Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(5): 1883 – 1896 (2025)
www.ejabf.journals.ekb.eg

Optimizing Liquid Bioslurry Use for Enhancing the Nutritional Quality of Fish Feed

Andi Masriah[1*], St. Zaenab[2], Soraya Ramadani[3], Nurfadilah[2], Nurilmi Achmad[2]

[1] PSDKU Aquaculture, Faculty of Fisheries and Marine Science, Brawijaya University, Kediri, East Java 64111, Indonesia
[2] Study Program of Aquaculture, Faculty of Fisheries, Cokroaminoto Makassar University, Jl. Perintis Kemerdekaan KM.11, Makassar 90245, South Sulawesi, Indonesia
[3] Study Program of Capture Fisheries, Werisar University, Campus Complex 1, Keyen Village, South Sorong Regency 98216, West Papua Province, Indonesia

[*]**Corresponding Author: andimasriah@ub.ac.id**

ARTICLE INFO

Article History:
Received: July 5, 2025
Accepted: Sep. 11, 2025
Online: Oct. 3, 2025

Keywords:
Fish feed,
Liquid bioslurry,
Nutrition,
Protein,
Proximate

ABSTRACT

Nutritional quality of fish feed remains a major challenge in aquaculture, particularly in balancing protein content and feed efficiency while reducing dependence on expensive commercial ingredients. Liquid bioslurry, a by-product of biogas production, has the potential to be used as a sustainable feed additive to improve nutritional quality due to its probiotic content. This study aimed to evaluate the effect of various concentrations of liquid bioslurry on the proximate composition of fish feed. The study was designed using a Completely Randomized Design (CRD) consisting of four treatments (20, 30, 40, and 50 % (*v/w*) of feed) and three replicates. The results of the analysis of variance showed that an increase in bioslurry concentration had a significant effect ($P<0.05$) on the nutrient content of the feed. Higher concentrations increased the moisture, ash, protein, and nitrogen-free extract (NFE) content, while the crude fiber content decreased. The highest fat content was obtained at a concentration of 40 % (*v/w*) of feed before decreasing at higher concentrations. This study shows that the 50 % (*v/w*) of feed is the optimal dose for improving feed nutritional quality, particularly through increased protein and NFE and decreased crude fiber. The use of liquid bioslurry as a fish feed additive is still relatively limited, so this study provides a new contribution to the field of aquaculture feed nutrition. The results of this study have practical implications for the formulation of more economical and environmentally friendly aquaculture feed.

INTRODUCTION

Aquaculture has great potential to address global challenges related to food and nutritional security **(Chizhayeva *et al.*, 2022)**. One of the main challenges in developing efficient and sustainable aquaculture is its dependence on commercial feed. This feed is generally formulated with specific compositions that contain complete nutrients to support optimal fish growth **(Nargesi *et al.*, 2020)**. However, its primary component is often fish meal, which is derived from limited marine resources that are vulnerable to

Indexed in Scopus ELSEVIER DOAJ IUCAT

overexploitation **(Abd Elzaher *et al.*, 2023)**. In addition, the high cost of commercial feed, which accounts for 60–70% of the total production costs, limits profit margins, especially for small- and medium-scale farmers.

To address this challenge, various innovations have been developed, one of which is the addition of feed additives, such as probiotics, in feed formulation development **(El-Saadoni *et al.*, 2021)** to support the expansion of sustainable and environmentally friendly farming practices. The addition of probiotic microorganisms to feed can assist the digestive process in the fish gastrointestinal tract. Probiotics are useful for inhibiting pathogenic microorganisms in the intestines and improving feed efficiency by releasing enzymes that aid digestion **(Wuertz *et al.*, 2021)**. The use of probiotics in aquaculture activities has been widely adopted, for example, the use of *Bacillus* species, as these bacterial strains can serve as effective probiotics for large-scale fish feed production **(Azad *et al.*, 2019; Liaqat *et al.*, 2024)**. The microorganisms in biogas bioactivators are genetically original and not genetically engineered. The effective microorganisms in the bioactivator include *Bacillus* spp. and lactic acid bacteria (*Lactobacillus*) **(Kumar *et al.*, 2023)**.

One potential source of probiotic bacteria that has begun to receive considerable research attention is liquid bioslurry, a byproduct of the biogas fermentation process based on organic waste. Bacterial exploration results showed that the bioslurry contained *Bacillus* strains with probiotic potential and enzymatic activities such as amylase, lipase, and protease **(Zaenab *et al.*, 2025)**. Liquid bioslurry also contains various nutrients, such as nitrogen, phosphorus, and potassium, as well as beneficial probiotic microorganisms **(Zhao *et al.*, 2013)**. These components have the potential to be used as additives in fish feed formulations to enhance the nutritional value, improve digestion, and support fish growth and immunity. Some preliminary studies have indicated that the addition of bioslurry to feed can increase feed utilization efficiency and fish growth performance, although its effectiveness greatly depends on the dosage and processing of the bioslurry prior to application **(FAO, 2013; Zaenab *et al.*, 2022)**. Previous research has shown that feeding fish with feed containing bioslurry results in the best growth rates and feed efficiency in tilapia raised in floating net cages **(Jamaluddin *et al.*, 2024)**.

