Article

Not peer-reviewed version

Replacement of Fish Meal with Crustacean Meals in Diets for Long Snouted Seahorse, Hippocampus guttulatus: Digestibility and Growth Performance

Jorge Palma *, Miguel Correia, José Pedro Andrade, Dominique Bureau

Posted Date: 15 August 2024

doi: 10.20944/preprints202408.1089.v1

Keywords: Seahorse; Hippocampus guttulatus; digestibility; fish meal; crustacean meals

 doi:10.20944/preprints202408.1089.v1

Article

Replacement of Fish Meal with Crustacean Meals in Diets for Long Snouted Seahorse, *Hippocampus guttulatus*: Digestibility and Growth Performance

Jorge Palma [1,*], Miguel Correia [2,3], José Pedro Andrade [1] and Dominique Bureau [4]

[1] Centre of Marine Sciences (CCMAR), University of the Algarve, 8005-139 Faro, Portugal; jandrade@ualg.pt
[2] Seahorse, Pipefish, and Seadragon Specialist Group, IUCN Species Survival Commission, Gland, Switzerland
[3] Project Seahorse, Institute for the Oceans and Fisheries, The University of British Columbia, Vancouver, British Columbia, Canada; m.correia@oceans.ubc.ca (M.C.)
[4] UG/OMNR Fish Nutrition Research Laboratory, Department of Animal and Poultry Science, University of Guelph, ON, N1G 2W1, Canada; dbureau@uoguelph.ca (D.B.)
* Correspondence: jpalma@ualg.pt

Abstract: This study investigated the effect of partially replacing fish meal with krill and copepod meals in inert diets, co-fed with shrimp, on the growth and nutrient digestibility of long-snout seahorse (*Hippocampus guttulatus*). A control diet (Diet 1) using raw starch and four experimental diets with similar protein (≈44.8%) and energy (≈15.1 MJ/kg) levels were tested. Diet 2 used fish meal as the sole protein source, while in Diets 3-5, krill and copepod meals replaced 44% of the fish meal. Seahorses fed Shrimp + Diets 2-5 showed significantly higher growth rates ($P < 0.05$) than those fed Shrimp + Diet 1, though there were no significant growth differences among Diets 2-5. Digestibility of dry matter (46.1% to 72.2%), lipids (73.3% to 85.5%), crude protein (89.8% to 95.8%), energy (82% to 92.2%), and phosphorus (28.7% to 64.4%) varied with diet, being consistently lower in seahorses fed Shrimp + Diet 1. As an agastric species, *H. guttulatus* did not exhibit impaired digestibility for any of the tested nutrients, minerals, or energy. This study suggests that crustacean meals can effectively substitute fish meal in inert diets for this species, contributing to the sustainability and optimization of captive seahorse husbandry practices.

Keywords: seahorse; *Hippocampus guttulatus*; digestibility; fish meal; crustacean meals

Key Contribution: This study demonstrates that seahorse species have a digestive capacity comparable to other fish species, with a high nutrient digestibility rate, despite their agastric physiology. When formulating inert diets for seahorses, fish meal can be successfully replaced by crustacean meals without compromising nutrient digestibility or growth performance.

1. Introduction

In marine aquaria, seahorses (*Hippocampus* spp.) are highly sought due to their distinctive morphology and unique mating behaviors [1–3]. However, the escalating demand for live and dried seahorses for use in Traditional Chinese Medicine (TCM) across several Asian countries [4,5] has led to decline in wild populations and their overexploitation [5]. In response, seahorse aquaculture emerges as the potential to meet the demand for the marine ornamental aquarium trade [6] while simultaneously alleviating pressure on wild populations. According to [7], the sale of live seahorses dominates the market for aquaculture-sourced seahorses. Nonetheless, seahorses remain as candidate species for aquaculture due to lingering uncertainties surrounding their nutritional requirements, with feeds representing a crucial bottleneck in their cultivation. Seahorses are agastric teleosts (lack a stomach) [8], and in such species, digestion primarily occurs in the intestine [9].

 doi:10.20944/preprints202408.1089.v1

Seahorses whose offspring undergo direct development in the male's brood pouch hatch with a fully developed digestive tract [10,11], enabling exogenous feeding within a few hours after release from the male's pouch [11,12]. In aquaculture, high seahorse mortality rates at early life stages have been linked to nutritional factors, diet digestibility [11,13,14], and low digestive efficiency and nutrient absorption due to the incomplete functionality of the digestive tract at those stages [13,15].

Regardless of their life stage, however, seahorses have a requirement to be fed natural diets, whether live or frozen, driven by their imprinted feeding behavior, which compels them to accept and consume only feed items that resemble recognizable prey shapes [14]. Although adult seahorses can be fed natural frozen diets [14,16,17], these can be enriched to enhance and meet the seahorse's nutritional requirements [14]. In the wild, regardless of the species' geographic distribution, seahorses primarily feed on available crustacean species [18–20], a dietary specificity that can be replicated using crustacean meals.

One of the first critical steps in developing diets for candidate aquaculture species is determining the digestibility coefficients (ADC) for various potential feed ingredients [21,22]. Assessing ingredient digestibility is essential for understanding the nutritional value of a feed ingredient and formulating research or commercial diets based on digestible nutrients [23]. Historically, fish meal has been the primary diet ingredient owing to its undeniable qualities, including high protein content, a well-balanced amino acid profile, high digestibility and palatability, and the absence of anti-nutritional factors [24,25]. However, in the contemporary pursuit of more sustainable and cost-effective food sources, numerous studies have focused on identifying alternatives to fish meal. Progress has been made in replacing fish meal with plant protein ingredients like soybeans, lupins, peas, and canola [24,26]. However, plant-based meals may exert detrimental effects on growth performance [27–30], feed efficiency [28,31], nutrient digestibility [32,33], digestive enzyme activities [32,34], and overall health condition [24,30]. This is mainly due to the fact that the use of plant ingredients by carnivorous species may be inadequate due to their high carbohydrate content, unbalanced amino acid profiles, and the presence of anti-nutritional factors [35], such as protease/trypsin inhibitors, saponins, or mycotoxins.

Unlike fish meal, untapped marine resources with substantial biomass can be found at lower trophic levels. This means that sustainable sources of crustacean meals can be obtained, with Antarctic krill (*Euphausia superba*) emerging as a promising candidate [36–39]. Current estimates indicate a standing biomass of 379 million tons [40], with just over 300,000 tons being harvested in the Atlantic Sector (Area 48) in 2018 [41]. Krill meal provides a high protein content, favorable amino acid, and fatty acid profiles, as well as enhanced palatability properties [39]. Thus, this study aimed to assess the efficacy of crustacean proteins as a replacement for fish meal in practical co-feed diets for the long-snout seahorse, *Hippocampus guttulatus*.

