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Enrichment of Cattle Rumen Probiotics in Artificial Diets to Optimize Survival, Growth, and Feed Efficiency of Giant Gourami Sago (*Osphronemus goramy* Lac)

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ABSTRACT

The study was conducted to analyze the effects of supplementing artificial diets with cattle rumen probiotics at different dosages on the growth performance of giant gourami fingerlings (*Osphronemus goramy Lac*) over a 60-day rearing period. A completely randomized design (CRD) was employed, consisting of five treatments with five replicates each. Microbial identification revealed that cattle rumen contained *Bacillus* sp. as the dominant bacterium with probiotic potential, whereas *Listeria* sp. was only sporadically present and exhibited pathogenic characteristics, thus requiring caution. Proximate analysis indicated that cattle rumen had a high moisture content (94.97%), along with protein (0.64%), lipid (0.14%), carbohydrate (3.07%), and ash (1.18%). These results suggest that rumen material constitutes a nutrient-rich substrate that supports the proliferation of fermentative microbes. Furthermore, amino acid profiling demonstrated the presence of essential amino acids such as leucine (2.54%), methionine (2.00%), proline (2.21%), alanine (1.71%), and glutamate (1.21%), which play important roles in enhancing probiotic performance as well as serving as nutritional sources for fish. The highest absolute weight gain was observed in treatment E, with an average of 124.83 ± 5.53^{c}. The greatest absolute length gain was recorded in treatment D, with an average of 0.63 ± 0.07^{a}. The highest mean specific growth rate (SGR) was also obtained in treatment E, averaging 2.70 ± 0.09^{c}. In contrast, the highest survival rate was achieved in treatment A, with an average of 94.44 ± 1.92^{a}. Overall, the results indicate that the optimal growth performance of giant gourami fingerlings was obtained when artificial diets were supplemented with cattle rumen probiotics at a dosage of 50mL/ kg feed, while the lowest performance was observed in the control treatment without probiotic supplementation (0mL/ kg feed).

INTRODUCTION

Food security remains a strategic issue that continues to receive significant attention in national development agendas **(Samara *et al.*, 2024; Islamy *et al.*, 2025;**

Juliannisa *et al.*, 2025). One sector with considerable potential to support food security is aquaculture **(Azrita *et al.*, 2020**; **Serdiati *et al.*, 2024)**. However, various challenges—such as production efficiency, feed quality, and environmental sustainability—continue to hinder the optimal development of this sector **(Bohnes *et al.*, 2022**; **Dalbem Barbosa *et al.*, 2024)**. Therefore, innovation and technological advancement in aquaculture practices are urgently needed, particularly through the utilization of biological resources, including feed enrichment **(Azrita *et al.*, 2018**; **Grayson & Dabrowski, 2022)** and natural probiotics, to enhance productivity and efficiency in fish farming **(Calcagnile *et al.*, 2024; Mohammed *et al.*, 2025)**.

Natural probiotics derived from cattle rumen represent a valuable form of livestock waste, since they contain a diverse array of beneficial microorganisms and are rich in nutrients such as digestive enzymes **(Wang & McAllister, 2002**; **Astuti *et al.*, 2020)**, proteins and amino acids **(Henao *et al.*, 2023)**. The utilization of cattle rumen not only contributes to the management of organic waste but has also been widely applied in agricultural and livestock sectors—for instance **(Oltjen *et al.*, 1996)**, as a component of organic fertilizers fermented animal feed, and probiotic agents aimed at enhancing the health and productivity of both livestock and aquaculture species **(Santra *et al.*, 2003; Edvan & Carneiro, 2011)**. Furthermore, the exploration and identification of indigenous bacteria from such natural sources provide multiple benefits, including the discovery of locally adapted strains with high resilience and biotechnological potential for sustainable applications **(Pardamean *et al.*, 2021)**.

