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RESEARCH ARTICLE
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INSECT BASE DIETARY CRUDE PROTEIN AS A SUBSTITUTION OF FISH MEAL AND DIETARY NUTRIENT EFFICIENCY IN GROWING OF *CHANNA MARULIUS* AND *CTENOPHARYNGODON IDELLA*

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Abstract
The Black solider fly *Hermetia illucens*, larvae meal (BSFLM) is a good source of protein alternative to fish meal (FM). The quality of fish fishmeal has been challenged economically in the aquaculture feed industry. The current research was designed to identify the varying ingredient content levels of black soldier fly products (BSFP), recorded highest crude protein 42% and moisture 28.5% in mature fly, crude fiber 20.5%, and total Ash 14.5% in BSFL shell, crude fat 25.5%, in the pupa. The Black soldier fly larvae meal was tested 6-week experiments on *Ctenopharyngodon idella* 60 fingerlings (initial average length 1.58inch, 3.4gm weight and final body length and weight (4.38±0.10[a,] 15.00±0.00 [a]). Respectively, *Channa marulius* fingerlings initial average length is 6.52cm, weight 3.52gm and gain final body length and weight (8.50±0.18 [a,] 6.00±0.16 [a]). The Sixty fingerlings of each fish were divided into three groups: commercial and BSFLM feed. The feeds conversion ratio (FCR) of *C. idella* (2.564) and *C. marulius* (2.155) was calculated with the commercial base diet. The BSFLM (FCR) value of *C. idella* (1.4368) and *C. marulius* (2.106). The growth rate has a significant difference (P <0.05) and the strongest correlation recorded, temperature, ammonia, total dissolved solids, and water pH to affect the fish growth. No adverse effects were noted from the utilization of BSFLM, the formula was recommended to be used for *C. idella* and *C. marulius* fish. We concluded from our study that the BSFLM supplementation shown promising biological effects and cost-effective alternative protein and energy sources for *C. idella* and *C. marulius* fish in term of their growth traits.

Keywords: diet, *Ctenopharyngodon idella*, *Channa marulius* larvae meal, water quality

1. Introduction

The swift expansion and heightened intensity within the aquaculture sector contribute to an approximate annual growth rate of 5.8% (1). The balanced essential amino acids, easy digestibility, and palatable taste of fishmeal (FM) are crucial factors for improving the digestion and absorption of nutrients (2). Consequently, the decline in wild fish catches and the surge in aquaculture feed demand led to a notable decrease in fishmeal (FM) supply, consequently driving up diet prices (3,4). There is a growing belief that fish meal may not be sustainable enough to adequately support the future needs of the aquaculture industry (5,6).
Insect meal (IM) and other animal-derived protein sources present viable alternatives to fish meal in aquaculture feed formulations (7,8). Research on insect meal (IM) as a viable substitute for fish feed in aquaculture has seen a notable increase in their demands in recent years (9,10). Like fish meal (FM), insect meal (IM) is abundant in protein, vitamins, and minerals (11). Furthermore, it contains many essential amino acids, notably lysine, methionine, and leucine, and lacks anti-nutritional elements (12,13). The black soldier fly larvae (BSFL) are increasingly recognized as a highly promising insect species because they convert food waste into high-quality protein. This has led to a surge in mass production in recent years (14,7). The larvae of the black soldier fly (BSFL) contain a protein content ranging from 30 to 58%, along with lipids and essential amino acids constituting 10 to 30% of their composition. This nutritional profile is comparable to that observed in fish raised in aquaculture (15,16).

Additionally, they encompass a plethora of micro and macro-minerals, alongside valuable vitamins (17, 37). Substituting some or all of the dietary fish meal (FM) with black soldier fly larvae (BSFL) has been demonstrated to be effective across various fish species, including rainbow trout (*Oncorhynchus mykiss*). (18), Japanese seabass, *Lateolabrax japonicas* (19), European sea bass, *Dicentrarchus labrax* (14), Atlantic salmon, Salmo salar (7,8), hybrid tilapia (Nile × Mozambique, *Oreochromis niloticus* × *O. mozambique*) (20).

Despite the high demand, rapid growth, and competitive pricing that have propelled Nile tilapia to the second-highest position in world production, there remains a scarcity of information regarding the effects of black soldier fly larvae meal (BSFLM) on the growth traits of the fish (21). The present study was designed to assess the effects of substituting black soldier fly larvae meal (BSFLM) as a full replacement for dietary fish meal (FM) on the growth of Grass carp (*Ctenopharyngodon idella*) and Sol fish (*Channa marulius*). Investigate the standard ranges of physiochemical parameters of water to ensure optimal fish growth and prepare newly formulated diets for *C. idella* and *C. marulius* fish; used Black soldier fly larvae meal which increases growth rate and good nutritious source of *C. idella* and *C. marulius* fish feed, contributing to the development of environmentally friendly and economically viable aquafeed solutions.

