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Original Research

Nutritional Optimization for Zero Water Exchange Aquaculture: A Study of Dietary Protein Levels for *Pangasionodon Hypopthalmus*

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Abstract

The protein utilization in feed significantly contributes to the preservation and bolstering of the sustainability of aquaculture endeavors. This study uses a zero-water exchange system to evaluate the effects of three commercial feeds with varying protein content on the nursery of *Pangasionodon hypopthalmus*. The research employed fish with an initial size of 11.54 ± 0.04 g, which were reared for 84 days using a closed system pond. The experimental treatments in this study encompass feeds containing protein contents of 20% (F20), 25% (F25), and 30% (F30). Findings revealed that the F30 treatment yielded the best production performance, with a specific growth rate and survival rate measuring 2.81 ± 0.08% day^{-1} and 99.83 ± 0.29%, respectively. Additionally, utilizing F30 resulted in the lowest Feed Conversion Ratio (FCR) at 1.75 ± 0.11. Employing lower protein content in the feed led to improved water quality. Nevertheless, employing F30 feed still maintained water quality within the acceptable standards for the *P. hypophthalmus* juvenile cultivation.

Keywords: *Pangasionodon hypopthalmus*, protein, feed, production performance, water quality

Introduction

By 2030, freshwater carp species and Pangas catfish are projected to constitute 62% of global aquaculture production [1]. *Pangasianodon hypophthalmus*, commonly known as striped catfish, is a precious species in freshwater aquaculture. The production of *P. hypophthalmus* has demonstrated substantial and consistent growth throughout the years. In 2020, the production figure witnessed a remarkable escalation, attaining a significant milestone of 2.520,4 thousand metric tons. This upward trajectory in fish production indicates a rising demand and highlights the promising economic prospects inherent in aquaculture endeavors [2].

Feed plays a pivotal role in catfish growth, health, and productivity. Feed constitutes the foremost expenditure in aquaculture production, accounting for approximately 40–60% of production costs and the principal waste generator within the system [3, 4]. Proteins represent the most expensive component of feed [5, 6],

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and this factor significantly impacts the sustainability of aquaculture operations due to its influence on profit margins. Given that feed constitutes the most significant expenditure in fish farming, inefficient utilization of feed proteins results from poor feeding management, low feed digestibility, and inefficient metabolism, thereby not only escalating feed costs but also contributing to deteriorating water quality [7–9].

Residual feed protein, feces rich in high-protein content, and metabolically excreted ammonia constitute waste derived from feed protein [10, 11]. Efforts to improve feed protein efficiency can focus on selecting protein source materials with high digestibility and a balanced composition of essential amino acids [12, 13]. Moreover, equally crucial is ensuring that the protein content in the feed is optimized to meet growth requirements [14, 15]. Low protein levels can lead to detrimental growth, while excessively high levels would be futile as they do not provide additional positive impacts on growth [16, 17] and could also exacerbate environmental pollution. Research on optimizing the protein content in the feed for *P. hypopthalmus* and several other catfish species has been conducted, with the optimal protein content ranging from 30 to 37% for *P. hypopthalmus* [18–21] and between 29 and 55% for other catfish species [7, 22–24].

Improving profit margins can be accomplished by optimizing feed protein levels and implementing sustainable environmental technologies, such as zero water exchange (ZWE) systems. ZWE holds the potential to minimize water resource usage, maintain optimal water quality, and reduce the risk of disease transmission [25–29]. Nevertheless, aquaculture employing ZWE methods may result in the build-up of feed waste, feces, and metabolic byproducts within the system [27, 30].

In general, waste management in ZWE systems typically involves the utilization of Recirculating Aquaculture Systems (RAS) or the application of biofloc methods [26, 31]. However, applications of RAS and biofloc methods are sometimes considered expensive and complex. Fortunately, catfish species such as the striped catfish exhibit a high tolerance to ammonia concentrations compared to other fish species [32, 33]. Additionally, striped catfish can adapt to low oxygen conditions by extracting oxygen directly from the air [34, 35]. Cultivation of ZWE systems without RAS installations or biofloc methods has been successfully implemented at striped catfish breeding centers in Sumatra Island, Indonesia.

Applying ZWE without RAS or biofloc methods requires intensive monitoring to ensure that the water quality in the cultivation system remains optimal and that the generated waste does not pollute the environment. One method can be employed by controlling the protein levels in the feed formulation [27]. To date, no research has examined this aspect. Considering the importance of reducing nitrogen waste in ZWE systems, this study tests protein levels in feeds within a low range or below the optimal levels investigated in previous studies. This research aims to test several protein levels in feeds, hoping to provide the best growth for catfish while minimizing the nitrogen waste produced, thus maintaining environmental sustainability in ZWE-based striped catfish cultivation.

Material and Methods

Fish Preparation

The study was conducted at the Jambi Freshwater Aquaculture Center in Indonesia, with the juvenile *P. hypophthalmus* procured from the establishment above. Prior to the initiation of the research, the juvenile underwent a pretreatment phase encompassing acclimatization within concrete ponds for two weeks. Throughout this acclimatization period, the juvenile was fed a commercial diet boasting 31% crude protein and 6% crude lipid. Following the acclimatization phase, the juvenile underwent a 24-hour fasting period and was ready to use in the experiment.

Experimental Design

The study employed a completely randomized design (CRD) featuring three treatments and three replicates, encompassing varying feed protein levels: 20% (F20), 25% (F25), and 30% (F30) protein content. Subsequently, juvenile fish with an average initial weight of 11.54 ± 0.04 g were stocked uniformly in nine gray concrete ponds measuring 6 m × 2.5 m × 1.0 m, with a stocking density of 400 juveniles per pond and reared for 84 days.

Throughout the research duration, the fish were cultivated within a closed system characterized by zero water exchange, devoid of any water renewal. Supplementary water was added to the system to balance out the water loss due to evaporation. The juvenile fish were nourished twice daily at 8 am and 4 pm, with a feeding rate of 5% of the total daily biomass. This feeding was administered manually, ensuring the thorough digestion of feed by the fish. The proximate composition analysis of the employed commercial feeds is presented in Table 1.

Water Quality and Fish Sampling

The water quality parameters including, pH, dissolved oxygen, and temperature, were recorded daily using a multi-parameter analyzer (Hanna HI9829). At intervals of 7 days, measurements of ammonia, nitrite, nitrate, and phosphorus levels in the water were conducted, followed by subsequent analyses employing the APHA method [36].

Proximate fish samples were collected on both day zero (comprising 6 fish) and on the 84th day of the study (encompassing 6 fish from each pond). The fish's weight was logged at 15-day intervals. The survival rates of the juvenile were recorded at the end of the experimental period.

Laboratory Analysis

Commencing the study, anesthetized lethal doses with MS 222 [37] were administered to six fish from the initial tank and pooled for proximate analysis. Concluding

Table 1. Fish feed proximate composition, juvenile fish proximate composition, and juvenile fish hepatosomatic index result from all experiments.

