

Research Article

Formulation of Fish Feeds using Indigenous Plant Raw Materials and their Impacts on Growth and Maturity of Small Indigenous Fish Species (SIS) *Mystus cavasius* (Hamilton, 1822)

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Received: 14 June, 2025

Accepted: 27 June, 2025

Published: 28 June, 2025

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Keywords: Nutrient; Indigenous plants; Growth rate; Reproduction; Protein level; Feed formulation; Native fish

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Abstract

A feeding trial lasting 120 days was conducted in earthen ponds to evaluate the effects of different dietary protein levels on the growth performance of *Mystus cavasius* (Hamilton, 1822) broodstock reared in hapas. Five dietary treatments were tested: four iso-caloric experimental feeds containing 30%, 35%, 40%, and 45% crude protein, and one commercial feed containing 33% protein as a reference. All diets were provided at 5% of total body weight, twice daily at 07:30 and 17:30 h. Each treatment was replicated three times. Growth performance was evaluated based on Average Weight Gain (AWG), body Weight Gain (BWG), Percentage Body Weight Gain (PBWG), Specific Growth Rate (SGR), and Average Daily Weight Gain (ADWG) in both male and female fish. Water quality parameters were monitored throughout the trial. Fish fed the 40% protein diet (MF3) exhibited the highest growth, with AWG of 22.80 ± 2.05 g (male) and 42.2 ± 1.05 g (female), significantly higher ($p < 0.05$) than other treatments. The 35% protein diet (MF2) followed in performance, with the commercial feed showing comparable results. The Gonadosomatic Index (GSI) values of both male and female were higher in MF3 (40% protein) compared to the other four feeds. Furthermore, Statistical analysis confirmed that the 40% protein diet significantly enhanced all growth metrics. Results suggest that 40% dietary protein is optimal for improving the growth of *M. cavasius* under hapa-based pond culture conditions.

Introduction

The global population is projected to reach 9 billion by 2050, leading to a substantial increase in the demand for food, particularly animal-based protein sources [1]. Meeting this demand presents a critical challenge for the global food production sector, which must ensure the provision of safe, affordable, and sustainable food. To address this issue, sustainable food production systems must be developed that rely on alternative feed ingredients with low human consumption value and reduced requirements for land, water, and energy.

In response to increasing demand for food fish, Bangladesh's aquaculture industry is rapidly shifting toward more intensive farming practices [2]. The sector has experienced considerable

expansion, both in production volume and farming intensity, transitioning from traditional to semi-intensive and intensive systems. This intensification has greatly increased the demand for fish feed, contributing significantly to operational costs [2]. Feed alone accounts for approximately 56.45–58.49% of total production expenses in aquaculture [3], with costs in certain cases rising to 60% – 70% [4–7]. The high cost is largely driven by the demand for dietary protein, which is the most expensive component of manufactured fish feed [8–10]. Fishmeal (FM), commonly used as a primary protein source in aquafeeds, contributes substantially to feed cost and is subject to supply fluctuations [11]. Despite its cost, FM remains essential for providing balanced nutrients that support fish growth and health [9,10,12]. Consequently, replacing FM with more affordable alternative protein sources that offer comparable amino acid profiles has become a key objective in aquaculture

nutrition research [13–15]. Among these alternatives, Plant Protein Ingredients (PPI) offer promising potential as cost-effective, sustainable feed components [16].

In parallel, increasing anthropogenic pressures and ecological changes in aquatic ecosystems have resulted in the degradation of natural breeding and feeding habitats for many indigenous fish species. *Mystus cavasius* (Hamilton, 1822), locally known as Gulsha, is a small indigenous catfish species (SIS) of the Bagridae family that has been categorized as Near Threatened by the IUCN [17] due to overfishing and habitat loss. This species is popular for its palatability and high nutritional value, being rich in proteins, lipids, essential minerals (such as calcium, iron, zinc, magnesium), and vitamins [18].