2. Materials and Methods

2.1. Study Area

The experiment was done in the Fish Pathology laboratory of College of Veterinary Sciences & Animal Husbandry, at Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa, Pakistan.

2.2. Black Soldier Fly Meal Preparation

Black soldier fly meal products like a black soldier fly (*Hermetia illucens*) larvae (BSFL) pre pupa 21 grams, BSFL pupa 28 grams, BSFL shell 24 grams, and die adult BSF 53 grams were collected from the farming site of the fish pathology laboratory. The collected instar pupa and pre-pupa were washed in tap water and then dried in a micro-oven at 90℃ for 10 minutes. The remaining two products were directly collected from love-cage and dark cage units. These four products were powdered in a grinding machine and stored in a sterilized plastic box for further use.

2.3. Proximate chemical composition of BSF products

The products were analyzed at (Feed & Water Testing Laboratory Poultry Research Institute, Murree Road Rawalpindi) Link https://poultry.punjab.gov.pk/ (Table 1).

Table 1. Chemical composition analysis of BSF products

Name of Ingredients	Name of Samples			
	BSF	**BSFL Pre-pupa**	**BSFL pupa**	**BSFL- Shell**
Moisture%	28.5	18.4	27.1	8.2
Crude-Protein %	42	27.13	27.13	41.13
Crude-Fat %	14	27.9	25.5	12.4
Crude-Fiber %	9.5	14.5	13.5	20.5
Total Ash %	3	9	7.5	14.5

2.4. Experimental Diets

The diet has been prepared for *Channa marulius* and *Ctenopharyngodon idella* fish. Which has been designed; Control Group A, Group B commercial formulated 18% crude protein(Cp), and Group C for BSFLM formulated feed. Diet was included in 11 ingredients with 18 and 30cp (crude protein) of BSFL meal alternative of fish meal. 6 ingredients were added to BSFL in a specific ratio regarding formula (22) (Table 2). Mixing the ingredients with the help of a unidirectional electric mixture machine (capacity 100kg/hour) added 20% tap water to the used floating pellet extruder at a temperature120◦C model type 60 Lima (Floating pellet machine). The floating pellet sizes from (0.5mm-1mm) for fingerlings were prepared and then stored in plastic bags at room temperature for further use.

Table 2. (A) Feed formulation for *Channa marulius* (B) Feed formulation for *Ctenopharyngodon idella*

A) *Channa marulius* Fish Formula	
Name of ingredients	**%**
BSFL Meal	30
Crude Fat	10
Crude fiber	7
Ash	4.5
Moisture	9.1
Vitamin Premixes	2
BSF oil	2
Gluten 60	4
wheat Bran	10
Rice Bran	4.4
Maize	17

B) *Ctenopharyngodon idella* Fish Formula	
Name of ingredients	**%**
BSFL Meal	18
Crude Fat	14.4
Crude fiber	7
Ash	4.5
Moisture	9.1
Vitamin Premixes	4
BSF oil	2
Gluten 60	6
wheat Bran	10
Rice Bran	8
Maize	17

2.5. Experimental Procedure

The total 60 fingerlings (*C. marulius*) were collected through the cast net at the Indus River. Transported to Fish pathology laboratory. The total 60 fingerlings (*C. idella*) were purchased from a government carp hatchery Mardan, and identification was done with the help of standard keys (23, 24). The initial weight and Length of *C. marulius* (Table 3) and C. Idella(Table 4) were calculated. Before stocking fingerlings were disinfected by KMNO4. Add 1 spoon KMNO4 in 10 liter tap water and proper mixed for 5 minutes. The fingerlings were dipped 10 times for 10 seconds and then transferred to each tank.

Table 3. Determined the initial average length and weight of *C. marulius*

Group A control G		**Group B commercial feed**		**Group C BSFL Meal**	
Initial Average L	6.52cm	Initial Average L	6.52cm	Initial Average L	6.52cm
Initial Average W	3.52 gram	Initial Average W	3.52 gram	Initial Average W	3.52 gram

Table 4. Determined the initial average length and weight of *C. idella*

Group A control G		**Group B (commercial feed**		**Group C (BSFL Feed)**	
Initial Average L	1.58 inch	Initial Average L	1.58 inch	Initial Average L	1.58 inch
Initial Average W	3.4 gram	Initial Average W	3.4 gram	Initial Average W	3.4 gram

The diet Experiment was done for 42 days. Two tanks left and right size 4×4 feet, depth 5 feet, and water volume 2265 liters. Central tank size 3×3 feet, depth 5 feet, and water volume 1274 liters. Each tank was changed to 30% water every day. Diet was provided three times a day. Maintain clear and healthy water throughout the experimental period.

