BIODIVERSITAS
Volume 26, Number 9, September 2025
Pages: 4333-4345

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260908

Fermentation of duck bone meal using *Bacillus* sp., *Saccharomyces* sp., and *Rhizopus* sp. for sustainable shrimp (*Litopenaeus vannamei*) feed

SURIANTI[1,2], ZAINUDDIN[3,♥], SITI ASLAMYAH[3], HASNI YULIANTI AZIS[3]

[1]Doctoral Program of Agricultural Science, Graduate School, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, South Sulawesi, Indonesia. Tel.: +62-411-585034, Fax.: +62-411-585868, ♥email: zainuddinlatief@gmail.com

[2]Department of Fisheries, Faculty of Science and Technology, Universitas Muhammadiyah Sidenreng Rappang. Jl. Angkatan 45 No. 1A, Sidenreng Rappang 91651, South Sulawesi, Indonesia

[3]Department of Fisheries, Faculty of Marine Science and Fisheries, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, South Sulawesi, Indonesia

Manuscript received: 17 April 2025. Revision accepted: 5 September 2025.

Abstract. *Surianti, Zainuddin, Aslamyah S, Azis HY. 2025. Fermentation of duck bone meal using* Bacillus *sp.,* Saccharomyces *sp., and* Rhizopus *sp. for sustainable shrimp* (Litopenaeus vannamei) *feed. Biodiversitas 26: 4333-4345.* Sustainable aquaculture necessitates alternatives to fishmeal because of its elevated cost and environmental impact. Duck bone waste, an underutilized by-product of poultry processing, can be valorized into feed ingredients, reducing organic waste and supporting a circular bioeconomy. This is the first study to assess Fermented Duck Bone Meal (FDBM) using mixed microbes (*Bacillus* sp., *Saccharomyces* sp., and *Rhizopus* sp.) as a fishmeal substitute in vannamei shrimp diets. Duck bone meal was fermented at inoculum doses of 0.5, 1, and 1.5 mL/100 g and fermentation durations of 12, 24, and 36 h. A control diet contained 100% fishmeal, while four experimental diets substituted fishmeal with optimally fermented duck bone meal (1.5 mL inoculum, 36 hours) at inclusion rates of 25%, 50%, 75%, and 100%, were fed to juvenile shrimp (0.19±0.01 g) for 60 days. Growth, survival, proximate composition, and amino acid profiles were analyzed. Proximate analysis of optimally fermented FDBM (1.5 mL, 36 h) revealed increased crude protein (42.72%), reduced crude fiber (1.49%), and elevated crude lipid (23.46%). Essential amino acids, such as lysine (2.77%) and glutamate (3.54%), were enhanced in the feed, aligning with higher amino acid retention in shrimp muscle (lysine: 4.85%, leucine: 4.57%). Shrimp fed the 75% FDBM diet (Feed D) had the highest final weight (23.04±0.09 g) and biomass weight gain (20.63±0.47 g), compared to the control diet (19.74±1.03 g) and the 25% FDBM diet (19.61±0.53 g), which exhibited the lowest values. Survival rates showed no significant differences among treatments ($p>0.05$). The 75% substitution of fishmeal with fermented duck bone meal improves prawn growth and feed quality, while reducing reliance on fishmeal, offering a cost-effective and environmentally responsible strategy for sustainable shrimp aquaculture.

Keywords: Amino acid, duck bone meal, *Litopenaeus vannamei*, proximate, shrimp feed

INTRODUCTION

Protein in modern aquaculture applications provides essential amino acids for tissue growth, enzyme activity, and physiological functions in white shrimp (*Litopenaeus vannamei* (Boone, 1931)) aquaculture (Wang et al. 2019; Chen et al. 2022). Traditional sources like fishmeal and soybean meal offer high digestibility and balanced amino acid content (Shi et al. 2016; Shao et al. 2025). However, fishmeal faces challenges from overfishing, supply fluctuations, and high costs (Yuan et al. 2023; Siddik et al. 2024). The sector is shifting toward sustainable alternatives utilizing underutilized biomass, food processing by-products, insect meal, and microbial fermentation (Sampathkumar et al. 2023). Developing nutritionally appropriate, economically viable fishmeal alternatives is critical for sustainable global aquaculture practices.

Bioprocessing technologies, including microbial fermentation, enzyme hydrolysis, and biowaste utilization, transform waste streams such as slaughterhouse by-products, fish processing waste, poultry products, agricultural by-products, and oyster shells, considered as recent aquaculture nutrition innovations, utilize agricultural and fishery food products as sustainable feed ingredients into valuable protein and mineral sources (Nisar et al. 2022). These methods reduce fishmeal reliance while mitigating environmental impacts through organic waste conversion and reduced nutrient emissions (Lal et al. 2024). Circular bioeconomy principles in feed production enhance environmental sustainability and economic resiliency through improving feed efficiency, gut health, and disease resistance (Zhang et al. 2020a).

Duck Bone Meal (DBM), a poultry processing by-product, provides vital protein and minerals for aquaculture feeds. It contains 40-41% protein and high calcium and phosphorus levels essential for skeletal construction, exoskeleton development, and optimal growth (Qisti et al. 2021; Saputra et al. 2023). However, DBM use is limited by low digestibility, which is requires biopreparations like microbial fermentation to improve digestibility, due to high mineral (ash) content and dense collagen matrix, hindering enzymatic nutrient access and reducing bioavailability (Vasyliuk et al. 2023). High ash content reduces growth performance and impairs nutrient absorption in shrimp and fish (Saputra et al. 2023). Fermentation enhances the digestibility of low-value animal by-products like DBM. Mixed microbial cultures, including filamentous fungi

(*Rhizopus* sp.), lactic acid bacteria, yeast (*Saccharomyces* sp.), and proteolytic bacteria (*Bacillus* sp.), improve nutritional quality by increasing protein digestibility, releasing bound minerals, and degrading antinutritional factors (Nkhata et al. 2018; Dawood and Koshio 2020). *Bacillus* sp. produces proteases, amylases, and cellulases (Salim et al. 2017; Pham et al. 2022), enhancing nutrient utilization and weight gain in carp (Mingmongkolchai and Panbangred 2018). *Saccharomyces cerevisiae* improves fish growth performance through better gut health (del Valle et al. 2023). *Rhizopus* sp. produces enzymes that hydrolyze structural polysaccharides (Canoy et al. 2024). Fermented proteins show improved digestibility and enhanced nutrient absorption (Siddik et al. 2024; Li et al. 2025). The use of this approach promotes aligning aquaculture with environmental conservation goals as a sustainable resource.

Despite growing interest in alternative protein sources, a knowledge gap exists regarding microbial fermentation of mixed bone-derived by-products for shrimp feed. Recent studies demonstrated single-strain fermentation effectiveness for plant proteins (Wang et al. 2024) and animal by-products (Wei et al. 2023). The integrated approach between bacteria, yeast, and fungi represents a novel bioprocessing strategy that leverages their distinct metabolic capabilities for comprehensive substrate conversion. However, no research addresses microbial consortia enhancement for duck bone meal in *L. vannamei* feed. Bone-based substrates require specialized microbial consortia producing diverse enzymes, including proteases and collagenases (Gariglio et al. 2019; Siddik et al. 2024). Single-strain fermentation lacks enzymatic diversity for complex substrates (Casillas-Hernández et al. 2022). This study uses strategically selected *Bacillus* sp., *Saccharomyces* sp., and *Rhizopus* sp. based on complementary enzymatic profiles (Mohammed et al. 2024).

This study hypothesizes that mixed microbial fermentation of DBM will revolutionize shrimp farming by significantly increasing crude protein content, enhancing essential amino acid bioavailability, and eliminating antinutritional factors, ultimately resulting in superior growth and higher survival rates in Vannamei shrimp. This innovative fermentation process is expected to contribute to reducing problematic ash content while enhancing mineral properties to match shrimp nutritional requirements, solving a critical challenge in aquaculture feed sustainability and poultry waste enhancement. Therefore, this study aims to evaluate the effect of mixed microbes fermentation of DBM on nutrient contents and vannamei shrimp growth performance, for sustainable aquaculture feed applications.

MATERIALS AND METHODS

Sample collection and preparation

Preparation of Duck Bone Meal (DBM)

Duck bones were collected from a local Palekko duck food vendor in Sidrap Regency, South Sulawesi, Indonesia. The preparation of duck bone meal followed the protocol described by Putranto et al. (2016), with modifications. The bones were manually cleaned, rinsed with distilled water, chopped into small pieces, and boiled in a pressure cooker at 121°C for 60 minutes to soften the matrix. The softened bones were then oven-dried at 60°C for 20 hours or sun-dried under hygienic conditions. Once dried, the bones were pulverized using a high-speed blender and sieved through a 0.25 mm mesh to obtain uniform Duck Bone Meal (DBM) particles.

Microbial strains and activation

Three microbial strains were used for fermentation: *Bacillus* sp., *Rhizopus* sp., and *Saccharomyces* sp. All strains were commercially obtained and selected based on their well-documented proteolytic and fermentative capacities. The *Bacillus* sp strain (1.5×10^8 CFU/mL) was provided by Biopocall MSME, Makassar. This strain was isolated from the hepatopancreas of healthy wild shrimps. *Rhizopus* sp. (1.6×10^7 CFU/g) was obtained from PT Aneka Fermentasi Industri (AFI), Bandung, and was isolated from traditional tempe fermentation. *Saccharomyces* sp. (1.2×10^8 CFU/g) was obtained from PT Sangra Ratu Boga, Indonesia, and isolated from a common baker's yeast strain. All strains were characterized by the suppliers using standard microbiological methods, including bacterial identification, colony morphology assessment, and microscopic spore identification for fungal strains. The selection leveraged strains adapted to protein-rich environments (shrimp hepatopancreas), cellulose-rich substrates (soybean fermentation), and carbohydrate metabolism, providing complementary enzymatic profiles for bone meal processing.