2. Materials and Methods

Ninety F3 generation *H. guttulatus* individuals were selected from a captive stock. Initially, the fish were manually sorted to determine their sex. Only males showing no visible signs of pregnancy were included to prevent initial wet weight bias. The fish were acclimated to the rearing conditions for one week and fed frozen Atlantic ditch shrimp (*Palaemonetes varians*) during this period.

The digestibility trial followed a completely randomized design, with three replicate tanks assigned to each of the five dietary treatments. Each tank was a 110-L fiberglass square bottom unit assembled in a flow-through system. Fifteen tanks were used in total, and each tank housed six animals (three males and three females). The tanks had a constant water flow of 110 L/h and moderate aeration. Seawater, filtered through continuous sand and biological filtration, entered the tanks through a black polystyrene tubing placed at the surface level in the corner of the tank. The water outflow structure, located in the centre of the tanks, consisted of a PVC tube structure covered with a mesh screen (500 μm diameter) at the water surface. Three nylon nautical rope holdfasts (1 cm ø) were placed inside each tank.

The temperature and salinity of the water were maintained at 20.5 ± 0.3 ˚C and 36.2 ± 0.1 ppt, respectively. The tanks were illuminated from above using two 36 W fluorescent tubes, providing an

 doi:10.20944/preprints202408.1089.v1

intensity of 547.5 ± 20.5 lux at the water surface. The photoperiod was controlled by a timer set to a 14-hour light and 10-hour dark cycle (0600–2000 h). Water quality parameters, including ammonia, nitrates, and nitrites, were measured twice a week, and remained stable throughout the experiment. Ammonia levels were consistently below detectable levels, nitrate levels were below 0.3 mg/L, and nitrite levels were below 1.25 mg/L. The experiment was conducted over a period of 90 days.

2.1. Experimental Diet Preparation

One control diet (Diet 1) consisting of raw starch without any protein or lipid sources was prepared to serve as the baseline diet for later analysis of apparent digestibility coefficients (ADC) for the non-supplemented shrimp diet (Table 1). Four additional isoproteic (≈44.8% crude protein [CP]) and isoenergetic (≈15.1 MJ digestible energy [DE]/kg) experimental inert diets were formulated, using different animal protein including fish meal, krill meal, and copepod meal (Cyclop-eeze®; Argent Chemical Laboratories, Redmond, WA, USA). In Diet 2, fish meal was included as the sole animal protein source. In Diets 3 to 5, krill and copepod meals were added at varying inclusion rates, resulting in a 44% replacement of fish meal (Table 1). All other ingredients remained consistent across the four diets (Table 1).

Table 1. Ingredients and proximate composition of the experimental diets (g/100g dry diet).

Ingredient (g/100 g)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal, herring	0	45	25	25	25
Krill meal	0	0	20	0	10
Cyclop-eeze	0	0	0	20	10
Soy protein concentrate	0	10	10	10	10
Starch, raw	95	0	0	0	0
Wheat flour	0	23.5	24	26	25
Wheat gluten	0	10	10	10	10
Krill oil	0	6.5	6	4	5
Vitamin premix[1]	1	1	1	1	1
Mineral premix[2]	1	1	1	1	1
$CaHPO_4$	2	2	2	2	2
Marker (Cr_2O_3)	1	1	1	1	1
Carophyl pink	0.1	0.1	0.1	0.1	0.1
Analyzed composition (g/100g)					
Dry matter	90.5	91.1	91.1	90.9	91.0
Crude Protein	0	46.7	45.1	43.3	44.2
Lipid	0	11.8	12.3	14.3	13.3
Ash	2.5	8.7	8.6	6.9	7.7
Gross energy (MJ/kg)	16.7	16.0	14.9	13.5	14.2

To evaluate the ADC, 10 g/kg of chromic oxide (Cr_2O_3; Merck KGaA, Germany) was added to each diet as an inert marker. Once the ingredients were mixed, the diets were steam pelleted using a laboratory pellet mill (California Pellet Mill, San Francisco, CA, USA), dried overnight under forced air at 35°C, and stored at 4 °C until use.

2.2. Shrimp Culture

Supplemented shrimps were prepared following the method outlined by [14]. In brief, Atlantic ditch shrimp (*Palaemonetes varians*) were captured in a single fishing event and sorted to an average size of 18.4 ± 1.8 mm (total length from the tip of the rostrum to the end of the telson). These shrimps were then distributed among five fiberglass tanks, with one tank assigned to each diet. The tanks were set up under the same conditions as described earlier.

 doi:10.20944/preprints202408.1089.v1

The shrimps were starved for 24 hours to ensure empty stomachs and then fed with each of the five diets (Diets 1 to 5) until they reached satiation. The feeding duration was approximately 15 minutes, during which the shrimps consumed their fill, and their stomachs became full. Following this feeding period, the shrimps were collected, immediately frozen, and stored at a temperature of -18 °C until further use. Prior to freezing, a sample weighing 100 g (wet weight) from each dietary treatment was preserved for future proximate analysis (Table 2).

Table 2. Proximate composition (dry matter basis) of the tested diets (shrimp + each of the inert diets).

Diets	Shrimp+D1	Shrimp+D2	Shrimp+D3	Shrimp+D4	Shrimp+D5
% Dry Matter	96.8	96.9	95.7	93.4	97.2
Ash %	20.0	20.4	20.3	20.5	20.1
Lipid %	5.4	5.6	5.7	5.5	5.7
Protein %	69.0	73.2	72.4	74.2	72.5
Phosphorus%	1.1	1.2	1.2	1.2	1.2
Gross Energy (kJ/g)	17.9	18.6	18.8	19.2	18.5

2.3. Digestibility and Growth Trial

The seahorses were fed the experimental diets once a day at approximately 6% body weight day^{-1}, ensuring a slight excess of feed. The daily shrimp ration was thawed in seawater before use. Once defrosted, excess water was drained from the diets, and they were gently dried, weighed, and fed to the seahorses. After a period of 2 hours, any remaining food was collected and subtracted from the initial amount offered. Seahorses produce fecal pellets that remain relatively intact, allowing for collection with minimal loss of dry matter. Fecal samples were collected in the morning before feeding, using a siphoning method. The fecal collections from each tank were combined daily and stored frozen at -18 °C until a sufficient quantity was obtained for chemical analyses. The apparent digestibility coefficients (ADC) of dry matter, energy, and protein were determined using the following formulas:

$$\text{ADC of dry matter (\%)} = (100 - (\text{dietary } Cr_2O_3/\text{fecal } Cr_2O_3) \times 100) \quad (1)$$

$$\text{ADC of nutrients or energy (\%)} = 100 \times [1 - (Cr_2O_3 \text{ in diet}/ Cr_2O_3 \text{ in feces}) \times (Y \text{ in feces}/Y \text{ in diet})]. \quad (2)$$