2.6. Water Quality Assessments

The water quality was tested at the start and end of every week. The water analysis was done through API FRESHWATER MASTER KIT (MARS) Fish Care North America; included a high range of pH, normal pH, Ammonia (mg/L), Nitrate and nitrite, TDS meter model 98302 (mg/L), Ph. meter model 98081 (belong the tester family) and thermometer (0˚C).

2.7. Growth Parameter Calculations

Every week determine parameter according to using formula; (mean final weight − mean initial weight) ÷ t (days); Weight gain (WG) = (mean final weight − mean initial weight); formula applied for determination of specific growth rate Specific (SGR %/day) = 100 × (lnWt − lnWo) ÷ t (days); Relative growth rate (RGR = [Wf (final weight) − Wi (initial weight)]/Wf × 100.

2nd formula for specific growth rate (SGR) as follows: $\text{SGR } (\%/\text{day}) \frac{\log(\text{wt})\text{-}\log(\text{wi})}{t} \times 100$

Wt for final length/weight

Wi for initial length/ weight

T for the time in days

2.8. Statistical analysis

Group means were compared using one-way ANOVA (SPSS Version 20.0). Tukey's test was used for mean separation and comparisons across treatments. Differences were considered significantly different at P<0.05.

3. Results

3.1. Feed formula for *C. idella* and *C. marulius*

The fish feed formula included 11 ingredients. Six such as BSFLM, Crude Fat, Crude Fiber, Ash, BSF oil and moisture were present in BSFL and five such as Vitamin premixes, Gluten 60, Wheat bran, Rice bran and Maize were added (Fig. 3,4).

