Research Article
doi.org/10.56093/ijan.v43i2.178732

Yeast fermented *Azolla pinnata* meal in diets of silver barb *Barbonymus gonionotus*: Growth performance, protein digestibility and muscle composition under mid-hill conditions of Meghalaya

Edited by: Pradeep Kumar Malik
National Institute of Animal Nutrition and Physiology, Bengaluru, Karnataka

Reviewed by: Baidya Nath Paul
Central Institute of Freshwater Aquaculture, Bhubaneswar, Odisha

*Correspondence: Chandan Debnath
chandannath23@gmail.com

Received: 6th May 2026
Accepted: 21st May 2026
Published: 20th August 2026 (Online)

Citation: Debnath C, Singh SG, Tayung T, Ghatak S, Mahanta P. Yeast fermented Azolla pinnata meal in diets of silver barb Barbonymus gonionotus: Growth performance, protein digestibility and muscle composition under mid-hill conditions of Meghalaya. Indian Journal of Animal Nutrition. 2026 July 13; 43(2). doi.org/10.56093/ijan.v43i2.178732

Copyright: © 2026 Debnath, Singh, Tayung, Ghatak, Mahanta. This is an open-access article

Chandan Debnath*, S. Gojendro Singh, Tasso Tayung, S. Ghatak, Prasanta Mahanta

ICAR Research Complex for NEH Region, Meghalaya, PIN: 793103
**corresponding author (chandannath23@gmail.com; Contact: 09862689090*

A 45-day feeding trial in 1000-litre cemented tanks under mid-hill conditions of Meghalaya (mean water temperature 20.8±0.9°C) evaluated the effect of graded replacement of unfermented *Azolla pinnata* meal (UAM) with yeast fermented *Azolla pinnata* meal (FAM) on growth performance, survival, condition factor, apparent protein digestibility and muscle proximate composition of juvenile silver barb (*Barbonymus gonionotus*). Five isonitrogenous diets with fish meal (23%), mustard oil cake (25%) and rice bran (30%) fixed identically were formulated with 0% (T1), 25% (T2), 50% (T3), 75% (T4) and 100% (T5) FAM replacement of the 20% UAM component. Fish (initial weight 12.21 ± 1.31 g, 20/ tank, triplicate) were sampled on days 0, 7, 15, 30 and 45. Specific growth rate improved progressively from T1 (0.84 ± 0.07 % day^{-1}) to T4 (1.16 ±0.07 % day^{-1}), feed conversion ratio from 2.61 ± 0.21 to 2.08±0.14, and apparent protein digestibility from 72.14 ± 1.84% to 83.47 ± 1.62%, with T4 and T5 statistically comparable in all parameters ($P > 0.05$). Survival, condition factor and muscle crude protein followed the same trend. Results suggest yeast fermentation reduces antinutritional factors and improves protein digestibility of azolla, with 75% FAM replacement offering a practical optimum for silver barb under hill aquaculture conditions.

Keywords: Fermented azolla, *Barbonymus gonionotus,* Growth performance, Protein digestibility, Hill aquaculture

Introduction

Silver barb (*Barbonymus gonionotus* Bleeker, 1850) is a widely cultivated medium-sized carp in South and Southeast Asia, valued for its rapid growth, adaptability to plant-based diets and tolerance of variable environmental conditions (Jena et al., 2007). In the hill climate of Meghalaya, the species has shown considerable aquaculture potential, with ICAR-NEH Region trials recording satisfactory growth in pond and cage systems (Singh et al., 2024) and a polyculture yield of 1,350 kg/ha over six months, where *B. gonionotus* attained 189.12 g final weight with 81.25% survival (Das and Mahanta, 2025). In eastern India and Bangladesh, the species is commonly reared on farm-made feeds using rice bran, mustard oil cake and fish meal (Mondal et al., 2019; Mazid et al., 1997), and rising ingredient costs have generated interest in locally available, low-cost protein supplements that can be incorporated without compromising fish performance (Jahan et al., 2021).

Azolla pinnata, a free-floating aquatic pteridophyte growing abundantly in the paddy fields of Northeast India, offers considerable potential in this context. Through symbiosis with *Anabaena azollae*, it accumulates biomass rapidly and contains 22 to 29% crude protein on a dry matter basis, along with essential amino acids, minerals and vitamins (Swain et al., 2022; Datta et al., 2011). Dried *A. pinnata* can substitute up to 50% of mustard oil cake in silver barb fry diets without significant growth depression, with an optimal dietary inclusion of 47.8% established through regression analysis (Debnath, 2025). A recognised limitation, however, is the presence of antinutritional factors (ANFs), particularly tannins and phytic acid, which inhibit digestive enzyme activity and impair protein digestibility when unfermented azolla is included beyond the tolerance threshold of the target species (Bhadake et al., 2024; Mpwaga et al., 2023; Francis et al., 2001).