To protect and restore natural stocks of *M. cavasius*, the development of captive breeding and culture systems is essential. Nutrition plays a fundamental role in enabling fish to reach their genetic growth and reproductive potential. Optimal growth performance depends not only on feed composition and nutrient balance but also on factors such as feeding behavior, feed intake, ration size, water quality, and temperature [19–21].

In developing countries, feed quality and affordability remain major constraints in aquaculture development [22]. High-quality, nutritionally balanced diets are essential for rapid and efficient fish growth. Therefore, the production of cost-effective fish feeds using locally available plant-based ingredients is crucial for the long-term sustainability of aquaculture in Bangladesh, especially in rural areas. A wide range of agricultural by-products and indigenous raw materials can be utilized to formulate economically viable and nutritionally adequate feeds. The effectiveness of such feeding strategies will directly impact the growth and productivity of *M. cavasius* [23].

Although several studies have focused on the biology, fecundity, induced spawning, and larval rearing of *M. cavasius*, limited research exists on feed formulation and nutritional requirements. Notably, Shaha, et al. [24] evaluated vitamin E-supplemented diets for broodstock performance. Building on this, the present study aimed to formulate high-quality, plant-based feeds using indigenous ingredients and evaluate their effects on the growth performance of *M. cavasius* reared under hapa-based pond culture conditions. It was hypothesized that plant-based formulated diets would not only support comparable growth to conventional feeds but also positively influence the maturation and reproductive potential of *M. cavasius*, owing to their balanced protein content and locally available nutrients.

Materials and methods

Corn gluten, soybean meal, rice bran, lentil bran, and molasses were collected from the local market in Mymensingh. A vitamin premix was obtained from a sales agent of Bangladesh Pharmaceutical Industries Ltd. All feed ingredients were analyzed for their proximate composition—namely, crude protein, lipid, ash, crude fiber, and moisture content—following the standard methods of the Association of Official Analytical Chemists [25], as presented in Table 1.

Calculation of Protein: (%) of Nitrogen = (Titration Reading–Blank Reading) × Strength of Acid × 100 / 5 × 100 / Weight of the Sample. In this case empirical factor was 6.25 for the fish. Protein (%) = % of Total N₂ × 6.

Chemical analysis of fish feed

Chemical analysis of the raw fish was estimated according to different standard analytical methods developed for proximate analysis:

Determination of the moisture of raw fish paste was conducted following the method [26].

$$\text{Moisture content (\%)} = \frac{\text{Loss of weight}}{\text{Weight of sample taken}} \times 100$$

The crude protein of the fish pest powders was determined by the Micro-Kjeldahl method [25]. Briefly, the percentage of nitrogen in fish samples was calculated, and the percentage of protein in the samples was calculated by multiplying the percentage of N by an empirical factor of 6.25.

$$\text{Percentage of Nitrogen (\%)} = \frac{\text{Mili-equivalent of N}_2(0.014) \times \text{N HCl} \times \text{Titration on value (ml)}}{\text{Weight of sample (gm)}} \times 100$$

Total lipid content of fish powders was determined by the method as described in the Bligh and Dyer method [27] and calculated using the following equation.

$$\text{Total lipid content (\%)} = \frac{\text{Weight of fat residue}}{\text{Weight of sample taken}} \times 100$$

The ash content of the fish samples was determined as the inorganic residues, oxides, sulphates, silicates, and chlorides in the dry muscle. The samples were heated to temperatures of 500 °C – 600 °C in a muffle furnace for about 3 hours. Afterwards, the percentage of ash content was calculated according to [25,28].

