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USE OF BY-PRODUCTS AND WASTE IN THE MANUFACTURE OF ARTISANAL FEED FOR HATCHLINGS OF GIANT SOUTH AMERICAN TURTLE (*Podocnemis expansa*) AND YELLOW-SPOTTED RIVER TURTLE (*Podocnemis unifilis*) AND IN JURUA COMMUNITY BREEDING SYSTEMS

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ABSTRACT: The breeding of turtles has been stimulated in the Amazon state as a conservation strategy and, in 2017, the State Enviromental Council regulated the community rearing of hatchlings of the Giant South American river turtle (*Podocnemis expansa*) and Yellow-spotted river turtle (*P.unifilis*). This study aimed to evaluate the natural feeding of hatchlings of these species in order to find local by-products and residues that could be used in artisanal feeds, assess their nutritional values, and test them in the diet of hatchlings in community breeding in the Middle Juruá River. First, samples of the stomach contents of animals in nature were collected using the flushing technique, the capture was made with nets (100mX3m) to identify the food items. Food and by-products usually provided in the creations were also collected and analyzed bromatologically. Sixty-one turtles were captured and 19 samples of stomach contents were collected. Twenty-three types of leaves, stems and fruits with 13.1 to 17% crude protein (CP) were identified, and pirarucu (*Arapaima gigas*) viscera byproducts / residues, 49-62% CP, muru-muru pie (*Astrocaryum murumuru*), 9 to 13% CP, cassava pulp (*Manihot esculenta*), 1.9% CP, which were later used as ingredients for making artisanal feed. Afterwards, an competition trial was carried out between turtle hatchlings fed on artisanal and commercial food (Nutripiscis TC-45©), and a control experiment with hatchlings fed on TC45 and with three different artisanal diets (varying the alternative ingredients and protein level 5.8% to 40.1% CP). It was found that, in 5 months, the largest and heaviest hatchlings were fed with TC45 (*P.expansa*=54.7 ± 4.6g; *P.unifilis*=45±2.3g) and artisanal food T2=35% pirarucu viscera+35 % murumuru pie (*P.expansa* =42.5 ± 3.1g; *P.unifilis* =26.7±1.4g) than those fed with artisanal diets of lower

protein level (*P.expansa*=27.3 to 28.1g; *P.unifilis* =16, 1 to 16.5 g).

KEYWORDS: Animal feeding; Chelonia (Genus); Aquaculture.

USO DE SUBPRODUTOS E RESÍDUOS NA ELABORAÇÃO DE RAÇÃO ARTESANAL PARA FILHOTES DE TARTARUGAS (*Podocnemis expansa*) E TRACAJÁS (*Podocnemis unifilis*) EM SISTEMAS DE CRIAÇÃO COMUNITÁRIA NO MÉDIO JURUÁ

RESUMO: A criação de quelônios tem sido estimulada no Amazonas como uma estratégia de conservação e em 2017, o Conselho Estadual de Meio Ambiente regulamentou a criação comunitária de filhotes de tartaruga (*Podocnemis expansa*) e tracajás (*P.unifilis*). Este estudo objetivou avaliar a alimentação natural de filhotes dessas espécies, buscar subprodutos e resíduos locais que pudessem ser utilizados em rações artesanais, avaliar seus valores nutricionais, e testá-los na dieta de filhotes em criações comunitárias no Médio rio Juruá. Primeiro, foram coletadas amostras do conteúdo estomacal dos animais na natureza pela técnica de flushing, sendo a captura feita com malhadeiras (100mX3m) para identificação dos itens alimentares. Também foram coletados os alimentos e subprodutos fornecidos nas criações, e analisados bromatologicamente. Foram capturados 61 quelônios e coletadas 19 amostras de conteúdo estomacal. Foram identificados 23 tipos de folhas e frutos com 13,1-17% de proteína bruta (PB) e analisados subprodutos: vísceras de pirarucu (*Arapaima gigas*), 49-62%PB, torta de muru-muru (*Astrocaryum murumuru*), 9-13%PB, polpa de macaxeira (*Manihot esculenta*), 1,9%PB; que foram, posteriormente, usados na elaboração de rações artesanais. Depois, realizou-se ensaio de competição entre filhotes de tracajá e tartarugas alimentados com ração artesanal e com a ração comercial (Nutripiscis TC-45©), e um experimento controle com filhotes alimentados com TC45 e com três diferentes de rações artesanais (variando os ingredientes alternativos e nível proteico 5,8% a 40,1%PB). Verificou-se que, em 5 meses, os filhotes maiores e mais pesados foram os alimentados com TC45 (tartarugas=54,7 ± 4,6g; tracajás=45±2,3g) e ração artesanal T2=35% vísceras pirarucu+35% torta murumuru (tartarugas=42,5 ± 3,1g; tracajás=26,7±1,4g) do que os alimentados com rações artesanais de menor nível proteico (tartarugas=27,3 a 28,1g; tracajás=16,1 a 16,5 g).

PALAVRAS-CHAVE: Alimentação dos animais; Quelônio; Aquicultura.