Conversely, no standardized procedures currently exist for the processing of cattle rumen—whether through fermentation, drying, or formulation—to guarantee its stability and effectiveness **(Lee *et al.*, 2023)**. The determination of optimal dosage also remains largely exploratory and lacks a strong scientific foundation. Moreover, the limited number of long-term studies has left the potential positive and negative impacts of rumen application on fish health insufficiently understood. Safety concerns must also be addressed, particularly regarding the possible presence of pathogenic microorganisms or antinutritional compounds if the rumen is improperly processed. These gaps highlight the urgent need for comprehensive and standardized research to fully realize the potential of cattle rumen as a safe and effective source of probiotics or feed enrichment material for aquaculture.

Research on the use of microorganisms as probiotics in aquaculture has continued to evolve, particularly as an alternative to antibiotics and as a means to improve feed efficiency. Numerous studies have identified various microorganisms—such as bacteria, fungi, and protozoa—as potential probiotic agents **(Jiang *et al.*, 2024; Palmonari *et al.*, 2024)**. In parallel, the cattle rumen has been recognized as a natural reservoir of complex microbial communities with significant probiotic potential due to its anaerobic resilience and its capacity to ferment fibrous substrates **(Guo *et al.*, 2018)**.

However, most research on the utilization of cattle rumen has thus far been concentrated in the livestock sector, particularly in the production of silage or fermented animal feed. Its application in aquaculture—especially as a natural probiotic source for freshwater fish—remains highly limited. Moreover, few studies have specifically targeted *Osphronemus goramy* (sago gourami) as an experimental species, despite its status as a high-value aquaculture commodity and its relatively slow growth rate compared to other cultured fish species **(Azrita *et al.*, 2021; Syofriani *et al.*, 2025)** In addition, the majority of probiotic-related studies have not explored the use of locally available organic waste such as cattle rumen, leaving the innovation potential of organic waste-based probiotics largely untapped.

MATERIALS AND METHODS

1. Time and location of the study

This study, which examined the chemical composition of cattle rumen and its potential function as a probiotic ingredient in fish feed formulation, was conducted between June and July 2025. The fermentation process of cattle rumen to produce probiotic material was carried out at the Aquaculture Laboratory of SUPMN Pariaman. Bacteriological isolation and identification were performed at the Veterinary Center **(**Balai Veteriner**)** in Bukittinggi, Agam Regency, West Sumatra Province. The chemical analysis of the fermented cattle rumen was conducted at the General Laboratory Services of CV. Vahana Scientific, Padang City.

2. Tools and materials

The equipment used in this study included 15 aquaria, gauze, fry nets, buckets, a Sojikyo 2000 HPS2 digital scale with a precision of 0.01g, millimeter paper, 30-liter jerry cans, a Lutron WA-2017SDPH water quality measurement kit, a thermometer, a complete aeration system, and a spray bottle. The materials used consisted of cattle rumen, fermented cattle rumen probiotics, commercial feed (PF-500), and 450 sago gourami (*Osphronemus goramy*) juveniles with an average body weight of 2.34 grams and an average length of 4.41cm. The fish were evenly distributed across all treatments and replications, with 30 individuals per group.

3. Research design

This study employed an experimental method using a completely randomized design (CRD) consisting of five treatments with three replications each. The treatments involved the supplementation of cattle rumen probiotics into the feed at varying doses: 0, 20, 30, 40, and 50mL/ kg feed.

4. Research procedure

Cattle rumen samples were collected from a slaughterhouse located in Pariaman City. The samples underwent bacteriological analysis at the Veterinary Center (Balai

Veteriner) in Bukittinggi, Agam Regency, West Sumatra Province, and were also examined for proximate composition and amino acid content at the General Laboratory Services, CV. Vahana Scientific, in Padang City. Subsequently, the cattle rumen was formulated into a probiotic mixture by combining it with palm sugar, finely ground rice bran, and water, followed by a four-day fermentation process.

The resulting probiotic preparation was then mixed with commercial feed at various dosage levels and was allowed to ferment for seven days. The fermented feed was analyzed for proximate composition and amino acid content at the same laboratory (CV. Vahana Scientific, Padang City). Following quality assessment, the fermented feed was administered to juvenile sago gourami (*Osphronemus goramy*).

Feed was provided at a rate of 5% of the total body weight of the fish, with a feeding frequency of three times per day over a 60-day rearing period.

