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Nutrient profile and performance of commercially available grower feeds in Brazil for the semi-intensive culture of juvenile *Penaeus vannamei* raised across different water salinities

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ABSTRACT - This study assessed the nutrient profiles of major grower shrimp feeds in Brazil used in semi-intensive farming of *Penaeus vannamei* and their impact on shrimp growth under optimal (15.8 ± 0.14 ppt), hypoosmotic (3.9 ± 0.57 ppt), and hyperosmotic (41.8 ± 0.62 ppt) salinities. Juvenile shrimp (0.85–1.60 g) were stocked at 50 animals/m^2 in 40 outdoor tanks (1 m^3) and fed across three consecutive trials lasting 63 to 86 days. Dietary nutrient composition varied widely, with crude protein ranging from 38.2 to 41.2%, carbohydrates from 28.5 to 43.8%, crude fat from 6.7 to 12.1%, and ash content from 10.7 to 20.2%. There were also variations in gross energy (16.2–18.5 MJ/kg), macrominerals, amino acids (AA), and essential fatty acids (EFA). Principal Component Analysis and hierarchical clustering identified EFA, AA, and minerals as key factors driving feed variability. Growth performance under optimal and hypoosmotic salinities was largely attributed to EFA levels. Feed E, with the highest EFA content, supported superior growth, followed by feeds D and A. This ranking (feed E > feed D > feed A > feed F, feed B, and feed C) aligned with observed growth, highlighting the critical role of EFA in shrimp performance under these salinities. Under hyperosmotic conditions, shrimp growth was similar across most feeds, indicating high salinity limited growth. The study suggests that specific nutrient profiles, particularly those enriched in EFA, support growth more effectively under optimal and hypoosmotic conditions, while others are less effective under high salinity stress. The findings emphasize the need for adaptive feed formulations to meet metabolic demands, offering valuable insights for feed selection in regions with suboptimal salinity conditions.

Keywords: amino acids, essential fatty acids, nutrient variability, osmotic stress, shrimp feed formulation

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1. Introduction

After years of fluctuating production levels and market challenges, recent industry reports indicate a resurgence in the Brazilian shrimp aquaculture sector. In 2023, the industry achieved a production milestone of 127.5 thousand metric tons, generating approximately R$ 2.63 billion in revenue (IBGE, 2024). Of this total, 99.6% originated from the Northeast region, with the state of Ceará contributing 57% and the state of Rio Grande do Norte 19.4%. Shrimp farming has now expanded across Brazil's diverse environments, including the oligohaline waters of the semi-arid interior and coastal areas where seasonal droughts and shallow estuaries can drive salinity to extreme levels. These salinity variations present unique challenges for shrimp farmers, as the metabolic demands of shrimp shift significantly under different osmotic conditions (Spanopoulos-Hernández et al., 2005; Wang et al., 2013).

Feed quality is central to the success of shrimp farming, directly influencing their growth, feed conversion efficiency, and survival, all critical for competitive production costs (Villarreal, 2023). Although previous studies have underlined the impact of environmental factors, such as salinity, on *Penaeus vannamei* growth and survival (Ponce-Palafox et al., 1997; Tseng and Hwang, 2008; Ern et al., 2014), limited data exist on the performance of commercial feeds across the range of salinities encountered in shrimp farming. Given the strong reliance of the industry on commercial feeds, understanding their effectiveness under both optimal and suboptimal salinity conditions is essential for farmers seeking to maximize yield and economic efficiency.

This study characterized and compared the nutritional profiles of six major commercial shrimp feeds sourced from a variety of leading multinational and local manufacturers in Brazil. We hypothesize that, due to variability in feed composition across manufacturers and the influence of salinity on shrimp metabolism and nutrient demands, differences in the nutritional composition of these feeds could significantly impact the survival, growth, and feed utilization of *P. vannamei* under varying salinity conditions. By identifying key nutrients that influence performance, this research aims to support effective feed formulation, enhancing both productivity and sustainability in Brazil's expanding shrimp aquaculture sector.

2. Material and methods

2.1. Experimental design

The study was conducted in the outdoor tank facilities of the Laboratório de Nutrição de Organismos Aquáticos (3°50'01.55" S, 38°25'22.74" W), located in Eusébio, Brazil. This laboratory is part of the Instituto de Ciências do Mar (Institute of Marine Sciences; LABOMAR) at the Universidade Federal do Ceará, Fortaleza, CE, Brazil. Three separate experiments were conducted to evaluate the effect of different salinity conditions on the growth, survival, and feed utilization of juvenile *P. vannamei*. Each experiment was carried out using six commercial grower shrimp feeds under optimal, hypoosmotic, and hyperosmotic conditions. The summary of key experimental conditions across three different salinity trials is shown in Table 1.

Table 1 - Experimental design across the different salinity experiments

	Optimal	Hypoosmotic	Hyperosmotic
Experiment number	1	2	3
Number of tanks	40	40	40
Number of tank replicates	6 - 7	6 - 7	6 - 7
Salinity (ppt)	15.8 ± 0.14	3.9 ± 0.57	41.8 ± 0.62
Temperature (°C)	28.5 ± 0.2	28.1 ± 0.1	26.7 ± 0.1
pH	8.28 ± 0.02	8.17 ± 0.02	7.96 ± 0.02
Post-larvae trait	Fast growth	Fast growth	Resistance
Initial body weight (g)	1.60 ± 0.16	1.53 ± 0.15	0.85 ± 0.10
Coefficient of variation	9.7%	10.0%	12.4%
Stocking - Harvest date	Nov. 21st, 23 – Jan. 23rd, 24	Feb. 22nd – May 3rd, 24	Jun. 14th – Sep. 6th, 24
Duration (days)	63	86	84
Weather conditions[1]	Hot, dry	Hot, humid	Windy, dry
Raining days	9	51	8
Rainfall (mm)	0.86 ± 3.53 (0 - 26)	13.9 ± 20.3 (0 - 111)	1.79 ± 10.8 (0 - 98)
Air temperature (°C)	28.6 ± 0.5 (27 - 31)	28.1 ± 0.8 (25 - 31)	27.2 ± 0.4 (24 - 29)
Air relative humidity (%)	77.3 ± 2.8 (68 - 84)	83.1 ± 3.4 (71 - 93)	74.5 ± 3.7 (61 - 85)
Average wind speed (km/h)	16.6	10.4	18.4

[1] Weather data were collected from a meteorological station located 5 km from the research site. Data were provided by the Plataforma de Coleta de Dados (PCD) of the Fundação Cearense de Meteorologia e Recursos Hídricos (FUNCEME).

2.2. Shrimp and culture system

Shrimp used in the study were obtained at the post-larval stage (PL10, 10-day-old PL) from commercial hatcheries (Table 1). Each experiment received a separate batch of PL10 with genetic traits focused on fast growth or resistance. Upon arrival, shrimp were subjected to quarantine, during which samples were screened using qPCR (quantitative real-time polymerase chain reaction) for white spot syndrome virus (WSSV) and infectious myonecrosis virus (IMNV). The qPCR analysis confirmed that shrimp were free of both pathogens. Following quarantine, shrimp were transferred to five nursery tanks (23 m^3 each) and reared until they reached the target size for each specific experiment. Prior to stocking in rearing tanks, shrimp were size-graded to ensure uniform body weight across all treatments.

Outdoor tanks used for experiments had a total volume of 1 m^3 and a bottom surface area of 1.02 m^2. Shrimp were stocked at a density of 50 shrimp/m^2, corresponding to 51 shrimp per tank. Tanks were operated under nutrient-rich water conditions typical of shrimp farming, resulting in a brownish water color promoted through fertilization. Tanks were continuously exposed to natural weather fluctuations, which promoted natural productivity associated with sunlight-exposed aquaculture systems. While the tanks were covered with lids, open sections allowed sunlight to enter. These openings were fitted with screens, permitting air circulation while preventing shrimp from escaping the tanks. Aeration was supplied by a 2.5 hp blower, with air distributed through micro-perforated hoses installed at the bottom of each tank.

2.3. Water source, fertilization, monitoring, and feeding

Culture water originated from two different sources, depending on the salinity required for each specific experiment: estuarine and deep well water. Estuarine water was collected through a system of pipes and pumps, with the collection point located 215 m from the experimental site, along the margins of the Pacoti River estuary. This estuary is situated 2.6 km from its mouth at the Atlantic Ocean and has an average salinity of 35 ppt. The deep well water used has an average salinity of 5 ppt. A 20-m^3 header tank was used to prepare the target salinity in advance. For hyperosmotic conditions (40 ppt), salinity was increased by dissolving crude sea salt (996.4 g/kg sodium chloride) into freshwater. Salinity adjustments were made prior to each trial, and the water was disinfected and filtered through sand for one week before being transferred to the experimental tanks. Ready-to-use water was kept available throughout the trials to correct water salinity or quality as needed.

