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Impact of Multiple Proteinacious Diet Formulations on Digestive Enzymes Activity of *Sperata Seenghala*

Muhammad Asif Abbas Tahir[1*], Muhammad Naeem[2]

[1*,2]Institute of Zoology, Bahauddin Zakariya University, Multan-60800, Pakistan

***Corresponding Author:** Muhammad Naeem
*Institute of Zoology, Bahauddin Zakariya University, Multan-60800, Pakistan

Abstract
In present study, maximum value of Relative gut mass (R.G.M.) was perceived in T_3 while least relative gut mass (RGM) was observed in T_4 (0.039±0.0005). Highest mean value of Relative gut length (RGL) was observed as 1.45±0.05 in T_3 and minimum (RGL) was determined as 1.22±0.03 in T_6. Least value of Zihler's index was observed in T_4 as 0.023±0.002 ($p>0.01$) while maximum Zihler's index as 0.08±0.01 in T_1 as a small fish exhibits large Zihler's index. The increasing trend of digestive somatic index (DSI) was observed as $T_3>T_2>T_6>T_1>T_5>T_4$. Amylase secretion of *Sperata seenghala* reported significant discrimination with different crude protein (CP) showing prominent variations. Higher most Amylase action was determined in T_1 (30% CP) having 0.50±0.09 (U/ml. Min^{-1}) ($p<0.001$) as its mean value with range of 0.40-0.58. However minimum amylase activity was witnessed in T_6 and T_5 as 0.35 ± 0.7 and 0.38 ± 0.85 correspondingly. With increased crude proteins action of protease reported positive relation with various feeding groups. Lowest crude protein percentage in diet (30% CP) depicted lower most protease actions (1.48 ± 0.22) while higher crude protein T_5 (50% CP) and fish meal (T_6) displayed greater concentration as 5.71±0.33 and 5.07±0.24 respectively. All experimental groups reported highest lipase activity than other enzymes.

Key Words: Relative gut mass, Relative gut length, *Sperate Seenghala*, Crude protein, protease, amylase, lipase, Zihler's index.

INTRODUCTION

Catfish culture has its widespread attributes across the world because of its rapid growth and enhanced commercial demands. Catfish species represent a significant and speedy developing assemblage of protein in aquaculture as compared to Tilapia and Carps (Phan *et al*., 2009; Lakra and Singh, 2010).

Sperata seenghala is predatory (Shammi and bhatnagar, 2002), carnivorous (Rehman, 2005; Babare *et al*., 2013) and omnivorous fish (Yeragi and Yeragi, 2014). *Sperata seenghala* feeds on significant amount of insects, larvae of insects, crustaceans, shrimps, prawns, molluscs, worms, rarely algae and on aquatic weeds (Arif, 2012). Seasonal variation in feeding habits with breeding has been observed, generally with poor feeding intensity in reproducing time whereas vigorous eating afterwards hatching (Arif, 2012). When body size of *Sperata seenghala* increases represent increased organic material in food as compared to vegetal stuff (Babare *et al*., 2013).

Variations in enzyme configurations are significant poisonousness directories and have been utilized to estimate the biochemical and physical health of important tissues of body in fish (Van der Oost *et al*., 2000; Gabriel and George, 2005). Therefore, it is crucial to study Digestive enzyme and their actions in various portions of intestine to understand mechanism of feed digestion and its adaptations to fluctuations in fish feeding surroundings (Romarheim *et al.,* 2007; Santigosa *et al.,* 2008). Digestive enzyme is a trustworthy apparatus to realize activities of digestion and fish dietary position (Johnston *et al.,* 2004). Proteolytic enzymes and Amylase actions can unveil proficiency of numerous species of fish to utilize protein and Carbohydrates (Hidalgo *et al.,* 1999). Lipases are also inducible enzymes (Aliyu-Paiko *et al.,* 2010) and can be inspired by the nutritional fat concentration (Li *et al.,* 2012; Buchet *et al.,* 2000).

The herbivore and omnivore fishes can digest starchy ingredients of plants more effectively than carnivorous fishes. Carbohydrate actions (Alpha amylase) are more developed in carnivorous fishes than herbivorous and omnivorous fishes (Fernandez *et al*., 2001). Numerous researchers have reported that alpha-amylase activity has disclosed high activity of amylase in omnivore and herbivore fish than carnivorous fishes (Drewe *et al*., 2004; Horn *et al*., 2006). Fishes cannot create cellulase enzyme and are not able to assimilate cellulose directly (Li *et al*., 2009).