Parameters	Experimental Feeds		
	F20	F25	F30
Fish Feed:			
Crude protein P (%)	20.92	27.80	30.80
Crude fat F (%)	4.97	6.06	6.06
Moisture content M (%)	8.15	8.75	9.25
Ash A (%)	9.04	9.45	12.33
Crude fiber CF (%)	3.3	1.51	1.22
Nitrogen-free extract NFE*	53.62	46.43	40.34
Gross energy (kcal/100 g of feed)**	379.64	400.06	392.65
Energy/protein ratio (kcal/g)	18.15	14.39	12.75
Juvenile Fish:			
Moisture (%)	79.15 ± 0.33^{ab}	78.39 ± 0.14^{b}	79.32 ± 0.40^{a}
Crude protein (%)	16.97 ± 0.32^{b}	18.30 ± 0.40^{a}	17.58 ± 0.38^{ab}
Crude lipid (%)	9.26 ± 1.02^{a}	7.65 ± 1.44^{a}	7.09 ± 1.15^{a}
Ash (%)	1.01 ± 0.04^{a}	1.07 ± 0.02^{a}	1.07 ± 0.07^{a}
Hepatosomatic Index (%)	1.94 ± 0.65^{a}	1.81 ± 0.24^{a}	1.91 ± 0.17^{a}

Means in the same line with different letters are significantly different ($p < 0.05$).

Note: * was calculated by difference: 100 - (P + F + M + A + CF)

** was calculated according to NRC (2011)

the research, six fish were sampled from each pond for proximate analysis, subsequently pooled, and stored at −20°C for further analysis.

Chemical analyses of both feed and fish adhered to the AOAC method [38]. Water content was determined through sample drying in a 105°C oven until constant weight was achieved. Incinerating samples in a muffle furnace measured ash content at 450°C for 24 hours. The Kjeldahl method was employed for crude protein analysis, involving digestion and distillation units, while lipid content was ascertained through petroleum ether extraction using a soxhlet apparatus.

Production Performance and Nutrient Utilization Observation

The observed parameters for assessing fish production performance and nutrient utilization in this study encompassed the specific growth rate (SGR), weight gain (WG), total feed consumption (TFC), feed conversion ratio (FCR), survival rate (SR), protein retention (PR), and lipid retention (LR). The formulas employed to calculate these parameters were performed as follows:

$$WG = (FW - IW) \quad (1)$$

$$SGR = 100 \times (\ln FW - \ln IW) / T \quad (2)$$

The specific growth rate (SGR) is expressed in % day-1.

The weight gain (WG) is expressed in g $fish^{-1}$. IW is the average initial weight of the fish, recorded on day 1, FW represents the average weight recorded at the conclusion of the experimental trial, and T is the study period (days).

$$FCR = \text{Total Feed Consumption (g)} / \text{Weight Gain in Wet Weight (g)} \quad (3)$$

The total feed consumption value is based on the daily feeding records accumulated during the study.

$$SR\ (\%) = 100 \times (\text{Final number of fish} / \text{initial number of fish}) \quad (4)$$

$$PR\ (\%) = 100 \times (FP - IP) / P\ Intake \quad (5)$$

$$PER\ (\%) = WG / PI \quad (6)$$

$$LR\ (\%) = 100 \times (FL - IL)/LI \quad (7)$$

FP stands for the final protein content of the fish's whole body, IP denotes the initial protein content of the entire

Table 2. Production performance and Nutrient utilization of *P. hypophthalmus* on Varied Levels of Protein Diets.

Parameters	Experimental Feeds		
	F20	F25	F30
Initial weight (g)	11.53 ± 0.02[a]	11.54 ± 0.10[a]	11.52 ± 0.02[a]
Final weight (g)	88.34 ± 7.16[b]	107.64 ± 0.44[a]	122.52 ± 7.85[a]
Weight gain (g)	76.81 ± 7.15[b]	96.10 ± 0.55 [a]	111.01 ± 7.87[a]
Specific growth rate (SGR) (% day^{-1})	2.42 ± 0.10[b]	2.66 ± 0.02[a]	2.81 ± 0.08 [a]
Feed conversion ratio (FCR)	2.10 ± 0.10[a]	1.88 ± 0.07[ab]	1.75 ± 0.11[b]
Total feed consumption (TFC) (g)	63.98 ± 2.97[b]	71.68 ± 2.63[a]	77.31 ± 1.57[a]
Protein efficiency ratio (PER)	2.27 ± 0.11[a]	1.92 ± 0.07 [ab]	1.86 ± 0.11 [b]
Protein retention (PR) (%)	38.65 ± 1.62[a]	35.42 ± 1.64[ab]	32.79 ± **2**.02[b]
Lipid retention (LR) (%)	88.81 ± **14**.25[a]	65.56 ± **14**.80[a]	64.44 ± **9**.13[a]
Survival rate (SR) (%)	99.67 ± **0**.58[a]	99.70 ± **0**.36[a]	99.83 ± **0**.29[a]

Means in the same line with different letters are significantly different ($p < 0.05$).

fish's body, and P intake represents the quantity of protein within the total feed ingested by the fish. In the provided equation, FL represents the final lipid content of the fish's entire body, IL signifies the initial lipid content of the fish's whole body, and L intake quantifies the lipid content within the total feed ingested by the fish.

Data Analysis

The data were analyzed using one-way ANOVA through the JMP® 8 software package (SAS Institute Inc., 2009). A significance level of 0.05 was adopted for null hypothesis rejection. When significant disparities were identified, Tukey's multiple range test was subsequently employed to ascertain the distinctions between means. The production performance, nutrient utilization, and water quality data were subjected to statistical analysis.

Results and Discussion

Fish Production Performance and Nutrient Utilization

The results of juvenile production performance and nutrient utilization after the study period are detailed in Table 2. Scientific literature extensively confirms that dietary protein levels substantially influence growth parameters, including final weight, weight gain, and SGR in numerous fish species [39]. Elevating dietary protein levels is frequently associated with enhanced growth performance [21, 22, 40]. An optimal dietary protein level is imperative to furnish the requisite amino acids for maximal growth. Nevertheless, continued growth becomes untenable beyond a certain protein threshold and may even decline. Notably, it is extensively documented that growth parameters rise in parallel with escalating dietary protein levels until an optimum point, beyond which they decline [21, 41, 44].

The growth depression observed at elevated protein levels could be attributed to the insufficiency of non-protein energy needed for the deamination and excretion of excess ingested amino acids, possibly resulting in a diminished energy allocation for growth [21, 41]. An alternate hypothesis suggests that the body's accumulation of free amino acids might become detrimental at excessive dietary protein levels, hampering growth and normal metabolic processes [21, 40, 44–46].

In this study, the final weight, weight gain, and SGR of *P. hypophthalmus* exhibited a significant increment with the augmentation of dietary protein levels from F20 to F30 treatment. The result indicates that the highest protein level treatment provided in this study (F30) showed the best production performance for striped catfish in a zero water exchange (ZWE) culture system. This observation underscores that the growth of P. *hypophthalmus* was influenced by the dietary protein level. This pattern aligns with investigations conducted on European grayling *Thymallus thymallus* [47] and striped catfish *Pangasianodon hypophthalmus* [21]. Where growth parameters witnessed a substantial increase up to the optimal point as dietary protein levels were elevated, followed by a plateauing effect at higher protein levels.