Therefore, it is necessary to evaluate the capability of liquid bioslurry waste as a probiotic source in fish feed to hydrolyze the nutritional components of fish feed, especially its ability to hydrolyze protein, which is the most expensive raw material in fish feeds. This study aimed to evaluate the effectiveness of various doses of liquid bioslurry as a probiotic source to improve the nutritional quality of fish feed. Specifically, the research focused on assessing changes in proximate composition, including crude protein, crude lipid, crude fiber, and nitrogen-free extract (NFE).

MATERIALS AND METHODS

Research tools and materials

The materials used in this study were commercial fish feed and liquid bioslurry waste obtained as byproducts from a biogas reactor in Pangkajene and Islands Regency, South Sulawesi, Indonesia. The equipment used in this research included an autoclave, oven, Petri dishes, digital scales, syringe, and Laminar Air Flow (LAF).

Research design

This study employed a Completely Randomized Design (CRD) with four treatments and three replicates, resulting in a total of 12 experimental units. The treatments tested involved fermenting commercial feed with various concentrations of liquid bioslurry at room temperature for approximately 24h. The incubation period of 24 hours was chosen because fermentation can proceed optimally during this time frame, thereby inhibiting the growth of undesirable microorganisms, preventing the risk of contamination, and increasing the nutrient and metabolite content of feed ingredients that are beneficial to fish **(Boonmee *et al.*, 2024)**. The treatments tested were as follows **(Zaenab *et al.*, 2022)**:

(A) 20 % (*v/w*) of feed
(B) 30 % (*v/w*) of feed
(C) 40 % (*v/w*) of feed
(D) 50 % (*v/w*) of feed

Work procedure

The research procedure began with the preparation of liquid bioslurry waste obtained from a biogas reactor. The waste was first filtered using cotton to separate coarse solids and insoluble impurities, resulting in a more homogeneous filtrate. The filtrate was then left at room temperature (±27–30 °C) for several hours to stabilize the conditions before use. One hundred grams of commercial fish feed was carefully weighed using an analytical balance, then placed in a Petri dish that had been sterilized beforehand with 70% alcohol. Next, liquid bioslurry waste is added to the feed by spraying it evenly using a sprayer, according to the dosage in the experimental design. The volume of solution for each treatment is adjusted by adding distilled water to reach 50 % (*v/w*) of feed to standardize the liquid input across the entire surface of the feed.

The feed and bioslurry mixture was then incubated in a closed, airtight incubation container that still allowed minimal gas exchange. Incubation was carried out at room temperature (±27–30°C) for approximately 24 hours. After incubation, the fermented feed is then removed and dried using an oven at a low temperature (40– 50°C) until the moisture content is stable, to prevent the growth of contaminating microbes after fermentation. The incubated dry feed is then analyzed using proximate analysis to determine its nutritional quality.

Observed variables

This study observed the nutrient composition of feed after fermentation using liquid bioslurry waste. The nutrient composition of the feed was analyzed using the proximate method, which includes analysis of moisture content, protein, fat, crude fiber, ash, and Nitrogen-free extract (NFE), according to the Association of Official Analytical Chemists standard **(AOAC, 2005)**.

Data analysis

The proximate data of fish feed, consisting of moisture, ash, fat, protein, and crude fiber content after fermentation, were analyzed using analysis of variance (ANOVA) and, if there was a significant effect ($P< 0.05$), further tested with the W test -Tukey HSD test. In addition, a linear regression test was performed to examine the correlation between the concentration of liquid bioslurry in feed and the protein content and feed fiber content using the SPSS application.

RESULTS AND DISCUSSION

The proximate composition of feed nutrients fermented with various concentrations of liquid bioslurry is shown in Table (1).

Table 1. Proximate composition of feed nutrients fermented with various concentrations of liquid bioslurry

Treatment	Parameter (%) ±std					
	Water	Ash	Fat	Protein	Crude fiber	NFE
Without incubation	19.22	10.08	4.25	20.1	15.71	30.02
20% (*v/w*) of feed	$19.26±0.145^{a}$	$10.48±0.195^{a}$	$4.52±0.100^{a}$	$17.95±0.035^{a}$	$15.72±0.100^{c}$	$45.38±0.300^{a}$
30% (*v/w*) of feed	$26.57±0.700^{b}$	$11.83±0.050^{b}$	$4.31±0.135^{ab}$	$19.62±0.040^{b}$	$15.14±0.100^{b}$	$46.47±0.200^{b}$
40% (*v/w*) of feed	$34.53±0.110^{c}$	$11.51±0.120^{b}$	$4.53±0.104^{a}$	$21.81±0.100^{c}$	$14.99±0.038^{b}$	$49.27±0.266^{a}$
50% (*v/w*) of feed	$39.99±0.566^{d}$	$13.25±0.100^{c}$	$4.15±0.100^{b}$	$22.08±0.064^{d}$	$13.57±0.100^{a}$	$53.48±0.895^{c}$

Note: Different superscript letters in the same column indicate significant differences between treatments ($P<0.05$) at a 95% confidence level.

