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Original Article

Partial replacement effect of dietary fish meal with blood meal on growth performance, body composition, and haematology of butter catfish *Ompok bimaculatus*

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Abstract
Escalating costs and the constrained supply of fish meal have underscored the imperative to identify alternative, economically viable protein sources for aquafeeds. This study evaluates the feasibility of incorporating blood meal as a partial replacement for fish meal in the diet of butter catfish (*Ompok bimaculatus*). Five iso-nitrogenous (35.0%) and iso-lipidic (7.0%) diets, designated T_0 (control), T_5, T_{10}, T_{15}, and T_{20}, were prepared by substituting fish meal protein at 0, 5, 10, 15, and 20% levels with blood meal protein, respectively. Fish groups were assigned to each of the formulated diets in triplicate. Each of the 15 glass aquariums (180 L) was stocked with 50 fish (mean±SD 1.5±0.03 g) and fed with experimental diets for eight weeks. The fish fed the T_{15} diet achieved the highest weight gain (4.61±0.22 g), percent weight gain (305.30±1.20%), and specific growth rate (1.42±0.21% day^{-1}) compared to fish fed all other diets. The lowest feed conversion ratio (1.85±0.08) was observed in fish fed T_{10} diet. The highest protein efficiency ratio (1.88±0.02) was also observed in fish fed T_{15} diet. Replacing fish meal with blood meal positively impacted the haematological profile and whole-body proximate composition. Additionally, the cost of the experimental diets was reduced compared to the control diet. Regression analysis indicated that the optimal replacement level of fish meal protein with blood meal protein ranged from 10.8% to 12.6%. We suggest that blood meal can be effectively used as a partial fish meal substitute in the aquafeed, offering a cost-effective and sustainable alternative.

Keywords: alternative protein; blood meal; butter catfish; feed utilization; growth performance

1 | INTRODUCTION

Fish farming is important in strengthening economies and ensuring nutritional security in low-income regions because essential fatty acids, vitamins, minerals, and amino acids can all be found in large quantities in fish (Beveridge *et al.* 2013). Feed accounts for approximately 50 – 70% of the total production costs in aquaculture, placing considerable economic pressure on producers (Tacon and Metian 2008). Fish culture vitally requires high-quality feeds that are nutritionally balanced (Hixon 2014). Protein is

one of the most important elements in the feed that affects fish growth, survival, and production because it provides essential and non-essential amino acids needed to synthesize body protein and energy for maintenance (Jena *et al.* 2012). The higher percentage of animal protein required in fish diets significantly increases feed input costs, thereby challenging the economic sustainability in intensive aquaculture unsustainable (Woodgate *et al.* 2022). Fish meal is a major protein source used in feed formulation by most aquafeed companies (Serra *et al.* 2024). Fish meal is generally added to animal diets to increase feed efficiency and growth through better feed palatability; it enhances nutrient uptake, digestion, and absorption (Hardy 2010; Ajani *et al.* 2016). Its high protein quality of fish meal is attributed to a well-balanced amino acids profile, making it ideal for inclusion in animal diets (Cho and Kim 2011). However, the supply of fish meal is not always sufficient. On the other hand, the demand for fish meal is increasing with the expansion of the aquaculture sector (Olsen and Hasan 2012; Saha *et al.* 2022). Therefore, a crucial aspect of sustainable aquaculture production is the careful selection of alternative feed ingredients to serve as protein sources in fish feed.

Considering the increasing cost of fish meal, researchers are interested in finding ways to replace fish meal totally or partially with other less expensive protein-rich ingredients (Kirimi *et al.* 2016; Lu *et al.* 2020). A large number of indigenous raw materials, mainly poultry by-product meals (Dawood *et al.* 2020), insect meal (Lu *et al.* 2020), worm meal (Chakraborty *et al.* 2021), cereal by-products (He *et al.* 2021), and leaf meal (Zhang *et al.* 2020) can be used in developing supplemental feed for the rearing of different fish species.

In this context, blood meal can be regarded as a highly valuable alternative protein source for many fish species due to its high protein content, reasonable price, and steady supply (Kirimi *et al.* 2016; Twahirwa *et al.* 2020). It is generally accepted that the costliest protein source in aquafeeds is fish meal. By comparison, blood meal, which is a by-product of slaughterhouses, is significantly less expensive (Twahirwa *et al.* 2020). It has been shown that, when inclusion levels are optimized, partially substituting blood meal for fish meal can lower feed production costs without compromising fish performance (Nogueira *et al.* 2012; Twahirwa *et al.* 2020). The weight of all the blood from domestic animals makes up six to seven percent of the carcass' lean flesh (Chiroque *et al.* 2023). The cooked sun-dried blood meal processed from cattle blood contains 80 – 85% crude protein (Ndelekwute *et al.* 2008). The amino acid composition of blood meal protein closely resembles that of the ideal protein, which has a higher lysine content than other dietary proteins like meat and bone meal (Gatnau *et al.* 2001). This protein can be fed as a substitute source of protein to animals, poultry, and other species where protein is a necessary component of their diets.