5. Measured parameters

Growth performance and feed utilization of juvenile sago gourami were assessed using several parameters, including final length (cm), final weight (g), weight gain (%), daily weight gain (mg day^{-1}), specific growth rate (SGR; % day^{-1}), net yield (g L^{-1}), coefficient of weight variation (%), coefficient of length variation (%), total feed intake (g), feed conversion ratio (FCR) and feed efficiency **(Effendie, 2002**; **Aryani *et al.*, 2017a)**. Supporting parameters measured in this study included survival rate (%), condition factor, and water quality **(Effendie, 2002**; **Aryani *et al.*, 2017a, b; Syandri & Azrita, 2021)**.

6. Chemical composition analysis of cattle rumen probiotics

The chemical composition of the cattle rumen probiotics was analyzed following the procedures described by **Pratama *et al.* (2019)**, **Islamy *et al.* (2024, 2025)**, with minor modifications to suit the characteristics of the sample. The parameters analyzed included moisture, crude protein, crude lipid, ash, crude fiber, and carbohydrate contents.

Moisture content was determined by oven-drying the samples at 105°C until a constant weight was achieved. Crude protein was measured using the Kjeldahl method, involving digestion, distillation, and titration steps to quantify total nitrogen, which was then converted to protein using a factor of 6.25. Crude lipid was determined using Soxhlet extraction with n-hexane as the solvent. Ash content was obtained by incinerating samples in a muffle furnace at 550 °C for 5 h, while crude fiber was analyzed through sequential acid and alkaline digestion. Carbohydrate content was calculated by difference: 100 – (% moisture + % protein + % lipid + % ash + % fiber). All analyses were conducted in triplicate, and results were expressed as percentages of dry weight. The analytical procedures adhered to standard protocols for proximate composition analysis, as reported described in related studies **(Pratama *et al.*, 2019; Islamy *et al.*, 2024, 2025)**.

7. Data analysis

The research data were compiled and processed using Microsoft Office 2010. The effects of the treatments were analyzed using a one-way analysis of variance **(**One-Way ANOVA**)**. If a significant difference was detected, Duncan's multiple range test was performed to determine differences among treatment groups **(Duncan, 1955)**, with the aid of SPSS software version 20 **(**SPSS Inc., Chicago, USA**)**. Statistical significance was accepted at a 95% confidence level ($P< 0.05$), and all mean values are presented with their corresponding standard deviation (SD).

RESULTS

1. Microbiological analysis of cattle rumen

Laboratory results revealed that isolates obtained from cattle rumen samples contained two dominant bacterial genera: *Bacillus* sp. and *Listeria* sp.

2. Chemical composition analysis of cattle rumen probiotics

The chemical analysis revealed that cattle rumen probiotics contain essential nutrients that are highly beneficial for fish growth.

Table 1. Proximate composition of cattle rumen and fermented rumen probiotics

Component	Value **(**%**)**	
	Raw Cattle Rumen	Fermented Cattle Rumen Probiotic
Moisture	89.49±0.08	94.97±0.18
Ash	2.09±0.01	1.18±0.02
Fat	0.76±0.01	0.14±0.01
Protein	1.56±0.00	0.64±0.00
Carbohydrates	6.10±0.08	3.07±0.17

3. Amino acid profile and nutritional shift in fermented cattle rumen probiotics

The protein content showed a decline from 1.56% to 0.64%, and carbohydrate content decreased from 6.10% to 3.07%. Overall, the fermentation process led to a reduction in ash, fat, protein, and carbohydrate content.

4. Amino acid analysis of fermented cattle rumen probiotics

Subsequent analysis was conducted to determine the amino acid profile of the probiotic product. The amino acid assessment was performed using the HPLC-FLD method.