Likewise, a corresponding absence of enhancement in the growth performance of *P. hypophthalmus* was noted when isolipid diets (5%) were administered, and the dietary protein content was escalated from 34%, 38%, and 42% to 46% [19]. Conversely, a notable growth increase was observed in *P. hypophthalmus*-fed isolipid diets (6%) with 30% to 40% dietary protein content [18]. Notably, in the present study, no marked decline in growth parameters was evident

at higher dietary protein levels, likely since the evaluated dietary protein levels remained within a range below excessive impact.

The feed conversion ratio (FCR) enhancement was demonstrated alongside the feed protein content elevation in this study, with the F30 group displaying the lowest FCR. The lowest FCR value reflects the highest feed conversion efficiency in achieving optimal fish weight gain. Numerous studies have reported an elevation in FCR as dietary protein content rises, followed by an eventual increase or deterioration beyond a certain protein threshold [41]. However, in this study, no discernible trend of FCR elevation was observed; this could be attributed to the low level of protein content within the feed, which cannot express the attainment of maximum response. This observation also implies that subsequent experiments encompassing protein content beyond the 30% threshold are necessary.

In this study, the protein efficiency ratio (PER) and protein retention (PR) values declined with increasing dietary protein levels. However, the PER and PR of the fish fed F20 were notably higher than those of F30, though not significantly different from those of F25. These findings align with research conducted on many fish species, including European grayling [47], yellow catfish [48], and red-spotted grouper *Epinephelus akaara* [49], which also result in declining PER and PR value as the protein content of the feed increases.

Dietary formulations with lower protein content commonly enhance utilization when contrasted with higher protein content feed [47], leading to PER and PR values on less protein content being relatively augmented. It is well established that fish cannot synthesize surplus protein from excessive dietary protein but utilize it as an energy source, which may lead to a decline in protein efficiency ratio (PER) at elevated dietary protein levels [21, 47].

This study observed an elevation in whole-body protein content as the dietary protein levels increased; the corresponding data are delineated in Table 1. These findings concur with outcomes documented for various species, including bullseye snakehead *Channa marulius* [50], *Sebastes schlegeli* [51], *Thymallus thymallus* [47], and *Pangasionodon hypophthalmus* [18, 21].

Table 1 presented fish's whole-body crude protein content, which exhibited a trend of incremental growth in proportion to the dietary protein levels, progressing from F20 to F25 treatment. However, it reached a plateau after that and displayed a declining tendency during the F30 treatment. Furthermore, the F20 treatment showcased the lowest whole-body protein content. This study's observed plateauing pattern at higher dietary protein levels suggests that the protein requirement for *P. hypophthalmus* was sufficient and that any surplus dietary protein is not utilized for body protein synthesis. A comparable pattern of responses has been evidenced in other species, such as Ide *Leuciscus idus* [39], Atlantic salmon *Salmo salar* [52], Black Sea Bream *Acanthopagrus schlegelii* [53], abalone *Haliotis discus hannai* [54], and pufferfish *Takifugu* sp. [55]. In these cases, the whole-body protein content plateauing phenomenon is observed upon surpassing the optimal dietary protein range. Likewise, augmenting dietary protein beyond the optimal requirement does not further enhance growth and overall bodily performance.

The dietary protein levels had no significant effect on the whole-body lipid composition of fish in the experimental diets, as indicated by the data presented in Table 1. This finding aligns with similar observations in Grouper [56] and *Silago sihama* [57]. Conversely, contrasting findings have been found in *Spinibarbus hollandi*, grouper *Epinephelus coioides*, Nile tilapia *Oreochromis niloticus*, Obscure puffer *Takifugu obscurus*, Ide *Leuciscus idus*, and bullseye snakehead *Channa marulius*. In these cases, an increase in dietary protein levels corresponds to a reduction in whole-body lipid content [39, 50].

In this study, no significant differences in Hepatosomatic Index (HSI) values were observed among treatments. Our study diverges from findings in Ide *Leuciscus idus* and red swamp crayfish *Procambarus clarkii*, where a correlation between decreasing HSI values and increasing dietary protein levels was established [39, 44].

Another study found that the decline in whole-body lipid content and HSI could be attributed to lower dietary carbohydrates (starch or dextrin) as dietary protein levels increased, aiming to create isoenergetic diets [39]. Carbohydrates can be readily converted into lipids and glycogen, subsequently stored in the liver [42, 43]. The augmentation of water content linked to glycogen accumulation within the liver contributes to an elevated HSI value [39]**.** Moreover, carbohydrates can undergo conversion to lipids and be stored in adipose tissues [58, 59], potentially leading to an elevation in whole-body lipid content in fish. In the case of *S. hollandi*, lipids stemming from dietary carbohydrates predominantly accumulate in the liver, perivisceral adipose tissue, and carcass [60, 61]. Furthermore, higher HSI, elevated whole-body lipid content, or a combination of both have been noted in response to low protein or high carbohydrate diets [44, 62, 63].

Moreover, a similar pattern has been observed in *Nibea miichthioides*, where elevating dietary lipid levels have been found to correspond with heightened body lipid content, consistent with the studies conducted on *P. hypophthalmus* [19], tilapia [64] and *Cyclopterus lumpus* [65].

Furthermore, increased dietary lipid and carbohydrate content has been linked to lipid deposition in the body of *P. hypophthalmus* [19]. No significant differences were found in HSI and body lipid content in this study; this could be attributed to the variation in dietary lipid (4.97 – 6.06%) and carbohydrate (presented as NFE 40.43 – 53.62%) content across the tested diets, which likely remained below the threshold needed to impact HSI and whole-body lipid content of *P. hypophthalmus* juveniles.

Based on the growth data and FCR, among the three dietary protein treatments (which were accompanied by lipid levels ranging from 4.97% to 6.06%), it becomes apparent that F30 provided the best and most efficient performance. Notably, the protein content value in this study is comparatively lower than that observed in prior research concerning the same fish species. Earlier investigations have established that a 12% lipid content

in the feed corresponds to an optimal protein requirement of 30% for fish growth [18]. Furthermore, analogous findings emerge from other *P. hypophthalmus* studies, reaffirming that an optimal protein requirement of 45% is essential when the feed's lipid content is 9% [19]. The latest studies reported the optimal protein requirements for *P. hypophthalmus* were 30%, 35%, and 37,1% [21, 66]**.** The divergence in feed lipid content between this study and the two preceding investigations likely contributes to the variability in the necessary optimal protein levels. Furthermore, the spectrum of applied protein content in this study remains beneath the optimal protein requirement for striped catfish reported in previous studies. Nevertheless, the F30 treatment in our study demonstrated the best performance. This notion is supported by optimal growth performance data (final weight, weight gain, and SGR) and feed performance (FCR).

Compared to other catfish species, the dietary protein requirement for juvenile fish established in this study is generally lower than the requirements observed for *Rhamdia quelen* (34%) [23], African catfish *Clarias gariepinus* (35–40%) [7], *Clarias magur* (55%) [67], and stinging catfish *Heteropneustes fossilis* (40–41%) [24]. Variations in the optimal dietary protein requirement observed within the catfish across these studies might be attributed to differences in feed formulation, environmental conditions, applied methodologies, stocking sizes, age classes, and species. [22, 64].