USO DE SUBPRODUCTOS Y RESIDUOS EN LA PREPARACIÓN DE ALIMENTOS ARTESANALES PARA JUVENILES DE TORTUGA

(*Podocnemis expansa*) Y TARICAYA (*Podocnemis unifilis*) EN SISTEMAS DE CRÍA COMUNITARIOS DEL RÍO MEDIO JURUÁ

RESUMEN: Se ha estimulado la creación de tortugas en la Amazonía como estrategia de conservación, y en 2017, el Consejo Estatal de Medio Ambiente regulóla crianza comunitaria de tortugas (*Podocnemis expansa*) y taricayas (*P.unifilis*). Este estudio tuvo como objetivo evaluar la alimentación natural de los juveniles de estas especies, y buscar subproductos y residuos locales que pudieran ser utilizados en raciones artesanales, evaluar sus valores nutricionales y probarlos en la dieta de las crías en creaciones comunitarias en el Medio Río Juruá. En primer lugar, se recolectaron muestras del contenido estomacal de los animales en la naturaleza mediante la técnica de flushing, la captura se realizó con redes de enmalle (100mX3m) para identificar los alimentos. También se recolectaron y analizaron bromatológicamente los alimentos y subproductos, generalmente suministrados en las creaciones. Se capturaron 61 tortugas y se recolectaron 19 muestras de contenido estomacal. Identificamos 23 tipos de hojas y frutos con 13.1-17% de proteína cruda (PC) y analizamos los subproductos: pirarucu (*Arapaima gigas*) viscera, 49-62% PB, pastel de muru-muru (*Astrocaryum murumuru*), 9-13% PB, pulpa de yuca (*Manihot esculenta*), 1,9% de PB; que se utilizaron en la preparación de raciones artesanales. Posteriormente, se realizó un ensayo de competencia entre crías de tortuga y taricaya alimentadas con racione artesanal y comercial (Nutripiscis TC-45 ©), y un experimento de control con crías alimentadas con TC45 y con tres dietas artesanales diferentes (variando los ingredientes alternativos y el nivel de proteína 5.8% al 40,1% PC). Se encontró que, en 5 meses, las crías más grandes y pesadas fueron alimentadas con TC45 (tortugas = 54.7 ± 4.6g; taricayas = 45 ± 2.3g) y alimento artesanal T2 = 35% vísceras de pirarucu + 35% pastel de murumuru (tortugas = 42.5 ± 3.1g; taricayas = 26.7 ± 1.4g) que los alimentados con dietas artesanales de menor nivel proteico (tortugas = 27.3 a 28.1g; taricayaspel = 16, 1 a 16.5 g).

PALABRAS CLAVES: Alimentación de animales, Quelonio; Acuicultura.

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INTRODUCTION

Chelonians are considered important items of food security by the riverine communities of the Amazon since they are an alternative source of animal protein (ANDRADE, 2017). However, its predatory use and illegal trade (PANTOJA-LIMA et al., 2014;

STANFORD et al., 2020), even today, endanger species such as the giant Amazon river turtle (*Podocnemis expansa*), yellow-spotted river turtle (*P. unifilis*) and six-tubercled Amazon river turtle (*P. sextuberculata*), which are the species most commonly seized by environmental agencies in the region (CHARITY; FERREIRA, 2020).

Community-based conservation projects have helped in the recovery of chelonian populations and, together with environmental agencies, have acted efficiently in the co-management of this faunal resource in the protection of nests and hatchlings (LIMA et al., 2017; CAMPOS-SILVA et al., 2018; FORERO-MEDINA et al., 2019) in more than 80% of the protected nesting areas (ANDRADE, 2017; FAGUNDES et al., 2021). Conservation programs, such as the "Pé-de-Pincha" project of the Federal University of Amazonas, have already released more than 5.5 million hatchlings into the wild, in 123 communities in the state of Amazonas and western Pará (ANDRADE,2017).

In 2017, the Amazonas State Council for the Environment (CEMAAM) officially recognized the efforts of the communities by creating 265 chelonian protection areas in the state (CEMAAM Resolution No. 25/2017– DOE, 2017). It also regulated the community system for raising chelonians in the state (CEMAAM Resolution No. 26/2017), allowing communities to raise, breed and fatten a percentage of giant Amazon river turtle (*P. expansa*) (10%) and yellow-spotted river turtle (*P. unifilis*) (20%) hatchlings born on the beaches protected by them. This was innovative because, previously, these communities that provided the protection did not conform to and could not be registered under the normative instruction MMA Nº7/2015, which regulates the breeding of wild animals in Brazil.

The possibility of community breeding of chelonians (intensive *ex situ* management) seems to be an alternative to generate income and cover part of the expenses for the protection of the species (CAMPOS SILVA et al., 2018) and has shown to be

a promising investment, not only because of the economic aspect, but also because of the relevance it assumes in the food security and culture of the peoples of the Amazon (DANTAS-FILHO et al., 2020). The development of community breeding systems, in excavated tanks or net tanks, can contribute to reducing the exploitation of these animals in the wild (GARCEZ et al., 2021).

Amazonas state has the largest number of commercial chelonian farms in Brazil, with a population of more than 155,000 animals (IBAMA, 2019; Dantas-Filho et al., 2020), and more than 61,000 animals and 302 tons of meat were legally marketed between 1999 and 2019, making the turtle the 5th most farmed aquatic organism in the state (ANDRADE et al., 2021).

Little is known about the feeding of young giant South American turtles and yellow-spotted river turtles in the wild, since most of the studies on feeding of these species were carried out involving adult animals (EISEMBERG et al., 2017; GARCEZ et al., 2020). In the communities of the Andirá River, in Barreirinha, Amazonas, Oliveira et al. (2020) researched the possible foods consumed by chelonians and their nutritional value. In captivity, they are omnivorous, but the young demonstrate a greater preference for foods of animal origin (ALMEIDA; ABE, 2009; ANDRADE et al., 2021).

In the feeding of the hatchlings up to juveniles of 3 years (raising and breeding), the commercial breeders of chelonians used, initially, mainly protein by-products of animal origin, such as bovine viscera (20%) and fish filleting residues (40%), but also spoilt vegetables from markets, fruits and tubers (20%), and only 20% provided commercial fish feed (fry), with 38-45% protein (ARAÚJO et al., 2013; GARCEZ et al., 2021). Currently, 55% of chelonian farmers use fish feed to feed their animals (Andrade et al., 2021). In the farms that provided feed with a higher animal protein content, the animals gained more weight than those raised with vegetable protein sources (SÁ et al., 2004; ALMEIDA; ABE, 2009; ANDRADE et al., 2021).