Table 2. Amino acid composition of cattle rumen before and after fermentation

No.	Amino Acid	Unit	Raw cattle Rumen	Fermented Cattle Rumen Probiotic
			Average	
I	**Essential Amino Acid (EAA):**			
1	L-Histidine	%	0.02±0.00	0.10±0.00
2	L-Threonine	%	0.06±0.00	0.10±0.00
3	L-Valine	%	0.00±0.00	0.00±0.00
4	L-Methionine	%	0.07±0.00	2.00±0.00
5	L-Lysine	%	0.08±0.00	0.71±0.00
6	L-Isoleusine	%	0.00±0,00	0.00±0.00
7	L-Leusine	%	0.01±0.00	2.54±0.00
8	L-Phenylalanine	%	0.00±0.00	0.00±0.00
	Total EAA	%	0.24±0.00	5.45±0.00
II	**Non-Essential Amino Acid (NEAA):**			
9	L-Aspartic Acid	%	0.06±0.00	1.20±0.05
10	L-Serine	%	0.06±0.00	0.23±0.01
11	L-Glutamic acid	%	0.24±0.00	1.21±0.00
12	Glycine	%	0.08±0.00	0.23±0.00
13	L-Alanine	%	0.00±0.00	1.71±0.04
14	L-Proline	%	0.12±0.00	2.21±0.00
15	L-Tyrosine	%	0.03±0.00	0.70±0.00
	Total NEAA	%	0.59±0.00	7.49±0.00

However, the dominant amino acids identified in the fermented product were L-leucine (2.54%), L-proline (2.21%), and L-methionine (2.00%) (Fig. 1).

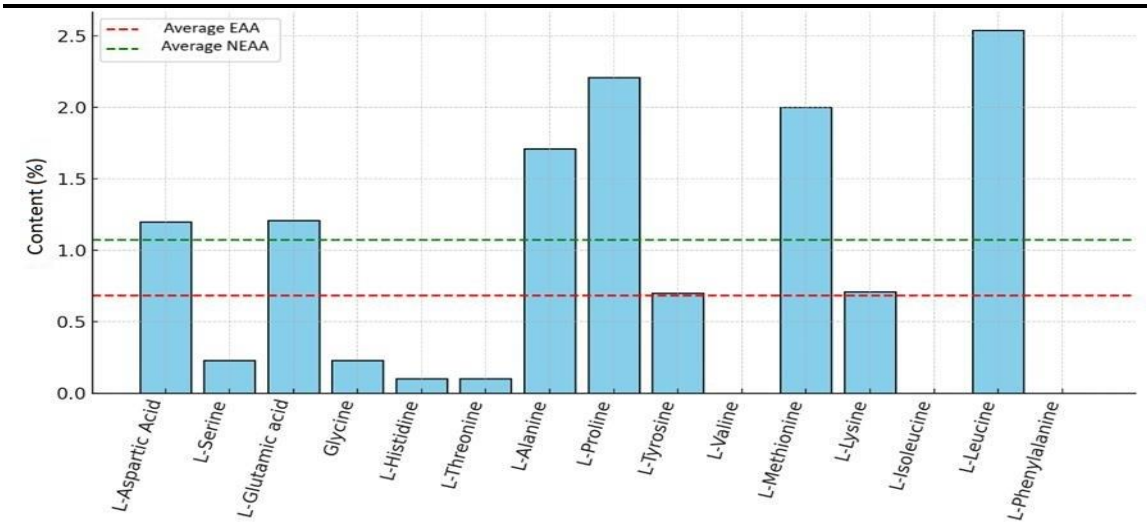

Fig. 1. Amino acid distribution in fermented cattle rumen probiotic

5. Chemical composition and amino acid analysis of experimental feeds

Following the proximate and amino acid analyses of the probiotic product, the probiotic was incorporated into commercial fish feed (PF 500) at different dosages (20, 30, 40, and 50mL/ kg). After four days of fermentation, proximate and amino acid analyses were conducted, as shown in Tables (3, 4).