The microbial activation protocol was adapted from Aslamyah et al. (2017), with slight modifications. Each microbe was cultured separately: 2 mL of *Bacillus* sp. was cultured in a growth medium containing 2 L of coconut water and 500 g of sugar, then incubated in a glass jar at room temperature for 24 hours. *Rhizopus* sp. and *Saccharomyces* sp. were cultured by mixing 10 g of sugar dissolved in 100 mL of sterile distilled water and stirred using a magnetic stirrer at 120 rpm at room temperature for one hour. Microorganisms were mixed using the method described by (AOAC 2016) with some modification, homogenized, and diluted according to the following treatments: (i) Treatment A: 0.5 mL *Bacillus* sp. + 0.5 g *Saccharomyces* sp. + 0.5 g *Rhizopus* sp., (ii) Treatment B: 1 mL *Bacillus* sp. + 1 g *Saccharomyces* sp. + 1 g *Rhizopus* sp., (iii) Treatment C: 1.5 mL *Bacillus* sp. + 1.5 g *Saccharomyces* sp. + 1.5 g *Rhizopus* sp.

Fermentation of duck bone meal

Fermentation was conducted to improve the nutritional profile of Duck Bone Meal (DBM). This study used a factorial completely randomized design with two factors: microbial dosage (A1: 1.5 mL/100 g DBM, A2: 3.0 mL/100 g DBM, A3: 4.5 mL/100 g DBM) and incubation time (B1: 12 hours, B2: 24 hours, B3: 36 hours), which resulted in 9 treatment combinations with three replicates (27 experimental units). For each treatment, pre-weighed DBM was placed in sterile polyethylene bags, and the microbial mixture was applied using a fine mist sprayer to ensure uniform distribution. The bags were sealed to maintain semi-anaerobic conditions and incubated at room

temperature (28-30°C) for the specified fermentation duration. The moisture content was carefully adjusted to 45-50% to support optimal microbial metabolism. At the end of each incubation period, the fermented material was briefly steamed at 60°C for one minute to inactivate the microbial enzymes, then cooled at room temperature (25°C) before storage. Chemical composition of crude protein, crude fat, crude fiber, ash, and nitrogen-free extract was evaluated at the Feed Chemistry Laboratory, Faculty of Animal Science, Universitas Hasanuddin, using proximate analysis according to AOAC (2016) methods.

Test feed manufacturing

The test feed used was pellet feed formulated according to the experimental treatments. The raw materials and their compositions (dry matter basis) for each test feed. The feed preparation process began with drying and grinding the ingredients into a powder. The raw materials used included fishmeal, fermented duck bone meal, soybean meal, shrimp head meal, corn meal, CMC (Carboxymethyl Cellulose), vitamin and mineral mix, and fish oil. Each ingredient was weighed according to the composition listed in Table 1.

These ingredients were then mixed until homogeneous, starting with the smallest percentage of ingredients and gradually adding the larger percentage. The homogeneous mixture was then moistened with 6% water (by weight) and kneaded into dough, which was extruded using a pellet-making machine. The pellets were cut into small pieces approximately ±0.5 cm in length and then dried. This size was adjusted according to the age and mouth gape of the juvenile vannamei shrimp to ensure proper ingestion and reduce feed loss.

Physical test

The physical evaluation of the feed included water stability, hardness level, sinking speed, and attractiveness. The sinking speed test was conducted by dropping 5 feed pellets into a 500 mL graduated cylinder filled with water to a height of 20 cm. The time required for the feed to reach the bottom of the container was recorded using a stopwatch (Aslamyah and Karim 2012).

Organoleptic test

The organoleptic evaluation of the test feed, included texture, aroma, and color. Feed texture was assessed based on its smoothness, fiber content, and porosity, which were influenced by the fineness of raw materials, fiber content, and type of binder used. The aroma of feed determined its quality, as it was closely related to the feed's attractiveness to shrimp. Furthermore, the colour of feed depended largely on the type of raw materials used.

Chemical analysis

The chemical evaluation of the feed included the analysis of its nutritional composition through proximate analysis and amino acid content analysis in both the feed and shrimp body.

Proximate analysis

The proximate analysis of feed was carried out according to the standard methods by the Association of Official Analytical Chemists (AOAC 2016). The following nutrients were analyzed: moisture, Crude Protein (CP), Crude Fat (CF), and ash. Energy content (Kcal/100 g). Moisture content was estimated by gravimetric analysis after oven drying at 105°C for 12 hr. Crude Protein (CP) was determined by Kjeldahl method (N × 6.25) after acid hydrolysis. Crude fat was calculated gravimetrically after extraction with petroleum ether in a Soxhlet system. Total ash was determined gravimetrically by ignition at 600°C for 6 hr in a muffle furnace. Crude fiber was estimated gravimetrically after acid and alkali digestion and loss in mass by combustion at 600°C for 3 hr. Nitrogen Free extract (NFE) was calculated from 1000-(crude protein + crude lipid + crude fibre + total ash). The Gross Energy (GE) content of the diets was estimated according to the following equation: Feed Gross Energy (GE) (kcal/100 g Dry Matter) = (protein content × 5.64) + (lipid content × 9.44) + (carbohydrate (NFE) content x 4.11) (NRC 2011).

Ethical statement

This study has been approved by the Ethics Committee at Universitas Muslim Indonesia, Makassar, Indonesia. (protocol number: 551/A.1/KEP-UMI/XII/2024).

Table 1. Percentage of test feed ingredients for each treatment

Raw materials	Percentage (%)				
	Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
Fish meal	34	25.5	17	8.5	0
Fermented duck bone meal	0	8.5	17	25.5	34
Shrimp head meal	31	18	14	10	14
Soybean meal	9	33	40	45	40
Corn flour	20	9	6	5	6
CMC	2	2	2	2	2
Fish oil	2	2	2	2	2
Vitamins and minerals	2	2	2	2	2

Note: The tested treatments involved substituting a fishmeal with duck bone meal, which was explained below: A. Control diet (100% fishmeal and 0% duck bone meal), B. (75% fishmeal and 25% duck bone meal), C. (50% fishmeal and 50% duck bone meal), D. (25% fishmeal and 75% duck bone meal), E. (0% fishmeal and 100% duck bone meal). The 0-100% substitution approach was selected to assess the nutritional viability of DBM as a fishmeal substitute across a full gradient, facilitating the identification of both optimal and limiting inclusion levels

Experimental animal and protocol

The experimental design was a Completely Randomized Design (CRD) with five treatments and triplicate. A total of three hundred juvenile vannamei shrimp (*L. vannamei*), (±0.19 g) were obtained from PL 25 stage originating from Barru Regency, South Sulawesi, Indonesia. The shrimp were adapted for one week and then divided randomly into five experimental groups with three replicates at a rate of 20 juveniles/unit. The study used plastic basins with a recirculation system, measuring 23 cm in height with a diameter of 58 cm. Each unit was filled with 45 L brackish water at a salinity of ±20 ppt. Feeding frequencies were three times a day (7:00 AM, 11:00 AM, 15:00 PM, and 19:00 PM). The feeding rate was adjusted according to the size of the shrimp (10% biomass per day), throughout 60 days in the experimental period.

Growth parameters

The shrimp were weighed in grams using the electronic digital scale (0.01 mg). Every week, the total biomass in each basin was counted to assess their survival and growth.

$$\text{Biomass weight gain}\left(\frac{\text{g}}{\text{fish}}\right) = \text{Final weight (g)} - \text{initial weight (g)}$$

$$\text{Survival rate (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}}$$

Amino acid analysis

Amino acid analysis followed the AOAC (2023) method using High-Performance Liquid Chromatography (HPLC) with a C18 reversed-phase column (4.6 × 150 mm). The mobile phase consisted of a mixture of water and methanol, with added Trifluoroacetic Acid (TFA) to enhance separation efficiency. Furthermore, in the gradient elution profile**,** 95% water and 5% methanol, gradually shifted to 30% water and 70% methanol over 30 minutes. Injection volume: 5 µL, flow rate: 0.5 mL/min, detection: UV-Vis detector at 280 nm. The HPLC system was calibrated using albumin standards, and standard amino acid solutions were used to validate separation and detection, which ensured accuracy and precision. A calibration curve was generated using different amino acid standard concentrations, and multiple analyses were conducted to ensure result reliability.

Data analysis

The analysis of proximate composition and amino acid contents were analyzed descriptively to illustrate qualitative differences in amino acid profiles. The results were presented as a means with Standard Deviations (SD) (mean±SD). After checking for normality (Shapiro-Wilk test) and homogeneity (Levene's test) when assumptions were met the data of growth performance and survival rate were analyzed by One-Way Analysis of Variance (ANOVA) to determine if significant differences occured among the dietary treatments followed by Tukey's test to compare differences between treatment means when significant values were observed. The mean values were considered significantly different when $p<0.05$. Statistical analysis was performed using SPSS (Statistical Package for Social Sciences, Version 25, IBM Corporation, New York, USA).

RESULTS AND DISCUSSION

Proximate compositions of fermented duck bone meal

The proximate composition result are presented in Table 2. The results showed that microbial inoculum dosage and fermentation duration significantly affected the nutritional quality of Duck Bone Meal (DBM) ($p<0.05$). Crude protein levels increased from 40.43% in A1B1 to 42.72% and 42.70% in A3B2, which were considerably higher ($p<0.05$) compared to treatments with reduced microbial inoculum. The lowest crude protein content was recorded in treatment A1B1 (40.43%). Similarly, crude fat also differed significantly, with the highest value found in A3B3 (23.46%) and the lowest in A1B1 (20.63%). Crude fibre reduced significantly across treatments, with the lowest value in A3B1 (1.40%), A3B2 (1.56%), and A3B3 (1.49%), whereas A1B1-A1B3 had higher values (2.31-2.48%). Nitrogen-Free Extract (NFE) values also varied significantly ($p<0.05$), with the highest value found in A1B1 (32.41%) and the lowest in A3B2 (29.84%). The ash and calcium concentration showed no significant differences among treatments ($p>0.05$).