where Y is the nutrient or energy content

Fish sampling was conducted every 2 weeks to record weight and length measurements, as well as to adjust the daily feed ration accordingly. After each sampling event, the daily wet weight of each ration was modified based on the average wet weight increase observed in each treatment group. This adjustment ensured that appropriate feed rations were maintained throughout the study period (i.e., "2-week ration adjustment"). To minimize stress during the sampling process, seahorses were measured differently than the three-length measurements proposed by [42] for obtaining total length. Instead, seahorses were measured by summing the head length (from the tip of the snout to the midpoint of the cleithral ring) and the body height (from the same point on the cleithral ring to the tip of the outstretched tail). This measurement approach aimed to minimize stress on the seahorses during sampling. Data was used to calculate: (1) weight gain (WG, g/fish) = $(W_f - W_i)/W_i$, where W_f is the final seahorse wet weight and W_i is the initial wet weight, (2) growth rate of the fish was calculated using the thermal-unit growth coefficient (TGC) = $([W_f^{1/3} - W_i^{1/3}]/\Sigma[T \times D]) \times 1000$, where W_f is the final seahorse wet weight and W_i is the initial wet weight, T the water temperature (C), and D is the number of days, (3) food conversion rate (FCR) = estimated individual feed consumption (dry weight)/increase in the individual wet weight, and (4) condition factor (CF) = (wet weight (g)/height^3 (cm)) ×100.

 doi:10.20944/preprints202408.1089.v1

2.4. Chemical Analyses

Diets and feces were analyzed following the procedures outlined in AOAC (1995). In brief, the dry matter was analyzed by drying samples at 105 °C to constant weight; ash by incineration of samples at 550 °C for 5 h; crude protein (N × 6.25) using a Leco® nitrogen analyzer (Leco Corporation, St. Joseph, USA); crude lipid by extraction with methyl-ether (ANKOM® XT10 Extractor); crude fiber by acid and basic digestion (Fibertec® System M., 1020 Hot Extractor, Tecator). The inert marker in diets and feces was quantified according to [43] protocol. Feeds and feces were incinerated at 650 °C for 16 h, followed by 10-min acid digestion in boiling HCl (1 N), and a second incineration at 650 °C for 16 h. All analyses were performed in triplicate.

2.5. Statistical Analyses

The data is presented as mean ± standard deviation (mean ± s.d). Growth analysis involved testing the differences in length, wet weight, CF, TGC, and FCR using nested ANOVA, followed by post hoc Neuman-Keul's (NK) multiple comparison test ($P = 0.05$). For the digestibility analysis, the mean values, and standard deviations of the digestibility of the five compound diets were calculated. Analysis of variance and the Tukey test were employed to determine the significance of differences ($P < 0.05$) between the digestibility of the diets. All statistical analyses were performed using GraphPad Prism® (version 6.00 for Windows; GraphPad Software, San Diego, CA, USA) software package.

3. Results

The data on final length, final weight, WG, TGC, CF, FCR, and survival are presented in Table 3. The growth rate was significantly higher ($P<0.05$) in seahorses fed shrimp + Diets 2 to 5 compared to those fed shrimp + Diet 1. However, there were no significant differences ($P > 0.05$) in growth rate between seahorses fed each of the four shrimp supplement diets (Table 3).

Table 3. Growth performance of adult *H. guttulatus* fed different diets (shrimp + each of the inert diets) at the end of growth trial (mean± s.d., n= 3). Different superscripts in the same row denote statistically significant differences ($P < 0.05$).

	Shrimp+D1	Shrimp+D2	Shrimp+D3	Shrimp+D4	Shrimp+D5
Initial length (cm)	15.9±0.3^a	15.7±0.3^a	15.7±0.2^a	15.8±0.2^a	15.9±0.3^a
Final length (cm)	17.4±0.5^a	17.5±0.4^a	17.7±0.7^a	17.5±0.7^a	17.7±0.6^a
Initial weight (g)	9.2±0.6^a	9.2±0.5^a	9.3±0.4^a	9.3±0.5^a	9.2±0.7^a
Final weight (g)	13.2±1.1^b	14.9±1.3^a	15.3±1.4^a	15.4±1.6^a	15.2±1.5^a
WG (g/fish)	0.43±0.21^b	0.62±0.2^a	0.65±0.18^a	0.66±0.24^a	0.65±0.27^a
TGC	0.07±0.02^b	0.10±0.01^a	0.11±0.01^a	0.11±0.02^a	0.11±0.02^a
Initial CF	0.23±0.02^a	0.24±0.02^a	0.24±0.03^a	0.24±0.02^a	0.23±0.03^a
Final CF	0.25±0.05^a	0.28±0.04^a	0.28±0.06^a	0.29±0.05^a	0.27±0.06^a
FCR	3.8±0.5^b	2.7±0.3^a	2.6±0.5^a	2.5±0.5^a	2.6±0.4^a
% survival	100	100	100	100	100

Similarly, WG, TGC, and FCR values were significantly higher ($P < 0.05$) in seahorses fed shrimp with supplemented diets. At the end of the experiment, there were no significant differences ($P > 0.05$) in CF among the animals fed each of the tested diets.

Table 4 presents the apparent digestibility coefficients of the tested diets and the derived coefficients from the test feed ingredients for long-snout seahorse. The apparent digestibility of dry matter (46.1-72.2%), lipids (73.3-85.5%), crude protein (89.8-95.8%), energy (82-92.2%), and phosphorus (28.7-64.4%) was influenced by the test ingredients ($P < 0.05$). Dry matter and nutrient digestibility were consistently lower ($P < 0.05$) for Shrimp+Diet 1 compared to all other tested diets.

 doi:10.20944/preprints202408.1089.v1

Table 4. Apparent digestibility of nutrients, energy, and phosphorus in the test ingredients for *H. guttulatus*. Different superscripts in the same row denote statistically significant differences (P < 0.05).

	Shrimp+D1	Shrimp+D2	Shrimp+D3	Shrimp+D4	Shrimp+D5
Dry Matter	46.1±0.7^{b}	72.1±1.1^{a}	72.3±2.6^{a}	71.5±2.6^{a}	72.2±0.5^{a}
Lipid	73.3±0^{b}	85.5±0.7^{a}	85±2.9^{a}	83.9±1.1^{a}	83.9±1.6^{a}
Protein	89.8±0.3^{b}	95.8±0.2^{a}	95.2±0.5^{a}	95.6±0.4^{a}	95.3±0.1^{a}
Energy	82±0.5^{b}	92.2±0.4^{a}	90.5±0.6^{a}	91.5±0.5^{a}	90.6±0.3^{a}
Phosphorus	28.7±0.1^{b}	64.4±1.7^{a}	59.1±2.7^{a}	63±3.2^{a}	61.6±2^{a}

Crude protein digestibility was generally high and consistent across the four shrimp + supplemented diets, regardless of the protein source (fish, krill, or copepod meal). Gross energy digestibility followed a similar trend to protein, with similarly high values. However, phosphorus digestibility was the lowest among the nutrients tested, with particularly poor digestibility in Shrimp+Diet 1 (28.7±0.1) compared to Shrimp+D2 (64.4±1.7).