Following tank filling, water preparation began following the methodology described by Nunes et al. (2023). Water was fertilized using a commercial probiotic (BM-Pro, Biotrends Soluções Tecnológicas Ltda., Eusébio, CE, Brazil) composed of a consortium of microorganisms (*Bacillus* spp., *Lactobacillus* spp., and *Saccharomyces cerevisiae*). The probiotic was initially activated with tap water for 8 h, and then boiled sugar-cane molasses was added at 1:2 (probiotic:molasses). The mixture was fermented in a bucket without aeration for 24 h. This fermented preparation was applied at 53 mL per tank once a day for a continuous three-day period before shrimp stocking.

Feeding was conducted using feeding trays, with four daily feedings at 07:00 and 10:00 h, and at 13:00 and 16:00 h. The daily feed ration was divided into four portions, corresponding to 25, 15, 15, and 45% of the total daily ration. The maximum daily feed intake (MM) was calculated using the equation $MM = 0.0931 \times BW^{0.62}$ (Nunes and Parsons, 2000), and a 30% reduction was applied to optimize feed efficiency and prevent excess feed waste. Previous work had shown that the MM can be restricted by 28.8% (Nunes et al., 2006). without any detrimental effect on shrimp growth performance. Feeding adjustments were also made based on expected survival rates and daily weight gains determined on a biweekly basis.

Water quality parameters, including pH, temperature, and salinity, were monitored daily. A portable pH meter (H98107 pHEP, Hanna Instruments Brasil) was used to record pH and temperature, while a refractometer (RTS-101ATC, Instrutherm Instrumentos de Medição Ltda) was employed to measure salinity at 01:00 pm in all tanks.

All shrimp rearing practices were carried out in compliance with relevant national laws and institutional guidelines. Ethical approval was not required for this study, as shrimp do not belong to phylum Chordata and subphylum Vertebrata, which are subject to ethical approval requirements under Brazilian Federal Law.

2.4. Feeds: chemical and physical analyses

Six commercial shrimp feeds were obtained from different feed companies operating within the state of Ceará, Brazil. Ceará is the largest shrimp-producing state in the country, with an estimated annual production of *P. vannamei* reaching approximately 73,000 mt in 2023, which accounted for about 57% of Brazil's total shrimp production (IBGE, 2024). The feeds were sourced from the leading feed manufacturers in the region, representing both multinational and local companies. The selection of these specific feeds was based on their significance in the national aquaculture sector. These manufacturers also supply feeds to other key shrimp-producing regions across Brazil, ensuring that the tested feeds provide a representative sample of those commonly used in the Brazilian shrimp production industry. All feeds in this study were designated for the grow-out phase. For confidentiality reasons, the names of the companies and their respective feed brands are not disclosed.

All chemical analyses were carried out using methods consistent with AOAC (2023). Dry matter (DM) and moisture content was determined by drying the samples in a convection oven for 24 h at 105 °C. Crude protein (CP) was quantified using the Dumas combustion method. The amino acid (AA) composition was analyzed using ion-exchange chromatography with post-column derivatization with ninhydrin. Prior to analysis, AA were oxidized with performic acid and neutralized with sodium metabisulfite. Protein-bound AA were released through hydrolysis with 6 N HCl at 110 °C for 24 h and subsequently quantified by the internal standard method, measuring the absorbance of the ninhydrin reaction products at 570 nm. This method captures both protein-bound and free AA present in the sample. Crude fat was determined using acid hydrolysis, while the fatty acid (FA) profile was assessed through high-resolution gas chromatography (GC) with flame ionization detection using a capillary GC column. Ash content was measured by burning the samples in a muffle furnace at 600 °C for 2 h. Crude fiber content was determined using enzymatic-gravimetric analysis. Gross energy content was analyzed using a bomb calorimeter (IKA Model 5000), following the traditional bomb calorimetry method. Minerals, including calcium, potassium, phosphorus, and magnesium, were measured using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Other essential nutrients, including cholesterol, phospholipids, vitamins, and trace minerals were not determined.

In addition to the chemical composition, key physical properties of the commercial feeds were also analyzed. Physical parameters included pellet diameter, length, hardness, number of pellets per 10 g, and water stability. Physical water stability of pellets was measured with an orbital horizontal shaker in accordance to the method described by Nunes et al. (2019). The length, diameter, and hardness of 30 pellets from each commercial feed were measured. A digital caliper (Starrett, Model No.: 799A-6/150) was used to determine the length and diameter of the pellets, while a hardness manual tester (Amandus Kahl GmbH & Co. KG) was employed to measure pellet hardness. To assess pellet size uniformity and bulk density, the number of pellets in 10 g of feed was counted in triplicate.

2.5. Shrimp performance and statistical analyses

At shrimp harvest, animals were counted and weighed individually on a precision scale with 0.01-g accuracy. Survival, growth performance, feed utilization and daily nutrient intake calculations were as follows:

$$\text{Survival (\%)} = \frac{\text{Final } N}{\text{Initial } N} \times 100 \quad (1)$$

$$\text{Weekly growth (g/week)} = \frac{(\text{FBW} - \text{IBW})}{\text{No. days}} \times 7 \quad (2)$$

$$\text{Specific growth rate (SGR, \%/day)} = \frac{\ln \text{FBW} - \ln \text{IBW}}{\text{No. days}} \times 100 \quad (3)$$

$$\text{Weight gain (\%)} = \frac{(\text{FBW} - \text{IBW})}{\text{IBW}} \times 100 \quad (4)$$

$$\text{Yield (MT/ha)} = \frac{\text{BIOg (MT)}}{\text{Tank bottom area (ha)}} \quad (5)$$

$$\text{Total feed intake (g, DM)} = \text{amount of feed distributed} - \text{amount of uneaten feed} \quad (6)$$

$$\text{Daily feed intake (g, DM/kg BW per day)} = \frac{\text{Total feed intake}}{\text{Average BW} \times \text{average } N \times \text{No. days}} \quad (7)$$

$$\text{Daily nutrient intake (g, DM/kg BW per day)} = \text{Daily feed intake} \times \% \text{ Nutrient content} \quad (8)$$

$$\text{Average BW (kg)} = \frac{(\text{IBW} + \text{FBW})}{(2 \times 1000)} \quad (9)$$

$$\text{Average } N = \frac{(\text{Initial } N + \text{Final } N)}{2} \quad (10)$$

$$\text{FCR} = \frac{\text{Total feed intake}}{(\text{BIOg})} \quad (11)$$

$$\text{Feed efficiency (FE)} = \frac{\text{BIOg} + \text{dBIO}}{\text{Total feed intake}} \quad (12)$$

$$\text{dBIO} = (\text{FBW} - \text{IBW}) \times (\text{Initial } N - \text{Final } N) \quad (13)$$

in which Initial *N* = number of shrimp at stocking, Final *N* = number of shrimp at harvest, IBW = initial body weight (g), FBW = final body weight (g), BIOi (g) = initial biomass, BIOf (g) = final biomass, BIOg (g, wet weight) = gained biomass (BIOf − BIOi), FCR = feed conversion ratio, and dBIO (g) = dead biomass.

All values are reported as mean ± standard deviation, unless otherwise specified. For each experiment, a one-way ANOVA was conducted to assess the effects of different commercial feeds on shrimp survival, growth performance, feed utilization, and daily feed/nutrient intake under specific salinity conditions. Experiments were conducted consecutively across different seasons, exposing the shrimp to varying weather conditions. As these seasonal effects were not controlled across experiments, it became challenging to isolate the effects of diet and salinity without weather-related confounding factors. Therefore, each experiment was analyzed independently, allowing the evaluation of feed performance within the unique salinity and environmental conditions of each experiment. The following mathematical model was adopted:

$$Yij = \mu + \tau i + \epsilon ij, \quad (14)$$

in which Yij is the j-th observation of feed type i; μ is the general mean response; τi is the non-random effect of feed type, in which $\sum_{i=1}^{k} \tau i = 0$; and ϵij is the random feed type error. When significant differences were detected, Tukey's Honest Significant Difference (HSD) test was applied for post-hoc comparisons between treatment means. The significant level of 5% was set in all statistical analyses.