Degree of hydrolysis of fermented duck bone meal

The degree of hydrolysis of fermented duck bone meal is presented in Table 3. The results revealed significant variations in the hydrolysis of crude fiber, protein, fat, and NFE across treatments ($p<0.05$). The most effective hydrolysis consistently occurred in A3B3 (1.5 mL/36 h), yielding the highest rates for protein (4.93%), crude fiber (49.05%), and NFE (12.41%), alongside the lowest residual fat (5.91%). Conversely, the lower hydrolysis was recorded in A1B2 for protein (0.51%) and fat (16.74%), while crude fiber (16.66%) and NFE (7.09%) were lowest in A1B1. Fermentation with 1.5 mL inoculum for 36 h proved most effective, maximizing protein, fiber, and carbohydrate (NFE) hydrolysis while lowering residual fat, thus enhancing the nutritional quality of duck bone meal.

Table 2. Proximate composition of duck bone meal fermented with different microbial doses and incubation times

Treatments	Protein %	Crude fat %	Crude fiber %	NFE %	Ash %	Calcium %
A1_B1	40.43^{d}	20.63^{d}	2.47^{a}	7.85^{ab}	28.89^{a}	9.50^{a}
A1_B2	40.49^{d}	20.75^{cd}	2.48^{a}	7.91^{a}	28.34^{ab}	9.80^{a}
A1_B3	40.93^{c}	20.85^{d}	2.31^{a}	7.36^{ab}	28.54^{a}	9.55^{a}
A2_B1	41.32^{bc}	21.53^{cd}	2.28^{ab}	7.23b^{c}	27.63^{b}	9.68^{a}
A2_B2	41.01^{bc}	20.85^{d}	2.27^{ab}	7.52^{ab}	28.34^{ab}	10.03^{a}
A2_B3	41.09^{bc}	22.56^{ab}	1.51^{ab}	6.53^{d}	28.30^{ab}	10.41^{a}
A3_B1	41.34^{b}	22.29^{bc}	1.40^{b}	6.75^{cd}	28.21^{ab}	9.55^{a}
A3_B2	42.72^{a}	22.83^{ab}	1.56^{ab}	6.47^{d}	26.40^{c}	10.25^{a}
A3_B3	42.70^{a}	23.46^{a}	1.49^{ab}	6.47^{d}	25.93^{c}	10.46^{a}

Note: Different letters in the same column indicate significant differences between treatments at the 95% confidence level. ($P<0.05$). A1_B1: 0.5mL/_12h, A1_B2: 0.5mL/_24h, A1_B3: 0.5mL/_36h, A2_B1: 1mL/_12h, A2_B2: 1 mL/_24h, A2_B3: 1mL/_36h, A3_B1: 1.5 mL/_12h, A3_B2: 1.5 mL/_24h, A3_B3: 1.5 mL/_36h

Table 3. Degree of hydrolysis of fermented duck bone meal

Treatments	Degree of protein hydrolysis (%)	Degree of fat hydrolysis (%)	Degree of hydrolysis of crude fibre (%)	Degree of NFE hydrolysis (%)
A1_B1	0.66±0.27^{a}	17.24±1.25^{d}	16.66±7.53^{a}	7.09±0.96^{ab}
A1_B2	0.51±0.10^{a}	16.74±1.38^{d}	14.77±3.40^{a}	7.91±0.19^{ab}
A1_B3	1.15±0.03^{a}	16.36±0.28^{d}	17.69±3.64^{a}	4.16±0.67^{a}
A2_B1	1.52±1.04^{a}	13.61±1.50^{cd}	18.89±1.46^{a}	3.41±1.92^{a}
A2_B2	0.76±0.17^{a}	16.36±2.21^{d}	30.40±4.61^{b}	2.66±0.86^{a}
A2_B3	0.97±0.05^{a}	9.52±1.61^{ab}	35.90±4.13^{b}	10.84±6.85^{b}
A3_B1	1.57±0.07^{a}	10.58±0.79^{bc}	51.71±0.24^{c}	7.91±3.09a^{b}
A3_B2	4.35±1.50^{b}	8.40±1.04^{ab}	46.10±2.82^{c}	11.73±0.77^{b}
A3_B3	4.93±0.37^{b}	5.91±2.58^{a}	49.05±0.60^{c}	12.41±1.73^{b}

Note: Different letters in the same column indicate significant differences between treatments at the 95% confidence level ($P<0.05$). A1_B1: 0.5mL/_12h, A1_B2: 0.5mL/_24h, A1_B3: 0.5mL/_36h, A2_B1: 1mL/_12h, A2_B2: 1 mL/_24h, A2_B3: 1mL/_36h, A3_B1: 1.5 mL/_12h, A3_B2: 1.5 mL/_24h, A3_B3: 1.5 mL/_36h

Table 4. Data on various organoleptic and physical test parameters for each experimental feed

Parameters	Treatments				
	Feeds A	Feeds B	Feeds C	Feeds D	Feeds E
Organoleptic testing					
Aroma	3.8	3.8	3.6	3.9	2.5
Color	3.1	3.8	3.8	3.7	2.7
Texture	2.4	2.8	3.7	3.9	2.3
Physical testing					
Water stability (minutes)	3.5	3.1	3.1	3.2	3.9
Feed hardness level (%)	2.9	2.6	3.1	3.2	2.7
Sinking speed (cm/sec)	3.7	3.4	3.7	3.8	4
Attractability (cm/sec)	3	3.2	3.8	3.8	3

Note: A. 100% fishmeal and 0% duck bone meal), B. 75% fishmeal and 25% duck bone meal, C. 50% fishmeal and 50% duck bone meal, D. 25% fishmeal and 75% duck bone meal, E. 0% fishmeal and 100% duck bone meal. Color Scale: 1. Light Brown, 2. Brown, 3. Dark Brown, 4. Blackish Brown, 5. Black. Aroma Scale: 1. Odorless, 2. Slightly Aromatic, 3. Fish-Scented, 4. Strongly Fish-Scented, 5. Very Strong Fish Aroma. Texture Scale: 1. Very Coarse, 2. Coarse, 3. Moderately Fine, 4. Fine, 5. Very Fine. Water Stability Scale: 1. Feed dissolved quickly in water, 2. The feed started dissolving within 30 minutes, 3. The feed started dissolving within 60 minutes, 4. Feed remained intact for about 2 hours, 5. Feed remained stable and intact for more than 3 hours. Feed Hardness Scale: 1. Very Hard, 2. Hard, 3. Moderate, 4. Soft, 5. Very Soft. Sinking Speed Scale: 1. Very Slow, 2. Slow, 3. Moderate, 4. Fast, 5. Very Fast. Attractability Scale: 1. Very Low, 2. Low, 3. Moderate, 4. High, 5. Very High

Organoleptic and physical tests

Organoleptic and physical tests are methods to assess the sensory quality and physical characteristics of artificial feed. The results of various organoleptic and physical test parameters on each experimental feed that has been fermented using mixed microbes are presented in Table 4. The organoleptic assessment indicated distinct variations among the treatments. Feeds A-D resulted in higher scores for aroma, color, and texture compared to Feed E, which consistently showed the lowest values, indicating reduced sensory acceptability. Texture was particularly improved in Feeds C and D, reaching scores of 3.7-3.9, suggesting better pellet integrity. Physical testing further supported these findings, where Feeds C and D demonstrated superior hardness, sinking speed, and attractability, highlighting their higher functional quality. Conversely, Feed E showed better water stability but lower overall performance in organoleptic traits and physical strength. These results suggest that fermentation and formulation processes in Feeds C and D produced diets with more desirable sensory and physical properties, making them more suitable for practical feeding applications.

Proximate analysis of feed

The results of proximate analysis of the treatment feed that substituted fishmeal with duck bone meal are presented in Table 5. The crude protein content was highest in Feed A at 40.15% and showed a gradual decline with increasing inclusion of duck bone meal, reaching the lowest level in Feed E (37.18%). Conversely, crude fat content increased notably with the incorporation of duck bone meal, peaking in Feed D (15.04. Crude fiber and Nitrogen-Free Extract (NFE) exhibited minimal variation across treatments, indicating that these components were not substantially affected by the replacement strategy. Interestingly, ash content was consistently elevated in feeds containing higher proportions of duck bone meal, with the highest value recorded in Feed E (24.80%). Crude fat content increased notably with the incorporation of duck bone meal, peaking in Feed D (15.04%).

Amino acid profile of feed

Amino acid composition is an important indicator to assess the quality of protein in feed. The result of amino acid compositions of the experimental diets is presented in Table 6. Essential amino acids including lysine, histidine, and arginine increased markedly with higher inclusion of duck bone meal, with Feed E containing the highest concentrations (lysine: 2.77%; histidine: 0.88%; arginine: 1.74%). In contrast, threonine, methionine, and isoleucine were relatively consistent throughout treatments. Among the non-essential amino acids, glutamic acid and aspartic acid increased consistently with higher inclusion of duck bone meal, peaking in Feed E (3.54% and 2.75% respectively). Proline levels also increased significantly (from 1.26% in Feed A to 1.92% in Feed E).

