Chapter 4

Results

4. Results

4.1. Nutritional characteristics of the two freshwater macrophytes, *Lemna minor* and *Ipomoea aquatica*

4.1.1. Proximate composition

The proximate composition analysis of the two freshwater macrophytes, *L. minor* and *I. aquatica*, is given in Table 4. *L. minor* exhibited a higher moisture content (6.70 ± 0.01%) than *I. aquatica* (5.53 ± 0.01%). However, *I. aquatica* demonstrated significantly ($P < 0.05$) higher protein content of 22.52 ± 0.03%, while in *L. minor* it was 20.53 ± 0.01%. The lipid content recorded was 7.53 ± 0.01% and 7.34 ± 0.01% for *L. minor* and *I. aquatica,* respectively. The ash content was higher in *L. minor* (16.51 ± 0.01%) than in *I. aquatica* (15.03 ± 0.01%). *I. aquatica* had a higher fibre content (6.04 ± 0.02%) than *L. minor* (5.14 ± 0.02%). The carbohydrate content was significantly higher in *L. minor* (43.60 ± 0.02%) than in *I. aquatica* (43.55 ± 0.01%).

Table 4. Proximate composition (%) of *Lemna minor* and *Ipomoea aquatica* (dry weight basis).

Parameters	***Lemna minor***	***Ipomoea aquatica***	***P* value**
Moisture	6.70 ± 0.01*	5.53 ± 0.01	< 0.001
Protein	20.53 ± 0.01	22.52 ± 0.03*	< 0.001
Lipid	7.53 ± 0.01*	7.34 ± 0.01	< 0.001
Ash	16.51 ± 0.01*	15.03 ± 0.01	< 0.001
Fibre	5.14 ± 0.02	6.04 ± 0.02*	< 0.001
Carbohydrate	43.60 ± 0.02*	43.55 ± 0.01	0.006

Notes: Data are expressed as mean ± SD. * Denotes significantly higher value within the same row at $P < 0.05$. n=3

4.1.2. Amino acid composition

The amino acid composition highlighted differences between the two species (Table 5). Total essential amino acid (EAA) content was significantly ($P < 0.05$) higher (38.56 ± 0.11 mg g^{-1}) in *I. aquatica* compared to *L. minor* (35.12 ± 0.35 mg g^{-1}). Notably, *I. aquatica* exhibited higher levels of arginine (6.75 ± 0.02 mg g^{-1}) and lysine (7.80 ± 0.04 mg g^{-1}), while *L. minor* exhibited higher leucine (8.65 ± 0.01 mg g^{-1}). Both species displayed comparable methionine concentrations, with *L. minor* at 1.00 ± 0.00 mg g^{-1} and *I. aquatica* at 0.97 ± 0.01 mg g^{-1}. However, total non-essential amino acid (NEAA) was significantly ($P < 0.05$) higher in *L. minor* (23.42 ± 0.00 mg g^{-1}) compared to *I. aquatica* (21.29 ± 0.00 mg g^{-1}) in *I. aquatica*. Proline was found to be significantly higher in *I. aquatica* (4.98 ± 0.01 mg g^{-1}) and serine in *L. minor* (5.52 ± 0.03 mg g^{-1}).

Table 5. Amino acid composition (mg g^{-1} dry weight) of *Lemna minor* and *Ipomoea aquatica was* used in the study.

Amino acids (mg g^{-1})	***Lemna minor***	***Ipomoea aquatica***	***P* value**
EAA			
Arginine	4.16 ± 0.02	6.75 ± 0.02*	< 0.001
Histidine	2.32 ± 0.23	2.01 ± 0.01	0.071
Lysine	6.63 ± 0.03	7.80 ± 0.04*	< 0.001
Leucine	8.65 ± 0.01*	7.72 ± 0.07	< 0.001
Methionine	1.00 ± 0.00*	0.97 ± 0.01	0.016
Phenylalanine	7.21 ± 0.03	8.52 ± 0.08*	< 0.001
Threonine	1.71 ± 0.00	2.03 ± 0.02*	< 0.001
Tryptophan	0.42 ± 0.00*	0.12 ± 0.00	< 0.001
Valine	3.01 ± 0.03*	2.63 ± 0.00	< 0.001
Total EAA	35.12 ± 0.35	38.56 ± 0.11*	< 0.001
NEAA			
Alanine	2.46 ± 0.03	2.41 ± 0.10	0.477
Aspartic acid	3.68 ± 0.09	3.21 ± 0.05	0.002
Cysteine	0.04 ± 0.00	0.04 ± 0.01	0.120

Glycine	0.02 ± 0.00	0.03 ± 0.00	0.143
Glutamic acid	3.11 ± 0.01*	2.71 ± 0.00	< 0.001
Proline	4.47 ± 0.04	4.98±0.01*	< 0.001
Serine	5.52 ± 0.03*	4.16±0.05	< 0.001
Tyrosine	3.29 ± 0.03*	2.74 ± 0.05	< 0.001
Citrulline	0.01 ± 0.00	0.01 ± 0.00	0.374
Asparagine	0.83 ± 0.05*	0.20 ± 0.00	0.019
Beta 3-4 dihydroxy phenylalanine	0.01 ± 0.00	0.01 ± 0.00	0.116
Total NEAA	23.42 ± 0.00*	21.29 ± 0.00	< 0.001
Total amino acids	58.53 ± 0.35	59.84 ± 0.11*	0.004

Notes: Data are expressed as mean ± SD. * Denotes significantly higher value at $P < 0.05$. EAA: Essential amino acid, NEAA: Non-essential amino acid

4.1.3. Fatty acid composition

Both the plants' fatty acid composition analyses revealed the presence of 7 SFAs, 4 MUFAs and 6 PUFAs (Table 6). Variations between the two species were also recorded. Total saturated fatty acid (SFA) content was higher in *L. minor* (34.94 ± 0.05%) compared to *I. aquatica* (33.92 ± 0.04%). Conversely, *I. aquatica* displayed a marginally higher total monounsaturated fatty acid (MUFA) content (16.40 ± 0.05%) compared to *L. minor* (14.85 ± 0.06%). Polyunsaturated fatty acids (PUFA) were prevalent in both species, with *L. minor* containing 50.21 ± 0.01% and *I. aquatica* at 49.68 ± 0.05%. The PUFA-to-SFA ratios were recorded as 1.44 ± 0.00 and 1.46 ± 0.00 for *L. minor* and *I. aquatica*, respectively. Both species showed an identical ω6/ω3 ratio of 0.84, indicative of a balanced fatty acid profile. Additionally, the combined content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was significantly ($P > 0.05$) higher in *I. aquatica* (2.37 ± 0.01%) than *L. minor* (2.31 ± 0.01%).

Table 6. Fatty acid composition (% of total fatty acid) of *Lemna minor* and *Ipomoea aquatica*.

Fatty acid	***Lemna minor***	***Ipomoea aquatica***	***P* value**
C13:0	0.85 ± 0.01	0.91 ± 0.00*	< 0.001
C14:0	1.98 ± 0.01	2.01 ± 0.01*	0.001
C15:0	0.48 ± 0.01	0.47 ± 0.02	0.116
C16:0	16.40 ± 0.01	16.50 ± 0.03*	< 0.001
C17:0	12.01 ± 0.01*	11.05 ± 0.02	< 0.001
C18:0	2.10 ± 0.02*	1.98 ± 0.01	< 0.001
C20:0	1.12 ± 0.01*	1.00 ± 0.01	< 0.001
Σ SFA	34.94 ± 0.05*	33.92 ± 0.04	< 0.001
C16:1n-5	5.10 ± 0.03	5.52 ± 0.02*	< 0.001
C18:1n-9	3.35 ± 0.01*	3.20 ± 0.01	< 0.001
C20:1n-9	4.30 ± 0.01	5.66 ± 0.01*	< 0.001
C15:1n-5	2.10 ± 0.02*	2.02 ± 0.01	< 0.001
Σ MUFA	14.85 ± 0.06	16.40 ± 0.05*	< 0.001
C20:5n-3	1.12 ± 0.02	1.25 ± 0.01*	< 0.001
C22:6n-3	1.19 ± 0.01*	1.12 ± 0.01	< 0.001
C18:3n-3	24.95 ± 0.02	27.21 ± 0.03*	< 0.001
C18:2n-6	20.41 ± 0.02*	16.62 ± 0.41	< 0.001
C18:3n-6	1.23 ± 0.01	1.97 ± 0.01*	< 0.001
C20:4n-6	1.31 ± 0.01	1.51 ± 0.01*	< 0.001
Σ PUFA	50.21 ± 0.01*	49.68 ± 0.05	< 0.001
PUFA/SFA	1.44 ± 0.00	1.46 ± 0.00*	< 0.001
ω6/ω3	0.84 ± 0.02	0.84 ± 0.01	0.131
EPA+DHA	2.31 ± 0.01	2.37 ± 0.01*	0.001

Notes: Data are expressed as mean ± SD. * Denotes significantly higher value at $P < 0.05$. SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid.

4.1.4. Antinutritional factors (ANFs)

The analysis of ANFs in *L. minor* and *I. aquatica* is given in Table 7. *L. minor* exhibited higher alkaloid content (1.00 ± 0.02%) than *I. aquatica* (0.93 ± 0.03%), while *I. aquatica* contained a significantly higher amount of tannic acid (0.21 ± 0.02%) than *L. minor* (0.10 ± 0.01%). Regarding saponin, *I. aquatica* showed a markedly elevated concentration (1.00 ± 0.03%) compared to *L. minor* (0.60 ± 0.01%). Phytic acid and oxalate were significantly ($P < 0.05$) higher in *I. aquatica* (0.88 ± 0.01% and 0.26 ± 0.02%, respectively) than in *L. minor* (0.48 ± 0.01% and 0.20 ± 0.02%, respectively).

Table 7. Composition (%) of Antinutritional factors (ANFs) in the two experimental plants.

ANFs (%)	***Lemna minor***	***Ipomoea aquatica***	***P* value**
Alkaloids	1.00 ± 0.02*	0.93 ± 0.03	< 0.001
Tannic acid	0.10 ± 0.01	0.21 ± 0.02*	< 0.001
Phytic acid	0.48 ± 0.01	0.88 ± 0.01*	< 0.001
Oxalate	0.20 ± 0.02	0.26 ± 0.02*	< 0.001
Saponin	0.60 ± 0.01	1.00 ± 0.03*	< 0.001

Notes: Data are expressed as mean ± SD. * Denotes significantly higher value at $P < 0.05$.

4.2. Effect of *Lemna minor* supplemented feeds on the growth performance, digestive enzymes and biochemical parameters of *Anabas testudineus*

4.2.1. Growth performance

The growth performance of *Anabas testudineus* (initial weight: 0.70 ± 0.01 g) fed the diet supplemented with different percentages of *Lemna minor* over a 60-day period is presented in Table 8. The final weights (FW) were 3.31 ± 0.02, 3.44 ± 0.02, 3.61 ± 0.01, 3.83 ± 0.01, and 3.70 ± 0.02 g for LM0, LM5, LM10, LM15 and LM20, respectively. Fish in the control group (LM0) recorded the lowest FW, while the fish-fed diets incorporating *L. minor* (LM5-LM20) showed higher FW than the control group. LM5 and LM10 groups showed incremental improvements. Notably, the highest FW was observed in the LM15 group, which represented a 1.16-fold increase compared to LM0 and was significantly greater than all other groups ($P < 0.05$). Although the LM20 group had a slight decrease in FW compared to LM15, it still achieved a 1.12-fold increase over the control. Correspondingly, body weight gain (BWG) percentages were 373.51 ± 1.85, 396.16 ± 2.31, 417.43 ± 2.92, 449.55 ± 2.72, and 427.84 ± 2.14% for LM0, LM5, LM10, LM15, and LM20, respectively. The LM0 group recorded the lowest BWG, while LM5 and LM10 showed gradual increases. The LM15 group recorded the highest BWG, corresponding to a 1.20-fold increase over the control group and significantly exceeding the BWG of all other groups ($P < 0.05$). The LM20 group showed a slight reduction in BWG compared to LM15 but maintained a 1.15-fold increase relative to LM0.

The specific growth rates (SGR) were 2.59 ± 0.01, 2.67 ± 0.01, 2.74 ± 0.01, 2.84 ± 0.01, and 2.77 ± 0.01 for LM0, LM5, LM10, LM15, and LM20, respectively. An increasing trend in SGR was noted, with higher inclusion levels of *L. minor* being up to 15%, where LM15 showed a 1.10-fold increase compared to the control (LM0), making it significantly higher than all other groups ($P < 0.05$). In contrast, the SGR of the LM20 group showed a slight decline, representing a 1.07-fold increase over the control. The feed conversion

ratios (FCR) were 1.56 ± 0.03, 1.52 ± 0.01, 1.46 ± 0.02, 1.39 ± 0.01, and 1.44 ± 0.01 for LM0, LM5, LM10, LM15 and LM20, respectively. A decreasing trend in FCR was observed with increasing levels of *L. minor* up to 15%, indicating improved feed utilisation. The LM15 group achieved the lowest FCR, indicating a 1.12-fold improvement over the control, significantly better than all other groups ($P < 0.05$). Survival rates were consistently 100% across all dietary treatments (LM0-LM20), suggesting that varying percentages of *L. minor* did not adversely affect fish survival.

The feed efficiency (FE) percentages were 63.97 ± 1.04, 65.99 ± 0.44, 68.32 ± 0.77, 71.69 ± 0.66, and 69.33 ± 0.47% for LM0, LM5, LM10, LM15, and LM20, respectively. The control group recorded the lowest FE, whereas the FE increased successively with higher inclusion levels of *L. minor* up to 15% (LM5-LM15). Specifically, LM15 exhibited a 1.12-fold increase in FE compared to LM0, making it significantly higher than all other groups ($P < 0.05$). However, the LM20 group showed a slight decline, with a 1.08-fold increase over the control. The protein efficiency ratios (PER) were 1.60 ± 0.03, 1.65 ± 0.01, 1.71 ± 0.02, 1.79 ± 0.02, and 1.73 ± 0.01 for LM0, LM5, LM10, LM15, and LM20, respectively. An upward trend in PER was observed, with *L. minor* levels increasing by up to 15%. However, there was a decrease in the LM20 group. The PER in the LM15 group was significantly higher ($P < 0.05$) than in the control and other dietary groups, with a 1.12-fold increase compared to LM0. Polynomial regression analysis of the SGR and FCR data indicated that optimal growth occurred when *L. minor* inclusion in the diet was between 16.25% and 17.10%, as shown in Figure 6 (a-b). This range of *L. minor* supplementation provided the best balance for maximising growth rates and improving feed utilisation efficiency. The study showed that the LM15 diet, containing 15% *L. minor*, was the most effective in enhancing growth performance in *A. testudineus*. Fish in this group exhibited the highest FW, BWG, SGR, FE, and PER while having the lowest FCR.

Table 8. The nutritional efficiency and growth performance of *Anabas testudineus* fed with varying percentages of *Lemna minor* diets (LM0: 0% *L. minor* incorporated diet, LM5: 5% *L. minor*, LM10: 10% *L. minor*, LM15: 15% *L. minor*, and LM20: 20% *L. minor*).

Parameters	**LM0**	**LM5**	**LM10**	**LM15**	**LM20**	***P* value**
IW (g)	0.70 ± 0.01	0.70 ± 0.02	0.70 ± 0.01	0.70 ± 0.02	0.70 ± 0.01	
FW (g)	3.31 ± 0.02^{e}	3.44 ± 0.02^{d}	3.61 ± 0.01^{c}	3.83 ± 0.01^{a}	3.70 ± 0.02^{b}	< 0.001
BWG (%)	373.51 ± 1.85^{e}	396.16 ± 2.31^{d}	417.43 ± 2.92^{c}	449.55 ± 2.72^{a}	427.84 ± 2.14^{b}	< 0.001
SGR (% day^{-1})	2.59 ± 0.01^{e}	2.67 ± 0.01^{d}	2.74 ± 0.01^{c}	2.84 ± 0.01^{a}	2.77 ± 0.01^{b}	< 0.001
FCR	1.56 ± 0.03^{a}	1.52 ± 0.01^{b}	1.46 ± 0.02^{c}	1.39 ± 0.01^{e}	1.44 ± 0.01^{d}	< 0.001
Survival (%)	100	100	100	100	100	
FE (%)	63.97 ± 1.04^{e}	65.99 ± 0.44^{d}	68.32 ± 0.77^{c}	71.69 ± 0.66^{a}	69.33 ± 0.47^{b}	< 0.001
PER	1.60 ± 0.03^{d}	1.65 ± 0.01^{c}	1.71 ± 0.02^{b}	1.79 ± 0.02^{a}	1.73 ± 0.01^{b}	< 0.001

Note: Different superscript letters denote statistically significant differences within a common row (n = 30, $P < 0.05$). IW: Initial weight, FW: Final weight, BWG: Body weight gain, SGR: Specific growth rate, FCR: Feed conversion ratio, FE: Feed efficiency, PER: Protein efficiency ratio.

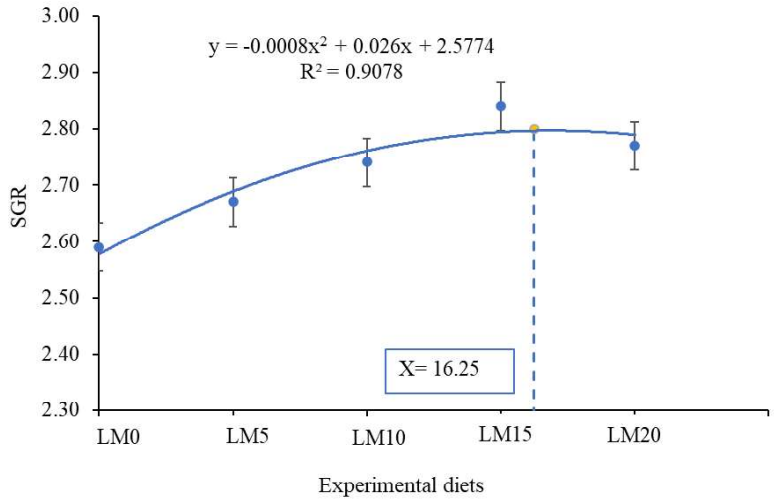


Figure 6 (a)

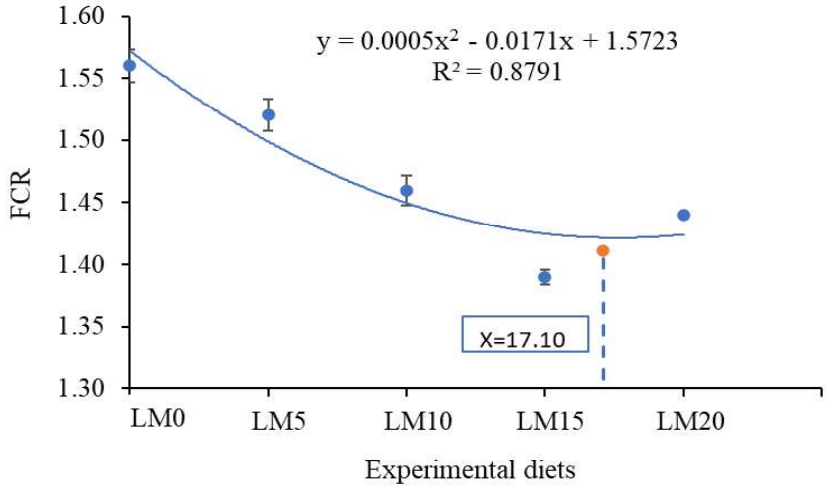


Figure 6 (b)

Figure 6. Polynomial regression analysis based on (a) SGR and (b) FCR of *Anabas testudineus* fed with different % inclusion of *Lemna minor* in the diet (LM0: 0% *L. minor*, LM5: 5% *L. minor*, LM10: 10% *L. minor*, LM15: 15% *L. minor*, and LM20: 20% *L. minor*).

4.2.2. Proximate composition

The proximate composition analysis of the muscle of *A. testudineus* fed varying levels of *L. minor*-incorporated diets for 60 days is presented in Table 9. The moisture content was 16.12 ± 0.01, 15.86 ± 0.01, 15.64 ± 0.02, 15.23 ± 0.01, and 15.48 ± 0.01% for LM0, LM5, LM10, LM15, and LM20 group, respectively. Fish-fed diets incorporating *L. minor* (LM5-LM20) showed lower moisture content than the control group. Notably, the LM15 group recorded a moisture content significantly lower ($P < 0.05$) than all other groups. Correspondingly, the protein contents were 64.40 ± 0.01, 64.47 ± 0.03, 64.56 ± 0.03, 64.75 ± 0.03, and 64.60 ± 0.02% for LM0, LM5, LM10, LM15, and LM20, respectively. The lowest protein content was recorded in the LM0 group. The LM15 group exhibited a significantly higher protein content ($P < 0.05$) than the control and other dietary groups, indicating that 15% *L. minor* supplementation optimises protein accumulation in *A. testudineus* muscle. The lipid contents were 7.85 ± 0.02, 7.92 ± 0.01, 8.02 ± 0.06, 8.16 ± 0.03, and 8.11 ± 0.01% for LM0, LM5, LM10, LM15, and LM20, respectively. The lowest lipid content was recorded in LM0. The LM15 group achieved the highest lipid content, significantly greater ($P < 0.05$) than all other groups.

The ash contents were 10.30 ± 0.03, 10.40 ± 0.01, 10.44 ± 0.05, 10.52 ± 0.04, and 10.47 ± 0.03% for LM0, LM5, LM10, LM15, and LM20, respectively. The ash content of the LM10 and LM20 groups was not significantly different ($P > 0.05$). The LM0 group recorded the lowest ash content, whereas the LM15 group exhibited a significantly higher ash content ($P < 0.05$) than the control and other dietary groups. Fibre content was 0.12 ± 0.01, 0.13 ± 0.02, 0.11 ± 0.01, 0.12 ± 0.01 and 0.11 ± 0.01% for LM0, LM5, LM10, LM15 and LM20 showing no significant differences ($P = 0.342$). The carbohydrate contents were 1.21 ± 0.02, 1.22 ± 0.02, 1.23 ± 0.01, 1.22 ± 0.03, and 1.23 ± 0.01% for LM0, LM5, LM10, LM15, and LM20, respectively. While differences in carbohydrate content were minimal, they were statistically significant ($P < 0.05$). The LM15 group exhibited significantly improved proximate composition ($P < 0.05$) compared to the control and other dietary

groups, indicating that 15% *L. minor* supplementation optimises the muscle quality of *A. testudineus*.

4.2.3. Digestive enzyme activity

The digestive enzyme activities of *A. testudineus* fed varying levels of *L. minor*-incorporated diets for 60 days are shown in Figure 7 (a-f). Fish fed the *L. minor* supplemented diets exhibited significantly higher ($P < 0.05$) enzyme activities compared to the control group (LM0) across all measured enzymes, including amylase, trypsin, chymotrypsin, pepsin, total protease, and lipase. Amylase activity in the control group (LM0) was 2.06 ± 0.01 U mg^{-1}, while the fish fed the *L. minor*-supplemented diets showed higher amylase activity (2.21 ± 0.03, 2.38 ± 0.01, 2.56 ± 0.01 and 2.44 ± 0.01 U mg^{-1} for LM5, LM10, LM15 and LM20, respectively) than the control group LM0. An increasing trend in amylase activity was observed across the *L. minor*-supplemented groups, peaking in the LM15 group, which showed a 1.24-fold increase compared to the control. However, a slight decrease was noted in the LM20 group compared to the LM15.

The trypsin activity was recorded as 15.45 ± 0.19, 17.23 ± 0.13, 20.59 ± 0.29, 23.51 ± 0.36, and 21.54 ± 0.21 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. The control group (LM0) recorded the lowest trypsin activity ($P < 0.05$), while the *L. minor*-supplemented groups showed a progressively increasing trend. The highest trypsin activity was observed in the LM15 group, showing a 1.52-fold increase compared to the control ($P < 0.05$). Conversely, a slight decline was noted in the LM20 group, where trypsin activity dropped to 1.39-fold compared to the control. Chymotrypsin activities were recorded as 110.5 ± 5.45, 125.14 ± 6.41, 152.43 ± 5.25, 182.50 ± 5.56 and 175.45 ± 6.15 U mg^{-1} for LM0, LM5, LM10, LM15 and LM20, respectively. An increase in chymotrypsin activity was observed with the increasing concentration of the *L. minor*. Higher activities were recorded in the LM15 and LM20 groups, with LM15 showing a 1.65-fold increase and LM20 showing a 1.59-fold increase compared to the control (LM0). Notably, no significant

difference ($P > 0.05$) was observed in chymotrypsin activity between the LM15 and LM20 groups. Total protease activity increased significantly ($P < 0.05$) in all *L. minor*-supplemented diet groups compared to the control (LM0, 1.36 ± 0.03 U mg^{-1}). The recorded activities were 1.43 ± 0.05, 1.56 ± 0.02, 1.82 ± 0.03, and 1.67 ± 0.02 U mg^{-1} for the LM5, LM10, LM15, and LM20 groups, respectively. The LM15 group exhibited the highest increase, with a 1.34-fold rise, followed by LM20, with a 1.23-fold increase. Both LM15 and LM20 groups demonstrated significantly elevated activity compared to the control, with the LM15 group showing the highest total protease activity among all groups ($P < 0.05$).

Pepsin activity increased consistently across all *L. minor*-supplemented groups compared to the control (LM0, 1105.96 ± 6.15 U mg^{-1}), with recorded values of 1177.42 ± 13.24, 1222.08 ± 3.74, 1270.52 ± 5.63, and 1237.13 ± 6.08 U mg^{-1} for LM5, LM10, LM15 and LM20, respectively. The highest activity was observed in the LM15 group, showing a 1.15-fold increase over the control. Although LM20 also demonstrated a notable 1.12-fold increase, there was a slight decline compared to the peak observed in the LM15 group ($P < 0.05$). Similarly, lipase activity followed an increasing trend across the *L. minor*-supplemented groups compared to the control (LM0, 29.13 ± 0.27 U mg^{-1}), with values of 33.14 ± 0.18, 35.20 ± 0.05, 38.09 ± 0.05 and 36.60 ± 0.04 U mg^{-1} for LM5, LM10, LM15 and LM20, respectively. The LM15 group showed the highest lipase activity, representing a 1.31-fold increase over the control. However, a slight decline was observed in the LM20 group, which recorded a 1.26-fold increase compared to LM15, indicating a peak in lipase activity at LM15 followed by a decrease.

The digestive enzyme activities for amylase, trypsin, chymotrypsin, total protease, pepsin and lipase were all significantly higher ($P < 0.05$) in fish-fed diets containing *L. minor* compared to the control. Among the different diet groups, the LM15 group consistently exhibited the highest enzyme activities, demonstrating that incorporating 15% *L. minor* in the diet significantly enhances the digestive capacity of *A. testudineus*.

