet, resulting in altered microbial fauna, intestinal pH, and death of certain types of bacteria, leading to the release of endotoxins that can cause bloating and death of the animal.

Second step

Although not suitable for extrusion due to its high fiber content, after adjustments, the commercial feed selected for this experiment had a mean length of 5.0 ±1.2 cm and a diameter of 2.0 ± 0.2 cm and was found to be quite favorable for consumption by the manatees. The floating duration of this feed without dissolving (in standing water) was 18 ± 2 hours.

Animals I and III quickly accepted the feed and maintained continuous and progressive intake throughout the experiment. Animal II exhibited uninterrupted intake, but intake that was less intense than that of the other two animals. Animal IV completely rejected the feed, not eating it even once, and thus was removed from the experiment soon after the end of the adaptation phase. The younger animals were quite interested and noticeably curious regarding the new feed. They consumed it faster, trying to grasp it without much control, often by putting more than one pellet in their mouths and feeling the texture with the tactile hairs present in the upper lips before eating the feed. Ramos & Stein (2000) reported that feeding habits form during infancy. Thus, it is suggested that new feed should be initially supplied to young animals that are more receptive to new food than animals that have already a formed dietary profile.

Because individuals I and II lived together with their mothers throughout infancy, they had the opportunity to develop their cognitive social skills and foraging techniques through the bond between mother and pup (Hayssen, 1993). However, this experience was not considered a determining factor for feed intake by manatee II. This finding reinforces the hypothesis that animal age is directly related to higher feed acceptability and consequently increased intake.

Throughout the experiment, feed was wasted when animals chewed the pellets. However, it was not possible to estimate this waste because although the tank was completely clean when the feed was supplied, the animals released small jets of feces that contaminated the water, making it impossible to differentiate between feces and feed. However, the feed was provided pellet by pellet, according to the animal's individual intake, which alleviated waste.

Nevertheless, this method may become infeasible when supplying feed to more animals in the same tank.

The animals did not exhibit constipation because the release of feces and flatus was observed daily.

The feces remained a color and consistency that were considered normal for the species, and no clinical signs of acute abdominal discomfort were observed, such as abdominal pain visually indicated by a curved body position or bloating evidenced by difficulty in submersion, which are both observed in calves at the INPA manatees with gastrointestinal disorders.

Conclusion

Adding feed to the diet of captive manatees is a new dietary alternative, given that these animals accept the inclusion of concentrate in the diet as long as the size is suitable for capturing. However, more studies are necessary regarding the addition of concentrates to the diet of *T. inunguis* to formulate a balanced and low-cost diet to maintain this species in captivity.

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