Based on the results of the ANOVA, the application of various concentrations of liquid bioslurry had a significant effect ($P<0.05$) on the moisture, ash, fat, protein, crude fiber, and NFE contents of fish feed. Table (1) shows that the higher the concentration of bioslurry in the fish feed, the higher the moisture, ash, protein, and NFE contents, with successive values of 39.99±0.566%, 13.25±0.100%, 22.08±0.064%, and 53.48±0.895%, respectively. However, this was not the case for fat, crude fiber, and NFE content. The highest fat content was found in the 40ml/ 100g feed treatment, with a value of 4.53±0.104%, whereas the crude fiber content decreased with increasing bioslurry concentration, with the lowest value in the 50% (*v/w*) of feed treatment at 13.57±0.100%. More specifically, the linear regression of the relationship between the concentration of liquid bioslurry in the feed and the change in protein content in the fish feed is presented in Fig. (1), while the linear regression of the relationship between the concentration of liquid bioslurry in the feed and the change in crude fiber content in the fish feed is shown in Fig. (2).

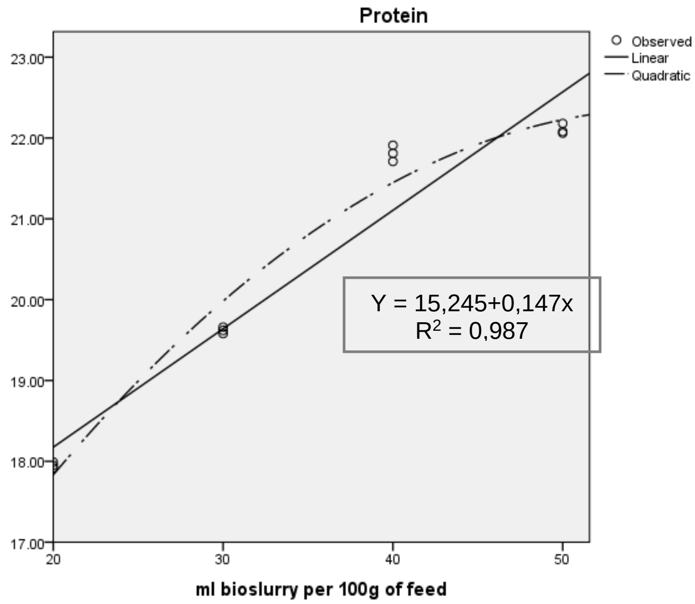

Fig. 1. Linear regression of the relationship between bioslurry concentration (% (*v/w*) of feed) and protein content in fish feed (%)

The graph in Fig. (1) shows that the increase in the dose of liquid bioslurry added to fish feed was directly proportional to the increase in protein content. Linear regression analysis revealed a very strong positive relationship between bioslurry concentration and protein content, with a coefficient of determination (R^2) of 0.987, indicating that approximately 98.7% of the variation in protein content can be explained by variations in bioslurry concentration. Based on observational data, the protein content increased from 17.95% at a dose of 20% (*v/w*) of feed to 19.62% at a dose of 30% (*w/v*), and reached approximately 21–22% at a dose of 40 % (*v/w*) of feed. At the highest dose (50 % (*v/w*) of feed), the protein content reached a maximum value of 22.08%.

The linear model showed a consistent upward trend, whereas the quadratic model indicated an optimum point, where further increases in dosage tended to result in smaller increases in protein. The increase in protein content in feed fermented with liquid bioslurry is related to the activity of microorganisms contained in the bioslurry, such as lactic acid bacteria and cellulose-degrading bacteria. During the fermentation process, these microbes synthesize cell biomass that is rich in protein (microbial protein), which contributes to an increase in total protein in the feed material. This is in line with the findings of **Fachri *et al*. (2024)**, who reported that the addition of probiotics or organic fermentation products can improve the nutritional quality of feed through enzymatic activity, particularly protease, which enhances the availability of nitrogen and essential amino acids **(Melo *et al.*, 2020; Zhang *et al.*, 2021)**.

This increase was likely influenced by microbial activity in the bioslurry, especially *Bacillus* spp., which are known to produce various hydrolytic enzymes, including amylase, protease, and lipase **(Kuebutornye *et al.*, 2019; Zaenab *et al.*, 2025)**. The fermentation process can also increase protein availability and improve the amino acid profile, as reported by **Bernardes *et al*. (2022)**, who showed that enzyme supplementation (phytase and protease) could enhance the digestibility of proteins and amino acids in animal feed ingredients. This confirms that the nutritional quality of feed can be improved, whether through fermentation or enzyme addition, making it more efficiently utilized by livestock or fish. The use of probiotics in fish feed can increase feed utilization efficiency by improving the nutritional value and digestibility **(Irshath *et al.*, 2023)**. Thus, the results of this study confirm that liquid bioslurry can be effectively used to increase the protein content of fish feed, thereby potentially supporting optimal growth, feed efficiency, and fish health in aquaculture. These findings affirm that liquid bioslurry can be effectively utilized to boost the protein content of fish feed, thus supporting optimal fish growth and health in aquaculture.