4. Discussion

Digestibility studies play a critical role in understanding nutrient utilization and facilitate precise formulation of diets for specific fish species [44]. Seahorses remain promising candidates for aquaculture due to their dependence on natural prey. Consequently, there is limited knowledge concerning seahorse nutrient requirements, retention of major nutrients, and energy utilization. Providing a mixed prey diet to closely resemble their natural diet is challenging, if not impossible. Hence, natural prey supplementation becomes essential in their production and as carnivorous species, dietary supplementation should aim to closely mimic their natural diet. In carnivorous fish diets, fish meal (FM) is often used as main ingredient, as different fish meals, which provide high levels of essential amino acids, are typically well digested, and contain few anti-nutritional factors [45]. However, the extensive use of FM in aquafeed production exerts significant pressure on global fish stocks. Consequently, reducing its usage is imperative to bolster the sustainability of aquaculture feeds, especially in formulating diets for candidate species like seahorses.

Various animal-protein ingredients have been tested as partial or complete alternatives to FM in diets for different fish species, with varying success [46]. Replacement of FM with alternative protein sources has proven effective in supporting or even enhancing growth performance in numerous fish species including European seabass (*Dicentrarchus labrax*) [26,47,48], largemouth bass (*Micropterus salmoides*) [49,50], white snook (*Centropomus viridis*) [51], rainbow trout (*Oncorhynchus mykiss*) [52,53], California yellowtail (*Seriola dorsalis*) [54], red tilapia (*Oreochromis niloticus* × *O. mossambicus*) [55], Atlantic salmon (*Salmo salar*) [56], gilthead sea bream (*Sparus aurata*) [57], and olive flounder (*Pleuronectes platessa*) [58]. In the present study, FM was partially replaced with crustacean meals without compromising the growth performance and digestibility of the supplementary diets. Concordantly, seahorses fed shrimp with diet supplementation exhibited significantly higher growth rates ($P<0.05$) compared to those fed on a non-supplemented shrimp diet. Notably, all four supplemented shrimp diets significantly improved growth performance ($p<0.05$) compared to the non-supplemented shrimp diet, with no significant differences ($p>0.05$) observed among the supplemented diets. This suggests that using alternative protein sources is a viable option, allowing for an effective reduction in the amount of fish meal (FM) used. These findings offer valuable insights, supporting the conclusion that more sustainable crustacean meals can be a feasible alternative to minimize, or even replace, FM in seahorse diets. These results align with those obtained by [14] who reported increased growth, and positive effects on reproductive rate and brood quality of *H. guttulatus* when fed a similar diet supplementation procedure. Similarly, [48] noted improved growth performance and feed utilization by European seabass (*D. labrax*) when using Antarctic krill meal as fish meal replacement. Furthermore, the use of crustacean meals in seahorse diets is fully justified to mimic the natural crustacean-based diet of these species.

The chromic oxide method [59] has been extensively utilized to assess the apparent digestibility of fish feeds. In the present study, the concentration of chromium oxide (in g/kg of diet) in the inert

 doi:10.20944/preprints202408.1089.v1

diets was intentionally higher than typically observed in most studies. This adjustment was made to account for the natural diet portion and ensure an adequate level of chromium oxide in the overall diet provided. This enabled accurate quantification of nutrients in both the diets and feces. This approach was successfully validated, as the quantity of chromic oxide employed allowed for the calculation of apparent digestibility coefficients (ADC) for the tested feed ingredients.

Protein digestibility ranged between 89.8% and 95.8%, with the non-supplemented shrimp diet displaying the lowest digestibility. This suggests that the long-snout seahorse possesses a notable capacity to digest protein despite its agastric physiology. In agastric fish species, an intestinal bulb or expansion in the anterior intestine, which serves as a food storage area [60], is present, functioning like a stomach. In the absence of pyloric caeca, agastric fish may have relatively longer intestines, measuring several times the length of the animal's body, which compensates for the lack of a stomach and increases the overall surface area of the intestine [61]. Therefore, these aforementioned agastric physiological characteristics do not impair protein digestibility. In fact, it was even higher than observed in other species (e.g., *M. salmoides* by [62], *Morone saxatilis* by [63] and similar to *O. mykiss* [64], and *Bidyanus bidyanus* [65] when testing FM digestibility).

However, the apparent digestibility of dry matter (DM) and phosphorus was generally lower, particularly for the control shrimp diet without dietary supplementation. Phosphorus is a crucial mineral required for normal growth, reproduction, and overall health in fish [66]. It serves as a major component of the skeleton, nucleic acids, and cell membranes, and plays a direct role in all energy-producing cellular reactions [67]. While fish can absorb phosphorus from water, its concentration is typically low in both fresh water and seawater, making food the primary source for fish [68].

The apparent digestibility coefficient (ADC) of phosphorus from fish meal has been reported to range from 17% to 81% for rainbow trout [69–72], from 0% to 61% for carp species [69,73,74], from 27% to 65% for tilapia species [75–78], and 58% for the Senegalese sole [79]. In the present study, the ADC of phosphorus ranged from 28.7±0.1% to 64.4±1.7%, falling within the reported range for other species. Despite the fact that the phosphorus percentage of inclusion in each of the tested diets was quite similar, the ADC of phosphorus increased in fish fed shrimp with supplemented diets (59.1±2.7% to 64.4±1.7%) compared to those fed the non-supplemented shrimp diet (28.7±0.1%). Therefore, phosphorus digestibility seems to be directly dependent on its source, increasing in direct relation to the inclusion of the tested ingredients (fish, krill, and copepod meals).

Overall, the *H. guttulatus*, being an agastric species, did not exhibit impaired digestibility for any of the nutrients, minerals, or energy tested. Thus, their apparent digestibility of the tested diets was proportional to the nutritional quality of the diets themselves.

Author Contributions: Conceptualization, J.P. and D.B.; methodology, J.P. and M.C.; formal analysis, J.P.; writing—original draft preparation, J.P.; writing—review and editing, all authors; project administration, J.P.; funding acquisition, J.P. and J.P.A.. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by project HIPPONUTRE—"Cultivo do cavalo marinho de focinho comprido, *Hippocampus guttulatus*: Optimização zootécnica e avaliação de requisitos nutricionais" (Programa Operacional MAR2020, 16-02-01-FMP-54); project HIPPOCAMPUS_RIA – "Avaliação e implementação de conservação ecossistémica de cavalos-marinhos" and received Portuguese national funds from FCT - Foundation for Science and Technology through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020.