The digestion process indicates requirement of nutrients for enzymatic activity and whole body functions. It is the elementary device to detect feeding suitability and its impact associated to fish growth and maintenance (Gisbert *et al.,* 2009). Chemical alterations in varying food components induced enzymatic exudations showed better feed utility (Caruso *et al.,* 2009). Activity of Digestive enzyme may alter in different species of fish because of variations in their digestive ability and nourishing habitats. The study of enzymatic function is useful for accepting procedure of fish digestion and fluctuations in surrounding vicinity (Sunde *et al.,* 2004).

Bano *et al.* (2023) studied that in Striped Catfish (*Pangasius hypophthalmus*) it was observed that by increasing dietary protein also raised protease and amylase activities however Lipase activity was reduced significantly ($P<0.05$).

MATERIALS AND METHODS

On the completion of experimental trail, five fish samples were randomly collected from each group and their bellies were excised to evaluate activity of digestive enzymes after weighing and measuring. Entire gut was detached and sweep away by child Tris HCl Buffer in ice tray. All these fish illustrations were measured, weighed then enclosed in aluminium foil to froze at 1 ℃ to withdraw enzymes safely. For withdrawal of enzymes, preserved fish illustrations were uniformed in child tris HCl buffer in Homogenizer. Then homogenized matter was Centrifuged at 15000 cycles/minute for 30 Minutes in Ultracentrifuge machine on 4° C. Collected supernatant and kept in Freezer under 0 ℃ until completion of exploration.

Analysis of Amylases

By consuming resolvable starch as a substrate, amylase action was done. Starch solution of 5 ml was taken in Experimental and control tubes. Enzyme homogenate of 1 ml was added and mixed in experimental tubes. Mixture was placed in Water bath under incubation at 37°C for 30 minutes and furthermore 1 ml of (1N) HCl was poured in it. Tubes were removed from water bath and mixed vigorously and in each controlled tube enzyme homogenate of 1 ml was poured. Blank with 5 ml purified water was made. in 0.5 ml aliquot removed from control as well as experimental groups were added by 0.2 ml of (1N) HCl and 0.1 ml of Iodine solution, dilute to 10 ml distilled water, mixed and its Absorbance was read on wavelength of 540 nm.

Analysis of Lipases

Gut homogenate of 2 ml was taken in two test tubes (Test and Blank). Blank test tube was retained in boiling Water bath for five minutes and cooled at Room temperature. Both test and blank tubes were added by 2 ml of olive oil and 0.5 ml of Phosphate Buffer having pH 7.4, further shaked by hands and placed in incubation on 37°C for 24 hours. After completion of incubating procedure, 1ml of Acetic acid and two drops of Phenolphthalein indicator were inserted in tapering Flask and Titrated by standardized solution of (2N) NaOH until appearance of pink colour.

Lipase activity was observed as,

Units/ml of Enzyme = Volume of NaOH x Normality of NaOH x 40/Volume of Used sample

= Y μ M ethanol released/Min.

Enzyme Activity = Y x 1000/254 (Molecular Weight of oleic acid) x 30 min

= (Z) U/mL. Min^{-1}

Analysis of Proteases

For protease activity of gut homogenate 0.65% casein was used. Two tubes (Test and blank) were arranged for each specimen. Then 5 ml Casein solution was inserted in both tubes and made equilibration on 37°C. Enzyme homogenate of 1 ml was inserted only in test tube and placed in incubation for ten minutes on 37 °C. After incubated procedure, both tubes (Test and blank) were inserted with 5 ml of 110 mm Tri Chloro Acetic acid (TCA) to stop reactivity and also mixed 1 ml of enzyme Homogenate in blank tube. Agitated both tubes by spinning and made its incubation at 37 °C for thirty minutes. Furthermore, mixture was filtered through Whatman # 50 filters and exploited in colour expansion. Standardized curve was organized by adopting L-tyrosine, by using sodium carbonate (Na_2CO_3) and Folin and ciocalteus Phenol reagent for colour expansion and recorded the absorbance of the filtrated material on wavelength of 660 nm.

RESULTS

Digestive enzyme activities of *Sperata seenghala* with different crude protein are specified in Table 1.1. Amylase secretion of *Sperata seenghala* reported significant discrimination with different crude protein (CP) showing prominent variations. Higher most Amylase action was determined in T_1 (30% CP) having 0.50±0.09 (U/ml. Min^{-1}) ($p<0.001$) as its mean value with range of 0.40-0.58. However minimum amylase activity was witnessed in T_6 and T_5 as 0.35 ± 0.7 and 0.38 ± 0.85 correspondingly. Amylase action showed an increased pattern from T_6 to T_3 (Table 1.1).

