

Review

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Review

# Digestive Physiology, Nutrition and Feeding of *Arapaima gigas*; A Review

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**Abstract:** The *Arapaima gigas*, a native fish species in the Amazon basin, possesses intriguing biological and zootechnical characteristics, along with substantial economic value, rendering it a promising candidate for development within intensive aquaculture systems. Numerous studies have been conducted to deepen the understanding of its digestive physiology based on its feeding habits, determine its nutritional requirements corresponding to different developmental stages, assess alternative ingredients to enhance feeding strategies, and elucidate its feeding behavior patterns in captivity. However, there remain gaps in information and a deficiency in various aspects of nutritional physiology, particularly as it relates to the formulation of efficient and balanced aquafeeds across different production phases for this species. This article provides a comprehensive review of the current state of knowledge regarding digestive physiology, advancements in nutrition and feeding strategies developed over the past two decades. Furthermore, it aims to pinpoint areas that require further research to enhance the understanding of *A. gigas* and its application in sustainable aquaculture practices.

**Keywords:** enzymatic activity; diets; digestibility; feeding strategies; nutritional requirements; neotropical fish

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**Key Contribution:** This review paper provides a comprehensive review of the current state of knowledge regarding digestive physiology, advancements in nutrition and feeding strategies developed over the past two decades in *Arapaima gigas*.

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## 1. Introduction

Aquaculture offers a sustainable source of fish while leveraging the Amazonian region's favorable conditions, such as climate, soil quality, and access to agricultural inputs for feed production. More specifically, the cultivation of *A. gigas* plays a vital role in mitigating various negative impacts. These include the reduction of wild fish populations, primarily due to overfishing in rivers, the expansion of agricultural land, and other human activities. However, despite these advantages, the cultivation of this and other native species has not yet reached its full potential and challenges hinder the consolidation of aquaculture in the Amazon region. *Arapaima gigas*, a native species of the Amazon River basin, holds the distinction of being the world's largest freshwater fish. Its suitability for aquaculture is underscored by a range of favorable traits, including rapid growth, ease of adaptation to commercial aquafeeds, good meat quality, high fillet yield without intramuscular bones, strong market demand, and widespread consumer acceptance [1–5]. Despite these promising biological and zootechnical features, its production under various intensive systems

remains at an early stage of development, primarily due to a host of limitations that hinder its commercial advancement [4].

One main constraint pertains to the limited knowledge concerning fundamental and applied aspects of the digestive physiology of the species, as well as its nutritional requirements in captivity. It must be pointed out that nutritional knowledge should be tailored to the specific species, taking into account its unique physiological and behavioral characteristics, with minimal room for generalizations [6]. In this regard, in recent years, several studies have contributed valuable insights, including the characterization of the gastrointestinal tract, determination of optimal protein and energy levels, formulation of feed rations, and assessment of ingredient digestibility in *A. gigas* [7–10]. However, there remains substantial gaps in knowledge regarding other aspects of digestive physiology relevant to nutrition. These aspects include some addressing fundamental research (characterization of gastrointestinal tract), applied research (determination of optimal protein and energy levels, assessment of ingredient digestibility, nutritional requirements for lipids, carbohydrates, vitamins, and minerals,) as well as practical applications of such knowledge to the formulation of feed rations, incorporation of functional additives and the use of non-traditional ingredients, since a comprehensive understanding of digestive mechanisms and effective nutritional management is essential to formulate efficient aquafeeds capable of meeting the species' diverse nutritional needs throughout its life stages [11,12]. This approach not only may contribute to the species' well-being but also should enhance the efficiency of its cultivation from both environmental and economic perspectives [8].

Considering all the above mentioned, the primary objective of this paper is to offer an up-to-date review of existing information on these critical aspects concerning digestion and feeding of *A. gigas*. Additionally, it aims to identify areas that warrant further investigation, with the ultimate goal of contributing to establish strategies for the sustainable development of the species in commercial aquaculture.

## 2. Literature Review Methodology

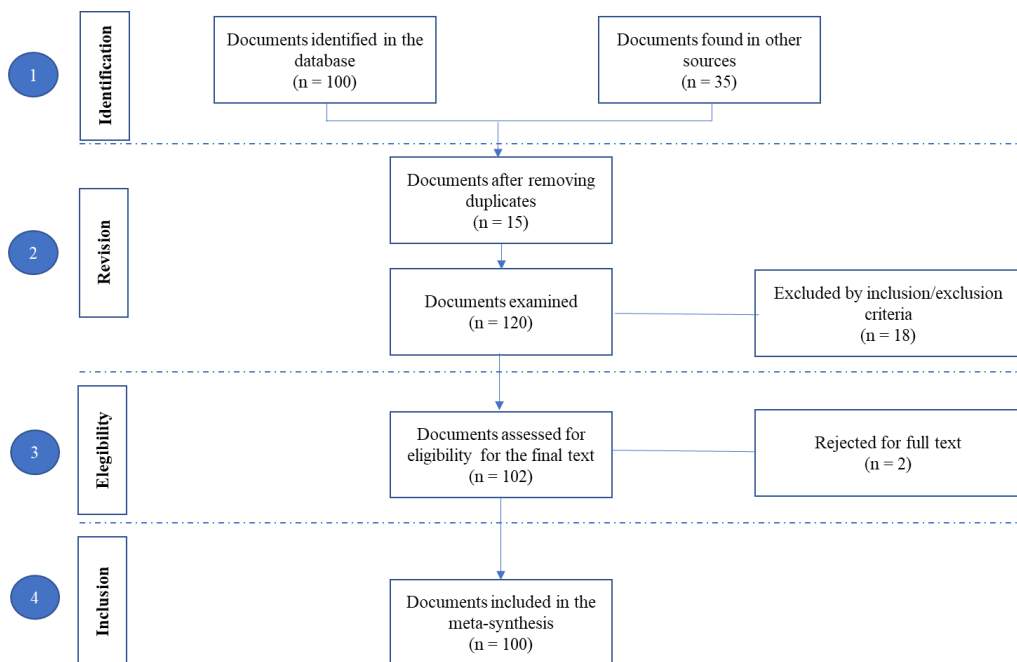
A systematic research of scientific articles and technical papers published in English, Spanish and Portuguese in Google Scholar, Scielo, Scindirect, Scopus, Springer Link and Wiley Online Library databases was carried out using the Preferred Reporting Items for Systemic Reviews and Metaanalysis (PRISMA). The words "Arapaima, diets, feed, nutritional requirements, enzymes, digestibility and culture" were used in the bibliographic search. This search was carried out from January to March 2023, with a time horizon of 2002 - 2022. Inclusion and exclusion criteria were used to process the information obtained. Firstly, all articles or documents related to the species, nutritional physiology and feeding were considered. The main exclusion criteria referred to documents that did not present a direct relationship with the established objective, i.e., they did not focus on the particular aspects of digestive physiology applied to the nutrition and feeding of the species. A flowchart of the PRISMA method applied is detailed in Figure 1.

A total of 100 articles, research works and technical documents were finally considered. The articles were organized into five categories: general review of species, digestive physiology, nutrition and nutritional requirements, digestibility and feeding strategies. After categorizing the documents, the information was reviewed and analyzed, with the aim of systematizing and identifying the gaps in knowledge still existing in the aspects studied.

## 3. General Aspects of the Species

### 3.1. Distribution and Habitat

*Arapaima gigas* is a native teleost native in the South American Amazon basin, with a length of up to 3 m and 200 kg of body weight [13–15], and is considered the largest freshwater scale fish. Commonly called "paiche" in Peru and Ecuador, it is also known as "pirarucu" in Brazil, "warapaima" in Colombia and "arapaima" or "de-chi" in Guyana [15–17].



**Figure 1.** PRISMA flowchart for literature searches and article inclusion.

The taxonomic classification of this species, according to Bezerra et al. [18], is as follows:

Division: Teleostei

Subdivision: Osteoglossoporma

Order: Osteoglossiformes

Family: Arapamidae (Osteoglossidae)