Table 3. Proximate composition of feed supplemented with varying doses of probiotic

Nutrient Component	Unit	Probiotic Dosage (mL/kg Feed)				
		0	20	30	40	50
Moisture	%	19.90±0.30^{a}	20.16±0.03^{a}	24.52±0.14^{b}	21.29±0.15^{c}	21.30±0.15^{d}
Protein	%	30.55±0.96^{a}	32.09±0.15^{b}	27.06±0.19^{c}	31.46±0.29^{d}	33.01±0.15^{e}
Fat	%	3.98±0.04^{a}	4.12±0.01^{b}	3.68±0.05^{c}	3.63±0.06^{d}	3.68±0.03^{e}
Ash	%	8.14±0.02^{a}	8.78±0.07^{b}	8.37±0.05^{c}	8.76±0.08^{d}	8.82±0.06^{e}
Carbohydrates	%	33.89±0.75^{a}	34.86±0.12^{b}	36.37±0.37^{c}	34.87±0.06^{d}	33.19±0.38^{a}

Note: Different superscript letters (a, b, c, etc.) within the same row indicate statistically significant differences ($P< 0.05$) among treatments.

The amino acid analysis revealed that the highest combined content of essential and non-essential amino acids was observed in the feed supplemented with 50mL/ kg probiotic, with an average concentration of 2.54%. The lowest amino acid content occurred in the 30mL/ kg treatment. L-aspartic acid was the most abundant (3.28%), and L-histidine had the lowest concentration (1.00%).

Table 4. Amino acid composition **(**%**)** of feed supplemented with different probiotic dosages

No.	Amino Acid **(**%**)**	Probiotic Dosage **(**mL/kg Feed**)**				
		0	20	30	40	50
I	**Essential Amino Acids (EAA)**					
1	L-Histidine	0.76±0.01^{a}	0.87±0.02^{b}	0.81±0.00^{c}	1.15±0.02^{d}	1.18±0.00^{e}
2	L-Threonine	1.76±0.04^{a}	1.85±0.01^{b}	1.83±0.00^{b}	2.42±0.03^{c}	2.54±0.01^{d}
3	L-Valine	2.62±0.00^{a}	2.70±0.00^{b}	2.49±0.00^{c}	2.80±0.10^{d}	2.91±0.00^{e}
4	L-Methionine	1.46±0.00^{a}	1.57±0.00^{b}	1.89±0.04^{c}	1.80±0.05^{d}	1.62±0.04^{e}
5	L-Lysine	2.63±0.05^{a}	2.74±0.04^{b}	2.39±0.00^{c}	3.12±0.04^{d}	2.67±0.04^{b}
6	L-Isoleusine	2.72±0.05^{a}	2.81±0.03^{b}	1.73±0.05^{c}	3.04±0.05^{d}	3.24±0.02^{e}
7	L-Leusine	2.71±0.00^{a}	2.83±0.00^{b}	3.16±0.00^{c}	3.08±0.01^{d}	2.58±0.01^{e}
8	L-Phenylalanine	2.14±0.00^{a}	2.27±0.00^{b}	2.32±0.05^{b}	2.26±0.00^{b}	3.84±0.05^{c}
	Total EAA	17.80±0.15	17.64±0.10	16.62±0.14	19.67±0.30	20.58±0.17
II	**Non-Essential Amino Acids (NEAA)**					
1	L-Aspartic Acid	3.14±0.00^{a}	3.26±0.00^{b}	3.27±0.02^{b}	3.29±0.00^{bc}	3.31±0.00^{c}
2	L-Serine	2.49±0.00^{a}	2.61±0.00^{b}	2.33±0.00^{c}	2.60±0.00^{d}	2.61±0.00^{b}
3	L-Glutamic acid	3.50±0.00^{a}	3.60±0.00^{b}	3.62±0.03^{b}	3.59±0.00^{b}	3.56±0.05^{b}
4	Glycine	1.71±0.00^{a}	1.84±0.00^{b}	1.84±0.00^{b}	1.85±0.01^{b}	1.83±0.01^{c}
5	L-Alanine	1.05±0.04^{a}	1.12±0.01^{b}	0.12±0.00^{c}	2.04±0.01^{d}	1.87±0.01^{e}
6	L-Proline	2.30±0.25^{a}	2.45±0.20^{a}	2.55±0.10^{a}	2,65±0.00^{c}	2.05±0.00^{d}
7	L-Tyrosine	1.63±0.00^{a}	1.76±0.00^{b}	1.86±0.03^{c}	2.28±0.03^{d}	2.29±0.03^{e}
	Total NEAA	15.82±0.29	16.64±0.21	16.59±0.18	18.30±0.29	17.52±0.09

Note: Superscript letters **(**^{a}, ^{b}, ^{c}, etc.**)** indicate significant differences **(***P*<0.05**)** between treatments within the same row.