Table 5. Results of proximate analysis of treatment feed (%)

Treatments feed	Compositions				
	Crude protein %	Crude fat %	Crude fiber %	NFE %	Ash %
Feed A	40.15	8.49	6.16	19.82	25.38
Feed B	39.7	11.1	5.56	20.35	23.29
Feed C	39.55	14.75	5.49	18.84	21.37
Feed D	38.24	15.04	5.26	19.49	21.98
Feed E	37.18	14.23	5.05	18.74	24.8

Note: A. 100% fishmeal and 0% duck bone meal), B. 75% fishmeal and 25% duck bone meal, C. 50% fishmeal and 50% duck bone meal, D. 25% fishmeal and 75% duck bone meal, E. 0% fishmeal and 100% duck bone meal

Table 6. Amino acid composition at different feed levels (% g/100 g feed)

Amino acids	Treatments				
	Feed A (%)	Feed B (%)	Feed C (%)	Feed D (%)	Feed E (%)
Aspartic acid	2.12	2.43	2.65	2.71	2.75
Glutamic acid	3.11	3.2	3.26	3.33	3.54
Serine	1.28	1.26	1.33	1.37	1.42
Glycine	1.55	1.56	1.64	1.71	1.71
Histidine	0.44	0.71	0.83	0.81	0.88
Arginine	1.36	1.44	1.42	1.74	1.74
Threonine	0.75	0.79	0.77	0.75	0.75
Alanine	1.34	1.36	1.38	1.42	1.45
Proline	1.26	1.46	1.84	1.88	1.92
Tyrosine	0.92	0.95	0.99	0.88	0.88
Valine	1.53	1.27	1.33	1.31	1.31
Methionine	0.66	0.67	0.66	0.68	0.68
Cysteine	0.57	0.74	0.87	0.91	0.91
Isoleucine	1.18	1.18	1.22	1.18	1.18
Leucine	2.33	2.44	2.47	2.52	2.62
Phenylalanine	1.03	1.21	1.26	1.29	1.32
Lysine	2.26	2.31	2.36	2.52	2.77

Note: A. 100% fishmeal and 0% duck bone meal), B. 75% fishmeal and 25% duck bone meal, C. 50% fishmeal and 50% duck bone meal, D. 25% fishmeal and 75% duck bone meal, E. 0% fishmeal and 100% duck bone meal

Table 7. Growth performance and survival rate of vannamei shrimp

Treatments	Initial weight	Final weight	Weight gain	Survival rate (%)
A	3.03 ± 0.04^{a}	19.74 ± 1.03^{a}	16.44 ± 0.60^{a}	86.66 ± 2.88^{a}
B	2.85 ± 0.09^{a}	19.61 ± 0.53^{a}	16.75 ± 0.63^{a}	88.66 ± 2.88^{a}
C	2.94 ± 0.08^{a}	21.90 ± 0.04^{bc}	18.95 ± 1.00^{bc}	90.00 ± 0.00^{a}
D	3.15 ± 0.33^{a}	23.04 ± 0.09^{c}	20.63 ± 0.47^{c}	95.00 ± 0.00^{a}
E	3.06 ± 0.55^{a}	21.01 ± 0.17^{ab}	17.34 ± 0.62^{ab}	86.66 ± 2.88^{a}

Table 8. Range of water quality analysis during the experimental period

Treatments	Temperature	pH	DO (ppm)	Ammonia (ppm)	Salinity
A	26.9	7.8	5.85	0.079	26
B	27	7.8	5.97	0.038	26
C	27	7.8	6.02	0.023	26
D	27	7.8	6.08	0.022	27
E	26.9	7.8	5.92	0.017	27

The graphical representation reveals a strong positive relationship between duck bone meal inclusion and amino acid concentration (Figure 1). The trend line demonstrates a a clear upward trend in amino acid levels as duck bone meal content and fermentation increase in the feed mixtures. This pattern indicates that duck bone meal, when fermented, has a more concentrated amino acid profile than fish meal.

Growth performance and survival rate of vannamei shrimp

The results of the growth performance of vannamei shrimp under different treatments presented in Table 7. Initial fish weights were not significantly different among feeding treatments ($p>0.05$). Growth parameters of vannamei shrimp were significantly influenced by the level of fishmeal replacement with Fermented Duck Bone Meal (FDBM) ($p<0.05$). Shrimp fed the 75% FDBM diet (Feed D) exhibited the highest final weight (23.04±0.09 g) and weight gain (20.63±0.47 g). The lowest final weight and weight gain were observed in control diet and the 25% FDBM diet (Feed B) (19.74±1.03 g and 19.61±0.53 g, respectively). The survival rates of the experimental fishes were not significantly different among treatments ($P>0.05$), varying in the ranges of 86.66-95.00%.

Water quality parameters

Water quality parameters recorded in this trial are shown in Table 8. The averages of water temperature, and water pH recorded in this trial were in the range of (26-27°C) and (pH 7.8), respectively. Dissolved Oxygen (DO), total ammonia, and salinity in the range of (5.85-6.08 ppm), (0.017-0.079 ppm), and (26-27) respectively. The results of the measurement showed that water quality parameters were within the range of acceptable limits for vannamei shrimp during the experimental period (60 days).

Amino acid profile of vannamei shrimp

The amino acid composition of vannamei shrimp under different treatments is presented in Table 9. Vannamei D consistently had the highest concentrations of several essential amino acids, including lysine (4.85%), leucine (4.57%), and phenylalanine (4.35%), indicating high protein quality. Similarly, Vannamei A also showed elevated levels of lysine (4.65%) and leucine (4.21%). In contrast, Vannamei E had the lowest amino acid concentrations across most metrics, with significantly lower values for lysine (2.70%), phenylalanine (0.98%), and methionine (0.86%).

Figure 2 demonstrates that vannamei shrimp amino acid concentrations decrease with increased duck bone meal in the feed. The results showed that treatments B and D demonstrated the highest amino acid content compared to other treatments. The lowest amino acid concentrations were observed in treatment E.

Discussion

The application of microbial fermentation in feed biotechnology offers the combined advantages of improving nutritional quality and promoting environmental sustainability. This study investigated Fermented Duck

Bone Meal (FDBM) using a microbial consortium consisting of *Bacillus* sp., *Rhizopus* sp., and *Saccharomyces* sp. as a promising replacement for fishmeal in aquaculture feed, revealing significant improvements through optimized fermentation parameters and demonstrating its potential contribution to sustainable aquaculture practices.

Fermentation with mixed microorganisms improved proximate composition by facilitating the enzymatic breakdown of complex proteins and fibres as observed in the treatments with higher microbial inoculum concentrations and longer fermentation times, particularly treatments A3B2 and A3B3. The major process involves microbially generated phytase enzymes that catalyze the stepwise dephosphorylation of phytic acid, resulting in stable chelate complexes with important minerals such as calcium, magnesium, iron, and zinc (Nkhata et al. 2018). This enzymatic breakdown, together with the acidic pH environment formed during fermentation, enhances the dissociation of mineral-phytate complexes, resulting in a significant increase in soluble calcium, iron, and zinc (Gupta et al. 2015). Simultaneously, the diverse enzymatic profile of fermenting microorganisms, particularly *Bacillus* sp. with extracellular lipolytic, proteolytic, cellulolytic, and amylolytic activities, degrades antinutritional factors such as tannins and additional phytate while breaking down cell wall polysaccharides that physically entrap minerals (Samtiya et al. 2020; Sharma et al. 2020). The fermentation process further improves feed quality through partial protein hydrolysis into digestible peptides and amino acids, reduction of crude fiber and non-starch polysaccharides, and production of beneficial metabolites including digestive enzymes (proteinase, amylase, cellulase), organic acids that improve palatability and gut health, vitamins, and bioactive compounds that collectively optimize nutrient utilization and explain the superior growth performance (Siddik et al. 2024).

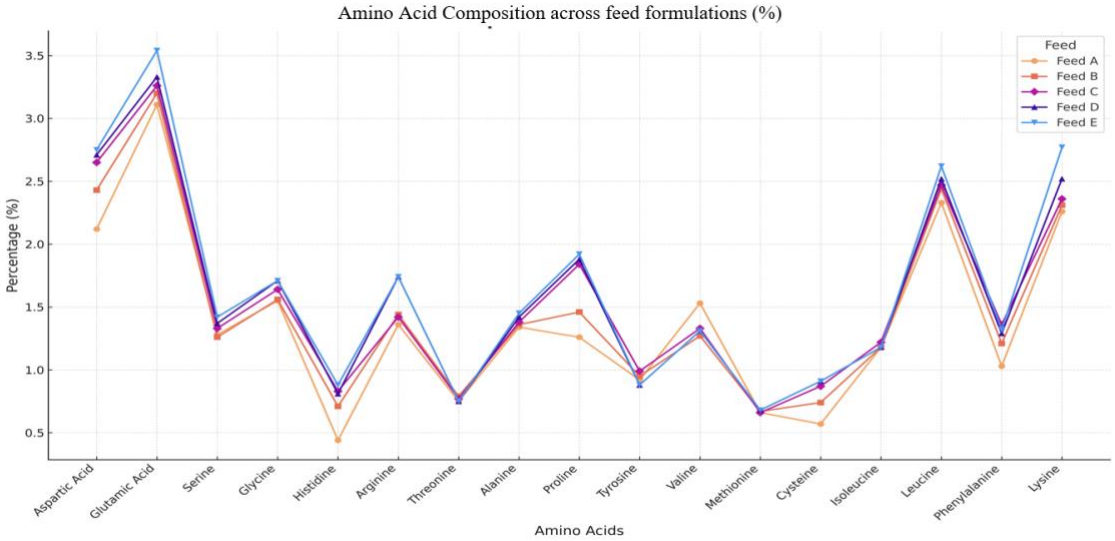

Figure 1. Trend of increasing amino acid levels in various feed formulations fermented using mixed microbes

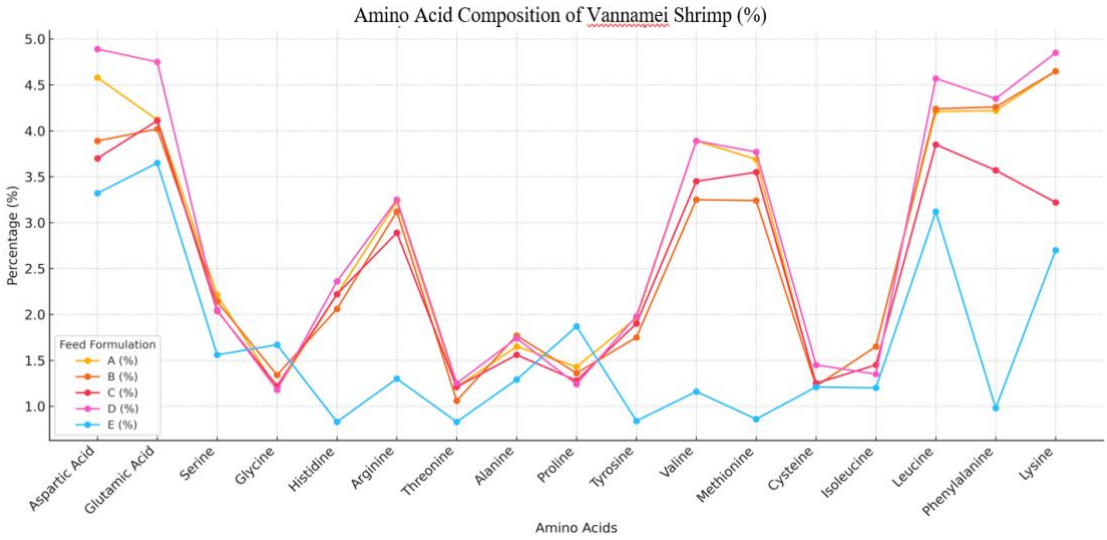

Figure 2. Trend of increasing levels of amino acids in vannamei shrimp in various feed formulations fermented using mixed microbes