With increased crude proteins action of protease reported positive relation with various feeding groups. Lowest crude protein percentage in diet (30% CP) depicted lower most protease actions (1.48 ± 0.22) while higher crude protein T_5 (50% CP) and fish meal (T_6) displayed greater concentration as 5.71±0.33 and 5.07±0.24 respectively. The order of protease activity in present study was observed as $T_5>T_6>T_4>T_3>T_2>T_1$ (Table 1.1).

All experimental groups reported highest lipase activity than other enzymes. Lipase activity showed an increased trend with increased crude protein (CP) percentage. Highest amount of lipase (16.04±2.73) was secreted with T_5 and lowest in T_1 (13.03±1.36) (Table 1.1). The pattern of increased lipase activity was observed as $T_5>T_4>T_6>T_3>T_2>T_1$ (Table 1.1).

Table 1.1: Effect of various dietary proteins on the activities of three digestive enzymes in *Sperata seenghala*; (a) Protease, (b) Amylases and (c) Lipases.

Feeding groups	Replicates	n	Protease Activity (U/mL.min^{-1})		Amylase Activity (U/mL.min^{-1})		Lipase Activity (U/mL.min^{-1})	
			Av. ±S.D	Range	Av. ±S.D	Range	Av. ±S.D	Range
T_1	A_1	7	1.44±0.21	1.21-1.76	0.46±0.07	0.40-0.58	12.34±1.24	10.50-13.88
	A_2	7	1.52±0.23	1.23-1.79	0.49±0.09	0.42-0.57	13.82±1.45	11.29-14.72
	A_3	7	1.47±0.22	1.20-1.81	0.52±0.11	0.45-0.55	12.93±1.32	10.49-13.88
	Overall	**21**	**1.48±0.22**	**1.20-1.81**	**0.50±0.09**	**0.40-0.58**	**13.03±1.36**	**10.49-13.88**
T_2	B_1	7	2.76±0.19	2.33-2.84	0.42±0.05	0.39-0.44	12.78±2.92	10.32-14.82
	B_2	7	2.63±0.21	2.48-2.74	0.40±0.08	0.37-0.45	13.12±1.17	10.83-15.01
	B_3	7	2.71±0.23	2.59-2.92	0.45±0.07	0.42-0.47	13.84±1.37	11.98-15.22

	Overall	**21**	**2.73±0.22**	**2.33-2.92**	**0.43±0.06**	**0.37-0.47**	**13.66±1.75**	**10.32-15.22**
	C_1	7	2.89±0.20	2.62-2.98	0.51±0.09	0.46-0.57	13.98±2.73	11.67-16.03
T_3	C_2	7	2.88±0.21	2.75-3.01	0.45±0.05	0.42-0.49	14.24±2.98	11.03-16.88
	C_3	7	3.02±0.25	2.89-3.19	0.48±0.09	0.45-0.53	14.61±2.25	12.04-16.01
	Overall	**21**	**2.94±0.22**	**2.62-3.19**	**0.48±0.07**	**0.42-0.57**	**14.29±2.78**	**11.03-16.88**
	D_1	7	4.25±0.29	3.58-4.75	0.43±0.05	0.39-0.47	15.82±2.89	13.89-17.73
T_4	D_2	7	3.98±0.24	3.87-4.11	0.39±0.09	0.35-0.42	14.19±2.34	13.54-16.89
	D_3	7	3.78±0.25	3.56-4.24	0.49±0.06	0.38-0.55	15.96±2.57	13.98-17.94
	Overall	**21**	**4.00±0.26**	**3.56-4.75**	**0.46±0.07**	**0.35-0.55**	**15.48±2.47**	**13.54-17.94**
	E_1	7	5.78±0.32	4.12-6.22	0.39±0.08	0.37-0.42	15.77±2.67	14.72-16.92
T_5	E_2	7	5.25±0.27	4.17-5.64	0.36±0.09	0.33-0.38	16.02±2.88	13.89-17.02
	E_3	7	6.11±0.35	5.11-6.89	0.40±0.08	0.37-0.43	16.33±2.91	14.65-17.56
	Overall	**21**	**5.71±0.33**	**4.12-6.89**	**0.38±0.85**	**0.33-0.43**	**16.04±2.73**	**13.89-17.56**
	CNT_1	7	5.18±0.24	4.01-5.98	0.33±0.06	0.31-0.36	15.34±2.03	14.15-16.01
T_6 (Fish Meal)	CNT_2	7	4.32±0.21	3.63-5.12	0.34±0.07	0.33-0.36	14.78±2.27	12.98-15.23
	CNT_3	7	5.72±0.25	4.64-5.84	0.38±0.09	0.35-0.39	14.86±2.19	12.23-15.82
	Overall	**21**	**5.07±0.24**	**3.63-5.98**	**0.35±0.7**	**0.31-0.39**	**14.99±2.16**	**12.23-16.01**