Table 1: Proximate composition of feed ingredients (Dry basis).

| Ingredients | Crude Protein | Crude Fat | Moisture | Fiber | Ash | Carbohydrate |
|--------------|---------------|-----------|----------|--------|--------|--------------|
| Corn gluten | 62.00% | 04.45% | 5.45% | 12% | 2.6% | 14% |
| Soybean meal | 44.00% | 14.84% | 7.53% | 18.49% | 4.87% | 37.39% |
| Rice bran | 10.26% | 10.45% | 11.67% | 20.85% | 16.4% | 42% |
| Lentil bran | 19.45% | 0.48% | 16.67% | 28.86% | 7.33% | 46.89% |
| Molasses | 04.45% | 0.00% | 31.67% | 11.93% | 11.93% | 86.32% |

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

Experimental fish feed at different protein levels

Four different iso-caloric feeds denoted by MF-1, MF-2, MF-3, and MF-4 were prepared by mixing in various combinations of the plant ingredients to give four different protein levels, viz, 30%, 35%, 40% and 45%. Various combinations of the ingredients in such a manner that the total metabolized energy per 100 g of feeds was about 340 Kcal (Table 2) maintained the protein level of 30%, 35%, 40% and 45%. The energy content of the feeds was calculated based on 4.0 Kcal/g carbohydrates, 4.0 Kcal/g proteins, and 9.0 Kcal/g lipids [29]. The feeds were made into pellets by adding the starch liquid and drying in an oven at 45 °C for two days. The company supplies fish feed and also tests a group of fish (CF) in parallel to compare the feed value with locally prepared feed.

Fish feed formulation

The main ingredients used for fish feed production are corn gluten (20–40 percent inclusion), rice bran (20–50 percent inclusion), wheat meal (5–20 percent inclusion), soybean meal (10–30 percent inclusion), lentil meal (5–15 percent inclusion) and molasses (5–15 percent inclusion). Feeds play a key role in the development of fish farming. The major constraints to the emergence of aquaculture in developing countries are feed quality and its cost [22]. Fish require a high-quality and nutritionally balanced diet for adequate growth within the shortest time. Therefore, local production of fish feed using locally available ingredients at low cost is crucial to the development and sustainability of aquaculture in Bangladesh, especially in the rural areas. The ingredient availability, its accessibility, and nutrient composition are of prime importance. Bhilave, et al. [30] indicated that the basic nutrient that cannot be compromised on in the choice of ingredients for feed formulation and production is protein. The present study screened local potential fish feed ingredients available in Bangladesh for their nutritional quality, availability, and cost. The aim is to provide information that can help in incorporating any of these ingredients in the local production of formulated fish feed.

Experimental fish and feeding regime

A total 150 numbers of brood *Mystus cavasius* (weight: 23.0–32.7g) were obtained from Shornolota Agro Fish Hatchery in Fulbaria, Mymensingh, Bangladesh, and were transferred to

the place of experiment and acclimated for 2 weeks. Young *M. cavasius* (gulsha) were fed a supplementary (basal diet) for 2 weeks while acclimating to experimental conditions. A total of one hundred fifty (150) uniform male and female fish were randomly selected and stocked into fifteen (15) net hapa aquarium with a mesh size of 1.5mm and surface area dimension of 0.95×0.50m with a stocking ratio of ten fish (male and female) per hapa. Hapas were suspended and placed in an experimental earthen pond to maintain a water depth of 0.6m inside the hapa. The hapas served as the culture medium for the experiment. Each treatment was replicated thrice. Hapas were tied to a stake with a rope. Sinkers were tied at the bottom edges of each hapas to allow stability in water. Water was pumped into the pond regularly to maintain the desired level of 0.6m in the hapas. Fishes are supplied with feed approximately 5% of their body weight daily, two equal feedings times (8.0 am and 18.0pm) for 90 days. Feed preparation was carried out bi-weekly to prevent long storage.

Growth performance

For evaluating the different growth parameters, such as length gain (cm), weight gain (g), percent (%) weight gain, specific growth rate (SGR% % per day), food conversion ratio (FCR), and protein efficiency ratio (PER) were taken into consideration.