Butter catfish (*Ompok bimaculatus*), a member of the family Siluridae, is an omnivorous freshwater fish species (Arthi *et al.* 2011). It is highly preferred as a table fish due to its soft meat texture, delicious taste, and high nutritional value (Alam *et al.* 2020; Akter *et al.* 2023). Butter catfish shows strong potential for commercial aquaculture; however, feed price will be one of the main obstacles because feed price accounts for the maximum of all operational costs in butter catfish culture (Arifa *et al.* 2021). Therefore, the current study was planned to examine the use of blood meal as a substitute for fish meal in the diet of butter catfish.

2 | METHODOLOGY

2.1 Preparation of blood meal

Fresh cow blood was obtained from a nearby slaughterhouse after the animal was sacrificed. A 0.2% diluted citric acid was added as an anticoagulant agent in the collection container to prevent blood clotting (Bari *et al.* 2015; Scaravilli *et al.* 2018). The blood was then cooked for 30 minutes to eliminate microbes (Kirimi *et al.* 2016). Subsequently, cooked blood was oven-dried at 60°C for 6 hours to remove moisture content at 10 – 12% level. Finally, the dried blood was crushed into tiny particles and kept in an airtight container before usage (Ogunji and Iheanacho 2021).

2.2 Preparation of experimental diet

Five experimental diets were formulated with purified ingredients (Table 1). Feed ingredients used in this study were procured from local feed market. In the control (T_0) diet, fish meal, soybean meal, and mustard oil cake were used as the protein sources. Fish oil was employed as the lipid supply, whereas wheat bran, rice bran, and wheat flour were used as the carbohydrate sources.

Fish meal protein was replaced with blood meal protein at 0, 5, 10, 15, and 20% levels and referred to as T_0, T_5, T_{10}, T_{15}, and T_{20}, respectively. The levels of blood meal inclusion were selected based on the previous studies where 5%, 10%, and 25% level of fish meal were successfully replaced with blood meal in the diet of hybrid catfish (*Clarias gariepinus* × *Heterobranchus longifilis*), hybrid clariid catfish (*Clarias gariepinus* × *Heterobranchus bidosarlis*), and *Heterobranchus bidorsalis* fingerling, respectively (Olukunle *et al.* 2002; Aliu and Dako 2018; Aliu and Ademiluyi 2020). After the ingredients were thoroughly mixed, a 3:1 ratio of water was added to create a dough. The dough was then allowed to air dry to around 10% moisture at room temperature after being run through a pelletizer fitted with a 2 mm diameter die. Ultimately, the test diets were placed into plastic bags, sealed, and kept at –20°C until use.

Although fish meal was replaced with blood meal, slight adjustments in other ingredients were made to

maintain balanced crude protein and nutrient levels across diets. These minor variations, common in aquaculture studies (Fagbenro and Jauncey 1995; Bureau *et al.* 1999), are unlikely to affect the results or compromise the validity of growth and health comparisons.

TABLE 1 Formulation and proximate combination of the experimental diets (dry weight basis).

Ingredients	Experimental diet (%)				
	T_0	T_5	T_{10}	T_{15}	T_{20}
Fish meal	34.00	32.31	30.61	28.91	27.20
Blood meal	0.00	1.14	2.30	3.42	4.56
Soybean meal	10.00	10.00	10.00	10.00	10.00
Mustard oil cake	11.00	10.50	10.10	9.90	9.60
Rice bran	30.00	29.05	30.08	30.77	31.60
Wheat bran	11.00	13.00	13.00	13.00	13.00
Wheat flour	2.00	2.00	2.00	2.00	2.00
Fish oil	1.00	1.00	1.00	1.00	1.00
Vitamin premix[1]	0.50	0.50	0.50	0.50	0.50
Mineral premix[2]	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100
Diet composition (%)					
Protein	35.40	35.10	35.18	35.35	35.36
Lipid	7.21	7.10	7.09	7.06	7.16
Ash	11.08	10.50	10.14	11.10	10.00
Moisture	11.30	11.36	11.43	10.92	10.87
Carbohydrate[3]	46.31	47.3	47.59	46.49	47.48
Energy (kJ g^{-1})[4]	16.36	16.43	16.49	16.32	16.53
Diet Price (USD kg^{-1})	0.47	0.46	0.44	0.43	0.41

[1]Vitamin premix supplied the following (mg g^{-1} mixture): thiamin hydrochloride, 5 mg; riboflavin, 5 mg; calcium pantothenate, 10 mg; nicotinic acid, 6.05 mg; biotin, 0.003 mg; pyridoxine hydrochloride, 0.825 mg; inositol, 10 mg; folic acid, 0.041 mg; L-ascorby1-2-Monophosphate-Mg, 2.025 mg; choline chloride, 44 mg; menadione, 4 mg; alpha-tocopherol acetate, 3.35 mg; para-aminobenzoic acid, 5 mg; myo-inositol, 20 mg; retinyl acetate, 0.4 mg; cholecalciferol, 0.0004685 mg. All ingredients were diluted with alpha-cellulose to 1 g (Hossain and Furuichi 1999; Hossain and Furuichi 2001). [2]Mineral premix supplied the following (mg g^{-1} mixture): $FeSO_4 \bullet 6H_2O$, 2.125 mg; $MgSO_4$, 137 mg; KCl, 75 mg; NaH_2PO_4, 87.2 mg; NaCl, 43.5 mg; $AlCl_3 \bullet 6H_2O$, 0.15 mg; KI, 0.15 mg; $CuCl_2 \bullet 2H_2O$, 0.1 mg; $ZnSO_4 \bullet 7H_2O$, 0.80 mg; $CoCl_2 \bullet 6H_2O$, 1 mg. All ingredients were diluted with alpha-cellulose to 1 g (Hossain and Furuichi 1999; Hossain and Furuichi 2001). [3]Carbohydrate was calculated by difference [100 - (crude protein + crude lipid + ash)]. [4]Gross energy was calculated by using the following coefficients: 16.7 kJ g^{-1} for protein and carbohydrate, and 37.5 kJ g^{-1} for lipid, respectively (Garling and Wilson 1976).