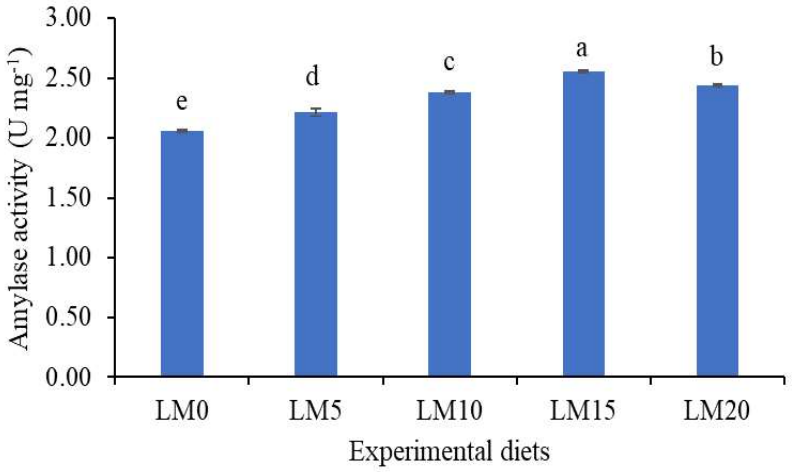


Figure 7 (a)

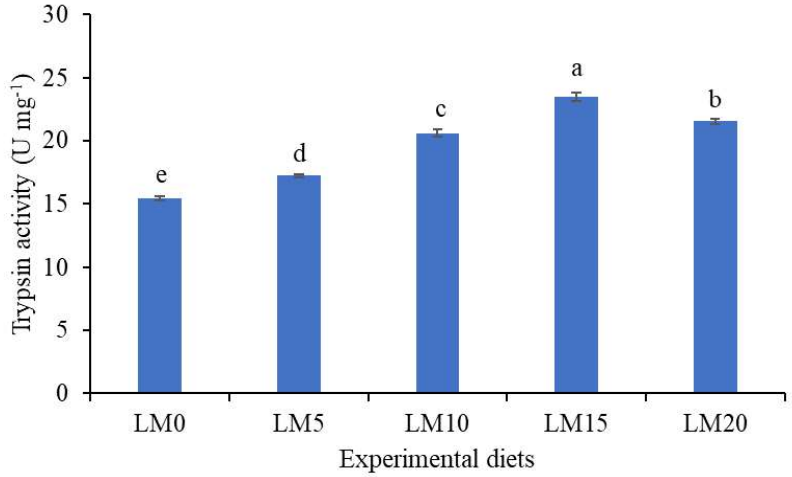


Figure 7 (b)

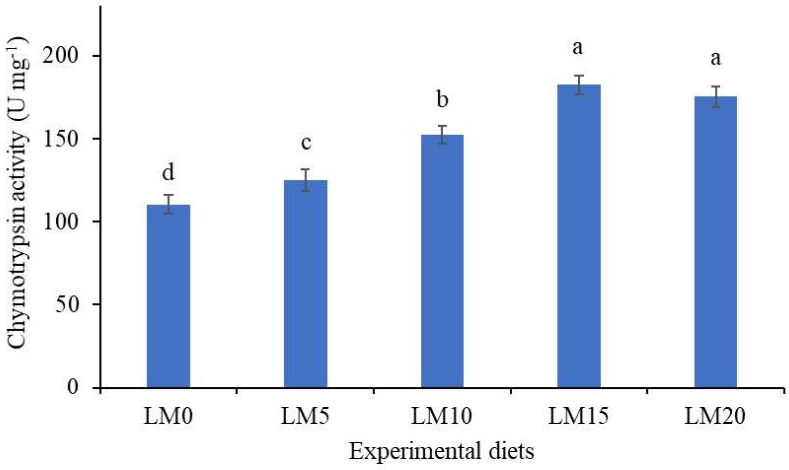
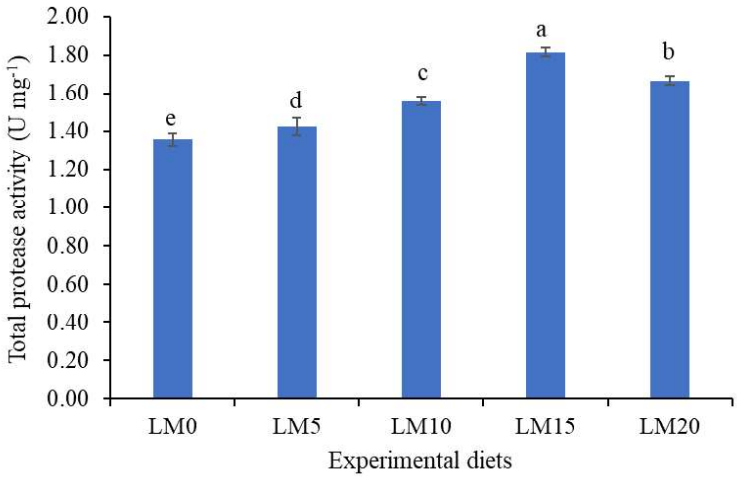


Figure 7 (c)



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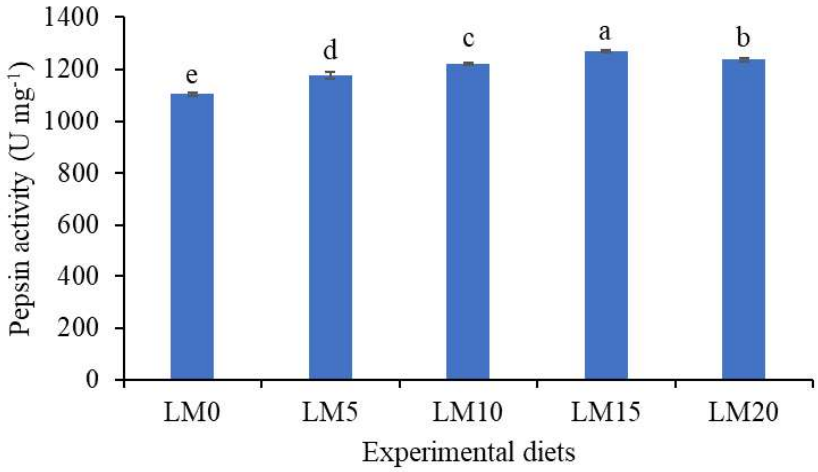

Figure 7 (e)

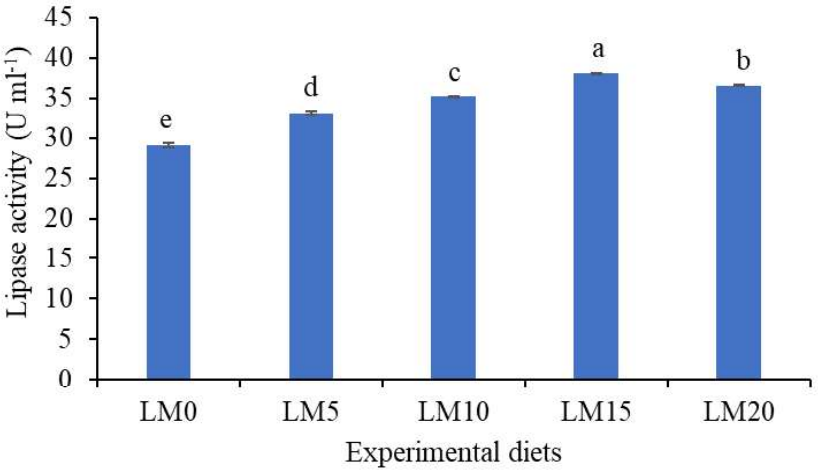

Figure 7 (f)

Figure 7. Digestive enzyme (a) Amylase, (b) Trypsin, (c) Chymotrypsin, (d) Total protease, (e) Pepsin, and (f) Lipase activity of *Anabas testudineus* fed with varying levels of *Lemna minor* incorporated diet (LM0: 0% *L. minor*, LM5: 5% *L. minor*, LM10: 10% *L. minor*, LM15: 15% *L. minor*, and LM20: 20% *L. minor*) for 60 days. Bars with different letters indicate statistically significant variations (n = 3, $P < 0.05$).

4.2.4. Amino acid composition

The amino acid composition of *A. testudineus* muscle tissue after 60 days of feeding with diets containing increasing percentages of *L. minor* is presented in Table 10. Both EAAs and NEAAs showed significant variations among the different dietary treatments ($P < 0.05$). Among the EAAs, the control group (LM0) and LM10 group had similar arginine levels (56.14 ± 0.33 and 55.65 ± 0.04 mg g^{-1}, respectively), both significantly higher than the LM5 (50.71 ± 0.34 mg g^{-1}) and LM20 (50.59 ± 0.03 mg g^{-1}) groups. However, it was significantly higher ($P < 0.05$) in the LM15 group (57.20 ± 0.05 mg g^{-1}, $P < 0.05$) compared to all other groups. Histidine content increased with higher inclusion levels of *L. minor*, peaking in the LM15 group (26.91 ± 0.21 mg g^{-1}), significantly exceeding all other groups ($P < 0.05$). Lysine content was notably higher ($P < 0.05$) in the LM5 group (81.17 ± 0.49 mg g^{-1}), representing the maximum among all treatments, followed by the LM10 group (70.54 ± 0.02 mg g^{-1}). The control group (LM0) exhibited a moderate lysine level (63.45 ± 0.06 mg g^{-1}). Leucine levels were highest in the LM15 group (58.48 ± 0.02 mg g^{-1}, $P < 0.05$), followed by the LM0 group (53.54 ± 0.17 mg g^{-1}), while the lowest level was recorded in the LM5 group (48.55 ± 0.00 mg g^{-1}).

Methionine was significantly higher ($P < 0.05$) in the LM10 group (66.63 ± 0.03 mg g^{-1}), followed by LM5 (56.98 ± 0.20 mg g^{-1}), while the lowest content was observed in LM0 (25.58 ± 0.39 mg g^{-1}). Phenylalanine content was significantly greater ($P < 0.05$) in the LM15 group (24.14 ± 0.01 mg g^{-1}) followed by the LM20 group (22.29 ± 0.02 mg g^{-1}). The lowest content was observed in the LM5 group (17.87 ± 0.05 mg g^{-1}). Threonine content was significantly higher ($P < 0.05$) in the LM15 group (27.25 ± 0.01 mg g^{-1}), whereas the lowest activity was observed in LM5 (21.06 ± 0.28 mg g^{-1}). Tryptophan content was significantly higher ($P < 0.05$) in the LM20 (5.78 ± 0.06 mg g^{-1}) group, followed by LM15 (5.54 ± 0.02 mg g^{-1}), compared to all other groups. The lowest activity was observed in the LM5 group (4.33 ± 0.01 mg g^{-1}). Valine content was significantly higher ($P < 0.05$) in LM15 (52.51 ± 0.33 mg g^{-1}) followed by LM5 (51.20 ± 0.62 mg g^{-1}) group, whereas the lowest content was

observed in the LM0 group (35.55 ± 0.30 mg g^{-1}). The total EAA recorded were 308.76 ± 0.71, 353.61 ± 0.48, 356.26 ± 0.08, 361.67 ± 0.39 and 336.00 ± 0.33 mg g^{-1} for LM0, LM5, LM10, LM15 and LM20, respectively. There was a clear upward trend in total EAA content, with an increase in *L. minor* inclusion by up to 15%. However, at 20% inclusion (LM20), the total EAA content decreased, though it remained higher than the control. The total EAA content was highest in the LM15 group, significantly exceeding the control group ($P < 0.05$).

Among the NEAAs, alanine content peaked in the LM10 group (83.91 ± 0.19 mg g^{-1}), significantly higher ($P < 0.05$) than all other treatments. The control group had the lowest alanine content (61.19 ± 0.08 mg g^{-1}). Aspartic acid content was significantly highest ($P < 0.05$) in the LM10 (47.62 ± 0.10 mg g^{-1}) group, followed by LM20 (42.40 ± 0.03 mg g^{-1}) group, while the LM5 (21.73 ± 0.15 mg g^{-1}) recorded the lowest content ($P < 0.05$). Both the LM10 and LM15 groups recorded significantly higher cysteine content (0.20 ± 0.00 and 0.21 ± 0.00 mg g^{-1}, respectively), with no significant difference ($P > 0.05$) observed between them. This was followed by LM5 (0.16 ± 0.01 mg g^{-1}) and LM20 (0.17 ± 0.01 mg g^{-1}), which also did not differ significantly ($P > 0.05$) from each other. The lowest cysteine content was observed in the LM0 group (0.13 ± 0.01 mg g^{-1}). The glycine content was significantly highest in LM15 (3.46 ± 0.00 mg g^{-1}, $P < 0.05$), followed by LM10 (3.19 ± 0.02 mg g^{-1}). In contrast, the lowest glycine content was observed in LM0 (1.53 ± 0.01 mg g^{-1}). Glutamic acid content was significantly higher in LM15 (97.54 ± 0.03 mg g^{-1}, $P < 0.05$), followed by LM5 (82.73 ± 0.09 mg g^{-1}). While the LM0 group recorded the lowest (68.19 ± 0.08 mg g^{-1}, $P < 0.05$).

Proline content was significantly higher in the LM5 group (100.35 ± 0.82 mg g^{-1}, $P < 0.05$), surpassing all other groups. The lowest proline level was observed in the LM0 group (65.86 ± 0.02 mg g^{-1}). The serine content was significantly higher in LM15 (99.32 ± 0.02 mg g^{-1}, $P < 0.05$), while it was lowest in LM0 (47.99 ± 1.27 mg g^{-1}). Tyrosine content was recorded as significantly highest in LM15 (34.58 ± 0.03 mg g^{-1}) followed by LM20 (32.42 ± 0.02 mg g^{-1}), and the lowest content was recorded in LM5 (25.29 ± 0.29 mg g^{-1})

group ($P < 0.05$). No significant difference was found in the citrulline content across the groups ($P > 0.05$). Asparagine content was significantly higher ($P < 0.05$) in LM20 (17.82 ± 0.01 mg g^{-1}), followed by LM5 (16.19 ± 0.02 mg g^{-1}) and LM15 (16.56 ± 0.01 mg g^{-1}). The lowest content was recorded in LM0 (11.31 ± 0.09 mg g^{-1}). The total NEAA content increased with higher *L. minor* inclusion, reaching the highest value in the LM15 group (427.00 ± 0.13 mg g^{-1}). This was significantly greater ($P < 0.05$) than the control group LM0 (320.92 ± 1.60 mg g^{-1}) and other treatments LM5 (400.61 ± 1.01 mg g^{-1}), LM10 (408.49 ± 0.42 mg g^{-1}) and LM20 (378.11 ± 0.06 mg g^{-1}).

The total amino acid content was 629.69 ± 1.89, 754.22 ± 0.53, 764.75 ± 0.50, 788.67 ± 0.52, and 714.10 ± 0.39 mg g^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. The lowest ($P < 0.05$) total amino acid content was recorded in the control group (LM0), while diets incorporating *L. minor* (LM5-LM20) resulted in higher total amino acid content compared to the control. The LM5 and LM10 groups showed incremental increases, with the highest total amino acid content observed in the LM15 group, which was significantly higher than all other groups ($P < 0.05$). However, the total amino acid content in the LM20 group was slightly lower than that of LM15.

4.2.5. Fatty acid composition

The fatty acid composition of *A. testudineus* fed with varying levels of *L. minor*-incorporated diets over 60 days is presented in Table 11. The analysis revealed significant differences ($P < 0.05$) among the dietary treatments across SFAs, MUFAs, and PUFAs. Among the SFAs, C13:0 was significantly ($P < 0.05$) higher in the control LM0 (0.47 ± 0.01%) and LM20 (0.45 ± 0.01%) groups compared to other treatments. C14:0 and C15:0 both showed a decreasing trend as *L. minor* levels increased, with the lowest values observed in LM15 at 1.38 ± 0.01% and 1.18 ± 0.01%, respectively. The most abundant SFA, C16:0, exhibited a significant reduction from LM0 (30.49 ± 0.01%) to LM15 (30.15 ± 0.01%), while C17:0 decreased consistently, reaching its lowest level in LM15 (2.40 ± 0.01%). Similarly, C18:0 and C20:0 showed significant decreases with

increasing *L. minor* levels, with their lowest values also found in LM15 (12.12 ± 0.02% and 1.19 ± 0.01%, respectively). Total SFA content were 50.01 ± 0.03, 49.41 ± 0.02, 49.10 ± 0.02, 48.84 ± 0.02 and 49.17 ± 0.04% for LM0, LM5, LM10, LM15 and LM20, respectively. The SFA content was significantly highest ($P < 0.05$) in the LM0 and decreased steadily across diets, reaching its lowest in LM15.

Among the MUFAs, C15:1n-5 showed a significant decline as *L. minor* levels increased, decreasing from 2.63 ± 0.01% in control (LM0) to 2.48 ± 0.01% in LM15. C16:1n-5 also exhibited a reduction, dropping from 8.04 ± 0.00% in LM0 to 7.93 ± 0.01% in LM15. Similarly, C18:1n-9 decreased consistently with increasing *L. minor* levels, starting at 14.21 ± 0.01% in LM0 and reaching 14.09 ± 0.01% in LM15. C20:1n-9 showed the most pronounced decrease among MUFAs, from 2.36 ± 0.00% in LM0 to 2.20 ± 0.01% in LM15. Total MUFA content were 27.24 ± 0.01, 27.02 ± 0.02, 26.86 ± 0.02, 26.70 ± 0.03, and 26.94 ± 0.08% for LM0, LM5, LM10, LM15, and LM20, respectively. The MUFA content was significantly highest ($P < 0.05$) in LM0 and decreased steadily across diets, reaching its lowest point in LM15, with a slight increase observed in LM20.

Among the PUFAs, C18:3n-3 showed a significant increase ($P < 0.05$) with increasing *L. minor* levels, peaking in LM15 (9.11 ± 0.03%). C20:5n-3 (EPA) also increased steadily, rising from 3.21 ± 0.01% in control (LM0) to 3.59 ± 0.01% in LM15. Similarly, C22:6n-3 (DHA) showed a notable rise, with its significantly highest ($P < 0.05$) concentration in LM15 (4.58 ± 0.02%), up from 4.08 ± 0.01% in LM0. Meanwhile, C18:2n-6 demonstrated a slight increase from 2.32 ± 0.00% in LM0 to 2.41 ± 0.01% in LM15, while C18:3n-6 also rose, reaching its highest level ($P < 0.05$) in LM15 (2.30 ± 0.01%). C20:4n-6 followed a similar trend, increasing from 2.31 ± 0.01% in LM0 to 2.47 ± 0.01% in LM15. Total PUFA content was 22.75 ± 0.01, 23.57 ± 0.03, 24.04 ± 0.09, 24.46 ± 0.03, and 23.89 ± 0.05 for LM0, LM5, LM10, LM15, and LM20, respectively. The PUFA content was significantly lowest ($P < 0.05$) in LM0 and increased consistently across diets, reaching its highest point in LM15.

The PUFA to SFA ratio was highest in the LM15 group (0.50 ± 0.01), showing a significant increase ($P < 0.05$) compared to the control group (0.45 ± 0.00). The ratio of ω6 to ω3 fatty acids showed no significant variations ($P > 0.05$) across the dietary treatments. The combined EPA and DHA content was highest ($P < 0.05$) in the LM15 group (8.17 ± 0.02%) compared to the control (7.29 ± 0.02%). These results indicate significant changes in fatty acid composition based on the inclusion levels of *L. minor* in the diet.

4.2.6. Biochemical parameters

The biochemical parameters of *A. testudineus* fed varying percentages of *L. minor* supplemented diets over 60 days are presented in Table 12. The total immunoglobulin (TIg) levels were 0.68 ± 0.02, 0.76 ± 0.01, 0.84 ± 0.01, 0.97 ± 0.02, and 0.91 ± 0.02 mg mL^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. Fish-fed diets incorporating *L. minor* (LM5–LM20) showed higher TIg levels compared to the control group. Notably, the LM15 group displayed the highest TIg level, which was significantly greater ($P < 0.05$) than all other groups. The lysozyme (LYZ) activity levels were recorded as 72.5 ± 0.02, 74.1 ± 0.03, 73.45 ± 0.04, 75.21 ± 0.02, and 76.14 ± 0.04 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. Fish fed with *L. minor*-supplemented diets (LM5–LM20) showed higher LYZ activity compared to the control (LM0). However, no significant differences were observed among the *L. minor*-supplemented groups, and LYZ activity in LM0 and LM5 was also not significantly different from each other.

Alkaline phosphatase (ALP) activity was recorded as 1.19 ± 0.03, 1.25 ± 0.04, 1.32 ± 0.02, 1.47 ± 0.03, and 1.43 ± 0.04 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. The LM15 group showed the highest ALP activity, which was significantly greater ($P < 0.05$) than the control (LM0). Catalase (CAT) activity was measured as 5.21 ± 0.02, 5.40 ± 0.03, 5.64 ± 0.02, 5.72 ± 0.02, and 5.57 ± 0.03 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. Higher CAT activity was observed in all *L. minor*-supplemented groups (LM5–LM20) compared to the control (LM0), which recorded the lowest

activity ($P < 0.05$). However, there were no significant differences in CAT activity among the *L. minor*-supplemented groups ($P > 0.05$). Superoxide dismutase (SOD) levels were recorded as 226.34 ± 7.5, 239.7 ± 4.3, 244.5 ± 5.1, 256.50 ± 5.30, and 255.12 ± 5.6 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. Although SOD activity was higher in the supplemented groups, no significant differences were observed ($P > 0.05$) among the dietary treatments.

Thiobarbituric acid reactive substances (TBARS) levels were recorded as 2.51 ± 0.03, 2.48 ± 0.04, 2.53 ± 0.05, 2.56 ± 0.06, and 2.54 ± 0.02 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. There were no significant differences ($P > 0.05$) in TBARS levels among the groups. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were consistent across all groups ($P > 0.05$). AST values recorded were 1.35 ± 0.02, 1.32 ± 0.01, 1.36 ± 0.03, 1.34 ± 0.02 and 1.35 ± 0.02 for LM0, LM5, LM10, LM15 and LM20, respectively. ALT values recorded were 1.21 ± 0.02, 1.24 ± 0.03, 1.25 ± 0.04, 1.21 ± 0.02 and 1.23 ± 0.01 U mg^{-1} for LM0, LM5, LM10, LM15 and LM20, respectively. No significant differences ($P > 0.05$) were observed in AST and ALT levels among the different dietary groups.

Table 9. Proximate analysis of the muscle of *Anabas testudineus* (% dry weight basis) fed varying levels of *Lemna minor* incorporated diet for 60 days.

Parameters	**LM0**	**LM5**	**LM10**	**LM15**	**LM20**	***P* value**
Moisture	16.12 ± 0.01^{a}	15.86 ± 0.01^{b}	15.64 ± 0.02^{c}	15.23 ± 0.01^{d}	15.48 ± 0.01^{d}	< 0.001
Protein	64.40 ± 0.01^{e}	64.47 ± 0.03^{d}	64.56 ± 0.03^{c}	64.75 ± 0.03^{a}	64.60 ± 0.02^{b}	< 0.001
Lipid	7.85 ± 0.02^{e}	7.92 ± 0.01^{d}	8.02 ± 0.06^{c}	8.16 ± 0.03^{a}	8.11 ± 0.01^{b}	< 0.001
Ash	10.30 ± 0.03^{d}	10.40 ± 0.01^{c}	10.44 ± 0.05^{b}	10.52 ± 0.04^{a}	10.47 ± 0.03^{b}	< 0.001
Fibre	0.12 ± 0.01	0.13 ± 0.02	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.342
Carbohydrate	1.21 ± 0.02^{a}	1.22 ± 0.02^{b}	1.23 ± 0.01^{cd}	1.22 ± 0.03^{c}	1.23 ± 0.01^{d}	< 0.001

Note. Rows with different superscript letters are significantly different (n = 3, $P < 0.05$). LM0: 0% *L. minor* supplemented feed, LM5: 5% *L. minor* supplemented feed, LM10: 10% *L. minor* supplemented feed, LM15: 15% *L. minor* supplemented feed, and LM20: 20% *L. minor* supplemented feed.

Table 10. Amino acid composition of *Anabas testudineus* fed diets incorporating increasing percentages of *Lemna minor* (LM0: 0% *L. minor*, LM5: 5% *L. minor*, LM10: 10% *L. minor*, LM15: 15% *L. minor*, and LM20: 20% *L. minor*) for 60 days.

Amino acids (mg g^{-1})	**LM0**	**LM5**	**LM10**	**LM15**	**LM20**	***P* value**
EAA						
Arginine	56.14 ± 0.33^{b}	50.71 ± 0.34^{c}	55.65 ± 0.04^{b}	57.20 ± 0.05^{a}	50.59 ± 0.03^{c}	< 0.001
Histidine	21.64 ± 0.39^{d}	21.73 ± 0.15^{d}	25.37 ± 0.06^{b}	26.91 ± 0.21^{a}	23.80 ± 0.01^{c}	< 0.001
Lysine	63.45 ± 0.06^{c}	81.17 ± 0.49^{a}	70.54 ± 0.02^{b}	56.06 ± 0.06^{e}	61.58 ± 0.04^{d}	< 0.001
Leucine	53.54 ± 0.17^{b}	48.55 ± 0.00^{e}	49.33 ± 0.02^{d}	58.48 ± 0.02^{a}	51.90 ± 0.03^{c}	< 0.001
Methionine	25.58 ± 0.39^{e}	56.98 ± 0.20^{b}	66.63 ± 0.03^{a}	53.60 ± 0.03^{d}	54.79 ± 0.08^{c}	< 0.001
Phenylalanine	21.48 ± 0.04^{c}	17.87 ± 0.05^{e}	21.15 ± 0.04^{d}	24.14 ± 0.01^{a}	22.29 ± 0.02^{b}	< 0.001
Threonine	26.44 ± 0.55^{b}	21.06 ± 0.28^{d}	24.05 ± 0.04^{c}	27.25 ± 0.01^{a}	23.58 ± 0.03^{c}	< 0.001
Tryptophan	4.95 ± 0.00^{d}	4.33 ± 0.01^{e}	5.12 ± 0.02^{c}	5.54 ± 0.02^{b}	5.78 ± 0.06^{a}	< 0.001
Valine	35.55 ± 0.30^{e}	51.20 ± 0.62^{b}	38.44 ± 0.04^{d}	52.51 ± 0.33^{a}	41.70 ± 0.03^{c}	< 0.001
Total EAA	308.76 ± 0.71^{e}	353.61 ± 0.48^{c}	356.26 ± 0.08^{b}	361.67 ± 0.39^{a}	336.00 ± 0.33^{d}	< 0.001
NEAA						
Alanine	61.19 ± 0.08^{e}	75.44 ± 0.25^{b}	83.91 ± 0.19^{a}	74.96 ± 0.02^{c}	72.58 ± 0.03^{d}	< 0.001
Aspartic acid	37.53 ± 0.14^{c}	21.73 ± 0.15^{e}	47.62 ± 0.10^{a}	23.66 ± 0.01^{d}	42.40 ± 0.03^{b}	< 0.001
Cysteine	0.13 ± 0.01^{c}	0.16 ± 0.01^{b}	0.20 ± 0.00^{a}	0.21 ± 0.00^{a}	0.17 ± 0.01^{b}	< 0.001
Glycine	1.53 ± 0.01^{e}	2.35 ± 0.02^{d}	3.19 ± 0.02^{b}	3.46 ± 0.00^{a}	2.78 ± 0.00^{c}	< 0.001
Glutamic acid	68.19 ± 0.08^{e}	82.73 ± 0.09^{b}	69.26 ± 0.03^{d}	97.54 ± 0.03^{a}	73.46 ± 0.00^{c}	< 0.001

Proline	65.86 ± 0.02^{e}	100.35 ± 0.82^{a}	95.71 ± 0.02^{b}	76.70 ± 0.02^{d}	81.05 ± 0.01^{c}	< 0.001
Serine	47.99 ± 1.27^{e}	51.30 ± 0.03^{d}	61.64 ± 0.08^{b}	99.32 ± 0.02^{a}	55.42 ± 0.01^{c}	< 0.001
Tyrosine	27.23 ± 0.08^{d}	25.29 ± 0.29^{e}	31.88 ± 0.06^{c}	34.58 ± 0.03^{a}	32.42 ± 0.02^{b}	< 0.001
Citrulline	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.147
Asparagine	11.31 ± 0.09^{d}	16.19 ± 0.02^{b}	15.07 ± 0.01^{c}	16.56 ± 0.01^{b}	17.82 ± 0.01^{a}	< 0.001
Beta 3-4 dihydroxy phenylalanine	0.02 ± 0.00^{a}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{b}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	< 0.001
Total NEAA	320.92 ± 1.60^{e}	400.61 ± 1.01^{c}	408.49 ± 0.42^{b}	427.00 ± 0.13^{a}	378.11 ± 0.06^{d}	< 0.001
Total amino acids	629.69 ± 1.89^{e}	754.22 ± 0.53^{c}	764.75 ± 0.50^{b}	788.67 ± 0.52^{a}	714.10 ± 0.39^{d}	< 0.001

Notes: Different superscript letters in the same row indicate significant differences (n= 3, $P < 0.05$). EAA: Essential Amino Acids, NEAA: Non-Essential Amino Acids.