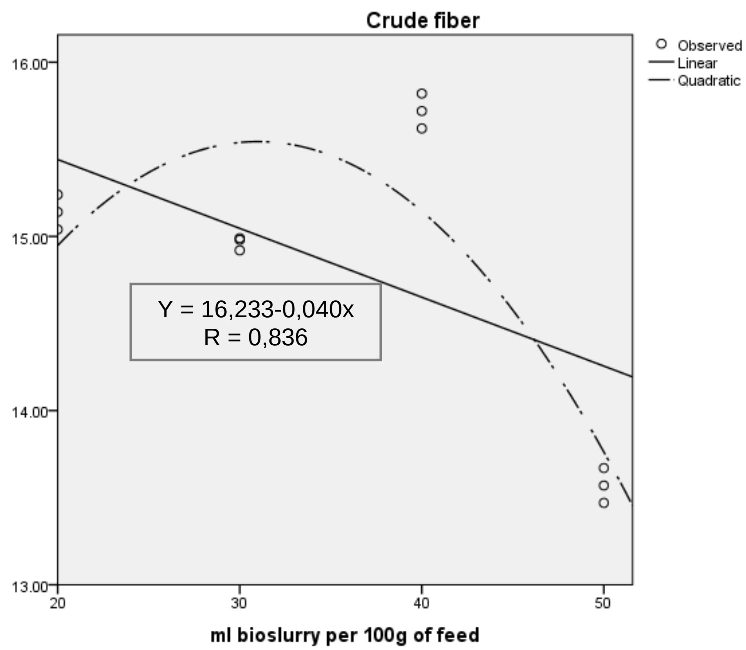

Fig. 2. Linear regression of the relationship between bioslurry concentration (% (*v/w*) of feed) and crude fiber content in the fish feed (%)

Fig. (2) shows that the addition of liquid bioslurry affected the crude fiber content of the fish feed. The linear model illustrated a downward trend in crude fiber as the bioslurry dose increased, whereas the quadratic model showed an initial increase before a sharp decline in crude fiber content at higher doses. This decrease in crude fiber content was caused by the activity of microorganisms in the bioslurry, which produced cellulolytic enzymes, thereby degrading fiber components into simpler molecules that were more easily digested. This finding is consistent with that of **Islam *et al*. (2019)**, who demonstrated that both probiotic and bioslurry fermentation processes can reduce crude fiber content in feed ingredients, thus increasing nutrient availability and digestibility in fish **(Amoah *et al.*, 2019)**.

The graph in Fig. (2) shows a strong negative relationship between bioslurry concentration and crude fiber content in fish feed, with an R^2 value of 0.836, indicating that approximately 83.6% of the variation in crude fiber content can be explained by the variation in bioslurry concentration. The data in the graph show that the higher the concentration of bioslurry, the lower the crude fiber content of the fish feed. Several marked data points indicate crude fiber content at certain concentrations, such as 15.72% at 20% (*v/w*) of feed, 15.14% at 30% (*v/w*) of feed, and 13.57% at 50% (*v/w*) of feed, respectively. These results indicate that liquid bioslurry can reduce the crude fiber content in fish feed, which may be beneficial for improving feed digestibility and nutrient utilization efficiency in fish. The decrease in crude fiber content occurs due to the biodegradation of fiber components (cellulose, hemicellulose, lignin) by microorganisms contained in the liquid bioslurry, which also produces several enzymes such as cellulase, hemicellulase, and lignase. This process breaks down crude fiber into simpler organic compounds, some of which are used as energy sources by microbes, while others contribute to the formation of metabolites such as organic acids. Thus, the measurable crude fiber content in feed materials decreases as the bioslurry dose increases. This is in accordance with **Kaur *et al.* (2018)**, who reported that digested biogas slurry can produce cellulolytic enzymes in the form of cellulose, hemicellulose, and lignin, which are fiber-degrading enzymes.

The significant increase in protein and decrease in fiber in the feed with increasing concentrations of bioslurry waste occur because the nitrogen content of the feed remains constant during the fermentation process, while the microbial metabolism consumes carbohydrates during fermentation, leading to a significant increase in protein content in the feed **(Jiang *et al.*, 2023)**. This is consistent with the findings of **Suardi *et al*. (2023)**, who reported that the soluble protein content in feed increases with an increase in the liquid bioslurry waste concentration in fish feed.

In contrast, the significant decrease in crude fiber content in the feed concurrently with the increase in bioslurry concentration is likely due to the digestion of cellulose by microorganisms capable of producing cellulase during the fermentation process **(Wang *et al.*, 2017; Jiang *et al.*, 2023)**, as bioslurry contains a diversity of bacteria. These bacteria include *Bacillus, Psychrobacter, Carnobacterium, Geobacillus, Acetomicrobium, Acinetobacter, Nitratireductor, Pseudomonas, Acidovorax, Niastella, Sphingobacterium komposti,* and *Acetomicrobium* **(Giri *et al.*, 2013; Apriantika *et al.*, 2022)**. In more detail, the visualization of fluctuations in nutrient content in feed incubated with various concentrations of liquid bioslurry waste is presented through a radar chart diagram in Fig. (3).