Previous research findings show that the optimal protein requirement range for striped catfish and other catfish species falls within the range of 30–40%. However, the results of this study indicate that a protein content of 30% is already capable of delivering the best performance in terms of specific growth rate (SGR), survival rate (SR), and the lowest feed conversion ratio (FCR). It is important to note that previous studies did not use the ZWE culture method. Therefore, using dietary protein levels above 30% would not significantly impact water quality, as water exchange is routinely performed in those previous studies (e.g., flow-through systems).

Water quality substantially impacts the growth, development, survival, physiology, and reproduction of fish. Deterioration in water quality parameters and escalating contamination present formidable obstacles to the efficiency and sustainability of aquaculture systems [28, 68, 69].

Water Quality Parameters

The water quality examination outcomes throughout the study are exhibited in Fig. 1. This study's ammonia, nitrite, and nitrate concentrations exhibited variations between 0.01–1.00, 0.04–0.37, and 0.01–0.30 mgL^{-1}. The cultivation of *P. hypophthalmus* exhibits a sufficiently high tolerance to ammonia levels, reaching up to 1.07 mgL^{-1} [70]. The established guidelines advise nitrite and nitrate levels below 50.00 and 100.00 mgL^{-1} [71].

The dissolved oxygen (DO) concentrations and temperature were noted throughout the study to be 2.67–4.58 mg L^{-1} and 22.8–26.7°C, respectively. Notably, *P. hypophthalmus* exhibited remarkable resilience to reduced dissolved oxygen levels, enduring concentrations as low as 0.05 mgL^{-1}. The observed pH and phosphorus concentration values spanned from 6.95–7.23 and 0.03–0.24 mgL^{-1}. The recommended pH range lies within 6.50–8.50 [72], while the suggested phosphorus concentration for catfish is less than 1.5 mgL^{-1} [73]. Overall, the water quality conditions within the cultivation pond align with the requisites for sustaining aquaculture.

The nitrogen content in fish feed can originate from various sources, primarily protein. Protein is a pivotal constituent within fish feed, embodying nitrogen in amino acids [74]. Amino acids serve as the fundamental building blocks of proteins and encompass nitrogen atoms. Conventional commercial fish feeds commonly encompass protein sources such as fishmeal, soybean meal [75, 76], rapeseed meal [77, 78], meat and bone meals [5, 79], and other plant-based materials. Each component bears distinct nitrogen content contingent upon its nutritional composition and protein concentration.

During fish digestion, the protein in feed undergoes enzymatic breakdown into amino acids. Subsequently, fish's intestines absorb these amino acids and utilize them to synthesize body proteins, growth, and other vital functions. Furthermore, amino acid metabolism liberates nitrogen in the form of ammonia as a byproduct. Over the cultivation period, poorly managed organic waste, such as unconsumed feed and feces, contributes to elevated ammonia levels in the aquatic environment.

The decomposition of organic matter by bacteria and microorganisms into ammonia compounds is recognized as the process of ammonification [80]. Within this process, microorganisms affect the hydrolysis of large feed and fecal molecules into more minor constituents. Consequently, the organic nitrogen within these molecules is transformed into ammonia through deamination by microorganisms. The resulting ammonia is subsequently released into the water as dissolved free ammonia. The higher protein content in the F30 treatment is the underlying factor contributing to the elevated concentration of produced ammonia compared to the other treatments.

An elevated ammonia concentration within aquatic environments can enhance augmented nitrite and nitrate levels. The F30 treatment also tends to yield higher nitrite and nitrate concentrations throughout the research. This phenomenon is attributed to the biological processes inherent in the nitrogen cycle. Ammonia, characterized as a nitrogenous compound, undergoes nitrification [81, 82], a transformative sequence facilitated by nitrifying microorganisms such as Nitrosomonas [71]. During this process, ammonia is initially converted into nitrite. Subsequently, nitrite is subjected to further oxidation, forming nitrate, under the influence of alternative nitrifying bacterial agents like Nitrobacter [71].

Nitrite and nitrate are nitrogen compounds integral to the nitrogen cycle's dynamics. Consequently, a surplus accumulation of ammonia within aquatic realms potentially precipitates heightened nitrite and nitrate production via these microbial transformations, thereby exerting ramifications

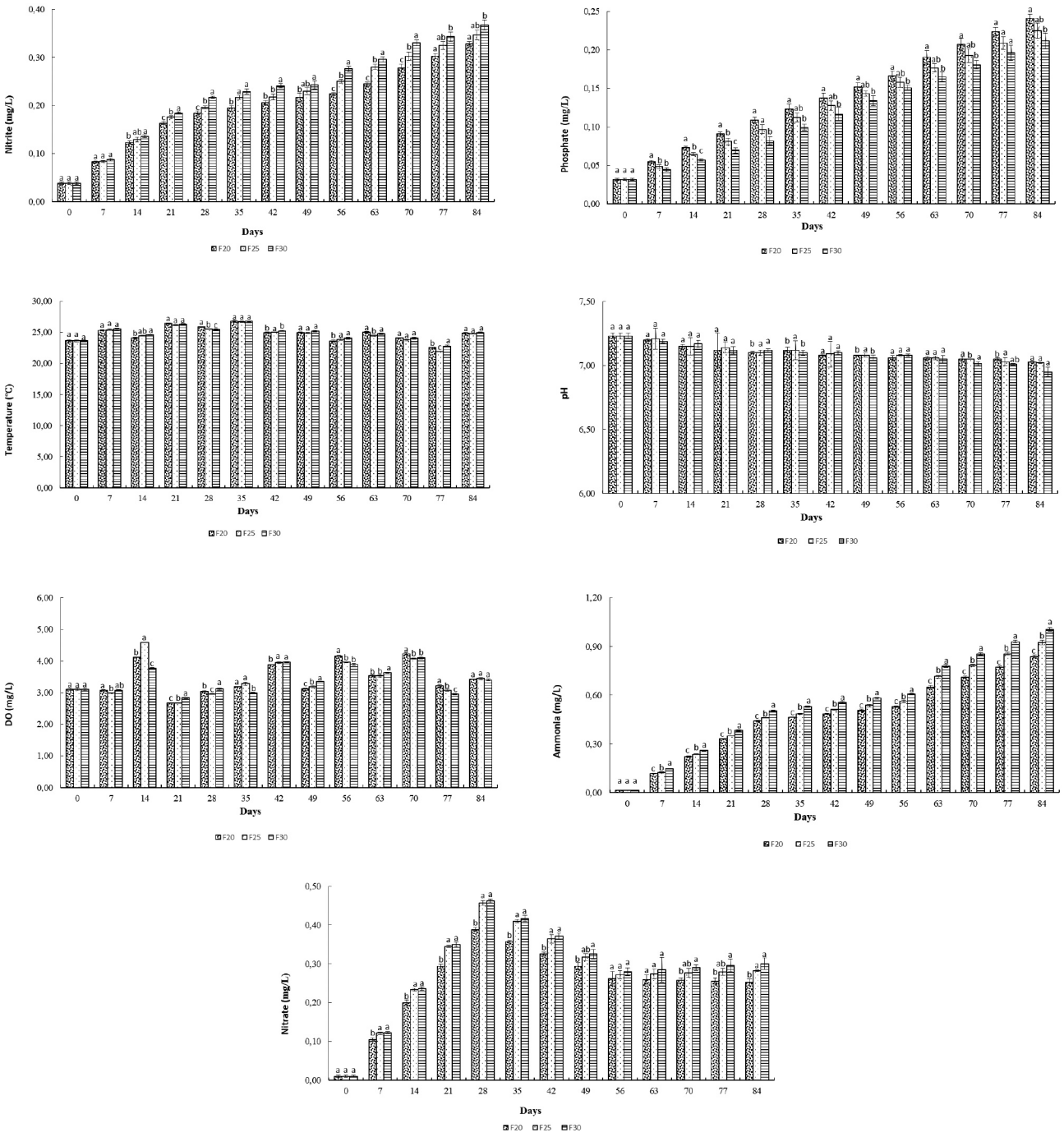

Fig. 1. Water quality conditions during the study: feed with a protein content of 20% (F20), feed with a protein content of 25% (F25), and feed with a protein content of 30% (F30). The use of distinct lowercase letters in the graph indicates a significant difference (p <0.05).

on the comprehensive quality of water and the equilibrium of the aquatic ecosystem.

