



DIGESTIVE ENZYMATIC AND GROWTH STUDY OF *Danio rerio* FED WITH *Agaricus bisporus* ENRICHED DIET

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author TA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author RJ managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The study serve the purpose to evaluate growth performance and digestive enzymatic study of the intestinal tissue of *Danio rerio* as it was measured function of quality of the test diets as the enzyme activity is affected by the proteins and energy densities. *A. bisporus* is a protein rich diet used as effective alternate to commercial diet. In the present investigation *A. bisporus* enriched diet is fed to zebra fish to study growth performance and digestive enzyme activity. A total of 80 zebra fish were divided into 4 groups. The experiment were performed in triplicates and one group is of control fed with commercial flakes. They are fed 10% of their body weight twice a day. The *A. bisporus* demonstrated that after 4th week the weight of the zebra is significantly increased to 0.90 ± 0.11 and the zebra fish has shown improvement in digestive enzyme activity Protease, Lipase, Amylase and Alkaline Phosphatase 270 ± 0.09 , 2.9 ± 0.3 , 91.11 ± 0.21 , 63.32 ± 0.66 respectively, as there was increase in the protein content in diet. Therefore *A. bisporus* diet can replaced fishmeal.

Keywords: *Agaricus bisporus*; *Danio rerio*; growth study; digestive enzyme; diet.

1. INTRODUCTION

Agaricus bisporus (*A. bisporus*), commonly known as the white button mushroom, is one of the most economically important edible mushrooms. It is considered as a valuable health food with high

contents of polyphenols, ergothioneine, vitamins, minerals and polysaccharides [1,2]. Mushroom Bran is derived from edible mushroom species and is a popular high-end product as a dietary supplement [3]. Bio active compounds of button mushroom have anti-fungal and anti-bacterial properties can prevent

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cardiovascular diseases; it is an ideal food for all patients, old people, pregnant women, and children [4,5]. Zebrafish represents an ideal organism to better understand fish physiological responses to new ingredients [6] and an emerging useful model organism for neuroscience/behavioral research [7], aquaculture [8,9], as a model for human diseases [10,11,12]. Zebrafish diets consist of manufactured feeds and/or live organisms. Manufactured feeds are often formulated for and sold to the food–fish aquaculture and aquarium industries. Live feeds consist of primarily brine shrimp (*Artemia sp.*), rotifers (*Brachionus sp.*), and paramecia. In the case of manufactured zebrafish feeds, since the product is marketed for aquaculture or aquarium use it is assumed that the feed is acceptable for animals used for research. This is a severe and potentially costly (money, time, loss of important lines etc.) oversight as the objectives of feeding food–fish, aquarium fish, and laboratory fish differ greatly (Marc et al., 2019). Zebrafish share a high degree of amino acid sequence and functionality with mammals including humans. They also possess characteristics, including simple husbandry, gregarious behaviour, small adult size, rapid growth, development and maturation, high fecundity and productivity, which make them an ideal model species for pilot feed studies on novel and difficult to source or synthesis ingredients [13,14]. Moreover, the assessment of presence and activity level of digestive enzymes may be used as a comparative indicator of developmental rate, food acceptance, digestive capacity, as well as survival rate of fish larvae [15]. However, very little investigation is done on enzymatic study of *A.bisporus* enriched diet fed to Zebra fish which plays an important role in studying the nutrition and growth of Zebra fish.

2. MATERIALS AND METHODS

Experimental diet: *Agaricus bisporus* (50%) was oven dried at 60° C until they turn crisp to prepare powder in order to prepare meal. Soy flour (20%), rice bran (15%), maize powder (10), cotton seed (2.5%), canola oil (1.5%) and NaCl (1%). All the solid ingredients were grinded, sieved to get consistency and even particle size palatable to *Danio rerio*. The homogenized mixture was squashed and compressed into cylindrical pellets and for a good shelf life the moisture content is removed to below 10%. For control commercial flakes Instincts is used which comprises of fishmeal, squid meal, vitamin premix and yeast extract. The protein is 42% and 16.70 J mg⁻¹ of energy in formulated diet. While in control the protein content is 39% and 13.31 J mg⁻¹.