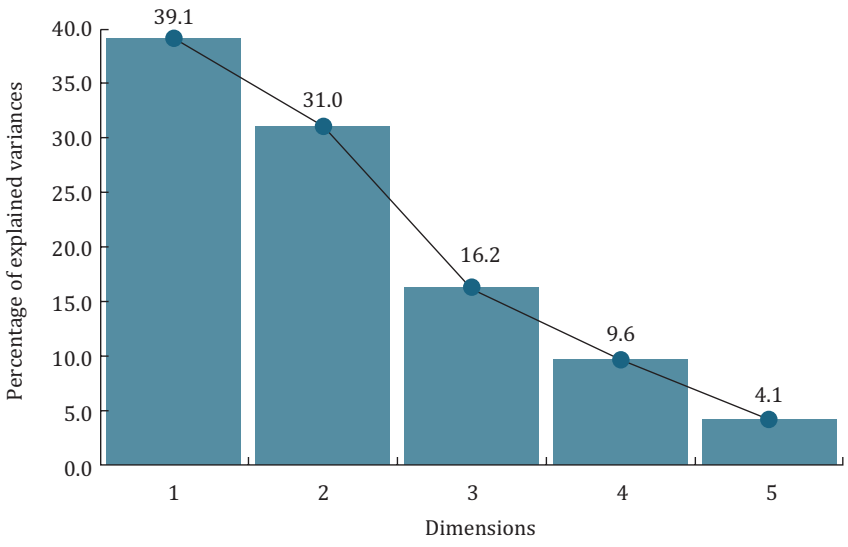
A Principal Component Analysis (PCA) was performed to reduce the dimensionality of the nutrient dataset and identify patterns across the six commercial shrimp feeds. The dataset included 77 variables, representing macro-, micronutrients, and nutrient ratios, such as AA, FA, minerals, and proximate composition. The PCA transformed the original variables into uncorrelated components

(principal components) that explain the variance within the dataset. Each feed was represented by its corresponding scores on the first five principal components. These scores served as input for the hierarchical cluster analysis using Ward's method to minimize within-cluster variance. Euclidean distance was used as the dissimilarity metric to measure the relative distance between feeds based on their PCA scores. The dendrogram obtained from this analysis provided a visual representation of the clusters, grouping feeds according to their similarity in nutrient profiles. All statistical analyses were performed using R software (version 3, 2024.04.2 Build 764).

3. Results

3.1. PCA and cluster analysis of feed nutrient profiles

The PCA of feeds revealed distinct clusters based on their nutrient profiles. The first two principal components (PC1 and PC2) accounted for 70.1% of the total variance, with PC1 explaining 39.1% and PC2 accounting for 31%. The scree plot confirmed that these two components captured most of the variability in the dataset (Figure 1). The PC1 primarily reflected differences in essential fatty acids (EFA), such as docosahexaenoic acid (DHA), which contributed 3.34% to the component, and eicosapentaenoic acid (EPA) + DHA (3.20%). Additionally, the proportions of EPA and DHA as part of the total fatty acids, represented as EPA_DHA_TFA (3.07%), DHA_TFA (3.03%), and EPA_TFA (2.84%), also strongly influenced this component. These variables had the highest loadings along the PC1 axis, meaning that differences in FA content were a key driver of the observed variability among the feeds. The PC2 captured variability related to both EFA and minerals. It was strongly associated with the total EFA as a percentage of total fatty acids (EFA_TFA), contributing 3.93% to PC2. Additionally, linoleic acid (LOA_TFA) and α-linolenic acid (ALA_TFA), two polyunsaturated fatty acids, accounted for 3.72 and 3.62% of this component, respectively. The mineral P contributed 3.56%, while the AA asparagine explained 3.14% of the variability along PC2. These variables highlight the role of FA and P in differentiating feed compositions. Figure 2 presents the biplot of the top nutrient contributors to PC1 and PC2, visually illustrating how these variables influenced feed separation along the two principal components. The hierarchical clustering analysis, based on PCA scores, further categorized the feeds into three distinct clusters (Figure 3). Feed E formed a unique single-member cluster, indicating that its nutrient composition was markedly different from the other feeds. This feed stood out for its higher scores on variables heavily weighted in PC1 and PC2, such as EFA both in absolute terms and as a



The first two components account for 70.1% of the total variance.

Figure 1 - Scree plot showing the proportion of variance explained by the first five principal components.

proportion of the total fatty acids. The second cluster included feeds A, F, B, and C, which showed greater similarity, though with some internal variability. Among them, feeds A and F exhibited closer alignment, sharing moderate levels of protein and higher fiber content. Feeds B and C, while part of the same cluster, showed slight differences in nutrient profiles. Feed D formed a separate cluster at a greater distance from the other feeds, reflecting its distinct nutrient profile. Its separation in the dendrogram was largely driven by its positioning along PC2, which reflected variability in minerals, particularly P. Specific AA also contributed to the unique profile of feed D. The clustering results emphasize the variability in nutrient composition among the feeds. The PC1 primarily captured differences related to EFA, while PC2 highlighted additional variability in minerals and AA. The dendrogram provided a clear visual representation of these patterns, illustrating how feeds grouped according to similarities in their nutrient compositions.

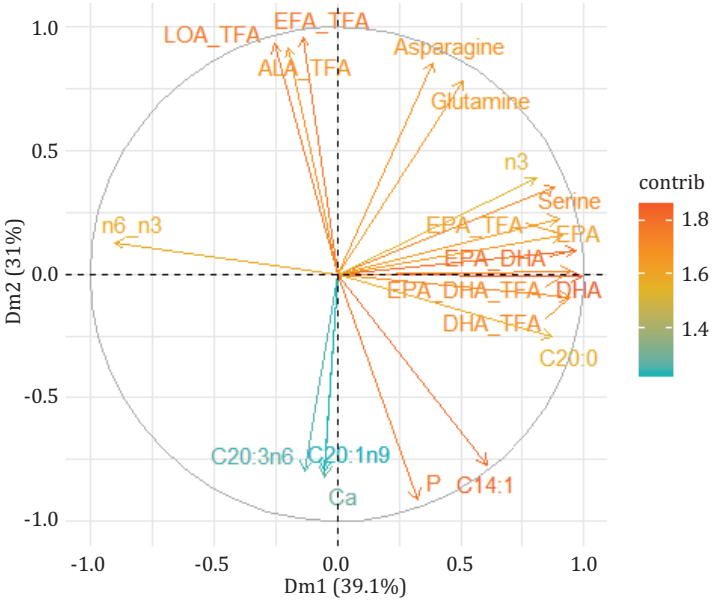


Figure 2 - Biplot of top nutrient contributors to principal components 1 and 2.

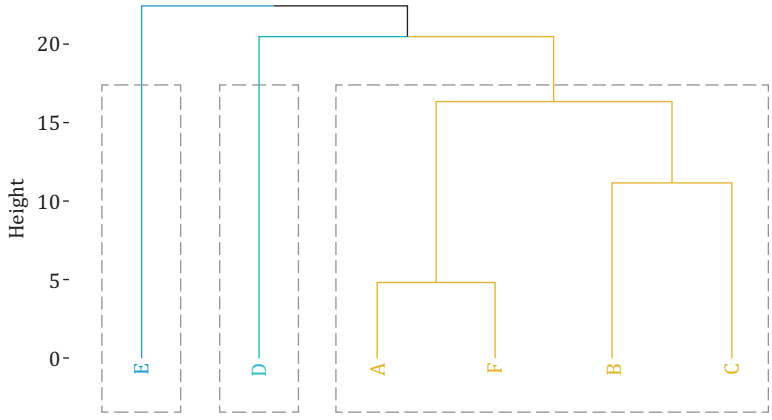


Figure 3 - Hierarchical cluster dendrogram of feeds based on nutrient profiles.

To build upon the insights from the PCA and clustering analysis, a detailed examination of the chemical and physical properties of the feeds was conducted. The following subsections provide an overview, highlighting subtle differences in nutrient composition that may not have been fully captured by the PCA but are likely to influence feed performance and nutrient utilization.

3.2. Proximate composition

Feed proximate composition included DM, CP, carbohydrates, crude fat, ash, and gross energy content (Table 2). Crude protein ranged from 38.2 (feed A) to 41.2% (feed C), with a coefficient of variation (CV) of 3%. Total carbohydrates varied widely, with feed C showing the lowest value (28.5%, DM basis) and feed D the highest (43.8%, DM basis), with non-fiber carbohydrates (e.g., starch) representing 39.2% (DM basis) of the composition in feed D. Crude fat was highest in feed E (12.1%, DM basis) and lowest in feed D (6.7%, DM basis). Ash content ranged from 10.7 (feed D) to 20.2% (feed C), and gross energy varied between 16.2 and 18.5 MJ/kg, with a CV of 5%.