Based on Fig. 1, an increase in the protein content of the feed utilized during the study results in a lower phosphorus concentration. This phenomenon can be attributed to various factors, including the efficiency of phosphorus digestion and absorption by the fish. Higher protein content in fish feed is typically associated with better amino acid profiles and enhanced digestibility for fish. During digestion, the phosphorus within the protein can be more effectively absorbed and utilized by the fish's body.

Furthermore, fish feed containing elevated protein content tends to exhibit a more balanced phosphorus-to-protein ratio; this implies that the phosphorus content within such feed is more concentrated and less excessive than its protein content. With this balanced ratio, the available phosphorus within the fish feed can be utilized more efficiently by the fish's body, resulting in decreased phosphorus excretion into the water. This assertion is

bolstered by further studies indicating that the utilization of feed with a protein content of 40% in the cultivation of *L. vannamei* shrimp yields a diminished phosphorus concentration compared to the use of feeds with protein contents of 30% and 35% [83].

The decline in pH levels observed during the study period is attributed to several contributing factors. One of the primary contributing factors is carbon dioxide (CO_2) production through various metabolic activities of fish, encompassing respiration and excretion. During respiration, fish release CO_2 into the water, which dissolves, forming carbonic acid (H_2CO_3) [84]. Furthermore, the breakdown of organic substances, such as unconsumed food remnants and fish waste, by alternative bacteria and microorganisms releases CO_2 and organic acids into the aquatic environment. Furthermore, nitrification, encompassing the conversion of ammonia to nitrite and subsequently to nitrate by nitrifying bacteria, can also reduce pH levels. This phenomenon arises from the nitrification process yielding protons (H^+) as byproducts, contributing to an overall increase in water acidity [84].

Conclusions

The study's results indicate that the F30 treatment outperformed others in production performance, with a specific growth rate of 2.81 ± 0.08% day^{-1} and a survival rate of 99.83 ± 0.29%. Moreover, F30 yielded the highest outcomes for the Feed Conversion Ratio (FCR), achieving values of 1.75 ± 0.11. Lower protein content in feed led to improved water quality. Even when using F30 feed, the water quality consistently met acceptable standards for cultivating *P. hypophthalmus* juveniles.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. DAS R., DAS B.K., HASSAN M.A., KRISHNA G., CHADHA N.K., RAWAT K.D., JENA K. Valorization of the insect waste as a source of dietary protein in replacing the fishmeal protein for the cage reared *Pangasianodon hypophthalmus*: An approach to search the alternate non-conventional feed resource of animal origin. Animal Feed Science and Technology, **303**, 115691, **2023**.
2. FAO. The State of World Fisheries and Aquaculture (SOFIA)-towards blue transformation. Food and Agriculture Organization of the United Nations; 2022. Available online: https://www.fao.org/3/cc0874en/cc0874en.pdf. (accessed on 5th January 2024)
3. PAILAN G.H., BISWAS G. Feed and Feeding Strategies in Freshwater Aquaculture. In Transforming Coastal Zone for Sustainable Food and Income Security, 1st ed.; Lama T., Burman D., Mandal U.K., Sarangi S.K., Sen H., Eds., Springer Cham: Cham, Switzerland, pp. 455, **2022**.
4. NAYLOR R.L., HARDY R.W., BUSCHMAN A.H., BUSH S.R., CAO L., KLINGER D.H., LITTLE D.C., LUBCHENCO J., SHUMWAY S.E., TROELL M. Review a 20-year retrospective review of global aquaculture. Nature, **591**, 551, **2021**.
5. HUA K., COBCROFT J.M., COLE A., CONDON K., JERRY D.R., MANGOTT A., PRAEGER C., VUCKO M.J., ZENG C., ZENGER K., STRUGNELL J.M. The Future of Aquatic Protein: Implications for Protein Sources in Aquaculture Diets. One Earth, **1** (3), 316, **2019**.
6. JIA S., LI X., HE W., WU G. Protein-sourced feedstuffs for aquatic animals in nutrition research and aquaculture., Recent Advances in Animal Nutrition and Metabolism, Advances in Experimental Medicine and Biology, **1354**, 237, **2022**.
7. LANGI S., MAULU S., HASIMUNA O.J., KAPULA V.K., TJIPUTE M. Nutritional requirements and effect of culture conditions on the performance of the African catfish (*Clarias gariepinus*): a review. Cogent Food & Agriculture, **10** (1), 2302642, **2024**.
8. KONG W., HUANG S., YANG Z., SHI F., FENG Y., KHATOON Z. Fish feed quality is a key factor in impacting aquaculture water environment: evidence from incubator experiments. Nature Scientific Reports, **10** (187), 1, **2020**.
9. CHADLI H., BAHIDA A., NHHALA H., ABDELLAOUI B., ERRAIOUI H. Uneaten Feed Quantification in Fish Farming Growing Phase of *Dicentrarchus labrax*, Feeding Flow and Distribution Modes Effects. Egyptian Journal of Aquatic Biology & Fisheries, **27** (4), 905, **2023**.
10. MADIREDDY I., BRAYBOY L. Effects of Protein Concentration in Fish Feed on Physical and Chemical Water Pollution. Journal of Student Research, **11** (1), **2022**.
11. NGUYEN T.T.T., FOYSAL M.D.J., FOTEDAR R., GUPTA S.K., SIDDIK M.A.B., TAY C.Y. The Effect of Two Dietary Protein Sources on Water Quality and the Aquatic Microbial Communities in Marron (*Cherax cainii*) Culture. Microbial Ecology, **82** (2), 299, **2021**.
12. RADHAKRISHNAN G., SILVA M.S., LOCK E.J., BELGHIT I., PHILIP A.J.P. Assessing amino acid solubility of black soldier fly larvae meal in Atlantic salmon (*Salmo salar*) in vivo and in vitro. Frontier in Physiology, **13**, 1028992, **2022**.
13. NHU B.M., TRUONG D.V., THI T.T.N., DUY T.Q.N., THAT C.T., SERRANO A.E.J.R. Lysine requirement of the spotted scat *Scatophagus argus* (Linaeus, 1766). The Israeli Journal of Aquaculture – Bamidgeh, **73**, 1, **2021**.
14. HASSAN A.L.I., MOCK T.S., SEARLE K., ROCKER M.M., TURCHINI G.M., FRANCIS D.S. Optimal Dietary Protein Requirement of Subadult Australian Hybrid Abalone (*Haliotis rubra* × *Haliotis laevigata*) at Different Rearing Temperatures. Aquaculture Research, **2023**, 13, **2023**.
15. BAEK S.I., CHO S.H. Dietary protein requirements of abalone (*Haliotis discus*, Reeve 1846) depending on abalone size. Fisheries and Aquatic Sciences, **24** (3), 129, **2021**.
16. BIN MA Y., ZOU W.G., YOU W.W., AI C.X., CHEN Y.X., SU Y., LUO X., KE C.H. Evaluation of optimal dietary protein levels and meat quality of adult hybrid