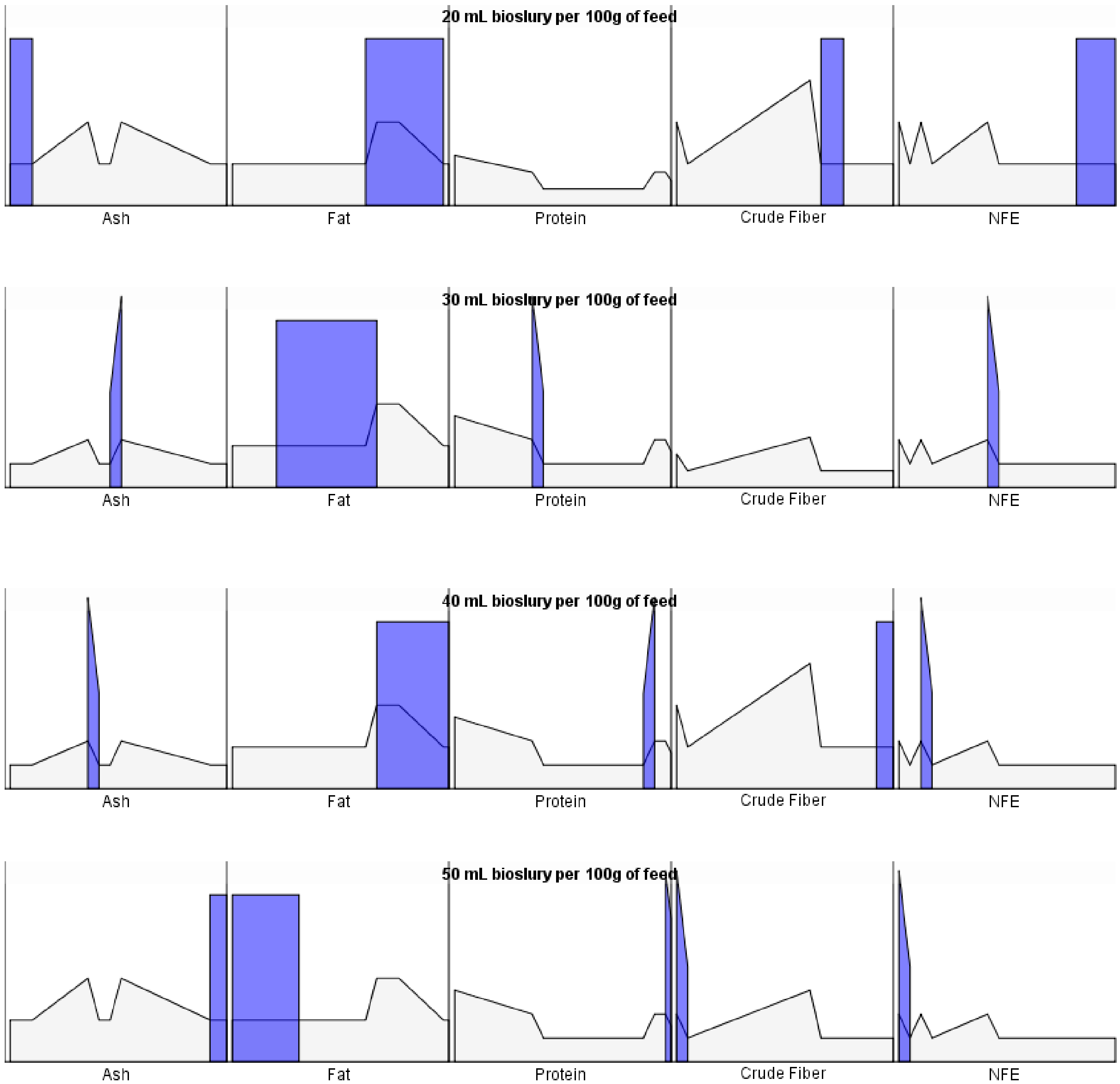

Fig. 3. Area chart diagram of nutrient profile in feed based on proximate analysis (ash, fat, protein, crude fiber, and NFE) in treatments with the addition of 20, 30, 40, and 50 % (*v/w*) of feed

As shown in Fig. (3), the addition of bioslurry to the feed had varying effects on the nutrient composition of the feed, depending on the dose of liquid bioslurry used. Based on the graph presented, administering 20% (*v/w*) of feed resulted in a significant increase in fat content, crude fiber, and nitrogen-free extract (NFE). The ash content also increased significantly, whereas the protein content did not change. However, at a higher dose (30 % (*v/w*) of feed), a greater increase in protein composition was observed, with the highest protein content found in the 50% (*v/w*) of feed treatment. However, at doses between 30 and 50% (*v/w*) of feed, there was a decrease in crude fiber content.

The figure shows the changes in the proximate composition of fish feed after the addition of liquid bioslurry at doses of 20, 30, 40, and 50% (*v/w*) of feed. In general, there was a noticeable shift in the nutrient content values, particularly in the protein and fat fractions.