Table 9. Amino acid composition of vannamei shrimp under different treatments (%)

Amino acids	Treatments				
	Vannamei A (%)	Vannamei B (%)	Vannamei C (%)	Vannamei D (%)	Vannamei E (%)
Aspartic acid	4.58	3.89	3.7	4.89	3.32
Glutamic acid	4.12	4.02	4.11	475	3.65
Serine	2.21	2.14	2.04	2.05	1.56
Glycine	1.19	1.34	1.22	1.18	1.67
Histidine	2.22	2.06	2.22	2.36	0.83
Arginine	3.23	3.12	2.89	3.25	1.3
Threonine	1.21	1.06	1.21	1.25	0.83
Alanine	1.65	1.77	1.56	1.74	1.29
Proline	1.43	1.36	1.28	1.24	1.87
Tyrosine	1.96	1.75	1.9	1.98	0.84
Valine	3.89	3.25	345	3.89	1.16
Methionine	3.69	3.24	3.55	3.77	0.86
Cysteine	1.22	1.22	1.25	1.45	1.21
Isoleucine	1.65	1.65	1.45	1.35	1.2
Leucine	4.21	4.24	3.85	4.57	3.12
Phenylalanine	4.22	4.26	3.57	4.35	0.98
Lysine	4.65	4.65	3.22	4.85	2.70

Note: Vannamei A: Shrimp treated with 100% fishmeal and 0% duck bone meal, Vannamei B: Shrimp treated with 75% fishmeal and 25% duck bone meal, Vannamei C: Shrimp treated with 50% fishmeal and 50% duck bone meal, Vannamei D: Shrimp treated with 25% fishmeal and 75% duck bone meal, Vannamei E: Shrimp treated with 0% fishmeal and 100% duck bone meal

The increase in crude fat content associated with increased microbial dosages and prolonged fermentation duration is likely attributable to microbial lipid metabolism and augmented lipase activity, which facilitate the liberation and synthesis of fatty acids during fermentation. This microbial synthesis aligns with deamination processes that divert carbon flow toward lipid biosynthesis pathways, as reported in fermented animal by-products including poultry and fish offal (Marcinčák et al. 2018; Wei et al. 2020; Djulardi et al. 2023; Weng et al. 2023). The notable decrease in crude fibre content indicates the effective action of microbial fibrolytic enzymes, including cellulase, hemicellulase, and xylanase, generated by species such as *Rhizopus* sp. and *Bacillus* sp. These enzymes hydrolyze complex polysaccharides, enhancing feed digestibility and palatability critical elements for optimizing aquaculture performance (Lynd et al. 2002; Mugwanya et al. 2022; Wlazło et al. 2022; Anwar et al. 2023). Fluctuations in nitrogen-free extract levels further indicate active microbial metabolism of soluble carbohydrates as fermentative microbes, including *S. cerevisiae* and *Bacillus* species, consume available sugars during substrate fermentation (Dewi et al. 2021).

Enhanced mineral parameters, specifically ash and calcium concentrations, result from heightened mineral solubilisation driven by organic acid production from fermentative yeasts and bacteria like *Saccharomyces* sp., thereby enhancing mineral bioavailability (Wang et al. 2022; Rizwanuddin et al. 2023). These findings are consistent with mineral concentration effects during organic matter degradation in fermented fish bone and meat waste substrates (Rakib et al. 2019; Eliopoulos et al. 2022; Kostova et al. 2022). While crude protein content slightly decreased owing to the inherently higher protein density of fish meal relative to duck bone meal, fermentation enhances protein availability through the proteolytic action of *Bacillus* spp., which secrete potent collagen-degrading enzymes that break down protein matrices into absorbable peptides, improving overall digestibility and nutritional quality (Bowzer et al. 2014; Salim et al. 2017; Coelho et al. 2019; Pham et al. 2022). Furthermore, fermentation improves amino acid composition by increasing important amino acids including glutamic acid, lysine, and proline, hence raising the feed's nutritional value (Gariglio et al. 2019). Overall, the results confirmed that the microbial dose of 1.5 mL/100 g with a fermentation time of 36 hours (A3B3) provided the most consistent and optimal improvement in all proximate parameters, thus making it the most effective treatment to improve the nutritional quality of duck bone meal as an alternative feed ingredient.

The combination of microbial inoculum concentration and incubation time has a significant impact on hydrolysis efficiency in Fermented Duck Bone Meal (FDBM). The degree of hydrolysis is mostly dependent on the microbial inoculum concentration. More hydrolytic enzymes can be produced as a result of enhanced microbial activity brought on by a higher concentration (Zinina et al. 2021). It has been shown that optimal hydrolysis conditions, such as those involving particular proteases, provide high protein content. High protein content and good digestibility have been shown to result from optimal hydrolysis conditions, such as those involving certain proteases, highlighting the significance of enzyme selection and process optimisation (Prandi et al. 2024). Fermentation processes not only enhance nutrient breakdown but also improve the nutritional value and digestibility of agricultural waste products, offering sustainable alternatives for animal feed (Yasmeen and Ahmad 2024). This highlights the potential for broader applications of optimized fermentation techniques beyond FDBM. This synergistic interplay is vital, as evidenced by the comparatively lower hydrolysis percentages recorded in treatments utilizing reduced inoculum levels and shorter

incubation periods. In those cases, limited microbial biomass and curtailed enzymatic activity fail to achieve optimal substrate degradation, as also reported in studies on the fermentation of other animal byproducts and plant materials (Wang et al. 2024).

The combined assessment of organoleptic and physical qualities demonstrates their importance in evaluating feed quality, palatability, and overall efficiency in shrimp farming. Organoleptic characteristics such as aroma, color, and texture are essential in assessing feed palatability and acceptance by shrimp. Fermentation-derived changes in these attributes can influence feed intake, as reported by Fahrudin et al. (2023), who noted that darker color and balanced texture are often associated with higher-quality ingredients. The shift in sensory attributes observed in feeds containing higher proportions of duck bone meal suggests that ingredient substitution alters the sensory cues used by shrimp to identify feed, potentially affecting feeding motivation and efficiency. Also, the physical properties, such as water stability and hardness, are crucial for preserving feed quality in aquatic ecosystems. Stability ensures that pellets remain intact long enough for shrimp to consume them, reducing nutrient leaching and environmental pollution, while appropriate hardness prevents feed disintegration or rejection during handling and ingestion. These principles are consistent with earlier reports highlighting that optimized texture and hardness directly support nutrient retention and efficient consumption, while reducing waste outputs in culture systems (Almuqaramah et al. 2018; Kurniawan et al. 2019). Furthermore, sinking speed and feed attractiveness also influence feeding performance, since pellets must reach prawns effectively while remaining detectable by chemical and physical cues. Prior research has shown that sinking dynamics affect both consumption rates and feed waste, with intermediate sinking velocities increasing feeding synchronisation and lowering competition (Walsh et al. 2022). Similarly, Nunes et al. (2024) stated that balanced protein formulations improve palatability, guaranteeing that prawns quickly consume the meal. The current findings support these observations, demonstrating that feed formulation techniques must optimise not only nutritional composition but also sensory and physical qualities in order to maximise feeding efficiency, growth, and sustainability. These findings suggest that sensory and physical characteristics must be examined concurrently, as their interactions ultimately impact feed acceptability, nutrient utilization, and environmental sustainability in shrimp farming.

The gradual substitution of fishmeal with FDBM consistently increased the essential amino acid content in all feed formulations. The fermentation process utilizes a synergistic microbial consortium consisting of *Bacillus* sp., *Rhizopus* sp., and *Saccharomyces* sp., all of which are producers of extracellular proteolytic enzymes known as proteases, peptidases, and aminopeptidases. These enzymes actively hydrolyze collagen and other structural proteins, accelerating the release of key amino acids, especially proline, glutamate, and glycine (Salim et al. 2017; Pham et al. 2022). In addition, *Rhizopus* sp. and *Saccharomyces* sp. contribute to mineral dissolution and degradation of residual fibrous tissues, further promoting amino acid liberation and nutrient bioaccessibility. Yeh et al. (2023) noted that *Bacillus subtilis* and *B. siamensis* showed high proteolytic efficiency in fermenting animal by-products such as meat and bone meal, increasing peptide production and improving feed digestibility.

The findings of this study are in line with previous studies by Nnaji (2014) and Swain et al. (2023), who reported that fermented poultry bone waste significantly improved the amino acid profile compared to conventional fishmeal. Similarly, Bowzer et al. (2014) highlighted the unique advantage of fermented duck bone in increasing the levels of glutamate and leucine, two amino acids essential for energy metabolism and muscle protein synthesis in aquatic organisms. These amino acids are physiologically indispensable for promoting growth, immune function, and tissue differentiation in *L. vannamei*, with lysine and leucine playing a central role in stimulating growth hormone secretion and facilitating muscle protein translation (Purba and Prasetyo 2014; Fauzi and Sari 2018). Fermentation using mixed microbial cultures not only enhances the levels of free amino acids but also improves overall protein quality through a multiphase enzymatic mechanism an advantage not typically achieved with single-strain fermentation. These results support the work of Guntari et al. (2022), which indicated that FDBM is a promising and cost-effective alternative protein source, rich in essential amino acids, compared to conventional fishmeal. This is further supported by the literature on duck blood plasma composition, which confirms the abundance of glutamate, arginine and lysine, three amino acids that play an important role in immune modulation and tissue development. Therefore, incorporating TTBF in feed formulations not only offers superior nutritional solutions, but also opportunities for the feed industry to develop more functional, efficient and sustainable products.