Table 11. Fatty acid composition (% of total fatty acid) in *Anabas testudineus* fed varying levels of *Lemna minor* incorporated diet (LM0: 0% *L. minor*, LM5: 5% *L. minor*, LM10: 10% *L. minor*, LM15: 15% *L. minor*, and LM20: 20% *L. minor*) for 60 days.

Fatty acid	**LM0**	**LM5**	**LM10**	**LM15**	**LM20**	***P* value**
C13:0	0.47 ± 0.01^{a}	0.42 ± 0.01^{b}	0.43 ± 0.01^{b}	0.42 ± 0.01^{b}	0.45 ± 0.01^{a}	< 0.001
C14:0	1.52 ± 0.01^{a}	1.45 ± 0.01^{b}	1.40 ± 0.01^{c}	1.38 ± 0.01^{d}	1.40 ± 0.01^{cd}	< 0.001
C15:0	1.36 ± 0.01^{a}	1.25 ± 0.01^{b}	1.21 ± 0.01^{c}	1.18 ± 0.01^{d}	1.22 ± 0.01^{c}	< 0.001
C16:0	30.49 ± 0.01^{a}	30.36 ± 0.00^{b}	30.21 ± 0.01^{c}	30.15 ± 0.01^{d}	30.23 ± 0.02^{c}	< 0.001
C17:0	2.61 ± 0.00^{a}	2.47 ± 0.01^{b}	2.44 ± 0.00^{c}	2.40 ± 0.01^{d}	2.50 ± 0.01^{b}	< 0.001
C18:0	12.27 ± 0.01^{a}	12.2 ± 0.01^{b}	12.18 ± 0.00^{b}	12.12 ± 0.02^{c}	12.12 ± 0.01^{b}	< 0.001
C20:0	1.29 ± 0.01^{a}	1.26 ± 0.01^{b}	1.23 ± 0.01^{c}	1.19 ± 0.01^{d}	1.24 ± 0.02^{d}	< 0.001
Σ SFA	50.01 ± 0.03^{a}	49.41 ± 0.02^{b}	49.10 ± 0.02^{d}	48.84 ± 0.02^{e}	49.17 ± 0.04^{c}	< 0.001
C15:1n-5	2.63 ± 0.01^{a}	2.56 ± 0.01^{b}	2.53 ± 0.01^{c}	2.48 ± 0.01^{d}	2.51 ± 0.01^{c}	< 0.001
C16:1n-5	8.04 ± 0.00^{a}	8.01 ± 0.00^{a}	7.97 ± 0.00^{ab}	7.93 ± 0.01^{b}	8.01 ± 0.06^{a}	0.003
C18:1n-9	14.21 ± 0.01^{a}	14.16 ± 0.01^{ab}	14.12 ± 0.01^{bc}	14.09 ± 0.01^{c}	14.14 ± 0.05^{bc}	0.001
C20:1n-9	2.36 ± 0.00^{a}	2.29 ± 0.01^{b}	2.25 ± 0.01^{c}	2.20 ± 0.01^{d}	2.28 ± 0.02^{b}	< 0.001
Σ MUFA	27.24 ± 0.01^{a}	27.02 ± 0.02^{b}	26.86 ± 0.02^{c}	26.70 ± 0.03^{d}	26.94 ± 0.08^{b}	< 0.001
C18:3n-3	8.73 ± 0.01^{e}	8.98 ± 0.01^{c}	9.01 ± 0.01^{b}	9.11 ± 0.03^{a}	8.95 ± 0.00^{d}	< 0.001
C20:5n-3	3.21 ± 0.01^{d}	3.36 ± 0.01^{c}	3.42 ± 0.00^{b}	3.59 ± 0.01^{a}	3.40 ± 0.01^{b}	< 0.001
C22:6n-3	4.08 ± 0.01^{d}	4.31 ± 0.01^{c}	4.53 ± 0.01^{b}	4.58 ± 0.02^{a}	4.54 ± 0.02^{b}	< 0.001
C18:2n-6	2.32 ± 0.00^{c}	2.26 ± 0.01^{d}	2.38 ± 0.01^{ab}	2.41 ± 0.01^{a}	2.36 ± 0.01^{b}	< 0.001

C18:3n-6	2.10 ± 0.01^{e}	2.21 ± 0.01^{d}	2.26 ± 0.01^{b}	2.30 ± 0.01^{a}	2.24 ± 0.01^{c}	< 0.001
C20:4n-6	2.31 ± 0.01^{d}	2.45 ± 0.01^{c}	2.44 ± 0.02^{bc}	2.47 ± 0.01^{ab}	2.40 ± 0.01^{a}	< 0.001
Σ PUFA	22.75 ± 0.01^{e}	23.57 ± 0.03^{d}	24.04 ± 0.09^{b}	24.46 ± 0.03^{a}	23.89 ± 0.05^{c}	< 0.001
PUFA/SFA	0.45 ± 0.00^{d}	0.48 ± 0.01^{c}	0.49 ± 0.00^{b}	0.50 ± 0.01^{a}	0.49 ± 0.00^{b}	< 0.001
ω6/ω3	0.42 ± 0.02^{a}	0.42 ± 0.00^{a}	0.42 ± 0.00^{a}	0.42 ± 0.02^{a}	0.41 ± 0.02^{a}	0.213
EPA+DHA	7.29 ± 0.02^{d}	7.67 ± 0.01^{c}	7.95 ± 0.01^{b}	8.17 ± 0.02^{a}	7.94 ± 0.03^{b}	< 0.001

Notes: Different superscript letters indicate significant differences in a shared row (n = 3, $P < 0.05$). SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid.

Table 12. Biochemical parameters of *Anabas testudineus* fed varying levels of *a Lemna minor*-incorporated diet for 60 days.

Parameters	**LM0**	**LM5**	**LM10**	**LM15**	**LM20**	***P* value**
TIg (mg mL^{-1})	0.68 ± 0.02^{e}	0.76 ± 0.01^{d}	0.84 ± 0.01^{c}	0.97 ± 0.02^{a}	0.91 ± 0.02^{b}	< 0.001
LYZ (U mg^{-1})	72.5 ± 0.02^{b}	74.1 ± 0.03^{ab}	73.45 ± 0.04^{a}	75.21 ± 0.02^{a}	76.14 ± 0.04^{a}	0.005
ALP (U mg^{-1})	1.19 ± 0.03^{b}	1.25 ± 0.04^{b}	1.32 ± 0.02^{ab}	1.47 ± 0.03^{a}	1.43 ± 0.04^{ab}	0.009
AST (U mg^{-1})	1.35 ± 0.02	1.32 ± 0.01	1.36 ± 0.03	1.34 ± 0.02	1.35 ± 0.02	0.117
ALT (U mg^{-1})	1.21 ± 0.02	1.24 ± 0.03	1.25 ± 0.04	1.21 ± 0.02	1.23 ± 0.01	0.240
CAT (U mg^{-1})	5.21 ± 0.02^{b}	5.40 ± 0.03^{a}	5.64 ± 0.02^{a}	5.72 ± 0.02^{a}	5.57 ± 0.03^{a}	< 0.001
SOD (U mg^{-1})	226.34 ± 7.5	239.7 ± 4.3	244.5 ± 5.1	256.50 ± 5.30	255.12 ± 5.6	0.395
TBARS (U mg^{-1})	2.51 ± 0.03	2.48 ± 0.04	2.53 ± 0.05	2.56 ± 0.06	2.54 ± 0.02	0.184

Notes: Values are represented as mean values ± SD. Means within the same column having different superscripts are significantly different ($P < 0.05$). T Ig: Total Immunoglobulin, ALP: Alkaline Phosphatase, LYZ: Lysozyme, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, CAT: Catalase, SOD: Superoxide dismutase, TBARS: Thiobarbituric acid reactive substances. LM0: 0% *L. minor*, LM5: 5% *L. minor*, LM10: 10% *L. minor*, LM15: 15% *L. minor*, and LM20: 20% *L. minor*.

4.3. Effect of *Lemna minor* supplemented feed on the growth performance, digestive enzymes and immune parameters of *Heteropneustes fossilis*.

4.3.1. Growth performance

The growth performance of *Heteropneustes fossilis* (initial weight: 0.51 ± 0.01 g) fed varying percentages of *Lemna minor* diets over 60 days is presented in Table 13. The FW recorded were 1.95 ± 0.10, 2.05 ± 0.02, 2.34 ± 0.05, 2.44 ± 0.08, and 2.06 ± 0.03 g for LM0, LM5, LM10, LM15, and LM20, respectively. Fish-fed diets containing *L. minor* (LM5-LM20) had higher FW than the control group, whereas the LM0 diet-fed fish recorded the lowest FW. The LM5 and LM10 groups exhibited a gradual increase, whereas the LM15 group had the highest FW, which was 1.25 times higher than LM0 and considerably higher than all other groups ($P < 0.05$). While FW was slightly lower in the LM20 group than in the LM15 group, it was still 1.06-fold higher than in the control group. Similarly, BWG percentages were 284.76 ± 16.69, 301.58 ± 2.34, 361.50 ± 10.13, 377.16 ± 18.07, and 311.94 ± 6.29% for LM0, LM5, LM10, LM15, and LM20, respectively. The LM0 group recorded the lowest BWG ($P < 0.05$), while LM5 and LM10 gradually increased. The highest BWG was recorded in the LM15 group, which represented a 1.32-fold increase over the control and significantly exceeded the BWG of all other groups ($P < 0.001$). The BWG of the LM20 group was marginally lower than the LM15 and was 1.10-fold higher than that of the LM0 group.

The SGR were 2.24 ± 0.07, 2.32 ± 0.01, 2.55 ± 0.04, 2.60 ± 0.06, and 2.36 ± 0.03% day^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. The control group (LM0) recorded the lowest SGR ($P < 0.05$), while diets containing *L. minor* (LM5–LM20) resulted in higher SGRs compared to the control. LM5 and LM10 groups showed gradual increases, with the highest SGR observed in the LM15 group, representing a 1.16-fold increase over LM0 and significantly greater than all other groups ($P < 0.05$). Although the LM20 group showed a slight decline in SGR compared to LM15, it still recorded a 1.05-fold increase over the control. The FCR were 1.25 ± 0.05, 1.15 ± 0.03, 0.98 ± 0.02,

0.93 ± 0.05, and 1.16 ± 0.03 for LM0, LM5, LM10, LM15, and LM20, respectively. The control group (LM0) exhibited the highest FCR ($P < 0.05$), while diets containing *L. minor* (LM5-LM20) showed progressively lower FCRs, indicating improved feed utilisation. The LM15 group achieved the lowest FCR, representing a 1.34-fold improvement over LM0 and significantly lower than all other groups ($P < 0.05$). Although the LM20 group had a slight increase in FCR compared to LM15, it still maintained a 1.08-fold improvement over the control. Fish survival was not adversely affected by varying amounts of *L. minor*; 100% survival rates were observed across all feeding groups (LM0-LM20).

PER were 2.00 ± 0.07, 2.17 ± 0.05, 2.55 ± 0.06, 2.68 ± 0.14, and 2.16 ± 0.05 for LM0, LM5, LM10, LM15, and LM20, respectively. The control group recorded the lowest PER while increasing levels of *L. minor* led to higher PERs of up to 15%. The LM15 group recorded the highest PER, representing a 1.34-fold increase over LM0 and significantly higher than all other groups ($P < 0.05$). Although the LM20 group showed a slight decrease compared to LM15, it still maintained a 1.08-fold increase over the control. FE percentages were 79.93 ± 2.88%, 86.96 ± 2.08%, 102.03 ± 2.52%, 107.29 ± 5.61%, and 86.47 ± 2.15% for LM0, LM5, LM10, LM15, and LM20, respectively. The LM0 recorded the lowest FE, while FE increased progressively with higher inclusion levels of *L. minor* up to 15%. The LM15 group demonstrated the highest FE, corresponding to a 1.34-fold increase over LM0 and significantly higher than all other groups ($P < 0.05$). Although the LM20 group showed a decline in FE compared to LM15, it still achieved a 1.08-fold improvement over the control.

The polynomial regression analysis of FCR and SGR suggested that optimal fish growth occurs when the *L. minor* inclusion level is between 11.89% and 12.30%, as shown in Figure 8 (a-b). The study showed that the LM15 diet, containing 15% *L. minor*, was the most effective in enhancing growth performance in *H. fossilis*. Fish in this group exhibited the lowest FCR and highest FW, BWG, SGR, FE, and PER.

Table 13. The growth performance of *Heteropneustes fossilis* fed with feed containing different percentage inclusion of *Lemna minor* for 60 days.

Parameters	LM0	LM5	LM10	LM15	LM20	*P* value
IW (g)	0.51 ± 0.01	0.51 ± 0.00	0.51 ± 0.01	0.51 ± 0.01	0.50 ± 0.01	
FW (g)	1.95 ± 0.10^{d}	2.05 ± 0.02^{c}	2.34 ± 0.05^{b}	2.44 ± 0.08^{a}	2.06 ± 0.03^{c}	< 0.001
BWG (%)	284.76 ± 16.69^{e}	301.58 ± 2.34^{d}	361.50 ± 10.13^{b}	377.16 ± 18.07^{a}	311.94 ± 6.29^{c}	< 0.001
SGR (% day^{-1})	2.24 ± 0.07^{e}	2.32 ± 0.01^{d}	2.55 ± 0.04^{b}	2.60 ± 0.06^{a}	2.36 ± 0.03^{c}	< 0.001
FCR	1.25 ± 0.05^{a}	1.15 ± 0.03^{b}	0.98 ± 0.02^{c}	0.93 ± 0.05^{d}	1.16 ± 0.03^{b}	< 0.001
Survival (%)	100	100	100	100	100	
FE (%)	79.93 ± 2.88^{d}	86.96 ± 2.08^{c}	102.03 ± 2.52^{b}	107.29 ± 5.61^{a}	86.47 ± 2.15^{c}	< 0.001
PER	2.00 ± 0.07^{d}	2.17 ± 0.05^{c}	2.55 ± 0.06^{b}	2.68 ± 0.14^{a}	2.16 ± 0.05^{c}	< 0.001

Note: Superscript letters denote statistically significant differences within a common row (n = 30, $P < 0.05$).). IW: Initial weight, FW: Final weight, BWG: Body weight gain, SGR: Specific growth rate, FCR: Feed conversion ratio, FE: Feed efficiency, PER: Protein efficiency ratio, LM0: 0% *L. minor*, LM5: 5% *L. minor*, LM10: 10% *L. minor*, LM15: 15% *L. minor*, and LM20: 20% *L. minor*.

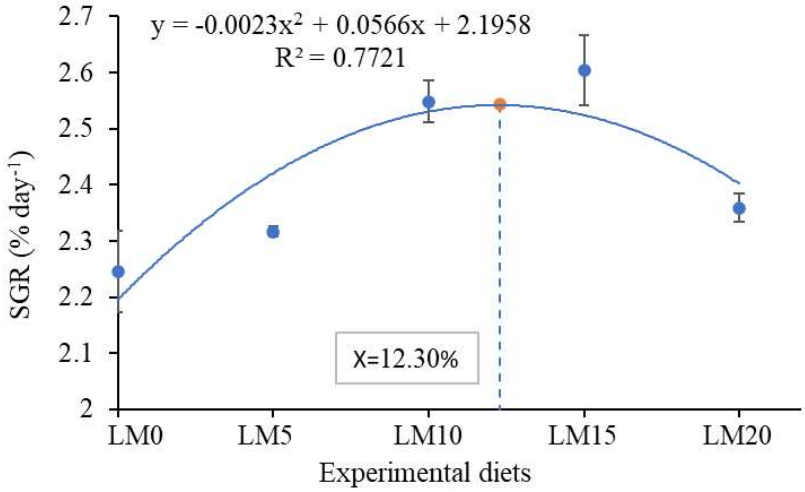

Figure 8 (a).

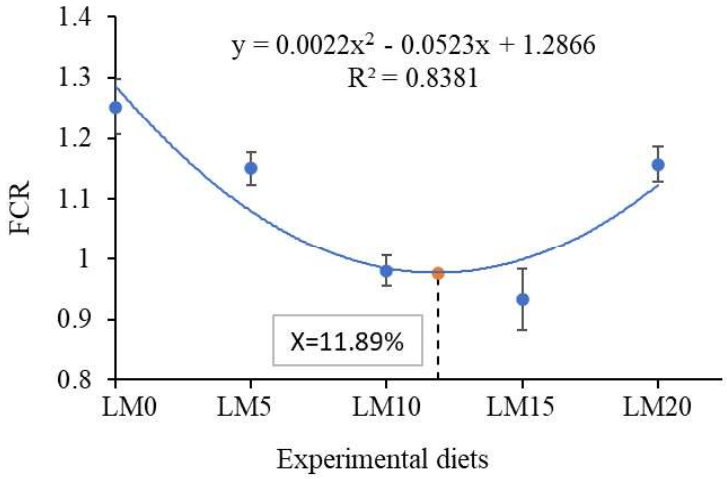

Figure 8 (b).

Figure 8. Optimising *Lemna minor* dietary intake via polynomial regression analysis based on (a) SGR and (b) FCR of *Heteropneustes fossilis*. LM0: 0% *L. minor*, LM5: 5% *L. minor*, LM10: 10% *L. minor*, LM15: 15% *L. minor*, and LM20: 20% *L. minor*.

4.3.2. Proximate composition

The proximate composition analysis of *H. fossilis* fed varying levels of *L. minor*-incorporated diets for 60 days is presented in Table 14. The protein contents were 63.54 ± 0.02, 63.66 ± 0.03, 63.84 ± 0.03, 63.95 ± 0.05, and $63.69 \pm 0.02\%$ for LM0, LM5, LM10, LM15, and LM20, respectively. The lowest protein content was observed in the LM0 group ($P < 0.05$). The LM15 group exhibited a significantly higher protein content ($P < 0.05$) than the control. The moisture content was 17.57 ± 0.01, 17.45 ± 0.01, 17.18 ± 0.01, 16.76 ± 0.01, and $17.53 \pm 0.01\%$ for LM0, LM5, LM10, LM15, and LM20 groups, respectively. Fish-fed diets with *L. minor* (LM5-LM20) showed lower moisture content than the control group. The LM15 group recorded a moisture content significantly lower ($P < 0.05$) than all other groups.

Carbohydrate contents were 1.44 ± 0.03, 1.28 ± 0.03, 1.09 ± 0.05, 1.15 ± 0.05, and $1.04 \pm 0.04\%$ for LM0, LM5, LM10, LM15, and LM20, respectively. Although differences in carbohydrate content were minimal, they were statistically significant ($P < 0.05$). The lipid contents were 7.97 ± 0.05, 8.07 ± 0.02, 8.27 ± 0.05, 8.44 ± 0.06, and $8.13 \pm 0.01\%$ for LM0, LM5, LM10, LM15, and LM20, respectively. The lowest lipid content was recorded in LM0, while the LM15 group achieved the highest lipid content, significantly greater ($P < 0.05$) than all other groups. Ash contents were 9.38 ± 0.02, 9.44 ± 0.01, 9.51 ± 0.03, 9.60 ± 0.05, and $9.50 \pm 0.06\%$ for LM0, LM5, LM10, LM15, and LM20, respectively. The ash content of the LM0 group was the lowest, whereas the LM15 group exhibited a significantly higher ash content ($P < 0.05$) than the control and other dietary groups. Fiber content remained consistent across all groups (LM0, LM5, LM10, LM15, and LM20) at $0.10 \pm 0.01\%$, showing no significant differences ($P = 0.499$). The LM15 group exhibited significantly improved proximate composition ($P < 0.05$) compared to the control and other dietary groups.

Table 14. Proximate analysis of *Heteropneustes fossilis* (% dry weight basis) fed varying levels of *Lemna minor* incorporated diet for 60 days.

Parameters	**LM0**	**LM5**	**LM10**	**LM15**	**LM20**	***P* value**
Moisture	17.57 ± 0.01^{a}	17.45 ± 0.01^{c}	17.18 ± 0.01^{d}	16.76 ± 0.01^{e}	17.53 ± 0.01^{b}	< 0.001
Protein	63.54 ± 0.02^{e}	63.66 ± 0.03^{d}	63.84 ± 0.03^{b}	63.95 ± 0.05^{a}	63.69 ± 0.02^{c}	< 0.001
Lipid	7.97 ± 0.05^{e}	8.07 ± 0.02^{d}	8.27 ± 0.05^{b}	8.44 ± 0.06^{a}	8.13 ± 0.01^{c}	< 0.001
Ash	9.38 ± 0.02^{d}	9.44 ± 0.01^{c}	9.51 ± 0.03^{b}	9.60 ± 0.05^{a}	9.50 ± 0.06^{b}	< 0.001
Fibre	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.499
Carbohydrate	1.44 ± 0.03^{a}	1.28 ± 0.03^{b}	1.09 ± 0.05^{cd}	1.15 ± 0.05^{c}	1.04 ± 0.04^{d}	< 0.001

Note. Superscript letters indicate significant differences in a shared row (n = 3, $P < 0.05$). LM0: 0% *L. minor*, LM5: 5% *L. minor*, LM10: 10% *L. minor*, LM15: 15% *L. minor*, and LM20: 20% *L. minor*.

4.3.3. Digestive enzyme activity

The digestive enzyme activity of *H. fossilis* fed varying levels of *L. minor*-incorporated diets is presented in Figure 9 (a-f). Amylase activity in the control group (LM0) was 2.02 ± 0.04 U mg^{-1}, while fish fed *L. minor*-supplemented diets exhibited increased amylase activity, with values of 2.14 ± 0.04, 2.30 ± 0.03, 2.33 ± 0.00, and 2.31 ± 0.01 U mg^{-1} for LM5, LM10, LM15, and LM20, respectively. An upward trend in amylase activity was observed across the *L. minor*-supplemented groups, reaching its peak in the LM15 group, which showed a 1.15-fold increase compared to the control ($P < 0.05$). Although no significant difference ($P > 0.05$) was found between the LM10 and LM15 groups, a slight decrease was observed in the LM20 group compared to LM15.

Lipase activity was recorded as 36.01 ± 0.63, 37.15 ± 0.51, 40.58 ± 0.14, 41.21 ± 0.86, and 39.13 ± 0.40 U min^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. The highest lipase activities ($P < 0,05$) were observed in the LM10 and LM15 groups, with no significant difference between them ($P > 0.05$). The LM10 group showed a 1.13-fold increase, while the LM15 group showed a 1.14-fold increase compared to the control. However, a slight decline was noted in the LM20 group. Pepsin activity was recorded as 1037.32 ± 17.54, 1094.55 ± 18.32, 1172.13 ± 17.68, 1173.20 ± 11.49, and 1151.33 ± 5.97 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. The highest pepsin activities ($P < 0.05$) were observed in the LM10, LM15, and LM20 groups, with no significant differences among them ($P > 0.05$). In contrast, the lowest pepsin activity was recorded in the control group (LM0).

Total protease activity was recorded as 0.80 ± 0.05, 0.82 ± 0.04, 0.83 ± 0.01, 0.83 ± 0.05, and 0.79 ± 0.05 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. However, there were no significant differences observed among the groups ($P > 0.05$). Trypsin activity was recorded as 41.39 ± 2.46, 42.37 ± 2.42, 43.43 ± 2.45, 44.35 ± 3.40, and 40.65 ± 4.84 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. However, no significant differences were observed among the groups ($P > 0.05$). Chymotrypsin activity was recorded as 68.72 ± 6.60, 69.33 ± 6.65, 79.13 ± 5.93, 68.99 ± 5.40, and

66.13 ± 6.70 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively, with no significant differences observed among the groups ($P > 0.05$).

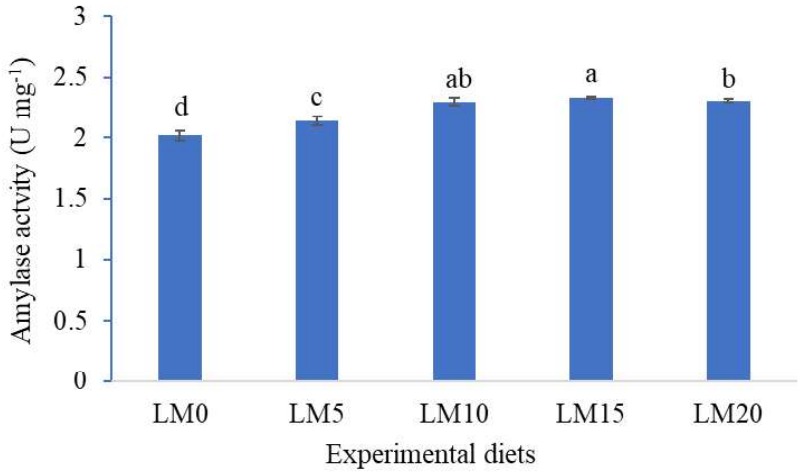

Figure 9 (a).