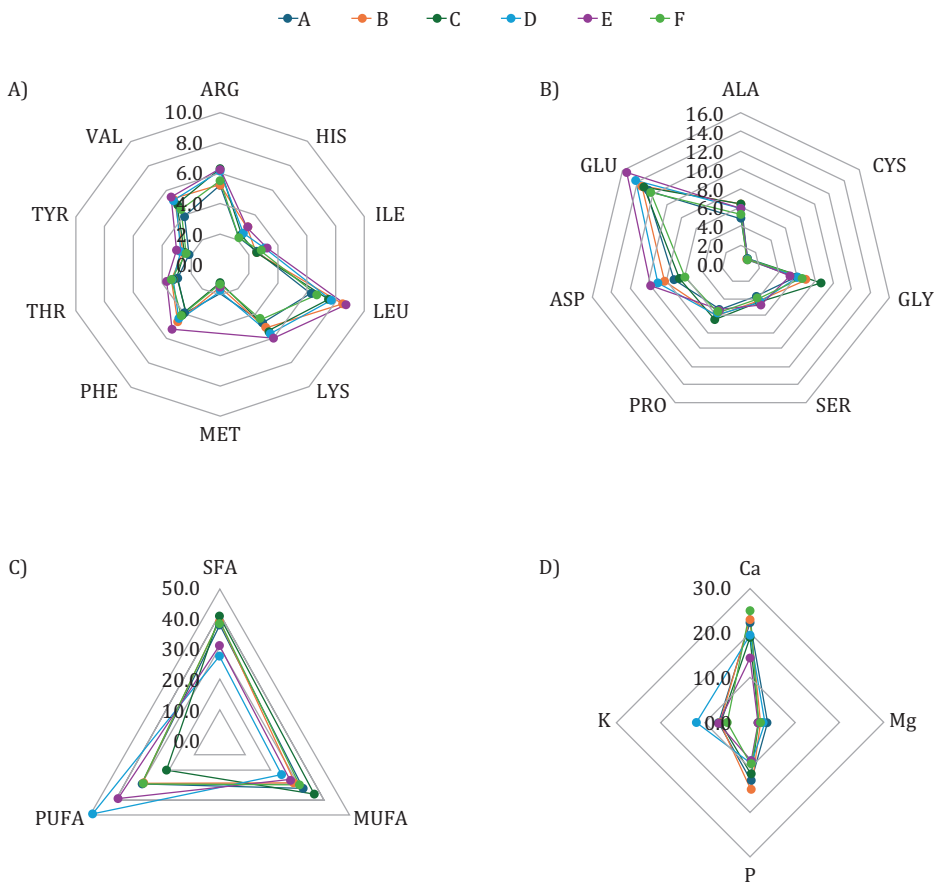
Table 2 - Proximate and macromineral composition of commercial feeds (%, dry matter basis)

Composition	Commercial feed						CV%
	A	B	C	D	E	F	
Dry matter	91.4	92.3	92.6	91.0	91.8	91.6	0.6
Crude protein	38.2	39.5	41.2	38.7	39.7	39.8	3
Carbohydrates[1]	38.2	41.6	28.5	43.8	32.1	33.4	16
Non-fiber carbohydrates[2]	33.9	39.4	25.4	39.2	29.2	29.0	18
Crude fiber	4.3	2.2	3.2	4.7	2.9	4.4	28
Ash	14.3	11.6	20.2	10.7	16.1	18.7	25
Crude fat	9.4	7.4	10.1	6.7	12.1	8.2	22
Gross energy (MJ/kg)	17.9	18.5	17.3	17.8	18.5	16.2	5
Macrominerals							
Calcium	3.2	2.7	3.9	2.1	2.3	4.7	32
Phosphorus	1.9	1.7	2.4	0.9	1.4	1.7	29
Ca:P ratio	1.7	1.5	1.7	2.3	1.7	2.8	26
Magnesium	0.5	0.3	0.3	0.3	0.3	0.4	28
Potassium	1.1	0.8	1.0	1.3	1.2	1.0	16

CV - coefficient of variation.
[1] Carbohydrates = non-fiber carbohydrates + crude fiber.
[2] Non-fiber carbohydrates = 100 – (crude protein + crude fat + ash + crude fiber).

3.3. Macrominerals

Mineral composition (Table 2) revealed substantial variability in macrominerals such as Ca, P, Mg, and K. Calcium content ranged from 2.1% (dry matter basis) in feed D to 4.7% in feed F, with an overall mean of 3.2% and a CV of 31.8%. Phosphorus levels were lowest in feed D (0.9%) and highest in feed C (2.4%), with a mean of 1.7% and a CV of 28.9%. The Ca:P ratio varied from 1.5 in feed B to 2.8 in feed F. Magnesium levels were relatively low, ranging from 0.3 to 0.5%, with a mean of 0.4% and a CV of 28.3%. Potassium content ranged from 0.8% in feed B to 1.3% in feed D, with a mean of 1.1% and a low CV of 16.1%. When considering minerals as a percentage of ash content (Figure 4D), Ca ranged from 14.4% in feed E to 25.3% in feed F, with a mean of 20.8% and a CV of 18%. Magnesium content showed higher variability (CV = 32%), ranging from 1.6% in feed C to 3.8% in feed A. Phosphorus expressed as a percentage of ash content ranged from 8.6% in feed D to 15.1% in feed B, with a mean of 11.0% and a CV of 25%. Potassium content had the highest variability when expressed as a percentage of ash (CV = 34%), ranging from 5.0% in feed C to 12.0% in feed D.



A) Essential amino acid profile (% of crude protein). B) Non-essential amino acid profile (% of crude protein). C) Fatty acid profile (% of crude fat). D) Macromineral profile (% of ash content).

Figure 4 - Nutritional profile of commercial feeds: amino acids, fatty acids, and macrominerals.

3.4. Amino acids

Amino acid composition includes both essential AA (EAA) and non-essential AA (NEAA; Table 3). Methionine showed the most variability among EAA, ranging from 0.47% in feed A to 0.80% in feed C, with a CV of 20%. Lysine content ranged from 1.79 (feed F) to 2.40% (feed E). Feed E had the highest total EAA content (18.26%), while feed A had the lowest (13.55%). Among NEAA, glutamine and asparagine were the most abundant. Glutamine reached 6.13% in feed E, showing moderate consistency with a CV of 9%. Across all AA, CV values ranged from 9 to 20%, indicating a moderate spread across feeds. The EAA profiles, expressed as a percentage of CP, reveals both similarities and differences among the six feeds, with each feed represented by a distinct line (Figure 4). Despite small variations, the overall EAA proportion to CP appears consistent across feeds. Lysine and leucine stood out, with CV of 11 and 12%, respectively. Lysine percentage to CP ranged from 4.5% (feed F) to 6.0% (feed E), while leucine levels varied from 6.4% (feed A) to 8.7% (feed E). The proportion of methionine to CP showed the greatest variability, with a CV of 19%. Feed C had the highest proportion, at 1.9% methionine of CP. Histidine showed the second highest variability (CV = 15%), while arginine was the most stable, with a CV of 7%. Other EAA, such as threonine, tyrosine, valine, and isoleucine, had moderate consistency, with CV ranging from 8 to 11%. The NEAA profiles displayed a similar pattern

to the EAA profiles though some individual NEAAs varied more (Figure 4B). Glutamine and asparagine remained the most abundant NEAA, with Glu peaking at 15.4% in feed E (CV = 9%). Asparagine showed higher variability (CV = 18%), followed by glycine with a CV of 17% and a peak of 8.6% in feed C. Alanine and cysteine exhibited low variability, both with CV of 11%. Serine and proline were the most consistent, with CV of 8%. Although taurine was excluded from the radar plot due to its low levels, it had the highest variability among all NEAA, with a CV of 92%, likely reflecting differences in the use of marine and animal-derived ingredients across feeds. The sum of EAA + NEAA (true protein) diverged considerably from the CP levels (Table 2) of each respective feed. The true protein content varied more significantly than the dietary CP content, from 29.70 to 37.47%, with a higher CV of 9%. Feed C carried the highest true protein content (36.92%), while feed A had the lowest true protein (29.70%).

Table 3 - Amino acid composition of commercial feeds (%, dry matter basis)

Amino acid composition	Commercial feed						CV%
	A	B	C	D	E	F	
Essential amino acids (EAA)							
Arginine (Arg)	2.01	2.14	2.58	2.35	2.42	2.18	9
Histidine (His)	0.83	1.17	1.00	1.01	1.22	0.86	16
Isoleucine (Ile)	1.00	0.99	1.19	1.20	1.29	1.12	11
Leucine (Leu)	2.44	3.34	3.08	3.02	3.46	2.67	13
Lysine (Lys)	1.81	2.06	2.25	2.20	2.40	1.79	12
Methionine (Met)	0.47	0.56	0.80	0.71	0.58	0.52	20
Phenylalanine (Phe)	1.48	1.87	1.76	1.80	2.10	1.59	12
Threonine (Thr)	1.12	1.31	1.37	1.34	1.46	1.20	9
Tyrosine (Tyr)	0.85	0.93	1.03	1.05	1.19	0.94	12
Valine (Val)	1.54	2.06	1.98	1.96	2.15	1.75	12
Non-essential amino acids (NEAA)							
Alanine (Ala)	1.86	2.52	2.63	2.26	2.36	2.07	13
Cysteine (Cys)	0.31	0.30	0.36	0.40	0.39	0.36	12
Glycine (Gly)	2.28	2.79	3.56	2.53	2.12	2.54	19
Serine (Ser)	1.44	1.66	1.84	1.68	1.88	1.58	10
Proline (Pro)	2.01	2.35	2.66	2.21	2.14	2.20	10
Asparagine (Asp)	2.74	3.24	2.75	3.48	3.87	2.43	17
Glutamine (Glu)	4.93	5.25	4.97	5.49	6.13	5.00	9
Taurine (Tau)	0.01	0.01	0.06	0.01	0.01	0.05	94
Hydroxyproline (Hyp)	0.57	0.74	1.06	0.62	0.29	0.64	38
∑ EAA	13.55	16.43	17.03	16.64	18.26	14.63	11
∑ NEAA	16.14	18.87	19.89	18.68	19.21	16.89	8
EAA + NEAA	29.70	35.30	36.92	35.32	37.47	31.53	9

CV - coefficient of variation.