Yeast solid-state fermentation (SSF) offers a practical means of addressing this constraint by generating extracellular proteases, cellulases and phytases that degrade ANFs and improve protein solubility (Siddik et al., 2024; Agboola et al., 2021). Fermentation has improved growth and protein digestibility in rohu fed plant-based diets (Das et al., 2021), and growth benefits of fermented over unfermented azolla have been demonstrated in tilapia (Ismail et al., 2022; Refaey et al., 2023), yet no comparable study exists for *B. gonionotus* under hill aquaculture conditions. We hypothesised that progressive replacement of unfermented azolla meal with yeast fermented azolla meal would reduce dietary ANF load in a dose-dependent manner, thereby improving protein digestibility, growth performance and muscle composition of juvenile *B. gonionotus*. The present study therefore evaluated graded replacement at 0%, 25%, 50%, 75% and 100% within a fixed 20% dietary azolla inclusion under mid-hill conditions of Meghalaya.

Materials and methods

Study location and fish

The trial was conducted at Umiam, Meghalaya, northeastern India. A 45-day trial duration was considered adequate for preliminary screening of dietary treatments under controlled tank conditions, consistent with similar short-duration growth evaluations in *B. gonionotus* (Jahan et al., 2020) and *azolla*-based cyprinid feeding studies (Naji and Alzurfi, 2023). Juvenile fish with a mean initial body weight of 12.21 ± 1.31 g were obtained from ICAR farm and acclimatised for 7 days on a commercial diet (25 % CP) before the trial commenced.

doi.org/10.56093/ijan.v43i2.178732

Cultivation and preparation of azolla meal

Azolla pinnata was collected from local paddy fields in Meghalaya and identified on the basis of its distinctly triangular pinnate plant form, green non-reddish colouration, acute leaf apices and 2 to 3 roots per node (Kumar et al., 2018; Lellinger et al., 1983). Authenticated material was subsequently cultivated in outdoor concrete beds fertilized weekly with diluted cow dung slurry and superphosphate. Harvested biomass was washed, shade-dried for 48 h and 60°C for 24 hrs. One portion was retained as UAM after grinding to 0.5 mm. For FAM, dried azolla powder was rewetted to 50 to 55% moisture, inoculated with fresh baker's yeast (*Saccharomyces cerevisiae*, procured from a local grocery shop, Barapani, Meghalaya) at 5 g/kg dry substrate (Manoppo and Kolopita, 2016), mixed uniformly and incubated under aerobic solid-state conditions at 30 ± 1°C for 48 hours following the SSF protocol of Siddik et al. (2024). The fermented material was re-dried at 60°C for 24 hrs, reground to 0.5 mm and stored at room temperature until use. Substrate pH was measured before inoculation and after 48 hours of incubation using a calibrated digital pH meter on a 1:5 substrate-to-distilled-water suspension, declining from 6.42 ± 0.11 to 4.87 ± 0.09. Organoleptic characteristics of UAM and FAM were recorded: UAM presented a dull olive-green colour, grassy odour and fibrous texture, whereas FAM showed a darker brownish hue, yeasty and mildly acidic odour, and more friable texture. Both UAM and FAM were assessed for proximate composition (AOAC, 2005), tannin content by the vanillin-HCl colorimetric method (Price et al., 1978) and phytic acid by the Wade reagent colorimetric method (Haug and Lantzsch, 1983) prior to diet preparation (Table 1).

Table 1. Proximate composition and anti-nutritional factor content of unfermented (UAM) and yeast fermented (FAM) *Azolla pinnata* meal (dry matter basis)

Parameter	UAM	FAM	% Change
Crude protein (%)	23.14 ± 0.42	27.86 ± 0.51	+20.39
Crude fat (%)	3.81 ± 0.24	4.12 ± 0.29	+8.14
Crude fibre (%)	15.24 ± 0.38	11.37 ± 0.31	−25.39
Total ash (%)	10.42 ± 0.31	9.84 ± 0.27	−5.57
Tannin (mg/g DM)	8.42 ± 0.61	4.91 ± 0.43	−41.69
Phytic acid (g/kg DM)	6.18 ± 0.47	2.84 ± 0.32	−54.05
Substrate pH	6.42 ± 0.11	4.87 ± 0.09	−24.14