Sub-family: Heterotidinae

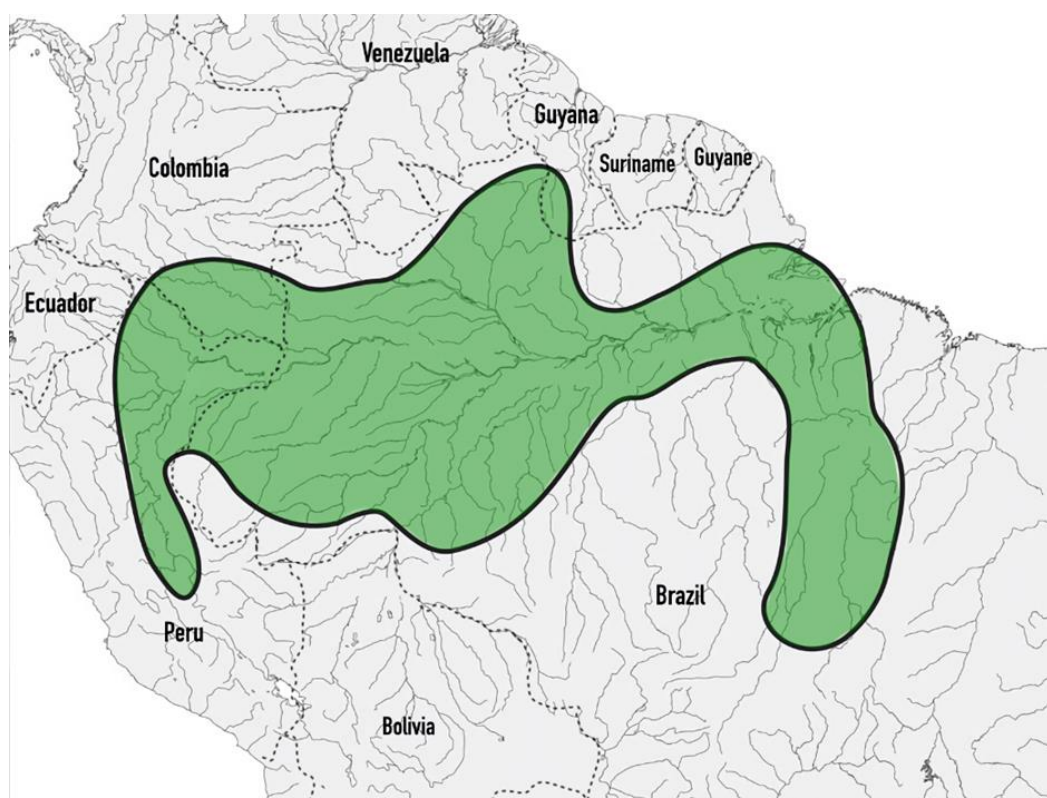
Genus: Arapaima

Species: *Arapaima gigas* (Cuvier 1829)

Species of the genus *Arapaima* have been traced back to the Cretaceous and Tertiary Amazon period, and likely evolved from primitive bony fish [19,20]. Although traditionally regarded as a monotypic genus, *Arapaima* was recently considered as having more than one species [21]. A distinctive characteristic of the Osteoglossiformes order, to which *A. gigas* belongs, is the ossification (hardening) of the tongue, which is made up of fine villi form lingual teeth [20–22]. This bony tongue enables the crushing of food and functions as an accessory organ of the gastrointestinal tract [22,23]. *A. gigas* is considered a tropical habitat species, thriving in water bodies with consistent temperatures ranging from 24 to 26 °C year-round [24]. It is naturally distributed in the sub-basin of the Amazon, Tocantins-Araguaia and Essequibo rivers, spanning Brazil, Ecuador, Colombia, Peru, and the rivers of Guyana [25–27] (Figure 2). Over time, it has been introduced to the Bolivian Amazon [16,28]. Presently, this species can be found in Central America, North America, and even Asia, including China, Indonesia, the Philippines, Malaysia, Singapore, and Thailand [29–32].

The preferred habitat are low-gradient aquatic environments of the Amazon River and its tributaries, mostly lakes and connecting channels during low water levels [13,33]. These environments are characterized by low water flow, depth, increased turbidity, abundant floating and emerging macrophytic vegetation, which in some cases can cover the entire body of water, and have hypoxic conditions [13,34]. This species can also be found in shallow areas in slow flowing rivers [18,35].





**Figure 2.** Map of the approximate natural distribution of *A. gigas* in South America [36].

### 3.2. Feeding Habits

As a carnivorous species, *A. gigas* has a natural diet that primarily consists of small fish, crustaceans, mollusks, and insects [20,34]. During its early stages, particularly as fry, *A. gigas* primarily consumes plankton, later transitioning to insects. In the juvenile stage, the diet mainly consists of small fish and micro crustaceans, and when they reach the adult stage, they feed exclusively on fish, crabs and prawns [20,37]. Studies suggest that there is no a particular “prey target – size” [20]. *A. gigas* captures its prey through a rapid movement of the head, often accompanied by a tail whip, producing a distinct high-pitched noise. This movement, involving the opercula lids, expels water taken in during the strike [38]. Fish prefer to feed during dawn or dusk, although they may also feed during the day. When temperatures rise, they seek refuge among aquatic vegetation to escape intense sunlight, often remaining stationary at the water's bottom but periodically surfacing to breathe atmospheric oxygen [39].

### 3.3. Culture and Production Cycle

*A. gigas*, known for its excellent meat quality, high fillet yield (up to 50%) and remarkable rapid growth, which is unique among freshwater fish species, can reach weights of up to 10 kg within a single year [4,14,40]. Consequently, the interest in cultivating this species has surged over the past two decades [41–43]. The production of *A. gigas* worldwide in volume totaled 2,113 tons in 2022, with Brazil (2,028 t) and Peru (85 t) being the two main producers worldwide [44,45] as detailed in Table 1.

*A. gigas* is typically farmed in earthen ponds, although floating cage systems are also utilized. Land-based pond culture involves one to three well-defined production phases: pre-growth, growth, and final fattening. However, the specific phases may vary based on the size of the fingerlings at the start of fry rearing and pre-growth processes. In some fish farms, particularly in Peru, where the emphasis is on fingerling growth, two main production phases are recognized: initial fattening and final fattening [39]. Nevertheless, an increasingly popular approach is direct fattening, which

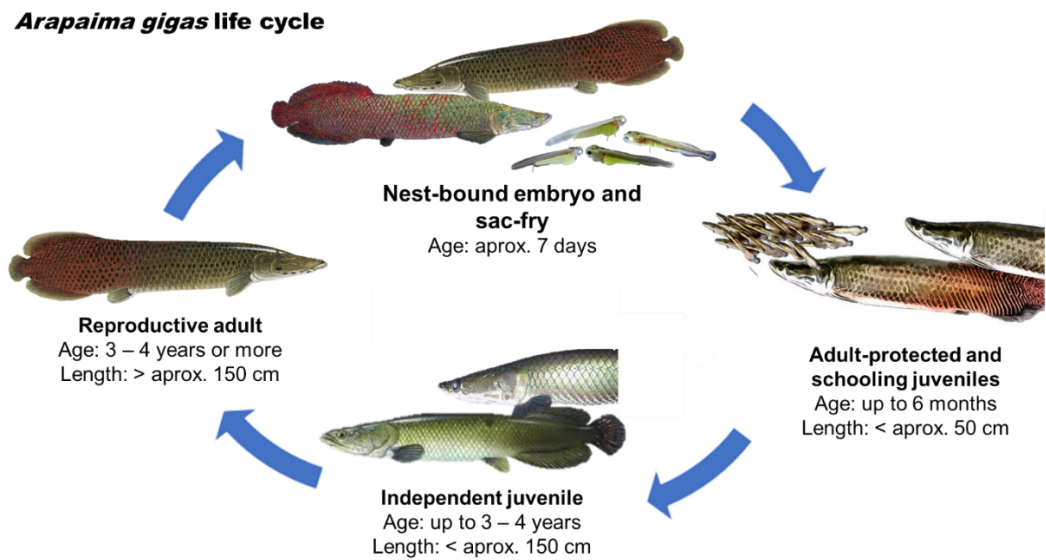
involves seeding fingerlings previously conditioned to commercial aquafeed (typically above 15 cm in size) for final harvesting in a single production system.

**Table 1.** Production of *A. gigas* in Brazil and Peru 2003 - 2022 (in tons) [44,45].

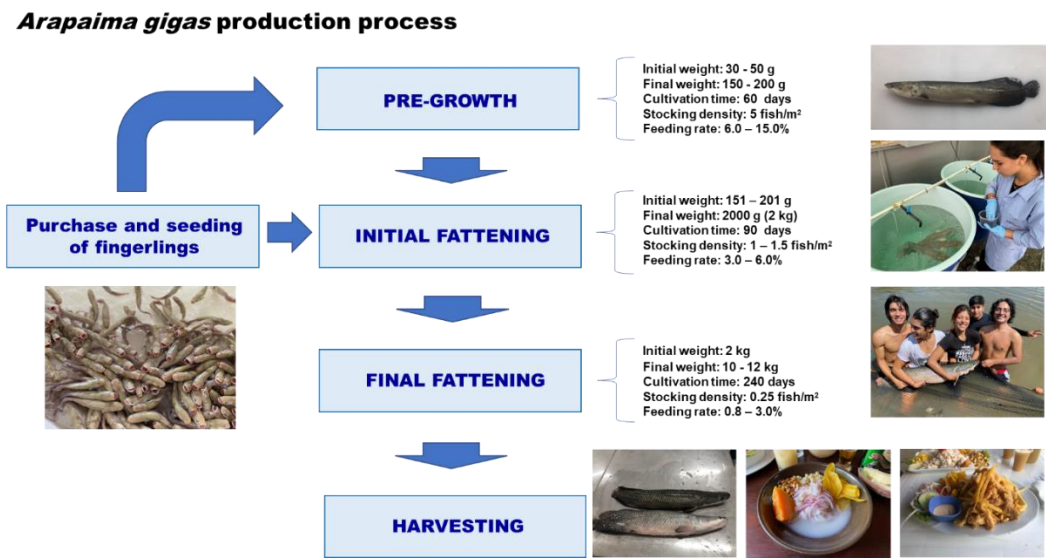
| Year | Brazil | Peru |
|------|--------|------|
| 2013 | 2301   | 94   |
| 2014 | 11763  | 55   |
| 2015 | 8389   | 135  |
| 2016 | 1266   | 142  |
| 2017 | 1259   | 218  |
| 2018 | 1832   | 295  |
| 2019 | 1893   | 86   |
| 2020 | 1886   | 99   |
| 2021 | 2137   | 81   |
| 2022 | 2028   | 85   |