Institutional Review Board Statement: CCMAR facilities and the research are certified to house and conduct experiments with live animals (Group-C licenses from the Direção Geral de Alimentação e Veterinária, Ministério da Agricultura, Florestas e Desenvolvimento Rural, Portugal). This study was performed by accredited researchers in laboratory animal science by the Portuguese Veterinary Authority (1005/92, DGV-Portugal, following FELASA category C recommendations) and conducted according to the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals for scientific purposes. The study adhered to the guidelines outlined by the European Union Council (86/609/EU) and the relevant Portuguese legislation concerning the use of laboratory animals. No animals were killed or died during the trials.

Data Availability Statement: The data used during the current study are available from the corresponding author upon reasonable request.

 doi:10.20944/preprints202408.1089.v1

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Masonjones, H.D.; Lewis, S.M. Differences in potential reproductive rates of male and female seahorses related to courtship roles. *Anim. Behav*. 2000, *59*, 11-20. [https://doi.org/10.1006/anbe.1999.1269]
2. Olivotto, I.; Planas, M.; Simões, N.; Holt, G.J.; Avella, M.A.; Calado, R. Advances in Breeding and Rearing Marine Ornamentals. *J. World Aquac. Soc.* 2011, *42*, 135-166. [https://doi.org/10.1111/j.1749-7345.2011.00453.x]
3. Wilson, M.J.; Vincent, A.C.J. Preliminary success in closing the life cycle of exploited seahorse species, *Hippocampus* spp., in captivity. *Aquar. Sci. Cons*. 1998, *2*, 179-196. [https://doi.org/10.1023/A:1009629130932]
4. Lourie, S.A.; Foster, S.J.; Cooper, E.W.T.; Vincent, A.C.J. *A Guide to the identification of seahorses*. Project Seahorse and TRAFFIC: University of British Columbia and World Wildlife Fund: Washington D.C., USA, 2004; ISBN 0 89164 169 6.
5. Vincent, A.C.J. *The international trade in seahorses*; TRAFFIC International: Cambridge, UK, 1996; ISNB 1 85850 098 2.
6. Martin-Smith, K.M.; Vincent, A.C.J. Exploitation and trade of Australian seahorses, pipehorses, sea dragons and pipefishes (Family Syngnathidae). Oryx 2006, *40*, 141-151. [https://doi.org/10.1017/S003060530600010X]
7. Koldewey, H.J.; Martin-Smith, K.M. A global review of seahorse aquaculture. Aquaculture 2010, *302*, 131-152. [https://doi.org/10.1016/j.aquaculture.2009.11.010]
8. Wilson, A.B.; Orr, J.W. The evolutionary origins of Syngnathidae: pipefishes and seahorses. *J. Fish Biol*., 2011, *78*, 1603-1623. [https://doi.org/10.1111/j.1095-8649.2011.02988.x]
9. Rønnestad, I.; Yúfera, M.; Ueberschär, B.; Ribeiro, L.; Sæle, Ø.; Boglione, C. Feeding behaviour and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research. Reviews in Aquaculture 2013, *5*, S59-S98. [https://doi.org/10.1111/raq.12010]
10. Ofélio, C.; Cohen, S.; Adriaens, D.; Radaelli, G.; Díaz, A.O. Histochemistry of goblet cells and micro-computed tomography to study the digestive system in the long-snouted seahorse *Hippocampus guttulatus*. *Aquaculture* 2019, *502*, 400-409. [https://doi.org/10.1016/j.aquaculture.2018.12.048]
11. Palma, J.; Bureau, D.P.; Andrade, J.P. The effect of diet on ontogenic development of the digestive tract in juvenile reared long snout seahorse *Hippocampus guttulatus*. *Fish Physiol. Biochem.* 2014, *40*, 739-750. [https://doi.org/10.1007/s10695-013-9881-8]
12. Roos, G.; Van Wassenbergh, S.; Aerts, P.; Herrel, A.; Adriaens, D. Effects of snout dimensions on the hydrodynamics of suction feeding in juvenile and adult seahorses. *J. Theor. Biol.* 2011, *269*, 307-317. [https://doi.org/10.1016/j.jtbi.2010.10.023]
13. Blanco, A.; Planas, M.; Moyano, F.J. Ontogeny of digestive enzymatic capacities in juvenile seahorses *Hippocampus guttulatus* fed on different live diets. *Aquac. Res*. 2016 *47*, 3558-3569. [https://doi.org/10.1111/are.12806]
14. Palma, J.; Andrade, J.P.; Bureau, D.P. Growth, Reproductive performances, and brood quality of long snout seahorse, *Hippocampus guttulatus*, fed enriched shrimp diets. *J. World Aquac. Soc*. 2012, *43*, 802-813. [https://doi.org/10.1111/j.1749-7345.2012.00611.x]
15. Palma, J.; Lima, R.; Andrade, J.P.; Lança, M.J. Optimization of live prey enrichment media for rearing juvenile short-snouted seahorse, *Hippocampus hippocampus*. *Fishes* 2023, *8*, 494. [https://doi.org/10.3390/fishes8100494]
16. Palma, J.; Stockdale, J.; Correia, M.; Andrade, J.P. Growth and survival of adult long snout seahorse (*Hippocampus guttulatus*) using frozen diets. *Aquaculture* 2008, *278*, 55-59. [https://doi.org/10.1016/j.aquaculture.2008.03.019]
17. Woods, C.M.C.; Valentino, F. Frozen mysids as an alternative to live Artemia in culturing seahorses *Hippocampus abdominalis*. *Aquac. Res*. 2003, *34*, 757-763. [https://doi.org/10.1046/j.1365-2109.2003.00882.x]
18. Kitsos, M.S.; Tzomos, T.; Anagnostopoulou, L.; Koukouras, A. Diet composition of the seahorses, *Hippocampus guttulatus* Cuvier, 1829 and *Hippocampus hippocampus* (L., 1758) (Teleostei, Syngnathidae) in the Aegean Sea. *J. Fish Biol*. 2008, *72*, 1259–1267. [https://doi.org/10.1111/j.1095-8649.2007.01789.x]
19. Woods, C.M.C. Natural diet of the seahorse *Hippocampus abdominalis*. *N. Z. J. Mar. Freshw. Res.* 2002, *36*, 655-660. [https://doi.org/10.1080/00288330.2002.9517121]
20. Yip, M.Y.; Lim, A.C.O.; Chong, V.C.; Lawson, J.M.; Foster, S.J. Food and feeding habits of the seahorses *Hippocampus spinosissimus* and *Hippocampus trimaculatus* (Malaysia). *J. Mar. Biol. Assoc. U. K.* 2014, *95*, 1033-1040. [https://doi.org/10.1017/S0025315414001660]
21. Burr, G.S.; Barrows, F.T.; Gaylord, G.; Wolters, W.R. Apparent digestibility of macro-nutrients and phosphorus in plant-derived ingredients for Atlantic salmon, *Salmo salar* and Arctic charr, *Salvelinus alpinus*. *Aquac. Nut*. 2011, *17*, 570-577. [https://doi.org/10.1111/j.1365-2095.2011.00855.x]