2.3 Fish rearing

Five treatments, comprising one control group and four experimental groups with three replications each, were used in the completely randomized design (CRD) experiment. Butter catfish fry were procured from a commercial hatchery. Fish that were in good health were gathered and transported to the wet lab in polythene bags with oxygen. Fish were housed in circular tanks with sufficient aeration upon arrival, wherein they were given two weeks to become used to the experimental settings.

Prior to their discharge into the experimental aquaria, the fish had a salt treatment using a 1% salt solution for 3 minutes to reduce external parasites and enhance acclimatization. A total of 750 fish, with an initial weight of 1.5 ± 0.03 g (mean ± SD), were chosen, weighed, and then divided into 15 aquaria, each holding 50 fish, and with a 180 L water capacity, such that the biomass of each aquarium was identical. The fish were given experimental diets two times a day, at 8 and 17 hours, until they reached near satiation for eight weeks. The daily feed supply to each tank was recorded.

Every morning, fish excrement was cleaned by siphoning. Each aquarium's water level was replenished by about 30% every day. A RESUN ACO-001 air pump was used to continuously aerate each aquarium. Throughout the trial, it was kept at a natural photoperiod of about 10:14 light to dark. The water quality parameters were measured regularly twice in a day. Regular maintenance of the water temperature (28 – 30°C) was accomplished by installing an external water heater (Sobo HF-300). A Celsius thermometer (Digi-thermo WT-2) was used to measure the water temperature (°C) in each tank. A digital pH meter (Hach Co., Colorado, USA) was used to measure the pH. The dissolved oxygen (DO) content of water was measured using a digital DO meter (Lutron DO-5509). An ammonia measuring kit (HANNA Instrument Test Kit) was used to determine ammonia (mg L^{-1}). On a sample day during the experimental period, there was no discernible difference in the water quality metrics. Ammonia (0.40 – 0.98 mg L^{-1}), dissolved oxygen (5.45 – 7.15 mg L^{-1}), pH (6.83 – 7.65), and temperature (28.03 – 30.53°C) were found to be within ranges that were appropriate for butter catfish during the trial period. The feeding trial and subsequent handling and sampling of experimental fish were carried out as per the ethical guideline of Gazipur Agricultural University, Gazipur-1706, Bangladesh.

2.4 Fish sampling and weighing

Following an eight weeks feeding trial, the fish were given a dose of tricaine methanesulfonate (MS-222) at a concentration of 100 mg L^{-1} for 2 – 3 minutes as an anesthetic and starved for 24 hours (Neiffer and Stamper 2009). MS-222 was used in this study as it is the only anaesthetic agent approved by the United States Food and Drug Administration (FDA) for use in food fish and also improves fish welfare during invasive or stressful procedures (Topic Popovic *et al.* 2012). Then, all the fish in each aquarium

were collected using a scoop net and counted, and their body weight was recorded using a digital electric balance (model EK600i). Blood samples were collected from 10 randomly selected fish from each tank using the standard methods (Mrong *et al.* 2021; Neepa *et al.* 2022). In brief, blood samples were drawn from the caudal vein using a 1 ml tuberculin syringe and immediately transferred into EDTA (Ethylene Diamine Tetra Acetic Acid) tubes (BD Microtainer®, UK) for anticoagulation and analysed immediately after collection for subsequent hematological analysis. After collection of blood samples, the fish were carefully dissected to collect the liver individually. Livers were blotted with filter paper to remove excess moisture and weighed separately on a digital balance to obtain individual liver weights for accurate assessment. The blood samples were analyzed immediately after collection to minimize degradation and ensure accuracy of hematological parameters. Finally, the fish samples were homogenized and stored in the refrigerator for proximate composition analysis.

2.5 Fish growth parameters

At the end of the trial period, growth performance and feed utilization parameters were determined using the following equations.

Weight gain (g) = mean final weight (g) – mean initial weight (g);

Weight gain (%) = [mean final fish weight (g) – mean initial fish weight (g)] / mean initial fish weight (g) × 100;

Specific growth rate (SGR; % d^{-1}) = [Ln final weight (g) − Ln initial weight (g)] / days × 100;

Food conversion ratio (FCR) = feed consumption (g) / body weight gain (g);

Hepatosomatic index (HSI; %) = liver weight (g) × 100 / fish weight (g);

Survival rate (%) = (final number of fish survived / number of actual fish stocked) × 100.