Following the description made by Chu-Koo et al. [39], the fry rearing stage begins with the capture of individuals from breeding ponds when they reach a minimum size of 2 cm. During this phase, fry initially consume zooplankton, followed by *Artemia nauplii*, and gradually transition to a balanced diet as part of a feeding training protocol. This phase typically spans between 17 to 32 days, depending on the specific protocol used, and concludes when the fish reach sizes ranging from 7 to 8 cm [39]. Moving on to the pre-growth stage, fingerlings, with sizes between 8 to 15 cm (having completed the rearing phase), continue their adaptation to balanced aquafeed and initiate their development toward the fattening stage. This phase takes place in earthen ponds, typically measuring between 300 to 500 m<sup>2</sup> (or other suitable culture infrastructures), over a period of 2 months. During this time, the fish grow to weigh between 150 to 200 g and reach sizes ranging from 24 to 30 cm [39].

In the initial fattening phase, juveniles from the pre-growth stage (typically weighing 150 to 200 g) are stocked at a density of 1 to 1.5 fish per square meter. This phase occurs in ponds measuring 500 m<sup>2</sup>, and the fish remain in this stage for approximately 3 months or until they reach 2 kg in weight, at which point they are selected and transferred to the second phase. In the final phase, fish are stocked at a density of 0.25 fish per square meter, typically in ponds ranging from 1,000 to 2,000 m<sup>2</sup> in size. During this stage, *A. gigas* can reach a weight of 10 to 12 kg after approximately 8 months, or other weights dictated by market demand [39]. The life cycle and development of *A. gigas*, from larval stage to breeding, and production process from purchase and seeding of fingerlings to final harvesting, are summarized in Figures 3 and 4.



**Figure 3.** Life cycle of *Arapaima gigas* can be divided into four main stages: (1) nest-bound embryo and sac fry, (2) adult-protected, schooling juveniles, (3) independent juveniles, and (4) reproductive adults [46].



**Figure 4.** *Arapaima gigas* production stages: purchase and seeding of fingerlings, pre-growth, initial fattening, final fattening and harvesting.

3.4. Rearing Conditions

Optimal water quality conditions are crucial to ensure the optimal growth of *A. gigas* and hence, is essential to monitor parameters such as temperature and pH in the culture units. It is worth noting that *A. gigas* relies on obligatory aerial respiration, obtaining the majority of its oxygen through its swim bladder [4,14,40]. This adaptation allows the species to tolerate waters with low levels of dissolved oxygen, providing an advantage over species that primarily respire through gills [1,11]. *A. gigas* can thrive even in environments with dissolved oxygen levels below 2 mg/L [47]. Despite its reliance on pulmonary respiration, this species still excretes CO<sub>2</sub> through its gills, necessitating low levels of this gas in the water for effective gas exchange. Elevated CO<sub>2</sub> levels above 20 mg/L can adversely affect animal health and increase stress, particularly in juveniles. *A. gigas* also exhibits notable tolerance to high concentrations of total ammonium. Research has shown that levels ranging from 0.8 to 2.4 mg/L do not significantly impede the development of the species in culture units [40]. Table 2 provides detailed information on some of the optimal water parameters for the culture of *A. gigas*.

**Table 2.** Suitable water quality ranges for the production of *A. gigas* [47,48].

| Parameters                                 | Stage: Fingerling |               | Stage: Fattening  |               |
|--|-------------------|---------------|-------------------|---------------|
|  | Permissible range | Optimal range | Permissible range | Optimal range |
| Temperature (°C)                           | 26.0 - 30.0       | 27.0 - 28.0   | 25.0 - 31.0       | 27.0 - 29.0   |
| Dissolved oxygen (mg/L)                    | 4.0 - 7.0         | > 5.0         | 4.0 - 7.0         | > 5.0         |
| Ammonium (mg/L)                            | < 0.05            | < 0.02        | < 0.05            | < 0.02        |
| Nitrites (mg/L)                            | < 0.05            | absent        | < 0.05            | absent        |
| pH   | 6.0 - 8.0         | 6.0 - 7.0     | 5.0 - 8.0         | 6.5 - 7.0     |
| Total alkalinity (mg/L CaCO <sub>3</sub> ) | > 30.0            | -             | > 20.0            | -             |
| Total hardness (mg/L CaCO <sub>3</sub> )   | > 30.0            | -             | > 20.0            | -             |
| Transparency (cm)                          | 30.0 - 60.0       | -             | 30.0 - 60.0       | -             |
| Carbon dioxide (mg/L)                      | < 20.0            | -             | < 20.0            | -             |

4. Nutritional Requirements

Conducting experimental work with this species is inherently challenging for several reasons: the large size of the specimens, the considerable cost associated with using *A. gigas* as biological material and the limited availability of laboratories equipped with suitable facilities and experimental systems for the study of large fish. Considering that nutritional requirements can vary depending on factors such as the size or stage of the species, protein source in the diet, production system, experimental conditions and environmental factors, there is a pressing need to complement existing research with information on nutrition at different stages of development. In terms of nutritional requirements, studies have primarily focused on determining optimal levels of protein, energy, and the energy/protein ratio.

4.1. Protein and Amino Acids

Different studies have assessed that requirements of crude protein in *A. gigas*, that can vary between 56% and 30%, depending on the age/size of the individuals, although several other parameters may have influenced the results obtained, like the type of rearing facility (floating cage, earthen pond), the duration of the experiment (from 4 weeks to 12 months) or feeding system (Table 3). In small juveniles of less than 100 g, some studies suggest optimal levels of around 50% CP for achieving the best weight gain [49,50] while others concluded that the best growth was achieved using diets containing 40% and 45% protein [51]. Even more, in a recent work conducted by Casado del Castillo et al. [52], authors demonstrated that fish fed diets ranging from 44% to 48% protein exhibited better growth and protein efficiency, but also that diets with 52% CP induced stress, affecting hemoglobin concentration and increasing oxygen demand.

The levels of CP can be reduced as fish grow up; in juveniles of 120 g Ituassú et al. [12] determined an optimal CP level of 48.6%, although they found that feed conversion and protein efficiency indices were not affected by reducing protein levels up to 33%. Juveniles of around 500 – 650 g present a requirement of around 40 – 45% CP [54,55]. On the other hand, protein requirements for fish of a higher size, over 1.5 to 4.0 kg seem to be around 36% [9,43].

Both producers of commercial feeds and technical documents for rearing of *A. gigas* have established protein levels of 50% to 55% for the pre-starting stage (fish between 12 to 15 cm and weighing less than 15 g), 48% to 50% for the starting stage (fish larger than 16 cm and weighing between 15 to 20 g), 45% for the growing stage (fish in the juvenile stage, between 300 to 500 g), and 40% for the fattening stage (fish larger than 1,000 g until harvest) [40,48].

Table 3. Optimal protein levels determined at different sizes and experimental conditions for *A. gigas*.

| Initial weight (g) | Optimal protein (%) | Diet       | Evaluated parameters | Facility  | References |
|--------------------|---------------------|------------|----------------------|---|------------|
| 40.72              | 53.76               | Pelletized | WG                   | Fiberglass tank for 6 weeks                             | [50]       |
| 54.00              | 50.00               | Pelletized | CF, FCR, SGR         | Floating cage   | [49]       |
| 68.75              | 44.00 – 45.80       | Pelletized | FCR, PER, WG         | Fiberglass conical tank 75 days                         | [52]       |
| 86.84              | 40.00               | Extruded   | FL, LG, FW, WG       | Rectangular cement tank for 84 days                     | [51]       |
| 120.60             | 48.60               | Extruded   | FCR, SGR, WG         | Floating cage for 45 days                               | [12]       |
| 133.00             | 40.00               | Extruded   | FB                   | Earthen pond for 12 months                              | [53]       |
| 500.00             | 40.00               | Extruded   | FCR, WG              | Earthen pond for 110 days                               | [54]       |
| 654.44             | 44.53               | Extruded   | FCR, WG              | Self-feeding system for 28 days (nutritional challenge) | [55]       |



|          |       |            |                |  |      |
|----------|-------|------------|----------------|--|------|
| 1,573.30 | 56.30 | Pelletized | Not determined | Self- feeding system for 23 days (nutritional challenge) | [43] |
| 2,000.00 | 36.00 | Extruded   | FCR, WG        | Tank system for 18 weeks                                 | [9]  |
| 2,025.00 | 37.40 | Extruded   | PER            | Floating cage for 88 days                                | [56] |

Adapted from López – Vásquez [57] and Guevara – Gutiérrez [58]. Abbreviations: CF, condition factor; FB, final biomass; FCR, feed conversion rate; FL, final length; FW, final weight; LG, length gain; SGR, specific growth rate; PER, protein efficiency rate; WG, weight gain.