Basic biometric guides of exploring fish considered at the time of their dissection were articulated in Table 1.2. Condition factor (K) reported non- significant discrimination with different groups of crude protein other than T_5 (0.43± 0.018). Maximum value of condition factor was observed in T_3 (40%CP) as 0.49±0.04 while its minimum in T_5 as 0.43 ± 0.018. Total length, percent body weight, Intestinal length, Intestinal weight and digestive somatic index (D.S.I.) explored significant impact with different crude proteins (Table 1.2)

In present study, maximum value of Relative gut mass (R.G.M.) was perceived in T_2(0.046±0.01) while least relative gut mass (RGM) was observed in T_4 (0.039±0.0005). An increased trend was practiced up to T_3 regarding relative gut mass. Highest mean value of Relative gut length (RGL) was observed as 1.45±0.05 in T_3 and minimum (RGL) was determined as 1.22±0.03 in T_6. (Table 1.2)

Zihler's Index exposed contrary relation with Fish weight and Gut length. Least value of Zihler's index was observed in T_4 as 0.023±0.002 ($p>0.01$) while maximum Zihler's index as 0.104±0.013 in T_2 as a small fish exhibits large Zihler's index. Zihler's index trend observed in present study was $T_2>T_1>T_6>T_5>T_3>T_4$. Maximum value of Digestive somatic index (DSI) was observed as 4.68±0.14 in T_3 and minimum (DSI) as 3.96±0.05 in T_4. The increasing trend of digestive somatic index (DSI) was observed as $T_3>T_2>T_6>T_1>T_5>T_4$ (Table 1.2).

Table 1.2: Biometric parameters measured in experimental fish (*Sperata seenghala*) reared at various Dietary proteins for 90 days.