2.6 Proximate composition analysis

Proximate compositions of feed ingredients, experimental diets, and fish carcasses were determined according to standard procedures by the Association of Official Analytical Chemists (AOAC 2000). Crude protein content was determined by the Auto Kjeldahl System (Model UDK159, VELP, Italy), and crude lipid content was assessed by ether-extraction method using a Soxhlet Extractor (Model SER 148, Velp, Italy). Moisture content was evaluated by oven (Model JSON-030S, JSR, Korea) drying at 105°C for 24 hours, and ash content was assessed by using a muffle furnace (Carbolite RHF 17/6S, Carbolite Ltd., England) at 550°C for 4 hours. All proximate analyses were performed in triplicate for each sample to ensure accuracy and reproducibility of the results.

2.7 Haematological parameters

Blood samples were gently inverted several times to ensure proper mixing of anticoagulant and blood before analysis. A fully automatic haematology analyzer (Model DH36, Dymind Biotechnology, China) was calibrated following manufacturer's instruction and used to examine the blood samples immediately after collection and determine haematological parameters such as leukocyte (WBC), erythrocyte (RBC), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Before determination of the samples parameters, the machine was calibrated for getting actual results.

2.8 Economic analysis

Economic benefit analysis was carried out to determine the economic implications of feeding fish meal and blood meal comprised diets following Ndelekwute *et al.* (2016). The cost per kilogram (USD kg^{-1}) for each ingredient was as follows: fish meal = 1.00, blood meal = 0.13, soybean meal = 0.33, mustard oil cake = 0.25, rice bran = 0.13, wheat bran = 0.13, wheat flour = 0.33, fish oil = 0.67, vitamin premix = 0.83, mineral premix = 0.83.

Cost kg^{-1} feed = Summation of price per kg of feed ingredients × their proportions in the feed formula ÷ 100

2.9 Data analysis

All data were gathered, entered, and stored on a computer spreadsheet throughout the experiment. Before statistical analysis, the Shapiro-Wilk and Levene tests were used to determine each data set's normality and homogeneity, respectively. Statistical software Statistix 10 (2013) was used to evaluate the data using one-way analysis of variance (ANOVA) to identify significant differences between the treatments at an α level of significance of 0.05. Tukey's multiple range test was performed to compare the mean values between individual treatments if the main effects were statistically significant.

3 | RESULTS

3.1 Growth and feed utilization performance of butter catfish

Table 2 shows the growth performance and feed utilization of butter catfish fed with different experimental diets. There were variation in fish growth and feed utilization indices in respect to replacement of fish meal with blood meal. The inclusion of blood meal protein in the diet at levels up to 15% was associated with increased weight gain (WG) and specific growth rate (SGR). Replacement of fish meal protein with blood meal protein also showed a positive impact on WG (%), and maximum value (305.30 ± 1.20%) was observed in T15, where 15% fish meal protein was replaced by blood meal protein.

Conversely, a further increase in replacement level tends to decrease butter catfish's WG (%) value. Regarding SGR, the effects of substituting fish meal protein with blood meal were also observed. It was found that the highest SGR value (1.42 ± 0.21%) was achieved when 15% of fish meal was replaced by blood meal.

TABLE 2 Growth and feed utilization performance of butter catfish *Ompok bimaculatus* feed with the experimental diets (mean ± SD; *n* = 3).

Parameter	Treatment				
	T_0	T_5	T_{10}	T_{15}	T_{20}
Initial weight (g)	1.51 ± 0.03	1.52 ± 0.04	1.50 ± 0.02	1.51 ±0.05	1.52 ± 0.02
Final weight (g)	5.35 ± 0.11^{b}	5.23 ± 0.13^{c}	5.87 ± 0.21^{ab}	6.12 ± 0.16^{a}	5.42 ± 0.09^{b}
Weight gain (g)	3.84 ± 0.21^{b}	3.71 ± 0.18^{c}	4.37 ± 0.11^{ab}	4.61 ± 0.22^{a}	3.90 ± 0.14^{b}
Weight gain (%)	254.30 ± 1.21^{b}	244.08 ± 1.27^{b}	291.33 ± 1.81^{ab}	305.30 ± 1.20^{a}	256.58 ± 1.75^{b}
SGR (%/day)	1.22 ± 0.15^{b}	1.18 ± 0.19^{b}	1.37 ± 0.18^{a}	1.42 ± 0.21^{a}	1.23 ± 0.12^{b}
FCR	2.24 ± 0.11^{a}	2.06 ± 0.01^{b}	1.85 ± 0.08^{c}	1.94 ± 0.07^{c}	2.02 ± 0.05^{b}
PER	1.74 ± 0.03^{b}	1.78 ± 0.03^{a}	1.82 ± 0.04^{a}	1.88 ± 0.02^{a}	1.71 ± 0.02^{b}
HSI	1.44 ± 0.03^{a}	1.24 ± 0.02^{b}	1.26 ± 0.05^{b}	1.46 ± 0.04^{a}	1.40 ± 0.06^{a}
Survivability (%)	100	100	100	100	100

Data in the same row bearing different superscript letters indicate a significant difference ($p < 0.05$). SGR, Specific growth rate (% day-1); FCR, Feed conversion ratio; PER, Protein efficiency ratio; HSI, Hepatosomatic index.