At a dose of 20% (*v/w*) of feed, the protein and fat contents showed a significant initial increase compared to the control, indicating that bioslurry contributes to improving the nutritional value of the feed. A more pronounced increase occurred at 30–40% (*w/v*), where the protein content reached its optimum point with a sharper rise, in line with the trend of fat, which tended to be stable. This suggests that a moderate dose of bioslurry can enhance enzymatic activity, especially of protease and lipase enzymes **(Zaenab *et al.*, 2025)**, which play a role in breaking down complex substrates into simpler compounds that are more easily utilized by the organism. The bacteria contained in bioslurry are efficient in improving nutritional quality, such as increasing protein content, which can enhance nutrient utilization **(Li *et al.*, 2019; Costa *et al.*, 2020)**.

However, at the highest dose (50% (*w/v*)), although the protein content continued to increase, the trend indicated a tendency toward decreased effectiveness. This suggests the possibility of substrate saturation or the accumulation of metabolites that may inhibit optimal nutrient availability **(Nikoo *et al.*, 2023)**. Meanwhile, the ash, crude fiber, and nitrogen-free extract (NFE) fractions showed relatively minor fluctuations, indicating that bioslurry has a more pronounced effect on protein and fat components than on carbohydrates and minerals. Probiotics can improve the nutritional quality of feed, primarily through the role of certain microorganisms **(Luo *et al.*, 2021)** that produce enzymes that break down complex compounds into simpler, more digestible forms **(Wang *et al.*, 2023)**. The interactions among various types of microorganisms in bioslurry indicate that, in general, the inoculation of mixed probiotics is usually more effective than single probiotic fermentation **(Smid & Lacroix, 2013)**.

These results are consistent with previous findings that reported that supplementation with probiotics or organic fermentation products in feed can improve nutritional quality, enhance digestibility, and modify the chemical profile of feed by improving microbial composition and enzymatic activity **(Jaziri *et al.*, 2023)**. Thus, the addition of liquid bioslurry can be viewed as a potential approach to improving the nutritional value of fish feed, although its use should consider the optimum dosage to achieve more effective and sustainable results.

CONCLUSION

Based on the results of this study, it can be concluded that the use of liquid bioslurry waste as an inoculum for commercial fish feed fermentation can significantly improve feed nutritional quality. The optimal dosage is 50 % (*v/w*) of feed, which can increase crude protein content to 22.08 ± 0.064% while reducing crude fiber content to 13.57 ± 0.100%, thereby improving protein availability and feed digestibility by fish. The use of liquid bioslurry in feed also provides economic benefits by reducing dependence on expensive high-protein feed ingredients and supports sustainability through the utilization of biogas waste. Its potential for large-scale application in aquaculture shows that liquid bioslurry is not only practical but also provides original and significant scientific contributions to the development of sustainable feed innovations.

REFERENCES

Abd Elzaher, H.A.; Ibrahim, Z.A.; Ahmed, S.A.; Salah, A.S.; Osman, A.; Swelum, A.A. and Abd El-Hack, M.E. (2023). Growth, carcass criteria, and blood biochemical parameters of growing quails fed *Arthrospira platensis* as a feed additive. Poultry Science, 102(12): 103205. https://doi.org/10.1016/j.psj.2023.103205

Amoah, K.; Huang, Q.C.; Tan, B.P.; Zhang, S.; Chi, S.Y.; Yang, Q.H.; Liu, H.Y. and Dong, X.H. (2019). Dietary supplementation of probiotic *Bacillus coagulans* ATCC 7050 improves the growth performance, intestinal morphology, microflora, immune response, and disease confrontation of Pacific white shrimp (*Litopenaeus vannamei*). Fish & Shellfish Immunology, 87: 796–808. DOI:10.1016/j.fsi.2019.02.029

AOAC. 2005. Official Methods of Ana lysis of The Association of Official Analytical Chemist. AOAC Inc., Washington.

Apriantika, A.P.; Anwari, R.; Janah, C.N. and Syaichurrozi, I. (2022). Review: Biogas production from cow dung and its potential in Indonesia. World Chemical Engineering Journal, 6(2): 50–56. https://doi.org/10.48181/wcej.v6i2.17994

Azad, M.A.K.; Islam, S.S.; Sithi, I.N.; Ghosh, A.K.; Banu, G.R.; Bir, J. and Huq, K.A. (2019). Effect of probiotics on immune competence of giant freshwater prawn *Macrobrachium rosenbergii*. Aquaculture Research, 50(2): 644–657. https://doi.org/10.1111/are.13942

Bernardes, R.; de Oliveira, C.; Calderano, A.; Ferreira, R.; Dias, K.; de Almeida, B.; Aleixo, P. and Albino, L. (2022). Effect of phytase and protease combination on performance, metabolizable energy, and amino acid digestibility of broilers fed nutrient-restricted diets. Revista Brasileira de Zootecnia, 51. https://doi.org/10.37496/rbz5120210211