Regarding the essential amino acid requirements for *A. gigas*, two studies have been conducted based on the essential amino acid composition found in muscle tissue (Table 4). These suggest the percentage of amino acids relative to dietary protein for specimens at various developmental stages and from both natural and aquaculture settings. Rodrigues et al. [59] estimated amino acid requirements by the analysis of muscle composition in specimens sampled in the wild and in farms considering two size classes ( $1.66 \pm 0.22$  kg and  $10.49 \pm 1.07$  kg, respectively). The estimated essential amino acid requirements were very similar between both groups, despite the differences in the muscle amino acid profile. Their results suggest that the highest estimated requirements for *A. gigas* should be for leucine, phenylalanine + tyrosine, arginine, and valine, mainly. In a different study, developed using juvenile specimens with an average weight of nearly 1 kg, the amino acids with the highest estimated requirements were arginine, phenylalanine + tyrosine, leucine, and isoleucine [60]. Both studies report close estimated requirements for the amino acids, except for arginine, leucine, lysine and threonine, with marked differences probably due to the calculation methodology used by the authors.

On the other hand, in the few studies existing about requirements of functional amino acids, Ramos et al. [61] determined that inclusion of 1.02% of glutamine in the diets of juvenile pirarucu (82.12 g) improved growth performance and influenced intestinal villi height and activity of important digestive enzymes, favoring nutrient digestion and absorption. Glutamine plays a role as a regulator of essential metabolic pathways and has potential to enhance the nutrition of neotropical carnivorous fish [62,63].

**Table 4.** Estimated essential amino acid requirement (as % of dietary protein) of *A. gigas* based on the amino acid profile of muscle tissue following Rodrigues et al. [59] and Orosco-Napan [60].

| Weight                   | 1.66 kg |            | 10.49 kg |            | 0.94 kg    |
|--------------------------|---------|------------|----------|------------|------------|
| Environment              | Natural | Controlled | Natural  | Controlled | Controlled |
| Arginine                 | 3.66    | 3.93       | 3.72     | 3.74       | 6.77       |
| Histidine                | 1.14    | 1.26       | 1.09     | 1.12       | 1.03       |
| Isoleucine               | 2.74    | 2.48       | 3.02     | 2.96       | 2.47       |
| Leucine                  | 5.25    | 5.00       | 5.31     | 5.40       | 3.11       |
| Lysine                   | 6.10    | 6.03       | 5.95     | 6.03       | 5.00       |
| Methionine               | 1.80    | 1.81       | 1.81     | 1.81       | N.D.       |
| Methionine + Cysteine    | 2.53    | 2.70       | 2.36     | 2.42       | 2.16       |
| Phenylalanine            | 2.73    | 2.75       | 2.64     | 2.66       | N.D.       |
| Phenylalanine + Tyrosine | 4.65    | 4.76       | 4.46     | 4.42       | 4.16       |
| Threonine                | 2.68    | 2.72       | 2.65     | 2.62       | 1.39       |
| Tryptophan               | 0.54    | 0.49       | 0.55     | 0.53       | 0.43       |
| Valine                   | 2.90    | 2.79       | 3.06     | 2.93       | 2.21       |

4.2. Lipids and Carbohydrates

Different studies have assessed that requirements of crude protein in *A. gigas*, that No specific research has been found on lipid requirements in *A. gigas*, although this information is important because carnivorous fish can digest unsaturated lipids more easily than long-chain saturated lipids (C18:0), which are deficient due to the interference of saturated fatty acids with digestibility [64,65].

Rojas et al. [66] suggested that the recommended level of total lipids in diets should be around 20% inclusion in diets with 4,000 kcal DE for the postlarvae and fry stages. This inclusion ensures an adequate energy/protein ratio (an average of 10 to 11 kcal DE/g CP) and optimal utilization of dietary protein. de Mattos et al. [43], conducting experiments in self-feeding systems and subjecting juvenile *A. gigas* to nutritional challenges, observed that fish set their target lipid intake at 19.5%.

Some technical studies consider lipid levels between 8% and 12% for the different stages of *A. gigas* production [48]. Commercial diets available on the market also vary in their lipid content, ranging from 9% to 12% for the pre-starter stage, 8% to 12% for the starter stage, 8% to 10% for the grower stage, and 7% to 10% for the fattening stage, respectively (Table 5).

**Table 5.** Requirements of the main nutrients for *A. gigas* according to culture stage (in g/100 g diet) [48].

| Nutrient | Fry/Fingerling | Grower | Juvenile/Finisher | Broodstock |
|----------|----------------|--------|-------------------|------------|
| Protein  | 55             | 45     | 40                | 35         |
| Lipid    | 12             | 12     | 10                | 8          |
| Fiber    | < 2            | < 3    | < 4               | < 5        |
| Ash      | < 10           | < 9    | < 9               | < 7        |

Requirements of carbohydrates are also scarcely studied in this species. The information available indicates that the species has a reduced ability to use dietary carbohydrates [67]. The above mentioned study by de Mattos et al. [43], determined that juvenile specimens set their target carbohydrate intake at 24.2% when subjected to nutritional challenges in self-feeding systems.

4.3. Energy and Protein: Energy Ratio

López-Vásquez [57] evaluated the effects of two protein levels (40% and 44%) and two digestible energy (DE) levels (4.0 and 4.4 Mcal/kg feed) in fingerlings weighing 12.42 g. After 42 days of evaluation, the best results were obtained for weight gain, biomass gain, and feed conversion factor with diets containing 44% protein, 4 Mcal/kg digestible energy. In bigger juvenile fish of around 170 g Vergara et al. [68] analyzed the effects of five levels of digestible energy in the diet (4.4, 4.6, 4.8, 5.0, and 5.2 Mcal/kg) and they determined optimal response when using 4.80 Mcal/kg feed.

In another study, conducted with juveniles of nearly 350 g Guevara-Gutiérrez [58] concluded that diets with 50% CP and 8.5 kcal DE/g CP showed better values of feed efficiency. However, with a ratio of 10 kcal DE/g CP and the same protein level, improvements were observed in gross body energy and body lipids. Table 6 summarizes the optimal energy and energy:protein ratio values obtained in the experimental trials.

**Table 6.** Optimal energy/protein ratios for different sizes of *A. gigas*.

| Initial weight (g) | Optimal energy  | Energy/protein ratio | References |
|--------------------|-----------------|----------------------|------------|
| 12.42              | 4.0 Mcal DE /kg | 9.0 Mcal/kg          | [57]       |
| 169.81             | 4.8 Mcal DE/kg  | 9.0 Mcal/kg          | [68]       |
| 345.7              | -               | 8.5 kcal/g           | [58]       |

Ono et al. [65] conducted experiments with juvenile *A. gigas* (96.8 ± 2.3 g) to evaluate the digestibility of four diets with different energy-to-protein ratios (11, 10.1, 9, and 8 kcal DE/g CP) and two lipid sources (soybean oil and poultry fat). The diets with ratios of 11 and 10.1 kcal/g exhibited the best apparent digestibility coefficients for dry matter (68.3 ± 0.9%), crude protein (73.4 ± 2.6%),

ether extract ( $98.8 \pm 0.5\%$ ), and gross energy ( $74.5 \pm 0.9\%$ ). This suggests that the energy-to-protein ratio significantly influences diet digestibility. Regarding the lipid sources, the diet with soybean oil showed better digestibility of ethereal extract ( $98.5 \pm 0.5\%$ ) compared to poultry fat, indicating that *A. gigas* digests unsaturated fats better than saturated fats.

#### 4.4. Micronutrients

Really there is a lack of information available on *A. gigas* vitamin and mineral requirements and the only available studies evaluated the use of vitamins C and E at levels that may be well above such requirements. In this sense, de Menezes et al. [69] conducted an evaluation of the efficacy of dietary supplementation with either vitamin C, vitamin E or both in juvenile fish of nearly 500 g kept in cages and fed with diets containing 40% CP. Their findings indicated that the inclusion of 800 mg/kg of vitamin C, as well as the combination of 800 mg/kg of vitamin C and 500 mg/kg of vitamin E, led to increased weight gain and improved survival rates after a 45-day trial period. Nevertheless, in a similar study developed by the same research group, using smaller fish of about 115 g, weight gain and survival rates remained unaffected by the inclusion of vitamin C and E at levels of 500, 800, and 1,200 mg/kg after a 2-month experimental period [70].

