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Effects of exogenous xylanase supplementation in plant-based diets on growth performance, nutrient digestibility and digestive enzyme activities of juvenile mori (*Cirrhinus mrigala*)

Hafsa Nadeem [a], Syed Zakir Hussain Shah [a,*], Mahroze Fatima [b], Maryam [a], Muhammad Mudassar Shahzad [c]

[a] *Department of Zoology, University of Gujrat, Gujrat, Pakistan*
[b] *Department of Fisheries & Aquaculture, University of Veterinary & Animal Sciences, Lahore, Pakistan*
[c] *Department of Zoology, Division of Science and Technology, University of Education Township Campus Lahore, Pakistan*

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A B S T R A C T

The present experimental study was conducted to determine the effects of xylanase supplementation on growth performance, nutrient digestibility and digestive enzyme activities of mori, *Cirrhinus mrigala*. Mori with an initial body weight of 8.34 ± 0.23 g were fed seven experimental diets: a diet with high fish meal content (positive control, PC), a diet with high plant content (negative control, NC), and five other diets with increasing levels of xylanase (680, 1273, 1882, 2486 and 3091 U/kg). Each diet was fed to triplicate groups of 15 fish each for 60 days. After the trial termination, supplementation of xylanase significantly improved final weight (FW), weight gain (WG) and specific growth rate (SGR) compared to NC diet. The best feed conversion ratio (FCR) was observed at 1882 U/kg of xylanase supplementation level which was non-significant ($P > 0.05$) to PC diet (high fish meal content). Whole body proximate composition and apparent digestibility coefficient (ADC%) for dry matter (DM), crude protein (CP), crude fat (CF), crude ash (CA) and gross energy (GE) were significantly affected by xylanase supplementation. Activities of hepatopancreatic and intestinal amylase, protease and lipase showed an increasing trend with the increase in xylanase supplementation level, peaking at 1882 U/kg of xylanase (non-significant ($P > 0.05$) to PC diet) and declining thereafter (2486 U/kg-3091 U/kg). Quadratic regression analysis showed that 1948 U/kg of xylanase was the optimum value for supplementation of xylanase in plant-based diet. In conclusion, exogenous xylanase supplementation improved the growth performance, nutrient digestibility and digestive enzyme activities in juvenile mori fed plant-based diet.

1. Introduction

A global increase in population requires a substantial food supply. A portion of this increasing food demand is being fulfilled by increasing the aquaculture production (FAO, 2020). Aquaculture is gaining momentum in giving the continuous supply of fish and fish products. However, this industry requires a large quantity of sustainable, cost-effective and environment friendly fish feed ingredients

* Corresponding author.
E-mail address: dr.zakirhussain@uog.edu.pk (S.Z.H. Shah).

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for expansion (Tacon, 2020). Plant-based protein sources have gained popularity in this context (Lee et al., 2020). As the plant ingredients are locally available at a low cost so their usage has rapidly gained attention among the fish nutritionists (Napier et al., 2020). Plant sources such as corn meal (Khalifa et al., 2018), sunflower seed meal (Hassaan et al., 2018), soy protein concentrate (Ribeiro et al., 2016), jatropha meal (Hassaan et al., 2017), okara meal (El-Saidy, 2011), fermented soybean meal (Hassaan et al., 2015), cottonseed meal (El-Saidy and Gaber, 2004), wheat middlings, rice gluten meal and rapeseed meal (Sinha et al., 2011) are commonly used in herbivorous fish feed formulation. However, plant products contain anti-nutritional factors (ANFs) like phytates, saponins, lectins, protease inhibitors and non-starch polysaccharides (NSPs) that hinder their usage as an alternative to fish meal (Hassaan et al., 2019; Lee et al., 2020). The NSP part of plant meal limits the availability of nutrients to the fish (Dawood and Koshio, 2020).