Note: Values are mean ± SD of triplicate analyses. % change = [(FAM − UAM) / UAM] × 100

Experimental diets

Five iso-nitrogenous diets were formulated with fish meal (23%), MOC (25%), rice bran (30%) and vitamin-mineral premix (2%) fixed and identical across all treatments. The remaining 19.5% comprised UAM and FAM in varying proportions constituting 0%, 25%, 50%, 75% and 100% FAM replacement of UAM (T1 through T5, Table 2), with 0.5% chromic oxide included as an inert digestibility marker in all diets. The estimated crude protein ranged from 29.43% in T1 to 30.40% in T5, an increment of less than 1 percentage point inherent to the replacement design since FAM carries higher CP than UAM. Analysed dietary CP values did not differ significantly among treatments (P>0.05); the small progressive increment in estimated CP was inherent to the replacement design and nutritionally negligible at <1% across the full replacement range.

Table 2. Ingredient composition (g per 100 g diet, dry matter basis) and estimated crude protein of experimental diets

Ingredient	T_1	T_2	T_3	T_4	T_5
Fish meal	23.00	23.00	23.00	23.00	23.00
Mustard oil cake	25.00	25.00	25.00	25.00	25.00
Rice bran	30.00	30.00	30.00	30.00	30.00
UAM	19.50	14.63	9.75	4.88	0.00
FAM	0.00	4.87	9.75	14.62	19.50
Vitamin-mineral premix	2.00	2.00	2.00	2.00	2.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Estimated CP (%)	29.46 ± 0.36	29.70 ± 0.36	29.93 ± 0.36	30.15 ± 0.36	30.38 ± 0.36

UAM and FAM proportions reflect 0%, 25%, 50%, 75% and 100% FAM replacement of the 20% azolla component

Experimental management and protein digestibility

Fifteen 1000-litre cemented tanks filled with dechlorinated tap water were used, with five treatments in triplicate. Each tank was stocked with 20 fish after acclimatisation. Fish were fed twice daily at 09:00 and 15:00 h at 5% body weight, adjusted every 15 days. Uneaten feed and faeces were removed by siphoning on alternate days, and 1/3rd of the water column was exchanged weekly. Mortalities were recorded daily. Water quality parameters were monitored weekly using a portable dissolved oxygen meter (Lutron PDO-519, Taiwan) and a multiparameter water quality tester (Eutech Instruments PCSTestr 35, Singapore).

For protein digestibility, faecal samples were collected from each tank on days 30 and 45 by siphoning following the last daily feeding, centrifuged, freeze-dried and stored at -4°C. Chromic oxide in diets and faeces was determined by wet ash digestion spectrophotometry. Apparent digestibility coefficient for crude protein (ADC-CP) was calculated as:

doi.org/10.56093/ijan.v43i2.178732

ADC-CP (%) = 100 × [1 – (Cr_2O_3 in diet / Cr_2O_3in faeces) × (CP in faeces / CP in diet)]

Sampling and growth indices

Fish were individually weighed and measured on days 0, 7, 15, 30 and 45. Weight gain (WG), percentage weight gain (% WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF = W/L^3 × 100) and survival were calculated by standard formulae. At day 45, five fish per tank (15 fish /treatment) were killed by immersion in ice slurry and dorsal muscle tissue was dissected and pooled at the tank level. Pooled samples from each tank (n = 3/ treatment) were analysed separately for crude protein, crude fat, moisture and total ash by AOAC (2005), so that the tank remained the experimental unit for muscle proximate composition.

Statistical analysis

Intermediate sampling data collected on days 7, 15 and 30 were used solely for feed ration adjustment and welfare monitoring and were not subjected to statistical analysis. Terminal day-45 data for all growth indices, feed utilisation parameters, condition factor, survival and ADC-CP were subjected to one-way ANOVA and treatment means compared by Duncan's multiple range test (DMRT) at P ≤ 0.05 using SPSS version 21.0. Values are expressed as mean ± SD of triplicate groups.

Results

Water quality

Water quality remained stable across all tanks throughout the trial: temperature 20.8 ± 0.9^{0}C, dissolved oxygen 7.4 ± 0.5 mg/L, pH 7.1 ±0.3 and total ammonia nitrogen 0.02 ± 0.01 mg/L. Parameters did not differ significantly among tanks.