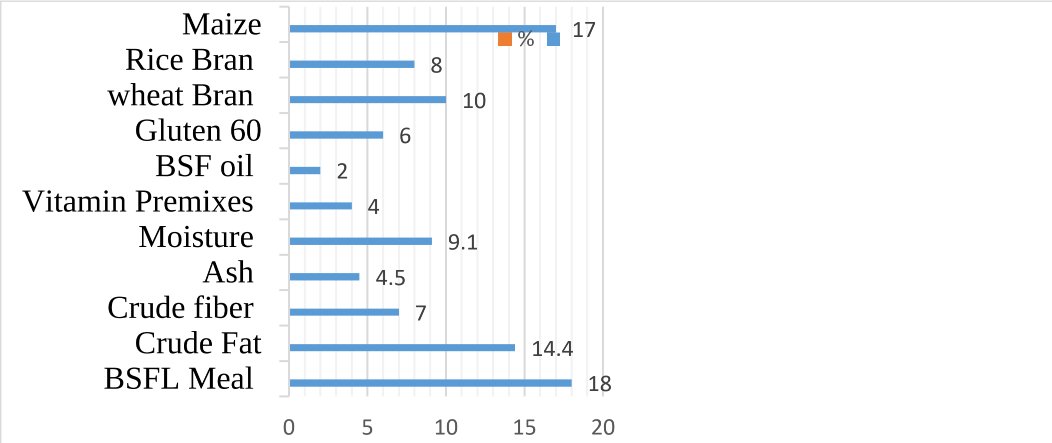

Figure 3. Shown commercial optimized feed formula for *C. Idella*

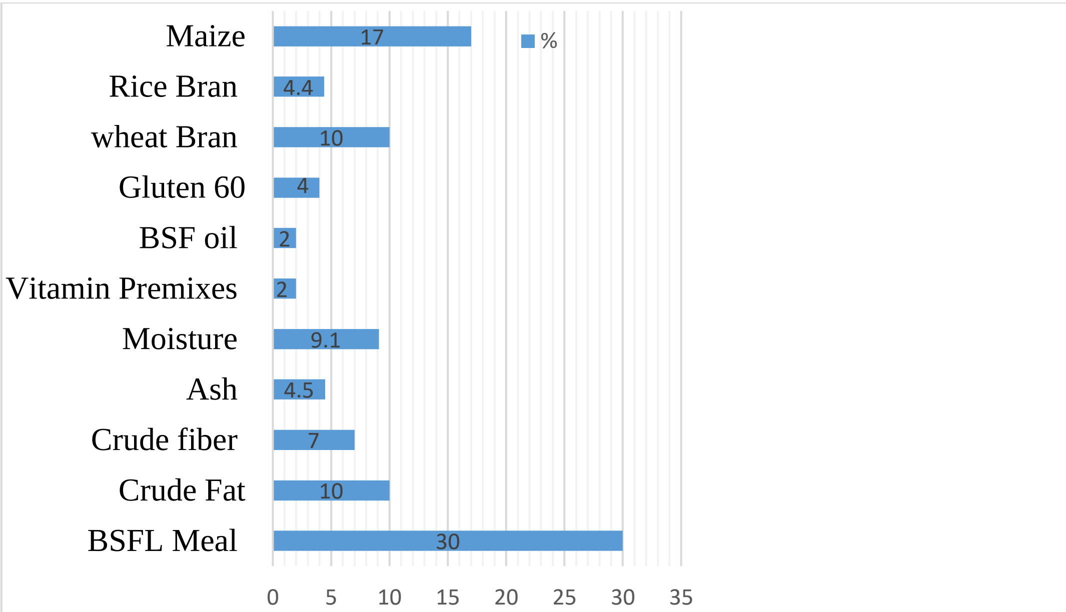

Figure 4. Commercial optimized formula for *C. marulius*.

3.2. Analysis of Growth Performance Variability

Table 5 shown the growth parameter, indicating the highest growth rates observed for *C. idella* and *C. marulius* in Diet 2, specifically in group C/3rd on Black Soldier Fly Larvae Meal (BSFLM) feed. Notably, a significant disparity was noted when compared to groups B and A. In group C, *C. idella* gain final body length (FBL) at week 6 was notably higher at 4.38±0.10a, in contrast to Group B's 3.92±0.06^{a} and Group A's 3.30±0.09^{a}. Similarly, the final body weight (FBW) of group C in week 6 was significantly higher at 15.00±0.00a compared to both groups B 9.90±0.10 ^{a} and A 8.88±0.06 ^{a}. Regarding *C. marulius*, both final body length (FBL) and final body weight (FBW) in group C were significantly greater at 8.50±0.18^{a} and 6.00±0.16^{a}, respectively, compared to groups A and B (Table 5).

Table 5. The *C. idella* and *C. marulius*, growth on Black soldier fly larvae meal feed alternative of fish meal uses in fresh water.

		weeks 1	weeks 2	weeks 3	weeks 4	weeks 5	weeks 6	MR %	SR%
Ctenopharyngodon idella	**Inch**	**I BL**	**TBL**	**TBL**	**TBL**	**TBL**	**FBL**		
	group A	1.58±0.05^{e}	1.76±0.05^{de}	1.98±0.02^{d}	2.48±0.05^{c}	2.90±0.06^{b}	3.30±0.09^{a}	0	100
	Group B	1.58±0.05 ^{c}	1.78±0.10 ^{c}	2.50±0.18 ^{b}	3.00±0.00 ^{b}	3.60±0.24 ^{a}	3.92±0.06 ^{a}	0	100

	Group C	1.58±0.05 ^{d}	1.92±0.08 ^{d}	2.84±0.18 ^{c}	3.92±0.04 ^{b}	4.16±0.09 ^{ab}	4.38±0.10 ^{a}	0	100
	Gram	**IBW**	**TBW**	**TBW**	**TBW**	**TBW**	**FBW**		
	group A	3.40±0.24 ^{e}	4.80±0.37 ^{cd}	5.40±0.24 ^{c}	7.40±0.51 ^{b}	8.00±0.32 ^{ab}	8.88±0.06 ^{a}	0	100
	Group B	3.40±0.24 ^{d}	5.20±0.20 ^{c}	6.40±0.40 ^{c}	8.40±0.51 ^{b}	9.00±0.32 ^{ab}	9.90±0.10 ^{a}	0	100
	Group C	3.40±0.24 ^{f}	6.00±0.00 ^{e}	9.00±0.00 ^{d}	10.00±0.00 ^{c}	12.40±0.40 ^{b}	15.00±0.00 ^{a}	0	100
Channa marulius	**Inch**	**I BL**	**TBL**	**TBL**	**TBL**	**TBL**	**FBL**		
	group A	6.52±0.18 ^{a}	6.54±0.18 ^{a}	6.56±0.19 ^{a}	6.58±0.20 ^{a}	6.58±0.20 ^{a}	6.58±0.08 ^{a}	0	100
	Group B	6.52±0.18 ^{a}	6.64±0.15 ^{a}	6.78±0.12 ^{a}	6.86±0.14 ^{a}	6.98±0.15 ^{a}	7.08±0.15 ^{a}	0	100
	Group C	6.52±0.18 ^{c}	6.76±0.14 ^{bc}	7.04±0.12 ^{bc}	7.32±0.14 ^{b}	8.00±0.08 ^{a}	8.50±0.18 ^{a}	0	100
	gram	**IBW**	**TBW**	**TBW**	**TBW**	**TBW**	**FBW**		
	group A	3.52±0.18 ^{c}	3.64±0.16 ^{bc}	3.74±0.16 ^{bc}	3.94±0.11 ^{bc}	4.18±0.11 ^{b}	4.88±0.10 ^{a}	0	100
	Group B	3.52±0.18 ^{d}	3.86±0.15 ^{cd}	4.18±0.12 ^{bc}	4.64±0.14 ^{b}	5.28±0.04 ^{a}	5.84±0.14 ^{a}	0	100
	Group C	3.52±0.18 ^{d}	3.96±0.15 ^{cd}	4.26±0.15 ^{bc}	4.92±0.08 ^{b}	5.86±0.19 ^{a}	6.00±0.16 ^{a}	0	100