The FCR value (1.85 ± 0.08) was significantly lower in T_{10} treatment, where 10% of fish meal in the diet was replaced with blood meal protein, compared to control treatment (2.24 ± 0.11) and FCR value in T_{15} treatment was similar to T_{10} treatment. However, a further increase in dietary blood meal level increases the FCR value. In contrast, the T_0 treatment showed a poor FCR value (2.24 ± 0.11). PER increased with the increase in the replacement levels of fish meal with blood meal up to 15% levels. However, a further rise in replacement negatively affected the PER. The highest HSI value of 1.46% was found in the T_{15} treatment, whereas the lowest value (1.24%) was recorded in the treatment T_5. The HSI values were also significantly different ($p < 0.05$) among the treatments. The survival rate of butter catfish was not affected by the replacement of fish meal protein with blood meal protein in the experimental diet, as the survival rate was 100% in all the treatments.

In regression analysis, a quadratic model was found to be the best fit model for the replacement of fish meal with blood meal in the butter catfish with WG (%) ($Y = -0.3151x^2 + 7.6173x + 241.41$ $R^2 = 0.465$, Y_{max} = X value of 12.1%), SGR (%) ($Y = -0.0013x^2 + 0.0303x + 1.1691$ $R^2 = 0.4709$, Y_{max} = X value of 11.7%), PER ($Y = -0.0011x^2 + 0.0237x + 1.7209$ $R^2 = 0.6467$, Y_{max} = X value of 10.8%), and FCR ($Y = 0.0023x^2 - 0.0581x + 2.2511$ $R^2 = 0.9309$, Y_{max} = X value of 12.6%) (Figure 1). The results indicated that butter catfish required 10.8 – 12.6% replacement of fish meal protein with blood meal protein for gaining optimum WG (%), SGR, FCR, and PER.

3.2 Proximate composition

The whole-body proximate composition of butter catfish was determined after feeding experimental diets for eight weeks (Table 3). The whole-body composition of fish varied among different the treatments in respect to replacement of fish meal with blood meal. The replacement of fish meal with blood meal increased whole-body crude protein content. The crude protein content of the T_{10}, T_{15}, and T_{20} treatments was significantly ($p < 0.05$) higher than others. However, moisture content was reduced with fish meal's replacement level with blood meal. There was no significant difference ($p > 0.05$) in whole-body fish's lipid and ash content.

TABLE 3 Proximate composition of butter catfish *Ompok bimaculatus* fed with different experimental diets for eight weeks (mean ± SD; *n* = 3).

Parameter	Treatment				
	T_0	T_5	T_{10}	T_{15}	T_{20}
Moisture (%)	77.22 ± 0.56^{a}	76.49 ± 0.42^{a}	75.01 ± 0.43^{ab}	74.80 ± 0.55^{b}	74.93 ± 0.33^{b}
Crude protein (% dm)	13.35 ± 0.06^{b}	14.32 ± 0.05^{ab}	15.07 ± 0.04^{a}	15.31 ± 0.03^{a}	14.92 ± 0.06^{a}
Crude lipid (% dm)	4.15 ± 0.03	4.05 ± 0.06	4.28 ± 0.09	4.21 ± 0.04	3.98 ± 0.12
Crude ash (% dm)	2.05 ± 0.02	1.97 ± 0.03	1.98 ± 0.03	2.19 ± 0.02	2.09 ± 0.03

Data in the same row bearing different superscript letters differ significantly ($p < 0.05$). dm = dry matter.