In the Middle Juruá region, there are several community-based chelonian conservation beaches. It was on these

beaches that the community rearing of giant Amazon river turtles and yellow-spotted river turtles in the Amazonas state began. In this region, there are also some production chains, such as the extraction of oil from forest seeds for cosmetics or the processing of pirarucu (*Arapaima gigas*) (Campos-Filho et al., 2020), that generate by-products and waste that could be used to feed chelonian hatchlings in community farms in the region.

This study aimed to evaluate the natural feeding habits of chelonians and seek information on local foods, by-products and residues that could be used to prepare artisanal feed for giant South American turtle (*P. expansa*) and yellow-spotted river turtle (*P. unifilis*) hatchlings, evaluate their nutritional values, and test them in the diet of these animals in community breeding units in the Middle Juruá region, in Carauari, Amazonas.

MATERIAL AND METHODS

The present study was developed in communities that participate in the Chelonians Community Management Program/Pé-de-Pincha/UFAM, in the Middle Juruá region, namely the communities of SDR Uacari and RESEX Middle Juruá, in Carauari (5º 28′ 35.7″ S; 67º 28′ 41.7″ W): Manarian (05º 25' 16,3" S; 67º17' 21.4″ W), São Raimundo (5° 25′ 2" S; 67° 31′ 38.2" W), Xibauzinho (05º 59′ 35.3″ S; 67º 47′ 37.0″ W) and Vila Ramalho (05º 29′ 43.4″ S; 067º 28' 39.3″ W).

The methodology for identifying the types of food and possible by-products or local residues used in feeding giant South American turtle (*P. expansa*) and yellow-spotted river turtle (*P. unifilis*) hatchlings in nurseries was done through interviews with beach monitors and the collection of samples of these foods during six collection expeditions in the periods of low water (September, 2018, July and September, 2019), rising water (November, 2018), high water (February, 2019) and falling water (May, 2019). Photos and samples were taken of each food, by-product or residue, which were georeferenced, and bromatological analysis was performed using the methodology described by the AOAC

(2016) in the Laboratory of Forrage Agriculture and Pastures (FCA - UFAM).

SAMPLING OF FOOD ITEMS FROM ANIMALS IN THE WILD

For the capture of giant South American turtle (*P. expansa*) and yellow-spotted river turtle (*P. unifilis*) hatchlings and juveniles to obtain stomach contents, two trammel nets (100 m x 3 m and 60-70 mm mesh) and a net obtained from the residents (40 line, mesh 13 or 18, 100 m X 5 m) were used, which were placed in different environments (rivers, lakes, terraces and flooded floodplain forests), with a continuous capture effort of 48 to 72 hours, checked every two hours (BALESTRA et al., 2016). From the captured animals, rectilinear measurements of carapace length, width and height (RCL, RCW and CH) were taken; plastron length and width (PL and PW) were measured with aluminum calipers of 18 and 80 cm. The animals were weighed on digital scales with a capacity of 10 kg and salter type scales of 100 g, 2,500 g and 10,000 g capacities.

The collection of stomach contents was performed using the Legler (1977) or flushing technique, which consists of stomach washing of the chelonian through the introduction of a probe through the mouth (nasopharyngeal probe No. 4, 8, 10, 14), passing through the esophagus until reaching the stomach, with water being injected via a 5 to 20 mL syringe to make the animal regurgitate. The regurgitated material was filtered through a milk strainer and sieved. The samples were stored in a Petri dish and then frozen for later identification and quantification of food items using a steroscopic magnifying glass. The potential food sources for the chelonians were divided into: 1) items of animal origin such as fish (FSH), insects (INS), crustaceans (CRU) and molluscs (MOL); 2) plant material items such as fruits (FRU), seeds (SDS), flowers (FLW), stem and stalks (ST), roots (RTS), leaves (LEA), algae and/or periphytons (AL+PER) and unidentified plant material (UPM).

EVALUATION OF ALTERNATIVE FEED FOR CAPTIVE ANIMALS

To evaluate some of the by-products and residues available in Carauari, an experiment was conducted between hatchlings fed with

an artisanal feed formulated and prepared by technicians from IFAM (Federal Institute of Technology of Amazonas) and by the residents at SDR Uacari. The following items were used: residues from the evisceration of pirarucu (*Arapaima gigas*) – REPir - from management areas in SDR Uacari/Resex Middle Juruá; the cake by-product of the extraction of murumuru oil (*Astrocaryum murumuru*) - REMur; and the pulp of macaxeira (*Manihot esculenta*) as a binder in the manufacture of an artisanal feed – TArt1 (composition in Table 1).

Table 1. Composition of commercial feed for feeding chelonian hatchlings in Carauari.

Artisanal Feed - Composition (%)				TArt1	T1	T2	T3
Ingredients				%	%	%	%
Pirarucu viscera meal (*Arapaima gigas*)				33.3	70	35	17.5
Murumuru residue cake (*Astrocaryum murumuru*)				33.3	0	35	52.5
Cassava (*Manihot esculenta*)				33.3	30	30	30
Nutrients	DM(%)	MM(%)	CP(%)	EE(%)	NDF(%)	ADF(%)	CF(%)
Commercial Feed							
TC45*	90	14	45	12	41.2	4.4	4.5
T0*	94.6	11	40.1	6.2	41.2	4.4	4.5
Artisanal Feed							
TArt1	93.5	1.6	27.3	9.0	60.3	18.4	
T1	78.2	1.7	27.7	5.2	51.4	31.7	
T2	93.5	1.6	27.3	9.0	60.3	18.4	
T3	73.1	1.8	5.8	5.3	60	31.7	

Acronyms: TC45 = commercial feed Nutripiscis© TC 45 and TArt1: artisanal feed used in the experiment; T0 = control commercial feed and T1, T2 and T3 = artisanal feeds tested in the laboratory experiment. DM=dry matter; MM=mineral matter; CP=crude protein; EE=ether extract; NDF=neutral detergent fiber; ADF=acid detergent fiber; CF=crude fiber.