22. NRC 2011. *Nutrient Requirements of Fish and Shrimp*. The National Academic Press; Washington, D.C., USA. [https://doi.org/10.17226/13039]
23. Booth, M.A.; Allan, G.L.; Smullen, R.P. Digestibility of common feed ingredients by juvenile mulloway *Argyrosomus japonicus*. *Aquaculture* 2013, *414-415*, 140-148. [https://doi.org/10.1016/j.aquaculture.2013.07.04]
24. Gatlin, D.M.; Barrows, F.T.; Brown, P.; Dabrowski, K.; Gaylord, T.G.; Hardy, R.W.; Herman, E.; Hu, G.; Krogdahl, Å.; Nelson, R.; Overturf, K.; Rust, M.; Sealey, W.; Skonberg, D.; J Souza, E.; Stone, D.; Wilson, R.; Wurtele, E. Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquac. Res*. 2007, *38*, 551-579. [https://doi.org/10.1111/j.1365-2109.2007.01704.x]
25. Tacon, A.G.J.; Hasan, M.R.; Metian, M. Demand and supply of feed ingredients for farmed fish and crustaceans: Trends and prospects. *FAO Fish. Tech. Pap.* 2011, *564*, 71-87. [https://www.fao.org/4/ba0002e/ba0002e.pdf]
26. Torrecillas, S.; Mompel, D.; Caballero, M.J.; Montero, D.; Merrifield, D.; Rodiles, A.; Robaina, L.; Zamorano, M.J.; Karalazos, V.; Kaushik, S.; Izquierdo, M. Effect of fishmeal and fish oil replacement by vegetable meals and oils on gut health of European sea bass (*Dicentrarchus labrax*). *Aquaculture* 2017, *468*, 386-398. [https://doi.org/10.1016/j.aquaculture.2016.11.005]
27. Lim, S.J.; Kim, S.S.; Ko, G.Y.; Song, J.W.; Oh, D.H.; Kim, J.D.; Kim, J.U.; Lee, K.J. Fish meal replacement by soybean meal in diets for Tiger puffer, *Takifugu rubripes*. *Aquaculture* 2011, *313*, 165-170. [https://doi.org/10.1016/j.aquaculture.2011.01.007]
28. Silva-Carrillo, Y.; Hernández, C.; Hardy, R.W.; González-Rodríguez, B.; Castillo-Vargasmachuca, S., The effect of substituting fish meal with soybean meal on growth, feed efficiency, body composition and blood chemistry in juvenile spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869). *Aquaculture* 2012, *364-365*, 180-185. [https://doi.org/10.1016/j.aquaculture.2012.08.007]
29. Song, Z.; Li, H.; Wang, J.; Li, P.; Sun, Y.; Zhang, L. Effects of fishmeal replacement with soy protein hydrolysates on growth performance, blood biochemistry, gastrointestinal digestion and muscle composition of juvenile starry flounder (*Platichthys stellatus*). *Aquaculture* 2014, *426-427*, 96-104. [https://doi.org/10.1016/j.aquaculture.2014.01.002]
30. Ye, J.; Liu, X.; Wang, Z.; Wang, K. Effect of partial fish meal replacement by soybean meal on the growth performance and biochemical indices of juvenile Japanese flounder *Paralichthys olivaceus*. *Aquac. Int*. 2011, *19*, 143-153. [https://doi.org/10.1007/s10499-010-9348-1]
31. Tantikitti, C.; Sangpong, W.; Chiavareesajja, S. Effects of defatted soybean protein levels on growth performance and nitrogen and phosphorus excretion in Asian seabass (*Lates calcarifer*). *Aquaculture* 2005, *248*, 41-50. [https://doi.org/10.1016/j.aquaculture.2005.04.027]
32. Tibaldi, E.; Hakim, Y.; Uni, Z.; Tulli, F.; de Francesco, M.; Luzzana, U.; Harpaz, S. Effects of the partial substitution of dietary fish meal by differently processed soybean meals on growth performance, nutrient digestibility and activity of intestinal brush border enzymes in the European sea bass (*Dicentrarchus labrax*). *Aquaculture* 2006, *261*, 182-193. [https://doi.org/10.1016/j.aquaculture.2006.06.026]
33. Zhang, Y.; Ji, W.; Wu, Y.; Han, H.; Qin, J.; Wang, Y. Replacement of dietary fish meal by soybean meal supplemented with crystalline methionine for Japanese seabass (*Lateolabrax japonicus*). *Aquacult. Res*. 2016, *47*, 243-252. [https://doi.org/10.1111/are.12486]
34. Li, Y.; Ai, Q.; Mai, K.; Xu, W.; Deng, J.; Cheng, Z. Comparison of high-protein soybean meal and commercial soybean meal partly replacing fish meal on the activities of digestive enzymes and aminotransferases in juvenile Japanese seabass, *Lateolabrax japonicus* (Cuvier, 1828). *Aquacult. Res*. 2014, *45*, 1051-1060. [https://doi.org/ 10.1111/are.12042]
35. Oliva-Teles, A.; Enes, P.; Peres, H. Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish, In *Feed and Feeding Practices in Aquaculture*; Davis, D.A. (Ed.); Woodhead Publishing, Oxford, UK, 2015; pp. 203-233. [https://doi.org/10.1016/B978-0-08-100506-4.00008-8]
36. Bui, H.T.D.; Khosravi, S.; Fournier, V.; Herault, M.; Lee, K.J. Growth performance, feed utilization, innate immunity, digestibility and disease resistance of juvenile red seabream (*Pagrus major*) fed diets supplemented with protein hydrolysates. *Aquaculture* 2014, *418-419*, 11-16. [https://doi.org/10.1016/j.aquaculture.2013.09.046]
37. Hansen, J.Ø.; Penn, M.; Øverland, M.; Shearer, K.D.; Krogdahl, Å.; Mydland, L.T.; Storebakken, T. High inclusion of partially deshelled and whole krill meals in diets for Atlantic salmon (*Salmo salar*). *Aquaculture* 2010, *310*, 164-172. [https://doi.org/10.1016/j.aquaculture.2010.10.003]
38. Olsen, R.E.; Suontama, J.; Langmyhr, E.; Mundheim, H.; Ringo, E.; Melle, W.; Malde, M.K.; Hemre, G.I. The replacement of fish meal with Antarctic krill, *Euphausia superba* in diets for Atlantic salmon, *Salmo salar*. *Aquacult. Nut*. 2006, *12*, 280-290. [https://doi.org/10.1016/j.aquaculture.2018.12.032]
39. Saleh, R.; Burri, L.; Benitez-Santana, T.; Turkmen, S.; Castro, P.; Izquierdo, M. Dietary krill meal inclusion contributes to better growth performance of gilthead seabream juveniles. *Aquacult. Res*. 2018, *49*, 3289-3295. [https://doi.org/10.1111/are.13792]