The research indicated that the partial substitution of fishmeal with Fermented Duck Bone Meal (FDBM) markedly enhanced the growth of vannamei shrimp. Research indicates that incorporating Fermented Soybean Meal (FSBM) and other fermented plant proteins as substitutes for fishmeal in whiteleg shrimp (*L. vannamei*) consistently enhances growth performance, feed efficiency, and immune parameters when partially replacing 30-75% of the fishmeal, compared to control diets devoid of fermented protein or with minimal substitution levels (Shao et al. 2018; Hamidoghli et al. 2020). Vannamei shrimp survival rates across all FDBM treatments were high (>86.66%) and did not differ substantially, despite variations in growth performance. This suggests that FDBM inclusion does not jeopardize shrimp health or survivability. This is consistent with research employing alternative fermented protein sources, which revealed higher survival rates and no negative impacts on animal wellbeing (Huervana et al. 2024).

The amino acid profiles of *L. vannamei* exhibited substantial variation across feed treatments, clearly influenced by the progressive substitution of fishmeal with Fermented Duck Bone Meal (FDBM). This is in accordance with the opinion of Purba and Prasetyo (2014) that amino acids are

the building blocks of protein, and protein is essential for various biological functions of shrimp, including growth and tissue repair. This finding is in line with previous studies showing that fish meal is a rich source of essential amino acids, which are important for the growth and development of aquatic species (Klahan et al. 2023; Tejeda-Miramontes et al. 2023; Bøgwald et al. 2024). The improvement was mechanistically linked to microbial fermentation during TTBF production, where mixed cultures of *Bacillus* sp., *Rhizopus* sp., and *Saccharomyces* sp. introduce broad enzymatic repertoires enhancing protein digestibility and nutrient bioavailability. *Bacillus* sp. secretes extracellular alkaline and neutral proteases hydrolyzing macromolecular proteins into dipeptides and free amino acids (Salim et al. 2017), while *Rhizopus* sp. contributes to collagen fiber degradation through collagenolytic activity, releasing structural amino acids such as proline, glutamate, and glycine (Pham et al. 2022). *Saccharomyces cerevisiae* plays a dual role by fermenting residual carbohydrates and producing low molecular weight peptides while facilitating calcium and phosphorus solubilization through acidification and organic acid production (Swain et al. 2023). Lysine, crucial for muscle tissue formation and enzyme synthesis, showed a significant reduction that could slow growth when not compensated by other nutrient sources, as this essential amino acid plays key roles in protein synthesis and overall growth performance (Allen et al. 2019; Medeiros et al. 2022; Li et al. 2023). Glutamic acid is essential for osmotic control and antioxidant defense (Jin et al. 2022). As a key component of the urea cycle and semicarbazide production, arginine supports osmoregulation and adds to shrimp taste and nitrogen metabolism (Zhang et al. 2022). The decline in amino acid levels observed in Feed E, indicates a biological threshold in TTBF inclusion where excessive substitution may disrupt nutrient balance, affect feed palatability, or result in incomplete fermentation leading to less digestible residues or potential antinutritional factors. These findings agree with Bowzer et al. (2014) and Nnaji (2014), who reported that fermented animal by-products such as poultry bone and duck offal could improve feed quality up to certain inclusion rates, beyond which performance plateaued or declined. Comparable trends have been reported in studies involving fermented poultry offal (Swain et al. 2023), fish viscera (Obirikorang et al. 2018), and animal blood meals (Luthada-raswiswi et al. 2022), all demonstrating improved amino acids and growth response in aquaculture species when fermentation was optimized.

Aquafeed containing Fermented Duck Bone Meal (FDBM) provides a variety of benefits for aquaculture sustainability. Nutritionally, microbial fermentation greatly improves the digestibility and quality of animal byproducts, boosting nutrient bioavailability and encouraging improved feed efficiency and growth performance in cultured species (Zhang et al. 2020b). Environmentally, the usage of FDBM promotes waste valorization by transforming chicken industry leftovers into high-value feed ingredients, lowering organic waste and fostering a circular bioeconomy. Improved digestibility of fermented components also results in lower nitrogen and phosphorus excretion, reducing eutrophication hazards and improving the environmental sustainability of aquaculture operations (Macusi et al. 2023). Poultry by-products are an economically viable alternative to traditional fishmeal, with the potential to reduce feed costs and increase shrimp farming profitability (Siddik et al. 2024). Although no Life Cycle Assessment (LCA) data were obtained in this study, further evaluations using tools such as the Global Feed LCA Institute (GFLI) database might be useful in assessing the environmental advantages and carbon footprint reduction related to FDBM incorporation in shrimp diets. Together, these qualities make FDBM a viable ingredient that supports both the environmental and economic pillars of sustainable aquaculture.

This study concluded that Fermentation of Duck Bone Meal (FDBM) with *Bacillus* sp., *Rhizopus* sp., and *Saccharomyces* sp. at 1.5 mL/100 g for 36 hours significantly improved its nutritional profile, including protein hydrolysis, amino acid enrichment, and mineral solubilisation ($P<0.05$). When used in prawn diets, partial replacement of fishmeal with FDBM resulted in significant benefits: the 75% inclusion level optimised growth performance, maintained a high survival rate, and provided acceptable feed quality in terms of stability, texture, and appeal. These findings confirm that FDBM can successfully minimise dependency on traditional fishmeal while maintaining Vannamei shrimp performance and feed functionality, making it an attractive alternative for environmentally friendly aquafeeds.

To advance application, future research should include digestibility tests to confirm nutrient utilization, cost-benefit assessments to determine economic feasibility, and long-term or farm-scale feeding studies to validate performance under commercial production conditions. These follow-ups will be crucial in determining the scalability and practical sustainability of FDBM as a substitute for fishmealin aquaculture.

ACKNOWLEDGEMENTS

This study was funded by The Center for Higher Education Funding and Assessment (PPAPT) through the Indonesia Endowment Fund (LPDP) with the Indonesian Educational Scholarship (BPI) No. 202209091626.

REFERENCES

Allen KM, Habte-Tsion H-M, Thompson KR, Filer K, Tidwell JH, Kumar V. 2019. Freshwater microalgae (*Schizochytrium* sp.) as a substitute to fish oil for shrimp feed. Sci Rep 9 (1): 6178. DOI: 10.1038/s41598-019-41020-8.

Almuqaramah TMH, Setiawati M, Priyoutomo NB, Effendi I. 2018. Pendederan udang vaname *Litopenaeus vannamei* dengan teknologi bioflok untuk meningkatkan pertumbuhan dan efisiensi pakan. Jurnal Ilmu Teknologi Kelautan Tropis 10 (1): 143-152. DOI: 10.29244/jitkt.v10i1.21671. [Indonesian]

Anwar A, Zainuddin Z, Djawad MI, Aslamyah S. 2023. Fermentation of rain tree (*Samanea saman*) seed meal using mixed microbes to improve its nutritional quality. Biodiversitas 24 (11): 5863-5872. DOI: 10.13057/biodiv/d241104.

AOAC [Association of Official Agricultural Chemists]. 2016. Official methods of analysis of AOAC International (20th eda). AOAC International, Gaithersburg, Maryland.

AOAC [Association of Official Agricultural Chemists]. 2023. Official methods of analysis of the association. Official Analytical Chemists 22th eds (22nd eds). AOAC International, Rockville, Maryland.

Aslamyah S, Karim MY, Badraeni. 2017. Fermentation of seaweed meal with various fermenters to improve the quality of fish feed ingredients. Jurnal Akuakultur Indonesia 16: 8-14. DOI: 10.19027/jai.16.1.8-14.

Aslamyah S, Karim MY. 2012. Uji organoleptik, fisik dan kimiawi pakan buatan untuk ikan bandeng yang disubstitusi dengan tepung cacing tanah (*Lumbricus* sp.). Jurnal Akuakultur Indonesia 11 (2): 124-131. DOI: 10.19027/jai.11.124-131. [Indonesian]

Bøgwald I, Herrig S, Pedersen AM, Wubshet SG, Eilertsen K-E. 2024. Effect of *Calanus finmarchicus* hydrolysate inclusion on diet attractiveness for whiteleg shrimp (*Litopenaeus vannamei*). Fishes 9 (4): 134. DOI: 10.3390/fishes9040134.

Bowzer J, Bergman A, Trushenski J. 2014. Growth performance of Largemouth Bass fed fish meal derived from Asian carp. N Am J Aquac 76 (3): 185-189. DOI: 10.1080/15222055.2014.893473.

Canoy TS, Wiedenbein ES, Bredie WLP, Meyer AS, Wösten HAB, Nielsen DS. 2024. Solid-state fermented plant foods as new protein sources. Ann Rev Food Sci Technol 15 (1): 189-210. DOI: 10.1146/annurev-food-060721-013526.

Casillas-Hernández R, Gonzalez-Galaviz JR, Rodriguez-Anaya LZ, Gil-Núñez JC, Del Carmen Rodríguez-Jaramillo M. 2022. Dietary use of methionine sources and *Bacillus amyloliquefaciens* CECT 5940 influences growth performance, hepatopancreatic histology, digestion, immunity, and digestive microbiota of *Litopenaeus vannamei* fed reduced fishmeal diets. Animals 13 (1): 43. DOI: 10.3390/ani13010043.

Chen J, Wang H, Yuan H, Hu N, Zou F, Li C, Shi L, Tan B, Zhang S. 2022. Effects of dietary *Clostridium autoethanogenum* protein on the growth, disease resistance, intestinal digestion, immunity and microbiota structure of *Litopenaeus vannamei* reared at different water salinities. Front Immunol 13: 1034994. DOI: 10.3389/fimmu.2022.1034994.

Coelho RTI, Yasumaru FA, Passos MJACR, Gomes V, Lemos D. 2019. Energy budgets for juvenile Pacific whiteleg shrimp *Litopenaeus vannamei* fed different diets. Braz J Oceanogr 67: e19243. DOI: 10.1590/S1679-87592019024306701.