			Fish Biometric indices						**Gut morphometric indices**							
Feeding groups	**Replicates**	**n**	**Fish Body Mass (g)**		**Standard Length (cm)**		**Fulton's Condition factor (K)**		**Relative Gut Mass (g)**		**Relative Gut Length (cm)**		**Zihler's Index (ZI)**		**Digesto Somatic Index (DSI)**	
			Av.±S.D	**Range**	**Av.±S.D**	**Range**	**Av.±S.D**	**Range**	**Av.±S.D**	**Range**	**Av.±S.D**	**Range**	**Av.±S.D**	**Range**	**Av.±S.D**	**Range**
	A_1	7	28.13 ±0.92	26.68-29.45	13.95±0.34	13.42-14.34	0.47±0.01	0.46-0.49	0.04±0.001	0.039-0.044	1.31±0.03	1.28-1.39	0.08±0.005	0.076-0.092	4.369±0.128	4.16-4.53
T_1 (30% CP)	A_2	7	28.31 ±0.77	27.33-29.42	14.06±0.16	13.83-14.28	0.47±0.02	0.44-0.50	0.04±0.001	0.040-0.042	1.35±0.01	1.324-1.366	0.08±0.01	0.077-0.092	4.11±0.09	4.01-4.28
	A_3	7	28.51 ±0.72	27.65-29.67	14.67±0.38	14.11-15.1	0.47±0.01	0.46-0.48	0.04±0.0004	0.039-0.041	1.29±0.02	1.27-1.33	0.08±0.005	0.074-0.088	4.04±0.04	3.98-4.09
	Overall	**21**	**28.32 ±0.79**	**26.68-29.67**	**14.23±0.44**	**13.42-15.1**	**0.47±0.01**	**0.44-0.50**	**0.041±0.001**	**0.039-0.044**	**1.32±0.03**	**1.27-1.39**	**0.08±0.01**	**0.07-0.09**	**4.09±0.10**	**3.97-4.37**
	B_1	7	25.71 ±0.95	24.33-26.85	13.21±0.13	13-13.4	0.48±0.01	0.469-0.498	0.04±0.001	0.041-0.045	1.31±0.01	1.28-1.33	0.10±0.01	0.091-0.117	4.34±0.13	4.19-4.50
T_2 (35% CP)	B_2	7	25.95 ±1.48	23.45-27.86	13.33±0.18	13.1-13.6	0.47±0.01	0.45-0.49	0.04±0.001	0.041-0.045	1.30±0.01	1.29-1.32	0.101±0.02	0.08-0.13	4.29±0.14	4.13-4.56
	B_3	7	25.08 ±0.91	23.9-26.64	13.23±0.18	13-13.5	0.46±0.01	0.453-0.472	0.045±0.001	0.042-0.046	1.29±0.02	1.28-1.32	0.11±0.01	0.091-0.123	4.46±0.12	4.28-4.58
	Overall	**21**	**25.58 ±1.15**	**23.45-27.86**	**13.26±0.17**	**13-13.6**	**0.47±0.01**	**0.45-0.49**	**0.046±0.001**	**0.041-0.046**	**1.30±0.01**	**1.28-1.33**	**0.104±0.013**	**0.08-0.13**	**4.36±0.14**	**4.13-4.58**
	C_1	7	36.04 ±1.34	33.54-37.81	14.75±0.77	13.6-15.7	0.49±0.04	0.43-0.55	0.05±0.001	0.045-0.047	1.46±0.05	1.41-1.54	0.05±0.004	0.041-0.055	4.68±0.08	4.56-4.77
T_3 (40% CP)	C_2	7	35.94 ±1.59	33.73-38.18	14.81±0.37	14.2-15.2	0.49±0.04	0.44-0.54	0.05±0.002	0.04-0.05	1.45±0.04	1.41-1.51	0.047±0.005	0.041-0.053	4.69±0.18	4.44-5.03
	C_3	7	35.69 ±1.79	32.86-38.19	14.43±0.71	13.2-15.1	0.51±0.05	0.44-0.61	0.05±0.002	0.044-0.049	1.47±0.06	1.39-1.55	0.05±0.005	0.040-0.054	4.66±0.17	4.41-4.92
	Overall	**21**	**35.89 ±1.51**	**32.86-38.19**	**14.67±0.63**	**13.2-15.7**	**0.49±0.04**	**0.43-0.61**	**0.05±0.001**	**0.04-0.05**	**1.45±0.05**	**1.39-1.55**	**0.05±0.004**	**0.04-0.05**	**4.68±0.14**	**4.41-5.03**
T_4 (45% CP)	D_1	7	45.58 ±1.86	42.83-47.89	16.47±0.46	15.6-17	0.47±0.02	0.46-0.504	0.04±0.0003	0.039-0.041	1.38±0.02	1.35-1.4	0.02±0.002	0.0215-0.0276	4.002±0.039	3.92-4.04
	D_2	7	46.04 ±1.01	44.47-47.56	16.45±0.36	16-17.1	0.48±0.02	0.44-0.49	0.039±0.0004	0.038-0.040	1.39±0.03	1.32-1.41	0.023±0.001	0.021-0.025	3.95±0.04	3.89-4.01

	D_3	7	46.01 ±1.06	44.24-47.46	16.39±0.26	16.1-16.8	0.48±0.01	0.47-0.51	0.039±0.0005	0.038-0.040	1.39±0.01	1.37-1.41	0.023±0.001	0.021-0.025	3.93±0.05	3.85-4.02
	Overall	**21**	**45.87 ±1.31**	**42.83-47.89**	**16.44±0.35**	**15.6-17.1**	**0.48±0.02**	**0.439-0.505**	**0.039±0.0005**	**0.038-0.040**	**1.39±0.019**	**1.33-1.41**	**0.023±0.002**	**0.0215-0.0276**	**3.96±0.05**	**3.85-4.04**
T_5 (50% CP)	E_1	7	31.89 ±0.99	30.28-33.26	15.1±0.22	14.8-15.4	0.43±0.02	0.38-0.44	0.040±0.004	0.037-0.047	1.27±0.02	1.22-1.29	0.06±0.006	0.05-0.07	4.05±0.36	3.69-4.68
	E_2	7	32.48 ±1.40	30.23-34.45	15.54±0.21	15.3-15.8	0.45±0.01	0.43-0.46	0.041±0.002	0.039-0.044	1.21±0.02	1.17-1.24	0.055±0.008	0.045-0.069	4.10±0.20	3.88-4.37
	E_3	7	31.83 ±1.22	30.26-33.44	14.94±0.17	14.7-15.2	0.43±0.012	0.41-0.44	0.04±0.002	0.037-4.28	1.28±0.03	1.25-1.33	0.06±0.006	0.05-0.07	4.10±0.23	3.77-4.36
	Overall	**21**	**32.07 ±1.19**	**30.23-34.45**	**15.19±0.32**	**14.7-15.8**	**0.43±0.018**	**0.38-0.46**	**0.041±0.003**	**0.037-0.047**	**1.25±0.04**	**1.17-1.33**	**0.06±0.007**	**0.04-0.07**	**4.08±0.25**	**3.69-4.68**
T_6 (Fish Meal)	F_1	7	27.62 ±2.29	24.95-31.82	13.99±0.62	13.2-15.2	0.47±0.009	0.46-0.49	0.043±0.001	0.041-0.045	1.26±0.03	1.21-1.30	0.09±0.02	0.057-0.111	4.37±0.13	4.16-4.53
	F_2	7	27.99 ±1.63	25.79-30.27	14.26±0.44	13.8-14.9	0.48±0.01	0.47-0.49	0.04±0.001	0.042-0.044	1.20±0.02	1.17-1.22	0.08±0.01	0.07-0.09	4.30±0.05	4.23-4.38
	F_3	7	28.93 ±1.28	27.22-30.82	14.54±0.46	13.8-15	0.50±0.009	0.49-0.52	0.041±0.001	0.040-0.043	1.22±0.03	1.19-1.26	0.073±0.008	0.062-0.086	4.14±0.098	4.044-4.31
	Overall	**21**	**28.18± 1.78**	**24.95-31.82**	**14.26±0.54**	**13.2-15.2**	**0.48±0.02**	**0.45-0.52**	**0.042±0.001**	**0.04-0.45**	**1.22±0.03**	**1.17-1.30**	**0.079±0.014**	**0.06-0.11**	**4.27±0.133**	**4.04-4.53**