abalone (*Haliotis discus hannai*♀ × *H. fulgens*♂) under two representative water temperatures. Aquaculture, **577**, 739907, **2023**.
17. WANG L., YAO Y., ZHAO Z., LI L., GAN H., DESOUKY H., WANG X., ZHANG Z. Effect of dietary protein, vitamin E, and vitamin C levels on the growth and gonad development of hybrid male abalone (Haliotis fulgens× H. discus hannai) by the orthogonal array design. Aquaculture International, **31**, 2581, **2023**.
18. PHUMEE P., HASHIM R., PAIKO M.A., CHIEN A.C.S. Effects of dietary protein and lipid content on growth performance and biological indices of iridescent Shark (*Pangasius hypophthalmus*, Sauvage 1878) fry. Aquaculture Research, **40**, 456, **2009**.
19. LIU X.Y., WANG Y., JI W.X. Growth, feed utilization and body composition of Asian catfish (*Pangasius hypopthalmus*) fed at different dietary protein and lipid levels. Aquaculture Nutrition, **17**, 578, **2011**.
20. KABIR M.A., GHAEDI A., TALPUR A.D., HASHIM R. Effect of dietary protein levels on reproductive development and distribution of amino acids in the body tissues of female *Pangasianodon hypophthalmus* (Sauvage, 1878) broodstock in captivity. Aquaculture Research, **46**, 1736, **2015**.
21. JAYANT M., MURALIDHAR A.P., SAHU N.P., JAIN K.K., PAL A.K., SRIVASTAVA P.P. Protein requirement of juvenile striped catfish, *Pangasianodon hypophthalmus*. Aquaculture International, 1, **2017**.
22. RADHAKRISHNAN G., SHIVKUMAR MANNUR V.S., YASHWANTH B.S., PINTO N., PRADEEP A., PRATHIK M.R. Dietary protein requirement for maintenance, growth, and reproduction in fish: A review. Journal of Entomology and Zoology Studies, **8** (4), 208, **2020**.
23. FREITAS L.E.L.DE, SILVA T.S., DE C., FRACALOSSI D.M. Protein to energy ratios and cost, performance, and ammonia excretion in juvenile jundiá (*Rhamdia quelen*), South American catfish. Scientia Agricola, **78** (Suppl.), e20200288, **2021**.
24. FATMA S., AHMED I. Effect of water temperature on protein requirement of *Heteropneustes fossilis* (Bloch) fry as determined by nutrient deposition, hemato-biochemical parameters and stress resistance response. Fisheries and Aquatic Sciences, **23** (1), **2020**.
25. AHMAD A., ABDULLAH S.R.S., HASAN H.A., OTHMAN A.R., ISMAIL N.I. Aquaculture industry: Supply and demand, best practices, effluent and its current issues and treatment technology. Journal of Environmental Management, **287**, 112271, **2021**.
26. NIU S., ZHANG K., LI Z., WANG G., LI H., XIA Y., TIAN J., YU E., GONG W., XIE J. Nitrification and denitrification processes in a zero-water exchange aquaculture system: characteristics of the microbial community and potential rates. Frontiers in Marine Science, **10**, 1072911, **2023**.
27. ALI M.A.M., KHURAIBA H.M., ELSAYED N.E.G., SHARAWY Z.Z. The Effect Of Different Stocking Densities Of Marine Shrimp Larvae *Litopeneaus vannamei* On Water Quality Using Biofloc Technology. Egyptian Journal of Nutrition and Feeds, **23** (1), 183, **2020**.
28. SETIJANINGSIH L., ADIYANA K., THESIANA L., ARDI I., PUSPANINGSIH D., SETIADI E., TAUFIK I., YAMIN M., KURNIASIH T. Study of Bottom Substrate Variation in Zero Water Discharge Aquaculture for Mahseer Fish (*Tor soro*) Nursery. Polish Journal of Environmental Studies, **33** (1), 341, **2024**.
29. SUPRIYONO E., RASUL, BUDIARDI T., HASTUTI Y.P., ADIYANA K., THESIANA L. A study on the effect of different colours of culture tanks in nursery, on the production performance, biochemical composition of flesh and physiological responses of whiteleg shrimp (*Litopenaeus vannamei*). Aquaculture Research, **52**, 4086, **2021**.
30. VASAVA R.J., HODAR A.R., ELCHELWAR V.R., DEEPAK A.P., PATEL K.M., SHRIVASTAVA V., YUSUFZAI S.I., ACHARYA P., PRABHAKAR P. Biofloc technology: An innovative approach to zero-water exchange and tentative zero-feed system: A review Journal of Entomology and Zoology Studies, **8** (2), 1036, **2020**.
31. KASAN N.A., DAGANG A.N., ABDULLAH M.I. Application of biofloc technology (BFT) in shrimp aquaculture industry. IOP Conference Series: Earth and Environmental Science, **196**, 012043, **2018**.
32. FRANKLIN D.A., EDWARD L.L. Ammonia toxicity and adaptive response in marine fishes-a review. Indian Journal of Geo Marine Sciences, **48** (03), 273, **2019**.
33. CHEW S.F., TAN S.Z.L., IP S.C.Y., PANG C.Z., HIONG K.C., IP Y.K. The Non-ureogenic Stinging Catfish, *Heteropneustes fossilis*, Actively Excretes Ammonia with the Help of Na+/K+-ATPase When Exposed to Environmental Ammonia. Frontiers in Physiology, **10**, 1615, **2020**.
34. SULARTO, HADIE L.E., MARNIS H., DARMAWAN J. Adaptation strategy for Jambal Catfish (*Pangasius djambal*) to stress the aquatic environment. IOP Conference Series: Earth and Environmental Science, **789**, 012025, **2021**.
35. SEGARAN T.C., AZRA M.N., PIAH R.M., LANANAN F., TÉLLEZ-ISAÍAS G., GAO H., TORSABO D., KARI Z.A., NOORDIN N.M. Catfishes: A global review of the literature. Heliyon, **9** (9), e20081, **2023**.
36. APHA. Standard methods for the examination of water and wastewater, 22nd ed.; American Public Health Association, Water Environment Federation; Washington DC, USA, **2012**.
37. SCHROEDER P., LLOYD R., MCKIMM R., METSELAAR M., NAVARRO J., O'FARRELL M., READMAN G.D., SPEILBERG L., MOCHO J.P. Anaesthesia of laboratory, aquaculture and ornamental fish, Proceedings of the first LASA-FVS Symposium. Laboratory Animals, **55** (4), 317, **2021**.
38. LATIMER G.W. Official Methods of Analysis,22nd ed.; George W. Latimer, Jr., Eds., AOAC Publications: New York, USA, **2023**.
39. REN M., JI K., LIANG H., GE X., MI H. Dietary Protein Requirement of Juvenile Ide, *Leuciscus idus* in Relation to Growth Performance, Whole-body Composition and Plasma Parameters. The Israeli Journal of Aquaculture – Bamidgeh, **69** (1424), 1, **2017**.
40. MANAM V.K. Fish feed nutrition and its management in aquaculture. International Journal of Fisheries and Aquatic Studies, **11** (2), 58, **2023**.
41. HUSSAIN M., UL-HASSAN H., SIDDIQUE M.A.M., MAHMOOD K., ABDEL-AZIZ M.F.A., LAGHARI M.Y., ABRO N.A., GABOL K., NISAR RIZWAN S., HALIMA Effect of varying dietary protein levels on growth performance and survival of milkfish *Chanos chanos* fingerlings reared in brackish water pond ecosystem. Egyptian Journal of Aquatic Research, **47**, 329, **2021**.
42. TALAL S., CEASE A., FARINGTON R., MEDINA H.E., ROJAS J., HARRISON J. High carbohydrate diet ingestion increases post-meal lipid synthesis and drives respiratory