The analysis of variance showed significant ($P< 0.05$) value of BSFLM feed alternative of commercial fish feed of the mention target fish, yellow highlighted shown significant value in all groups C see in table 5. Growth parameters of *C. idella* and *C. marulius*, IBL (initial body length), TBL (Total body length), FBL (final body length), IBW (initial body weight), TBW (total body weight) FBW (final body weight), MR (Mortality rate) and SR (Survival rate).

3.3. Variable selection for the modelling framework

The presence of collinearity among the explanatory variables (predictors) can lead to instability in model performance, resulting in uncertain predictions. Therefore, it is advisable to exclude highly correlated variables from model fitting to ensure robust results. To tackle the collinearity issue, we employed two methods: Pearson correlation and variance inflation factor (VIF) analysis, to identify and eliminate correlated variables. The VIF indicates the extent to which standard errors inflate due to multicollinearity among variables included in the model. By detecting collinearity among variables, the VIF-based approach excludes those with high VIF values.

Additionally, we utilized Pearson correlation to evaluate collinearity among predictors, excluding variables with a correlation coefficient $|r| \geq 0.7$ from the model. The dark-shaded blue and shaded red colours represent high collinearity among the variables, while the light-shaded ones represent low collinearity. Similarly, the bigger the circle, the higher the correlation value see in figure 5. and 6.