Average Weight Gain (AWG): This was determined by calculating the difference between the initial weight and the final weight of the fish [31].

$$\text{AWG} = W_2 - W_1, \text{ Where } W_1 = \text{initial weight } W_2 = \text{final weight.}$$

Average Daily Weight Gain (ADWG): This was determined by calculating the difference between the initial weight and the final weight divided by time [4].

$$\text{Average Daily Weight Gain (ADWG)} = \frac{\text{Average final weight}(W_1) - \text{Average initial weight}(W_0)}{\text{time}(t)/\text{day}}$$

Percentage Weight Gain (PWG): This will be determined from the relationship between the total weight gains of the fish expressed as a percentage of the initial weight.

$$\text{Percentage Weight Gain (PWG)} = \frac{\text{Average final weight} - \text{Average initial weight}}{\text{Average initial weight}} \times 100 \quad [32]$$

Table 2: Composition of prepared fish feed at different protein levels.

| Name of Ingredients | Feed-MF1 (30%) protein | | Feed-MF2 (35%) Protein | | Feed-MF3 (40%) protein | | Feed-MF4 (45%) protein | |
|---------------------|------------------------|----------------------------|------------------------|----------------------------|------------------------|----------------------------|------------------------|----------------------------|
| | Weight in g of feed | Total protein in feed (gm) | Weight in gm of feeds | Total protein in feed (gm) | Weight in g of feeds | Total protein in feed (gm) | Weight in g of feeds | Total protein in feed (gm) |
| Corn gluten | 25 | 15.5 | 35 | 21.7 | 40 | 24.8 | 50 | 31.0 |
| Rice bran | 40 | 4.1 | 30 | 3.07 | 20 | 2.05 | 10 | 1.03 |
| Soybean | 20 | 8.8 | 20 | 8.8 | 25 | 11.0 | 25 | 11.0 |
| Lentil bran | 10 | 1.95 | 10 | 1.95 | 10 | 1.95 | 10 | 1.95 |
| Molasses | 5 | 0.23 | 5 | 0.23 | 5 | 0.23 | 5 | 0.23 |
| Vitamin premix | 0.5 | | 0.5 | | 0.5 | | 0.5 | |

Specific Growth Rate (SGR): This will be determined from the relationship of the differences in weight of the fish within the experimental period.

$$\text{Specific Growth Rate (SGR)} = \frac{[\ln W_t - \ln W_0]}{t(\text{time of days})} \times 100 \quad [33]$$

Where \ln natural logarithm, W_0 = initial weight (g), W_t = final weight (g), and t = time of day.

$$\text{Body weight gain (BWG)} = (W_t - W_0) \times N_t \quad [34]$$

Feed conversion ratio FCR = total feed intake (g)/total weight gain (g) [35].

$$\text{PER} = \text{total gain (g)/protein intake (g)} \quad [21].$$

Where W_t and W_0 were final and initial fish weights (g), respectively; N_t and N_0 were final and initial numbers of fish in each replicate, respectively; L (cm) was final length; and t is the experimental period in days.

Gonadosomatic index

The fish was dissected with scissors, cutting from the anus to the lower jaw, exposing the belly. Fine forceps were utilized to gently extract the stomach and intestine. The ovary was delicately transferred to a Petri dish. With distilled water, the ovary was cleaned and washed [11]. The ovary's length and weight were measured, and the color of the ovary was examined and documented. Gonadosomatic Index (GSI) values of different feeding trials were collected, and data on different parameters of male and female ($n = 30$) from fifteen (15) net hapa were recorded and analyzed. The relationship between gonadal maturity and the growth of fish is directly proportional. The GSI therefore rises gradually before the gonads mature into ripening. GSI values were estimated as the ratio of the wet gonad weight to somatic weight expressed in percentage by using the following formula: [36]:

$$\text{GSI} = \frac{\text{Weight of the gonad}}{\text{Weight of fish}} \times 100$$

Water quality parameters

Temperature, pH, and Dissolved Oxygen (DO) were monitored during the period of the experiment. The temperature of the water was taken twice a day (8 pm and 4 pm) using a mercury-in-glass thermometer calibrated in degrees Celsius. Dissolved oxygen was measured using the titration method. pH was measured with a digital pH meter (model checker I produced by Hanna Instrument Company). The pH and Dissolved Oxygen were measured weekly.