exchange ratios above 1. Journal of Experimental Biology, **224**, jeb240010, **2021**.
43. TAPPY L. Metabolism of sugars: A window to the regulation of glucose and lipid homeostasis by splanchnic organs. Clinical Nutrition, **40**, 1691, **2021**.
44. LU X., PENG D., CHEN X., WU F., JIANG M., TIAN J., LIU W., YU L., WEN H., WEI K. Effects of dietary protein levels on growth, muscle composition, digestive enzymes activities, hemolymph biochemical indices and ovary development of pre-adult red swamp crayfish (*Procambarus clarkii*). Aquaculture Reports, **18**, 100542, **2020.**
45. FURUYA W.M., CRUZ T.P.D., GATLIN D.M.III. Amino Acid Requirements for Nile Tilapia: An Update. Animals, **13**, 900, **2023**.
46. XING S., LIANG X., ZHANG X., OLIVA-TELES A., PERES H., LI M., WANG H., MAI K., KAUSHIK S.J., XUE M. Essential amino acid requirements of fish and crustaceans, a meta-analysis. Reviews in Aquaculture, **16** (3), **2024**.
47. RAHIMNEJAD S., DABROWSKI K., IZQUIERDO M., MALINOVSKYI O., KOLÁROVÁ J., POLICAR T. Effects of dietary protein and lipid levels on growth, body composition, blood biochemistry, antioxidant capacity and ammonia excretion of European Grayling (*Thymallus thymallus*). Frontiers in Marine Science, **8**, 715636, **2021**.
48. CHEN Z., FEI S., DUAN Y., LIU C., LIU H., HAN D., JIN J., YANG Y., ZHU X., XIE S. Effects of dietary protein level on the growth, reproductive performance, and larval quality of female yellow catfish (*Pelteobagrus fulvidraco*) broodstock. Aquaculture Reports, **24**, 101102, **2022**.
49. WANG J.T., HAN T., LI X.Y., YANG Y.X., YANG M., HU S.X. Effects of dietary protein and lipid levels with different protein-to-energy ratios on growth performance, feed utilization and body composition of juvenile red-spotted grouper, *Epinephelus akaara*. Aquaculture Nutrition, **23**, 994, **2017**.
50. NAZIR S., KHAN N., FATIMA M., AZMAT H., NAVEED S., RAMZAN M.M., ASGAR M., BANO S., KHIZER A., WAN A.H.L. The influence of dietary protein concentration on digestive enzyme activities, growth, and body composition in juvenile bullseye snakehead (*Channa marulius*). PLoS ONE, **18** (2), e0281274, **2023**.
51. LI P., SONG Z., HUANG L., SUN Y., SUN Y., WANG X., LI L. Effects of dietary protein and lipid levels in practical formulation on growth, feed utilization, body composition, and serum biochemical parameters of growing rockfish *Sebastes schlegeli*. Aquaculture Nutrition, 9970252, **2023**.
52. MOCK T.S., FRANCIS D.S., JAGO M.K., GLENCROSS B.D., SMULLEN R.P., KEAST R.S.J., TURCHINI G.M. The impact of dietary protein: lipid ratio on growth performance, fatty acid metabolism, product quality and waste output in Atlantic salmon (*Salmo salar*). Aquaculture, **501**, 191, **2019**.
53. WANG L., ZHANG W., GLADSTONE S., NG W.K., ZHANG J., SHAO Q. Effects of isoenergetic diets with varying protein and lipid levels on the growth, feed utilization, metabolic enzymes activities, antioxidative status and serum biochemical parameters of black sea bream (*Acanthopagrus schlegelii*). Aquaculture, **513**, 734397, **2019**.
54. MA S., GUO Y., SUN L., FAN W., LIU Y., LIU D., HUANG D., LI X., ZHANG W., MAI K. Over high or low dietary protein levels depressed the growth, TOR signaling, apoptosis, immune and anti-stress of abalone *Haliotis discus hannai*. Fish and Shellfish Immunology, **106**, 241, **2020**.
55. PARK I.S., LEE S., YOO G.Y. Impact of dietary protein levels on growth, feed utilization, body composition, and hematological characteristics of juvenile hybrid pufferfish (*Takifugu obscurus* × *T. rubripes*). Aquaculture Reports, **22**, 100994, **2022.**
56. YE H., XU M., CHEN L., TAN X., CHEN S., ZOU C., SUN Z., LIU Q., YE C., WANG A. Effects of dietary plant protein sources influencing hepatic lipid metabolism and hepatocyte apoptosis in hybrid grouper (*Epinephelus lanceolatus* ♂ × *Epinephelus fuscoguttatus* ♀). Aquaculture, **506**, 437, **2019**.
57. LIU H., DONG X., TAN B., DU T., ZHANG S., YANG Y., CHI S., YANG Q., LIU H. Effects of dietary protein and lipid levels on growth, body composition, enzymes activity, expression of IGF-1 and TOR of juvenile northern whiting, Sillago sihama. Aquaculture, **533**, 736166, **2021**.
58. FELIX J.B., COX A.R., HARTIG S.M. Acetyl-CoA and metabolite fluxes regulate white adipose tissue expansion. Trends in Endocrinology & Metabolism, **32** (5), 320, **2021**.
59. MORIGNY P., BOUCHER J., ARNER P., LANGIN D. Lipid and glucose metabolism in white adipocytes: Pathways, dysfunction and therapeutics. Nature Reviews Endocrinology, **17**, 276, **2021.**
60. ROUMANS K.H.M., BASSET S.J., PETERS H.P.F., SCHRAUWEN P., SCHRAUWEN H.V.B. Liver fat storage pathways: Methodologies and dietary effects. Current Opinion in Lipidology, **32** (1), 9, **2021**.
61. XU D., GONG Y., XIANG X. Discovery, characterization, and adipocyte differentiation regulation in perirenal adipose tissue of large yellow croaker (*Larimichthys crocea*). Fish Physiology and Biochemistry, **49**, 627, **2023**.
62. ZHAO L., LIAO L., TANG X., LIANG J., LIU Q., LUO W., ADAM A.A., LUO J., LI Z., YANG S., RAHIMNEJAD S. High-carbohydrate diet altered conversion of metabolites, and deteriorated health in juvenile largemouth bass. Aquaculture, **549**, 737816, **2022**.
63. FAN Z., WU D., LI C., LI J., WANG L., ZHENG X., ZHANG H., ZHOU M. Alternate feeding between high- and low-carbohydrate based diets improves the growth digestive capacity, liver glycometabolism for common carp (*Cyprinus carpio*). Aquaculture Reports, **34**, 101920, **2024**.
64. PAUL M., SARDAR P., SAHU N.P., JANA P., DEO A.D., HARIKRISHNA V., VARGHESE T., SHAMNA N., KUMAR P., KRISHNA G. Effect of dietary lipid level on growth performance, body composition, and physiometabolic responses of genetically improved farmed tilapia (GIFT) juveniles reared in inland ground saline water. Aquaculture Nutrition, 5345479, **2022**.
65. BERGE G.M., ZHOU W., JOHANSEN L.H., CHIKWATI E., KORTNER T.M., LEIN I., KROGDAHL Å. Effects of dietary lipid level on growth, digestive physiology and disease resistance in lumpfish (*Cyclopterus lumpus*). Aquaculture, **566**, 739209, **2023**.
66. KHATTABY A.E.R.A. Effect of varying levels of dietary protein and stocking density on performance and feed utilization of Pangasius Catfish (*Pangasius hypophthalmus*) fingerlings. Egyptian Journal for Aquaculture, **11** (1), **2021.**
67. MIR I.N., SRIVASTAVA P.P., BHAT I.A., JAFFAR Y.D., SUSHILA N., SARDAR P. KUMAR S., MURALIDHAR A.P., JAIN K.K. Optimal dietary lipid and protein level for growth and survival of catfish *Clarias magur* larvae. Aquaculture, **520**, 734678, **2020**.
68. SUPRIYONO E., ADIYANA K., THESIANA L. A Study of Environmentally Friendly Recirculating Aquaculture System on Lobster Panulirus homarus Nursery. Polish Journal of Environmental Studies, **32** (5), **2023**.