3.5. Fatty acids

Table 4 outlines the detailed composition of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in the six commercial shrimp feeds, while Figure 4C summarizes the relative proportions of these fatty acid categories as percentages of crude fat. The SFA content was highest in feed C (4.07%) and lowest in feed D (1.84%), with a CV of 24% across all feeds. In Figure 4C, this pattern is reflected, where feed C also shows the highest SFA proportion at 40.8% of crude fat, while feed D has the lowest at 27.7%. This variation highlights the potential differences in fat sources, such as animal fats or plant-based oils, used in these feeds. The MUFA were most abundant in feed C (3.60%) and least abundant in feed D (1.59%) with a CV of 28% (Table 4). Similarly, Figure 4C shows

feed C with the highest MUFA proportion at 36.1%, while feed D has the lowest at 23.7%. The PUFA displayed the highest variability (CV = 35%), with feed E having the highest PUFA content (5.12%) and feed B the lowest (2.27%). In Figure 4C, PUFA proportions also vary widely, with feed D standing out with the highest PUFA percentage (48.4%) and feed C showing the lowest (20.4%). This large variation in PUFA content reflects different levels of EFA, such as omega-3 and omega-6 fatty acids.

Table 4 - Fatty acids composition of commercial feeds (%, dry matter basis)

Fatty acid composition	Commercial feed						CV%
	A	B	C	D	E	F	
Saturated fatty acids (SFA)							
C14:0	0.16	0.13	0.21	0.03	0.22	0.15	46
C15:0	0.02	0.03	0.03	0.01	0.03	0.02	35
C16:0	2.19	1.75	2.49	1.21	2.24	1.98	23
C17:0	0.04	0.05	0.06	0.02	0.05	0.04	32
C18:0	1.05	0.97	1.11	0.47	1.03	0.89	25
C20:0	0.03	0.02	0.05	0.02	0.05	0.03	41
C21:0	0.02	0.01	0.03	0.01	0.03	0.02	45
C22:0	0.02	0.02	0.03	0.03	0.05	0.02	41
C23:0	0.01	0.01	0.01	0.01	0.01	0.01	0
C24:0	0.02	0.02	0.05	0.03	0.03	0.02	41
Monounsaturated fatty acids (MUFA)							
C14:1	0.01	0.01	0.02	0.00	0.01	0.01	63
C16:1n7	0.24	0.15	0.26	0.04	0.26	0.23	44
C18:1n9t (*trans*)	0.02	0.02	0.02	0.01	0.01	0.01	37
C18:1n9	2.57	1.88	3.24	1.52	2.85	2.14	27
C20:1n9	0.09	0.03	0.06	0.02	0.05	0.07	48
C22:1n9	0.01	0.01	0.01	0.00	0.02	0.01	63
C24:1n9	0.01	0.01	0.01	0.00	0.01	0.01	49
Polyunsaturated fatty acids (PUFA)							
C18:2n6 (LOA)*	2.46	1.93	1.88	2.97	4.23	2.15	34
C20:2n6	0.02	0.01	0.02	0.01	0.02	0.02	31
C18:3n6	0.03	0.01	0.01	0.00	0.01	0.02	77
C18:3n3 (ALA)*	0.21	0.16	0.13	0.27	0.41	0.19	44
C20:3n6	0.02	0.01	0.01	0.00	0.01	0.02	65
C20:3n3	0.01	0.00	0.00	0.00	0.00	0.00	245
C20:4n6	0.04	0.02	0.05	0.01	0.07	0.05	55
C20:5n3 (EPA)*	0.00	0.04	0.06	0.00	0.17	0.03	126
C22:2n6	0.00	0.00	0.00	0.00	0.00	0.00	0
C22:6n3 (DHA)*	0.01	0.09	0.15	0.00	0.20	0.05	95
∑ SFA	3.56	3.01	4.07	1.84	3.74	3.18	24
∑ MUFA	2.94	2.10	3.60	1.59	3.20	2.47	28
∑ PUFA	2.80	2.27	2.31	3.26	5.12	2.53	35
∑ EFA[1]	2.68	2.22	2.22	3.24	5.01	2.42	36
EPA + DHA	0.01	0.13	0.21	0.00	0.37	0.08	105
∑ n-3	0.23	0.29	0.34	0.27	0.78	0.27	57
∑ n-6	2.57	1.98	1.97	2.99	4.34	2.26	33
∑ n-9	2.70	1.95	3.34	1.55	2.94	2.24	27
n-6/n-3	11.2	6.8	5.8	11.1	5.6	8.4	31

CV - coefficient of variation.
[1] EFA - essential fatty acids: linoleic acid (LOA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).

3.6. Essential fatty acid profile

The EFA profile focused on linoleic acid (LOA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA; Table 5). Linoleic acid was most abundant in feed D (44.4% of total fatty acids) and least abundant in feed C (18.8% of total FA), with a CV of 30%. This high content of LOA in feed D indicates a greater inclusion of vegetable oils rich in omega-6 fatty acids, such as soybean oil. This is observed in the high PUFA content in feed D, which correlates with its elevated LOA level (Figure 4C). Alfa-linolenic acid, an omega-3 FA, exhibited high variability (CV = 38%), with feed D showing the highest proportion (4.00% of total FA) and feed C the lowest (1.30% of total fatty acids). Eicosapentaenoic acid and DHA, crucial long-chain omega-3 FA often derived from marine sources, were most abundant in feed E (3.10% EPA + DHA to total fatty acids), while some feeds had very low or non-detectable amounts. The EPA + DHA content significantly differentiated feed E from the others (Table 5 and Figure 4C). The CV for EPA alone was particularly high (107%), reflecting a considerable discrepancy in the use of marine oils across the feeds. The n-6:n-3 ratio was highest in feed A (11.2) and lowest in feed E (5.6; Table 4). This ratio is a key indicator of the balance between pro-inflammatory omega-6 and anti-inflammatory omega-3 FA. The observed variability in FA profiles aligns with the PCA results, in which PC1 highlighted the differentiation of feeds based on their EFA content. Feed E, with the highest EPA + DHA levels and the most balanced n-6:n-3 ratio, clustered distinctly in the PCA, further emphasizing the importance of these FA in the differentiation of feed nutritional profiles.

Table 5 - Essential fatty acid (EFA) profile of commercial feeds

EFA% of total fatty acids	Commercial feed						CV%
	A	B	C	D	E	F	
C18:2n6 (LOA)	26.5	26.2	18.8	44.4	35.1	26.3	30
C18:3n3 (ALA)	2.3	2.2	1.3	4.0	3.4	2.3	38
C20:5n3 (EPA)	0.0	0.5	0.6	0.0	1.4	0.4	107
C22:6n3 (DHA)	0.1	1.2	1.5	0.0	1.7	0.6	84
EPA + DHA	0.1	1.8	2.1	0.0	3.1	1.0	90
Total	28.8	30.1	22.2	48.4	41.5	29.6	29

LOA - linoleic acid; ALA - α-linolenic acid; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid.

3.7. Feed physical properties

Statistical analyses revealed significant differences ($P<0.05$) across all physical parameters, including pellet diameter, length, hardness, the number of pellets in 10 g, and pellet stability in water (Table 6). However, it is important to note that while these differences are statistically significant, their practical relevance for shrimp growth performance and pellet quality is less clear, especially given the CV and the inherent variability of these properties in real-world conditions. Pellet diameters ranged from 1.85 mm in feed C to 2.17 mm in feed A, with statistical differences observed between the feeds ($P<0.001$). Despite these differences, the CV of 3.9% suggests low variation, indicating that these statistical differences may not translate into meaningful practical outcomes. Significant differences in pellet length were observed among feeds, with feed D having the longest pellets (6.97 mm) and feed C the shortest (2.36 mm, $P<0.001$). Pellet hardness varied significantly across feeds, with feed F being the hardest (4.40 kg) and feed C the softest (1.00 kg, $P<0.001$). A high CV (36.9%) was observed, indicating considerable variability. The number of pellets per 10 g varied greatly across feeds, with feed C having significantly more pellets (1252) compared to feed A (453, $P<0.001$). The high CV (45.3%) underscores the variability in this parameter. Pellet stability in water showed significant differences among feeds ($P = 0.009$), with Feed B demonstrating the highest stability (89.7%) and feed E the lowest (81.7%).