Statistical analysis

The data obtained from the trial were subjected to one-way analysis of variance (ANOVA) (using SPSS 16.0 programme) to test for effects of dietary treatments. When ANOVA identified a significant difference among groups, multiple comparison tests among means were performed using Duncan's new multiple range test. For each comparison, statistically significant

differences were determined by setting the aggregate type I error at 5% ($p < 0.05$).

Ethics approval

The experiment was conducted based on the guidelines of the Ethical Committee of the Bangladesh Agricultural University Research System (BAURES), Bangladesh Agricultural University, Mymensingh, Bangladesh.

Results

Physico-chemical parameters of water

Experimental pond water temperature, pH, dissolved oxygen, and dissolved ammonia during the experimental period in all the hapa were found within the desirable range for the fish rearing. Average Temperature, pH, dissolved oxygen, and dissolved ammonia of water in all hapa ranged from 26.8–29.8 °C, 6.7–7.6, 5.2–6.1 mg/L, and 0.25–0.5 mg/L, respectively.

Effect of feed ingredients on the growth performance of Gangetic Mystus (*M. cavasius*):

At the end of 90 days, experimental feed and company supply fish feed treatment, the Body Weight Gain (BWG), Percent of Body Weight Gain (PBWG), Daily Growth Rate (DGR) and Specific Growth Rate (SGR), Food Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) of *M. cavasius* (gulsha) were determined and presented in Tables 3–5.

The Gonado-somatic Index values (GSI) of *M. cavasius* of different feeds are shown in Figure 1. It is observed from (Figure 1, that the gonado-somatic index values of both male and female were found to be higher in MF3 (40% protein). The average values of gonado-somatic index values for males in CF, MF1, MF2, MF3, and MF4 were 1.58, 1.74, 2.05, 2.35, and 1.93, respectively. The gonado-somatic index values for females were also higher in MF3 compared to the other four feeds.

Discussion

Water quality parameters play a vital role in the successful rearing of fry and fingerlings, directly influencing their growth, survival, and physiological well-being. Among these, water temperature is a critical factor affecting both the physico-chemical and biological properties of the aquatic environment. In the present study, recorded water temperatures ranged from 26.8 °C to 29.8 °C, which is well within the optimal thermal tolerance limits (14 °C–38 °C) reported for tilapia and *Mystus gulio* [37]. This temperature range supports optimal metabolic activity and growth in freshwater fish species.

The water pH observed during the study ranged from 6.7 to 7.6, aligning with the optimal range (6.5–8.5) recommended for aquaculture [38]. Maintaining pH within this range is essential, as deviations can lead to physiological stress, impaired metabolism, and reduced growth performance in cultured species, including *Mystus cavasius*.

Dissolved oxygen (DO) concentrations fluctuated slightly between 5.2 and 6.1 mg/L⁻¹, remaining within the

Table 3: Body Weight Gain (BWG), Percentage of Weight Gain (% BWG), Daily Growth Rate (DGR), and Specific Growth Rate (SGR) of *Mystus cavasius* male and female broodstock after 90 days of experimental feed and company supply fish feed treatment (Mean \pm SD $n = 15$)