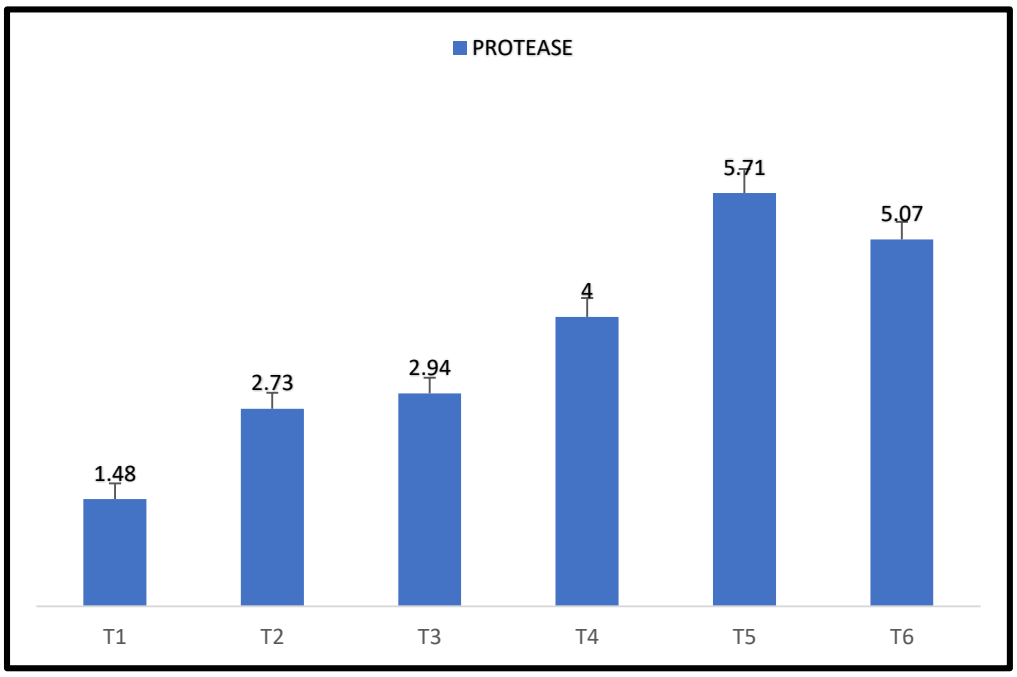

Figure 1.1: The protease activity of experimental fish (*Sperata seenghala*) reared at various protein diets for 90 days.