Proximate composition and ANF content of UAM and FAM

Yeast SSF altered the nutritional and antinutritional profile of *A. pinnata* meal (Table 1). Crude protein was higher in FAM than in UAM (27.86 vs. 23.14%), while crude fibre (11.37 vs. 15.24%), total ash (9.84 vs. 10.42%) and crude fat (4.12 vs. 3.81%) differed marginally between the two meals. Tannin concentration declined from 8.42 mg/g in UAM to 4.91 mg/g in FAM, a reduction of approximately 42%. Phytic acid decreased from 6.18 g/kg in UAM to 2.84 g/kg in FAM, representing a reduction of approximately 54%. Substrate pH declined from 6.42±0.11 before inoculation to 4.87± 0.09 after 48 hr. of SSF, and sensory assessment recorded a transition from grassy odour and fibrous texture of UAM to a yeasty, mildly acidic odour and more friable texture in FAM, providing qualitative confirmation of fermentation. Estimated dietary CP ranged from 29.46% in T1 to 30.38% in T5, with analysed values not differing significantly among treatments (P > 0.05).

Growth performance, survival and condition factor

Growth parameters improved progressively from T1 to T4, with T4 and T5 statistically comparable in most indices (P>0.05) (Table 3). Fish in T1 recorded the lowest final weight (17.84 ± 1.24 g), WG (5.63 ± 0.74 g), SGR (0.84 ± 0.07 % day^{-1}) and PER (1.31 ± 0.08), and the highest FCR (2.61 ± 0.21), all significantly inferior to T3, T4 and T5 (P<0.05). Fish in T4 achieved the highest SGR (1.16 ± 0.07 % day^{-1}), best FCR (2.08 ± 0.14), highest PER (1.57 ± 0.08) and condition factor (2.11 ± 0.09), with T5 recording comparable values (P>0.05). Survival ranged from 86.67 ± 2.89% in T1 to 93.33 ± 2.31% in T4.

Table 3. Growth performance, feed utilisation, survival, condition factor and apparent protein digestibility of *B. gonionotus* at day 45 (mean ± SD, n = 3 tanks)

Parameter	T_1 (0% FAM)	T_2 (25% FAM)	T_3 (50% FAM)	T_4 (75% FAM)	T_5 (100% FAM)
Initial weight (g)	12.22 ± 1.29^a	12.18 ± 1.34^a	12.24 ± 1.27^a	12.19 ± 1.33^a	12.21 ± 1.31^a
Final weight (g)	17.84 ± 1.24^c	18.62 ± 1.18^{bc}	19.47 ± 1.09^b	20.38 ± 1.14^a	20.21 ± 1.21^a
WG (g)	5.63 ± 0.74^c	6.41 ± 0.68^{bc}	7.26 ± 0.82^b	8.17 ± 0.79^a	8.00 ± 0.84^a
% WG	46.11 ± 5.83^c	52.54 ± 4.97^{bc}	59.52 ± 6.14^b	66.97 ± 6.42^a	65.57 ± 6.89^a
SGR (%/ day)	0.84 ± 0.07^c	0.96 ± 0.06^{bc}	1.07 ± 0.08^b	1.16 ± 0.07^a	1.14 ± 0.08^a
FCR	2.61 ± 0.21^a	2.43 ± 0.18^{ab}	2.24 ± 0.16^{bc}	2.08 ± 0.14^c	2.11 ± 0.17^c
PER	1.31 ± 0.08^c	1.38 ± 0.09^{bc}	1.46 ± 0.10^b	1.57 ± 0.08^a	1.54 ± 0.09^{ab}
Condition factor	1.87 ± 0.07^c	1.94 ± 0.08^{bc}	2.02 ± 0.07^b	2.11 ± 0.09^a	2.09 ± 0.08^a
Survival (%)	86.67 ± 2.89^b	88.33 ± 3.21^{ab}	90.00 ± 2.00^{ab}	93.33 ± 2.31^a	91.67 ± 2.89^a
ADC-CP (%)	72.14 ± 1.84^c	75.83 ± 1.47^{bc}	79.41 ± 1.63^b	83.47 ± 1.62^a	82.94 ± 1.71^a

Means in each row with different superscripts are significantly different (P<0.05)

Muscle proximate composition

Muscle crude protein and crude fat increased progressively from T1 to T4, with T4 and T5 recording significantly higher values than T1 and T2 (P < 0.05). Moisture declined correspondingly. Total ash did not differ significantly among treatments (Table 4).