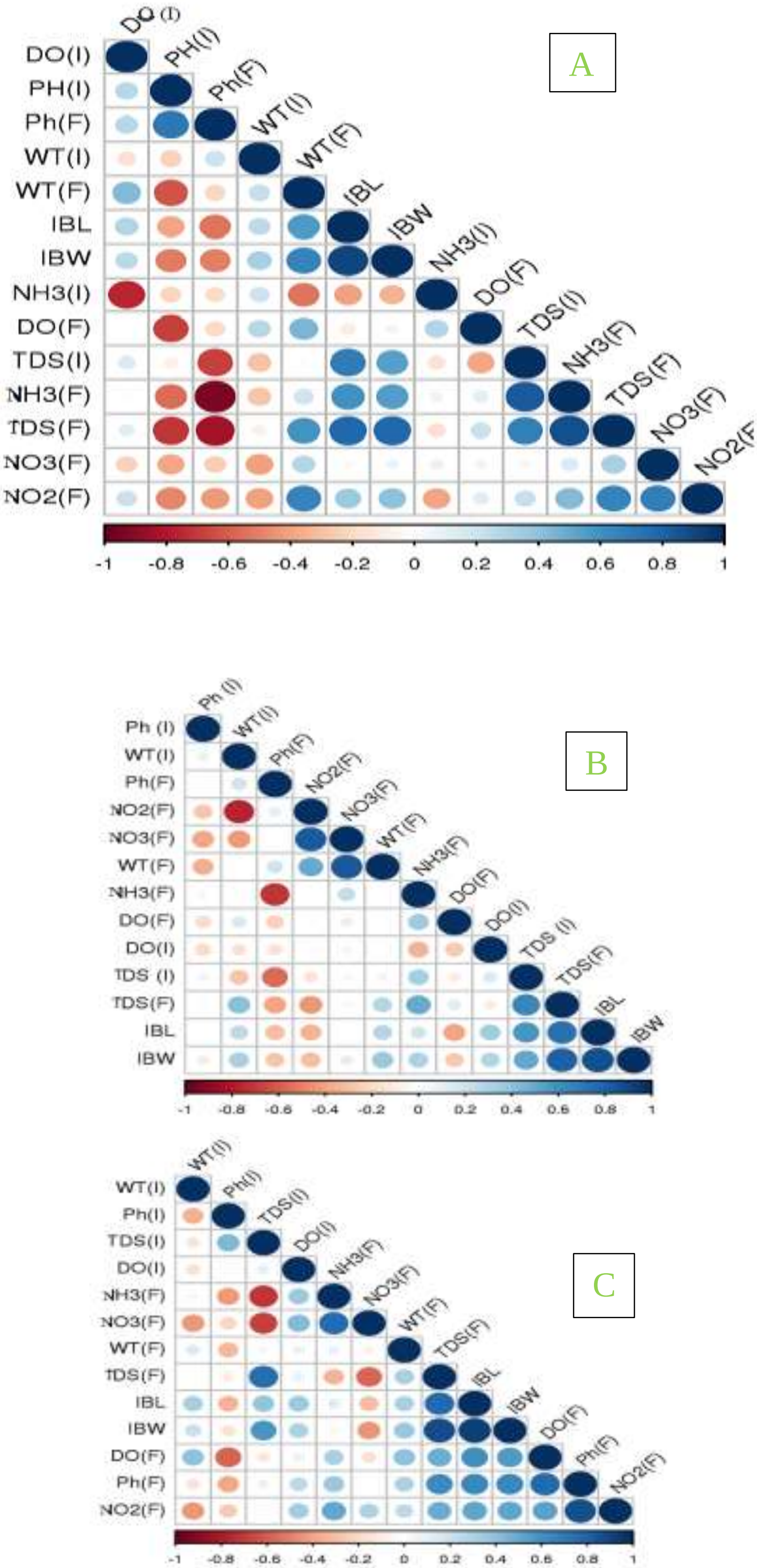

Figure 5. The Pearson correlation matrix to assess the collinearity among the *C. idella* variables of the Group A, B and C of physiochemical water parameters effects on Growth *C. idella*.WT(I): Water temperature initial, pH(I): pH initial, TDS (I): Total Dissolved Solid initial, DO (I): Dissolved oxygen initial, NH_3 (F): ammonia final, NO_3 (F): Nitrate final, WT (F): Water

temperature final, TDS (F): Total Dissolved solid final, IBL: Initial body length, IBW: Initial body weight, DOF: Dissolved oxygen final, pH (F): pH final, NO_2 (F): Nitrite final.

When compared all the parameters, the most independent variables that had the lowest VIF values were WT(I); and DO(F) in group A. WT(I), pH(F), DO(I&F) in group B, and DO(I), WT(F), WT(I) in group C. the strongest correlation had shown in group A, ph (I &F) and NH_3 (F). in group B shows WT (I & F), NH_3 (F), TDS (F), pH (F) and Group C has pH (F), TDS (I &F).