| Type | Parameters | The company supplies fish feed | Prepared fish feed | | | |
|--------------|-------------------------|--------------------------------|--------------------|-------------------|-------------------|-------------------|
| | | (CF) 33% protein | (MF1) 30% protein | (MF2) 35% protein | (MF3) 40% protein | (MF4) 45% protein |
| Male Brood | Initial Body Weight (g) | 33.15 \pm 0.15 | 31.7 \pm 0.19 | 33.5 \pm 0.14 | 36.3 \pm 0.10 | 34.5 \pm 0.12 |
| | Final Body Weight (g) | 49.40 \pm 0.14 | 46.40 \pm 0.18 | 52.40 \pm 0.17 | 59.1 \pm 0.19 | 52.7 \pm 0.11 |
| | Body Weight Gain (g) | 16.25 \pm 0.95 | 14.7 \pm 1.49 | 18.9 \pm 1.10 | 22.80 \pm 2.05 | 18.2 \pm 1.49 |
| | % Body Weight Gain | 49.4 \pm 0.49 | 46.37 \pm 0.35 | 56.41 \pm 0.50 | 62.8 \pm 0.50 | 52.75 \pm 0.50 |
| | Daily Growth rate (g) | 0.180 \pm 0.07 | 0.163 \pm 0.15 | 0.21 \pm 0.36 | 0.25 \pm 0.09 | 0.20 \pm 0.25 |
| | Specific Growth Rate | 0.44 \pm 0.85 | 0.42 \pm 0.92 | 0.497 \pm 0.65 | 1.54 \pm 0.28 | 0.47 \pm 0.55 |
| Female Brood | Initial Body Weight (g) | 32.8 \pm 1.05 | 33.2 \pm 1.95 | 31.9 \pm 1.90 | 32.9 \pm 1.01 | 33.1 \pm 1.00 |
| | Final Body Weight (g) | 52.1 \pm 1.15 | 53.7 \pm 1.05 | 62.1 \pm 1.00 | 75.1 \pm 1.06 | 62.9 \pm 1.04 |
| | Body Weight gain (g) | 20.1 \pm 2.05 | 20.5 \pm 1.49 | 30.2 \pm 1.10 | 42.2 \pm 1.05 | 29.8 \pm 1.49 |
| | % Body Weight Gain | 61.28 \pm 1.49 | 61.75 \pm 1.32 | 94.67 \pm 1.50 | 128.26 \pm 2.50 | 90.03 \pm 1.50 |
| | Daily growth Rate | 0.23 \pm 0.15 | 0.23 \pm 0.55 | 0.34 \pm 0.25 | 0.47 \pm 0.45 | 0.33 \pm 0.15 |
| | Specific Growth Rate | 0.51 \pm 0.07 | 0.53 \pm 0.09 | 0.74 \pm 0.35 | 0.91 \pm 0.05 | 0.71 \pm 0.15 |

Table 4: Body Weight Gain (BWG), Food Conversion Ratio (FCR), and Protein Efficiency Ratio (PER) of *M. cavasius* Brood-fish after 90 days fed with experimentally prepared fish feed (Mean \pm SD $n = 15$)

| Parameters | 72g company supply fish feed | 72g prepared fish feed | | | |
|------------------------------------|-----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| | (33% Protein) fed /fish/ 90 days. | (30% protein) fed /fish/ 90 days | (35% protein) fed /fish/ 90 days | (40% protein) fed /fish/ 90 days | (45% protein) fed /fish/ 90 days |
| Male Body Weight Gain (g) | 16.25 \pm 0.95 | 14.7 \pm 0.49 | 18.9 \pm 0.80 | 22.8 \pm 0.95 | 18.2 \pm 0.49 |
| Feed conversion ratio (FCR) | 4.43 | 4.89 | 3.80 | 3.15 | 3.95 |
| Female Body Weight Gain (g) | 20.1 \pm 1.12 | 20.5 \pm 1.14 | 30.2 \pm 2.02 | 42.2 \pm 2.09 | 29.8 \pm 1.10 |
| Female Feed conversion ratio (FCR) | 3.58 | 3.51 | 2.30 | 1.70 | 2.41 |
| Male Body Weight Gain (g) | 16.25 \pm 0.55 | 14.7 \pm 0.89 | 18.9 \pm 0.80 | 22.8 \pm 0.65 | 18.2 \pm 0.45 |
| Protein efficiency ratio (PER) | 1.46 \pm 0.60 | 1.47 \pm 0.90 | 1.33 \pm 0.30 | 1.26 \pm 0.85 | 1.78 \pm 0.90 |
| Female Body Weight Gain (g) | 20.1 \pm 1.12 | 20.5 \pm 1.14 | 30.2 \pm 2.02 | 42.2 \pm 2.09 | 29.8 \pm 1.10 |
| Protein efficiency ratio (PER) | 1.18 \pm 0.70 | 1.05 \pm 0.90 | 0.83 \pm 0.70 | 0.68 \pm 0.80 | 1.08 \pm 0.90 |