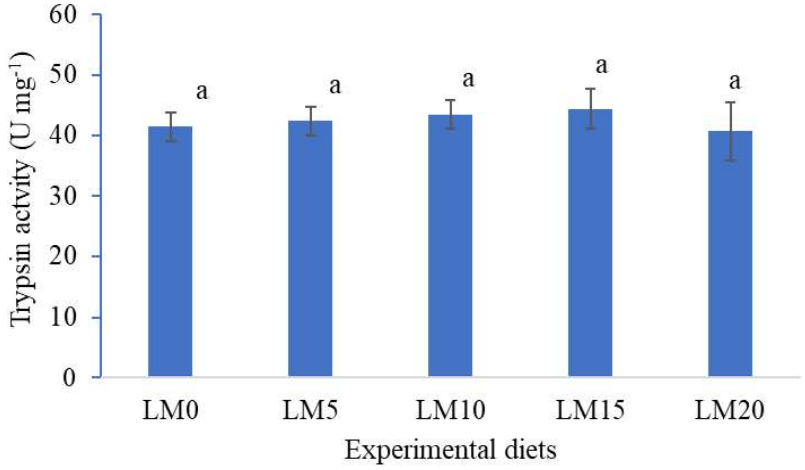

Figure 9 (b).

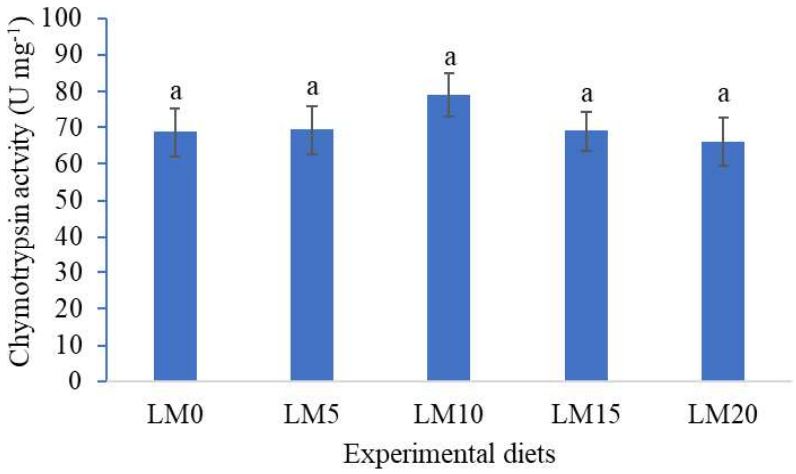

Figure 9 (c).

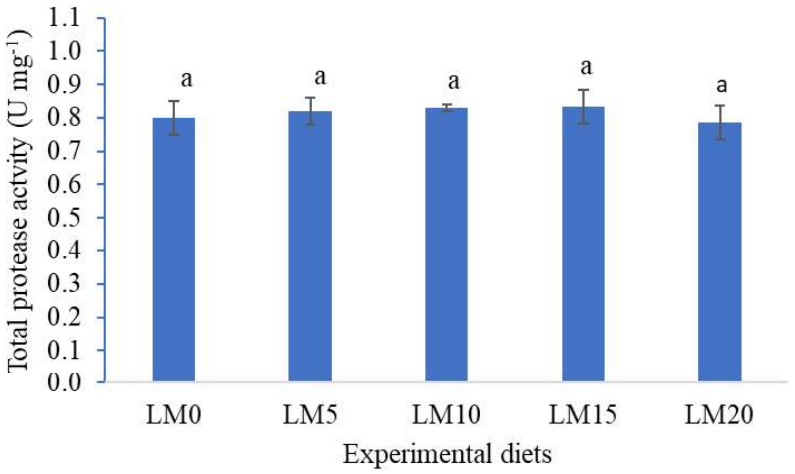

Figure 9 (d).

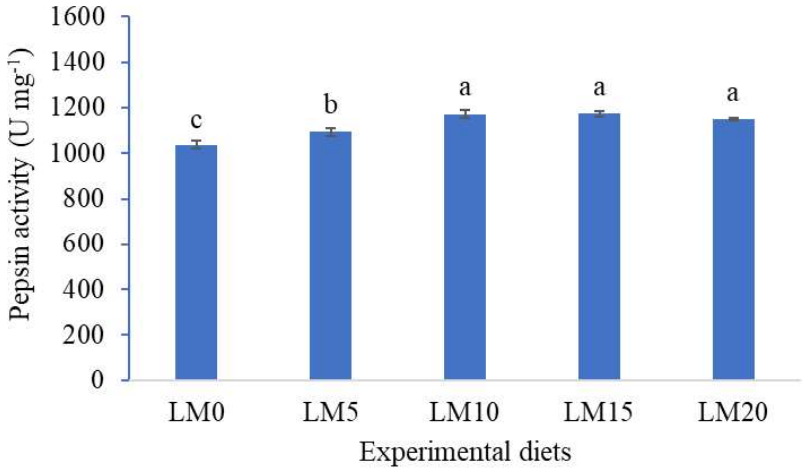

Figure 9 (e).

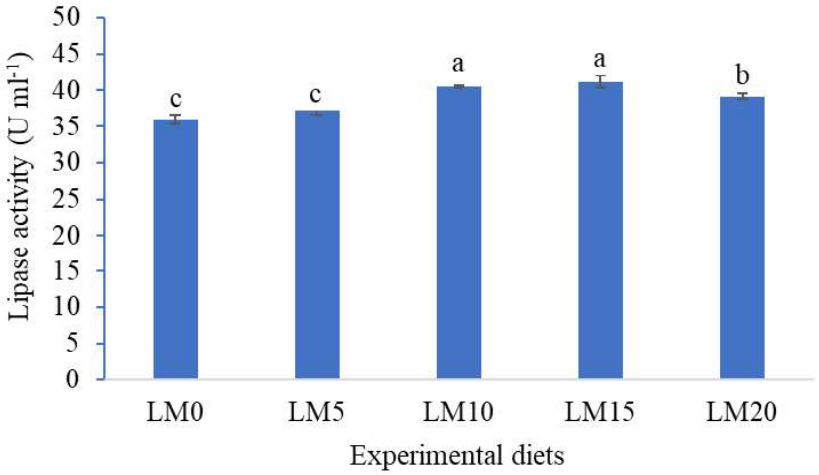

Figure 9 (f).

Figure 9. Digestive enzyme (a) Amylase, (b) Trypsin, (c) Chymotrypsin, (d) Total protease, (e) Pepsin, and (f) Lipase activity of *Heteropneustes* fed with varying levels of *Lemna minor* incorporated diet for 60 days. Different letters indicate statistically significant variations (n = 3, $P < 0.05$). LM0: 0% *L. minor*, LM5: 5% *L. minor*, LM10: 10% *L. minor*, LM15: 15% *L. minor*, and LM20: 20% *L. minor*.

4.3.4. Amino acid composition

The amino acid composition of *H. fossilis* fed varying levels of *L. minor*-incorporated diets for 60 days is presented in Table 15. Significant variation was observed in both EAAs and NEAAs composition among the dietary groups. Among the EAAs, arginine levels were highest ($P < 0.05$) in the LM15 group (32.31 ± 0.01 mg g^{-1}), significantly exceeding all other groups. The control group (LM0) had the lowest arginine content (24.94 ± 0.26 mg g^{-1}). Histidine peaked in the LM15 group (20.25 ± 0.13 mg g^{-1}), significantly higher than all other treatments ($P < 0.05$), while the lowest level was observed in LM0 (14.99 ± 0.41 mg g^{-1}) and LM20 (14.92 ± 0.10 mg g^{-1}). Lysine content was highest ($P < 0.05$) in the LM5 group (90.83 ± 0.21 mg g^{-1}), followed by the LM10 group (82.15 ± 0.20 mg g^{-1}). The LM20 group had the lowest ($P < 0.05$) lysine content (70.39 ± 0.07 mg g^{-1}). Leucine levels were highest in the LM0 group (114.61 ± 0.50 mg g^{-1}), followed by LM15 (106.04 ± 0.12 mg g^{-1}) and the lowest level was recorded in LM10 (59.68 ± 0.04 mg g^{-1}). Methionine was significantly higher ($P < 0.05$) in LM15 (63.50 ± 0.58 mg g^{-1}) compared to all other groups, while the lowest level ($P < 0.05$) was found in LM10 (25.91 ± 0.06 mg g^{-1}).

Phenylalanine level was highest ($P < 0.05$) in LM0 (70.86 ± 0.93 mg g^{-1}) and lowest in LM20 (27.69 ± 0.40 mg g^{-1}). Threonine was highest in LM10 (46.66 ± 0.10 mg g^{-1}), with the lowest levels observed in the LM0 group (25.60 ± 0.28 mg g^{-1}). Tryptophan peaked in LM10 (6.60 ± 0.01 mg g^{-1}), while the lowest content was found in LM15 (3.85 ± 0.01 mg g^{-1}). Valine content was highest in the LM15 group (22.36 ± 0.11 mg g^{-1}), which was not significantly different from the LM0 group (22.29 ± 0.08 mg g^{-1}). In contrast, the lowest valine content was recorded in the LM5 group (16.20 ± 0.70 mg g^{-1}). Total EAA content was 403.75 ± 0.53, 354.21 ± 1.03, 323.56 ± 0.53, 417.30 ± 1.52, and 307.70 ± 0.46 mg g^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. The LM15 group exhibited the highest total EAA content ($P < 0.05$), followed by the LM0 group, while the lowest value was recorded in the LM20 group.

For NEAAs, alanine content was highest ($P < 0.05$) in the LM20 group (79.44 ± 1.07 mg g^{-1}), followed by the LM10 group (73.47 ± 0.05 mg g^{-1}). The lowest alanine levels were observed in the control group (LM0, 43.84 ± 0.18 mg

g^{-1}) and LM15 (44.40 ± 1.00 mg g^{-1}), with no significant difference between these two groups ($P > 0.05$). Aspartic acid content was highest in LM10 (92.89 ± 0.82 mg g^{-1}) and lowest in LM5 (59.25 ± 1.29 mg g^{-1}). Cysteine content was highest in LM0 and LM5 (0.66 ± 0.01 and 0.72 ± 0.05 mg g^{-1}, respectively), with no significant differences between them ($P > 0.05$). Glycine content was highest in the LM20 group (0.64 ± 0.00 mg g^{-1}) and lowest in the LM15 group (0.34 ± 0.02 mg g^{-1}). Glutamic acid content peaked in LM10 (110.46 ± 0.87 mg g^{-1}), while the lowest was observed in LM0 (45.56 ± 0.11 mg g^{-1}). Proline content was highest ($P < 0.05$) in the control group (LM0, 90.39 ± 0.15 mg g^{-1}), whereas the lowest was recorded in LM5 (53.71 ± 0.15 mg g^{-1}). Serine content was highest in LM5 (96.91 ± 2.01 mg g^{-1}) and LM10 (93.57 ± 1.12 mg g^{-1}), with no significant difference between them ($P > 0.05$), and the lowest content was found in LM0 (52.20 ± 0.23 mg g^{-1}). Tyrosine content was also highest in the control group (LM0, 22.64 ± 0.05 mg g^{-1}), while LM20 had the lowest value (8.40 ± 0.02 mg g^{-1}). Citrulline content peaked in LM20 (2.93 ± 0.05 mg g^{-1}), while the lowest content was recorded in both LM0 (1.30 ± 0.05 mg g^{-1}) and LM10 (1.29 ± 0.03 mg g^{-1}) with no significant difference between them ($P > 0.05$). Asparagine content was significantly highest ($P < 0.05$) in LM0 (1.40 ± 0.01 mg g^{-1}) and lowest in LM10 (0.08 ± 0.00 mg g^{-1}). Beta 3-4 dihydroxy phenylalanine was most abundant in LM0 (0.07 ± 0.01 mg g^{-1}) and lowest in LM10 (0.01 ± 0.00 mg g^{-1}). The total NEAA content was 331.70 ± 1.42, 349.42 ± 0.59, 449.61 ± 2.90, 310.26 ± 4.45, and 449.49 ± 1.21 mg g^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. The LM10 and LM20 groups showed significantly higher ($P < 0.05$) total NEAA content, with no significant difference between them ($P > 0.05$), while the lowest content was observed in the LM15 group. Overall, the total amino acid content was highest in the LM10 group (773.18 ± 3.43 mg g^{-1}), followed by LM20 (757.19 ± 0.75 mg g^{-1}). In contrast, the lowest content was observed in LM5 (703.63 ± 1.62 mg g^{-1}), indicating significant variations in amino acid composition based on diet ($P < 0.05$). The total amino acid content in LM0 and LM15 did not differ significantly from each other ($P > 0.05$).

Table 15. Amino acid composition of *Heteropneustes fossilis* fed varying levels of *Lemna minor* incorporated diet for 60 days.

Amino acids (mg g^{-1})	**LM0**	**LM5**	**LM10**	**LM15**	**LM20**	***P* value**
EAA						
Arginine	24.94 ± 0.26^{e}	27.79 ± 0.10^{d}	28.81 ± 0.19^{c}	32.31 ± 0.01^{a}	31.54 ± 0.10^{b}	< 0.001
Histidine	14.99 ± 0.41^{d}	17.33 ± 0.07^{c}	18.03 ± 0.23^{b}	20.25 ± 0.13^{a}	14.92 ± 0.10^{d}	< 0.001
Lysine	73.12 ± 0.12^{d}	90.83 ± 0.21^{a}	82.15 ± 0.20^{b}	81.61 ± 0.01^{c}	70.39 ± 0.07^{e}	< 0.001
Leucine	114.61 ± 0.50^{a}	78.58 ± 0.26^{c}	59.68 ± 0.04^{e}	106.04 ± 0.12^{b}	61.37 ± 0.27^{d}	< 0.001
Methionine	51.82 ± 0.11^{b}	33.19 ± 0.28^{c}	25.91 ± 0.06^{e}	63.50 ± 0.58^{a}	30.64 ± 0.12^{d}	< 0.001
Phenylalanine	70.86 ± 0.93^{a}	57.27 ± 0.66^{c}	35.14 ± 0.11^{d}	59.70 ± 0.25^{b}	27.69 ± 0.40^{e}	< 0.001
Threonine	25.60 ± 0.28^{d}	27.67 ± 0.09^{c}	46.66 ± 0.10^{a}	27.69 ± 0.36^{c}	44.86 ± 0.40^{b}	< 0.001
Tryptophan	5.51 ± 0.04^{b}	5.34 ± 0.03^{c}	6.60 ± 0.01^{a}	3.85 ± 0.01^{e}	4.85 ± 0.01^{d}	< 0.001
Valine	22.29 ± 0.08^{ab}	16.20 ± 0.70^{d}	20.58 ± 0.07c	22.36 ± 0.11^{a}	21.45 ± 0.06^{b}	< 0.001
Total EAA	403.75 ± 0.53^{b}	354.21 ± 1.03^{c}	323.56 ± 0.53^{d}	417.30 ± 1.52^{a}	307.70 ± 0.46^{e}	< 0.001
NEAA						
Alanine	43.84 ± 0.18^{d}	50.04 ± 0.80^{c}	73.47 ± 0.05^{b}	44.40 ± 1.00^{d}	79.44 ± 1.07^{a}	< 0.001
Aspartic acid	73.10 ± 1.13^{c}	59.25 ± 1.29^{e}	92.89 ± 0.82^{a}	64.57 ± 0.76^{d}	89.54 ± 0.47^{b}	< 0.001
Cysteine	0.66 ± 0.01^{a}	0.72 ± 0.05^{a}	0.60 ± 0.01^{b}	0.57 ± 0.02^{b}	0.56 ± 0.01^{b}	< 0.001
Glycine	0.55 ± 0.02^{b}	0.39 ± 0.02^{c}	0.58 ± 0.01^{b}	0.34 ± 0.02^{d}	0.64 ± 0.00^{a}	< 0.001

Glutamic acid	45.56 ± 0.11^{e}	74.73 ± 0.46^{c}	110.46 ± 0.87^{a}	47.43 ± 0.69^{d}	99.60 ± 0.81^{b}	< 0.001
Proline	90.39 ± 0.15^{a}	53.71 ± 0.15^{e}	63.01 ± 0.04^{d}	74.64 ± 0.18^{c}	81.42 ± 1.31^{b}	< 0.001
Serine	52.20 ± 0.23^{d}	96.91 ± 2.01^{a}	93.57 ± 1.12^{a}	57.00 ± 1.57^{c}	86.50 ± 1.58^{b}	< 0.001
Tyrosine	22.64 ± 0.05^{a}	11.29 ± 0.11^{d}	13.67 ± 0.03^{c}	18.14 ± 0.24^{b}	8.40 ± 0.02^{e}	< 0.001
Citrulline	1.30 ± 0.05^{d}	1.56 ± 0.03^{c}	1.29 ± 0.03^{d}	2.57 ± 0.01^{b}	2.93 ± 0.05^{a}	< 0.001
Asparagine	1.40 ± 0.01^{a}	0.80 ± 0.00^{b}	0.08 ± 0.00^{e}	0.58 ± 0.00^{c}	0.45 ± 0.00^{d}	< 0.001
Beta 3-4 dihydroxy phenylalanine	0.07 ± 0.01^{a}	0.03 ± 0.01^{b}	0.01 ± 0.00^{c}	0.03 ± 0.00^{b}	0.02 ± 0.00^{cb}	< 0.001
Total NEAA	331.70 ± 1.42^{c}	349.42 ± 0.59^{b}	449.61 ± 2.90^{a}	310.26 ± 4.45^{d}	449.49 ± 1.21^{a}	< 0.001
Total amino acids	735.45 ± 1.96^{c}	703.63 ± 1.62^{d}	773.18 ± 3.43^{a}	727.56 ± 5.96^{c}	757.19 ± 0.75^{b}	0.001

Notes: Superscript letters indicate significant differences in a shared row (n = 3, $P < 0.05$). EAA: Essential amino acids, NEAA: Non-essential amino acids. LM0: 0% *L. minor*, LM5: 5% *L. minor*, LM10: 10% *L. minor*, LM15: 15% *L. minor*, and LM20: 20% *L. minor*.

4.3.5. Fatty acid composition

The fatty acid composition of *H. fossilis* fed varying levels of *L. minor*-incorporated diets for 60 days is presented in Table 16. Significant differences ($P < 0.05$) were observed among the dietary treatments across SFAs, MUFAs, and PUFAs. Among the SFAs, C13:0 was significantly higher in the LM5 (2.88 ± 0.01%) and LM10 (2.87 ± 0.02%) groups compared to all other treatments, with the control (LM0) showing the lowest value (1.72 ± 0.01%). C14:0 displayed a clear decreasing trend as *L. minor* levels increased, reaching its lowest concentrations in LM15 (2.31 ± 0.01%) and LM20 (2.30 ± 0.02%), with no significant difference between these two treatments ($P > 0.05$). C17:0, the most abundant SFA, fluctuated across treatments, peaking at 15.96 ± 0.03% in LM15. In the case of C18:0, there was a marked reduction across LM5, LM10, LM15, and LM20 (0.08 ± 0.00%, 0.09 ± 0.01%, 0.09 ± 0.01%, and 0.08 ± 0.01%, respectively), with no significant differences ($P > 0.05$) among these treatments, but all were lower compared to the control (LM0, 0.14 ± 0.01%). C20:0 showed the lowest value in LM5 (0.37 ± 0.01%) but reached its highest level in LM20 (1.06 ± 0.01%). Similarly, C27:0 was highest in LM0 (2.77 ± 0.02%) and lowest in LM10 and LM15 (2.55 ± 0.02%). C34:0 also showed a decreasing trend, starting at 2.07 ± 0.01% in LM0 and reaching 1.72 ± 0.01% in LM15. The total SFA content was 26.01 ± 0.05, 25.96 ± 0.06, 25.96 ± 0.03, 25.84 ± 0.09, and 26.08 ± 0.04% for LM0, LM5, LM10, LM15, and LM20, respectively. The total SFA was highest in LM20, followed closely by LM0, while LM15 had the lowest total SFA content ($P < 0.05$).

In the case of MUFAs, C16:1n-7 exhibited a decreasing trend, dropping from 3.07 ± 0.01% in LM0 to 2.63 ± 0.02% in LM15. However, no significant differences ($P > 0.05$) were observed between LM10 (2.65 ± 0.01%), LM15 (2.63 ± 0.02%), and LM20 (2.67 ± 0.02%). C16:1n-5 showed a significant decline with increasing *L. minor* levels, reducing from 12.39 ± 0.01% in LM0 to 11.95 ± 0.03% in LM15. C18:1n-9 consistently decreased with increasing *L. minor* levels, starting at 1.53 ± 0.01% in LM0 and reaching 1.28 ± 0.02% in LM20. C18:1n-16 decreased, dropping from 14.45 ± 0.02% in LM0 to 14.18 ±

0.02% in LM15. Similarly, C20:1n-9 decreased from 3.96 ± 0.02% in LM0 to 3.81 ± 0.01% in LM15, although the difference between LM0 and LM15 (3.81 ± 0.01%) was not statistically significant ($P > 0.05$). The content of C18:1n-5 remained relatively stable across the treatments, with minor variations. Total MUFA content was 48.71 ± 0.06, 48.01 ± 0.08, 47.51 ± 0.08, 47.23 ± 0.12, and 47.32 ± 0.10% for LM0, LM5, LM10, LM15, and LM20, respectively. The MUFA content decreased steadily from LM0 to LM15, with LM0 showing the highest value and LM15 the lowest ($P < 0.05$). A slight increase was observed in LM20 compared to LM15.

Among the PUFAs, C20:5n-3 (EPA) increased steadily from 2.56 ± 0.01% in LM0 to 2.77 ± 0.02% in LM10, while C20:3n-3 increased consistently, reaching its highest value in LM20 (2.29 ± 0.02%). C22:6n-3 (DHA) also exhibited a significant increase, peaking in both LM15 (1.64 ± 0.03%) and LM20 (1.64 ± 0.03%) with no significant difference ($P > 0.05$) between the group, up from 1.40 ± 0.02% in LM0. C18:3n-3 showed a significant increase with increasing *L. minor* levels, peaking at 1.41 ± 0.02% in LM15 compared to 1.29 ± 0.02% in LM0. C20:4n-6 followed a similar pattern, rising from 3.91 ± 0.01% in LM0 to 4.11 ± 0.02% in LM20, while C20:2n-6 increased from 3.72 ± 0.01% in LM0 to 4.00 ± 0.01% in LM20. C18:2n-6 showed a slight increase, reaching its peak at 8.46 ± 0.01% in LM20 while remaining similar at 8.45 ± 0.02% in both LM10 and LM15. No significant differences were observed between these groups. C22:4n-6 reached its highest levels in LM15 (2.16 ± 0.01%) and LM20 (2.12 ± 0.01%), with no significant difference observed between the two groups ($P > 0.05$).

The total PUFA content was 25.17 ± 0.01, 25.90 ± 0.13, 26.41 ± 0.09, 26.69 ± 0.11, and 26.59 ± 0.06% for LM0, LM5, LM10, LM15, and LM20, respectively. It exhibited a general increasing trend across the treatments, reaching its highest levels in LM15 and LM20, with no significant difference ($P > 0.05$) between these two groups. The PUFA to SFA ratio was highest ($P < 0.05$) in LM15 (1.03 ± 0.01), indicating a significant increase compared to the control LM0 (0.97 ± 0.00). The ω6/ω3 ratio showed a slight decrease from 2.41

± 0.02 in LM0 to 2.33 ± 0.02 in LM15. However, there were no significant differences ($P > 0.05$) among LM10 (2.34 ± 0.01%), LM15 (2.33 ± 0.02%), and LM20 (2.36 ± 0.02%). The combined EPA and DHA content was highest in LM10, LM15, and LM20 (4.32 ± 0.03%, 4.36 ± 0.04%, and 3.45 ± 0.05%, respectively), with no significant differences ($P > 0.05$) observed among these groups.

4.3.6. Biochemical parameters

The biochemical parameters of *H. fossilis* fed varying percentages of *L. minor*-supplemented diets over 60 days are presented in Table 17. The TIg levels were consistent across the groups, measured at 0.21 ± 0.00, 0.22 ± 0.01, 0.22 ± 0.00, 0.21 ± 0.00, and 0.22 ± 0.00 mg mL^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively, with no significant differences ($P = 0.120$). LYZ activity varied significantly among the dietary groups ($P < 0.05$), with values of 94.26 ± 8.52, 105.56 ± 11.11, 80.37 ± 1.09, 80.40 ± 0.65, and 81.95 ± 11.36 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. The highest LYZ activity was observed in the LM5 group ($P < 0.05$). Alkaline phosphatase (ALP) activity also showed significant variation ($P < 0.001$), with the highest value recorded in the LM5 group (1.80 ± 0.10 U mg^{-1}), compared to 1.44 ± 0.04, 1.67 ± 0.21, 1.15 ± 0.06, and 1.28 ± 0.06 U mg^{-1} for LM0, LM10, LM15, and LM20, respectively.

AST activity levels were significantly different among the groups ($P < 0.05$), recorded at 2.20 ± 0.09, 2.13 ± 0.05, 2.11 ± 0.08, 2.16 ± 0.15, and 2.14 ± 0.05 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. Similarly, ALT levels varied significantly ($P < 0.05$), with values of 2.16 ± 0.35, 2.07 ± 0.09, 2.06 ± 0.14, 2.15 ± 0.13, and 2.14 ± 0.08 U mg^{-1} for the respective groups. Lower AST was observed in LM10, LM15 and LM20, and lower ALT activities were observed in LM5, LM10 and LM20 compared with other groups ($P < 0.05$). CAT activity did not differ significantly among the groups ($P = 0.210$), with levels recorded at 1.85 ± 0.44, 1.39 ± 0.69, 1.32 ± 0.34, 1.86 ± 0.44, and 1.60 ± 0.36 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. Superoxide dismutase (SOD) activity was also similar across the groups ($P =$

0.780), measured at 371.78 ± 92.39, 370.77 ± 66.59, 371.97 ± 92.64, 370.62 ± 35.12, and 371.63 ± 68.07 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. TBARS levels varied significantly among the groups ($P < 0.05$), with values recorded as 2.78 ± 0.07, 2.74 ± 0.09, 2.77 ± 0.12, 2.75 ± 0.03, and 2.76 ± 0.22 U mg^{-1} for LM0, LM5, LM10, LM15, and LM20, respectively. Notably, the LM5 group exhibited lower activity compared to the other groups.

Table 16. Fatty acid composition (% of total fatty acid) in *Heteropneustes fossilis* fed varying levels of *Lemna minor* incorporated diet for 60 days.