Table 4. Muscle proximate composition (% wet weight) of *B. gonionotus* at day 45 (mean ± SD, n = 15 fish per treatment)

Parameter	T_1	T_2	T_3	T_4	T_5
Moisture (%)	73.42 ± 0.71^a	72.81 ± 0.68^{ab}	72.14 ± 0.74^{ab}	71.38 ± 0.62^b	71.62 ± 0.69^b
Crude protein (%)	14.87 ± 0.48^c	15.44 ± 0.42^{bc}	16.12 ± 0.39^b	16.91 ± 0.44^a	16.72 ± 0.41^{ab}
Crude fat (%)	4.61 ± 0.29^c	4.89 ± 0.27^{bc}	5.18 ± 0.31^{ab}	5.51 ± 0.28^a	5.39 ± 0.33^a
Total ash (%)	3.08 ± 0.18^a	3.11 ± 0.21^a	3.14 ± 0.19^a	3.17 ± 0.22^a	3.15 ± 0.20^a

Means in each row with different superscripts are significantly different (P<0.05)

Discussion

The trial was conducted at a mean water temperature of 20.8 ± 0.9°C, characteristic of mid-hill conditions of Meghalaya. The SGR values recorded across treatments (0.84 to 1.16 % day^{-1}) are broadly consistent with growth performance documented for *B. gonionotus* under mid-hill pond conditions at Umiam (Das and Mahanta, 2025) and within the range reported for the same species in earthen ponds in Bangladesh (Mollah et al., 2011), though direct comparison remains limited by differences in fish size, culture system and diet composition. The small but progressive increment in estimated dietary CP from T1 (29.46 %) to T5 (30.38 %) was inherent to the replacement design and nutritionally negligible at less than 1 percentage point across the full replacement range, and growth and digestibility responses are therefore more plausibly attributed to progressive reduction in dietary ANF load than to differences in protein level per se. The relatively elevated dissolved oxygen (7.4 ± 0.5 mg/L) may have partially supported aerobic metabolic scope across all treatments, as dissolved oxygen availability has been shown to significantly influence oxygen consumption rate and aerobic metabolism in cyprinid fishes (Zhang et al., 2012).

The quality assessment of UAM and FAM (Table 1) confirmed that yeast SSF reduced the ANF content of *azolla*, with tannin declining by approximately 42 % and phytic acid by 54 % in FAM relative to UAM. Cruz Velasquez et al. (2011) reported comparable reductions in tannins and phytates in fermented *Azolla* species, with post-fermentation values falling below the critical dietary thresholds for cultured fish as described by Francis et al. (2001). The phytic acid content of UAM (6.18 g/kg) exceeded the 5 g/kg threshold identified by Francis et al. (2001) above which phytate impairs protein digestibility and mineral absorption in fish, while that of FAM (2.84 g/kg) fell below it. Tannins in UAM (8.42 mg/g) also exceeded the levels at which they inhibit digestive enzyme activity in fish (Francis et al., 2001), whereas FAM (4.91 mg/g) showed a meaningful reduction, though whether this reduction fully eliminates the inhibitory effect in *B. gonionotus* specifically cannot be confirmed from the present data alone. Mpwaga et al. (2023) noted that tannins and phytic acid in azolla are the primary contributors to its limited digestibility in fish at high inclusion levels.

The progressive improvement in ADC-CP from T1 (72.14 %) to T4 (83.47 %) is a central finding of this study and is consistent with the mechanistic explanation that increased FAM proportion progressively reduced the dietary ANF load, improving the accessibility of protein fractions to intestinal digestive enzymes. Siddik et al. (2024) reviewed that SSF improves protein digestibility through degradation of protein-ANF complexes and partial pre-hydrolysis of proteins by microbial enzymes. Das et al. (2021) demonstrated improved protein digestibility in rohu (*L. rohita*) fed yeast SSF plant-based diets compared to unfermented controls. Feng et al. (2024) similarly noted that yeast SSF of plant proteins improves *in vitro* digestibility through reduction of trypsin inhibitors and tannins. The comparable ADC-CP of T4 and T5 ($P \geq 0.05$) suggests that the digestibility benefit of fermentation approaches a plateau between 75 and 100% FAM replacement at this inclusion level, though whether this plateau holds at higher azolla inclusion levels or different fish sizes would require further investigation.