* Ingredients: broken rice, soybean meal, fish meal, blood meal, salmon meal, offal meal, corn, refined fish oil, salmon oil, soybean oil, salt, mineral premix (iron sulfate, copper, manganese monoxide, calcium iodate, zinc oxide, sodium selenite, cobalt sulfate) and vitamin supplement (C, D3, E, K3 and B1). Source: Presence (manufacturer).

As the control, the commercial feed TC-45 from Nutripiscis© with 45% crude protein (CP) in a 2-4 mm pellet was used. In this test, 200 giant South American turtle (*P. expansa*) and 200 yellow-spotted river turtle (*P. unifilis*) hatchlings of 15 days of age were used, which were housed in four 1,000-liter water tanks (50 animals of each species/box), two tanks located in the community of Manarian (SDR Uacari) and

two in São Raimundo (Resex Middle Juruá). In one tank, the animals received the commercial control feed (TC45) and in the other the artisanal feed (Tart1). The hatchlings were measured and weighed at the beginning (November, 2018) and at the end of the experiment, which lasted 90 days. The hatchlings were fed once a day in the morning based on 5% of the initial biomass.

A control experiment was also carried out with hatchlings fed with commercial control feed (T0) and fed with three different types of artisanal feeds (varying the percentage of alternative ingredients and protein level 5.8% to 40.1% CP) for 5 months. The experiment was carried out at the Laboratory of Nutrition of Aquatic Organisms - LAMPaq/UFAM, using a structure with twelve 60-liter plastic trays, with water inlet and outlet and lighting. In these facilities, 60 hatchlings of giant South American turtles (*P. expansa*) and 60 hatchlings of yellow-spotted river turtles (*P. unifilis*) were allocated, with 15 days of age, which were individually marked with nicks on the carapace, measured and weighed. Each plastic tray received five giant South American turtle hatchlings (*P. expansa*) and five yellow-spotted river turtle hatchlings (*P. unifilis*), and each plastic tray constituted an experimental unit (plot/repetition). The hatchlings were fed once a day in the morning (9 am) based on 5% of their initial biomass, which was adjusted monthly after each weighing.

The treatments were three different diets (T1=70% REPir; T2=35% REPir+35% REMur; T3= 17.5 REPir+52.5% REMur – see composition in Table 1) and the commercial control feed TC45 (T0). The experimental design was completely randomized with four treatments (T1, T2, T3 and T0/TC45) x three replications. The hatchlings were measured (rectilinear carapace length and width, RCL and RCW; plastron length and width, PL and PW; and height, CH) and weighed monthly for 150 days. The quantities of food provided were also measured, and the daily weight gain (GDP), apparent consumption and feed conversion (FC) were estimated. The mortality/survival of the hatchlings and the temperature, pH and BOD of the water were also recorded. The biometric variables of the

hatchlings in the experiment were analyzed using descriptive statistics and the Mann-Whitney test. In the control experiment, ANOVA, Tukey's test and regression analysis were applied to analyze the biometric and performance variables of the hatchlings. The statistical packages used were MINITAB and PAST.

RESULTS AND DISCUSSION

In the six expeditions carried out, twenty-three types of leaves, stems and fruits were identified and six types of by-products and residues used to feed the chelonian hatchlings. The following local foods were analyzed: purple potato stem (*Ipomea batata*), cecropia fruit (*Cecropia* sp.), murumuru (*Astrocaryum murumuru*), sawtooth coriander (*Erungium foetidum*) and red morning glory (*Ipomoea coccinea* and *I. cairica*) with crude protein (BP) ranging from 13.1 to 17%.; and *others such as watermelon rind* (*Citrullus lanatus*), banana leaf (*Musa paradisíaca*), cassava stem and root (*Manihot esculenta*), pineapple (*Ananas comosus*), wild yam (*Dioscorea trifida*), pacara earpod tree (*Enterolobium contortisiliquum*), water lettuce, water hyacinth leaves (*Eichhornia crassipes*, *Pistia stratiotes*), sedgegrass (*Paspalum repens*), watercress (*Nasturtium officinale*), pepper elder (*Peperomia pellucida*), purslane (*Portulaca oleracea*), flameflower (*Talinum triangulare*), cowpea (*Vigna unguiculata*) and camu-camu (*Myrciaria Dubia*) with 5.3 to 8.5% CP (Table 2).

It was observed that in communities with greater abundance of local foods or their by-products, the use of commercial feed for fish fry was suppressed (São Raimundo) or reduced to only twice a week (Xibauazinho). In addition to plant-based foods (leaves, stems and fruits) and the feed, the community members supplemented the diet of the hatchlings with various foods or residues of animal origin, such as viscera and fish filleting remains (flag-tailed prochilodus, *Semaprochilodus insignis* and *S. taeniurus*; piau, *Leporinus trifasciatus*; barred catfish, *Pseudoplatystoma fasciatum*; pacu, *Myleus sp*. and *Mylosoma sp*.; silver arowana, *Osteoglossum bicirrhosum*; and peacock bass, *Cichla ocellaris*), cooked or baked fish, chopped into small pieces. These

animal-based supplements had an average of 18.7±1.4% CP and 10.6±8.8% EE, adding important protein and energy support in the diet of the hatchlings in community farms.

Table 2. Bromatological composition of some of the leaves, stems, fruits, by-products and residues used to feed giant South American turtle (*P. expansa*) and yellow-spotted river turtle (P. unifilis) hatchlings in community farms in the Middle Juruá, Amazonas.