Fatty acid	**LM0**	**LM5**	**LM10**	**LM15**	**LM20**	***P* value**
C13:0	1.72 ± 0.01^{d}	2.88 ± 0.01^{a}	2.87 ± 0.02^{a}	2.70 ± 0.02^{b}	2.47 ± 0.02^{c}	< 0.001
C14:0	2.45 ± 0.01^{a}	2.39 ± 0.01^{b}	2.38 ± 0.01^{b}	2.31 ± 0.01^{c}	2.30 ± 0.02^{c}	< 0.001
C17:0	15.85 ± 0.01^{b}	15.69 ± 0.01^{c}	15.56 ± 0.02^{d}	15.96 ± 0.03^{a}	15.69 ± 0.02^{c}	< 0.001
C18:0	0.14 ± 0.01^{a}	0.08 ± 0.00^{b}	0.09 ± 0.01^{b}	0.09 ± 0.01^{b}	0.08 ± 0.01^{b}	< 0.001
C20:0	1.01 ± 0.01^{b}	0.37 ± 0.01^{e}	0.57 ± 0.02^{c}	0.53 ± 0.02^{d}	1.06 ± 0.01^{a}	< 0.001
C27:0	2.77 ± 0.02^{a}	2.58 ± 0.01^{bc}	2.55 ± 0.02^{c}	2.55 ± 0.02^{c}	2.62 ± 0.01^{b}	< 0.001
C34:0	2.07 ± 0.01^{a}	1.97 ± 0.01^{b}	1.96 ± 0.01^{b}	1.72 ± 0.01^{d}	1.86 ± 0.02^{c}	< 0.001
Σ SFA	26.01 ± 0.05^{ab}	25.96 ± 0.06^{ab}	25.96 ± 0.03^{ab}	25.84 ± 0.09^{b}	26.08 ± 0.04^{a}	0.009
C16:1n-5	12.39 ± 0.01^{a}	12.26 ± 0.01^{b}	12.10 ± 0.04^{c}	11.95 ± 0.03^{e}	12.03 ± 0.03^{d}	< 0.001
C16:1n-7	3.07 ± 0.01^{a}	2.75 ± 0.01^{b}	2.65 ± 0.01^{c}	2.63 ± 0.02^{c}	2.67 ± 0.02^{c}	< 0.001
C18:1n-9	1.53 ± 0.01^{a}	1.48 ± 0.02^{b}	1.42 ± 0.01^{c}	1.36 ± 0.01^{d}	1.28 ± 0.02^{e}	< 0.001
C18:1n-16	14.45 ± 0.02^{a}	14.32 ± 0.02^{b}	14.26 ± 0.02^{c}	14.18 ± 0.02^{d}	14.25 ± 0.01^{c}	< 0.001
C20:1n-9	3.96 ± 0.02^{a}	3.90 ± 0.02^{d}	3.85 ± 0.02^{c}	3.81 ± 0.01^{a}	3.82 ± 0.01^{b}	< 0.001
C18:1n-5	13.30 ± 0.01^{a}	13.29 ± 0.01^{a}	13.24 ± 0.03^{b}	13.30 ± 0.01^{a}	13.27 ± 0.01^{ab}	0.004
Σ MUFA	48.71 ± 0.06^{a}	48.01 ± 0.08^{b}	47.51 ± 0.08^{c}	47.23 ± 0.12^{d}	47.32 ± 0.10^{cd}	< 0.001
C18:3n-3	1.29 ± 0.02^{c}	1.35 ± 0.01^{b}	1.37 ± 0.01^{b}	1.41 ± 0.02^{a}	1.27 ± 0.01^{c}	< 0.001
C20:5n-3	2.56 ± 0.01^{c}	2.62 ± 0.02^{c}	2.77 ± 0.02^{a}	2.72 ± 0.01^{b}	2.70 ± 0.02^{b}	< 0.001

C20:3n-3	2.13 ± 0.02^{c}	2.16 ± 0.02^{c}	2.22 ± 0.01^{b}	2.24 ± 0.03^{b}	2.29 ± 0.02^{a}	< 0.001
C22:6n-3	1.40 ± 0.02^{d}	1.46 ± 0.01^{c}	1.55 ± 0.02^{b}	1.64 ± 0.03^{a}	1.64 ± 0.03^{a}	< 0.001
C20:4n-6	3.91 ± 0.01^{d}	3.97 ± 0.02^{c}	4.05 ± 0.02^{b}	4.07 ± 0.02^{ab}	4.11 ± 0.02^{a}	< 0.001
C20:2n-6	3.72 ± 0.01^{d}	3.93 ± 0.03^{c}	3.95 ± 0.02^{bc}	3.99 ± 0.02^{ab}	4.00 ± 0.01^{a}	< 0.001
C18:2n-6	8.24 ± 0.02^{c}	8.38 ± 0.02^{b}	8.45 ± 0.02^{a}	8.45 ± 0.02^{a}	8.46 ± 0.01^{a}	< 0.001
C22:4n-6	1.92 ± 0.02^{c}	2.03 ± 0.02^{b}	2.05 ± 0.01^{b}	2.16 ± 0.01^{a}	2.12 ± 0.01^{a}	< 0.001
Σ PUFA	25.17 ± 0.01^{d}	25.90 ± 0.13^{c}	26.41 ± 0.09^{b}	26.69 ± 0.11^{a}	26.59 ± 0.06^{ab}	< 0.001
PUFA/SFA	0.97 ± 0.00^{d}	1.00 ± 0.00^{c}	1.02 ± 0.00^{b}	1.03 ± 0.01^{a}	1.02 ± 0.00^{b}	< 0.001
ω6/ω3	2.41 ± 0.02^{a}	2.41 ± 0.01^{a}	2.34 ± 0.01^{b}	2.33 ± 0.02^{b}	2.36 ± 0.02^{b}	< 0.001
EPA+DHA	3.96 ± 0.01^{c}	4.08 ± 0.03^{b}	4.32 ± 0.03^{a}	4.36 ± 0.04^{a}	4.35 ± 0.05^{a}	< 0.001

Notes: Superscript letters indicate significant differences in a shared row (n = 3, $P < 0.05$). SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid. LM0: 0% *L. minor*, LM5: 5% *L. minor*, LM10: 10% *L. minor*, LM15: 15% *L. minor*, and LM20: 20% *L. minor*.

Table 17. Biochemical parameters of *Heteropneustes fossilis* fed with different percentage inclusion of *Lemna minor* diets for 60 days.

Parameters	LM0	LM5	LM10	LM15	LM20	*P* value
TIg (mg mL^{-1})	0.21 ± 0.00	0.22 ± 0.01	0.22 ± 0.00	0.21 ± 0.00	0.22 ± 0.00	0.120
LYZ (U mg^{-1})	94.26 ± 8.52^{ab}	105.56 ± 11.11^{a}	80.37 ± 1.09^{c}	80.40 ± 0.65^{b}	81.95 ± 11.36^{b}	< 0.001
ALP (U mg^{-1})	1.44 ± 0.04^{c}	1.80 ± 0.10^{a}	1.67 ± 0.21^{b}	1.15 ± 0.06^{d}	1.28 ± 0.06^{b}	< 0.001
AST (U mg^{-1})	2.20 ± 0.09^{a}	2.13 ± 0.05^{a}	2.11 ± 0.08^{b}	2.16 ± 0.15^{ab}	2.14 ± 0.05^{b}	< 0.001
ALT (U mg^{-1})	2.16 ± 0.35^{ac}	2.07 ± 0.09^{b}	2.06 ± 0.14^{b}	2.15 ± 0.13^{a}	2.14 ± 0.08^{ab}	0.002
CAT (U mg^{-1})	1.85 ± 0.44	1.39 ± 0.69	1.32 ± 0.34	1.86 ± 0.44	1.60 ± 0.36	0.210
SOD (U mg^{-1})	371.78 ± 92.39	370.77 ± 66.59	371.97 ± 92.64	370.62 ± 35.12	371.63 ± 68.07	0.780
TBARS (U mg^{-1})	2.78 ± 0.07^{ab}	2.74 ± 0.09^{b}	2.77 ± 0.12^{a}	2.75 ± 0.03^{ab}	2.76 ± 0.22^{a}	< 0.001

Notes: Values are represented as mean values ± SD. Means within the same column having different superscripts are significantly different ($P < 0.05$). TIg: Total Immunoglobulin, ALP: Alkaline Phosphatase, LYZ: Lysozyme, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, CAT: Catalase, SOD: Superoxide dismutase, TBARS: Thiobarbituric acid reactive substances. LM0: 0% *L. minor*, LM5: 5% *L. minor*, LM10: 10% *L. minor*, LM15: 15% *L. minor*, and LM20: 20% *L. minor*.

4.4. Evaluating the growth, digestive physiology, and biochemical parameters of *Anabas testudineus* fed with an *Ipomoea aquatica*-supplemented diet

4.4.1. Growth performance

The growth performance of *A. testudineus* (initial weight: 0.75 ± 0.01 g) fed varying percentages of *I. aquatica*-supplemented diets over 60 days is presented in Table 18. The FW of the fish were 3.72 ± 0.02, 3.86 ± 0.01, 4.04 ± 0.01, 4.27 ± 0.03, and 4.10 ± 0.01 g for IA0, IA5, IA10, IA15, and IA20, respectively. Fish-fed diets supplemented with *I. aquatica* (IA5–IA20) showed significantly higher FW than the control group. Among these groups, FW progressively increased in the IA5 and IA10 groups, peaking in the IA15 group ($P < 0.05$), which exhibited a 1.15-fold increase compared to the control (IA0). Although FW in the IA20 group slightly declined compared to IA15, it remained 1.10-fold greater than IA0. Similarly, BWG percentages were recorded as 397.78 ± 2.04, 414.67 ± 1.33, 439.11 ± 1.54, 469.78 ± 3.36, and 446.67 ± 1.33% for IA0, IA5, IA10, IA15, and IA20, respectively. Gradual increases in BWG were observed in the IA5 and IA10 groups, peaking in the IA15 group, which showed a 1.18-fold increase compared to the control (IA0) and significantly exceeded the BWG of all other groups ($P < 0.05$). While the BWG of the IA20 group slightly decreased compared to IA15, it still demonstrated a considerable improvement over the control. Furthermore, survival rates were consistently 100% across all dietary treatments (IA0–IA20), demonstrating that the different inclusion levels of *I. aquatica* had no adverse effects on fish survival or health during the experimental period.

FCR were 1.51 ± 0.01, 1.48 ± 0.01, 1.42 ± 0.01, 1.37 ± 0.02, and 1.41 ± 0.01 for IA0, IA5, IA10, IA15, and IA20, respectively. Increasing the inclusion level of *I. aquatica* up to 15% resulted in a consistent decline in FCR, reflecting enhanced feed efficiency. The IA15 group exhibited the lowest FCR, indicating a 1.10-fold improvement compared to the control and significantly outperforming all other groups ($P < 0.05$). SGR were 2.67 ± 0.01, 2.73 ± 0.00,

2.81 ± 0.00, 2.90 ± 0.01, and 2.83 ± 0.00 for IA0, IA5, IA10, IA15, and IA20, respectively. A trend of increasing SGR was observed with higher levels of *I. aquatica* inclusion up to 15%, where the IA15 group showed a 1.09-fold increase compared to the control (IA0) and was significantly higher than all other groups ($P < 0.05$). Although the SGR of the IA20 group slightly declined, it still represented a 1.06-fold increase compared to the control (IA0). These results demonstrate that the inclusion of *I. aquatica* enhances not only growth but also feed utilization efficiency.

PER were 1.66 ± 0.02, 1.69 ± 0.01, 1.76 ± 0.01, 1.82 ± 0.01, and 1.77 ± 0.00 for IA0, IA5, IA10, IA15, and IA20, respectively. A gradual increase in PER was observed as the levels of *I. aquatica* increased to 15%, followed by a slight decline in the IA20 group. The IA15 group recorded the highest PER, with a 1.10-fold improvement over the control, significantly surpassing all other groups ($P < 0.05$). FE percentages were recorded as 66.42 ± 0.60, 67.57 ± 0.58, 70.42 ± 0.58, 72.84 ± 0.33, and $70.80 \pm 0.09\%$ for IA0, IA5, IA10, IA15, and IA20, respectively. Higher FE values were noted in fish fed diets supplemented with *I. aquatica*, with the IA15 group achieving the highest FE, significantly higher ($P < 0.05$) than all other groups. The IA15 group exhibited a 1.10-fold increase in FE compared to the control (IA0). These improvements in PER and FE reinforce the role of *I. aquatica* in enhancing nutrient utilization and overall growth performance in *A. testudineus*.

A polynomial regression analysis of the SGR and FCR data revealed that optimal growth in *A. testudineus* was achieved when *I. aquatica* inclusion levels ranged from 16.64% to 17.50%, as illustrated in Figure 10 (a-b). This range represents the ideal level of *I. aquatica* supplementation for maximizing growth performance in *A. testudineus*. The maximum values for FW, BWG, SGR, PER, and FE, combined with improved FCR, were observed at the IA15 level of inclusion, which proved to be the most effective in enhancing overall growth performance. The findings suggest that 15% *I. aquatica* inclusion in diets provides optimal nutritional benefits for *A. testudineus*, offering a promising strategy for sustainable aquaculture practices.

Table 18. The nutritional efficiency and growth performance of *Anabas testudineus* fed with varying percentages of *Ipomoea aquatica* diets (IA0: 0% *I. aquatica* incorporated diet, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*).

Parameters	**IA0**	**IA5**	**IA10**	**IA15**	**IA20**	***P* value**
IW (g)	0.75 ± 0.01	0.75 ± 0.02	0.75 ± 0.01	0.75 ± 0.02	0.75 ± 0.01	
FW (g)	3.72 ± 0.02^{e}	3.86 ± 0.01^{d}	4.04 ± 0.01^{c}	4.27 ± 0.03^{a}	4.10 ± 0.01^{b}	< 0.001
BWG (%)	397.78 ± 2.04^{e}	414.67 ± 1.33^{d}	439.11 ± 1.54^{c}	469.78 ± 3.36^{a}	446.67 ± 1.33^{b}	< 0.001
SGR (% day^{-1})	2.67 ± 0.01^{e}	2.73 ± 0.00^{d}	2.81 ± 0.00^{c}	2.90 ± 0.01^{a}	2.83 ± 0.00^{b}	< 0.001
FCR	1.51 ± 0.01^{a}	1.48 ± 0.01^{b}	1.42 ± 0.01^{c}	1.37 ± 0.02^{e}	1.41 ± 0.01^{d}	< 0.001
Survival (%)	100	100	100	100	100	
FE (%)	66.42 ± 0.60^{d}	67.57 ± 0.58^{cd}	70.42 ± 0.58^{c}	72.84 ± 0.33^{a}	70.80 ± 0.09^{b}	< 0.001
PER	1.66 ± 0.02^{c}	1.69 ± 0.01^{bc}	1.76 ± 0.01^{b}	1.82 ± 0.01^{a}	1.77 ± 0.00^{a}	< 0.001

Notes: All data are represented as mean ± sd. Superscript letters denote statistically significant differences within a common row (n = 30, $P < 0.05$). IW: Initial weight, FW: Final weight, BWG: Body weight gain, SGR: Specific growth rate, FCR: Feed conversion ratio, FE: Feed efficiency, PER: Protein efficiency ratio. IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*.

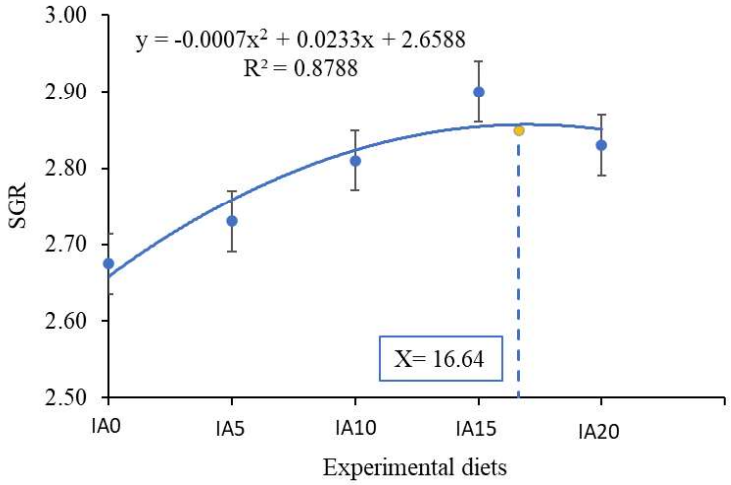

Figure 10 (a).

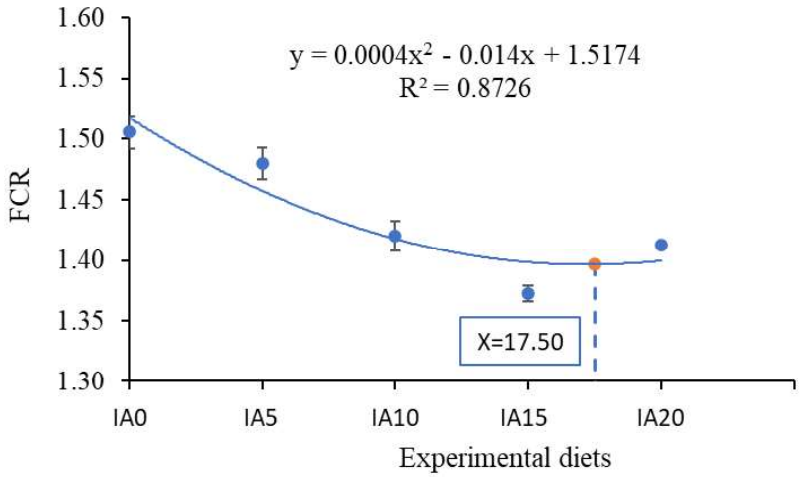

Figure 10 (b).

Figure 10. Polynomial regression analysis based on (a) SGR and (b) FCR of *Anabas testudineus* fed with different % inclusion of *Ipomoea aquatica* in the diet. IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*.

4.4.2. Proximate composition

The proximate composition analysis of *A. testudineus* fed varying levels of *I. aquatica*-incorporated diets over 60 days showed significant variations in several nutritional parameters (Table 19). The moisture contents were 15.42 ± 0.02, 15.15 ± 0.02, 14.80 ± 0.01, 14.23 ± 0.01, and $14.57 \pm 0.02\%$ for IA0, IA5, IA10, IA15, and IA20, respectively. Lower moisture content was recorded in the *I. aquatica* incorporated diet-fed fish compared to the control. The IA15 group recorded the lowest moisture content ($P < 0.05$) compared to all other groups. Correspondingly, the protein contents were 65.24 ± 0.01, 65.35 ± 0.02, 65.56 ± 0.02, 65.92 ± 0.02, and $65.67 \pm 0.02\%$ for IA0, IA5, IA10, IA15, and IA20, respectively. The IA15 group exhibited a significantly higher protein content ($P < 0.05$) than the control and other dietary groups, indicating that 15% *I. aquatica* supplementation optimizes protein accumulation in *A. testudineus* muscle.

Lipid contents were 8.22 ± 0.02, 8.35 ± 0.02, 8.42 ± 0.01, 8.56 ± 0.01, and $8.46 \pm 0.01\%$ for IA0, IA5, IA10, IA15, and IA20, respectively. Higher lipid content was observed in the *I. aqautica* incorporated diet-fed fish. the IA15 group achieved the highest lipid content, significantly greater ($P < 0.05$) than all other groups. Fibre content remained consistent across all groups (IA0, IA5, IA10, IA15, and IA20) at approximately 0.10 ± 0.01 to $0.11 \pm 0.01\%$, showing no significant differences ($P = 0.737$). The carbohydrate contents were 1.13 ± 0.01, 1.15 ± 0.02, 1.15 ± 0.02, 1.12 ± 0.01, and $1.17 \pm 0.01\%$ for IA0, IA5, IA10, IA15, and IA20, respectively. Although the IA20 group showed a slightly higher carbohydrate content, no significant difference was observed in IA10 and IA20 ($P > 0.05$). The ash contents were 9.89 ± 0.02, 9.89 ± 0.01, 9.97 ± 0.01, 10.06 ± 0.01, and $10.02 \pm 0.01\%$ for IA0, IA5, IA10, IA15, and IA20, respectively. The IA0 and IA5 groups recorded the lowest ash content ($P < 0.05$), whereas the IA15 group exhibited a significantly higher ash content ($P < 0.05$) than the control and other dietary groups.

Table 19. Proximate analysis of *Anabas testudineus* (% dry weight basis) fed varying levels of *Ipomoea aquatica* supplemented diet for 60 days (n=3).

Parameters	**IA0**	**IA5**	**IA10**	**IA15**	**IA20**	***P* value**
Moisture	15.42 ± 0.02^{a}	15.15 ± 0.02^{b}	14.80 ± 0.01^{c}	14.23 ± 0.01^{e}	14.57 ± 0.02^{d}	< 0.001
Protein	65.24 ± 0.01^{e}	65.35 ± 0.02^{d}	65.56 ± 0.02^{c}	65.92 ± 0.02^{a}	65.67 ± 0.02^{b}	< 0.001
Lipid	8.22 ± 0.02^{d}	8.35 ± 0.02^{c}	8.42 ± 0.01^{c}	8.56 ± 0.01^{a}	8.46 ± 0.01^{b}	< 0.001
Ash	9.89 ± 0.02^{d}	9.89 ± 0.01^{d}	9.97 ± 0.01^{c}	10.06 ± 0.01^{a}	10.02 ± 0.01^{b}	< 0.001
Fibre	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.737
Carbohydrate	1.13 ± 0.01^{bc}	1.15 ± 0.02^{ab}	1.15 ± 0.02^{ab}	1.12 ± 0.01^{bc}	1.17 ± 0.01^{a}	0.003

Note. Superscript letters indicate significant differences in a shared row (n = 3, $P < 0.05$). IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*.

4.4.3. Digestive enzyme activity

The digestive enzyme activity of *A. testudineus* fed with varying percentages of *I. aquatica*-incorporated diets for 60 days is presented in Figure 11 (a-f). Significantly higher ($P < 0.05$) amylase, trypsin, chymotrypsin, pepsin, total protease, and lipase activity were observed in *I. aquatica* supplemented fed diet fish compared to the control. Amylase activity was 2.16 ± 0.01, 2.35 ± 0.01, 2.49 ± 0.01, 2.61 ± 0.01, and 2.53 ± 0.02 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. An increasing trend was observed, with the highest amylase activity recorded in the IA15 group, representing a 1.21-fold increase compared to the control ($P < 0.05$). However, IA20 recorded a slight decrease compared to IA15. Trypsin activity followed a similar trend, with values of 17.72 ± 0.77, 19.14 ± 0.42, 23.48 ± 0.30, 26.16 ± 0.66, and 24.20 ± 0.60 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. The highest trypsin activity was observed in the IA15 group ($P < 0.05$), which showed a 1.48-fold increase compared to the control ($P < 0.05$), while a slight decline was noted in the IA20 group, where trypsin activity decreased to 1.37-fold compared to the control.

Chymotrypsin activity also increased with the rising levels of *I. aquatica* in the diet, recording 121.69 ± 6.80, 157.76 ± 9.05, 187.41 ± 9.08, 241.46 ± 11.54, and 223.60 ± 17.40 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. The IA15 group showed the highest chymotrypsin activity ($P < 0.05$), representing a 1.98-fold increase compared to the control, while the IA20 group recorded a 1.84-fold increase. Notably, there was no significant difference ($P > 0.05$) between IA15 and IA20. Lipase activity increased consistently across all *I. aquatica*-supplemented groups compared to the control (IA0, 33.35 ± 0.27 U mg^{-1}), with values of 35.11 ± 0.18, 37.15 ± 0.05, 39.44 ± 0.05, and 35.80 ± 0.04 U mg^{-1} for IA5, IA10, IA15, and IA20, respectively. The IA15 group showed the highest lipase activity ($P < 0.05$), representing a 1.18-fold increase over the control, while the IA20 group showed a slight decline compared to the IA15 group. Pepsin activity was recorded as 1060.95 ± 8.46, 1165.65 ± 3.29, 1213.42 ± 1.29, 1233.95 ± 4.18, and 1218.71 ± 2.22 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. The IA15 group recorded the

highest ($P < 0.05$) pepsin activity, with a 1.16-fold increase over the control, while a slight decrease was noted in the IA20 group. Total protease activity increased significantly ($P < 0.05$) in all *I. aquatica*-supplemented diet groups compared to the control (IA0, 1.44 ± 0.01 U mg^{-1}), with values of 1.49 ± 0.02, 1.71 ± 0.01, 1.93 ± 0.02, and 1.79 ± 0.01 U mg^{-1} for IA5, IA10, IA15, and IA20, respectively. The IA15 group exhibited the highest ($P < 0.05$) total protease activity, with a 1.34-fold increase over the control, while the IA20 group showed a slight decrease compared to IA15.

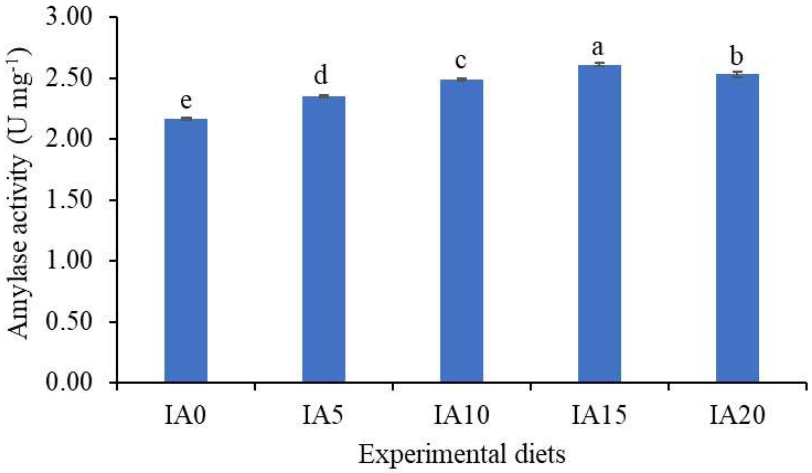

Figure 11 (a).

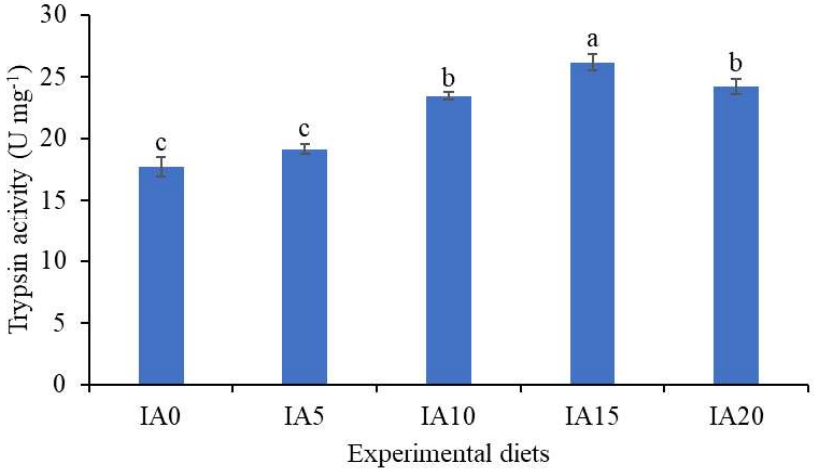

Figure 11 (b).