### 5. Digestive Physiology

#### 5.1. Morphology of the Gastrointestinal Tract

The morphology of a fish's digestive tract has evolved to ensure that the processes of ingestion, digestion, and nutrient absorption are well-adapted to the feeding habits of each species. Therefore, the development of species-specific feeds for different fish species and the establishment of appropriate feeding strategies must take into account not only their feeding habits and behaviors but also the anatomical and morphophysiological features of their digestive systems [71]. Regarding the gastrointestinal tract of *A. gigas*, various studies have explored aspects of its development and appearance in larval stages, ontogeny and morphology, and histology [73,74]. Ruíz-Tafur et al. [74] observed that newly hatched *A. gigas* larvae lacked a mouth and any vestiges of a digestive tract and the yolk sac accounted for 82.8% of their total body length. The buco-pharyngeal cavity appeared approximately 48 hours after hatching (HAH). Notably, as reported by the authors, the larvae began ascending to the water's surface at around 103 HPH, or approximately 4.3 days post-hatching. This timeframe suggests a high degree of independence in swimming activity, the ability to breathe atmospheric oxygen and the capability to capture and ingest exogenous food.

Saavedra and Collado [75] have noted that *A. gigas* larvae emerge to the surface of ponds once the yolk sac has been completely reabsorbed. They typically begin feeding on plankton around the fifth or sixth day post-hatching. Chu-Koo et al. [39], referring to Darias et al., suggest that when fry reach a length of approximately 1.7 to 2.0 cm and rise to the surface, their digestive system is fully developed and ready for the digestion of complex exogenous foods. In contrast, Ruíz-Tafur et al. [74] observed that the fish exhibit a fully developed digestive tract and initiate exogenous feeding at 146 hours after hatching (HAH), while the yolk sac is almost entirely absorbed by 194 HAH. de Alcântara et al. [29] conducted a study on the morphological and histochemical development of the gastrointestinal tract of larvae at the initial stage of swimming towards the water surface. At this stage, the larvae, with a weight of  $0.05 \pm 0.01$  g and a length of  $2.21 \pm 0.06$  cm, featured an open mouth and anus, no yolk sac, well-developed digestive organs, fully formed gastric glands, a folded intestinal tract, and a brush border. From days 11 to 14, there was an increase in the concentration of gastric glands and thickness of the stomach's muscular layer. Subsequently, during days 14 to 20, the larvae presented a more complex intestinal tract. Based on their observations, the authors suggest that *A. gigas* larvae can be effectively fed inert exogenous diets when they reach a size of approximately 2.0 cm.

As *A. gigas* matures, the morphology and thickness of its digestive tract gradually develop. The characteristics of the digestive tract exhibit typical morphological and histological traits seen in other carnivorous fish, as described by Rodrigues and Cargnin-Ferreira [73]. The esophagus is

characterized by a short, straight muscular tube with deep longitudinal folds of mucosa [72]. It presents a fan-shaped dilation at the beginning and continues with marked reduction of lumen in the medial portion, thus enabling distension during ingestion of large foods [72]. The mucosa and submucosa contain dense and thick connective tissue, which serves to protect the integrity of the esophageal wall against sudden distension during prey ingestion. The esophagus is known for its elasticity, a common feature in predatory fish. The stomach of *A. gigas* is elongated in "J" shape shaped muscle sac, characterized by its muscular and distensible nature [72,73]. This configuration has been described in other species with carnivorous and omnivorous feeding habits ones [76–78].

*A. gigas*'s stomach has divided into three regions: cardiac with a lighter aspect, fundus portion with few folds in the mucosa, and pyloric with deeper folds [72]. However, Rodrigues and Cargnin-Ferreira [73] identified two distinguishable regions, the "body" (proximal and glandular region, with folded mucosa) and the "pylorus" (distal and glandular region, relatively more muscular with shallower folds). The stomach's substantial volume, along with the presence of deep longitudinal folds and a well-developed muscular tunica, facilitates stomach distension and allows for the storage of large quantities of food in carnivorous fish as *A. gigas* [79].

The intestine in *A. gigas* is characterized by a relatively short tube, with an intestinal length:total length ratio close to 1.0. Near the beginning of the intestine, two pyloric caeca of different lengths can be observed. The rectum is identified by its flattened shape [73]. The midgut begins as a wide tube, gradually narrows in diameter until it stabilizes, and then widens relatively once more [73]. The intestinal mucosa predominantly features complex and transversely oriented folds, which are believed to optimize the processes of digestion and nutrient absorption, compensating for the relatively short length of the intestinal tract [73]. The liver is a single organ, and the pancreas is diffusely located within the liver. These exocrine glands are situated in the mesentery, near the pyloric region of the stomach, the pyloric caeca, and the initial portion of the intestine [73].

## 5.2. Digestive Biochemistry

Studies assessing the activity of digestive enzymes have been focused into the ability of *A. gigas* to modulate its digestive processes in response to changes in its diet. It's well-known that in fish, the activity of digestive enzymes can vary depending on factors such as the type of food offered, feeding conditions, or metabolic adaptations due to feeding schedules [80,81]. Based on the results reported by these studies, enzymatic digestion in *A. gigas* is mediated by the presence of acid proteases, alkaline proteases, lipases and amylases, mainly.

From the beginning of feeding, specimens of *A. gigas* with an average weight of 1.5 g present proteases, lipases and amylases, which increase their activity as a natural response to the digestion of commercial food [82]. The enzymatic activity is influenced by the type of live prey supplied, prior to the use of commercial food. Regardless of the type of food supplied, the proteolytic activity (around 25 and 60 IU/mg protein for acidic and alkaline protease) is higher than the lipolytic (less than 25 IU/mg protein) and amylolytic activity (less than 0.010 IU/mg protein) [82]. In the juvenile stage, the enzymatic activity becomes more evident depending on the characteristics of the diets supplied. Lima et al. [83] determined the activity of alkaline proteases ( $11.29 \pm 2.60$  U/mg), lipases ( $8.22 \pm 0.79$  U/mg) and amylases ( $16.76 \pm 1.36$  U/mg) in specimens with  $65.2 \pm 0.4$  g, which improve its activity with the inclusion of endogenous enzyme complexes. While Luz et al. [84] working with juvenile individuals ( $132.07 \pm 3.12$  g) and feeding them extruded diets (45% CP) and sodium butyrate, favored the adaptation of enzymatic activity, including amylase (1.26 IU), lipase (5.92 IU), and nonspecific alkaline protease (2.63 IU).

Maraví-Aguilar [85] found improved activity in digestive enzymes of juvenile *A. gigas* ( $127.5 \pm 28.41$  g) cultured in the biofloc system with significantly higher expression of lipases (1.60 U/mg protein) and amylases (0.04 U/mg protein). The authors suggested that the increase in lipases and amylases could be attributed to the contribution of exogenous enzymes from microorganisms associated with the bioflocs. In a study by Pedrosa et al. [86], different feeding strategies related to feed intake were tested with juveniles weighing  $500.0 \pm 50.9$  g in a recirculation system, no significant differences were observed in enzymatic activities of protease, amylase, and lipase, at the intestinal



level. It is suggested that those Amazonian fish have evolved to maintain a full set of digestive enzymes, enabling them to utilize all available food efficiently, despite the additional energy expenditure required [87].

In relation to alkaline proteases, studies have identified a type of trypsin in the pyloric ceca and dipeptidases throughout the intestinal tube. This trypsin is characterized by its high activity and stability in a wide range of pH (from 6.0 to 11.5, with maximum activity at pH 9.0), thermostability (22 to 55° C, with maximum activity at 65°C), and activity at high salt concentrations (up to 45%, w/v) [88]. The expression of dipeptidase in various segments of the digestive tract (anterior segment, middle segment, and pyloric caeca segments) was influenced by the type of diet. Specimens of *A. gigas* that were fed forage fish exhibited higher enzyme activity, showing increases of 19%, 16%, and 10% in these respective segments, as observed by Revilla-Aguirre (2009) [89].

Regarding the amylase activity, *A. gigas* has low activity [67,90]. Enzymatic action on starch was observed in juvenile specimens (131.34 ± 3.29 g) which, when fed with different vegetable inputs, showed surface erosion of starch granules in faeces [91]. Revilla-Aguirre [89] reported that maltase activity increased by 33.5% to 42.6% in intestinal segments when the fish were fed balanced diets with 43% crude protein (CP). This indicates that *A. gigas*, being a carnivorous species, exhibits a slight modulation and adaptation of enzymes in response to balanced feed which primarily contains plant ingredients. Regarding saccharase activity, the author found no presence of this enzyme.

5.3. Functional Parameters of the Gastrointestinal Tract

No information could be identified on key aspects of the functional parameters of the gastrointestinal tract of *A. gigas*, such as changes in the postprandial pH at the stomach and intestine or gastrointestinal transit rates.

6. Feed Management

Feeding constitutes the largest expense in intensive production systems, especially in carnivorous fish farming, primarily due to the high protein requirements in those species [92]. To establish efficient feed management strategies, it's essential to consider species-specific feed intake and feeding frequency, which can significantly impact the success of the culture [93,94]. This consideration can result in several benefits, including improved growth of cultured organisms, reduced feed wastage, and ultimately, maximized profitability. In the case of *A. gigas*, several studies have been conducted to determine optimal feed management practices [8].