Item and part used	Scientific Name	DM %	MM %	CP %	EE %	CF %	ADF %	NDF %
Plant-based foods: leaves, fruits and seeds								
Purple sweet potato stem and leaves	*Ipomoea batatas*	23.7	11.4	13.1	0.1	-	8.7	3.4
Cecropia fruits	*Cecropia sp.*	87.4	8.2	15.6	0.8	-	-	-
Murumuru seeds,	*Astrocaryum murumuru*	90.7	1.5	13.1	26.3	9.6	-	-
Sawtooth coriander leaves,	*Erungium foetidum*	6.7	28.3	15.3	0.2	-	-	-
Red morning glory leaves	*Ipomoea cairicica*	20.6	14.3	17	0.5	-	-	-
Water lettuce leaves	*Pistia stratiotes*		18.9	15.0	4.4	12	-	-
Water hyacinth leaves	*Eichhornia crassipes*		17.1	12.4	4.7	15	-	-
Camu-camu, fruit	*Myrcia dubia*	95.9	2.6	5.3	3.4	11.3	-	-
Wild yam roots	*Dioscorea sp*	73.7	0.9	2.3	0.1	7.3	-	-
Cowpea seeds	*Vigna unguiculata*	87.5	0.5	4.2	0.1	2.0	-	-
Butternut squash and its leaves	*Cucurbita moschata*	86.5	0.8	1.7	0.5	2.2	-	-
Pineapple, fruit	*Ananas comosus*	86.3	0.4	0.9	0.1	1	-	-
Watermelon rind, Casca	*Citrullus lanatus*	90.7	0.3	0.9	0.01	0.1	-	-
Papaya skin	*Carica papaya*	90.6	0.82	1.56	0.1	1.2	-	-
Banana tree leaves	*Musa paradisiaca*	16.4	8.9	12.6	2.4	-	27.4	45.3
Capim membeca	*Paspalum repens*	29.1	5.86	5.33	0.2	-	40.6	72.1
By-products and processing residues of animal and vegetable origin								
Viscera of pirarucu	*Arapaima gigas*	70.4	2.1	49.6	22.9	-	-	-
Pirarucu liver meal	*Arapaima gigas*	93.7	3.7	44.6	6.8	-	-	-
Pirarucu viscera meal	*Arapaima gigas*	92.2	1.7	62.3	2.5	-	-	-
Murumuru cake	*Astrocaryum murumuru*	80.2	2.1	9.7	3.1	25.9	-	-
Sweet cassava flour	*Manihot esculenta*	90.7	1.3	1.8	0.3	1.9	-	-
Bitter cassava skins	*Manihot esculenta*	80.5	24	4.5	0.8	-	28.7	42.9

*- DM=dry matter; MM=Mineral matter; CP=crude protein; EE=ether extract; CF=crude fiber; ADF=acid detergent fiber; NDF=neutral detergent fiber.

It was also observed that the ingredients of animal origin from waste by-products of the evisceration of pirarucu (*Arapaima gigas*) - liver meal (44.6% CP) and viscera meal (62.3% CP) - presented a higher level of protein than other ingredients such as fruits - 7.4±2.9% CP (4.3 to 14% CP); and leaves and seeds (13-17% CP). The artisanal feed that was elaborated had 14.3% liver meal, 28.6% viscera meal, 28.6% Sweet cassava and 28.6% murumuru cake (TArt1), balancing the protein sources of animal and vegetable origin.

EVALUATION OF FOOD ITEMS IN CHELONIANS CAPTURED IN THE WILD

During the expeditions, 61 chelonians were captured (36 giant South American turtles, *P. expansa*; 6 yellow-spotted river turtles, *P. unifilis*; and 19 six-tubercled Amazon river turtle, *P. sextuberculata*), from which 19 samples of stomach contents were collected, 5 samples in the low-water period, 4 in the high-water period, 2 in the rising-water period, and 8 in the falling-water period. The giant South American turtles measured 20.4±8.7 cm (min=12.8 cm; max=41.6 cm) of rectilinear carapace length (RCL), weighed 1,280±2,140 g (min=294 g; max=7,500 g), had an estimated age of between 3 and 8 years, and were 81% females (Table 3).

The yellow-spotted river turtles measured 13.5±0.3 cm, weighed 359±59 g, were aged 4 to 7 years, and were 50% females. The analyzed samples were collected only from juvenile giant South American turtles (12.8 to 22.4 cm in carapace length and 294 to 1,292 g in weight) and yellow-spotted river turtles (13.3-13.7 cm and 317-400 g) aged 4 to 6 years. The stomach contents weighed between 6.7 and 18.3 g, with the largest volumes found during rising-water, high-water or falling-water periods. In the low-water periods, the stomachs were almost always empty. Traces of seeds, fruits, leaves, roots and stems, fish scales and bivalve shells were found, which shows the omnivorous tendency of the hatchlings and juveniles.

Table 3. Biometric variables for weight, sex ratio and age of the chelonians (P. expansa, P. unifilis and P. sextuberculata) captured in Middle Juruá, Carauari, Amazonas.

Species		Carapace		Plastron		Weight (g)	Sex	Age (years)
		RCL (cm)	RC W (cm)	PL (cm)	PW (cm)			
Giant South American turtle N=36	Md	20.4±	15.6±	16.8±	7.8±	1280±	81% F; 6% M; 13%I	5.9± 3.2
	SD	8.7	6.1	6.9	4.3	2140		
	Max	41.6	33.2	38	22.8	7500		8
	Mín	12.8	9.8	11	4.6	294		3
Yellow-spotted river turtle N=6	Md	13.5±	10.15±	11.75±	5.0±	359±	50% F	6± 2
	SD	0.28	0.07	0.64	0.14	58.7		
	Max	13.7	10.2	12.2	5.1	400		7
	Mín	13.3	10.1	11.3	4.9	317		4
Six-tubercled Amazon river turtle N=19	Md	16.7±	13.9±	13.3±	6.3±	590±	65% F; 35% M	7.8±
	SD	4.9	5.0	3.8	2.2	420		4.2
	Max	25	21	20	10	1350		15
	Mín	10.4	8.2	8.2	3.8	130		3

* Md=mean; SD=standard deviation; Max=Maximum; Min=minimum. ** - RCL=rectilinear carapace length; RCW=rectilinear carapace width; PL=plastron length; PW=plastron width.