These NSPs belong to a complex carbohydrate group mainly containing linked monomers of pentoses and hexoses like galactose, mannose, xylose and arabinose. NSPs may constitute 25–50 % of the total plant component as in lupin and wheat where they act as a primary source of carbohydrates (van Barneveld, 1999). In monogastric animals, like fish, the enzymes to digest these NSPs are scarcely present or are completely absent (Kuz'Mina, 1996). As a result, the NSPs are not digested which leads to an increased viscosity in the gastrointestinal tract (Leenhouwers et al., 2007), this adversely impacts the growth rate. Evidence of poor growth performance due to low nutrient utilization was recorded for tilapia (*Oreochromis niloticus* x *O. aureus*) (Leenhouwers et al., 2007). The digestibility of these plant sources can be enhanced by overcoming the anti-nutritional effects of NSPs (Francis et al., 2001).

Supplementation of exogenous NSP hydrolyzing enzymes in plant meal diets increases the nutrient digestion and growth performance in fish. Xylanase belongs to the class of enzymes that causes the hydrolysis of β-1,4 bond in xylan, an NSP present in plant meals. They hydrolyze the xylan present in the cell wall of plant meals, reducing the molecular size of NSP, which otherwise would become viscous by absorbing water and confine nutrients as observed in white Pekin ducks (*Anas platyrinchos domesticus*) (Adeola and Bedford, 2004). This reduces gut viscosity and improves digestion in nonruminant animals (Adeola and Cowieson, 2011). Xylanase dietary supplementation has been shown to improve growth rate in fish such as catfish (*Clarias gariepinus*) (Babalola, 2006), sea bass (*Lateolabrax japonicus*) (Ai et al., 2007), tilapia (*Oreochromis niloticus* and *O. mossambicus*) (El-ashry et al., 2021; Hassaan et al., 2019; Hlophe-Ginindza et al., 2016) and large yellow croaker (*Larmichthys crocea*) (Luo et al., 2020). It also improves the activity of intestinal endogenous enzymes including amylase, lipase, chymotrypsin and trypsin in large yellow croaker (*Larmichthys crocea*) and Jian carp (*Cyprinus carpio*) (Luo et al., 2020; Jiang et al., 2014).

Cirrhinus mrigala, which is commonly known as mori or mrigal is a freshwater fish. It is extensively farmed in Pakistan, Bangladesh and India as a part of polyculture or in monoculture practices. It is omnivore and herbivore in nature. Due to its feeding behavior, a heterotrophic community of diverse bacteria is present in its gastrointestinal tract. These bacteria produce digestive enzymes and ANF degrading enzymes (phytase, tannase, xylanase, cellulase, chitinase) (Ray et al., 2012), consequently helping the fish in digesting its

Table 1
Formulated and analyzed chemical composition of experimental diets (g/kg).