Dawood MAO, Koshio S. 2020. Application of fermentation strategy in aquafeed for sustainable aquaculture. Rev Aquac 12 (2): 987-1002. DOI: 10.1111/raq.12368.

del Valle DC, Bonadero MC, Fernández-Gimenez AV. 2023. *Saccharomyces cerevisiae* as probiotic, prebiotic, synbiotic, postbiotics and parabiotics in aquaculture: An overview. Aquaculture 569: 739342. DOI: 10.1016/j.aquaculture.2023.739342.

Dewi ADT, Suhartanto B, Astuti A, Astuti D. 2021. The effect of sorghum varieties (*Sorghum bicolor* (L.) Moench) and protein levels on chemical composition and in vitro digestibility of fermented complete feed. Key Eng Mater 884: 171-177. DOI: 10.4028/www.scientific.net/kem.884.171.

Djulardi A, Mirnawati, Ciptaan G, Kurnia R, Srifani A, Adriani L, Makmur M. 2023. Improving the quality and nutritional value of a mixture of sago pith and indigofera leaves fermented with *Rhizopus oligosporus*. World Vet J 13: 580-586. DOI: 10.54203/scil.2023.wvj62.

Eliopoulos C, Markou G, Chorianopoulos N, Haroutounian SA, Arapoglou D. 2022. Preliminary research concerning the enrichment of industrial hemp extract residues via solid state fermentation with *Pleurotus ostreatus*. Appl Sci 12 (5): 2376. DOI: 10.3390/app12052376.

Fahrudin AM, Subandiyono S, Chilmawati D. 2023. Pengaruh protein dalam pakan terhadap efisiensi pemanfaatan pakan dan pertumbuhan juvenil vaname (*Litopenaeus vannamei*). Jurnal Sains Akuakultur Tropis 7 (1): 114-126. DOI: 10.14710/sat.v7i1.17284. [Indonesian]

Fauzi RUA, Sari ERN. 2018. Business analysis of maggot cultivation as a catfish feed alternative. Industria: Jurnal Teknologi Manajemen Agroindustri 7 (1): 39-46. DOI: 10.21776/ub.industria.2018.007.01.5. [Indonesian]

Gariglio M, Dabbou S, Biasato I, Capucchio MT, Colombino E, Hernández F, Madrid J, Martínez S, Gai F, Caimi C, Oddon SB, Meneguz M, Trocino A, Vincenzi R, Gasco L, Schiavone A. 2019. Nutritional effects of the dietary inclusion of partially defatted *Hermetia illucens* larva meal in muscovy duck. J Anim Sci Biotechnol 10: 37. DOI: 10.1186/s40104-019-0344-7.

Guntari PA, Lestariningsih L, Haryuni N. 2022. Evaluation of the utilization of maggot flour in feed on joper chicken performance. J Sci Nusantara 2 (2): 87-92. DOI: 10.28926/jsnu.v2i2.349. [Indonesian]

Gupta RK, Gangoliya SS, Singh NK. 2025. Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. J Food Sci Technol 52 (2): 676-684. DOI: 10.1007/s13197-013-0978-y.

Hamidoghli A, Won S, Farris NW, Bae J, Choi W, Yun H, Bai SC. 2020. Solid state fermented plant protein sources as fish meal replacers in whiteleg shrimp (*Litopaeneus vannamei*). Anim Feed Sci Technol 264: 114474. DOI: 10.1016/j.anifeedsci.2020.114474.

Huervana FS, Traifalgar RFM, Dionela CS. 2024. Solid-state fermentation converts rice bran into a high-protein feed ingredient for *Penaeus monodon*. Front Mar Sci 11: 1384492. DOI: 10.3389/fmars.2024.1384492.

Jin Y, Xu M, Jin Y, Deng S, Tao N, Qiu W. 2022. Simultaneous detection and analysis of free amino acids and glutathione in different shrimp. Foods 11 (17): 2599. DOI: 10.3390/foods11172599.

Klahan R, Deevong P, Wiboonsirikul J, Yuangsoi B. 2023. Growth performance, feed utilisation, endogenous digestive enzymes, intestinal morphology, and antimicrobial effect of pacific white shrimp (*Litopenaeus vannamei*) fed with feed supplemented with pineapple waste crude extract as a functional feed additive. Aquac Nutr 2023: 1160015. DOI: 10.1155/2023/1160015.

Kostova I, Sabeva R, Velyanova G, Isaeva E. 2022. Cadmium concentrations in coals and fly ashes from coal fired thermoelectric power plants in bulgaria. Rev Bulg Geol Soc 83 (3): 101-104. DOI: 10.52215/rev.bgs.2022.83.3.101.

Kurniawan LA, Arief M, Manan A, Nindarwi DD. 2019. Pengaruh pemberian probiotik berbeda pada pakan terhadap retensi protein dan retensi lemak udang vaname (*Litopenaeus vannamei*). J Aquac Fish Health 6 (1): 32-40. DOI: 10.20473/jafh.v6i1.11272. [Indonesian]

Lal J, Vaishnav A, Kumar D, Jana A, Jayaswal R, Chakraborty A, Kumar S, Devati, Pavankalyan M, Sahil. 2024. Emerging innovations in aquaculture: Navigating towards sustainable solutions. Intl J Environ Clim Change 14 (7): 83-96. DOI: 10.9734/ijecc/2024/v14i74254.

Li B, Boukhennou A, Shao J, Miao L, Du Y, Chen J. 2025. Application status and development prospect of fermented ingredients in aquaculture. Aquac Rep 42: 102842. DOI: 10.1016/j.aqrep.2025.102842.

Li X, Yang L, Jiang S, Zhou F, Jiang S, Li Y, Chen X, Yang Q, Duan Y, Huang J. 2023. Effect of fly maggot protein as dietary on growth and intestinal microbial community of pacific white shrimp *Litopenaeus vannamei*. Biology 12 (11): 1433. DOI: 10.3390/biology12111433.

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS. 2002. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiol Mol Biol Rev 66 (3): 506-577. DOI: 10.1128/mmbr.66.3.506-577.2002.

Luthada-raswiswi RW, Brien GO, Mukaratirwa S. 2022. Fishmeal replacement with animal protein source (*Crocodylus niloticus* meat meal) in diets of Mozambique tilapia (*Oreochromis mossambicus*) of different size groups. Appl Sci 12: 1-17. DOI: 10.3390/app12147211.

Macusi ED, Cayacay MA, Borazon EQ, Sales AC, Habib A, Fadli N, Santos MD. 2023. Protein fishmeal replacement in aquaculture: A systematic review and implications on growth and adoption viability. Sustainability 15 (16): 12500. DOI: 10.3390/su151612500.

Marcinčák S, Klempová T, Bartkovský M, Marcinčáková D, Zdolec N, Popelka P, Mačanga J, Čertík M. 2018. Effect of fungal solid-state fermented product in broiler chicken nutrition on quality and safety of produced breast meat. Biomed Res Intl 2018: 2609548. DOI: 10.1155/2018/2609548.

Medeiros L, Nornberg B, Azevedo R, Cardoso A, Rosas VT, Tesser MB, Pedrosa VF, Romano LA, Wasielesky W, Marins LF. 2022. Recombinant *Bacillus subtilis* expressing a fungal phytase as a probiotic additive in the diet of pacific white shrimp *Litopenaeus vannamei.* Res Sq 2022: 1-18. DOI: 10.21203/rs.3.rs-2234500/v1.

Mingmongkolchai S, Panbangred W. 2018. *Bacillus* probiotics: An alternative to antibiotics for livestock production. J Appl Microbiol 124 (6): 1334-1346. DOI: 10.1111/jam.13690.

Mohammed AA, Aslamyah S, Zainuddin Z, Djawad MI. 2024. In vitro evaluation of synbiotics combinations of different inulin concentrations and multi-strains probiotics based on microbial growth and digestive enzymes production. Biodiversitas 25 (10): 3693-3702. DOI: 10.13057/biodiv/d251031.

Mugwanya M, Dawood MAO, Kimera F, Sewilam H. 2022. Replacement of fishmeal with fermented plant proteins in the aquafeed industry: A systematic review and meta-analysis. Rev Aquac 15 (1): 62-88. DOI: 10.1111/raq.12701.