Total 72.0 g each prepared basal fish feed /fish/90 days and 23.76g protein/fish/90 days used for this experiment

Table 5: Average Initial length, Final length, Increase, and percentage of length of *M. cavasius* (Male and Female gulsha) fed with prepared experimental diet. n (15) (after 90 days).

| Type | Treatment | % of protein | Average Initial length (cm) | Average final length (cm) | Average length increase in cm | Percentage of length increase (%) |
|--------------|---------------------|--------------|-----------------------------|---------------------------|-------------------------------|-----------------------------------|
| Male Brood | Company feed (CF) | 33% | 15.30 \pm 0.16 | 20.46 \pm 0.14 | 5.16 \pm 0.87 | 37.72 \pm 0.62 |
| | Prepared feed(MF1) | 30% | 14.85 \pm 0.25 | 20.95 \pm 0.18 | 6.10 \pm 0.98 | 41.07 \pm 0.93 |
| | Prepared feed (MF2) | 35% | 14.75 \pm 0.35 | 20.97 \pm 0.41 | 6.22 \pm 0.87 | 42.16 \pm 0.52 |
| | Prepared feed(MF3) | 40% | 14.70 \pm 0.27 | 24.95 \pm 0.95 | 10.25 \pm 0.94 | 69.72 \pm 0.45 |
| | Prepared feed (MF4) | 45% | 15.20 \pm 0.80 | 23.05 \pm 0.75 | 7.85 \pm 0.77 | 51.64 \pm 0.82 |
| Female Brood | Company feed (CF) | 33% | 14.30 \pm 0.16 | 17.46 \pm 0.14 | 3.16 \pm 0.87 | 22.09 \pm 0.62 |
| | Prepared feed(MF1) | 30% | 14.85 \pm 0.25 | 18.95 \pm 0.18 | 4.10 \pm 0.98 | 27.60 \pm 0.93 |
| | Prepared feed (MF2) | 35% | 14.75 \pm 0.35 | 19.97 \pm 0.41 | 5.22 \pm 0.87 | 35.39 \pm 0.52 |
| | Prepared feed(MF3) | 40% | 14.10 \pm 0.27 | 20.95 \pm 0.95 | 6.85 \pm 0.94 | 48.58 \pm 0.45 |
| | Prepared feed (MF4) | 45% | 15.20 \pm 0.80 | 20.05 \pm 0.75 | 4.85 \pm 0.77 | 31.90 \pm 0.82 |

recommended range for freshwater fish culture (4.5–8.0 mg/L) as reported by Bhatnagar and Sangwan [39]. Adequate oxygen availability is crucial for aerobic respiration and overall fish health. Suboptimal DO levels (below 4 mg/L⁻¹) can result in reduced feeding, physiological stress, electrolyte imbalance,

and potentially mortality, which can negatively impact yield and production efficiency.

Un-ionized ammonia (NH₃-N) is another critical parameter, as elevated concentrations are highly toxic, particularly to fry

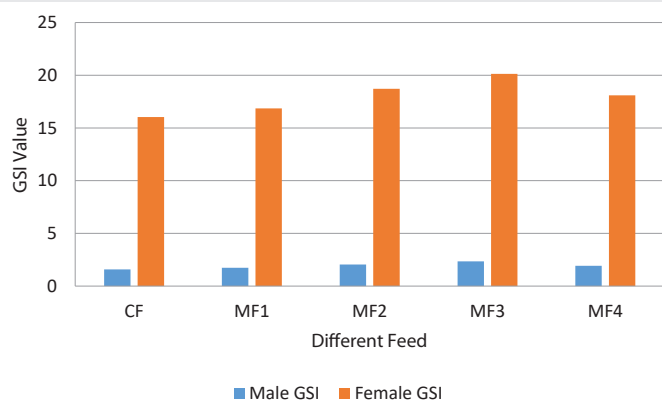


Figure 1: Effect of different dietary protein levels on the maturation of *M. cavasius* (CF: Company Feed; MF1: Manufactured feed-protein 30%; MF2: Protein 35%; MF3: Protein 40%; MF4: Protein 45%).