2.1 Experimental Setup

The experiment was of 4 weeks. *Danio rerio* were obtained from the hatchery center of Kalyan and acclimated to laboratory conditions. The average weight of *Danio rerio* was 0.25±0.13g was stocked in triplicate in 50L aquarium tank and the group of controlled. The fish was maintained under a 14 h/10 h day/night photoperiod cycle under controlled conditions of temperature 28-35°C. During the acclimatization period, the fish were fed twice a day with commercial fish food. The fish were acclimatized for 5 days. The fish were divided 20 fish each (04×20=80 fish). *A. bisporus* enriched diets at the rate of 10% of their body weight twice a day was fed to them, for control tank feeding was done three times a day with commercial flakes for four weeks. The respective diets in each group were continued till the end of experiment.

2.2 Enzymatic Studies

Homogenate preparation: For enzyme analysis 12 hours fasting zebra fish was used from each tank. Anesthetized and dissected on the ice plate. The tissue was homogenized and tissue concentration was made to 10 mg/ml with 0.09% chilled NaCl. The tissue homogenate was centrifuged at 3000 rpm for 10 minutes. Aliquot of supernatant was used as enzyme source. The homogenized was stored in freezer until used. The protease activity was determined by applying methods of Egauche and Iwamoto [16]. The activity of amylase enzyme was determined by using 3-5 dinitrosalicylic acid (DNSA) reagent [17] and lipase by Hayashi and Tappel, [18] while the remainder of intestines homogenized supernatant was kept for alkaline phosphatase analyses and purified according to Gisbert et al. [19].

2.3 Growth Performance

At the end of the every week weight has been recorded. Final weight (FW), weight gain (WG), survival rate were determined for each tank using the following formula: FW (mg) = Individual final wet weight. WG (%) = ((Individual final wet weight - Individual initial wet weight) / Initial wet weight) x 100. Survival rate (%) = 100 × (final fish number) / (initial fish number), where W1 and W2 are the initial and final weight (mg) [20].

2.4 Statistical Analysis

Treatments are assigned to experimental units completely at random. Data were analyzed in a paired t- test in order to determine the effect of treatments;

Microsoft Excel and graph pad prism 9.1 followed in order to detect significant differences between the groups. The results were considered significant at $P \leq 0.05$. The software used was graph pad prism 9.1. The differences between variables were tested by Monte Carlo test with 499 permutations ($P \leq 0.05$) [21].

3. RESULTS AND DISCUSSION

Based on the current study proved that the *A. bisporus* enriched diet produced different response levels in terms of growth, and digestive enzyme in the *Danio rerio* fish as compared to commercial flakes diet. as also reported by Eswari et al. [22]. Fish fed with *A. bisporus* diet shows a positive growth performance as summarized in the Table 1. The growth range from 0.29 ± 0.10 to final weight 90 ± 0.11 at the end of the experiment whereas the control growth ranges from 0.26 ± 0.13 to 0.59 ± 0.13 at the end of the experiment. The Fig. 1 shows the graphical representation of the growth positive response of the *Danio rerio* when fed with *A. bisporus* enriched diet.

Digestive enzymes play a fundamental role in the digestion process and are directly correlated to dietary fish nutritional status and adaptability to change [23, 24,25]. In the study the digestive enzyme shows an increasing trend in the gut tissue. The Protease value increases from 250 ± 0.21 to 270 ± 0.09 , Lipase 1.6 ± 0.1 to 2.9 ± 0.3 , Amylase from 80.32 ± 0.21 to 91.11 ± 0.21 and alkaline phosphatase from 41.52 ± 0.66 to 63.32 ± 0.66 (Fig. 4). The increment in the digestive enzyme is due to the increase in the protein content in the diet. The data has been summarized in Table 1 whereas the two way ANOVA found to be significant (Table 1a). The similar increasing trend is demonstrated by Guerrero, [26]. The folding of the intestinal mucosa, with the subsequent increase in the absorption surface, may explain the progressive increases in all enzymatic activities observed during zebrafish development, like those observed in other fish larvae i.e., common Pandora, [27] sharp snout sea bream, [28]. The increment in the digestive enzymes suggests the better digestion and the nutrition is much effectively available for the growth of the fish. As the duration of the of the experiment is increasing