69. MRAMBA R.P., KAHINDI E.J. Pond water quality and its relation to fish yield and disease occurrence in small-scale aquaculture in arid areas. Heliyon, **9** (6), e16753, **2023**.
70. ANDRIANI S., DEWI N.N., RAHARDJA B.S. Effectiveness of striped catfish (*Pangasianodon hypophthalmus*) cultivation in an aquaponic system with three different plants against ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3). IOP Conference Series: Earth and Environmental Science, **1036**, 012049, **2022**.
71. SUPRIYONO E., SOELISTYOWATI D.T., ADIYANA K., THESIANA L. The effects of alkalinity on production performance and biochemical responses of spiny lobster *Panulirus homarus* reared in a recirculating aquaculture system. Israeli Journal of Aquaculture, **74**, 1, **2022**.
72. PRATAMA A.R., SUPRIYONO E., NIRMALA K., WIDIYATI A. Effect of Filter Media Recirculation Differences on Water Quality, Growth and Survival Rate of Juvenile Soro Fish (*Tor soro*). Jurnal Salamata, **4** (1), **2022** [In Bahasa].
73. WANG M., FENG W., WANG Y., LI B., WANG J., ZHU X., ZHANG L. Water quality, plankton composition, and growth performance of juvenile yellow catfish (*Pelteobagrus fulvidraco*) in mono- and polyculture systems. Aquaculture, **552**, 738017, **2022**.
74. ZHENG J., ZHANG W., DAN Z., CAO K., CUI K., ZHU S., ZHUANG Y., MAI K., AI Q. Effects of fish meal replaced by methanotroph bacteria meal (Methylococcus capsulatus) on growth, body composition, antioxidant capacity, amino acids transporters and protein metabolism of turbot juveniles (*Scophthalmus maximus* L.). Aquaculture, **562**, 738782, **2023**.
75. LIU T., HAN T., WANG J., LIU T., BIAN P., WANG Y., CAI X. Effects of replacing fish meal with soybean meal on growth performance, feed utilization and physiological status of juvenile redlip mullet *Liza haematocheila*. Aquaculture Report, **20**, 100756, **2021**.
76. WU Y., MA H., WANG X., REN X. Taurine supplementation increases the potential of fishmeal replacement by soybean meal in diets for largemouth bass Micropterus salmoides. Aquaculture Nutrition, **27**, 691, **2021**.
77. BU X.Y., WANG Y.Y., CHEN F.Y., TANG B.B., LUO C.Z., WANG Y., YANG Y.H. An evaluation of replacing fishmeal with rapeseed meal in the diet of Pseudobagrus ussuriensis: growth, feed utilization, nonspecific immunity, and growth-related gene expression. Journal of World Aquaculture Society, **49**, 1068, **2018**.
78. DOSSOU S., KOSHIO S., ISHIKAWA M., YOKOYAMA S., DAWOOD M.A., EL BASUINI M.F., OLIVIER A. Effect of partial replacement of fish meal by fermented rapeseed meal on growth, immune response and oxidative condition of red sea bream juvenile, *Pagrus major*. Aquaculture, **490**, 228, **2018**.
79. GASCO L., GAI F., MARICCHIOLO G., GENOVESE L., RAGONESE S., BOTTARI T., CARUSO G. Fishmeal alternative protein sources for aquaculture feeds. In Feeds For the Aquaculture Sector: Current Situation and Alternative Sources, 1st ed.; Gasco L., Gai F., Maricchiolo G., Genoves L., Ragonese S., Bottari T., Eds., Springer Cham: Cham, Switzerland, pp. 1–28, **2018**.
80. HLORDZI V., KUEBUTORNYE F.K.A., AFRIYIE G., ABARIKE E.D., LU Y., CHI S., ANOKYEWAA M.A. The use of Bacillus species in maintenance of water quality in aquaculture: A review. Aquaculture Reports, **18**, 100503, **2020**.
81. ROETS-DLAMINI Y., LALLOO R., MOONSAMY G., KUMARI S., NASR M., RAMCHURAN S., BUX F. Development of Bacillus spp. consortium for one-step "Aerobic Nitrification-Denitrification" in a fluidized-bed reactor. Bioresource Technology Reports, **17**, 100922, **2022**.
82. HUANG F., PAN L., LV N., TANG X. Characterization of novel Bacillus strain N31 from mariculture water capable of halophilic heterotrophic nitrification–aerobic denitrification. Journal of Bioscience and Bioengineering, **124**, 564, **2017**.
83. STREBEL L.M., NGUYEN K., ARAUJO A., CORBY T., RHODES M., BECK B.H., ROY L.A., DAVIS D.A. On demand feeding and the response of Pacific white shrimp (Litopenaeus vannamei) to varying dietary protein levels in semi-intensive pond production. Aquaculture, **574**, 739698, **2023**.
84. SANTOS A.M., BERNARDINO L.F., ATTRAMADAL K.J.K., SKOGESTAD S. Steady-state and dynamic model for recirculating aquaculture systems with pH included. Aquacultural Engineering, **102**, 102346, **2023**.