and juvenile fish. In this study, $\text{NH}_4\text{-N}$ concentrations ranged from 0.25 to 0.5 mg L^{-1} , remaining within the acceptable limit ($< 0.5 \text{ mg L}^{-1}$) proposed by Santhosh and Singh [38]. As ammonia toxicity is often associated with gill damage and impaired osmoregulation, the levels recorded in the current study are not expected to contribute to fry mortality.

Total alkalinity, which reflects the buffering capacity of water, also influences water stability and productivity. The measured values in this study were within the suitable range for productive aquaculture systems. According to Alikunhi [40], total alkalinity above 100 mg/L^{-1} is considered beneficial for fish culture. Previous studies by Paul [41], Kohinoor [42], Garg and Bhatnagar [43], and Kohinoor, et al. [44] similarly reported alkalinity values conducive to fish growth.

Growth performance of *M. cavasius* broodstock fed diets with varying protein levels is summarized in Table 4. Fish fed with Feed-MF3 (40% crude protein) achieved the highest final weight ($105.37 \pm 8.16 \text{ g}$), followed by Feed-MF2 (35% protein; $86.58 \pm 3.26 \text{ g}$), Feed-MF1 (30% protein; $78.47 \pm 2.43 \text{ g}$), and commercial feed (CF; 33% protein), which yielded comparable results to Feed-MF1. Statistical analysis (ANOVA) revealed a significant difference ($p < 0.05$) in final weight, with Feed-MF3 supporting superior growth.

Growth indicators such as absolute growth, absolute growth rate, and Specific Growth Rate (SGR) were all higher in Feed-MF3 compared to Feed-MF2 and Feed-MF1. Feed-MF4 (45% protein) produced similar growth to Feed-MF3, indicating that protein levels beyond 40% may not result in significant additional growth benefits. These findings are consistent with previous studies demonstrating a positive correlation between dietary protein level and fish growth, up to an optimal threshold. Similar trends have been reported in Chinook salmon [45], common carp [46], and *Clarias batrachus* [47,48].

The highest reproductive development in both male and female *M. cavasius* occurred with the MF3 diet, suggesting that an optimal dietary protein level enhances gonadal maturation, with MF3 outperforming other feed formulations in promoting reproductive performance [49–51].

Overall, the results suggest that a dietary protein level of approximately 40% is optimal for maximizing growth performance in *M. cavasius* broodstock, without incurring unnecessary feed costs or compromising water quality due to excess nitrogenous waste.

Conclusion

This study demonstrates that formulating fish feeds using indigenous plant-based ingredients can effectively support the growth and maturation of *Mystus cavasius* under hapa-based pond culture conditions. A dietary protein level of approximately 40% was ideal for optimizing the growth performance of *M. cavasius* broodstock. Additionally, the GSI index indicated that the gonadal development of *M. cavasius* was more favorable at a 40% dietary protein level compared to others. The results highlight the potential of locally sourced plant proteins as viable alternatives to fishmeal, offering a cost-effective and nutritionally appropriate option for small-scale aquaculture systems. By improving feed efficiency and promoting reproductive performance, these formulations contribute directly to the sustainable intensification of aquaculture in Bangladesh. This approach not only enhances the production of small indigenous species but also strengthens food security and supports rural livelihoods through reduced dependence on imported feed inputs.

Acknowledgement

The authors would like to express their special gratitude to the farm manager and farmworker of Sharnalata Agro Fisheries Farm, Mymensingh, Bangladesh, for their collaboration and for providing necessary support in the form of infrastructural facilities made available for undertaking this study.

Authors' contributions

AS: Conceptualization, Funding acquisition, Data Collection and curation, Software, Writing – original draft, review & editing; AT: Data curation, Software, Writing – review & editing; MSI: Writing – review & editing.

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