Boonmee, T.; Deevong, P.; Rinthong, P.; Yuangsoi, B. (2024). Improvement of nutritive value of soybean meal by microbial hydrolysis with Bacillus subtilis Hs-2 for use as raw material in Nile Tilapia (*Oreochromis niloticus*) diet. Aquaculture Reports., 35. https://doi.org/10.1016/j.aqrep.2024.101943

Chizhayeva, A.; Amangeldi, A.; Oleinikova, Y.; Alybaeva, A. and Sadanov, A. (2022). Lactic acid bacteria as probiotics in sustainable development of aquaculture. Aquatic Living Resources, 35: 10. https://doi.org/10.1051/alr/2022011

Costa, M.; Costas, B.; Machado, M.; Teixeira, C.; Fernández-Boo, S.; Sa, T.; Batista, S.; Marques, A.; Miranda, F. and Valente, L. M. P. (2020). Anchovy and giant squid hydrolysates can enhance growth and the immune response of European seabass (*Dicentrarchus labrax*) fed plant-protein-based diets. Aquaculture., 523: 735182. https://doi.org/10.1016/j.aquaculture.2020.735182

El-Saadony, M.T.; Alagawany, M.; Patra, A.K.; Kar, I.; Tiwari, R.; Dawood, M.A.O.; Dhama, K. and Abdel-Latif, H.M.R. (2021). The functionality of probiotics in aquaculture: An overview. Fish & Shellfish Immunology, 117: 36–52. https://doi.org/10.1016/j.fsi.2021.07.007

Fachri, M.; Amoah, K.; Huang, Y.; Cai, J.; Alfatat, A.; Ndandala, C.B.; Shija, V.M.; Jin, X. and Chen, H. (2024). Probiotics and paraprobiotics in aquaculture: a sustainable strategy for enhancing fish growth, health and disease prevention – a review. Frontiers in Marine Science, 11:1499228. https://doi.org/10.3389/fmars.2024.1499228

Food and Agriculture Organization of the United Nations (FAO). (2013). Bioslurry = Brown Gold? A review of scientific literature on the co-product of biogas production. Environment and Natural Resources Management Working Paper, no. 55, Rome.

Giri, S.S.; Sukumaran, V.; Sen, S.S. and Jena, P.K. (2013). Effects of dietary supplementation of potential probiotic *Bacillus subtilis* VSG4 on the innate immunity and disease resistance of *Labeo rohita* fingerlings. Fish & Shellfish Immunology, 34(2): 660–666. https://doi.org/10.1016/j.fsi.2012.12.008

Irshath, A.; Rajan, A.; Vimal, S.; Prabhakaran, V. and Ganesan, R. (2023). Bacterial pathogenesis in various fish diseases: recent advances and specific challenges in vaccine development. Vaccines, 11(2): 470. https://doi.org/10.3390/vaccines11020470

Islam, M.A.; Biswas, P.; Sabuj, A.A.M.; Haque, Z.F.; Saha, C.K.; Alam, M.M.; Rahman, M.T. and Saha, S. (2019). Microbial load in bio-slurry from different biogas plants in Bangladesh. Journal of Advanced Veterinary and Animal Research, 6(3): 376–383. https://doi.org/10.5455/javar.2019.f357

Jamaluddin, R.; Suardi, A. H.; Zaenab, S.; Masriah, A. and Nurfadilah. (2025). Effectiveness on growth performance and feeding efficiency of tilapia (*Oreochromis niloticus*) through solid bioslurry feed in floating net cages. Journal of Aquaculture and Fish Health, 14(1): 79–90. https://doi.org/10.20473/jafh.v14i1.63715

Jaziri, A.; Shapawi, R.; Mokhtar, R.; Noordin, W. and Huda, N. (2023). Tropical marine fish surimi by-products: utilisation and potential as functional food application. Food Reviews International, 39: 3455–3480. https://doi.org/10.1080/87559129.2021.2012794

Jiang, W.; Jia, X.; Xie, N.; Wen, C.; Ma, S.; Jiang, G.; Li, X.; Chi, C.; Zhang, D. and Liu, W. (2023). Aquafeed fermentation improves dietary nutritional quality and benefits feeding behavior, meat flavor, and intestinal microbiota of Chinese mitten crab (*Eriocheir sinensis*). Animal Nutrition, 14: 1–19. https://doi.org/10.1016/j.aninu.2023.04.002

Kuebutornye, F. K. A.; Abarike, E. D. and Lu, Y. (2019). A review on the application of *Bacillus* as probiotics in aquaculture. Fish & Shellfish Immunology, 87: 820–828. https://doi.org/10.1016/j.fsi.2019.02.010

Kaur, A. and Phutela, U. P. (2018). Cellulolytic Enzyme Production by Trichoderma reesei MTCC 164 Using Cattle Dung and Cattle Dung Slurry of Biogas as a Substrate. International Journal of Pure & Applied Bioscience, 6(1) : 523-531. DOI: http://dx.doi.org/10.18782/2320-7051.5935

Kumar, A.; Verma, L. M.; Sharma, S. and Singh, N. (2023). Overview on agricultural potentials of biogas slurry (BGS): applications, challenges, and solutions. Biomass Conversion and Biorefinery, 13(15). https://doi.org/10.1007/s13399-021-02215-0