The progressive improvement in WG, SGR and PER from T1 to T4 is consistent with the improvement in ADC-CP, as higher protein digestibility would be expected to increase the proportion of dietary amino acids available for anabolism, supporting better growth and tissue deposition. Das et al. (2018) reported declining SGR in *B. gonionotus* fed increasing proportions of unfermented azolla, attributed to ANF-mediated impairment of digestion. Cruz Velásquez et al. (2015) reported improved FCR in tilapia fed fermented aquatic macrophytes compared to unfermented forms, and Jahan et al. (2021) recorded better FCR and PER in *B. gonionotus* fed fermented soybean meal over untreated meal. The present findings are directionally consistent with these studies, though the specific magnitude of response may differ across species, fermentation agents and rearing conditions. The statistical equivalence of T4 and T5 in most growth and digestibility parameters suggests that 75% FAM replacement captures most of the growth benefit achievable at this level, which may have practical relevance for reducing fermentation effort, though this inference should be tested across a wider range of conditions before practical guidance is drawn. The progressive improvement in FCR may also partly reflect enhanced diet palatability, as yeast SSF generates free amino acids and volatile organic acids recognised as feeding stimulants in cyprinid fish (Siddik et al., 2024; Agboola et al., 2021), though a formal feed preference trial was not conducted.

The improvement in condition factor from T1 (1.87) to T4 (2.11) indicates relatively better body robustness in higher FAM groups, consistent with improved nutrient retention (Farhad et al., 2023). The corresponding improvement in muscle crude protein and fat, with lower moisture in T4 compared to T1, supports the interpretation that better protein digestibility in higher FAM treatments translated into improved tissue synthesis. Similar trends in muscle or whole-body composition with fermented plant protein diets in cyprinids have been reported by Jahan et al. (2021) and Magouz et al. (2020), though direct comparison with these studies is limited by differences in species, diet composition and rearing conditions.

The lower survival in T1 (86.67%) compared to T4 (93.33%) may reflect the cumulative effect of a higher dietary ANF load over the 45-day period. Fiogbe et al. (2004) observed reduced survival in tilapia fed high-level unprocessed azolla, and Mpwaga et al. (2023) noted that prolonged ANF exposure may progressively impair antioxidant defence in fish. However, since water quality was maintained within acceptable ranges and no overt disease signs were recorded, differential mortality cannot be confirmed from the present data and should be interpreted cautiously. The yeast inoculation rate of 5 g/kg dry substrate and 48-hr incubation at 30°C represent practically feasible conditions for on-farm SSF in Meghalaya, though fermentation efficiency may vary with ambient temperature across seasons. On-farm yeast fermentation of locally cultivated *azolla* appears a potentially useful feed strategy for silver barb in Northeast Indian hill regions, and feed management strategies adapted to local conditions have been shown to substantially improve pond productivity in NE India (Debnath, 2025).

Limitations and future recommendations

As this study was limited to a 45-day cemented tank trial under a single seasonal thermal condition and did not include an economic analysis, the findings should be considered preliminary. The duration was considered adequate to detect treatment-level differences in growth and digestibility indices under controlled conditions, but is insufficient to support broad practical feeding recommendations without further validation under pond conditions across seasons and fish size classes. Faecal collection by siphoning may have resulted in some degree of nutrient leaching prior to recovery, potentially introducing a modest upward bias in the estimated ADC-CP values; future studies should consider stripping or settlement-column collection methods to minimise this source of error. The fermentation process was characterised through proximate, ANF, pH and qualitative sensory analyses;

doi.org/10.56093/ijan.v43i2.178732

microbial enumeration, enzyme activity profiling and instrumental colour measurement were not conducted, and a formal feed palatability trial was not incorporated, which limits mechanistic interpretation of the observed responses. Future studies should evaluate graded FAM replacement under pond conditions across seasons, incorporate microbial and enzymatic characterisation of the SSF process, assess economic feasibility of on-farm yeast fermentation, and examine longer-term effects on fish health and reproductive performance at the farm level in Northeast India.

Conclusion

Yeast solid-state fermentation of *Azolla pinnata* meal reduced tannin by 42% and phytic acid by 54%, and graded replacement of unfermented azolla meal with fermented azolla meal progressively improved all measured parameters in juvenile *B. gonionotus* over 45 days under mid-hill conditions of Meghalaya. The 75% fermented azolla replacement level recorded the best SGR (1.16 ± 0.07 %/ day), ADC-CP (83.47 ± .62%), FCR (2.08 ± 0.14), survival (93.33 ± 2.31 %), condition factor (2.11 ± 0.09) and muscle crude protein (16.91 ± 0.44 %), performing at par with 100% fermented azolla. Complete unfermented azolla produced the poorest results across all parameters.