The relatively low CV of 3.1% suggests that pellet stability was consistent across feeds. However, even though statistical differences exist, the range of variation is narrow, and the differences may not be practically relevant, as all feeds remained stable for the duration of typical shrimp feeding periods.

Table 6 - Physical properties of the commercial feeds

Feed	Pellet diameter (mm)	Pellet length (mm)	Pellet hardness (kg)	No. pellets in 10 g	Pellet stability in water
A	2.17 ± 0.09a	5.08 ± 1.40b	2.93 ± 1.20c	453 ± 6d	86.1 ± 2.78ab
B	1.99 ± 0.02c	6.00 ± 1.44ab	3.87 ± 1.28ab	584 ± 13b	89.7 ± 2.87a
C	1.85 ± 0.07e	2.36 ± 0.20c	1.00 ± 0.00d	1252 ± 20a	85.8 ± 3.13ab
D	1.91 ± 0.06d	6.97 ± 1.40a	3.70 ± 0.70ab	542 ± 25bc	84.1 ± 4.27ab
E	2.05 ± 0.10b	6.19 ± 1.91a	3.50 ± 0.90bc	513 ± 37cd	81.7 ± 2.14b
F	1.94 ± 0.06cd	6.27 ± 1.36a	4.40 ± 1.13a	587 ± 28b	86.9 ± 2.53ab
CV%	3.9	30.0	36.9	45.3	3.1
P-value	< 0.001	< 0.001	< 0.001	< 0.001	0.009

3.8. Shrimp growth performance and feed utilization

In Experiment 1 (optimal salinity) there were significant differences (P<0.05) in final body weight, weekly growth, weight gain, and SGR (Table 7). Feed E exhibited the highest performance across these parameters, with a FBW of 13.47 g, weekly growth of 1.31 g/week, weight gain of 835.1%, and an SGR of 3.33%/day. Intermediate performance was observed for feeds A and D, which were not significantly different from feed E or any other feed in terms of FBW (11.00 g and 10.22 g, respectively), weekly growth (1.04 g/week and 0.96 g/week), and SGR (3.02%/day and 2.92%/day). Feed E performed better than feeds B, C, and F, which had lower values for FBW (ranging from 9.18 g to 9.63 g), weekly growth (0.84 g/week to 0.89 g/week), and SGR (2.75%/day to 2.83%/day). Feed F showed the poorest overall growth performance. Feed utilization shows that feed F had the highest daily feed intake (34.32 g DM/kg BW/day) but the lowest feed efficiency (0.75), indicating poor nutrient utilization (Table 8). In contrast, feed E had the highest feed efficiency (1.02), while intermediate feed efficiency was observed in feeds A and D, which were not significantly different from feed E.

In Experiment 2 (hypoosmotic salinity), there were also significant differences in shrimp survival, FBW, weekly growth, weight gain, and SGR (Table 7). Feed E again stood out with the highest FBW (12.50 g), weight gain (820.0%), and SGR (2.45%/day). Again, intermediate performance was observed in feeds A, D, which were not significantly different from feed E or any other feed in terms of FBW (11.76 g and 12.29 g, respectively), weight gain, and SGR. Feeds A, B, D, and E outperformed feed F, which had the lowest FBW (9.72 g) and SGR (2.15%/day). Survival also varied significantly (P = 0.040), with feed F achieving the highest survival (97.6%) and feed E the lowest (84.6%). Feeds A, B, C, and D showed intermediate survival, with no significant differences among them. For feed utilization (Table 8), feed C had the highest daily protein intake (12.95 g). Feeds A, B, and D demonstrated intermediate feed efficiency, with no significant differences between them. Feed E exhibited the highest feed efficiency (0.64), while feed F showed the lowest (0.53).

Under hyperosmotic salinity (Experiment 3), feed B achieved the best growth performance. While most feeds showed no significant differences in growth, feed E was an exception, performing significantly worse than feeds A, B, and F (P<0.05). Survival remained high across all feeds, with no statistically significant differences (P = 0.662). Regarding feed utilization (Table 8), feed E had the highest daily protein intake (16.35 g of protein, DM/kg BW/day) and daily energy intake (760.9 KJ gross energy/kg BW/day) but demonstrated the lowest feed efficiency (0.48). Feed A showed the highest feed efficiency (0.55), followed by feeds B and F, which were not significantly different from feed A (P>0.05).

Table 7 - Survival, growth performance, and yield of shrimp fed six commercial diets across varying salinity conditions (data represents the means ± standard deviation)

Salinity condition	Parameter	A	B	C	D	E	F	P-value
Optimal (Exp. #1)	Survival (%)	83.65 ± 24.08	93.74 ± 4.48	96.48 ± 3.99	94.12 ± 4.99	72.27 ± 30.27	68.24 ± 35.91	0.207
	Initial body weight (g)	1.61 ± 0.01	1.62 ± 0.01	1.62 ± 0.01	1.62 ± 0.03	1.62 ± 0.02	1.61 ± 0.01	0.820
	Final body weight (g)	11.00 ± 2.25ab	9.63 ± 0.37b	9.18 ± 0.56b	10.22 ± 0.38ab	13.47 ± 3.22a	9.32 ± 1.16b	0.003
	Weekly growth (g/week)	1.04 ± 0.25ab	0.89 ± 0.04b	0.84 ± 0.06b	0.96 ± 0.04ab	1.31 ± 0.36a	0.86 ± 0.13b	0.004
	Weight gain (%)	682.5 ± 142.4ab	596.4 ± 23.6b	567.8 ± 37.4b	630.0 ± 27.7ab	835.1 ± 204.7a	578.4 ± 73.4b	0.004
	Specific growth rate (%/day)	3.02 ± 0.33ab	2.83 ± 0.06b	2.75 ± 0.1b	2.92 ± 0.07ab	3.33 ± 0.37a	2.78 ± 0.2b	0.002
	Yield (MT/ha)	3.58 ± 0.84	3.70 ± 0.10	3.61 ± 0.09	3.99 ± 0.21	3.65 ± 1.32	2.22 ± 1.38	0.061
Hypoosmotic (Exp. #2)	Survival (%)	90.58 ± 6.82ab	92.14 ± 7.35ab	90.2 ± 5.69ab	89.4 ± 7.02ab	84.65 ± 4.71a	97.64 ± 2.56b	0.040
	Initial body weight (g)	1.53 ± 0.03	1.53 ± 0.02	1.54 ± 0.03	1.54 ± 0.03	1.52 ± 0.03	1.53 ± 0.02	0.945
	Final body weight (g)	11.76 ± 0.26ab	11.32 ± 0.61bc	10.55 ± 0.63cd	12.29 ± 0.64ab	12.50 ± 0.78a	9.72 ± 0.62d	0.001
	Weekly growth (g/week)	0.83 ± 0.02abc	0.80 ± 0.05bc	0.74 ± 0.05cd	0.87 ± 0.05ab	0.89 ± 0.06a	0.67 ± 0.05d	0.001
	Weight gain (%)	766.6 ± 20.4ab	738.4 ± 33.7bc	686.4 ± 42.1cd	797.0 ± 34.3ab	820.0 ± 40.4a	636.8 ± 38.4d	0.001
	Specific growth rate (%/day)	2.37 ± 0.03ab	2.32 ± 0.05bc	2.24 ± 0.07cd	2.41 ± 0.05ab	2.45 ± 0.06a	2.15 ± 0.07d	0.001
	Yield (MT/ha)	4.56 ± 0.45ab	4.44 ± 0.24ab	3.98 ± 0.16b	4.72 ± 0.45a	4.52 ± 0.27ab	3.98 ± 0.21b	0.003
Hyperosmotic (Exp. #3)	Survival (%)	88.88 ± 5.07	97.07 ± 3.44	90.98 ± 2.25	82.63 ± 17.15	85.28 ± 8.03	97.26 ± 2.63	0.662
	Initial body weight (g)	0.84 ± 0.01	0.85 ± 0.01	0.85 ± 0.03	0.85 ± 0.01	0.85 ± 0.01	0.86 ± 0.01	0.660
	Final body weight (g)	8.58 ± 0.39a	8.61 ± 0.81a	8.11 ± 0.69ab	8.00 ± 0.81ab	7.16 ± 0.39b	8.45 ± 0.83a	0.009
	Weekly growth (g/week)	0.64 ± 0.03a	0.65 ± 0.07a	0.6 ± 0.06ab	0.59 ± 0.07ab	0.53 ± 0.04b	0.63 ± 0.07a	0.010
	Weight gain (%)	919.0 ± 51.6a	912.4 ± 91.89a	851.8 ± 83ab	839.8 ± 92.8ab	745.3 ± 45.3b	888.0 ± 96.8ab	0.008
	Specific growth rate (%/day)	2.77 ± 0.05a	2.77 ± 0.10a	2.70± 0.10ab	2.66 ± 0.11ab	2.53 ± 0.05b	2.72 ± 0.11a	0.001
	Yield (MT/ha)	3.82 ± 0.21a	3.87 ± 0.40a	3.59 ± 0.35ab	3.50 ± 0.41ab	3.09 ± 0.22b	3.79 ± 0.42a	0.005

Lowercase letters indicate the differences between feeds at the same salinity condition (One-way ANOVA, P<0.05).