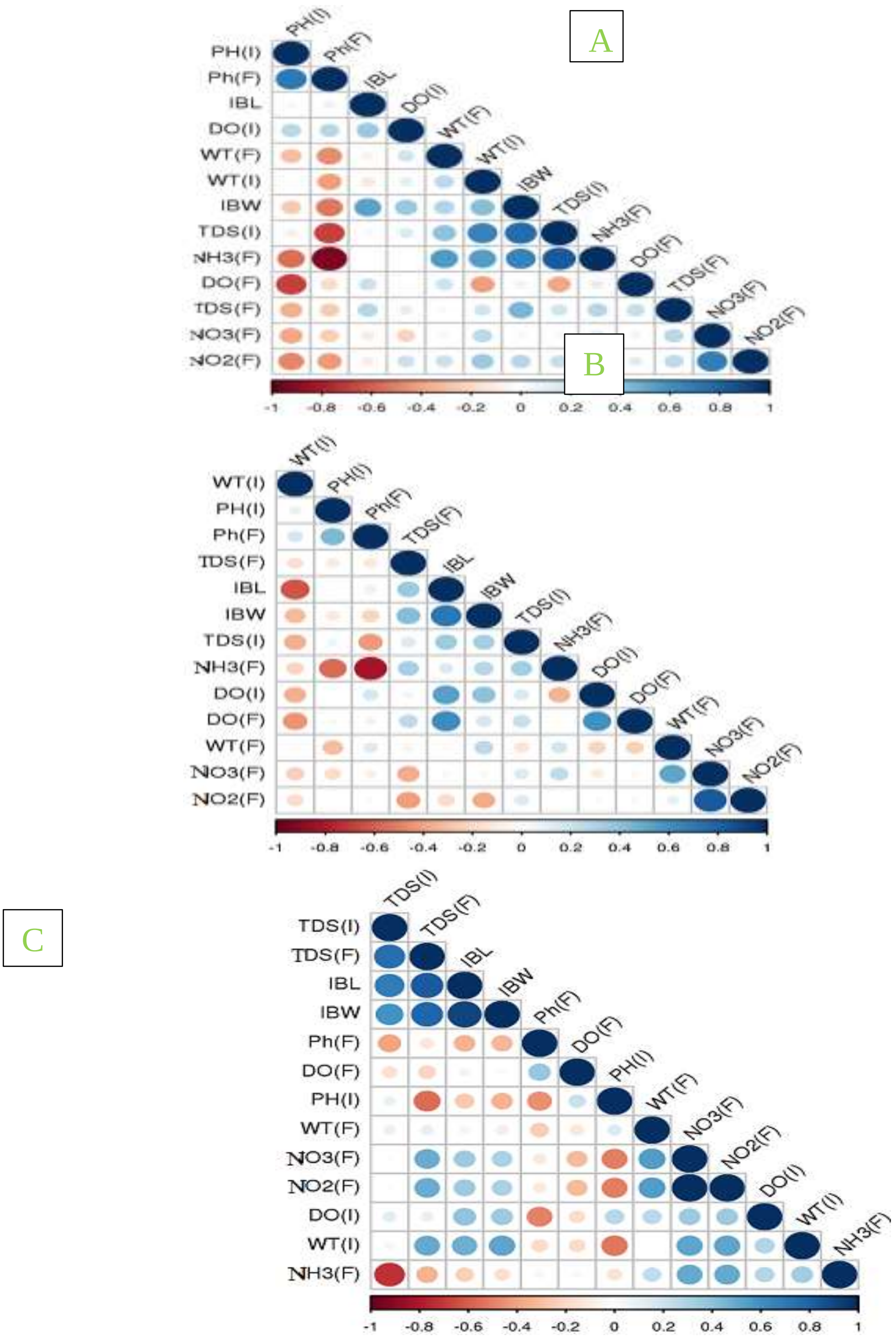

Figure 6. The Pearson correlation matrix to assess the collinearity among the *C. marulius* variables of the Group A, B and C of physiochemical water parameters effects on Growth *C. marulius*. WT(I): Water temperature initial, pH(I): pH initial, TDS (I): Total Dissolved Solid initial, DO (I): Dissolved oxygen initial, NH_3 (F): ammonia final, NO_3 (F): Nitrate final, WT (F): Water

temperature final, TDS (F): Total Dissolved solid final, IBL: Initial body length, IBW: Initial body weight, DO(F): Dissolved oxygen final, pH (F): ph final, NO_2 (F): Nitrite final.

Correlation analysis was used to determine the relationship of *C. marulius* growth response among the different physiochemical parameters of water. The most independent variables that had the lowest VIF values were IB(L), WT(I), TDS(F), NO3(F), NO_2(F) in group A, TDS(F), WT(F), NO3(I), NO2(F) in group B, and WT(F), DO(F), NH3(F) in group C. the strongest correlation had been shown in group A, pH (I&F). in group B, pH (F).in group C, TDS (I &F), NO_3 (F) and NH_3 (F).

3.4. Importance of variable

The contribution of each variable to the performance of each model. It identifies the variable that offers the highest contribution to feeding the BSFLM for *C. idella* and *C. muralluis* culture. Notably, in each group, the most independent variables, characterized by the lowest VIF values, differed, as depicted in Figure 5 and 6.

3.5. Feed conversion Ratio

The feeding conversion is an indicator of feeding efficiency, apply the given below formula for mathematical calculation

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$$\text{FCR} = \frac{Total\ feed\ consumed}{Total\ weight\ of\ products}$$

where the total weight of products = final weight of the products and starting weight of the products are followed by Fcr-feed-conversion-rate-formula. According to this formula was determine FCR of the *C. idella* FCR group B (Table 6) and group C in (Table 7) as well as the *C. marulius* (Tables 8 & 9).

Table 6. The FCR value of commercial feed on C. *idella*

Commercial feed for Group (B) *C. idella*	
FCR	**weight in gram**
Total feed consumed	2000
initial animals weight	408
final animals weight	1188
total weight gain by animals	780
FCR Value	2.564

Table 7. The FCR value of BSFLM feed on *C. idella*.

BSFLM feed for Group (C) *C. idella*	
FCR	**Weight in gram**
Total feed consumed	2,000
initial animals weight	408
final animals weight	1800
total weight gain by animals	1392
FCR Value	**1.4368**

Table 8: The FCR value of commercial feed on *C. marulius*.