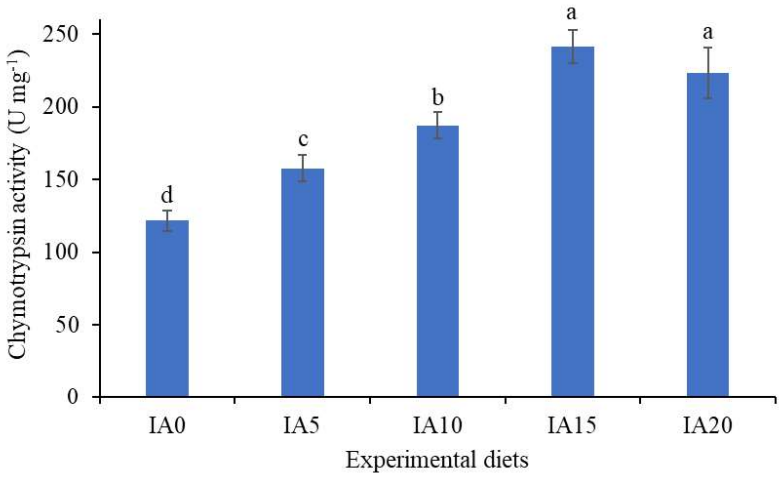

Figure 11 (c).

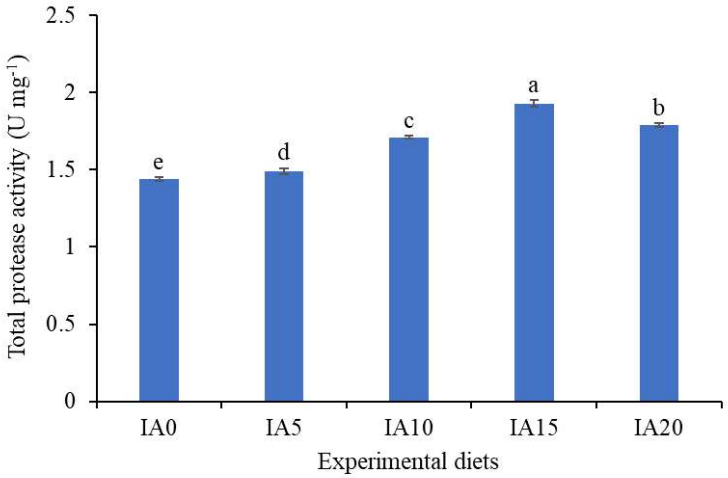

Figure 11 (d).

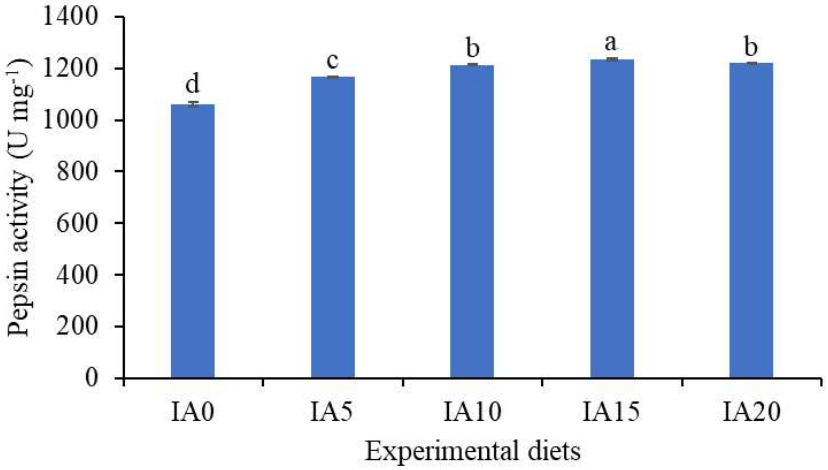

Figure 11 (e).

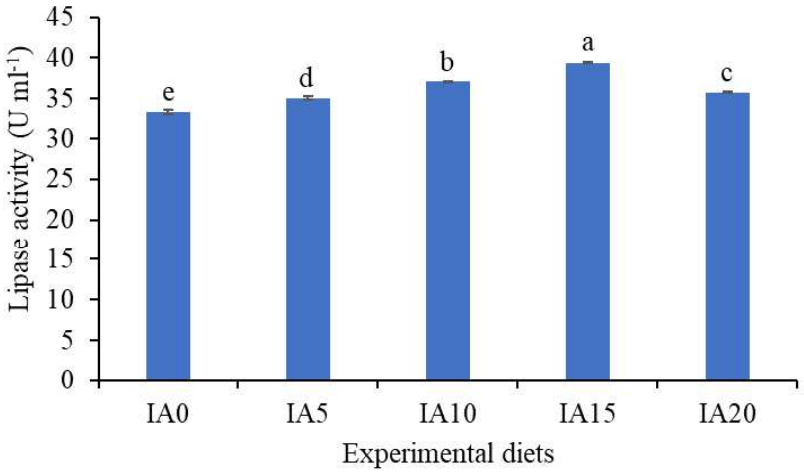

Figure 11 (f).

Figure 11. Digestive enzyme (a) Amylase, (b) Trypsin, (c) Chymotrypsin, (d) Total protease, (e) Pepsin, and (f) Lipase activity of *Anabas testudineus* fed with varying levels of *Ipomoea aquatica* incorporated diet for 60 days. Different letters indicate statistically significant variations (n = 3, $P < 0.05$). IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*.

4.4.4. Amino acid composition

The amino acid composition of *A. testudineus* muscle tissue after 60 days of feeding with diets containing increasing percentages of *I. aquatica* is presented in Table 20. Among the EAAs, arginine content was highest in the IA10 group (43.59 ± 0.09 mg g^{-1}), significantly surpassing all other groups ($P < 0.05$), while the lowest level was observed in the control group (IA0, 27.06 ± 0.06 mg g^{-1}). Histidine content increased with the inclusion of *I. aquatica*, peaking in the IA10 group (12.49 ± 0.47 mg g^{-1}), significantly higher than the other groups. The lowest histidine levels were found in the IA0 and IA5 groups (8.48 ± 0.03 and 8.63 ± 0.71 mg g^{-1}, respectively). Lysine content was notably higher ($P < 0.05$) in the IA10 group (38.43 ± 0.23 mg g^{-1}), representing the maximum among all treatments, followed by the IA15 group (32.81 ± 0.12 mg g^{-1}). In comparison, the control group (IA0) exhibited the lowest ($P < 0.05$) lysine level (23.06 ± 0.06 mg g^{-1}). Leucine levels were highest ($P < 0.05$) in the IA5 group (77.59 ± 1.55 mg g^{-1}), followed by the IA10 group (71.86 ± 1.17 mg g^{-1}). The lowest leucine level was recorded in the control group (58.08 ± 0.07 mg g^{-1}).

Methionine content was significantly higher ($P < 0.05$) in the IA15 group (38.18 ± 0.07 mg g^{-1}), followed by IA10 (33.07 ± 0.18 mg g^{-1}), while the lowest content was observed in the IA0 group (28.28 ± 0.23 mg g^{-1}). Phenylalanine content was greatest in the IA15 group (68.25 ± 0.42 mg g^{-1}), followed by IA10 (58.27 ± 0.35 mg g^{-1}). The lowest content was recorded in the IA20 group (49.64 ± 0.00 mg g^{-1}). Threonine was significantly higher ($P < 0.05$) in the IA15 group (22.98 ± 0.22 mg g^{-1}), whereas the lowest levels were observed in the IA0 group (15.08 ± 0.08 mg g^{-1}). Tryptophan content reached its highest levels in both the IA15 (51.72 ± 0.21 mg g^{-1}) and IA10 (51.04 ± 0.28 mg g^{-1}) groups, with no significant difference between these two groups ($P > 0.05$). These levels were significantly higher than those of all other groups, while the IA0 and IA20 exhibited the lowest tryptophan content at 42.01 ± 0.49 mg g^{-1} and 41.30 ± 0.17 mg g^{-1}, respectively. Valine content was significantly higher ($P < 0.05$) in the IA15 group (19.59 ± 0.33 mg g^{-1}), with the lowest content found in the control group (IA0, 16.81 ± 0.31 mg g^{-1}). The total EAA content was

272.93 ± 0.33, 311.14 ± 1.48, 348.41 ± 1.92, 350.61 ± 0.54, and 287.84 ± 1.49 mg g^{-1} for the IA0, IA5, IA10, IA15, and IA20 groups, respectively. The highest total EAA content was observed in the IA10 and IA15 groups, with no significant difference ($P > 0.05$) between these two groups. Both levels were significantly greater ($P < 0.05$) than the control (IA0), reflecting a clear upward trend with increasing *I. aquatica* inclusion up to 15%. However, at 20% inclusion (IA20), the total EAA content decreased, although it remained above the control level.

Among the NEAAs, Cysteine content showed no significant difference ($P > 0.05$) across all groups, remaining constant at 0.09 ± 0.01 mg g^{-1}. Alanine content was significantly higher ($P < 0.05$) in the IA15 group (91.68 ± 0.04 mg g^{-1}), while the LM20 group exhibited the lowest alanine content (64.50 ± 0.01 mg g^{-1}). Aspartic acid content was significantly higher ($P < 0.05$) in the IA10 group (34.22 ± 0.43 mg g^{-1}). In contrast, the lowest content was recorded in the IA0 (25.40 ± 0.20 mg g^{-1}) and IA5 (24.80 ± 0.61 mg g^{-1}) groups, with no significant difference ($P > 0.05$) between them. Glycine content was highest in the IA15 group (1.80 ± 0.16 mg g^{-1}, $P < 0.05$). In contrast, the lowest levels were recorded in the IA0 (1.04 ± 0.01 mg g^{-1}), IA5 (1.04 ± 0.07 mg g^{-1}), and IA20 (0.96 ± 0.01 mg g^{-1}) groups, with no significant difference ($P > 0.05$) among these groups.

Tyrosine content was significantly higher ($P < 0.05$) in the IA5 group (23.44 ± 0.02 mg g^{-1}) and lowest in the IA20 group (16.75 ± 0.03 mg g^{-1}). Glutamic acid content was significantly higher ($P < 0.05$) in the IA20 group (63.26 ± 0.14 mg g^{-1}), followed by the IA10 group (55.54 ± 1.21 mg g^{-1}). In contrast, the lowest levels were observed in the control group (IA0, 46.23 ± 0.23 mg g^{-1}) and IA5 (45.53 ± 0.25 mg g^{-1}), with no significant difference ($P < 0.05$) between these two groups. Proline content was significantly higher in the IA15 group (77.52 ± 0.52 mg g^{-1}, $P < 0.05$), surpassing all other groups. In contrast, the lowest proline level was recorded in the control group IA0 (60.09 ± 0.04 mg g^{-1}) and IA20 (58.03 ± 1.11 mg g^{-1}) with no significant difference between them ($P > 0.05$). Serine content was significantly higher ($P < 0.05$) in the IA20 group

(53.90 ± 0.19 mg g^{-1}), while it was lowest ($P < 0.05$) in the IA5 (31.25 ± 0.22 mg g^{-1}). Citrulline content remained consistent across all groups at 0.01 ± 0.00 mg g^{-1}, with no significant differences ($P > 0.05$) observed. Asparagine content was significantly higher ($P < 0.05$) in the IA5 group (0.28 ± 0.01 mg g^{-1}), with the lowest recorded in the control group (0.20 ± 0.00 mg g^{-1}) and IA20 (0.19 ± 0.00 mg g^{-1}). Beta 3-4 dihydroxy phenylalanine was consistent across the groups with no significant difference. The total NEAA content was 257.78 ± 0.50, 266.05 ± 0.04, 295.10 ± 1.65, 319.01 ± 0.83, and 289.16 ± 1.32 mg g^{-1} for the IA0, IA5, IA10, IA15, and IA20 groups, respectively. The highest total NEAA content was recorded in the IA15 group, while the lowest was observed in the IA0 group. The total amino acid content was 530.71 ± 0.84, 577.19 ± 1.44, 643.51 ± 3.57, 669.62 ± 1.37, and 577 ± 2.80 mg g^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. The control group (IA0) recorded the lowest ($P < 0.05$) total amino acid content, while diets incorporating *I. aquatica* (IA5-IA20) resulted in higher total amino acid content compared to the control. The highest total amino acid content was observed in the IA15 group, significantly higher than all other groups ($P < 0.05$).

4.4.5. Fatty acid composition

The fatty acid composition of *A. testudineus* fed with varying levels of *I. aquatica* over 60 days is presented in Table 21. Among the SFAs, C14:0 decreased as *I. aquatica* inclusion increased, dropping from 1.43 ± 0.03% in IA0 to 1.32 ± 0.01% in IA20. C15:0 followed a similar trend, reducing from 1.30 ± 0.00% in IA0 to 1.24 ± 0.02% in IA10 before slightly rising at higher *I. aquatica* levels. C13:0 was significantly higher in the control group, IA0 (0.54 ± 0.01%), IA5 (0.52 ± 0.01%), and IA15 (0.52 ± 0.01%), with no significant ($P > 0.05$) difference between these groups. C16:0 was the most abundant SFA, with the highest levels recorded in IA0 (30.13 ± 0.02%), IA5 (30.08 ± 0.03%), and IA20 (30.13 ± 0.03%), showing no significant ($P > 0.05$) differences among these groups. Similarly, C17:0 was highest in IA0 (2.53 ± 0.02%) and IA5 (2.52 ± 0.01%) with no significant difference between them, and it was found lowest in

IA10 (2.45 ± 0.03%) and IA15 (2.44 ± 0.02%), with no significant difference between these two groups. Stearic acid (C18:0) decreased gradually from 12.22 ± 0.01% in IA0 to 11.85 ± 0.01% in IA20. Meanwhile, C20:0 levels did not show a significant difference between IA0 (1.31 ± 0.01), IA5 (1.29 ± 0.01%), IA10 (1.23 ± 0.01%) and IA15 (1.31 ± 0.01%), whereas, lowest was found in IA20 (1.20 ± 0.02%). The total SFA content was 49.44 ± 0.01, 49.16 ± 0.03, 48.87 ± 0.04, 48.59 ± 0.01, and 48.67 ± 0.01% for IA0, IA5, IA10, IA15, and IA20, respectively. The total SFA content decreased significantly ($P < 0.05$) with increasing levels of *I. aquatica* inclusion, with the highest content observed in IA0 and the lowest in IA15.

In terms of MUFAs, the content of C16:1n-5 remained relatively stable across all treatments ($P > 0.05$). C18:1n-9 level showed the highest content in IA0 (14.66 ± 0.01%), IA5 (14.56 ± 0.04%) and IA20 (14.54 ± 0.00%). C18:1n-9 level showed a significant decline from 14.66 ± 0.01% in IA0 to 14.42 ± 0.09% in IA10. Similarly, C20:1n-9 decreased from 2.52 ± 0.01% in IA0 to 2.43 ± 0.01% in IA15, followed by a slight increase to 2.47 ± 0.01% in IA20. The C15:1n-5 was significantly higher in IA0 (2.81 ± 0.01%) and IA20 (2.78 ± 0.01%) groups. The MUFA content was 28.46 ± 0.06, 28.17 ± 0.05, 28.02 ± 0.15, 28.03 ± 0.07, and 28.24 ± 0.02% for IA0, IA5, IA10, IA15, and IA20, respectively. The MUFA content was significantly highest in IA0 and IA20, with no significant difference ($P > 0.05$) between these two groups. This was followed by IA5, IA10, and IA15, which also showed no significant differences ($P > 0.05$) among each other.

The C20:5n-3 content increased significantly from 3.10 ± 0.01% in IA0 to 3.34 ± 0.00% in IA15. IA20 recorded a 3.31 ± 0.03% value, with no significant difference ($P > 0.05$) from IA15. C22:6n-3 showed significantly higher ($P < 0.05$) in IA10 (4.25 ± 0.04%), IA15 (4.30 ± 0.03%) and IA20 (4.22 ± 0.02%). C18:3n-3 content increased significantly with higher *I. aquatica* levels, rising from 8.65 ± 0.01% in IA0 to a maximum of 8.92 ± 0.00% in IA15. The level decreased slightly in IA20 (8.81 ± 0.03%) but remained significantly higher than in IA0 ($P < 0.05$). C18:2n-6 content also increased, with the highest

levels recorded in IA15 (2.28 ± 0.00%) and IA20 (2.30 ± 0.04%), significantly greater than IA0 (2.12 ± 0.01%) ($P < 0.05$). The content of C18:3n-6 increased significantly from 2.01 ± 0.02% in IA0 to 2.24 ± 0.03% in IA10 and 2.25 ± 0.02% in IA15, with no significant difference ($P > 0.05$) between IA10 and IA15. Although IA20 slightly decreased to 2.18 ± 0.01%, it remained significantly higher than IA0 ($P < 0.05$).

Among PUFA, EPA and DHA were significantly higher in the IA15 group. EPA increased from 3.10 ± 0.01% in the control group to 3.34 ± 0.00% in IA15, while DHA increased from 4.00 ± 0.01% in the control group to 4.30 ± 0.03% in IA15 ($P < 0.05$). The total PUFA content was highest in the IA15 group (23.39 ± 0.05%) and lowest in the control group (22.10 ± 0.01%). The highest C20:4n-6 was recorded in IA5 (2.69 0.01), whereas no significant difference ($P > 0.05$) was found in IA10 (2.30 ± 0.01%), IA15 (2.31 ± 0.01%) and IA20 (2.30 ± 0.01%). The PUFA content was 22.10 ± 0.01, 22.67 ± 0.15, 23.11 ± 0.11, 23.39 ± 0.05, and 23.10 ± 0.03% for IA0, IA5, IA10, IA15, and IA20, respectively. PUFA levels increased steadily with higher inclusion levels of *I. aquatica*, reaching a peak in IA15. Although a slight decrease was observed in IA20, the PUFA content remained significantly higher than in the control (IA0).

The PUFA to SFA ratio increased progressively with higher levels of *I. aquatica*, reaching its highest value in IA15 (0.48 ± 0.01%) and IA20 (0.47 ± 0.00%) with no significant difference between them ($P > 0.05$). In contrast, the lowest ratio was observed in IA0 (0.45 ± 0.00%). The ω6 to ω3 ratio remained stable across all treatments ($P > 0.05$). The combined content of EPA and DHA increased significantly from 7.10 ± 0.01% in IA0 to 7.64 ± 0.04% in IA15. However, there was no significant difference ($P > 0.05$) among IA10 (7.51 ± 0.05%), IA15 (7.64 ± 0.04%), and IA20 (7.53 ± 0.01%).

4.4.6. Biochemical parameters

The biochemical parameters of *A. testudineus* fed different inclusion levels of *I. aquatica* over 60 days are presented in Table 22. LYZ activity levels were

64.37 ± 0.78, 67.55 ± 0.49, 71.21 ± 1.85, 70.19 ± 3.29, and 71.85 ± 1.96 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. The IA10, IA15, and IA20 groups demonstrated significantly higher LYZ activity compared to the control ($P < 0.05$). TIg levels were measured as 0.62 ± 0.02, 0.70 ± 0.01, 0.81 ± 0.01, 0.99 ± 0.02, and 0.85 ± 0.02 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. Fish-fed diets supplemented with *I. aquatica* (IA5–IA20) exhibited higher TIg levels than the control (IA0), with the IA15 group showing the highest level, which was significantly greater ($P < 0.05$) than all other groups. ALP activity was recorded as 1.27 ± 0.02, 1.29 ± 0.02, 1.38 ± 0.09, 1.45 ± 0.02, and 1.41 ± 0.07 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively, with the IA15 group showing the highest ALP activity, which was significantly greater than the control ($P < 0.05$). However, IA10, IA15 and IA20 did not differ significantly ($P < 0.05$).

AST and ALT activities showed consistency across all groups ($P > 0.05$). AST levels were recorded as 1.26 ± 0.03, 1.25 ± 0.02, 1.23 ± 0.02, 1.24 ± 0.02, and 1.23 ± 0.02 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. ALT levels were 1.14 ± 0.04, 1.11 ± 0.02, 1.10 ± 0.04, 1.12 ± 0.04, and 1.13 ± 0.02 U mg^{-1}, respectively. CAT activity levels were measured as 5.89 ± 0.01, 7.22 ± 0.03, 7.30 ± 0.87, 7.84 ± 0.66, and 7.50 ± 0.91 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. Fish-fed *I. aquatica*-supplemented diets (IA5–IA20) showed significantly higher CAT activity than the control group ($P < 0.05$). However, there were no significant differences ($P < 0.05$) in CAT activity among the supplemented groups (IA5, IA10, IA15, and IA20). SOD activity levels were recorded as 245.81 ± 36.54, 262.91 ± 29.56, 267.83 ± 7.49, 280.66 ± 5.39, and 275.04 ± 8.79 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively, with no significant differences observed among the groups ($P > 0.05$). Similarly, TBARS levels were 2.68 ± 0.04, 2.72 ± 0.13, 2.63 ± 0.05, 2.69 ± 0.05, and 2.67 ± 0.06 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively, also showing no significant differences across the dietary treatments ($P > 0.05$).

Table 20. Amino acid composition of *Anabas testudineus* fed diets incorporating increasing percentages of *Ipomoea aquatica*.

Amino acids (mg g^{-1})	**IA0**	**IA5**	**IA10**	**IA15**	**IA20**	***P* value**
EAA						
Arginine	27.06 ± 0.06^{e}	28.08 ± 0.04^{d}	43.59 ± 0.09^{a}	39.57 ± 0.08^{b}	28.64 ± 0.12^{c}	< 0.001
Histidine	8.48 ± 0.03^{d}	8.63 ± 0.71^{d}	12.49 ± 0.47^{a}	10.49 ± 0.06^{b}	9.11 ± 0.49^{c}	< 0.001
Lysine	23.06 ± 0.06^{e}	23.84 ± 0.20^{d}	38.43 ± 0.23^{a}	32.81 ± 0.12^{b}	30.85 ± 0.04^{c}	< 0.001
Leucine	58.08 ± 0.07^{d}	77.59 ± 1.55^{a}	71.86 ± 1.17^{b}	67.03 ± 0.80^{c}	60.29 ± 0.96^{e}	< 0.001
Methionine	28.28 ± 0.23^{d}	33.06 ± 0.19^{b}	33.07 ± 0.18^{b}	38.18 ± 0.07^{a}	29.19 ± 0.06^{c}	< 0.001
Phenylalanine	54.09 ± 0.04^{c}	57.95 ± 0.15^{b}	58.27 ± 0.35^{b}	68.25 ± 0.42^{a}	49.64 ± 0.00^{d}	< 0.001
Threonine	15.08 ± 0.08^{e}	16.24 ± 0.16^{d}	20.67 ± 0.12^{b}	22.98 ± 0.22^{a}	19.91 ± 0.08^{c}	< 0.001
Tryptophan	42.01 ± 0.49^{c}	46.81 ± 0.29^{b}	51.04 ± 0.28^{a}	51.72 ± 0.21^{a}	41.30 ± 0.17^{c}	< 0.001
Valine	16.81 ± 0.31^{c}	18.93 ± 0.21^{b}	18.99 ± 0.27^{b}	19.59 ± 0.33^{a}	18.90 ± 0.08^{b}	< 0.001
Total EAA	272.93 ± 0.33^{d}	311.14 ± 1.48^{b}	348.41 ± 1.92^{a}	350.61 ± 0.54^{a}	287.84 ± 1.49^{c}	< 0.001
NEAA						
Alanine	69.78 ± 0.22^{d}	70.83 ± 0.10^{c}	86.84 ± 0.10^{b}	91.68 ± 0.04^{a}	64.50 ± 0.01^{e}	< 0.001
Aspartic acid	25.40 ± 0.20^{c}	24.80 ± 0.61^{c}	34.22 ± 0.43^{a}	32.05 ± 0.07^{b}	31.45 ± 0.12^{b}	< 0.001
Cysteine	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.124
Glycine	1.04 ± 0.01^{c}	1.04 ± 0.07^{c}	1.39 ± 0.05^{b}	1.80 ± 0.16^{a}	0.96 ± 0.01^{c}	< 0.001
Glutamic acid	46.23 ± 0.23^{d}	45.53 ± 0.25^{d}	55.54 ± 1.21^{b}	52.01 ± 0.22^{c}	63.26 ± 0.14^{a}	< 0.001

Proline	60.09 ± 0.04^{c}	68.79 ± 0.82^{b}	57.13 ± 1.06^{d}	77.52 ± 0.52^{a}	58.03 ± 1.11^{cd}	< 0.001
Serine	35.36 ± 0.21^{d}	31.25 ± 0.22^{e}	39.74 ± 0.12^{c}	46.06 ± 1.03^{b}	53.90 ± 0.19^{a}	< 0.001
Tyrosine	19.55 ± 0.05^{c}	23.44 ± 0.02^{a}	19.91 ± 0.13^{b}	17.54 ± 0.05^{d}	16.75 ± 0.03^{e}	< 0.001
Citrulline	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	
Asparagine	0.20 ± 0.00^{d}	0.28 ± 0.01^{a}	0.23 ± 0.01^{c}	0.25 ± 0.01^{b}	0.19 ± 0.00^{d}	< 0.001
Beta 3-4 dihydroxy phenylalanine	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.210
Total NEAA	257.78 ± 0.50^{e}	266.05 ± 0.04^{d}	295.10 ± 1.65^{b}	319.01 ± 0.83^{a}	289.16 ± 1.32^{c}	< 0.001
Total amino acids	530.71 ± 0.84^{d}	577.19 ± 1.44^{c}	643.51 ± 3.57^{b}	669.62 ± 1.37^{a}	577 ± 2.80^{c}	< 0.001

Notes: Superscript letters indicate significant differences in a shared row (n = 3, $P < 0.05$). EAA: Essential Amino Acids, NEAA: Non-Essential Amino Acids. IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*.

Table 21. Fatty acid composition (% of total fatty acid) in *Anabas testudineus* fed with diet containing varying levels of *Ipomoea aquatica* incorporated diet for 60 days.