40. Atkinson, A.; Siegel, V.; Pakhomov, E.A.; Jessopp, M.J.; Loeb, V. A re-appraisal of the total biomass and annual production of Antarctic krill. *Deep-Sea Res PT I* 2009, *56*, 727-740. [https://doi.org/10.1016/j.dsr.2008.12.007]
41. CCAMLR. *Krill Fishery Report 2018*. CCAMLR; Hobart; Australia, 2018. [https://www.ccamlr.org/en/publications/fishery-reports-2018-0]
42. Lourie, S. *Measuring seahorses*. *Project Seahorse Technical Report No.4, Version 1.0*. Project Seahorse, Fisheries Centre; University of British Columbia, Canada, 2003. ISBN 0-89164-169-6
43. Atkinson, J. L.; Hilton, J.W.; Slinger S. J. Evaluation of Acid-Insoluble Ash as an Indicator of Feed Digestibility in Rainbow Trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci*. 1984, *41*, 1384-1386. [https://doi.org/10.1139/f84-170]
44. Lee, S. Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*). *Aquaculture* 2002, *207*, 79-95. [https://doi.org/10.1016/S0044-8486(01)00751-7]
45. Allan, G.L.; Parkinson, S.; Booth, M.A.; Stone, D.A.J.; Rowland, S.J.; Frances, J.; Warner-Smith, R. Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. *Aquaculture* 2000, *186*, 293-310. [https://doi.org/10.1016/S0044-8486(99)00380-4]
46. Rawles, S.D.; Fuller, A.; Green, B.W.; Abernathy, J.W.; Straus, D.L.; Deshotel, M.B.; McEntire, M.E.; Huskey, G.; Rosentrater, K.A.; Beck, B.H.; Webster, C.D. Growth, body composition, and survival of juvenile white bass (*Morone chrysops*) when dietary fish meal is partially or totally replaced by soybean meal, poultry by-product meal, an all-plant protein blend or a commercial plant-animal protein blend. *Aquacult. Rep*. 2022, *26*, 101307. [https://doi.org/10.1016/j.aqrep.2022.101307]
47. Kaushik, S.J.; Covès, D.; Dutto, G.; Blanc, D. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture* 2004, *230*, 391-404. [https://doi.org/10.1016/S0044-8486(03)00422-8]
48. Torrecillas, S.; Montero, D.; Carvalho, M.; Benitez-Santana, T.; Izquierdo, M., Replacement of fish meal by Antarctic krill meal in diets for European sea bass *Dicentrarchus labrax*: Growth performance, feed utilization and liver lipid metabolism. *Aquaculture* 2021, *545*, 737166. [https://doi.org/10.1016/j.aquaculture.2021.737166]
49. Xu, J.; Sheng, Z.; Chen, N.; Xie, R.; Zhang, H.; Li, S. Effect of dietary fish meal replacement with spray dried chicken plasma on growth, feed utilization and antioxidant capacity of largemouth bass (*Micropterus salmoides*). *Aquacult. Rep*. 2022, *24*, 101112. [https://doi.org/10.1016/j.aqrep.2022.101112]
50. Liao, H.; Liu, P.; Deng, Y.; Zhang, W.; Pan, C.; Jia, Y.; Long, F.; Tang, H. Feeding effects of low-level fish meal replacement by algal meals of *Schizochytrium limacinum* and *Nannochloropsis salina* on largemouth bass (*Micropterus salmoides*). *Aquaculture* 2022, 557. [https://doi.org/10.1016/j.aquaculture.2022.738311]
51. Arriaga-Hernández, D.; Hernández, C.; Martínez-Montaño, E.; Ibarra-Castro, L.; Lizárraga-Velázquez, E.; Leyva-López, N.; Chávez-Sánchez, M.C. Fish meal replacement by soybean products in aquaculture feeds for white snook, *Centropomus viridis*: Effect on growth, diet digestibility, and digestive capacity. *Aquaculture* 2021, 530, 735823. [https://doi.org/10.1016/j.aquaculture.2020.735823]
52. Slawskiref, H.; Nagel, F.; Wysujack, K.; Balke, D.T.; Franz, P.; Schulz, C. Total fish meal replacement with canola protein isolate in diets fed to rainbow trout (*Oncorhynchus mykiss* W.). *Aquacult. Nut*. 2013, *19*, 535-542. [https://doi.org/10.1111/anu.12005]
53. Brinker, A.; Reiter, R. Fish meal replacement by plant protein substitution and guar gum addition in trout feed, Part I: Effects on feed utilization and fish quality. *Aquaculture* 2011, *310*, 350-360. [https://doi.org/10.1016/j.aquaculture.2010.09.041]
54. Stuart, K.R.; Barrows, F.T.; Silbernagel, C.; Alfrey, K.; Rotstein, D.; Drawbridge, M.A. Complete replacement of fish oil and fish meal in the diet of juvenile California yellowtail *Seriola dorsalis*. *Aquacult. Res*. 2021, *52*, 655-665. [https://doi.org/10.1111/are.14923]
55. Abdelghany, A.E. Partial and complete replacement of fish meal with gambusia meal in diets for red tilapia '*Oreochromis niloticus* × *O. mossambicus*'. *Aquacult. Nut*. 2003, *9*, 145-154. [https://doi.org/10.1046/j.1365-2095.2003.00234.x]
56. Bruni, L.; Belghit, I.; Lock, E.J.; Secci, G.; Taiti, C.; Parisi, G. Total replacement of dietary fish meal with black soldier fly (*Hermetia illucens*) larvae does not impair physical, chemical or volatile composition of farmed Atlantic salmon (*Salmo salar* L.). *Sci. Food Agric*. 2020, *100*, 1038-1047. [https://doi.org/10.1002/jsfa.10108]
57. Gómez-Requeni, P.; Mingarro, M.; Calduch-Giner, J.A.; Médale, F.; Martin, S.A.M.; Houlihan, D.F.; Kaushik, S.; Pérez-Sánchez, J. Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). *Aquaculture* 2004 *232*, 493-493-510. [https://doi.org/10.1016/S0044-8486(03)00532-5]
58. Rahman, M.M.; Kim, K.W.; Lee, S.M. Apparent digestibility coefficients of animal feed ingredients for olive flounder (*Paralichthys olivaceus*). *Fish. Aquat. Sci*. 2022, *25*, 537-548. [https://doi.org/10.47853/FAS.2022.e48]