6. Growth performance

Growth performance parameters—including absolute weight gain, absolute length gain, specific growth rate **(**weight**)**, specific growth rate **(**length**)**, thermal growth coefficient **(**TGC**)**, harvest yield, and survival rate—showed significant differences **(***P*< 0.05**)** among treatments. The best performance was observed in the 50mL/ kg treatment **(**Treatment E**)**. During the study, rearing temperature ranged from 28.9– 29.8°C, and dissolved oxygen ranged from 7.0– 7.1mg/ L.

Table 5. Growth performance of giant gourami **(***Osphronemus goramy***)** juveniles during 60 days of rearing

Parameter	Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
	0 mL/kg **(**Control**)**	20 mL/kg Probiotic Dosage	30 mL/kg Probiotic Dosage	40 mL/kg Probiotic Dosage	50 mL/kg Probiotic Dosage
Initial Weight **(**g**)**	70.03±0.38 ^{a}	70.43±0.42 ^{a}	70.07±0.38 ^{a}	70.00±0.17 ^{a}	70.47±0.81 ^{a}
Final Weight **(**g**)**	130.40±4.61 ^{a}	138.17±2.19 ^{b}	127.77±2.65 ^{a}	136.53±1.42 ^{b}	**158.40±2.43** ^{c}
Initial Length **(**cm**)**	4.60±0.10 ^{b}	4.47±0.15 ^{ab}	4.20±0.10 ^{a}	4.13±0.15 ^{a}	**4.63±0.38** ^{b}

Final Length (cm)	6.67±0.15 [a]	6.83±0.32 [a]	6.70±0.17 [a]	6.73±0.38 [a]	6.97±0.21 [a]
Absolute Weight Gain (g)	86.22±7.31 [a]	96.17±2.75 [b]	82.36±4.03 [a]	95.05±1.69 [b]	**124.83±5.53 [c]**
Absolute Length Gain (cm)	0.45±0.05 [a]	0.53±0.07 [a]	0.59±0.08 [a]	0.63±0.07 [a]	0.51±0.17 [a]
Specific Growth Rate – Weight (%/day)	2.07±0.13 [a]	2.25±0.05 [b]	2.00±0.07 [a]	2.23±0.03 [b]	**2.70±0.09 [c]**
Specific Growth Rate – Length	1.24±0.13 [a]	1.42±0.16 [a]	1.56±0.16 [a]	1.62±0.14 [a]	1.37±0.38 [a]
Thermal Growth Coefficient (TGC)	1.96±0.13 [a]	2.15±0.05 [b]	1.89±0.08 [a]	2.13±0.03 [b]	**2.65±0.09 [c]**
Harvest Yield (g/fish)	4.08±0.17 [a]	4.41±0.11 [b]	3.99±0.13 [a]	4.39±0.04 [b]	**5.22±0.13 [c]**
Survival Rate (SR, %)	94.44±1.92 [a]	93.33±3.33 [a]	93.33±3.33 [a]	88.89±5.09 [a]	93.33±3.33 [a]

Note: Superscript letters ([a], [b], [c]) within the same row indicate statistically significant differences (P<0.05) among treatments based on post-hoc analysis.

DISCUSSION

The results of this study highlight the significant functional roles of *Bacillus* sp. as both enzymatic and probiotic agents in aquaculture systems. Members of this genus are widely recognized for their ability to produce extracellular enzymes—such as proteases, amylases, lipases, and cellulases—that catalyze the degradation of complex macromolecules in feed, including proteins, carbohydrates, and lipids **(Nirmala *et al.*, 2021; Yu *et al.*, 2022; Sawant *et al.*, 2025)**. In the context of sago gourami aquaculture, this enzymatic capacity is particularly relevant, as the species requires high-fiber diets and exhibits relatively slow digestion. The presence of *Bacillus*-based probiotics can enhance feed digestibility, thereby improving feed conversion efficiency (FCR) **(Atef *et al.*, 2024; Shija *et al.*, 2025)**