Fatty acid	**IA0**	**IA5**	**IA10**	**IA15**	**IA20**	***P* value**
C13:0	0.54 ± 0.01^{a}	0.52 ± 0.01^{ab}	0.49 ± 0.02^{b}	0.52 ± 0.01^{ab}	0.45 ± 0.02^{c}	< 0.001
C14:0	1.43 ± 0.03^{a}	1.35 ± 0.01^{bc}	1.37 ± 0.01^{b}	1.33 ± 0.01^{bc}	1.32 ± 0.01^{c}	< 0.001
C15:0	1.30 ± 0.00^{a}	1.27 ± 0.01^{ab}	1.24 ± 0.02^{c}	1.25 ± 0.01^{c}	1.26 ± 0.01^{bc}	< 0.001
C16:0	30.13 ± 0.02^{a}	30.08 ± 0.03^{a}	29.91 ± 0.04^{b}	29.76 ± 0.02^{c}	30.13 ± 0.03^{a}	< 0.001
C17:0	2.53 ± 0.02^{a}	2.52 ± 0.01^{ab}	2.45 ± 0.03^{c}	2.44 ± 0.02^{c}	2.48 ± 0.01^{b}	< 0.001
C18:0	12.22 ± 0.01^{a}	12.14 ± 0.04^{b}	12.13 ± 0.01^{b}	11.99 ± 0.12^{c}	11.85 ± 0.01^{d}	< 0.001
C20:0	1.31 ± 0.01^{a}	1.29 ± 0.01^{a}	1.23 ± 0.01^{a}	1.31 ± 0.01^{a}	1.20 ± 0.02^{b}	< 0.001
Σ SFA	49.44 ± 0.01^{a}	49.16 ± 0.03^{b}	48.87 ± 0.04^{c}	48.59 ± 0.01^{e}	48.67 ± 0.01^{d}	0.009
C15:1n-5	2.81 ± 0.01^{a}	2.72 ± 0.03^{bc}	2.73 ± 0.02^{bc}	2.70 ± 0.02^{dc}	2.78 ± 0.01^{ab}	< 0.001
C16:1n-5	8.48 ± 0.03	8.45 ± 0.03	8.43 ± 0.02	8.44 ± 0.02	8.45 ± 0.02	0.132
C18:1n-9	14.66 ± 0.01^{a}	14.56 ± 0.04^{ab}	14.42 ± 0.09^{c}	14.46 ± 0.01^{bc}	14.54 ± 0.00^{abc}	< 0.001
C20:1n-9	2.52 ± 0.01^{a}	2.45 ± 0.02^{bc}	2.45 ± 0.01^{bc}	2.43 ± 0.01^{c}	2.47 ± 0.01^{b}	< 0.001

Σ MUFA	28.46 ± 0.06^{a}	28.17 ± 0.05^{b}	28.02 ± 0.15^{b}	28.03 ± 0.07^{b}	28.24±0.02^{ab}	< 0.001
C20:5n-3	3.10 ± 0.01^{d}	3.23 ± 0.05^{c}	3.26 ± 0.01^{bc}	3.34 ± 0.00^{a}	3.31 ± 0.03^{ab}	< 0.001
C22:6n-3	4.00 ± 0.01^{c}	4.14 ± 0.04^{b}	4.25 ± 0.04^{a}	4.30 ± 0.03^{a}	4.22 ± 0.02^{ab}	< 0.001
C18:3n-3	8.65 ± 0.01^{d}	8.77 ± 0.04^{c}	8.86 ± 0.03^{b}	8.92 ± 0.00^{a}	8.81 ± 0.03^{bc}	< 0.001
C18:2n-6	2.12 ± 0.01^{d}	2.14 ± 0.02^{bc}	2.22 ± 0.01^{b}	2.28 ± 0.00^{a}	2.30 ± 0.04^{a}	< 0.001
C18:3n-6	2.01 ± 0.02^{d}	2.11 ± 0.02^{c}	2.24 ± 0.03^{a}	2.25 ± 0.02^{a}	2.18 ± 0.01^{b}	< 0.001
C20:4n-6	2.23 ± 0.02^{c}	2.69 ± 0.01^{a}	2.30 ± 0.01^{b}	2.31 ± 0.01^{b}	2.30 ± 0.01^{b}	< 0.001
Σ PUFA	22.10 ± 0.01^{d}	22.67 ± 0.15^{c}	23.11 ± 0.11^{b}	23.39 ± 0.05^{a}	23.10 ± 0.03^{b}	< 0.001
PUFA/SFA	0.45 ± 0.00^{d}	0.46 ± 0.00^{c}	0.47 ± 0.00^{b}	0.48 ± 0.01^{a}	0.47 ± 0.00^{ab}	< 0.001
ω6/ω3	0.40 ± 0.02	0.41 ± 0.01	0.41 ± 0.01	0.41 ± 0.02	0.41 ± 0.01	0.156
EPA+DHA	7.10 ± 0.01^{c}	7.37 ± 0.09^{b}	7.51 ± 0.05^{a}	7.64 ± 0.04^{a}	7.53 ± 0.01^{a}	< 0.001

Notes: Superscript letters indicate significant differences in a shared row (n = 3, $P < 0.05$). SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid. IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*.

Table 22. Biochemical parameters of *Anabas testudineus* fed varying levels of *Ipomoea aquatica*-incorporated diet for 60 days.

Parameters	**IA0**	**IA5**	**IA10**	**IA15**	**IA20**	***P* value**
TIg (mg mL^{-1})	0.62 ± 0.02^{e}	0.70 ± 0.01^{d}	0.81 ± 0.01^{c}	0.99 ± 0.02^{a}	0.85 ± 0.02^{b}	< 0.001
LYZ (U mg^{-1})	64.37 ± 0.78^{b}	67.55 ± 0.49^{ab}	71.21 ± 1.85^{a}	70.19 ± 3.29^{a}	71.85 ± 1.96^{a}	0.005
ALP (U mg^{-1})	1.27 ± 0.02^{b}	1.29 ± 0.02^{b}	1.38 ± 0.09^{ab}	1.45 ± 0.02^{a}	1.41 ± 0.07^{ab}	0.009
AST (U mg^{-1})	1.26 ± 0.03	1.25 ± 0.02	1.23 ± 0.02	1.24 ± 0.02	1.23 ± 0.02	0.117
ALT (U mg^{-1})	1.14 ± 0.04	1.11 ± 0.02	1.10 ± 0.04	1.12 ± 0.04	1.13 ± 0.02	0.240
CAT (U mg-1)	5.89 ± 0.01^{b}	7.22 ± 0.03^{a}	7.30 ± 0.87^{a}	7.84 ± 0.66^{a}	7.50 ± 0.91^{a}	< 0.001
SOD (U mg^{-1})	245.81 ± 36.54	262.91 ± 29.56	267.83 ± 7.49	280.66 ± 5.39	275.04 ± 8.79	0.395
TBARS (U mg^{-1})	2.68 ± 0.04	2.72 ± 0.13	2.63 ± 0.05	2.69 ± 0.05	2.67 ± 0.06	0.184

Notes: Values are represented as mean values ± SD. Means within the same column having different superscripts are significantly different ($P < 0.05$). T Ig: Total Immunoglobulin, ALP: Alkaline Phosphatase, LYZ: Lysozyme, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, CAT: Catalase, SOD: Superoxide dismutase, TBARS: Thiobarbituric acid reactive substances. IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*.

4.5. Evaluating the growth, digestive physiology and biochemical parameters of *Heteropneustes fossilis* fed with *Ipomoea aquatica* supplemented diet

4.5.1. Growth performance

Table 23 presents the growth performance of *Heteropneustes fossilis* (initial weight: 0.65 ± 0.01 g) fed varying percentages of *Ipomoea aquatica* diets over 60 days. The FW were 2.19 ± 0.00, 2.33 ± 0.02, 2.58 ± 0.01, 2.48 ± 0.01, and 2.37 ± 0.00 g for IA0, IA5, IA10, IA15, and IA20, respectively. An increase in FW was observed with increasing *I. aquatica* supplementation, peaking in IA10, representing a 1.18-fold increase over the control and significantly surpassing all other groups ($P < 0.05$). The lowest FW was recorded in the control group (IA0). The IA15 and IA20 groups showed slight declines compared to IA10 but maintained a 1.13-fold and 1.08-fold increase over the control, respectively. BWG percentages were 238.77 ± 2.62, 258.46 ± 4.35, 299.25 ± 5.47, 281.54 ± 2.18, and 267.46 ± 4.03% for IA0, IA5, IA10, IA15, and IA20, respectively. Higher BWG was observed in the *I. aquatica*-supplemented fed diet. The IA10 group recorded the highest BWG, corresponding to a 1.25-fold increase over the control, significantly exceeding all other groups ($P < 0.05$). The IA15 and IA20 groups experienced slight declines compared to IA10 but maintained increases of 1.18-fold and 1.12-fold relative to the control, respectively.

FCR were 1.50 ± 0.01, 1.42 ± 0.01, 1.30 ± 0.01, 1.34 ± 0.01, and 1.40 ± 0.01 for IA0, IA5, IA10, IA15, and IA20, respectively. Lower FCR was recorded in the *I. aquatica* incorporated diet-fed fish. The IA10 group recorded the lowest FCR, representing a 1.15-fold improvement over the control, significantly better than all other groups ($P < 0.05$). SGR were 2.03 ± 0.01, 2.13 ± 0.02, 2.31 ± 0.02, 2.23 ± 0.01, and 2.17 ± 0.02% day^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. An upward trend in SGR was observed with increasing levels of *I. aquatica* up to 10%, with the IA10 group showing a 1.14-fold increase compared to the control ($P < 0.05$). In contrast, the SGR of the IA20 group showed a slight decline, representing a 1.07-fold increase over the control.

Survival rates remained 100% across all dietary treatments (IA0–IA20), suggesting that varying percentages of *I. aquatica* did not negatively impact fish survival.

FE percentages were 66.76 ± 0.60, 70.20 ± 0.18, 77.20 ± 0.40, 74.39 ± 0.41, and 71.46 ± 0.63% for IA0, IA5, IA10, IA15, and IA20, respectively. Higher FE was recorded in the *I. aquatica*-supplemented fed diet compared to the control. The IA10 group exhibited a 1.16-fold increase in FE compared to the control, making it significantly higher than all other groups ($P < 0.05$). The IA15 and IA20 groups showed slight declines in FE, with increases of 1.11-fold and 1.07-fold relative to the control, respectively. PER were 1.67 ± 0.01, 1.75 ± 0.00, 1.93 ± 0.01, 1.86 ± 0.01, and 1.79 ± 0.02 for IA0, IA5, IA10, IA15, and IA20, respectively. The PER increased with the increase in *I. aquatica* levels by 10%, followed by slight declines in the IA15 and IA20 groups. The PER in the IA10 group was significantly higher ($P < 0.05$) than in the control and other groups, achieving a 1.16-fold increase compared to the control.

A polynomial regression analysis of the FCR and SGR data revealed that the optimal growth of *H. fossilis* was achieved when *I. aquatica* inclusion in the diet ranged from 11.73% to 11.97%, as illustrated in Figure 12 (a-b). This supplementation level provided the ideal balance between optimising growth rates and enhancing feed utilization efficiency. Among the diets tested, IA10, which included 10% *I. aquatica*, emerged as the most effective. It yielded the highest values for FW, BWG, SGR, FE, and PER while also achieving the lowest FCR, highlighting its efficiency in promoting better growth performance.

Table 23. The nutritional efficiency and growth performance of *Heteropneustes fossilis* fed with varying percentages of *Ipomoea aquatica* diets. All data are represented as mean ± sd.

Parameters	**IA0**	**IA5**	**IA10**	**IA15**	**IA20**	***P* value**
IW (g)	0.65 ± 0.01	0.65 ± 0.00	0.65 ± 0.01	0.65 ± 0.00	0.65 ± 0.01	0.737
FW (g)	2.19 ± 0.00^{e}	2.33 ± 0.02^{d}	2.58 ± 0.01^{a}	2.48 ± 0.01^{b}	2.37 ± 0.00^{c}	< 0.001
BWG (%)	238.77 ± 2.62^{e}	258.46 ± 4.35^{d}	299.25 ± 5.47^{a}	281.54 ± 2.18^{b}	267.46 ± 4.03^{c}	< 0.001
SGR (% day^{-1})	2.03 ± 0.01^{e}	2.13 ± 0.02^{d}	2.31 ± 0.02^{a}	2.23 ± 0.01^{b}	2.17 ± 0.02^{c}	< 0.001
FCR	1.50 ± 0.01^{a}	1.42 ± 0.01^{b}	1.30 ± 0.01^{e}	1.34 ± 0.01^{d}	1.40 ± 0.01^{c}	< 0.001
Survival (%)	100	100	100	100	100	
FE (%)	66.76 ± 0.60^{e}	70.20 ± 0.18^{d}	77.20 ± 0.40^{a}	74.39 ± 0.41^{b}	71.46 ± 0.63^{c}	< 0.001
PER	1.67 ± 0.01^{e}	1.75 ± 0.00^{d}	1.93 ± 0.01^{a}	1.86 ± 0.01^{b}	1.79 ± 0.02^{c}	< 0.001

Note: Superscript letters denote statistically significant differences within a common row (n = 30, $P < 0.05$). IW: Initial weight, FW: Final weight, BWG: Body weight gain, SGR: Specific growth rate, FCR: Feed conversion ratio, FE: Feed efficiency, PER: Protein efficiency ratio. IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*.

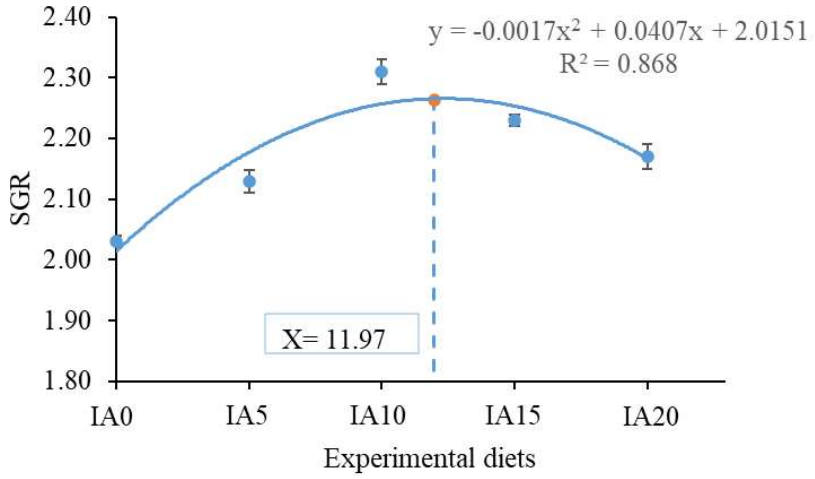

Figure 12 (a).

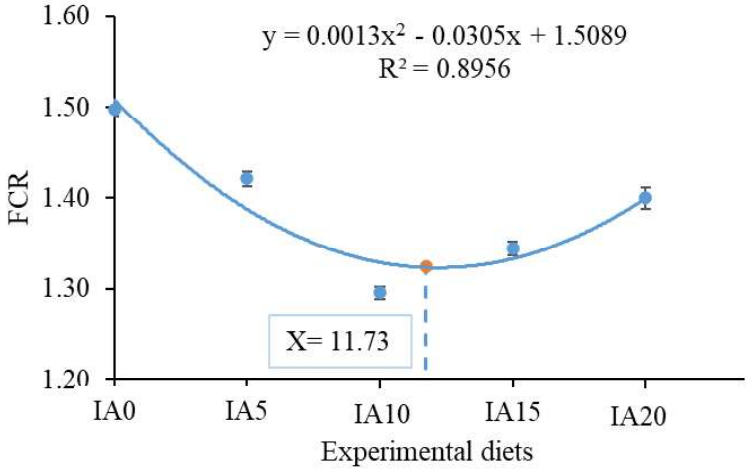

Figure 12 (b).

Figure 12. Polynomial regression analysis based on (a) SGR and (b) FCR of *Heteropneustes fossilis* fed with different % inclusion of *Ipomoea aquatica* in the diet. IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*.

4.5.2. Proximate composition

The proximate composition analysis of *H. fossilis*-fed diets with varying levels of *I. aquatica* over 60 days revealed significant differences across several nutritional parameters (Table 24). The moisture contents were 18.10 ± 0.02, 17.75 ± 0.03, 17.48 ± 0.02, 17.60 ± 0.03, and 18.00 ± 0.04% for IA0, IA5, IA10, IA15, and IA20 groups, respectively. Fish-fed diets containing *I. aquatica* (IA5–IA20) had lower moisture content than the control group. Notably, the IA10 group recorded the lowest moisture content, significantly lower ($P < 0.05$) than all other groups. The lipid contents were 7.15 ± 0.05, 7.23 ± 0.02, 7.32 ± 0.05, 7.28 ± 0.06, and 7.20 ± 0.01% for IA0, IA5, IA10, IA15, and IA20, respectively. The lowest lipid content was recorded in the control group (IA0) and IA20, while the IA10 group achieved the highest lipid content, which was significantly greater ($P < 0.05$) than all other groups.

Correspondingly, the protein contents were 62.51 ± 0.04, 62.67 ± 0.04, 62.74 ± 0.06, 62.70 ± 0.04, and 62.40 ± 0.07% for IA0, IA5, IA10, IA15, and IA20, respectively. The lowest protein content was recorded in the control group (IA0), while the IA10 group exhibited a significantly higher protein content ($P < 0.05$) than the control and other dietary groups. The ash contents were 10.84 ± 0.01, 10.93 ± 0.02, 11.01 ± 0.01, 10.96 ± 0.03, and 10.92 ± 0.02% for IA0, IA5, IA10, IA15, and IA20, respectively. The control group recorded the lowest ash content, while the IA10 group exhibited a significantly higher ash content ($P < 0.05$) than the control and other dietary groups. The carbohydrate contents were 1.31 ± 0.01, 1.32 ± 0.01, 1.34 ± 0.01, 1.34 ± 0.02, and 1.36 ± 0.01% for IA0, IA5, IA10, IA15, and IA20, respectively. While differences in carbohydrate content were minimal, they were statistically significant ($P < 0.05$). Fibre content remained consistent across all groups (IA0, IA5, IA10, IA15, and IA20) at 0.09 ± 0.01, 0.10 ± 0.01, 0.11 ± 0.01, 0.12 ± 0.01, and 0.12 ± 0.01%, respectively, showing no significant differences ($P > 0.05$).

Table 24. Proximate analysis of *Heteropnustes fossilis* (% dry weight basis) fed varying levels of *Ipomoea aquatica* incorporated diet for 60 days.

Parameters	**IA0**	**IA5**	**IA10**	**IA15**	**IA20**	***P* value**
Moisture	18.10 ± 0.02^{a}	17.75 ± 0.03^{c}	17.48 ± 0.02^{e}	17.60 ± 0.03^{d}	18.00 ± 0.04^{b}	< 0.001
Protein	62.51 ± 0.04^{e}	62.67 ± 0.04^{c}	62.74 ± 0.06^{a}	62.70 ± 0.04^{b}	62.40 ± 0.07^{d}	< 0.001
Lipid	7.15 ± 0.05^{d}	7.23 ± 0.02^{c}	7.32 ± 0.05^{a}	7.28 ± 0.06^{b}	7.20 ± 0.01^{d}	< 0.001
Ash	10.84 ± 0.01^{c}	10.93 ± 0.02^{b}	11.01 ± 0.01^{a}	10.96 ± 0.03^{b}	10.92 ± 0.02^{b}	< 0.001
Fibre	0.09 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.231
Carbohydrate	1.31 ± 0.01^{d}	1.32 ± 0.01^{c}	1.34 ± 0.01^{b}	1.34 ± 0.02^{b}	1.36 ± 0.01^{a}	< 0.001

Note. Superscript letters indicate significant differences in a shared row (n = 3, $P < 0.05$). IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica.*

4.5.3. Digestive enzyme activity

The digestive enzyme activities of *H. fossilis* fed diets containing varying concentrations of *I. aquatica* over 60 days are illustrated in Figure 13 (a-f). Trypsin activity was 20.19 ± 0.80 U mg^{-1} in the control, with progressively higher levels in the experimental groups, 22.19 ± 0.64, 29.01 ± 0.61, 25.22 ± 0.38, and 24.49 ± 0.87 U mg^{-1} for IA5, IA10, IA15, and IA20, respectively. The highest trypsin activity was seen in IA10, showing a 1.44-fold increase compared to the control ($P < 0.05$), followed by a slight decrease in IA15 and IA20. Amylase activity in the control group (IA0) was 1.86 ± 0.02 U mg^{-1}, while fish fed the *I. aquatica*-enriched diets demonstrated increased amylase activity, with values of 1.99 ± 0.10, 2.28 ± 0.02, 2.19 ± 0.03, and 2.11 ± 0.01 U mg^{-1} for IA5, IA10, IA15, and IA20, respectively. An upward trend was observed across the diet groups, peaking in IA10 (1.23-fold increase) before slightly declining in IA15 and IA20. Chymotrypsin activity increased significantly ($P < 0.05$) with *I. aquatica* supplementation, from 50.80 ± 0.83 U mg^{-1} in the control to 63.93 ± 0.66, 67.36 ± 1.04, 64.62 ± 1.67, and 60.31 ± 1.14 U mg^{-1} for IA5, IA10, IA15, and IA20, respectively. The peak chymotrypsin activity occurred in the IA10 group, showing a 1.33-fold increase over the control. A slight decline was noted in IA15 and IA20, but these groups maintained significantly higher activity than the control ($P < 0.05$).

Total protease activity was also significantly higher in the experimental groups, starting from 0.82 ± 0.01 U mg^{-1} in the control and rising to 0.96 ± 0.02, 1.24 ± 0.02, 1.13 ± 0.02, and 1.04 ± 0.01 U mg^{-1} for IA5, IA10, IA15, and IA20, respectively. The highest activity ($P < 0.05$) was recorded in the IA10 group, reflecting a 1.51-fold increase compared to the control. Pepsin activity consistently rose across the *I. aquatica*-fed groups compared to the control, which had a pepsin activity of 751.90 ± 12.16 U mg^{-1}. Pepsin activities for IA5, IA10, IA15, and IA20 were 778.75 ± 11.29, 860.08 ± 14.76, 828.67 ± 10.34, and 816.66 ± 13.07 U mg^{-1}, respectively. The highest pepsin activity was observed in the IA10 group, reflecting a 1.14-fold increase over the control. There was no significant difference ($P > 0.05$) in pepsin activity between the IA15 and IA20

groups. Lipase activity followed an upward trend, beginning at 43.91 ± 0.38 U mL^{-1} in the control group and increasing to 45.14 ± 0.86, 47.54 ± 0.41, 46.66 ± 1.04, and 45.45 ± 0.27 U mL^{-1} for IA5, IA10, IA15, and IA20, respectively. The IA10 and IA15 groups exhibited the highest lipase activity, with no significant difference ($P > 0.05$). A slight decline in lipase activity was noted in the IA20 group.

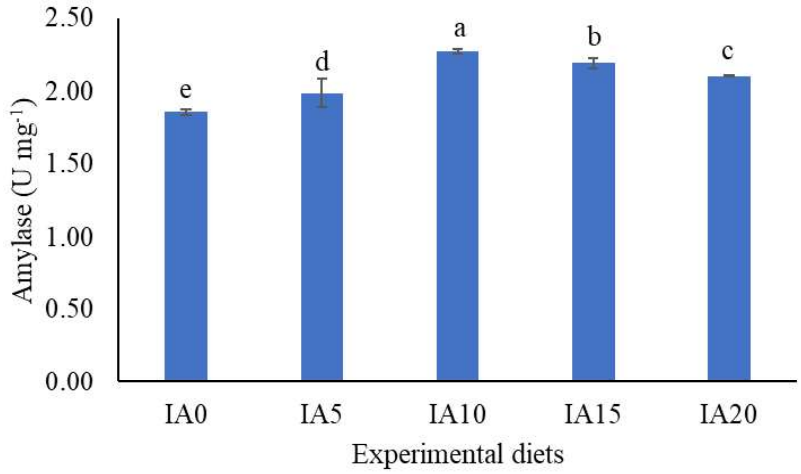

Figure 13 (a).

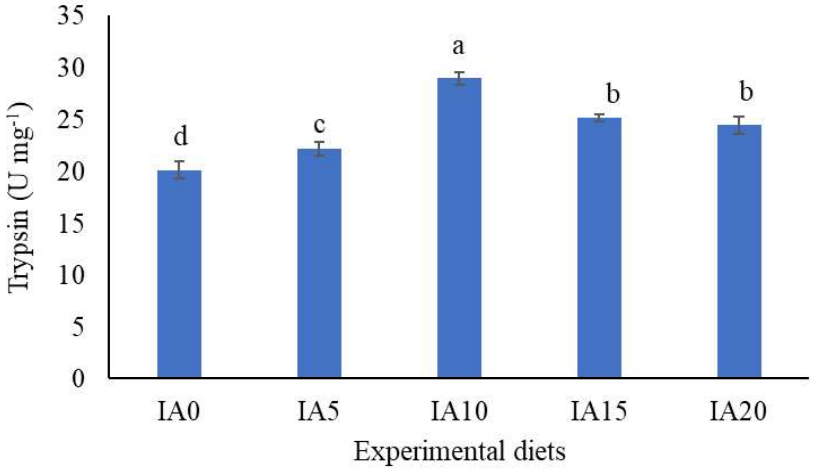

Figure 13 (b).

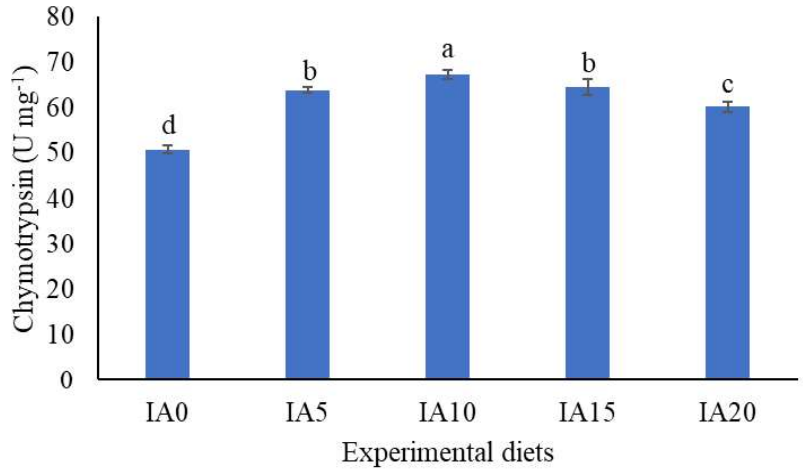

Figure 13 (c).

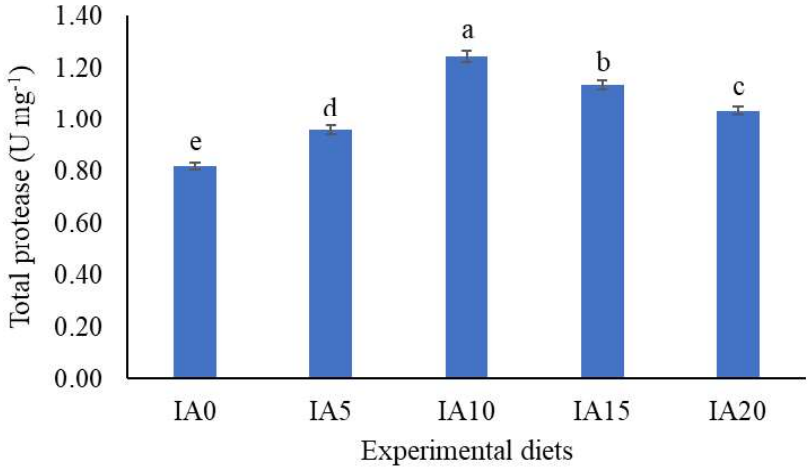

Figure 13 (d).