6.1. Feeding Rate

Studies on determining the feeding rate specifically for *A. gigas* are limited. The available information is typically found in technical documents or extension guides. As reported by Rodrigues et al. [95], *A. gigas* exhibits a feeding rate variation ranging from 10.0% to 0.8% relative to the fish size (Table 7).

**Table 7.** Feeding rates (g feed/100 g live fish) proposed by different authors for different sizes of *A. gigas*.

| Average weight (g) | Ono and Campos [47],<br>Rodrigues et al. [95] |         | Aquatech's feed chart |         |
|--------------------|---|---------|-----------------------|---------|
|                    | Minimum                                       | Maximum | Minimum               | Maximum |
| 5 – 10             | 6.0   | 10.0    | 10.0                  | 12.0    |
| 10 – 20            | 4.5   | 6.0     | 7.0                   | 10.0    |
| 20 – 50            | 3.7   | 4.5     | 7.0                   | 10.0    |
| 50 – 100           | 3.3   | 3.7     | 4.5                   | 7.0     |
| 100 – 700          | 2.4   | 3.3     | 3.5                   | 4.5     |
| 700 – 1,500        | 1.9   | 2.4     | 2.5                   | 3.5     |
| 1,500 – 4,000      | 1.5   | 1.9     | 1.5                   | 2.5     |

|                |     |     |     |     |
|----------------|-----|-----|-----|-----|
| 4,000 – 8,000  | 1.1 | 1.5 | 1.0 | 1.5 |
| 8,000 – 12,000 | 0.8 | 1.1 | 0.5 | 1.0 |

Oliveira-Tenazoa and Delgado Vargas [96] using an artificial diet containing 50% CP conducted an evaluation of three feeding rates (5%, 10%, and 15%) and their effects on the growth of juvenile *A. gigas* of less than 5 g. At the end of the 80-day experimental period, the 10% feeding rate demonstrated superior performance in terms of final weight, final length, weight gain and length increment. In another study by Cardoso [97], feeding rates ranging from 4% to 8% of live weight were evaluated in fish with an average weight of 480 g raised in pens and fed on diets containing 40% CP. The results showed no significant differences in performance among the various feeding rates after 60 days of experimentation and hence the authors recommended a feeding rate equal to or below 4% of live weight due to its cost-effectiveness. These amounts may be even lowered when using self-feeding systems.

de Mattos et al. [47] found that juvenile *A. gigas*, with an average weight of 310 g, fed diets containing 45% CP exhibited a daily feed consumption rate of approximately 2.35% of their body weight. After 30 days of experimentation, fish nearly doubled their initial weight and achieved a feed conversion rate of approximately 1. In another similar study carried out with bigger fish of nearly 650 g, authors observed a daily feed consumption representing 2.14% of body weight, and similar results in weight gain and feed efficiency after 28 days of culture [55].

6.1. Feeding Frequency and Feeding Time

In aquaculture feed management, feeding frequency holds significant importance as it directly impacts feed intake, digestion, absorption, and consequently, production efficiency and profitability of the culture [98,99]. The appropriate feeding frequency is species-specific, contingent upon feeding behavior and gut transit rates [5,100]. Furthermore, it can vary based on developmental stage [101,102] and growth conditions [103]. It is crucial to carefully consider feed supply because unsuitable feeding frequencies can potentially increase foraging and aggressive behavior in fish, leading to elevated energy expenditure. This, in turn, may alter growth and feed efficiency [100]. In addition, low feeding frequencies in some species may reduce the available energy for fish growth [104].

Numerous studies have been conducted to determine the optimal feeding frequency in *A. gigas*, as summarized in Table 8. However, it's important to note that results remain inconclusive. They exhibit variations even among fish at similar developmental stages, with no discernible trend according to growth. In the above mentioned study related to feeding rates, Oliveira Tenazoa and Delgado Vargas [96] conducted on fry below 5 g, the authors also evaluated the effect of two feeding frequencies; 4 and 6 times/day. They observed that feeding frequency did not have an impact on specific growth rate, feed conversion, condition factor or survival.

Similarly, Medeiros et al. [105] assessed the performance of fingerlings weighing 30 g using feeding frequencies of 2, 3, and 4 times/day with diets containing 40% CP and a feeding rate of 5% in an open water circulation system. After a 15-day evaluation, there was no discernible influence of feeding frequency on final weight, weight gain, or feed conversion. The authors suggested that the absence of differences might be attributed to the relatively short experimental period. However, from an economic perspective, feeding twice a day at this cultivation stage would be more advantageous as it requires less labor [105]. Pozo – Reyes [106] investigated three feeding frequencies (2, 4, and 6 times/day) in fingerlings weighing nearly 90 g raised in cages within earthen ponds and at a feeding rate of 8% of a commercial diet with 45% CP. After 56 days of experimentation, fish fed 6 times/day exhibited superior length gain, but other parameters such as weight gain, specific growth rate, feed conversion, condition factor and survival were not affected by feeding frequency.

Rodrigues et al. [107] suggested that feeding frequency for fish weighing 80 g should be between 3 to 4 times/day. Their recommendation was based on observations that this feeding frequency resulted in higher feed consumption, greater muscle growth, and increased body fat accumulation. They also noted that fish fed only once a day exhibited hyperphagic behavior. Such behavior is commonly observed in fish subjected to food deprivation or low feeding frequency, as documented

in previous studies [109,110]. Silva-Espín [108] conducted research on specimens weighing 240 g and used commercial trout feed with 50% CP. They examined three different feeding frequencies: 3, 5, and 7 times/day. After 6 months of experimentation, it was found that feeding 7 times/day had a more favorable impact on weight gain and length increase. A higher feeding frequency in fish of this size may result in more efficient feed utilization by the animals and generates less waste.

**Table 8.** Feeding frequencies (meals/day) proposed by different authors.

| Initial weight (g) | Optimal feeding frequency (meals/day) | Diet used                                     | Evaluated parameters                                    | Experimental condition   | References |
|--------------------|---------------------------------------|---|---|--|------------|
| 5                  | 6                                     | Commercial trout extruded (50% CP)            | FL, FW, LG, WG  | Circular tank system with continuous water flow for 80 days                  | [96]       |
| 30                 | 2                                     | Commercial feed (45% CP)                      | SGR   | Open water circulation system for 15 days                                    | [105]      |
| 78                 | 6                                     | Commercial feed (45% CP)                      | LG  | Cage system inserted in earthen ponds for 56 days                            | [106]      |
| 80                 | 3 – 4                                 | Mixture of two commercial extruded (41.8% CP) | FW, SGR, WG   | Circular tank system with continuous water flow for 63 days                  | [107]      |
| 240                | 7                                     | Extruded                                      | LG, WG  | Aquarium system for 13 months  | [108]      |
| 500                | 2                                     | Extruded                                      | Fish exhibited no differences for: FL, FL, FCR, FW, SGR | Recirculation systems for 8 weeks and different feeding strategies evaluated | [86]       |
| 1,000              | 2                                     | Commercial extruded (40% CP)                  | FW, WG  | Cage system inserted in ponds for 45 days                                    | [109]      |

Pedrosa et al. [86] evaluated various feeding strategies in juveniles weighing 500 g in a recirculation system. These strategies included feeding to apparent satiety 2 and 3 times/day, feeding at a rate of 2% of body weight, and automatic feeding, using diets with 40% protein. They found no significant differences between the treatments after 8 weeks, indicating that the evaluated feeding strategies did not compromise animal growth, biochemical parameters, or digestive enzymes. Similar results were reported by Gandra et al. [109] when they tested feeding strategies in juveniles weighing more than 1 kg. They used a commercial extruded diet with 40% CP and fed the fish at apparent satiety, either once or twice a day, or every other day. Their findings indicated that animals fed twice a day achieved higher weight gain and final weight after 45 days of the trial. Considering the aforementioned, recommended practical feeding frequencies recommendations typically range from 4 times/day for the early stages of growth to 1 time/day for the final or finishing stages, as detailed in Table 9.

**Table 9.** Feeding frequency and pellet size for *A. gigas* according to weight following Ono and Campos [47] and Rodrigues et al. [95].

| Average weight(g) | Feeding frequency | Pellet size (mm) |
|-------------------|-------------------|------------------|
| 5 – 10            | 4                 | 0.8 – 1.0        |
| 10 – 20           | 4                 | 1.5 – 1.7        |
| 20 – 50           | 4                 | 1.7 – 2.5        |
| 50 – 100          | 3                 | 2.5              |

|                |   |             |
|----------------|---|-------------|
| 100 – 700      | 3 | 4.0         |
| 700 – 1,500    | 3 | 6.0         |
| 1,500 – 4,000  | 2 | 8.0         |
| 4,000 – 8,000  | 2 | 10.0        |
| 8,000 – 12,000 | 1 | 10.0 – 15.0 |

To date, there have been very few studies aimed at identifying the preferential timing of food intake by *A. gigas* and the influence of different feeding shifts on its productive performance. Only three studies were found related to verifying daily food consumption and feeding behavior in specimens weighing between 318 to 1,500 g [42,43,111]. The results consistently show that juvenile *A. gigas* exhibit a diurnal feeding habit related to their periods of normal activity.