59. Hanley, F. The digestibility of foodstuffs and the effects of feeding selectivity on digestibility determinations in tilapia, *Oreochromis niloticus* (L). *Aquaculture* 1987, *66*, 163-179. [https://doi.org/10.1016/0044-8486(87)90229-8]
60. Guillaume, J.; Choubert, G. Digestive physiology and nutrient digestibility in fishes In *Nutrition and Feeding of Fish and Crustaceans*; Guillaume J.; Kaushik, S.; Berbot, P.; Métailler, R. Eds; Springer-Praxis: Chichester, UK, 2001; pp. 27-58, ISBN 10: 1852332417; ISBN 13: 9781852332419.
61. -Le, H.T.M.D.; Shao, X.; Krogdahl, Å.; Kortner, T.M.; Lein, I.; Kousoulaki, K.; Lie, K.K.; Sæle, Ø. Intestinal Function of the Stomachless Fish, Ballan Wrasse (*Labrus bergylta*). *Front. Mar. Sci.* 2019, *6*, 140. [https://doi.org/10.3389/fmars.2019.00140]
62. Portz, L.; Cyrino, P.J.E. Digestibility of nutrients and amino acids of different protein sources in practical diets by largemouth bass *Micropterus salmoides* (Lacepédè, 1802), *Aquacult. Res.* 2004 *35*, 312-320. [https://doi.org/10.1111/j.1365-2109.2004.00984.x]
63. Small B.C.; Austic R.E.; Joseph H.S. Jr Amino acid availability of four practical feed ingredients fed to striped bass *Morone saxatilis*. *J. World Aquac. Soc.* 1999, *30*, 58-64. [https://doi.org/10.1111/j.1749-7345.1999.tb00317.x]
64. Slawski, H.; Nagel, F.; Wysujack, K.; Balke, D.T.; Franz, P.; Schulz, C. Total fish meal replacement with canola protein isolate in diets fed to rainbow trout (*Oncorhynchus mykiss* W.). *Aquacult. Nut*. 2013, *19*, 535-542. [https://doi.org/10.1111/anu.12005]
65. Stone, D.A.J.; Allan, G.L.; Parkinson, S.; Frances, J. Replacement of fishmeal in diets for Australian silver perch B*idyanus bidyanus* (Mitchell). II. Effects of cooking on digestibility of a practical diet containing different starch products, *Aquacult. Res.* 2003, *34*, 195-204. [https://doi.org/10.1046/j.1365-2109.2003.00772.x]
66. Bai, S.C.; Choi, S.M.; Kim, K.W.; Wang, X.J. Apparent protein and phosphorus digestibilities of five different dietary protein sources in Korean rockfish, *Sebastes schlegeli* (Hilgendorf), Aquacult. Res. 2001, 32, 99-105. [https://doi.org/10.1046/j.1355-557x.2001.00009.x]
67. NRC. *Nutrient requirements of fish*. Committee on Animal Nutrition, Board on Agricultural National R.C. Academy Press, Washington DC, USA, 1993, ISBN 0 309 59629 7.
68. Lall, S.P. Digestibility, metabolism and excretion of dietary phosphorus in fish. In *Nutritional Strategies and Aquaculture Waste*; Cowey, C.B.; Cho, C.Y., Eds.; Proceedings of the 1st International Symposium on Nutritional strategies in Management of Aquaculture Waste. University of Guelph, Canada, 1991; pp. 21–36.
69. Ogino, C.; Takeuchi, L.; Taheda, H.; Watanabe, T. Availability of dietary phosphorus in carp and rainbow trout. *Bull. Jpn Soc. Sci. Fish*. 1979, *49*, 1527–1532. [https://doi.org/10.2331/suisan.45.1527].
70. Riche, M.; Brown, P.B. Availability of phosphorus from feedstuffs fed to rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 1996, *142*, 269–282. [https://doi.org/10.1016/0044-8486(95)01218-4]
71. Sugiura, S.H.; Dong, F.M.; Rathbone, C.K.; Hardy, R.W. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture* 1998 *159*, 177–202. [https://doi.org/10.1016/S0044-8486(97)00177-4]
72. Morales, G.A.; Azcuy, R.L.; Casaretto, M.E.; Márquez, L.; Hernández, A.J.; Gómez, F.; Koppe, W.; Mereu, A. Effect of different inorganic phosphorus sources on growth performance, digestibility, retention efficiency and discharge of nutrients in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 2018, *495*, 568-574 [https://doi.org/10.1016/j.aquaculture.2018.06.036].
73. Yone, Y.; Toshima, N. The utilization of phosphorus in fish meal by carp *Cyprinus carpio* and black sea bream *Mylio macrocephalus*. *Bull. Jpn Soc. Sci. Fish.* 1979, *45*, 753–756. [https://doi.org/10.2331/suisan.45.753]
74. Shcherbina, M.A.; Gamygin, E.A.; Sal'kova, I.A. Availability of phosphorus from main dietary sources in common carp, *Cyprinus carpio* L. *Živočišná Výroba* 1997, *42*, 125–130. [http://doi.org/10.5555/19981404929]
75. Watanabe, T.; Takeuchi, T.; Murakami, A.; Ogino, C. Availability to *Tilapia nilotica* of phosphorus in whitefish meal. *Bull. Jpn Soc. Sci. Fish*. 1980, *46*, 897–900. [https://doi.org/10.2331/suisan.46.897]
76. Miranda, E.C.; Pezzato, A.C.; Pezzato, L.E.; Furuya, W.M. Apparent phosphorus availability in food for the Nile tilapia (*Oreochromis niloticus*). *Acta Sci. Anim. Sci.* 2000, *22*, 669–675. [https://doi.org/10.4025/actascianimsci.v22i0.2910]
77. Furuya, W.M.; Pezzato, L.E.; de Miranda, E.C.; Furuya, V.R.B.; Barros, M.M. Apparent digestibility coefficients of energy and nutrients of some ingredients for Nile tilapia, *Oreochromis niloticus* (L.) (Thai strain). *Acta Sci. Anim. Sci*. 2001, *23*, 465–469.

 doi:10.20944/preprints202408.1089.v1

78. Köprücü, K.; Özdemir, Y. Apparent digestibility of selected feed ingredients for Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 2005, *250*, 308–316. [https://doi.org/10.1016/j.aquaculture.2004.12.003]
79. Dias, J.; Yúfera, M.; Valente, L.M.P.; Rema, P. Feed transit and apparent protein, phosphorus and energy digestibility of practical feed ingredients by Senegalese sole (*Solea senegalensis*). *Aquaculture* 2010, *302*, 94-99. [https://doi.org/10.1016/j.aquaculture.2010.02.013]