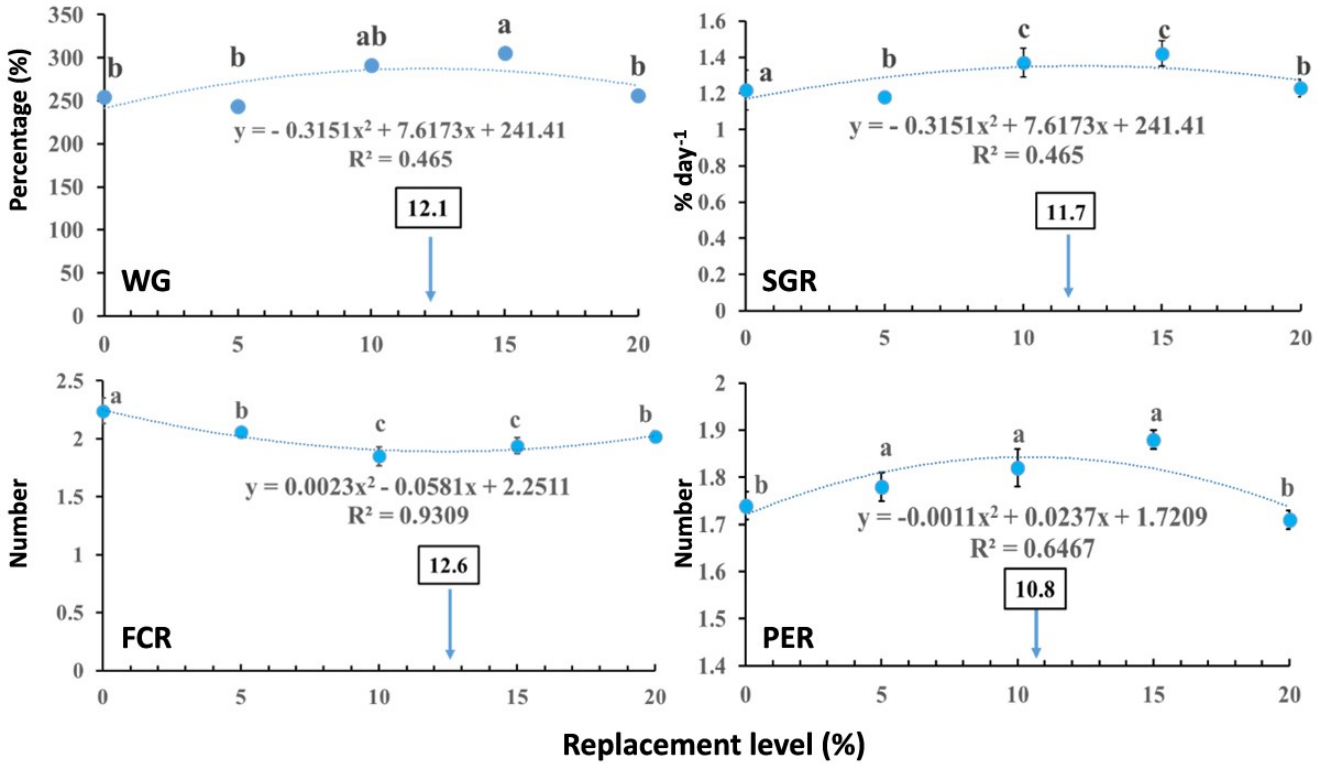

FIGURE 1 Polynomial regression analysis of weight gain (WG%), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) (dependent variables) against replacement level of fish meal with blood meal (independent variable) in the diet of butter catfish *Ompok bimaculatus*.

3.3 Haematological parameters

Haematological parameters of butter catfish, investigated in this study (Table 4). The RBC count (2.60 ± 0.22 ×10^6 μL^{-1}), WBC count (57.42 ± 0.30 ×10^3 μL^{-1}), Hb (8.80 ± 0.20 g dl^{-1}), LYMPH (39.31 ± 0.14%), NEUT (22.00 ± 0.09%), PCT (0.32 ± 0.01 ng mL^{-1}), MCH (66.91 ± 0.32 pg), and MCHC (43.94 ± 0.24%) were significantly ($p < 0.05$) higher in treatment T_{15} where 15% fish meal protein was replaced with blood meal protein. However, these values were reduced with further replacement of fish meal protein with blood meal protein. The MONO, EOS, and HCT count gradually decreased with the replacement of fish meal protein with blood meal protein. However, the EOS counts in T_5, T_{10}, and T_{15} treatments had no significant ($p > 0.05$) difference from the control (T_0) treatment.

TABLE 4 Haematological parameters in butter catfish *Ompok bimaculatus* in different treatments (mean ± SD; $n = 3$).

Blood parameters	Treatment				
	T_0	T_5	T_{10}	T_{15}	T_{20}
Hb (g dL^{-1})	7.90 ± 0.18^{b}	8.80 ± 0.20^{a}	8.00 ± 0.08^{ab}	8.30 ± 0.10^{ab}	6.90 ± 0.15^{c}
RBC (×10^6 μL^{-1})	1.72 ± 0.20^{bc}	2.10 ± 0.12^{a}	1.99 ± 0.08^{ab}	2.21 ± 0.22^{a}	1.05 ± 0.01^{c}
WBC (×10^3 μL^{-1})	41.34 ± 0.25^{d}	45.56 ± 0.15^{c}	48.48 ± 0.12^{b}	57.42 ± 0.30^{a}	39.44 ± 0.31^{d}
LYMPH (%)	35.42 ± 0.18^{ab}	36.78 ± 0.13^{ab}	37.41 ± 0.11^{b}	39.31 ± 0.14^{a}	29.6 ± 0.14^{c}
NEUT (%)	16.00 ± 0.09^{b}	17.00 ± 0.07^{b}	21.00 ± 0.08^{a}	22.00 ± 0.09^{a}	14.00 ± 0.06^{c}
MONO (%)	5.00 ± 0.06^{a}	3.00 ± 0.04^{b}	3.00 ±0.04^{b}	2.00 ± 0.03^{c}	2.00 ±0.03^{c}
EOS (%)	4.00 ± 0.22^{a}	4.00 ± 0.12^{a}	4.00 ± 0.15^{a}	4.00 ± 0.02^{a}	3.00 ± 0.02^{b}
PCT (ng mL^{-1})	0.08 ± 0.01^{bc}	0.06 ± 0.01^{c}	0.30 ± 0.02^{b}	0.32 ± 0.01^{a}	0.12 ± 0.01^{b}
HCT (%)	27.85 ± 0.29^{c}	29.5.41 ± 0.24^{b}	31.50 ± 0.22^{b}	33.95 ± 0.24^{a}	32.90 ± 0.19^{ab}
MCH (pg)	58.40 ± 0.31^{b}	58.70 ± 0.36^{b}	63.70 ± 0.46^{ab}	66.91 ±0.32^{a}	61.98 ± 0.49^{ab}
MCHC (%)	39.30 ± 0.22^{b}	41.70 ± 0.24^{ab}	42.50 ± 0.27^{ab}	43.94 ± 0.24^{a}	44.86 ± 0.28^{a}