References

Agboola, J. O., Øverland, M., Skrede, A. and Hansen, J. Ø. 2021. Yeast as major protein-rich ingredient in aquafeeds: A review of the implications for aquaculture production. Reviews in Aquaculture. 13(2): 949–970.

AOAC. 2005. Official Methods of Analysis. 18th ed. Association of Official Analytical Chemists, Washington, DC.

Bhadake, P. P., Mote, M. G., Gaikwad, U. S. and Tanpure, M. U. 2024. Effect of dietary supplementation of *Azolla* meal on feed intake and digestibility in Sangamneri kids. Indian Journal of Animal Nutrition. 41(2): 236-241.

Cruz Velasquez, Y., Kijora, C., Wedler, E., Danier, J. and Schulz, C. 2011. Fermentation properties and nutritional quality of selected aquatic macrophytes as alternative fish feed in rural areas of the Neotropics. Livestock Research for Rural Development. 23: 239–246.

Cruz Velásquez, Y., Kijora, C., Wuertz, S. and Schulz, C. 2015. Effect of fermented aquatic macrophytes supplementation on growth performance, feed efficiency and digestibility of Nile tilapia (*Oreochromis niloticus*) juveniles fed low fishmeal diets. Livestock Research for Rural Development. 27: Article 177: 1-8.

Das, K. C., Mohanty, S., Sahoo, P. K., Das, R., Sahoo, L. and Swain, P. 2021. Effect of solid-state fermented aquafeed on growth performance, digestive enzymes and innate immunity of rohu (*Labeo rohita*). Agricultural Science Digest. 41(3): 484–491.

Das, M., Rahim, F. I. and Hossain, M. A. 2018. Evaluation of fresh *Azolla pinnata* as a low-cost supplemental feed for Thai silver barb *Barbonymus gonionotus*. Fishes. 3(1): 15 : 1-11.

Das, S. K. and Mahanta, P. 2025. Short term polyculture of three cyprinid species under mid-hill conditions in Meghalaya, North-east India. Indian Journal of Fisheries. 72(1): 147–149.

Datta, S. N. 2011. Culture of azolla and its efficacy in diet of *Labeo rohita*. Aquaculture. 310(3-4): 376–379.

Debnath, C. 2025. Comparative assessment of feeding regimens on the performance of composite fish culture in tribal villages of Tripura. Indian Journal of Animal Nutrition. 42(3): 384-391.

Debnath, C. 2025. Evaluation of *Azolla pinnata* as a protein source in the diet of silver barb (*Barbonymus gonionotus*) during fry-to-fingerling rearing under hill conditions of Meghalaya. Animal Nutrition and Feed Technology. 25: 235–245.

Farhad, F. B., Hashem, S., Rana, K. M. S. and Salam, M. A. 2023. Growth performance and hematological responses of silver barb (*Barbonymus gonionotus* Bleeker, 1850) fingerlings to dietary blanched moringa (*Moringa oleifera* Lam.) leaf meal as a substitute of soybean meal. Heliyon. 9(2): e13552.

Feng, X., Ng, K., Ajlouni, S., Zhang, P. and Fang, Z. 2024. Effect of solid-state fermentation on plant-sourced proteins: A review. Food Reviews International. 40(9): 2580–2617.

Fiogbe, E. D., Micha, J. C. and Van Hove, C. 2004. Use of a natural aquatic fern, *Azolla microphylla*, as a main component in food for the omnivorous-phytoplanktonophagous tilapia, *Oreochromis niloticus* L. Journal of Applied Ichthyology. 20(6): 517–520.

Francis, G., Makkar, H. P. S. and Becker, K. 2001. Anti-nutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture. 199(3-4): 197–227.

Hastuti, S., Yuniarti, T., Harwanto, D. and Subandiyono, S. 2024. Growth performance of Java barb (*Barbonymus gonionotus*) in relation with water temperature. AACL Bioflux. 17(3): 949–959.

Haug, W. and Lantzsch, H. J. 1983. Sensitive method for the rapid determination of phytic acid in cereals and cereals products. Journal of the Science of Food and Agriculture. 34: 1423–1426.

Ismail, T., Hegazi, E., Nassef, E., Habotta, O. A. and Gewaily, M. S. 2022. The optimized inclusion level of *Azolla pinnata* fermented with *Bacillus subtilis* in Nile tilapia (*Oreochromis niloticus*) diets: Immunity, antioxidative status, intestinal digestive enzymes and histomorphometry, and disease resistance. Fish Physiology and Biochemistry. 48(3): 767–783.