Beyond their digestive contributions, *Bacillus* spp. exert pronounced biocontrol effects in aquaculture environments **(Hlordzi *et al.*, 2020; Keshmirshekan *et al.*, 2024)**. They synthesize a variety of antimicrobial compounds—bacteriocins, lipopeptides (iturin, fengycin, surfactin), and siderophores—that inhibit the proliferation of pathogenic microorganisms through competitive exclusion and interference with nutrient availability **(Markelova *et al.*, 2025)**. These mechanisms are effective against major fish pathogens such as *Aeromonas hydrophila*, *Edwardsiella tarda*, and *Vibrio* spp., thereby improving the microbial balance of the culture system and reducing disease incidence **(Purkait *et al.*, 2018**; **Santos *et al.*, 2021)**.

In contrast, *Listeria* sp. detected in rumen isolates are not considered beneficial microorganisms. Although present in the gastrointestinal tracts of ruminants, they constitute a minor portion of the rumen microbiome, which is typically dominated by fermentative bacteria such as *Ruminococcus*, *Fibrobacter*, and *Butyrivibrio* involved in fiber and carbohydrate metabolism **(Mousa *et al.*, 2025)**. *Listeria monocytogenes* may persist as a transient contaminant in asymptomatic cattle and is generally regarded as a potential zoonotic pathogen **(Ribeiro *et al.*, 2023)**. Factors such as physiological stress, immunosuppression, poor feed quality, or environmental contamination can promote its

survival. Therefore, its presence in rumen samples should be interpreted as incidental contamination rather than as an active or beneficial microbial component **(Cardenas-Alvarez *et al.*, 2022)**.

Proximate analysis revealed notable biochemical alterations following rumen fermentation. The moisture content increased markedly **(**89.49 to 94.97%**)**, which can be attributed to the accumulation of fermentation-derived metabolites such as organic acids, ethanol, and carbon dioxide **(Rahmadani *et al.*, 2025)**. Concurrently, reductions in ash **(**2.09% → 1.18%**)**, fat **(**0.76% → 0.14%**)**, protein **(**1.56% → 0.64%**)**, and carbohydrates **(**6.10% → 3.07%**)** indicate microbial utilization of these nutrients as substrates for metabolism and enzyme production **(Goodenough & Kleyn, 1976; Adebo *et al.*, 2022; Fu *et al.*, 2024; Zhang *et al.*, 2024)**. Although nutrient concentrations declined, microbial proliferation during fermentation increased the functional and probiotic value of the product, supporting its classification as a biological feed additive rather than a direct nutritional source **(Aliyu *et al.*, 2019; Anyiam *et al.*, 2023; Siddik *et al.*, 2024)**. Overall, the fermentation process led to a general reduction in the basic nutritional components—ash, fat, protein, and carbohydrates **(Anyiam *et al.*, 2023)**. However, this was accompanied by a significant increase in the functional value of the product due to the proliferation of live microbial populations and the accumulation of fermentation-derived metabolites. This observation reinforces the conclusion that fermented cattle rumen serves more effectively as a biological supplement to enhance the health and performance of cultured aquatic organisms, rather than as a primary source of nutrients **(Aliyu *et al.*, 2019; Siddik *et al.*, 2024)**.

The amino acid composition further underscores the nutritional enhancement achieved through fermentation. Dominant amino acids—including L-leucine (2.54%), L-proline (2.21%), and L-methionine (2.00%)—play critical physiological roles in muscle protein synthesis, antioxidant defense, and stress resilience **(Dhiman *et al.*, 2025; Yang *et al.*, 2025)**. Additional amino acids such as L-alanine, L-glutamic acid, L-aspartic acid, L-tyrosine, and L-lysine were also detected in moderate concentrations, supporting energy metabolism, neurotransmitter synthesis, and structural protein formation essential for fish growth and development **(Falco *et al.*, 2020; Dou *et al.*, 2023; Salamanca *et al.*, 2025; Wang *et al.*, 2025)**.