In juvenile giant South American turtles, the food items identified were 12.5% fish, 5% molluscs, 20% fruits, 20% seeds, 5.1% roots and 21.4% plant stems. For yellow-spotted river turtles, 20% of seeds, 40% of leaves and 40% of plant stems were found. In the six-tubercled Amazon river turtles, 100% seeds were found. In other words, the juvenile giant South American turtles were the only ones that presented food of animal origin in their diet (bivalve shells and scales of Characiforms) in the Middle Juruá.

Portal et al. (2002) studied the natural diet of yellow-spotted river turtles (*P. unifilis*) in Pracuúba, Amapá, and found thirty-five plant species (legumes 22.8%; grasses 8.6%), of which eight presented a good possibility of being ingredients for regional feed, due to their nutritional values being above 10% crude protein (CP) and their natural availability: *Commelina longicaulis*, dayflower (21%); *Polygonum acuminatum*, tapertip smartweed (20%); *Aschymene sensitiva*, sensitive jointvetch (20%); *Macrolobium acaiaefolium* (17%); *Oryza glandiglumes* (15%); *Thalia geniculata* (14%); *Nymphaea rudgeana* (11%); and *Hymenachne amplexicaulis* (10%).

In the present study, we also found eight foods of plant origin that have protein levels above 10% CP and that could be used in the manufacture of artisanal feeds.

Eisemberg et al. (2017) observed that chelonians of the Podocnemididae family are opportunistic herbivores, with a diet of 46 to 99% vegetables. Garcez et al. (2020) studied the feeding of giant South American turtles, yellow-spotted river turtles and six-tubercled Amazon river turtle in the Juruá River and observed a generalist feeding strategy and herbivorous habits in periods of high and low water, with greater consumption of fruits, leaves, seeds and stems of the families Bignoniaceae, Bombacaceae, Capparaceae, Fabaceae, Moraceae and Pontederiaceae.

In the blackwater flooded forests and lakes of the Andirá River, Oliveira et al. (2020) identified 55 fruits (74.5% in the high-water period) from 19 families being consumed by chelonians. The main ones being Fabaceae, Mirtaceae, Apocynaceae, Arecaceae, Bignoniaceae and Poaceae. The most consumed species were *Campsiandra comosa Benth*; *Peritassa dulcis* (Benth) Miers; *Tabebuia barbata* (E. Mey) Sandwith; and *Pouteria campanulata* Baehn, which presented higher values of crude protein (7.4±2.9%), ether extract (5.4±5%) and crude energy (4,338 to 5,357 cal/g).

Garcez et al. (2012) observed that during the high-water period giant South American turtles (*P. expansa*) and yellow-spotted river turtles (*P. unifilis*) consume a higher amount of fruits, leaves, seeds and stems, than in the low-water period. This was also described by Alho and Padua (1982) when analyzing the stomach contents of giant South American turtle adults. They observed that the stomachs contained little residues, which indicates that during the breeding period a lower food intake occurs.

For giant South American turtles (*P. expansa*), Garcez et al. (2020) found that the prevailing diet consists of seeds, fruits and a variety of invertebrates that inhabit aquatic environments. Moreover, Figueroa et al. (2012), observed that in the high-water period, fruits, leaves and scales of fish of the Characiformes family are easily found in the diet of *P. expansa*.

Fachin-Téran and Vogt (2014) also observed that the seeds ingested by six-tubercled Amazon river turtles (*P. sextuberculata*) were almost exclusively from the Poaceae family, as observed in this study.

EVALUATION OF ALTERNATIVE FEED FOR CHELONIANS IN CAPTIVITY

In Manarian, the experiment with different diets showed that the yellow-spotted river turtles hatchlings fed only with commercial feed TC45 had a final weight of 24.84±5.15 g, and the hatchlings fed with artisanal feed TArt1 had a final weight equal to 22.62±0.98 g, thus showing a tendency of the yellow-spotted river turtles hatchlings fed with feed TC45 to be heavier (F test: p=0.09), but there was no significant difference by the t tests (p=0.24) and Mann-Whitney (p=0.34) test (Table 4). However, in São Raimundo, the hatchlings fed with TC45 weighed 37.99±7.66 g, and were larger (P<0.0001) than those fed with artisanal feed (20.04±0.56 g). In Manarian, the giant South American turtle hatchlings fed only with TC45 feed presented a weight of 35.26±6.66 g, which was higher (P<0.0001 by T, Mann-Whitney tests) than the weight of those fed with artisanal feed (27.70±3.45 g). In São Raimundo, giant South American turtles fed with TC-45 weighed 39.78±1.10 g and were larger (P<0.0001) than those fed with TArt (25.41±0.51 g). The mortality of giant South American turtle hatchlings was higher in animals fed with artisanal feed (8 and 10%), with no mortality in hatchlings fed with TC45 or in yellow-spotted river turtles.

Table 4. Rectilinear carapace length (RCL) and weight of giant South American turtle (*P. expansa*) and yellow-spotted river turtle (*P. unifilis*) hatchlings fed different feed in community farms.

Species	Location	N	TC45 RCL (mm)	TArt1 RCL (mm)	TC45 Final weight (g)*	TArt1 Final weight (g)*
P. expansa *	Manarian	106	61.5±4.4[a]	56±2.3[b]	35.26±6.66[a]	27.70±3.45[b]
	S. Raimundo	97	62±4.8[a]	54.5±2.6[b]	39.78±1.10[a]	25.41±0.51[b]
P. unifilis *	Manarian	53	51.7±5.4[a]	50.6±1.9[a]	24.84±5.15[a]	22.62±0.98[a]
	S. Raimundo	100	59.0±4.3[a]	47.6±3.3[b]	37.99±7.66[a]	20.04±0.56[b]

* Means followed by different letters on the same line differ significantly by the Mann-Whitney test and t-test.