Data in the same row bearing different superscript letters differ significantly ($p < 0.05$). Hb: hemoglobin; RBC: red blood cell; WBC: white blood cell; LYMPH: lymphocyte; NEUT: neutrophil; MONO: monocyte; EOS: eosinophil; PCT: procalcitonin; HCT: hematocrit, MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

4 | DISCUSSION

The efficiency of various alternative protein sources as partial or complete dietary replacements for fish meal has been evaluated in fish diets (El-Sayed 2004). In the present study, replacing fish meal with blood meal improved growth performance (WG and SGR) and feed utilization up to a 15% inclusion level however performance declined beyond that level. Compared to the control diet, a similar growth performance was observed in *Clarias gariepinus* when fish were fed with a blood meal - replaced diet (Njieassam 2016). In another study, it was reported that replacing fish meal with 10% blood meal in pelleted feeds was adequate for promoting the growth of tilapia, *Oreochromis niloticus* (Otubusin 2001). Moreover, Aliu and Dako (2018) found optimal growth and no adverse effects on hybrid *Clarias gariepinus* and *Heterobranchus bidorsalis* up to 15 – 20 % fish meal replacement with blood meal. In the present study, the weight gain of butter catfish increased with the replacement of fish meal protein up to 15% with blood meal. However, further inclusion resulted in reduced weight gain in butter catfish as blood meal is recognized to have an imbalanced composition of amino acids (insufficient methionine) and some anti-nutritional factors. In the diet, substituting fish meal with blood meal reduced levels of methionine, lysine, isoleucine, leucine, proline, and valine (Kirimi *et al.* 2016). This study recommends utilizing a 15% replacement of fish meal with blood meal in the diet to achieve enhanced growth for butter catfish. Fish grown with blood meal protein at various inclusion levels showed significant changes in the mean value of FCR and PER. A low FCR value indicates better feed utilization and reduced production costs. In this study, the control group exhibited the highest FCR, whereas the groups fed with blood meal protein had lower FCR values. This lower FCR indicates the better nutritional value of the blood meal diet that was consumed and efficiently converted into muscle. The treatment with the best FCR value was T10, where 10% of fish meal protein was replaced with blood meal protein, followed by T_{15} with a 15% replacement. Lawal *et al.* (2017) reported achieving the best FCR value at 25% blood meal protein replacement in the *C. gariepinus* diet, whereas the highest FCR was found in the 50% blood meal-replaced diet. However, the present study's results differed from the above mentioned findings as our fish consumed less feed and provided better growth, indicating better feed utilization.

According to regression analysis, the ideal percentage of blood meal protein to replace fish meal protein in the butter catfish diet in order to achieve the best SGR, FCR, WG, and PER is 10.8 – 12.6%. Moreover, the replacement of fish meal protein with blood meal protein reduced the diet cost compared to the control diet. In a study, it was reported that replacing fish meal with 10% blood meal in pelleted feeds was adequate for promoting the growth of tilapia (Otubusin 2001). In the present study, the weight gain of butter catfish increased with the replacement of fish meal protein up to 12.1% with blood meal protein. However, further increase in inclusion level resulted in reduced weight gain in butter catfish as blood meal is recognized to have an imbalanced composition of amino acids (methionine, lysine, isoleucine, leucine, proline, and valine) (Kirimi *et al.* 2016). This study recommends utilizing a 12.1% replacement of fish meal with blood meal in the diet to achieve enhanced growth for butter catfish. Fish grown with blood meal protein at various inclusion levels showed significant changes in the mean value of FCR and PER. A low FCR value indicates better feed utilization and lower production costs. In this study, the control group exhibited the highest FCR, whereas the groups fed with blood meal protein had lower FCR values. This lower FCR indicates the better nutritional value of the blood meal diet that was consumed and efficiently converted into muscle. The treatment with the best FCR value was 12.6% of fish meal protein was replaced with blood meal protein. Lawal *et al.* (2017) reported achieving the best FCR value at 25% blood meal protein-replaced diet in the *C. gariepinus*, whereas the highest FCR was found in the 50% blood meal -replaced diet. However, the present study's results differed from the abovementioned findings as our fish consumed less feed and provided better growth, indicating better feed utilization.

Among all treatments, T_{15}, where 15% of fish meal protein was substituted with blood meal protein exhibited the highest hepatosomatic index (HSI) value. In comparison, the lowest value (1.24%) was recorded in treatment T_5, where 5% fish meal protein was replaced by blood meal protein. An experiment on gilthead sea bream (*Sparus aurata*) found the highest HSI value of 1.63 ± 0.39 when 10% blood meal was included in the diet (Nogueira *et al.* 2012). Similarly, another experiment was conducted with *Cyprinus carpio*, where 20% of the replacement of fish meal protein with blood meal protein in the diet showed the best suitable HSI value of 2.66 (Ngoc *et al.* 2016).