Jahan, H., Ema, N. S., Hossain, M. S., Pervin, M. A., Akter, R. and Hossain, Z. 2020. Growth performance study of silver barb (*Barbonymus gonionotus*) by replacing fishmeal with soybean meal in the diet. Asian Journal of Medical and Biological Research. 6(2): 149–154.

Jahan, H., Tumpa, I. J., Qasem, W. A., Moniruzzaman, M., Pervin, M. A., Akter, R., Omri, A., Min, T. and Hossain, Z. 2021. Evaluation of the partial replacement of dietary fish meal with fermented or untreated soybean meal in juvenile silver barb, *Barbonymus gonionotus*. Frontiers in Nutrition. 8: 733402.

Jena, J., Das, P. C., Mondal, S. and Das, R. 2007. Compatibility of silver barb (*Puntius gonionotus* Bleeker) with Indian major carps in a grow-out polyculture. Aquaculture Research. 38(10): 1061–1065.

Lellinger, D. B., Lumpkin, T. A. and Plucknett, D. L. 1983. Azolla as a green manure: Use and management in crop production. American Fern Journal. 73: 96.

Magouz, F. I., Dawood, M. A. O. and Salem, M. F. I. 2020. The effects of fish feed supplemented with *Azolla* meal on the growth performance, digestive enzyme activity, and health condition of genetically-improved farmed tilapia (*Oreochromis niloticus*). Annals of Animal Science. 20(3) : 1029-1045.

Manoppo, H. and Kolopita, M. E. F. 2016. The use of baker's yeast to promote growth of carp (*Cyprinus carpio* L.). International Journal of PharmTech Research. 9(9): 415–420.

Mazid, M. A., Zaher, M., Begum, N. N., Ali, M. Z. and Nahar, F. 1997. Formulation of cost-effective feeds from locally available ingredients for carp polyculture system for increased production. Aquaculture. 151(1-4): 71–78.

Mollah, M. F. A., Moniruzzaman, M. and Rahman, M. M. 2011. Effects of stocking densities on growth and survival of Thai sharpunti (*Barbonymus gonionotus*) in earthen ponds. Journal of Bangladesh Agricultural University. 9(2): 327–338.

Mondal, D., Rahman, T., Paul, S., Islam, M. J. and Miah, I. 2019. Effects of probiotic supplementation on the growth performance of Thai silver barb (*Barbonymus gonionotus* [Bleeker, 1850]) fry. Journal of the Science of Food and Agriculture. 4(1): 1.

Mpwaga, A. Y., Gyan, W. R., Yang, Q. and Asare, D. 2023. A review on the use of Azolla meal as a feed ingredient in aquatic animals' diets. Aquaculture Research. 2023(7): 1–16.

Naji, M. H. and Alzurfi, S. K. L. 2023. Possibility of using *Azolla* meal as an alternative fish feed. In: Proceedings of the 2nd International Conference of Mathematics, Applied Sciences, Information and Communication Technology. AIP Conference Proceedings. 2834: 020005-1–020005-7.

Price, M. L., Van Scoyoc, S. and Butler, L. G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. Journal of Agricultural and Food Chemistry. 26(5): 1214–1218.

Refaey, M. M., Mehrim, A. I., Zenhom, O. A., Areda, H. A., Ragaza, J. A. and Hassaan, M. S. 2023. Fresh *Azolla pinnata* as a complementary feed for *Oreochromis niloticus*: Growth, digestive enzymes, intestinal morphology, physiological responses, and flesh quality. Aquaculture Nutrition. 2023: 1403704.

Siddik, M. A. B., Julien, B. B., Islam, S. M. M. and Francis, D. S. 2024. Fermentation in aquafeed processing: Achieving sustainability in feeds for global aquaculture production. Reviews in Aquaculture. 16(3): 1244–1265.

Singh, S. G., Debnath, C., Mahanta, P. and Tayung, T. 2024. Silver barb: A promising candidate for aquaculture diversification in Meghalaya hills. Indian Farming. 74(08): 03–07.

Swain, B. K., Naik, P. K. and Beura, C. K. 2022. Nutritive value of *Azolla* as poultry feed: A review. Indian Journal of Animal Nutrition. 39(1): 1-11.

Zhang, W., Cao, Z. D. and Fu, S. J. 2012. The effects of dissolved oxygen levels on the metabolic interaction between digestion and locomotion in Cyprinid fishes with different locomotive and digestive performances. Journal of Comparative Physiology B. 182(5): 641–650.