Li, C. Q.; Zhang, B. L.; Zhou, H. H.; Wang, X.; Pi, X. E.; Wang, X.; Mai, K. S. and He, G. (2019). Beneficial influences of dietary Aspergillus awamori fermented soybean meal on oxidative homoeostasis and inflammatory response in turbot (*Scophthalmus maximus* L.). Fish & Shellfish Immunology, 93: 8–16. https://doi.org/10.1016/j.fsi.2019.07.037

Liaqat, R.; Fatima, S.; Komal, W.; Minahal, Q.; Kanwal, Z.; Suleman, M. and Carter, C. G. (2024). Effects of *Bacillus subtilis* as a single strain probiotic on growth, disease resistance and immune response of striped catfish (*Pangasius hypophthalmus*). PLoS ONE, 19(1): 1–20. https://doi.org/10.1371/journal.pone.0294949

Luo, J. C.; Long, H.; Zhang, J.; Zhao, Y. and Sun, L. (2021). Characterization of a deep sea *Bacillus toyonensis* isolate: genomic and pathogenic features. Frontiers in Cellular and Infection Microbiology, 11: 629116. https://doi.org/10.3389/fcimb.2021.629116

Melo, J.; Ruiz-Pardo, R.; Hume, M.; Sidjabat, H. and Villamil, L. (2020). Probiotics for cultured freshwater fish. Microbiology Australia, 41. https://doi.org/10.1071/MA20026

Nargesi, E.A.; Falahatkar, B.; and Sajjadi, M. M. (2020). Dietary supplementation of probiotics and influence on feed efficiency, growth parameters and reproductive performance in female rainbow trout (*Oncorhynchus mykiss*) broodstock. Aquaculture Nutrition, 26: 98–108. https://doi.org/10.1111/anu.12970

Nikoo, M.; Regenstein, J. and Yasemi, M. (2023). Protein hydrolysates from fishery processing by-products: production, characteristics, food applications, and challenges. Foods, 12(24): 4470. https://doi.org/10.3390/foods12244470

Smid, E. J. and Lacroix, C. (2013). Microbe–microbe interactions in mixed culture food fermentations. Current Opinion in Biotechnology, 24(2): 148–154. https://doi.org/10.1016/j.copbio.2012.11.007

Suardi, A. H. A.; Alpiani; Masriah, A.; Zaenab, S. and Syamsuddin. (2023). Effectiveness of liquid bioslurry dosage on dissolved protein content in fish feed. Jurnal Perikanan UNRAM., 13(3): 863–870. https://doi.org/10.29303/jp.v13i3.641 [in Indonesia]

Wang, C.; Qiu, X. Z.; Hou, R.; Liu, J.; Li, L. and Mao, X. (2023). Improvement of soybean meal quality by one-step fermentation with mixed-culture based on protease activity. Innovative Food Science and Emerging Technologies, 85: 103311. https://doi.org/10.1016/j.ifset.2023.103311

Wang, Y.; Deng, Q.; Song, D.; Wang, W.; Zhou, H.; Wang, L. and Li, A. (2017). Effects of fermented cottonseed meal on growth performance, serum biochemical parameters, immune functions, antioxidative abilities, and cecal microflora in broilers. Food and Agricultural Immunology, 28(4): 725–738. https://doi.org/10.1080/09540105.2017.1311308

Wuertz, S.; Schroeder, A. and Wanka, K. M. (2021). Probiotics in fish nutrition-long-standing household remedy or native nutraceuticals? Water MDPI., 13(10): 1348. https://doi.org/10.3390/w13101348

Zaenab, S.; Masriah, A.; Suryahman, A. (2022). Effect of bioslurry concentration in feed on the growth and survival of milkfish (*Chanos chanos* Forsskal). International Journal of Applied Biology., 6(2), 249–257. https://doi.org/10.29303/ijoab.v6i2.22150

Zaenab, S.; Zainuddin, Z.; Sriwulan, S.; Nisaa, K.; Haryati, H.; Karim, M. Y. and Anshary, H. (2025). Exploration of bioslurry bacteria candidate probiotics for fish feed: Identification, morphological characteristics and enzyme activity. Egyptian Journal of Aquatic Biology and Fisheries, 29(3): 2251–2268. https://doi.org/10.21608/ejabf.2025.432438

Zhang, Y.; Ishikawa, M.; Koshio, S.; Yokoyama, S.; Dossou, S.; Wang, W.; Zhang, X.; Shadrack, R.S.; Mzengereza, K.; Zhu, K. and Seo, S. (2021). Optimization of soybean meal fermentation for aqua-feed with *Bacillus subtilis natto* using the response surface methodology. Fermentation, 7(4): 306. https://doi.org/10.3390/fermentation7040306

Zhao, H.; Li, J.; Liu, J.; Lü, Y.; Wang, X. and Cui, Z. (2013). Microbial community dynamics during biogas slurry and cow manure compost. Journal of Integrative Agriculture, 12(6): 1087–1097. https://doi.org/10.1016/S2095-3119(13)60488-8