In this study, butter catfish's survival rate was 100% in all treatments after eight weeks of the rearing period. This result complies with another experiment conducted on *C. gariepinus*, which found a survival rate of 95 – 100% (Lawal *et al.* 2017). Similar survival rates using 0%, 50%, and 100% blood meal in the diet of catfish *C. gariepinus* and reported that fish meal could be replaced entirely (100%) without any adverse effect on survival (Ogunji and Iheanacho 2021). The impact of fish meal replacement with blood meal in fingerling rainbow trout (*Oncorhynchus mykiss*) was experimented and found that survival ranged from 99.72 to 100% in fish fed the test diets, and there were no differences among the treatments (Bahrevar and Faghani-Langroudi 2015).

In this experiment, the whole-body crude protein and moisture content of butter catfish were significantly different although the proximate composition of the experimental diets was similar. Therefore, the variation in the whole-body proximate composition is due to the replacement of fish meal with blood meal. It was observed that the fish fed diet with 15% blood meal inclusion exhibited the highest protein and ash content in the whole-body proximate composition analysis of butter catfish. Additionally, the diet with 10% blood meal inclusion showed the highest crude lipid content, while the diet with 5% blood meal inclusion had the highest moisture value. These results differed from the findings of the research on fingerling rainbow trout (*O. mykiss*) using blood meal in the diet. They found the highest moisture content (75.08%) and protein content (17.78%) in the diet containing 20% blood meal, whereas the lowest moisture and protein values were observed in the diet with 0% replacement of fish meal protein with blood meal protein (Bahrevar and Faghani-Langroudi 2015). They also reported that the highest lipid content (5.63%) and ash content (2.77%) were found when 10% fish meal protein was replaced by blood meal protein, while the lowest values of lipid and ash content were observed when 20% fish meal protein was replaced by blood meal protein. Research on *Oreochromis niloticus* was conducted and reported that the maximum ash content was observed when 60% fish meal protein was replaced by blood meal protein (Ambreen *et al.* 2023).

The health condition of fish can be determined by analyzing their haematological parameters (Harikrishnan *et al.* 2011). During this experiment, we observed the highest values for WBC count, RBC count, and Hb in treatment T_{15}, where 15% of fish meal protein was replaced with blood meal protein in the butter catfish diet. A rising pattern in RBC and WBC indicates a positive health condition, which aids fish in safeguarding against diseases and coping with stress through their nonspecific immune responses (Akter *el al.* 2021). Ambreen *et al.* (2023) reported a positive effect of blood meal supplementation on the RBC and WBC counts of genetically improved farmed tilapia (*O. niloticus*), with RBC and WBC levels recorded at $1.50 \pm 0.02 \times 10^6$ cells/mm³ and $117.36 \pm 0.84 \times 10^6$ cells/mm³, respectively. As the incorporation of blood meal protein exceeded 15%, these values gradually declined. No significant difference was observed among the T_0 and T_{10} treatments. In another study, the highest WBC count of 24.84×10^9 L^{-1} in *C. gariepinus* was observed when 50% of the fish meal protein was replaced with blood meal protein in the fish's diet (Hossain *et al.* 2002). Those findings were similar to the results reported by another researcher (Sogbesan *et al.* 2007). In a separate study, a total RBC count of $8.50 \pm 0.72 \times 10^{12}$ L^{-1} was recorded in *C. carpio* (Arthanari and Dhanapalan 2016). However, in another investigation, a total RBC count of $2.52 \pm 0.21 \times 10^{12}$ L^{-1} was recorded in *Mugilis cephalus* (Satheeshkumar *et al.* 2012). Regarding Hb levels, the highest Hb content (9.57 ± 0.24 g dl^{-1}) was reported in *C. carpio* when the diet contained 50% blood meal protein (Rawling *et al.* 2012). Our findings differed from those of the mentioned studies, which may be attributed to the different species used in the experiments. Furthermore, there was a gradual decrease in the MONO, EOS, and HCT counts as fish meal protein was replaced with blood meal protein. However, in treatment T_{15}, where 15% of fish meal protein was substituted with blood meal protein, the values of LYMPH, NEUT, PCT, and MCHC showed a significant increase ($p < 0.05$) indicating the favorable state of fish health, which helps them defend against illnesses using their generalized immune reactions.

5 | CONCLUSIONS

The substitution of 15% fish meal protein with blood meal protein resulted improved WG, SGR, and PER (20.0%, 16.4% and 9.3% higher compared to control, respectively) in butter catfish. It resulted in improved whole-body proximate composition of butter catfish and haematological parameters. Additionally, the diet cost also reduced in respect to substitution of fish meal with blood meal in all the experimental diets compared to the control diet. Therefore, formulating a diet with 15% blood meal protein as the primary protein source proved more cost-effective than the conventional commercial diet for butter catfish, as blood meal offers a readily available and inexpensive protein source. However, regression analysis based on weight gain, SGR, FCR, and PER indicated that the optimum replacement level of fish meal protein with blood meal protein for butter catfish was in a range of 10.8–12.6 %.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

Md. Mubarak Hossain: Data acquisition, analysis and interpretation of data, drafting of the article; Md. Amzad Hossain: Conceptualization and design of the study, project administration, fund acquisition, supervision; Fazla Rabby Udoy, Md. Rabiul Islam, and Md. Shahanoor Alam: Critical revision of the article; Taslima Akter, Mohammad Shafiqul Alam, and Md. Shah Alam Sarker: Supervision and scholastic revision of the article. The manuscript's final version was approved by all authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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