In the control experiment carried out in the laboratory with three artisanal feeds (T1, T2 and T3) and the commercial control feed T0 (TC45), using the F test, a significant difference (P<0.0001) was observed between the final weight, length and width of the carapace and plastron of giant South American turtles fed with the different types of feed tested (Figures 1: A and B). Using the Tukey test, it was noted that the best treatments were the commercial feed TC45 (weight=54.7±4.6 g) and T2 (42.5±3.0 g), which were significantly different from the weights of the animals fed with T1 (27.3±2.1 g) and T3 (28.1±7.06 g) (Table 5).

Table 5. Rectilinear carapace length (RCL), weight, daily weight gain (DWG) and feed conversion (FC) of giant South American turtles (P. expansa) and yellow-spotted river turtles (P. unifilis) fed different experimental feeds in captivity.

Treatment	Initial RCL (mm)	Final RCL (mm)**	Initial weight (g)	Final weight (g)**	DWG (g/day)*	FC**
Giant South American turtle (*Podocnemis expansa*)						
T0	46.8±0.1	69.6±1.7^{a}	20.0±0.3	54.7±4.6^{a}	0.21 ±0.07^{a}	1.6^{a}
T1	46.0±1.1	52.7±0.9^{c}	20.1±0.3	27.3±2.1^{c}	0.04 ±0.01^{c}	9.5^{c}
T2	45.8±0.2	63.6±1.5^{b}	19.6±0.4	42.5±3.0^{ab}	0.17 ±0.06^{b}	1.8^{a}
T3	46.3±1.3	53.5±3.4^{c}	19.4±0.7	28.1±7.1^{c}	0.04 ±0.01^{c}	5.5^{b}
Yellow-spotted river turtle (*Podocnemis unifilis*)						
T0	37.1±1.8	61.9±1.6^{a}	13.2±1.1	45.0±2.3^{a}	0.18 ±0.05^{a}	2.1^{a}
T1	36.4±2.0	42.4±0.0^{c}	12.9±0.2	16.5±0.3^{c}	0.02 ±0.03^{c}	14.7^{c}
T2	39.2±0.9	51.7±0.5^{b}	15.0±1.0	26.7±1.4^{b}	0.10 ±0.02^{b}	2.9^{a}
T3	37.8±0.2	42.6±0.2^{c}	13.6±0.3	16.1±0.3^{c}	0.02 ±0.01^{c}	9.1^{b}

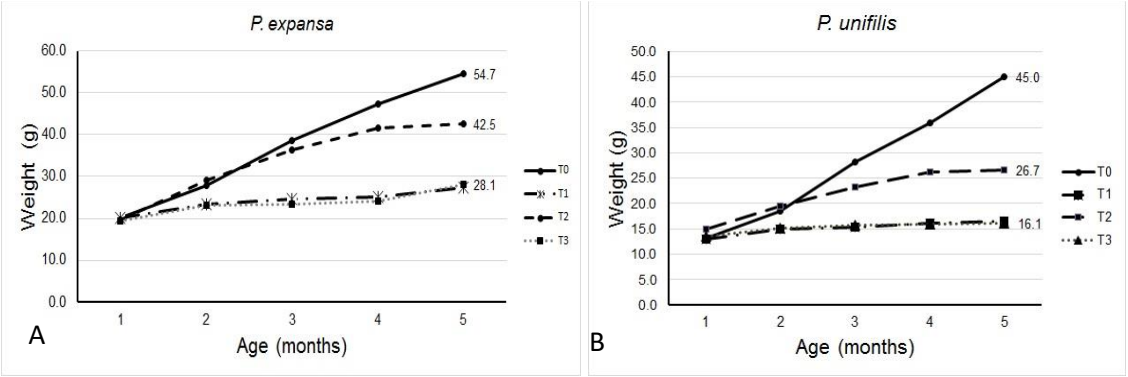
** Means followed by different letters in the same columns are significantly different by the Tuckey test (P<0.01).

The variables measured for yellow-spotted river turtles were also analyzed and verified using the F test, and a significant difference (P<0.0001) between the final weight, length and width of the carapace and plastron was found for the yellow-spotted river turtles hatchlings fed with the different types of feed tested. Using the Tukey test, it was observed that the best treatment was the commercial feed TC45 (45.0±2.3 g), followed by the artisanal feed T2 (26.7±1.4 g), in which the

weights of the animals were significantly different from the weights of the animals fed with T1 (16.5±0.3 g) and T3(16.1±0.3 g).

It was observed that the highest apparent feed intakes also occurred in animals fed with commercial feed TC45 (0.5±0.02 g/day) and T2 (0.4±0.02 g/day), while T1 (0.1±0.03 g/day) and T3 (0.1±0.01 g/day) showed increasingly lower intakes. Apparently, the hatchlings rejected the murumuru cake, making the selectivity of animals very clear. In the leftovers, small pieces of the murumuru cake were noted in treatments T1 and T3. Palatability of the product may be a problem, since it could have gone rancid, or because of the very large size and hardness of the cake pellets. Higher levels of fiber (ADF) and lower values of fat (EE) in the T1 and T3 diets also seem to have contributed to this lower consumption and lower digestibility, which may have affected the performance of hatchlings fed with these feeds.

Figure 1. Growth in weight of chelonian hatchlings fed with different types of artisanal and commercial feed: A) giant South American turtle (*P. expansa*); B) yellow-spotted river turtle (*P. unifilis*).