Table 8 - Feed utilization, daily protein, and energy intake of shrimp fed six commercial diets across varying salinity conditions (data represents the means ± standard deviation)

Salinity condition	Parameter	A	B	C	D	E	F	P-value
Optimal (Exp. #1)	Daily feed intake[1]	28.74 ± 1.21b	30.57 ± 0.43b	30.69 ± 0.69b	28.46 ± 0.43b	28.11 ± 1.4b	34.32 ± 3.7a	0.000
	Daily protein intake[2]	11.03 ± 0.41c	12.17 ± 0.39bc	12.49 ± 0.46ab	11.08 ± 0.17c	11.16 ± 0.55c	13.65 ± 1.47a	0.000
	Daily energy intake[3]	518.1 ± 19.3ab	557.9 ± 11.3ab	534.4 ± 13.4ab	511.0 ± 9.1ab	501.1 ± 39.0b	578.9 ± 84.6a	0.021
	Feed conversion ratio	1.68 ± 0.58	1.53 ± 0.05	1.53 ± 0.03	1.42 ± 0.04	2.00 ± 1.09	4.07 ± 3.67	0.080
	Feed efficiency	0.88 ± 0.15ab	0.75 ± 0.02b	0.73 ± 0.03b	0.83 ± 0.03ab	1.02 ± 0.21a	0.75 ± 0.08b	0.003
Hypoosmotic (Exp. #2)	Daily feed intake[1]	29.92 ± 1.64	31.39 ± 1.53	31.44 ± 0.60	31.35 ± 2.89	30.2 ± 1.06	32.3 ± 1.13	0.209
	Daily protein intake[2]	11.43 ± 0.63b	12.38 ± 0.6ab	12.95 ± 0.25a	12.14 ± 1.12ab	11.99 ± 0.42ab	12.84 ± 0.45a	0.008
	Daily energy intake[3]	536.7 ± 29.4	580.4 ± 28.2	544.3 ± 10.4	558.97 ± 51.6	557.78 ± 19.5	524.29 ± 18.3	0.073
	Feed conversion ratio	1.97 ± 0.22	2.03 ± 0.12	2.11 ± 0.09	2.06 ± 0.26	2.03 ± 0.09	2.11 ± 0.06	0.670
	Feed efficiency	0.62 ± 0.02ab	0.58 ± 0.04abc	0.57 ± 0.02bc	0.60 ± 0.05ab	0.64 ± 0.03a	0.53 ± 0.02c	0.000
Hyperosmotic (Exp. #3)	Daily feed intake[1]	37.2 ± 2.27ab	35.58 ± 1.28a	38.28 ± 1.8ab	39.21 ± 3.84ab	41.18 ± 2.29b	35.64 ± 3.05a	0.007
	Daily protein intake[2]	14.18 ± 0.88a	14.03 ± 0.54a	15.76 ± 0.75ab	15.17 ± 1.52ab	16.35 ± 0.88b	14.18 ± 1.2a	0.002
	Daily energy intake[3]	667.3 ± 41.1b	658.2 ± 24.2bc	662.4 ± 31.5bc	699.1 ± 68.7ab	760.9 ± 42.0a	578.7 ± 49.0c	0.000
	Feed conversion ratio	1.82 ± 0.13a	1.80 ± 0.10a	1.92 ± 0.11ab	1.90 ± 0.18ab	2.07 ± 0.07b	1.82 ± 0.18ab	0.019
	Feed efficiency	0.55 ± 0.04a	0.56 ± 0.03a	0.52 ± 0.03ab	0.53 ± 0.05ab	0.48 ± 0.02b	0.55 ± 0.05ab	0.025

[1] Expressed as g of feed in dry matter basis per kg of shrimp body weight per day.
[2] Expressed as g of protein in dry matter basis per kg of shrimp body weight per day.
[3] Expressed as KJ of dietary gross energy per kg of shrimp body weight per day.
Lowercase letters indicate the differences between feeds at the same salinity condition (One-way ANOVA, $P<0.05$).

4. Discussion

This study investigated the nutrient profiles of major grower shrimp feeds available in Brazil for semi-intensive shrimp farming and assessed their impact on the growth, feed utilization, and survival of *P. vannamei* under water salinity concentrations typical of the Northeastern Brazil, a region central to the shrimp production of the country (IBGE, 2024). By conducting trials over multiple seasons and salinity levels, this research captures the complexity of commercial farming environments, where diverse environmental and nutritional factors intersect. Additionally, to better contextualize these findings, we consider how the nutrient composition of the evaluated feeds compares with benchmark knowledge in shrimp nutrition, both domestically and internationally.

This study did not aim to directly assess the extent to which these commercial feed formulations align with the optimal dietary requirements of *P. vannamei* as reported in scientific literature. However, existing nutritional benchmarks, established through controlled research, emphasize specific dietary requirements for EAA, lipid composition, and digestible energy levels. In contrast, commercial feed compositions are often shaped by external factors, including raw material availability, regulatory policies, and cost considerations, leading to variations that may not always reflect strict nutritional optimization. Consequently, our focus was on characterizing compositional differences across feeds and evaluating their effects under different salinity conditions, our findings also highlight the balance that Brazilian commercial feeds must strike between adhering to nutritional standards and addressing economic and logistical constraints.

It is worth noting that while the scope of this study did not extend to evaluating specific feed characteristics such as feed digestibility, and the use of functional feed additives and nutrients, these are important aspects that could further influence shrimp performance. Future research could consider these elements to provide a more comprehensive understanding of feed efficiency and shrimp health. For example, understanding the digestibility of the diets, the use of feed additives, and bioactive compounds could offer further insights into how these factors impact shrimp growth under varying environmental stressors.

The CP content, a common measure of dietary protein levels, differed from true protein (the sum of EEA and NEAA). Our findings indicated that CP alone in the commercial feeds did not fully reflect protein quality and content, as variations in AA composition and the presence of non-protein nitrogen influenced true protein levels, which are more relevant for shrimp nutrition. Similarly, Carvalho et al. (2016) found discrepancies between protein and true protein apparent digestibility coefficients (ADC) in a study on commercial feedstuffs for juvenile *P. vannamei*. They suggested that higher endogenous losses of non-AA nitrogen from the tested ingredients contributed to lower protein ADC compared with true protein ADC.

In the present study, PCA and hierarchical clustering were applied to differentiate nutrient profiles across feeds effectively. The PCA distilled the dataset into essential components, emphasizing the nutrients driving variability, while hierarchical clustering organized feeds by nutrient similarity. This approach enabled a holistic examination of full nutrient profiles, aligning with commercial nutritional strategies.

The analyses identified EFA, minerals, and specific AA as primary contributors to variability. Proximate composition revealed differences in CP, carbohydrates, crude fat, and ash, reflecting varied formulation strategies. Crude protein levels, ranging from 38.2% (feed A) to 41.2% (feed C) with a CV of 3%, suggest a consistent industry standard. Additionally, AA:CP ratios were relatively uniform, indicating a common understanding.

The hierarchical clustering analysis categorized the six grower feeds into three distinct clusters, reflecting diverse formulation strategies. Feed E formed a unique cluster characterized by high EFA levels, particularly EPA and DHA, along with the highest crude fat (12.1%, DM basis) and gross energy (18.5 MJ/kg) contents. The high marine oil content of feed E suggests a formulation strategy aimed

at meeting higher energy and EFA demands, likely to support resilience under challenging farming conditions.

Feeds A, F, B, and C formed a broader cluster with moderate crude fat levels (9.5%–10.8%) and balanced nutrient profiles. Feeds C and B, with moderate EPA and DHA levels, suggest partial inclusion of marine-derived ingredients. Feed C, with its high CP (41.2%) and ash (20.2%), likely contains animal byproducts, such as fishmeal or others, enhancing mineral content, particularly P (2.4% DM basis). Feed D formed its own cluster, distinguished by its lack of EPA and DHA, and reliance on carbohydrates (43.8%, DM basis) for energy. The lower CP (38.4%) and ash (10.7%) contents of feed D suggest an economical, plant-based formulation with minimal animal-derived ingredients.

Under both hypoosmotic and hyperosmotic conditions, shrimp survival remained high (approximately 90%), although growth rates declined relative to optimal salinity, suggesting a trade-off in which energy was redirected from growth to meet osmoregulatory demands. Weekly growth rates dropped by 19% in hypoosmotic and 38% in hyperosmotic conditions compared with optimal salinity, with production yields reflecting these trends. These findings are consistent with previous studies, such as Bray et al. (1994), which reported optimal growth at lower salinities (5–15 ppt) and significantly reduced growth at higher salinities.