Table 1. Effect of *A. bisporus* enriched diet on growth performance of *Danio rerio* after feeding for four weeks. (The values in table are in the form of Mean \pm Standard error. Values in bracket are Percentage variation. Values are significant at $p < 0.05$. Mean and standard deviation are the pool of three individual measurements. The difference between control and exposure period (in days) were found to be statistically significant)

	Control	Initial	Final Weight	Weight gain %	Survival rate
Week 1	0.26 ± 0.13	0.25 ± 0.13	0.29 ± 0.10	16 ± 0.21	75 ± 0.09
Week 2	0.30 ± 0.13	0.29 ± 0.10	0.39 ± 0.11	32 ± 0.23	87 ± 0.09
Week 3	0.44 ± 0.11	0.39 ± 0.11	0.50 ± 0.11	30 ± 0.11	93 ± 0.09
Week 4	0.59 ± 0.13	0.50 ± 0.11	0.90 ± 0.11	80 ± 0.11	93 ± 0.09

Table 1a. Two way ANOVA for Table 1

Paired t test	
P value	0.0306
P value summary	*
Significantly different ($P < 0.05$)?	Yes
One- or two-tailed P value?	Two-tailed
t, df	$t=3.865$, $df=3$
Number of pairs	4

Table 2. Effect of *A. bisporus* enriched diet on growth performance of *Danio rerio* after feeding for four weeks. (The values in table are in the form of Mean \pm Standard error. Values in bracket are Percentage variation. Values are significant at $P \leq 0.05$. Mean and standard deviation are the pool of three individual measurements. The difference between control and exposure period (in days) were found to be statistically significant)

	Control	Test
Protease	250 ± 0.21	270 ± 0.09
Lipase	1.6 ± 0.1	2.9 ± 0.3
Amylase	80.32 ± 0.21	91.11 ± 0.21
Alkaline phosphatase	41.52 ± 0.66	63.32 ± 0.66

the survival rate was also increasing and found to be 75 ± 0.09 , 87 ± 0.09 , 93 ± 0.09 , 95 ± 0.09 (Fig. 2). The weight gain in % of four weeks demonstrated an increasing trend from 16 ± 0.21 , 32 ± 0.23 , 30 ± 0.11 to 80 ± 0.11 respectively (Fig. 3) is in agreement with [29]. The *A. bisporus* in combination of *Lactobacillus casei* shows up regulated the expression of growth-related genes [30]. The highest survival rate was

observed in week 4 with 95 ± 0.09). In *Paralichthys olivaceus* fish 74% and 79% survival rate was observed when fed with 1% and 10% *A. bisporus* diets [31]. Also the Effects of dietary white-button mushroom powder on mucosal immunity, antioxidant defense, and growth of common carp (*Cyprinus carpio*) [32].

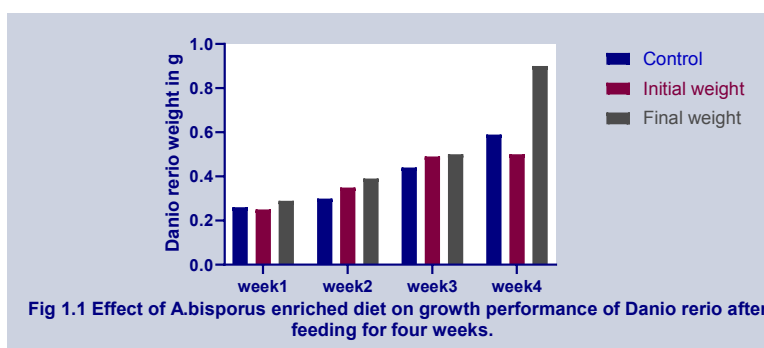


Fig. 1. Effect of *A. bisporus* enriched diet on growth performance of *Danio rerio* after feeding for four weeks