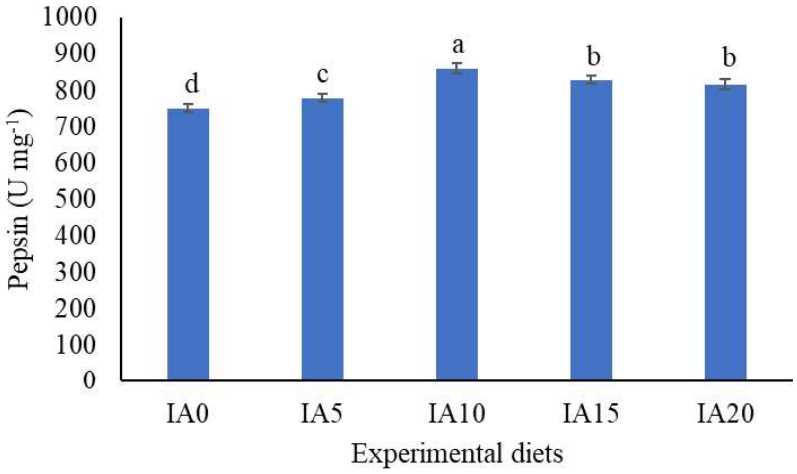

Figure 13 (e).

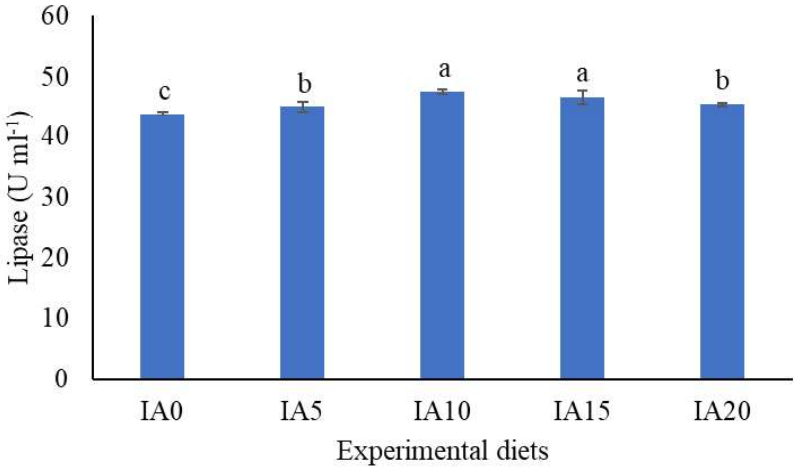

Figure 13 (f).

Figure 13. Digestive enzyme (a) Amylase, (b) Trypsin, (c) Chymotrypsin, (d) Total protease, (e) Pepsin, and (f) Lipase activity of *Heteropneustes fossilis* fed with varying levels of *Ipomoea aquatica* incorporated diet for 60 days. Different letters indicate statistically significant variations ($n = 3$, $P < 0.05$). IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*.

4.5.4. Amino acid composition

The amino acid composition of *H. fossilis* muscle tissue after 60 days of feeding with diets containing increasing percentages of *I. aquatica* is presented in Table 25. Among the EAAs, the IA5 and IA10 groups exhibited the highest arginine concentrations, 54.55 ± 0.21 and 53.89 ± 0.90 mg g^{-1}, respectively, surpassing other groups significantly, while the lowest concentration was found in the IA20 group (48.10 ± 0.17 mg g^{-1}). Histidine levels were elevated in the IA0 and IA5 groups (35.91 ± 0.59 and 36.75 ± 0.47 mg g^{-1}, respectively), with the lowest value in IA15 (30.40 ± 0.53 mg g^{-1}) and IA20 (29.37 ± 0.15 mg g^{-1}, $P < 0.001$). Lysine was notably high in the IA10 group (73.45 ± 0.05 mg g^{-1}), while the IA0 group showed the lowest concentration (44.73 ± 0.19 mg g^{-1}, $P < 0.001$). Leucine was most abundant in the IA10 group (51.15 ± 0.03 mg g^{-1}), and the lowest concentration was recorded in IA5 (41.68 ± 0.23 mg g^{-1}). Methionine content peaked in IA10 (66.10 ± 0.24 mg g^{-1}) and was lowest in the IA5 group (34.45 ± 0.38 mg g^{-1}). The highest phenylalanine level was observed in IA15 (24.15 ± 0.52 mg g^{-1}), significantly ($P < 0.05$) higher than in IA0 (19.31 ± 0.04 mg g^{-1}). Threonine levels declined with higher inclusion levels, highest in IA0 (26.71 ± 0.10 mg g^{-1}) and IA5 (26.57 ± 0.05 mg g^{-1}) and lowest in IA15 (17.25 ± 0.06 mg g^{-1}). Tryptophan peaked in IA15 (5.02 ± 0.02 mg g^{-1}), while IA20 recorded the lowest value (0.34 ± 0.02 mg g^{-1}). Valine was significantly higher in IA5 (48.71 ± 0.64 mg g^{-1}) and IA10 (48.34 ± 0.53 mg g^{-1}), with the lowest value in IA20 (40.10 ± 0.02 mg g^{-1}). The total EAA content ranged from 316.77 ± 0.01 mg g^{-1} in IA0 to 372.62 ± 1.72 mg g^{-1} in IA10, with the highest content observed in IA10 ($P < 0.05$).

Glycine was highest in IA10 (3.14 ± 0.11 mg g^{-1}) and lowest in IA15 and IA20 (1.40 ± 0.01 mg g^{-1}). Serine levels were relatively stable across groups. However, IA20 (61.70 ± 0.61%) recorded significantly lowest ($P < 0.05$). Alanine peaked in IA10 (59.77 ± 0.25 mg g^{-1}), and the lowest content was observed in IA5 (49.10 ± 0.44 mg g^{-1}). Aspartic acid was highest in IA10 (55.50 ± 0.50 mg g^{-1}), with the lowest value observed in IA0 (23.44 ± 0.03 mg g^{-1}). Cysteine content remained relatively stable across groups, with minor variations.

Glutamic acid was most abundant in IA0 (48.42 ± 0.08 mg g^{-1}) and IA10 (47.52 ± 0.26 mg g^{-1}), whereas IA5 recorded a lower value (39.42 ± 0.09 mg g^{-1}). Proline was elevated in IA5 (78.70 ± 1.88 mg g^{-1}), with a decline observed in IA20 (65.37 ± 0.32 mg g^{-1}). Tyrosine concentration was highest in IA5 (28.72 ± 0.07 mg g^{-1}) and lowest in IA0 (15.86 ± 0.17 mg g^{-1}). The total NEAA content increased with *I. aquatica* inclusion, peaking in IA10 (347.41 ± 0.67 mg g^{-1}), which was significantly ($P < 0.05$) more than all other groups. The total amino acid content was highest ($P < 0.05$) in the IA10 group (718.06 ± 2.01 mg g^{-1}) and lowest in the IA0 group (621.70 ± 0.59 mg g^{-1}). Significant increases in total amino acids were observed with increasing levels of *I. aquatica* up to the 10% inclusion, while the IA20 group exhibited a slight decrease relative to IA10.

4.5.5. Fatty acid composition

Table 26 presents the fatty acid composition of *H. fossilis* fed with varying levels of *I. aquatica*-incorporated diets over 60 days. Among the SFAs, C14:0 showed a decreasing trend with increasing *I. aquatica* levels, reaching its lowest in IA20 (2.22 ± 0.02%). C13:0 was significantly higher in the IA5 group (1.78 ± 0.01%) than other treatments, with the lowest level observed in IA20 (1.55 ± 0.12%). The most abundant SFA, C17:0, increased consistently across the diets, peaking in IA20 (15.91 ± 0.02%). C18:0 and C20:0 displayed varying trends. C18:0 reached its highest levels in both IA15 (0.15 ± 0.01%) and IA20 (0.16 ± 0.01%), with no significant difference ($P > 0.05$) between the two. Similarly, C20:0 peaked in IA0 (1.07 ± 0.01%) and IA20 (1.08 ± 0.01%), showing comparable values at these inclusion levels. C27:0 and C34:0 demonstrated a decreasing trend, with their highest levels observed in IA0, at 2.75 ± 0.02% and 2.01 ± 0.01%, respectively. Total SFA content was 25.74 ± 0.03%, 25.42 ± 0.05%, 25.10 ± 0.06%, 25.32 ± 0.07%, and 25.47 ± 0.05% for IA0, IA5, IA10, IA15, and IA20, respectively. The highest SFA content was observed in IA0, with a general decrease across diets, except for a slight rise in IA20.

Among the MUFAs, C18:1n-9 decreased from 1.50 ± 0.02% in IA0 to 1.44 ± 0.01% in IA15. C16:1n-5 significantly declined from 12.30 ± 0.01% in

IA0 to 12.10 ± 0.03% in IA20. C16:1n-7 followed a similar trend, dropping from 3.35 ± 0.01% in IA0 to 3.26 ± 0.01% in IA10. C18:1n-16 also decreased, with the highest value in IA0 (14.23 ± 0.02%) and the lowest in IA15 (14.04 ± 0.02%). C20:1n-9 showed a decline from 3.86 ± 0.01% in IA0 to 3.75 ± 0.02% in IA10, while C18:1n-5 decreased slightly from 13.58 ± 0.03% in IA0 to 13.50 ± 0.02% in IA10. Total MUFA content was 48.82 ± 0.08, 48.41 ± 0.05, 48.23 ± 0.08, 48.19 ± 0.12, and 48.44 ± 0.10% for IA0, IA5, IA10, IA15, and IA20, respectively. MUFA content was highest in IA0 and showed a consistent decline across diets, with a slight increase in IA20.

Among the PUFAs, C18:3n-3 increased significantly ($P < 0.05$) with *I. aquatica* incorporation, peaking in IA5 (1.39 ± 0.01%) and IA10 (1.38 ± 0.01%). C20:5n-3 (EPA) also increased consistently, with the highest level in IA10 (2.65 ± 0.02%) and IA15 (2.64 ± 0.01). C20:3n-3 peaked in IA10 (2.23 ± 0.01%) as well. C22:6n-3 (DHA) reached its maximum value in IA10 (1.62 ± 0.02%). C20:4n-6 rose to its highest level in IA10 and IA15 (4.14 ± 0.02% and 4.12 ± 0.02%, respectively). C18:2n-6 also showed an increasing trend, peaking in IA10 (8.51 ± 0.02%) and IA15 (8.48 ± 0.02%). Total PUFA content was 25.44 ± 0.05, 26.17 ± 0.10, 26.67 ± 0.09, 26.49 ± 0.06, and 26.09 ± 0.04% for IA0, IA5, IA10, IA15, and IA20, respectively. PUFA content was lowest in IA0 and increased steadily across diets, peaking in IA10 before a slight decline in IA15 and IA20. The PUFA to SFA ratio was highest in IA10 (1.06 ± 0.00), significantly greater than in the control (0.97 ± 0.00). The ω6 to ω3 ratio showed slight but significant variations across dietary treatments, with the highest value in IA0 (2.46 ± 0.02) and the lowest in IA10 (2.38 ± 0.01). The combined EPA and DHA content was highest in the IA10 group (4.27 ± 0.04%) and IA15 (4.24 ± 0.03%), significantly exceeding that of the control (3.93 ± 0.01%).

4.5.6. Biochemical parameters

The biochemical parameters of *H. fossilis* fed varying percentages of *I. aquatica*-incorporated diets over 60 days are presented in Table 27. ALP activity levels were recorded as 1.30 ± 0.02, 1.33 ± 0.02, 1.32 ± 0.11, 1.31 ± 0.01, and 1.31 ±

0.02 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. There were no significant differences in ALP activity among the dietary groups ($P > 0.05$), indicating stable ALP levels across diets. The TIg levels were recorded as 0.26 ± 0.01, 0.28 ± 0.01, 0.35 ± 0.01, 0.31 ± 0.01, and 0.30 ± 0.01 mg mL^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. Fish-fed diets incorporating *I. aquatica* (IA5–IA20) showed higher TIg levels than the control group (IA0). Notably, the IA10 group achieved the highest TIg level (0.35 ± 0.01 mg mL^{-1}), which was significantly greater ($P < 0.05$) than the control and all other dietary groups. LYZ activity levels were measured as 54.09 ± 2.64, 54.38 ± 0.60, 53.07 ± 2.82, 53.98 ± 3.11, and 54.40 ± 1.85 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. No significant differences were observed among the groups ($P > 0.05$), suggesting that LYZ activity was not influenced by *I. aquatica* supplementation.

AST levels were measured as 2.70 ± 0.05, 2.68 ± 0.01, 2.71 ± 0.52, 2.74 ± 0.06, and 2.72 ± 0.08 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. ALT levels were 2.62 ± 0.18, 2.56 ± 0.15, 2.58 ± 0.05, 2.52 ± 0.06, and 2.26 ± 0.08 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. No significant differences were found in AST ($P = 0.120$) and ALT ($P = 0.210$) activities among the groups, indicating no adverse effects on liver function from dietary treatments. CAT activity was recorded as 1.92 ± 0.18, 2.14 ± 0.20, 2.05 ± 0.17, 2.12 ± 0.04, and 1.22 ± 0.09 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. Although variations were noted in CAT activity across the treatments, no significant differences were observed ($P > 0.05$). SOD levels were measured as 201.25 ± 11.02, 205.18 ± 12.11, 203.45 ± 10.01, 202.12 ± 12.01, and 206.04 ± 8.01 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. The dietary groups observed no significant differences ($P = 0.243$) in SOD. TBARS levels were recorded as 2.89 ± 0.03, 2.75 ± 0.04, 2.80 ± 0.07, 2.91 ± 0.04, and 2.84 ± 0.06 U mg^{-1} for IA0, IA5, IA10, IA15, and IA20, respectively. Statistical analysis indicated no significant differences ($P = 0.185$).

Table 25. Amino acid composition of *Heteropneustes fossilis* fed diets incorporating increasing percentages of *Ipomoea aquatica* for 60 days.

Amino acids (mg g^{-1})	**IA0**	**IA5**	**IA10**	**IA15**	**IA20**	***P* value**
EAA						
Arginine	53.09 ± 0.13^{b}	54.55 ± 0.21^{a}	53.89 ± 0.90^{ab}	50.22 ± 0.20^{c}	48.10 ± 0.17^{d}	< 0.001
Histidine	35.91 ± 0.59^{a}	36.75 ± 0.47^{a}	32.49 ± 0.38^{b}	30.40 ± 0.53^{c}	29.37 ± 0.15^{c}	< 0.001
Lysine	44.73 ± 0.19^{e}	65.84 ± 0.10^{d}	73.45 ± 0.05^{a}	70.15 ± 0.05^{b}	67.63 ± 0.32^{c}	< 0.001
Leucine	43.45 ± 0.36^{c}	41.68 ± 0.23^{d}	51.15 ± 0.03^{a}	47.73 ± 0.27^{b}	47.27 ± 0.67^{b}	< 0.001
Methionine	44.57 ± 0.15^{c}	34.45 ± 0.38^{d}	66.10 ± 0.24^{a}	48.00 ± 0.30^{b}	45.20 ± 0.35^{c}	< 0.001
Phenylalanine	19.31 ± 0.04^{c}	17.06 ± 0.06^{d}	21.81 ± 0.19^{b}	24.15 ± 0.52^{a}	22.42 ± 0.07^{b}	< 0.001
Threonine	26.71 ± 0.10^{a}	26.57 ± 0.05^{a}	21.90 ± 0.02^{b}	17.25 ± 0.06^{d}	18.71 ± 0.22^{c}	< 0.001
Tryptophan	3.61 ± 0.01^{b}	2.38 ± 0.00^{d}	3.49 ± 0.01^{c}	5.02 ± 0.02^{a}	0.34 ± 0.02^{e}	< 0.001
Valine	45.39 ± 0.28^{b}	48.71 ± 0.64^{a}	48.34 ± 0.53^{a}	41.77 ± 0.54^{c}	40.10 ± 0.02^{d}	< 0.001
Total EAA	316.77 ± 0.01^{d}	327.98 ± 1.19^{c}	372.62 ± 1.72^{a}	334.69 ± 0.24^{b}	319.13 ± 0.64^{d}	< 0.001
NEAA						
Alanine	56.47 ± 0.62^{b}	49.10 ± 0.44^{e}	59.77 ± 0.25^{a}	55.40 ± 0.06^{c}	53.30 ± 0.26^{d}	< 0.001
Aspartic acid	23.44 ± 0.03^{d}	51.79 ± 1.06^{b}	55.50 ± 0.50^{a}	51.77 ± 0.68^{b}	48.17 ± 0.29^{c}	< 0.001
Cysteine	0.14 ± 0.02^{ab}	0.12 ± 0.01^{b}	0.18 ± 0.01^{ab}	0.15 ± 0.01^{ab}	0.15 ± 0.01^{ab}	0.016
Glycine	2.11 ± 0.03^{c}	2.25 ± 0.01^{b}	3.14 ± 0.11^{a}	1.40 ± 0.01^{d}	1.40 ± 0.01^{d}	< 0.001
Glutamic acid	48.42 ± 0.08^{a}	39.42 ± 0.09^{d}	47.52 ± 0.26^{a}	44.57 ± 0.67^{b}	40.50 ± 0.50^{c}	< 0.001

Proline	72.57 ± 0.17^{bc}	78.70 ± 1.88^{a}	70.33 ± 0.58^{c}	73.67 ± 0.58^{b}	65.37 ± 0.32^{d}	< 0.001
Serine	65.18 ± 0.75^{a}	65.92 ± 0.88^{a}	66.32 ± 0.20^{a}	65.27 ± 0.23^{a}	61.70 ± 0.61^{b}	< 0.001
Tyrosine	15.86 ± 0.17^{d}	28.72 ± 0.07^{a}	15.52 ± 0.11^{d}	26.12 ± 0.29^{b}	24.25 ± 0.23^{c}	< 0.001
Citrulline	0.01 ± 0.00^{ab}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.001
Asparagine	20.72 ± 0.06^{b}	20.46 ± 0.16^{b}	27.14 ± 0.25^{a}	19.70 ± 0.26^{c}	19.84 ± 0.06^{c}	< 0.001
Beta 3-4 dihydroxy phenylalanine	0.02 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{b}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.029
Total NEAA	304.93 ± 0.60^{d}	336.52 ± 2.92^{b}	347.41 ± 0.67^{a}	338.07 ± 0.97^{b}	314.72 ± 1.76^{c}	< 0.001
Total amino acids	621.70 ± 0.59^{e}	664.50 ± 1.79^{c}	718.06 ± 2.01^{a}	72.76 ± 0.80^{b}	633.85 ± 2.27^{d}	< 0.001

Note. Superscript letters indicate significant differences in a shared row (n = 3, $P < 0.05$). EAA: Essential amino acids, NEAA: Non-essential Amino acids. IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*.

Table 26. Fatty acid composition (% of total fatty acid) in *Heteropneustes fossilis* fed varying levels of *Ipomoea aquatica* incorporated diet for 60 days.

Fatty acid	**IA0**	**IA5**	**IA10**	**IA15**	**IA20**	***P* value**
C13:0	1.69 ± 0.02^{b}	1.78 ± 0.01^{a}	1.72 ± 0.01^{b}	1.77 ± 0.01^{c}	1.55 ± 0.12^{d}	< 0.001
C14:0	2.37 ± 0.01^{a}	2.34 ± 0.01^{b}	2.30 ± 0.01^{c}	2.33 ± 0.01^{c}	2.22 ± 0.02^{d}	< 0.001
C17:0	15.75 ± 0.01^{c}	15.81 ± 0.01^{b}	15.78 ± 0.02^{c}	15.82 ± 0.03^{b}	15.91 ± 0.02^{a}	< 0.001
C18:0	0.10 ± 0.01^{c}	0.11 ± 0.00^{b}	0.12 ± 0.01^{b}	0.15 ± 0.01^{a}	0.16 ± 0.01^{a}	< 0.001
C20:0	1.07 ± 0.01^{a}	0.82 ± 0.01^{b}	0.71 ± 0.02^{d}	0.78 ± 0.02^{c}	1.08 ± 0.01^{a}	< 0.001
C27:0	2.75 ± 0.02^{a}	2.61 ± 0.01^{b}	2.50 ± 0.01^{c}	2.58 ± 0.02^{b}	2.64 ± 0.01^{b}	< 0.001
C34:0	2.01 ± 0.01^{a}	1.95 ± 0.01^{b}	1.97 ± 0.01^{b}	1.89 ± 0.01^{c}	1.91 ± 0.02^{c}	< 0.001
Σ SFA	25.74 ± 0.03^{a}	25.42 ± 0.05^{b}	25.10 ± 0.06^{c}	25.32 ± 0.07^{b}	25.47 ± 0.05^{b}	0.004
C16:1n-5	12.30 ± 0.01^{a}	12.12 ± 0.01^{b}	12.09 ± 0.01^{c}	12.10 ± 0.02^{c}	12.10 ± 0.03^{c}	< 0.001
C16:1n-7	3.35 ± 0.01^{a}	3.28 ± 0.01^{b}	3.26 ± 0.01^{c}	3.27 ± 0.02^{c}	3.29 ± 0.02^{c}	< 0.001
C18:1n-9	1.50 ± 0.02^{a}	1.48 ± 0.01^{b}	1.45 ± 0.01^{c}	1.44 ± 0.01^{d}	1.47 ± 0.02^{b}	< 0.001
C18:1n-16	14.23 ± 0.02^{a}	14.20 ± 0.02^{a}	14.18 ± 0.02^{b}	14.04 ± 0.02^{c}	14.18 ± 0.01^{b}	< 0.001
C20:1n-9	3.86 ± 0.01^{a}	3.80 ± 0.01^{b}	3.75 ± 0.02^{c}	3.82 ± 0.01^{b}	3.87 ± 0.01^{a}	< 0.001
C18:1n-5	13.58 ± 0.03^{a}	13.53 ± 0.01^{b}	13.50 ± 0.02^{b}	13.52 ± 0.02^{b}	13.50 ± 0.03^{b}	0.006
Σ MUFA	48.82 ± 0.08^{a}	48.41 ± 0.05^{b}	48.23 ± 0.08^{c}	48.19 ± 0.12^{d}	48.44 ± 0.10^{b}	< 0.001
C18:3n-3	1.32 ± 0.02^{b}	1.39 ± 0.01^{a}	1.38 ± 0.01^{a}	1.36 ± 0.02^{b}	1.35 ± 0.01^{b}	< 0.001
C20:5n-3	2.49 ± 0.01^{d}	2.56 ± 0.02^{c}	2.65 ± 0.02^{a}	2.64 ± 0.01^{a}	2.61 ± 0.02^{b}	< 0.001

C20:3n-3	2.10 ± 0.02^{d}	2.17 ± 0.02^{c}	2.23 ± 0.01^{a}	2.20 ± 0.03^{b}	2.19 ± 0.02^{b}	< 0.001
C22:6n-3	1.44 ± 0.02^{c}	1.51 ± 0.01^{b}	1.62 ± 0.02^{a}	1.60 ± 0.03^{a}	1.50 ± 0.03^{b}	< 0.001
C20:4n-6	3.93 ± 0.01^{c}	4.05 ± 0.02^{b}	4.14 ± 0.02^{a}	4.12 ± 0.02^{a}	3.98 ± 0.02^{c}	< 0.001
C20:2n-6	3.84 ± 0.01^{d}	3.96 ± 0.05^{c}	4.01 ± 0.03^{bc}	3.98 ± 0.04^{ab}	3.95 ± 0.03^{a}	< 0.001
C18:2n-6	8.31 ± 0.01^{c}	8.44 ± 0.02^{b}	8.51 ± 0.02^{a}	8.48 ± 0.02^{a}	8.43 ± 0.01^{b}	< 0.001
C22:4n-6	2.01 ± 0.01^{c}	2.09 ± 0.02^{b}	2.13 ± 0.01^{b}	2.11 ± 0.01^{a}	2.08 ± 0.01^{a}	< 0.001
Σ PUFA	25.44 ± 0.05^{d}	26.17 ± 0.10^{c}	26.67 ± 0.09^{a}	26.49 ± 0.06^{b}	26.09 ± 0.04^{c}	< 0.001
PUFA/SFA	0.97 ± 0.00^{c}	1.03 ± 0.00^{b}	1.06 ± 0.00^{a}	1.05 ± 0.01^{a}	1.02 ± 0.00^{b}	< 0.001
ω6/ω3	2.46 ± 0.02^{a}	2.41 ± 0.01^{b}	2.38 ± 0.01^{c}	2.40 ± 0.02^{b}	2.41 ± 0.02^{b}	< 0.001
EPA+DHA	3.93 ± 0.01^{c}	4.07 ± 0.01^{b}	4.27 ± 0.04^{a}	4.24 ± 0.03^{a}	4.11 ± 0.02^{b}	< 0.001

Notes: Superscript letters indicate significant differences in a shared row (n = 3, $P < 0.05$). SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid. IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*.

Table 27. Biochemical of *Heteropneustes fossilis* fed with different percentage inclusion of *Ipomoea aquatica* diets for 60 days.

Parameters	IA0	IA5	IA10	IA15	IA20	*P* value
TIg (mg mL^{-1})	0.26 ± 0.01^{c}	0.28 ± 0.01^{c}	0.35 ± 0.01^{a}	0.31 ± 0.01^{b}	0.30 ± 0.01^{b}	< 0.001
LYZ (U mg^{-1})	54.09 ± 2.64	54.38 ± 0.60	53.07 ± 2.82	53.98 ± 3.11	54.40 ± 1.85	0.143
ALP (U mg^{-1})	1.30 ± 0.02	1.33 ± 0.02	1.32 ± 0.11	1.31 ± 0.01	1.31 ± 0.02	0.138
AST (U mg^{-1})	2.70 ± 0.05	2.68 ± 0.01	2.71 ± 0.52	2.74 ± 0.06	2.72 ± 0.08	0.120
ALT (U mg^{-1})	2.62 ± 0.18	2.56 ± 0.15	2.58 ± 0.05	2.52 ± 0.06	2.260 ± 0.08	0.210
CAT (U mg^{-1})	1.92 ± 0.18	2.14 ± 0.20	2.05 ± 0.17	2.12 ± 0.04	1.22 ± 0.09	0.176
SOD (U mg^{-1})	201.25 ± 11.02	205.18 ± 12.11	203.45 ± 10.01	202.12 ± 12.01	206.04 ± 8.01	0.243
TBARS (U mg^{-1})	2.89 ± 0.03	2.75 ± 0.04	2.80 ± 0.07	2.91 ± 0.04	2.84 ± 0.06	0.185

Notes: Values are represented as mean values ± SD. Means within the same column having different superscripts are significantly different ($P < 0.05$). T Ig: Total Immunoglobulin, ALP: Alkaline Phosphatase, LYZ: Lysozyme, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, CAT: Catalase, SOD: Superoxide dismutase, TBARS: Thiobarbituric acid reactive substances. IA0: 0% *I. aquatica*, IA5: 5% *I. aquatica*, IA10: 10% *I. aquatica*, IA15: 15% *I. aquatica*, and IA20: 20% *I. aquatica*.