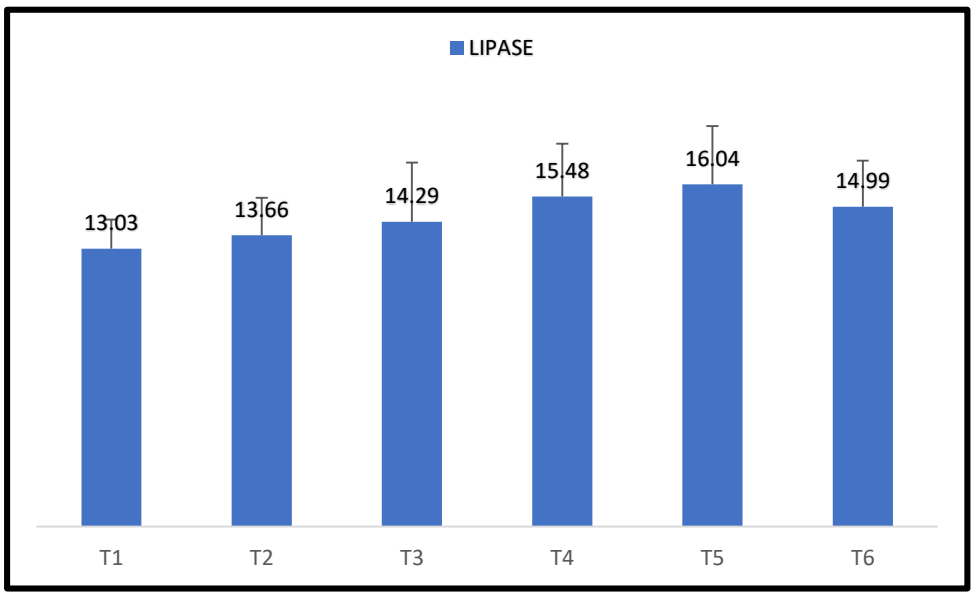

Figure 1.2: The lipase activity of experimental fish (*Sperata seenghala*) reared at various protein diets for 90 days.

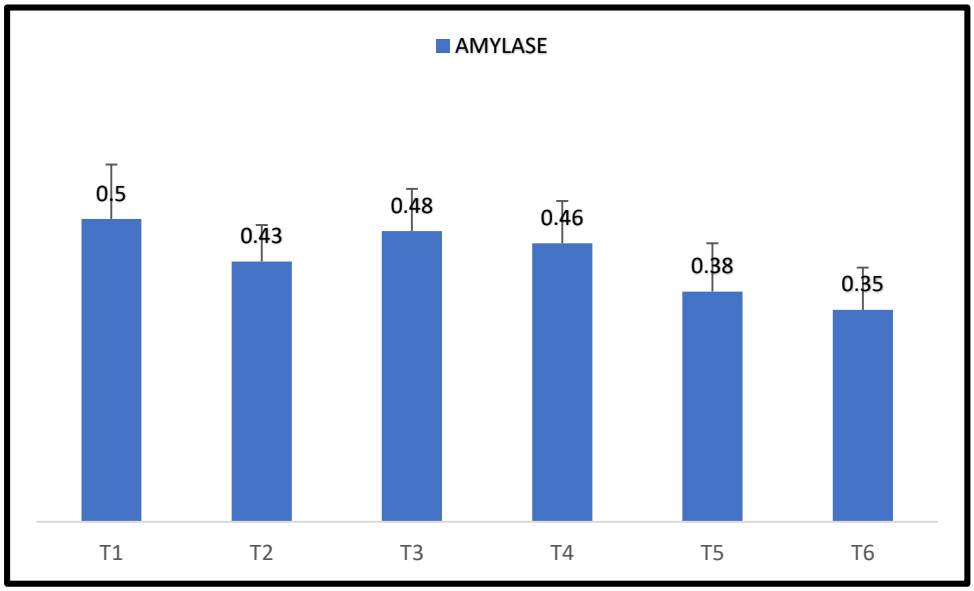

Figure 1.3: The amylase activity of experimental fish (*Sperata seenghala*) reared at various protein diets for 90 days.

DISCUSSION

Numerous studies exposed that variations in actions of digestive enzyme are because alterations in feeding constituents as observed in present study of *Sperata seenghala* (Table 1.1), undernourishment, feeding time, food manipulations and concentrations of protein (Hakim *et al.*, 2006).When fish were fed with cotton seed meal and fish meal improved protease activity was observed in the complete intestine which eventually improved fish growth representing cotton seed meal and fish meal as better components for feed formulation for *Labeo rohita* (Iqbal *et al.*, 2018). Significant differences in amylase and protease activity were determined in *Labeo rohita* in response to different feed constituents (Kumar *et al.*, 2011) that showed similar trend in protease and amylase activity of *Sperata seenghala* in present study (Table 1.1).