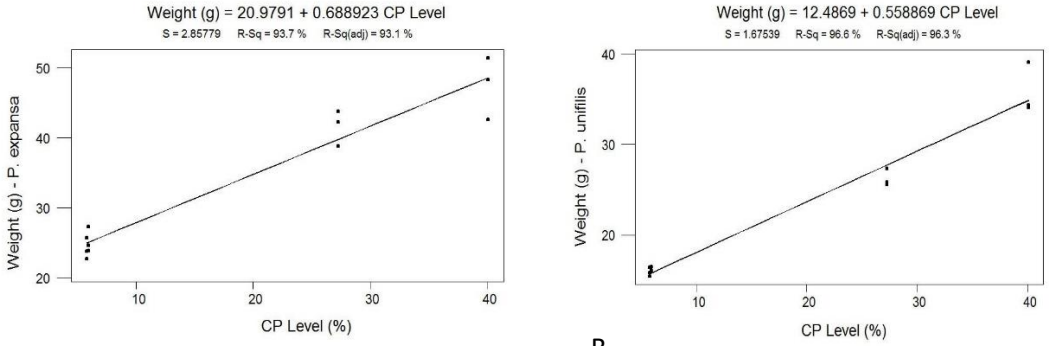


The best responses in relation to weight gain and feed consumption were found with TC45 and T2, which had more efficient feed conversion; respectively, 1.60 and 1.78 for giant South American turtles (*P. expansa*) and 2.06 and 2.93 for yellow-spotted river turtles (*P. unifilis*) (Table 5).

The difference between the final weights of giant South American turtles and yellow-spotted river turtles fed with

TC45 commercial feed and artisanal feeds with different levels of pirarucu viscera meal and murumuru is directly related to the crude protein level of each feed. Regression analysis was performed between the final weight of giant South American turtles and yellow-spotted river turtles versus the crude protein level of the feed they were given. A relationship of 93.1% ($P<0.0001$) was found for the weight of the giant South American turtles. a significant relationship ($P<0.0001$) was also found for yellow-spotted river turtles with R^2=96.6% (Figure 2).

Figure 2. Regression analysis between the final weight of chelonian hatchlings and the crude protein level of the artisanal feed used: A) giant South American turtles (*P. expansa*); B) yellow-spotted river turtles (*P. uniflis*).



When analyzing the mortality rates according to the treatment, it was observed that the T1 and T2 treatments presented a higher number of deaths for both the giant South American turtles (6.6 and 6.7%) and yellow-spotted river turtles (26.6 and 53.3%, respectively). There were no deaths of animals fed with commercial feed. Yellow-spotted river turtles presented, on average, higher mortality (23.3±22.7%) than giant South American turtles (3.35±3.8%). This may be related to a lower digestive capacity of yellow-spotted river turtles subjected to diets with higher levels of animal protein and higher fiber content, which could increase their susceptibility.

Sá et al. (2004) observed that *P. expansa* hatchlings fed diets with high protein levels (more than 27% BP) in the first months of life had better growth and weight gain than those fed diets with

lower protein values. They also found that hatchlings fed diets containing more animal protein showed better results than those fed wth protein of plant origin. Alho and Padua (1982) reported that hatchlings of this species have a preference for diets with a higher proportion of animal products than larger animals. Dias and Fachin-Téran (1998) showed that young yellow-spotted river turtles fed animal protein sources grow more than those fed a vegetarian diet.

Andrade et al. (2021) and Garcez et al. (2021) observed that in commercial breeding of giant South American turtles (*P. expansa*) in the Amazon, these animals grew and weighed more when fed animal by-products and feeds with high protein levels (40 to 45% CP) and higher energy levels (4,000 to 4,500 kcal/kg CE). However, yellow-spotted river turtles (*P. unifilis*) had lower protein (28-32%) and energy requirements (3,500 kcal/kg of CE) than giant South American turtles (Andrade et al., 2021).

Almeida and Abe (2009) conducted an experimente that tested different by-products as a protein source in diets for *P. expansa* hatchlings, and observed that fish meal and poultry viscera meal had better digestibility than meat and bone meal, which can be explained by the high content of mineral matter of the latter.

For Araújo et al. (2013), although the hatchlings and juveniles can process plant material, an animal diet allows greater growth, which in chelonians is linked to greater survival. Houssaine-Lima (1998), when evaluating the effects of isoprotein diets containing different protein sources (fish meal and soybean meal) and varying their percentage composition (100% vegetable to 100% animal) in the feeding of hatchlings of *P. expansa* in the first year of life, noted that the diet with 50% animal protein:50% vegetable protein provided better weight gain and homeostasis of blood biochemical parameters. However, in the diet with 100% animal protein, although the growth was good, there was a higher mortality rate.

Yoshioka et al. (2017) also observed that yellow-spotted river turtles fed with feed with high protein levels (above 40%) showed an increase in plasma urea and, as a consequence, there was an increase in the concentration of ammonia in the

animals' water, indicating that high protein levels in the feed of yellow-spotted river turtles hatchlings would not be adequate, since they could compromise the renal functions of these animals. They, therefore, recommended feed with 36% CP to ensure the animals' health.

CONCLUSION

In the communities of Carauari, 23 types of leaves, stems and fruits with 13.1 to 17% CP, and animal by-products, evisceration residues of the pirarucu (*Arapaima gigas*) with 49-62% CP were identified, which can be used in the elaboration of feed for feeding giant South American turtle and yellow-spotted river turtle hatchlings in community farms.

In the wild, juvenile giant South American turtles presented food items that were more than 15% in animal origin (fish and shells), while yellow-spotted river turtles presented a diet only with vegetable items (fruits, leaves, stems and seeds).

Giant South American turtle (*P. expansa*) hatchlings had better performance and higher consumption when fed with commercial feed and artisanal feeds with higher levels of animal-based protein (viscera meal) than vegetable-based (murumuru cake) proteins, and higher levels of protein and ether extract. To replace commercial feed, pirarucu viscera meal (between 33 to 50%) can be included in the diet of giant South American turtle hatchlings and improves growth, weight gain and feed conversion.

Yellow-spotted river turtle (*P. unfilis*) hatchlings also had greater growth when fed with commercial feed and artisanal feed with more balanced levels of animal and vegetable-based proteins, and were less interested in animal protein than the giant South American turtles. However, it should be noted that, for these animals, higher levels of animal protein (above 50%) in the diet seem to increase mortality.

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