Crescêncio et al. [111] examined feeding periods and their impact on the growth of juvenile specimens weighing 318 g. Fish were subjected to diurnal (feeding from 9 AM to 3 PM), nocturnal (feeding from 9 PM to 3 AM), and continuous (feeding at 9 AM, 3 PM, 9 PM, and 3 AM) feeding schedules. They observed that fish fed continuously exhibited better weight gain, biomass gain, specific growth rate, and total consumption. However, fish fed during the day and at night showed similar weight gain. In the previously mentioned studies carried out by de Mattos et al. [42,43] using a self-feeding system, results indicated that fish displayed a strict diurnal feeding pattern, with approximately 70% – 90% of their daily feeding activity occurring during the day.

4. Type of Ingredients and Their Digestibility

As any other fish species, the intensive culture of *A. gigas* requires the use of balanced diets. Although there are some specific feeds commercially available for this species, in practice, most producers in Peru and Brazil use trout feeds or general formulations for carnivorous fish, respectively. The more common ingredients used in commercial diets for *A. gigas* are fishmeal, soya cake, maize meal, rice, wheat middlings, fish oil and soybean oil, with some of them occasionally including beef meal, maize protein concentrates and maize gluten.

At the local level, in the case of the Peruvian Amazonia, agro-industrial by-products or cereals such as rice or ground maize are easily accessible while more conventional ingredients such as fishmeal and fish oil come from the coast, while soybean is imported, both them at a high cost. Thus, there is a strong interest in reducing such dependence by developing feeds based on local ingredients in such region. Nevertheless, there has been limited research into the utilization of non-traditional ingredients in the diets of *A. gigas*. These studies have explored the incorporation of ingredients both from animal sources, such as waste from the meat and fish industry, as well as some locally produced plant-based ingredients. In example, it has been demonstrated that the inclusion of blood meal and poultry by-product meal at levels of up to 9% and 15% respectively in diets for fingerlings of 5 – 35 g improved production parameters [112,113]. Also, it has been demonstrated that the proportion of fishmeal in the diet could be reduced by up to 30% without causing any adverse effects on production performance and feed intake in juveniles pirarucu (5 g), when replaced by poultry by-product meal and meat-and-bone meal [114].

Regarding the use of vegetable sources, soybean meal can be included in diets for *A. gigas* larvae (25-235 g) without compromising zootechnical performance and fish welfare, even with a 30% reduction in fish meal [115,116]. The inclusion of 5% sunflower cake resulted in good growth in juveniles around 430 g [117]. The good results in growth and feed efficiency obtained by Ribeiro [112] using a combination of chestnut residues with dehydrated blood, meat and bone meal and pork fat, in diets for 10 g fry suggest that there is a great potential to explore the use of alternative ingredients in feeds for this species. In this sense, detailed information on digestibility coefficients for common and alternative ingredients in *A. gigas* is required and results obtained in different experiments carried out to date are resumed in Table 10.

Sources from marine origin presented values of apparent digestibility coefficients above 75%, with fishmeal being the most prominent (89.2% for dry matter, 97.6% for protein and 89.1% for gross energy) [118]. Terrestrial animal sources, with the exception of blood meal, present a wide range of ADC values above 70%. Poultry by-product meal has the best digestibility (93.5 % for dry matter,



90.3 % for protein and 85.7 % for gross energy) [118], followed by meat and bone meal (70.8 % for dry matter, 89.4 % for protein and 75.4 % for gross energy) [118] and hydrolysed feather meal (79.5 % for dry matter, 79.7 % for protein and 91.1 % for gross energy) [119]. Sources of plant origin present a greater variability in their ADCs due to their diversity of types and processing methods. With the exception of soybean cake, oilseed meals and their processed products showed digestibilities higher than 70%, as in the case of soybean meal (79.0% for dry matter, 92.4% for protein and 83.7% for gross energy) [119] and soybean protein concentrate (71.6% for dry matter, 96.9% for protein and 65.9% for gross energy) [120], respectively. Although cereals such as corn or broken rice showed intermediate digestibility values, results suggest that these ingredients are well-utilized by *A. gigas*, exceeding the typical range of 70 to 75%. Wheat bran presented the lowest digestibility in the reported studies (< 70%) [7,91], probably due to its high contents in fiber [7].

In relation to local inputs in the Amazon, digestibility tests with raw sachá inchi cake (*Plukenetia volubilis* Linneo) and palm kernel cake (*Elaeis guineensis* Jacq) presented ADC values higher than 80%, both for dry matter, protein and gross energy [121,122]. These results suggest that raw sachá inchi and palm kernel cake has the potential to be used in *A. gigas* diets during the juvenile stage, as well as other local inputs that require study.

8. Use of Functional Feed Additives

This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex. To date, only a limited number of studies have explored the use of functional feed additives, such as amino acids, peptides, nucleotides or enzyme complexes in diets for *A. gigas*, these yielding diverse results. For this reason, there is a need for further research in this area to gain a better understanding of their potential benefits.

**Table 10.** Apparent digestibility coefficients (ADC) of dry matter, gross energy and protein from different animal and plant sources in the diets of “paiche” (*A. gigas*).

| Ingredients in experimental diets | ADC dry matter (%) | ADC protein (%) | ADC gross energy (%) | Fish weight (g) | Reference |
|-----------------------------------|--------------------|-----------------|----------------------|-----------------|-----------|
| Marine sources                    |                    |                 |                      |                 |           |
| Fish by-product meal              | 72.3               | 77.2            | 76.1                 | 204.45          | [117]     |
| Fish meal                         | 89.2               | 97.6            | 89.1                 | 235.00          | [118]     |
| Salmon by-product meal            | 83.6               | 83.9            | 75.6                 | 204.45          | [117]     |
| Terrestrial animal sources        |                    |                 |                      |                 |           |
| Blood meal                        | 42.1               | 38.6            | 56.0                 | 204.45          | [117]     |
| Feather meal                      | 79.5               | 80.4            | 83.3                 | 235.00          | [118]     |
| Hydrolyzed feather meal           | 79.5               | 79.7            | 91.1                 | 204.45          | [117]     |
| Meat and bone meal                | 62.4               | 72.0            | 70.1                 | 204.45          | [117]     |
| Meat and bone meal                | 70.8               | 89.4            | 75.4                 | 235.00          | [118]     |
| Poultry by-product meal           | 100.6              | 83.6            | 85.7                 | 204.45          | [117]     |
| Poultry by-product meal           | 93.5               | 90.3            | 96.2                 | 235.00          | [118]     |
| Poultry fat                       | 63.9               | 65.7            | 69.0                 | 96.80           | [65]      |
| Plant sources                     |                    |                 |                      |                 |           |
| Broken rice                       | 81.1               | 80.2            | 68.5                 | 131.34          | [91]      |
| Corn                              | 70.2               | 64.4            | 77.7                 | 131.34          | [91]      |
| Corn                              | 76.4               | 93.4            | 40.1                 | 235.00          | [7]       |
| Corn gluten feed                  | 65.9               | 77.1            | 63.9                 | 204.45          | [117]     |
| Corn gluten meal                  | 102.4              | 93.5            | 87.8                 | 204.45          | [117]     |
| Corn gluten meal                  | 61.2               | 74.2            | 59.8                 | 235.00          | [118]     |
| Corn starch                       | 70.7               | 90.9            | 47.9                 | 235.00          | [7]       |

|                         |      |      |      |        |       |
|-------------------------|------|------|------|--------|-------|
| Defatted rice bran      | 76.5 | 67.1 | 56.7 | 131.34 | [91]  |
| Low-tannin sorghum      | 77.7 | 65.9 | 54.9 | 131.34 | [91]  |
| Palm kernel cake        | 89.0 | 96.2 | 89.8 | 322.69 | [122] |
| Rice bran               | 46.2 | 68.2 | 42.2 | 235.00 | [7]   |
| Sacha inchi cake        | 83.0 | 86.4 | 84.9 | 180.00 | [121] |
| Soybean cake            | 61.4 | 80.8 | 71.3 | 279.22 | [123] |
| Soybean meal            | 76.7 | 83.8 | 58.0 | 235.00 | [118] |
| Soybean meal            | 79.0 | 92.4 | 83.7 | 204.45 | [117] |
| Soybean oil             | 65.3 | 68.5 | 70.6 | 96.80  | [65]  |
| Soy protein concentrate | 71.6 | 96.9 | 65.9 | 217.68 | [120] |
| Wheat bran              | 77.3 | 54.7 | 57.6 | 131.34 | [91]  |
| Wheat bran              | 45.1 | 68.6 | 47.4 | 235.00 | [7]   |

Several studies has demonstrated positive effects of including exogenous enzymes in diets for *A. gigas* on productive performance and nutrient digestibility. Cavero [90] working with specimens weighing  $6.6 \pm 0.5$  g, analyzed the effects of including proteases, lipases, and amylases at proportions of 0.0%, 0.1%, 0.2%, and 0.4% in commercial diets containing 45% CP over a period of 37 days. Diets containing exogenous proteases and lipases led to improvements in final weight, weight gain, and feed conversion. However, the addition of amylase to the diets did not result in any notable improvements in these parameters.