Highest lipase activity in *Catla catla* and lowest lipase activity in *Hypophthalmichthys molitrix* was determined when fed with Soya bean meal diets (Ismat *et al.*, 2013) while in present study maximum lipase activity was showed in T_5 (50%) crude protein diet and minimum lipase activity was observed with T_2 (35%) crude protein diets in *Sperata seenghala*.(Table 1.1) The intestinal amylase activity of *Hypophthalmichthys molitrix* fingerlings was observed significantly greater when fed with duck weed diet as compared to soya bean diet in polyculture system (Aslam *et al.*, 2018) however increased crude protein diet represented decreased amylase activity in *Sperata seenghala* in present study (Fig 1.3) (Table 1.1). In present study lower amylase activity was determined with different crude proteins in *Sperata seenghala* being a carnivorous fish that showed parallel findings with findings of Horn *et al.* (2006) and Drewe *et al.* (2004) and in which higher amylase activity was determined in herbivorous and omnivorous fishes as compared to carnivorous fishes.

Chan *et al.* (2004) has reported that herbivore fishes accomplish their little accessibility of protein by enhancing their enzyme activity. To enhance protein digesting efficiency, some herbivore fishes display Trypsin activity equivalent to carnivore species or even higher than it. Protease enzyme activity fluctuates with concentration of protein in diet (Haider *et al.*, 2018), similar findings were also observed in present exploration of *Sperata seenghala* with different protein diets (CP) confirming that crude protein diets affects digestive enzyme activity.

Lipid contents also varies due to variations in silage concentration, representing lipase enzyme activity among all treatment diets (Haider *et al.*, 2018) and similar findings were also defined by Klomklao *et al.* (2006). Present study also showed variations in lipase activity with different crude protein diets with maximum lipase activity in T_5.

Impact of processed wastes of fish on Digestive enzymes of *Cyprinus carpio* was studied and reported non-significant dissimilarity in protease activity however Lipase and amylase diverged considerably among entire experimental diets concluding that costly fish meal in fish diet can be replaced by waste of fish body viscera. Furthermore, protease activity was reported to fluctuate with proteinaceious diet (Haider *et al.*, 2018) similarly in present study it was observed that protease activity also fluctuates with different crude protein diets showing increased trend of protease activity with increased crude protein (Fig 1.1) (Table1.1). Sabapathy and Teo (1993) and Kapoor *et al.* (1975) conveyed as carnivore fish species depicts greater Protease activity than omnivore and herbivore species as witnessed in current work of *Sperata seenghala*.

Lopez-Lopez *et al.* (2005) reported insignificant relation in protease activity and protein diets whereas in present study a progressive relationship of protease enzyme was detected with increased crude protein (CP) disclosing lowermost protease activity was observed with T_1 (30% CP) and higher protease activity with T_5 (50% CP) and fish meal (T_6) respectively (Table 1.1).

Amylase and protease secretions were reported to alter with variations of amount of protein and carbohydrate containing diets (Le Moullac *et al.*,1994). However, extensive amount of protein and carbohydrates in diet resulted in reduced protease and amylase (Cara *et al.*, 2003) while in present study amount of amylase activity and protease activity also showed variations with different crude protein diets (CP), amylase activity was found to be decreased with increased crude protein and protease activity was reported to be increased with increased crude protein diets (Fig 1.1-1.3). *Homarus americanus* and *Cherax quadricarinatus* reported non-significant correlation between amylase and carbohydrate containing diet correspondingly (Lopez-Lopez *et al.*, 2005; Cahu *et al.*,1999) while in present study of *Sperata seenghala* both showed significant differences.

CONCLUSION

In present study, maximum value of Relative gut mass (R.G.M.) was perceived in T_3 while least relative gut mass (RGM) was observed in T_4 (0.039±0.0005). Highest mean value of Relative gut length (RGL) was observed as 1.45±0.05 in T_3 and minimum (RGL) was determined as 1.22±0.03 in T_6. Least value of Zihler's index was observed in T_4 as 0.023±0.002 ($p>0.01$) while maximum Zihler's index as 0.08±0.01 in T_1 as a small fish exhibits large Zihler's index. Amylase secretion of *Sperata seenghala* reported significant discrimination with different crude protein (CP) showing prominent variations. Higher most Amylase action was determined in T_1 (30% CP) having 0.50±0.09 (U/ml. Min^{-1}) ($p<0.001$). However minimum amylase activity was witnessed in T_6 and T_5 as 0.35 ± 0.7 and 0.38 ± 0.85 correspondingly. With increased crude proteins action of protease reported positive relation with various feeding groups All experimental groups reported highest lipase activity than other enzymes.

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