Commercial feed for Group (B) *C. marulius*	
FCR	**weight in gram**
Total feed consumed	1000
initial animals weight	704
final animals weight	1168
total weight gain by animals	464
FCR Value	2.155

Table 9: The FCR value of BSFLM feed on *C. marulius*.

BSFLM feed for Group (C) *C. marulius*	
FCR	**weight in gram**
Total feed consumed	1000
initial animals weight	704
final animals weight	1200
total weight gain by animals	496
FCR Value	2.106

The *C. idella* and *C. marulius* FCR value were recorded1.43and 2.106 on BSFLM in group C is notably higher than the FCR value of 2.564 and 2.155 observed in group B on commercial feed. This suggests that BSFLM feed results in a significantly higher growth rate compared to commercial feed for the *C. idella* and *C. marulius*.

4. Discussion

Nutrition plays a crucial role in facilitating the optimal growth of *C. idella* and *C. marulius*, which are significant and cost-effective sources of protein. (25). Utilizing Black Soldier Fly Larvae Meal (BSFLM) as a high-protein source has the potential to promote optimal growth in both *C. idella* and *C. marulius* (26). In the current study, the complete replacement of fish meal (FM) with Black Soldier Fly Larvae Meal (BSFLM) yielded noteworthy results.

The growth performance of both common grasses, fed with 18% crude protein (CP), and sole fish, fed with 30% CP, demonstrated significant improvement when fed with BSFLM. This improvement was evidenced by increased daily weight gain, a 100% survival rate, and no recorded mortality, contrasting with the use of FM. (27, 34). The feed conversion ratio (FCR) for *C. idella* fish, recorded at 1.4 on a diet formulated with BSFLM, indicates a notable efficiency in feed utilization. In comparison, the FCR for *C. marulius* was recorded at 2.106, demonstrating a significant improvement over commercial feed formulations containing fish meal (FM) (28). Similar to my current findings, no adverse effects were observed on the growth performance of the fish (29).

The measured feed efficiency indices, including total feed intake, rates of feed intake, feed conversion ratio, feed efficiency, and total digestibility, showed consistency among both *C. idella* and sole fish when fed with BSFLM. These findings mirror previous results reported in rainbow trout (*Oncorhynchus mykiss*) (30, 35). Black Soldier Fly Larvae Meal (BSFLM) is a rich source of omega-3, omega-6, and omega-9 fatty acids, which are essential for fish nutrition. Additionally, it has been observed to enhance the gut microflora of the fish. These benefits contribute to improved growth performance in fish (31,32).

However, based on analysis, the final body length (FBL) of *C. idella* in group C recorded a significant value of 4.38±0.10 in week 6th, compared to group B with 3.92±0.06 and group A with 3.30±0.09. Additionally, the final body weight (FBW) *C. idella* in group C was significantly higher at 15.00±0.00 in week 6th compared to groups B and A. Similarly, for *C. marulius*, both final body length (FBL) and final body weight (FBW) in group C were significantly higher at 8.50±0.18 and 6.00±0.16 respectively, compared to groups A and B see details in table no 5. A positive correlation was observed between mean fish length and temperature (r= 0.54232, p=0.0001) and a negative correlation between mean fish length and conductivity (r= −0.34323, p=0.0111) (33).

The results showed a highly significant correlation between the growth parameters and physio-chemical parameters recorded (figure 5 & 6). Quality of portentous feed and water contributes to both the quantity and quality of fish production compared to poor water quality and unpretentious protein. It's important to monitor feed and maintain optimum levels of pH, water temperature, dissolved oxygen (DO), and ammonia in the fishpond to ensure the best culture conditions.

5. Conclusion

In conclusion, Black Soldier Fly Larvae Meal (BSFLM) emerged as a promising and cost-effective protein source, serving as the optimal alternative to fish meal (FM) for *C. idella* and *C. marulius* fish. The inclusion of BSFLM in formulated diets resulted in improved feed efficiency and enhanced health conditions for the fish, without any adverse effects. Maintaining physio-chemical parameters of water within the optimum range is crucial for achieving maximum growth and high yields in pond farming. Poor water quality can induce stress in fish, leading to stunted growth, diseases, and mortality. Therefore, it is recommended to maintain good water quality with normal pH, adequate dissolved oxygen (DO), optimal water temperature, ammonia, and appropriate total dissolved solids (TDS) levels for *C. idella* and *C. marulius*. Providing a diet with 18% (CP) for *C. idella* and 30% CP for *C. marulius* is essential for achieving high yields and fast growth. This approach offers globally to produce BSFLM protein, using recommended fish feed formulas and adhering to optimal water quality parameters for enhanced production efficiency.

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