The addition of enzymes to the diet was evaluated in the work of Bordinhon [67], aimed to increase the low digestibility of the carbohydrate fraction of raw wheat meal. The author obtained an improvement in the digestibility coefficient, which was maximized with diets containing cooked wheat meal and supplemented with exogenous amylase. Alcântara [124] evaluated the addition of lipase and protease in diets with partial substitution of poultry viscera meal and meat and bone meal, and concluded that there was no increase in the apparent digestibility coefficients of nutrients in the diets, nor in the digestibility of these alternative ingredients. In contrast, Lima et al. [83] evaluated the effects of including an enzyme complex (Allzyme® SSF®, USA) at different concentrations (0.25, 0.50, 0.75, and 1 g/kg) in 40% CP extruded diets for fish with an average weight of  $65.2 \pm 0.4$  g. After a 30-day experiment, the inclusion of the enzyme complex led to an increase in the apparent digestibility of crude protein, crude energy, and dry matter, as well as an increase in liver glycogen and total protein content in the liver and intestine. The higher accumulation of dry matter, gross energy, and body fat indicated weight gain in fish treated with the enzyme complex.

Calderón-Espinoza [125] evaluated the inclusion of two nutritional supplements based on peptides and nucleotides (2% Fish 40® and 2% Fish 75®) in fry weighing 2.3 g and measuring 7.3 cm, and fed diets with 40% CP. After 45 days of evaluation, it was concluded that the inclusion of these additives had no effect on growth improvement or economic performance. However, they partially affected water chemistry parameters by increasing carbon dioxide and alkalinity levels. The use of fish protein hydrolysates has also been evaluated in *A. gigas*. Ribeiro et al. [10] analyzed diets containing different levels of inclusion of this functional additive derived from tilapia waste (0, 4, 8, 12, 16, and 20%). After an experimental period of 8 weeks, growth parameters were not significantly affected by the inclusion of the hydrolysate in juveniles with an average weight of  $91.4 \pm 2.7$  g, suggesting that it can be safely used up to an inclusion level of 20%.

Regarding the use or probiotics, in a study conducted by do Vale Pereira et al. [126], the effects of feeding diets containing two indigenous bacterial strains, *Lactococcus lactis* subsp. *lactis* ( $1 \times 10^8$  CFU-1) and *Enterococcus faecium* ( $1 \times 10^8$  CFU-1), previously isolated from the gastrointestinal tract of *A. gigas* [127], were analyzed in juveniles with an average weight of  $58.86 \pm 10.25$  g. After 21 days of feeding, the study observed that both bacterial strains could positively influence haematoimmunological parameters, modulate the gut microbiota, and increase antimicrobial capacity in the gut. This finding suggests that the use of specific probiotics could be beneficial for the production of *A. gigas*.

9. Final Considerations

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript. One major obstacle for the development of intensive culture of *A. gigas* is the incomplete validation of technological packages, which encompass various aspects of fish farming, including different aspects of its nutrition. While Amazonian aquaculture has the potential for dynamic growth, it faces significant hurdles related to technological validation and the cost of production, especially in terms of the formulation and production of fish feeds, since their high cost contributes to increased production expenses, making large-scale production less economically viable. Addressing these challenges will be crucial for the sustainable development of aquaculture in the region. Within this context, the present review is a attempt to present current knowledge on both basic and applied aspects of the nutrition and feeding of *A. gigas*, as well as to identify those aspects still requiring further research. In this sense, there is a lack of information regarding aspects such as times for gastric emptying and intestinal transit rates, postprandial pH variation and its influence on digestive biochemistry. Also, the modulation of enzyme activity by *A. gigas* is not yet fully understood, so further studies are needed to identify peaks of enzyme expression at the gastric and intestinal levels. The lack of comprehensive knowledge regarding the digestive physiology of *A. gigas* poses significant challenges in determining its nutritional requirements and the appropriate feeding practices. While there are studies on protein, lipid and carbohydrate requirements, these need to be considered for different stages of production and depending on the production system, nutritional challenge and environmental conditions. The inclusion of carbohydrates and the determination of the appropriate level are key to reducing feed costs, considering also the importance of the inclusion of micro-ingredients such as vitamins and minerals, of which little is known about their mode of action in *A. gigas*. Reducing feed costs is also linked to the implementation of appropriate feeding practices. Studies on this topic have given inconclusive results, making it imperative to determine the optimal feed dosage to deliver the appropriate amount of nutrients efficiently. Additionally, there is a need to assess the digestibility of non-traditional ingredients and understand how this species utilizes such ingredients in its diet. All those knowledge gaps making it difficult to formulate and produce well-balanced and efficient feed that can adequately meet the species' metabolic needs are resumed in Table 11.

**Table 11.** Knowledge gaps on the nutritional physiology and feeding strategies of *A. gigas*.

| Aspects                        | Comments  |
|--------------------------------|---|
| Gastrointestinal functionality | Further studies are required to comprehend the variations in postprandial gastrointestinal pH, gastric emptying, and intestinal transit. This will aid in understanding the physiological responses of <i>A. gigas</i> to feed intake.  |
| Digestive enzymes              | The modulation of enzymes in <i>A. gigas</i> during postprandial digestion is not yet unknown. This information is necessary to identify the peaks of enzyme expression in the stomach and intestines.  |
| Protein and amino acids        | Research on this subject has primarily focused on individuals from the juvenile stage onwards. However, there is a need to determine the nutritional requirements for the early stages. Additionally, further studies are required to include other variables that affect protein requirements, such as production systems, nutritional challenges, and environmental conditions. |
| Energy and P:E ratio           | To date, few studies have been conducted on the subject. It is necessary to determine the E/P ratio for different sizes of <i>A. gigas</i> , taking into account the various stages of production.  |
| Lipids and fatty acids         | The use of dose-response methodology, considered appropriate for determining nutritional requirements in fish, has not been employed to determine lipid levels. The available information is provided in technical documents for extension purposes, and in some cases, the   |

| recommended levels are only applicable to certain stages of the species. |  |
|--|--|
| Aspects  | Comments   |
| Carbohydrates  | The carbohydrate levels in <i>A. gigas</i> have not been determined using the dose-response methodology. Further studies are needed to observe the protein-sparing effect in this species and determine the maximum levels of inclusion in the diet.   |
| Vitamins   | Only the effects of two vitamins have been studied. Therefore, further research is necessary to determine the requirements of a larger number of vitamins. This will enable the formulation of vitamin premixes that can be included directly in feed.   |
| Feeding rates  | Producers make use of information that is available in technical extension manuals or information provided by the aquafeed company. Scientific information is currently only available for four specific sizes of <i>A. gigas</i> . To ensure efficient use of the provided feed, further studies on a larger range of sizes and culture stages are necessary.   |
| Feeding frequencies  | Producers make use of information that is available in technical extension manuals or information provided by the aquafeed company. The studies conducted have reported varying results for the same stages of <i>A. gigas</i> , indicating inconclusiveness. Further studies are necessary, with adjustments made to experimental design, to determine the optimal feed supply for the day.                 |
| Feeding time   | The studies focus solely on certain sizes of <i>A. gigas</i> . It is necessary to determine the optimal feeding time for the various sizes involved in the species' production cycle to ensure optimal feed intake and nutrient utilisation throughout the culture.  |
| Use of alternative ingredients   | Although some non-traditional inputs have been evaluated, further research is required to assess the effects of including local inputs, particularly waste generated by economic activity, such as beer bran, fish, poultry, livestock, and agro-industrial waste. Additionally, aspects such as nutritional quality, price, and availability of these inputs should be taken into consideration.            |
| Functional additives   | The focus of studies on functional additives has mainly been on improving the palatability of aquafeeds with a higher content of plant inputs or enhancing the absorption of nutrients. However, it is important to consider the effects of other functional additives such as acidifiers, gut conditioners, and phytases, which could improve the bioavailability of nutrients provided by aquafeed inputs. |
| Digestibility of ingredients   | Analyses of digestibility mainly focus on the dry matter digestibility of ingredients and/or feed, rather than the digestibility of N and P. This would enable an understanding of <i>A. gigas</i> ' ability to access the nutrients provided and could lead to the use of pre-digestive treatments or additives to enhance aquafeed ingredient digestibility.   |

**Supplementary Materials:** The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: title; Table S1: title; Video S1: title.

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**Conflicts of Interest:** Declare The authors declare no conflicts of interest.

## Appendix A

The appendix is an optional section that can contain details and data supplemental to the main text—for example, explanations of experimental details that would disrupt the flow of the main text but nonetheless remain crucial to understanding and reproducing the research shown; figures of replicates for experiments of which representative data is shown in the main text can be added here if brief, or as Supplementary data. Mathematical proofs of results not central to the paper can be added as an appendix.

## Appendix B

All appendix sections must be cited in the main text. In the appendices, Figures, Tables, etc. should be labeled starting with “A”—e.g., Figure A1, Figure A2, etc.

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