Reduced growth under hypoosmotic conditions, although evident, was less severe than under hyperosmotic stress. This aligns with Wang et al. (2013), who found that while osmoregulation at low salinities demands energy, it is less growth-limiting than the demands imposed by hyperosmotic environments. This moderate reduction in growth at low salinities indicates that, although osmoregulation requires energy, its impact on growth is comparatively mild relative to high-salinity stress.

The more pronounced growth reduction observed under hyperosmotic conditions may also stem from decreased digestive enzyme activity. Wang et al. (2013) reported reductions in enzymes such as amylase, pepsin, and trypsin in crustaceans at higher salinities, which likely affected digestive efficiency and contributed to lower growth and feed utilization in our study. Feed and nutrient intake rose in both hypoosmotic (3%) and hyperosmotic (26%) conditions compared with optimal salinity, likely driven by a feeding protocol based on maximum daily intake (Nunes and Parsons, 2000). However, this intake increase did not translate into growth improvements; feed efficiency dropped by 29% under hypoosmotic and by 36% under hyperosmotic conditions, indicating the challenges shrimp face in converting feed to growth when under osmotic stress.

Performance differences across feeds were most pronounced under optimal and hypoosmotic conditions (experiments 1 and 2). Feed E, enriched with marine oils high in EPA and DHA, demonstrated superior growth and feed efficiency, underscoring the established benefits of these long-chain n-3 highly unsaturated fatty acids (HUFA) for shrimp. The presence of EPA and DHA in feed E likely enhanced membrane stability, immune function, and metabolic resilience, supporting findings that such HUFA improve shrimp growth by promoting lipid digestibility and efficient energy utilization (Glencross et al., 2002). Evidence suggests that dietary HUFA enrichment can mitigate the negative effects of hypersalinity on juvenile *P. vannamei* growth performance.

Hurtado et al. (2006) examined the effects of HUFA supplementation (4.0% of an emulsion with 50% HUFA) on *P. vannamei* juveniles reared at low (5 ppt), medium (30 ppt), and high (50 ppt) salinities over 21 days. Authors reported that shrimp growth performance was negatively impacted at high salinity, but this was mitigated by the HUFA-enriched diet. In addition, shrimp fed HUFA-enriched diets had significantly higher EPA and DHA levels in the hepatopancreas and gills. Similar findings were reported by Castro et al. (2018), who raised *P. vannamei* of 2.79 ± 0.60 g for 64 days under isosmotic (ISO, 23 ± 1.2 ppt) and hyperosmotic (HOS, 44 ± 2.0 ppt) conditions. Diets included: control (3.5% soybean oil), fish (2.7% fish oil + 1.0% soybean oil), krill (4.8% krill oil + 4% soybean oil), krill- (1.5% krill oil + 2.1% soybean oil), and krill+ (5.5% krill oil + 0.4% soybean oil). At harvest, shrimp fed the krill diet exhibited the fastest growth (1.01 ± 0.01 g/week) and highest body weight (11.97 ± 2.01 g), regardless of water salinity.

In the present study, feeds A and D achieved growth outcomes comparable to feed E despite their minimal EPA and DHA levels. This performance highlights the effectiveness of alternative nutrient profiles in supporting shrimp growth. Feeds A and D contained the highest levels of LOA after feed E, at 2.46 and 2.97% DM basis, respectively, likely sourced from plant-based oils such as soybean oil. This LOA-rich composition provided a cost-effective solution, fulfilling energy requirements while maintaining adequate growth performance. Research by González-Félix et al. (2010) supports this view, demonstrating that while LOA cannot fully replicate the physiological benefits of EPA and DHA, it can sufficiently meet shrimp's EFA needs when balanced with appropriate n-3 sources. These findings indicate that under optimal to moderately stressful salinity conditions, diverse nutrient profiles can achieve growth rates comparable to those of feeds containing higher marine oil content.

Based on the total EFA content observed in each diet, the overall growth performance in experiments 1 and 2 can be largely attributed to these EFA levels. Feed E, with the highest EFA content, supported superior growth, followed by feeds D and A, which also performed well, underscoring the critical role of EFA in growth and feed utilization under optimal and hypoosmotic conditions. This ranking of EFA content, feed E > feed D > feed A > feed F, feed B, and feed C, closely aligns with the observed growth outcomes, emphasizing that while EPA and DHA provide unique physiological benefits, the total EFA concentration across diets may have been the main dietary factor influencing shrimp growth.

It is important to note that these results likely apply to the specific context and nutrient density of the diets analyzed here. Physiological responses such as growth are dynamic and can vary depending on how closely the dietary nutrient levels approach the species' optimal requirements. When nutrient levels are near optimal, growth responses may be less sensitive to small dietary differences. In contrast, when nutrient levels deviate significantly from optimal, growth and other physiological responses become more sensitive, with deficiencies in key nutrients exerting stronger limitations on performance. This highlights the importance of not only EFA content but also ensuring that the broader nutrient profile in shrimp diets is balanced and adequately meets species-specific requirements.

In experiment 3, conducted under hyperosmotic conditions, growth performance was generally similar across feeds, suggesting that high salinity imposed osmotic stress that constrained growth irrespective of diet. However, feed E, with its high-fat formulation, exhibited significantly lower FBW, weekly growth, and SGR compared with the other feeds. This poorer performance may be directly linked to the elevated fat levels in feed E, which likely imposed additional metabolic demands under high-salinity stress.

Shrimp under hyperosmotic conditions allocate a substantial portion of their metabolic energy to osmoregulation, leaving less capacity for processing and metabolizing excess dietary fat. Studies by Glencross et al. (2002) and González-Félix et al. (2002) indicated that shrimp digestibility of dietary fat diminishes as fat levels increase, particularly when exceeding the optimal range of around 8-10%. This reduction in lipid digestibility is likely exacerbated by the energy-intensive nature of hyperosmotic environments, where the combined burden of osmoregulation and lipid metabolism can compromise growth.

Additionally, excessive dietary fat has been shown to cause increased lipid deposition in the hepatopancreas, as observed in other studies like Chen et al. (2024). This lipid overload can lead to hepatopancreatic cell abnormalities, reducing the efficiency of the organ in supporting digestion and metabolism, further impairing growth under stress. These effects were likely more pronounced in feed E, where the high lipid content would have increased metabolic strain on shrimp in hyperosmotic conditions, explaining the reduced growth and feed efficiency observed in this treatment. These findings are consistent with Xu et al. (2018), who reported that shrimp fed a diet containing 12.0% lipid exhibited reduced growth performance accompanied by signs of oxidative stress.

Overall, these findings suggest that while high-fat feeds may support growth in lower salinities, they become counterproductive under hyperosmotic conditions. The poor performance of feed E under hyperosmotic stress is likely attributable to the added metabolic load of processing excess fat, which diverts energy away from growth and results in diminished performance outcomes.

Future studies should explore optimal EFA ratios and fat thresholds in high-salinity contexts and further validate LOA-rich oils as an alternative to EPA and DHA sources. This should help farmers and formulators in optimizing feed and raw materials selection for resilient, cost-effective shrimp production under diverse farming conditions.

5. Conclusions

This study provides insight into the impact of nutrient profiles on the growth and feed efficiency of *P. vannamei* under varying salinity conditions. Principal component analysis and hierarchical clustering identified EFA, minerals, and amino acids as key variables influencing feed variability and shrimp performance. Feed E, high in EPA and DHA, demonstrated superior growth under optimal and hypoosmotic conditions, highlighting the benefits of n-3 HUFA. In contrast, feeds A and D, though lower in EPA and DHA, achieved similar growth outcomes, suggesting the viability of LOA-rich plant oils as a cost-effective alternative under moderate salinity conditions.

Total EFA content was closely linked to growth, with feeds ranked in order of E > D > A > F, B, and C, indicating that while EPA and DHA offer unique benefits, total EFA levels are crucial for growth. However, under hyperosmotic conditions, the high-fat composition of feed E led to poorer growth, likely due to the additional metabolic demands imposed by excessive dietary fat, which hindered energy allocation to growth. These findings emphasize the need for targeted feed formulations that balance EFA and fat content based on environmental stressors.

Data availability

The entire dataset supporting the results of this study is available upon reasonable request to the corresponding author.

Author contributions

Conceptualization: Nunes, A. J. P. **Data curation**: Façanha, F. N. and Nunes, A. J. P. **Formal analysis:** Façanha, F. N. and Nunes, A. J. P. **Funding acquisition:** Nunes, A. J. P. **Investigation:** Nunes, A. J. P. **Methodology**: Nunes, A. J. P. **Project administration:** Nunes, A. J. P. **Resources:** Nunes, A. J. P. **Supervision:** Nunes, A. J. P. **Writing – original draft:** Façanha, F. N. and Nunes, A. J. P. **Writing – review & editing:** Nunes, A. J. P.

Conflict of interest

The authors declare no conflict of interest.

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