NRC [National Research Council]. 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington.
Nisar U, Peng D, Mu Y, Sun Y. 2022. A solution for sustainable utilization of aquaculture waste: A comprehensive review of biofloc technology and aquamimicry. Front Nutr 8: 791738. DOI: 10.3389/fnut.2021.791738.
Nkhata SG, Ayua E, Kamau EH, Shingiro J-B. 2018. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Sci Nutr 6 (8): 2446-2458. DOI: 10.1002/fsn3.846.
Nnaji J. 2014. Physico-chemical quality and plankton density of water in duck-fish production systems. Am Chem Sci J 4 (6): 975-982. DOI: 10.9734/acsj/2014/11604.
Nunes AJP, Leite JS, Gomes CGD, Dragøy R, Burri L. 2024. The minimum dietary level and mix ratio of krill meal and fishmeal to elicit feed intake and growth performance in juvenile *Penaeus vannamei*. Sustainability 16 (11): 4628. DOI: 10.3390/su16114628.
Obirikorang KA, Amisah S, Skov PV. 2018. Growth performance, feed utilization and sensory characteristics of nile tilapia, *Oreochromis niloticus* fed diets with high inclusion levels of copra meal. J Anim Res Nutr 1 (4): 2572-5459. DOI: 10.21767/2572-5459.100018.
Pham VHT, Kim J, Shim J, Chang S, Chung W. 2022. Purification and characterization of strong simultaneous enzyme production of protease and α-amylase from an extremophile-*Bacillus* sp. FW2 and its possibility in food waste degradation. Fermentation 8 (1): 12. DOI: 10.3390/fermentation8010012.
Prandi B, Samaei SP, Beninati F, De Nardi A, Tedeschi T, Sforza S. 2024. Exploitation of bones-rich poultry by-products to produce protein hydrolysates: Optimization of hydrolysis parameters and chemical characterization. Poult Sci 103: 103924. DOI: 10.1016/j.psj.2024.103924.
Purba M, Prasetyo LH. 2014. Growth and carcass production responses of epmp broiler ducks to various levels of crude fiber and protein in the diet. Jurnal Ilmu Ternak Veteriner 19 (3): 1085. DOI: 10.14334/jitv.v19i3.1085.
Putranto HF, Asikin AN, Kusumaningrum I. 2016. Karakterisasi tepung tulang ikan belida (*Chitala* sp.) sebagai sumber kalsium dengan metode hidrolisis protein. Ziraa'ah 41 (1): 11-20. [Indonesian]
Qisti N, Nugraha A, Najah Z. 2021. Effect of temperature and drying time on chemical characteristics of duck bone meal. Teknika J Sains Teknologi 17 (1): 15-20. DOI: 10.36055/tjst.v17i1.10608.
Rakib MK, Islam MS, Munira S, Koly SF, Biswas AC. 2019. In vitro nutraceuticals elemental research of two Bangladeshi plants. Intl J Unani Integr Med 3 (2): 21-23. DOI: 10.13140/RG.2.2.18306.84169.
Rizwanuddin S, Kumar V, Singh P, Naik B, Mishra S, Chauhan M, Saris PEJ, Verma A, Kumar V. 2023. Insight into phytase-producing microorganisms for phytate solubilization and soil sustainability. Front Microbiol 14: 1127249. DOI: 10.3389/fmicb.2023.1127249.
Salim AA, Grbavčić S, Šekuljica N, Stefanović A, Tanasković SJ, Luković N, Knežević-Jugović Z. 2017. Production of enzymes by a newly isolated *Bacillus* sp. TMF-1 in solid state fermentation on agricultural by-products: The evaluation of substrate pretreatment methods. Bioresour Technol 228: 193-200. DOI: 10.1016/j.biortech.2016.12.081.
Sampathkumar K, Yu H, Loo SCJ. 2023. Valorisation of industrial food waste into sustainable aquaculture feeds. Future Foods 7: 100240. DOI: 10.1016/j.fufo.2023.100240.
Samtiya M, Aluko RE, Dhewa T. 2020. Plant food anti-nutritional factors and their reduction strategies: An overview. Food Prod Proc Nutr 2: 6. DOI: 10.1186/s43014-020-0020-5.
Saputra S, Antoro S, Dhoe SB, Suprayudi MA, Widanarni W. 2023. Nutrient digestibility and enzyme activity of microbial floc, *Tetraselmis chuii*, and *Spirulina platensis* fed on juvenile cobia (*Rachycentron canadum*). E3S Web Conf 442: 02037. DOI: 10.1051/e3sconf/202344202037.
Shao J, Zhao W, Han S, Chen Y, Wang B, Wang L. 2018. Partial replacement of fishmeal by fermented soybean meal in diets for juvenile white shrimp (*Litopenaeus vannamei*). Aquac Nutr 25 (1): 145-153. DOI: 10.1111/anu.12838.
Shao J, Zheng Q, Chen Z, Zhu W, Ren Q, Yuan K, Yang L. 2025. Enzyme-treated soybean meal serves as an effective alternative to fishmeal in the diet of the shrimp *penaeus vannamei*. Aquac Nutr 2025 (1): 2312302. DOI: 10.1155/anu/2312302.
Sharma R, Garg P, Kumar P, Bhatia SK, Kulshrestha S. 2020. Microbial fermentation and its role in quality improvement of fermented foods. Fermentation 6 (4): 106. DOI: 10.3390/fermentation6040106.
Shi X, Luo Z, Chen F, Huang C, Zhu X-M, Liu X. 2016. Effects of dietary cellulase addition on growth performance, nutrient digestibility and digestive enzyme activities of juvenile crucian carp *Carassius auratus*. Aquac Nutr 23 (3): 618-628. DOI: 10.1111/anu.12429.
Siddik MAB, Julien BB, Islam SMM, Francis DS. 2024. Fermentation in aquafeed processing: Achieving sustainability in feeds for global aquaculture production. Rev Aquac 16 (3): 1244-1265. DOI: 10.1111/raq.12894.
Swain BK, Naik PK, Sahoo SK, Mishra SK, Kumar D, Beura CK. 2023. Effect of replacing fishmeal by soybean meal on the performance, nutrient utilization and egg quality of khaki campbell ducks in late laying phase. Indian J Anim Res B-5144: 1-6. DOI: 10.18805/ijar.b-5144.
Tejeda-Miramontes JP, García-Ulloa M, Rodríguez-Quiroz G, Rodríguez-González H. 2023. Substituting fishmeal with extruded cull chickpea meal in diets for the white leg shrimp (*Litopenaeus vannamei*, Boone): A preliminary study of the effect on production parameters. Israeli J Aquac Bamidgeh 75 (2): 1-8. DOI: 10.46989/001c.87519.
Vasyliuk OM, Skrotskyi SO, Khomenko LA, Babich TV. 2023. Probiotics based on lactic acid bacteria for aquaculture. Mikrobiol J 85 (2): 75-92. DOI: 10.15407/microbiolj85.02.075.
Walsh S, Nguyen K, Strebel L, Rhodes M, Davis DA. 2022. Utilising feed effectors and automated feeders for semi-intensive pacific white shrimp (*Litopenaeus vannamei*) production. Aquac Fish Fish 2 (6): 540-551. DOI: 10.1002/aff2.83.
Wang L, Ye L, Hua Y, Zhang G, Li Y, Zhang J, He J, Liu M, Shao Q. 2019. Effects of dietary-methionine (met-met) on growth performance, body composition and haematological parameters of white shrimp (*Litopenaeus vannamei*) fed with plant protein-based diets. Aquac Res 50 (6): 1718-1730. DOI: 10.1111/are.14064.
Wang P, Wang S, Zhu C, Sun Y, Yan Q, Yi G. 2024. *Monascus purpureus* M-32 fermented soybean meal improves the growth, immunity parameters, intestinal morphology, disease resistance, intestinal microbiota and metabolome in Pacific white shrimp (*Litopenaeus vannamei*). Anim Nutr 17: 283-296. DOI: 10.1016/j.aninu.2024.03.009.
Wang Q, Qi Z, Fu W, Pan M, Ren X, Zhang X, Rao Z. 2024. Research and prospects of enzymatic hydrolysis and microbial fermentation technologies in protein raw materials for aquatic feed. Fermentation 10 (12): 648. DOI: 10.3390/fermentation10120648.
Wang X, Song J, Liu Z, Zhang G, Zhang Y. Fermentation quality and microbial community of corn stover or rice straw silage mixed with soybean curd residue. Animals 12: 919. DOI: 10.3390/ani12070919.
Wei C, Wang X, Li C, Zhou H, Liu C, Mai K, He G. 2020. Replacement of fishmeal with *Shewanella* sp. MR-7 fermented soya bean meal in pacific white shrimp. Aquac Res 52: 2110-2120. DOI: 10.1111/are.15063.
Wei H, Tan B, Yang Q, Mau M, Lin Y, Chi S. 2023. Growth, nonspecific immunity, intestinal flora, hepatopancreas, and intestinal histological results for *Litopenaeus vannamei* fed with diets supplement with different animal by-products. Aquac Rep 29: 101521. DOI: 10.1016/j.aqrep.2023.101521.
Weng L, Wang Z, Zhuang W, Yang T, Xu X, Liu J, Liu J, Xu Z, Chen R, Wang Q, Wang S, Cai Y, Ying H. 2023. Effect of replacing fishmeal using fermented soybean meal on growth performance, intestine bacterial diversity, and key gene expression of largemouth bass (*Micropterus salmoides*). Fermentation 9 (6): 520. DOI: 10.3390/fermentation9060520.
Wlazło Ł, Nowakowicz-Dębek B, Ossowski M, Łukaszewicz M, Czech A. 2022. Effect of fermented rapeseed meal in diets for piglets on blood biochemical parameters and the microbial composition of the feed and faeces. Animals 12 (21): 2972. DOI: 10.3390/ani12212972.
Yasmeen R, Ahmad F. 2024. Microbial fermented agricultural waste-based broiler feed: A sustainable alternative to conventional feed. World's Poult Sci J 81 (1): 271-287. DOI: 10.1080/00439339.2024.2443222.
Yeh R-H, Hsieh C-W, Chen K-L. 2022. Two-stage fermented feather meal enhances growth performance and amino acid digestibility in broilers. Fermentation 9 (2): 128. DOI: 10.3390/fermentation9020128.
Yuan H, Hu N, Zheng Y, Hou C, Tan B, Shi L, Zhang S. 2023. A comparison of three protein sources used in medium-sized *Litopenaeus vannamei*: Effects on growth, immunity, intestinal digestive enzyme activity, and microbiota structure. Fishes 8 (9): 449. DOI: 10.3390/fishes8090449.
Zhang X, Sun Z, Cai J, Wang J, Wang G, Zhu Z, Cao F. 2020b. Effects of dietary fish meal replacement by fermented moringa (*Moringa oleifera* Lam.) leaves on growth performance, nonspecific immunity and disease resistance against *Aeromonas hydrophila* in juvenile gibel

carp (*Carassius auratus* gibelio var. CAS III). Fish Shellfish Immunol 102: 430-439. DOI: 10.1016/j.fsi.2020.04.051.

Zhang Y, Chen X, Yu H, Zhang X, Hu S, Chen X. 2022. Investigation of the conversion mechanism of endogenous semicarbazide in shrimp on the amino acid level. Ecotoxicol Environ Saf 249: 114393. DOI: 10.1016/j.ecoenv.2022.114393.

Zhang Y, Lu R, Qin C, Nie G. 2020a. Precision nutritional regulation and aquaculture. Aquac Rep 18: 100496. DOI: 10.1016/j.aqrep.2020.100496.

Zinina O, Merenkova S, Galimov D. 2021. Optimization of microbial hydrolysis parameters of poultry by-products using probiotic microorganisms to obtain protein hydrolysates. Fermentation 7 (3): 122. DOI: 10.3390/fermentation7030122.