Incorporation of the fermented rumen probiotic into commercial fish feed significantly influenced its proximate composition across different inclusion levels **(**20–50mL/ kg**)**. The 30mL/ kg treatment exhibited the highest moisture content, likely due to increased microbial metabolic activity, whereas the 50mL/ kg treatment produced the highest ash and protein contents, reflecting enhanced mineral solubilization and protein hydrolysis **(Chowdhury & Roy, 2020)**. Conversely, fat and carbohydrate levels declined with increasing probiotic dosage, suggesting active lipid and carbohydrate utilization by the microbial community during fermentation. These findings indicate that probiotic

supplementation can improve feed digestibility and nutrient bioavailability, particularly for protein and minerals—two essential components for optimal fish growth and physiological performance.

Amino acid enrichment was most pronounced at the 50mL/ kg dosage, where the combined essential and non-essential amino acid content reached 2.54%, dominated by L-aspartic acid **(**3.28%**)**. This improved amino acid profile likely enhanced digestive efficiency and metabolic energy balance, contributing to better growth outcomes **(Yang & Liao, 2019)**.

Growth performance data corroborated the biochemical findings, demonstrating significant improvements **(***P*< 0.05**)** in absolute weight gain, specific growth rate **(**SGR**)**, thermal growth coefficient **(**TGC**)**, and harvest yield, particularly in the 50mL/ kg treatment. Survival rate remained consistently high across treatments, indicating that probiotic inclusion did not induce physiological stress or adverse effects. The rearing conditions—temperature **(**28.9– 29.8°C**)** and dissolved oxygen **(**7.0– 7.1mg/ L**)**—were within the optimal range for *O. goramy* culture **(Syofriani *et al.*, 2025)**, ensuring that growth improvements were primarily attributable to probiotic supplementation.

Overall, these findings demonstrate that the incorporation of *Bacillus*-dominated, cattle-rumen-derived probiotics into commercial feed can effectively enhance nutrient digestibility, amino acid balance, and growth performance in giant gourami culture. This study provides empirical support for the use of livestock-derived probiotics as a sustainable and cost-effective biotechnological innovation to optimize aquaculture production and feed utilization efficiency while contributing to circular resource management within integrated aquaculture systems.

Future studies should integrate genomic approaches to elucidate the microbial composition and functional diversity of rumen-derived probiotics **(Wang *et al.*, 2024)**. High-throughput 16S rRNA, metagenomic and DNA metabarcoding analyses are recommended to identify key species **(Valen *et al.*, 2023**; **Nazran *et al.*, 2025)**, bacterial taxa, enzymatic genes, and antimicrobial biosynthetic pathways involved in fermentation **(Jiang *et al.*, 2025)**. Whole-genome sequencing of dominant *Bacillus* strains should be conducted to verify probiotic safety and genetic potential. Integrating multi-omics data—metagenomics, metatranscriptomics, and metabolomics—will further clarify the molecular mechanisms underlying probiotic efficacy, supporting the development of genetically characterized, safe, and sustainable microbial formulations for aquaculture applications **(Toropov *et al.*, 2020)**.

CONCLUSION

The identification results indicated that *Bacillus* sp. was the dominant bacterial genus present in cattle rumen, showing strong probiotic potential. In contrast, *Listeria* sp. was detected only sporadically and is considered pathogenic, warranting caution in its

application. Proximate analysis of the cattle rumen revealed high moisture content (94.97%), along with measurable levels of protein (0.64%), fat (0.14%), carbohydrates (3.07%), and ash (1.18%). These values suggest that the rumen substrate is nutrient-rich and suitable for supporting the growth of fermentative microorganisms. Furthermore, the amino acid profile showed the presence of several important amino acids, including leucine (2.54%), methionine (2.00%), proline (2.21%), alanine (1.71%), and glutamic acid (1.21%), which contribute to probiotic functionality and serve as essential nutrients for fish. Overall, the best growth performance of giant gourami (*Osphronemus goramy*) was observed in the treatment supplemented with 50mL/ kg of cattle rumen probiotic, while the lowest performance was recorded in the control group (0mL/ kg), indicating a dose-dependent effect of probiotic supplementation on fish growth.

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