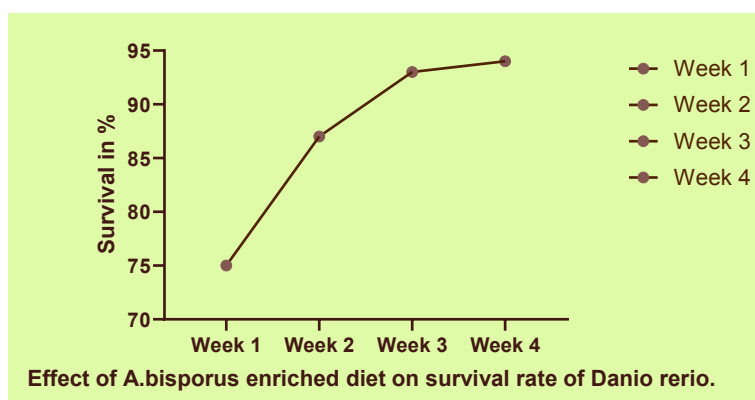


Fig. 2. Effect of *A. bisporus* enriched diet on survival of *Danio rerio* after feeding for four weeks

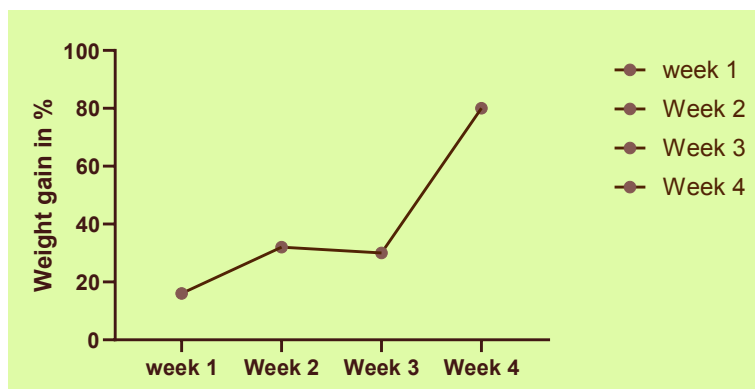


Fig. 3. Effect of *A. bisporus* enriched diet on weight gain of *Danio rerio* after feeding for four weeks

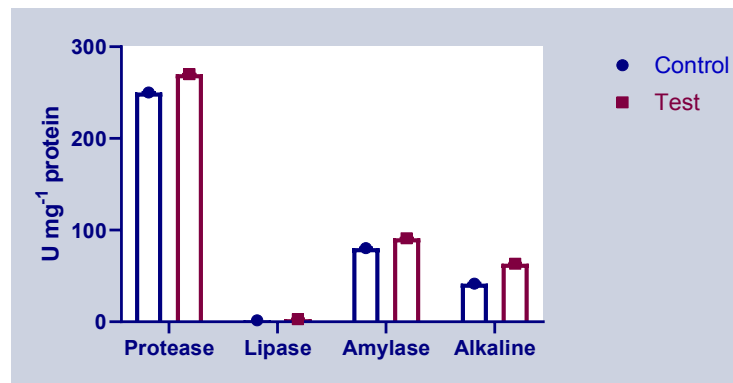


Fig. 4. Digestive enzyme activities in Zebra fish (*Danio rerio*) gut when fed with *A.bisporus* enriched diet

4. CONCLUSION

The consistent result of growth performance and digestive enzyme activity reveals the *A. bisporus* diet when fed to *Danio rerio* shows more positive growth when compared to commercial flake diet. Future studies should be comprehensive to reflect the use in commercial utilization.

Thus, *A. bisporus* enriched diet is a suitable replacement of the commercial diet and can be used as a potential feed in aquaculture.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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