Ingredients	PC diet	NC diet	Xylanase supplemented diets (U/kg)				
			600	1200	1800	2400	3000
Wheat	150.000	150.000	150.000	150.000	150.000	150.000	150.000
Rice polish	132.500	100.000	100.000	100.000	100.000	100.000	100.000
Cottonseed meal	100.000	150.000	150.000	150.000	150.000	150.000	150.000
Sunflower meal	50.000	200.000	200.000	200.000	200.000	200.000	200.000
Corn gluten 60%	50.000	182.500	182.500	182.500	182.500	182.500	182.500
Fish meal	400.000	100.000	100.000	100.000	100.000	100.000	100.000
Fish oil	70.000	70.000	70.000	70.000	70.000	70.000	70.000
Vitamin premix[a]	10.000	10.000	10.000	10.000	10.000	10.000	10.000
Mineral mixture[b]	10.000	10.000	10.000	10.000	10.000	10.000	10.000
Ascorbic acid	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Carboxymethyl cellulose	0.500	0.500	0.499	0.498	0.497	0.496	0.495
Xylanase[c]	–	–	0.001	0.002	0.003	0.004	0.005
Dicalcium phosphate	10.000	10.000	10.000	10.000	10.000	10.000	10.000
Chromic oxide	5.000	5.000	5.000	5.000	5.000	5.000	5.000
Lysine HCl	2.000	2.000	2.000	2.000	2.000	2.000	2.000
Dl-Methionine	4.000	4.000	4.000	4.000	4.000	4.000	4.000
L-Threonine	5.000	5.000	5.000	5.000	5.000	5.000	5.000
Analyzed Chemical Composition and Enzyme Activity							
DM (g/kg)	90.220	90.210	90.290	90.360	90.450	90.290	90.320
CF (g/kg)	14.230	14.450	14.950	15.000	14.400	14.460	14.350
CA (g/kg)	4.300	4.300	4.300	4.150	4.300	4.300	4.450
CP (g/kg)	31.300	31.450	31.450	31.400	31.300	31.250	31.250
GE (kcal/g)	3412.000	3418.000	3412.000	3422.0000	3428.000	3428.000	3424.000
Xylanase (U/kg)	75.000	72.000	680.000	1273.000	1882.000	2486.000	3091.000

PC= Positive control, NC= Negative Control, DM= Dry matter, CP= Crude protein, CF= Crude fat, CA= Crude ash, GE= Gross energy

[a] Vitamin premix (g/kg): vitamin A, 55,00,000 IU; vitamin D_3, 11,00,000 IU; vitamin B_2, 0.10; vitamin B_3, 0.20; vitamin B_5, 2500; vitamin B_6, 1.00; vitamin B_{12}, 0.006; vitamin E, 0.75; vitamin K, 1.00; choline chloride, 150.

[b] Mineral premix (g/kg): $MnSO_4$, 27.0; KI, 1.00; $FeSO_4.7\ H_2O$, 7.50; $ZnSO_4$, 5.00; $CuSO_4.5\ H_2O$, 2.00; $CoCl_2.6\ H_2O$, 0.45; $NaH_2PO_4.\ 2\ H_2O$, 7.80; $CaCO_3$, 2.48; selenium, 50 ppm.

[c] Xylanase, Econase – XT (1,4-Beta Xylanase), 600,000 BXU/g, AB Vista

natural diet. However, the amount of enzymes produced by the fish is not enough to help the fish to cope with the plant-based diet (Kuz'Mina, 1996). Hence, following the trend in the nutrition industry, it is inevitable to increase the utilization rate of carbohydrates for fulfilling fish nutritional demands, to conserve the protein reserves and increase the growth rate (Wu et al., 2014). As xylanase is an effective carbohydrate utilizing enzyme thus, it is necessary to determine its optimum level and effects in all aquaculture species. However, according to our knowledge no data regarding xylanase supplementation is available for *Cirrhinus mrigala* (mori), an important Indian major carp. Thus, to make its cultural practices more efficient it is necessary to determine the optimum xylanase supplementation level for plant-based diet in *Cirrhinus mrigala* juveniles and to evaluate its effects on growth performance, nutrient digestibility and digestive enzyme activities.

2. Material and methods

The experimental work was conducted in the Fish Nutrition Laboratory, Department of Zoology, University of Gujrat, Hafiz Hayat Campus, Gujrat, Pakistan and performed after the approval of Ethical Committee of University of Gujrat, Pakistan.

2.1. Feed preparation

The purpose of the present study was to determine the optimum xylanase inclusion in plant-based diet. Seven diets (sinking feed) were formulated to meet fish nutrient requirements. Xylanase (Econase – XT: 1,4-Beta Xylanase, 600,000 BXU/g: 1 BXU of xylanase produces 1 nmole of reducing sugars as xylose from birch xylan at 50 ℃ and pH 5.3 (De La Huebra, 2019), AB Vista), was added to experimental diets. A positive control (PC) diet (high fish meal content), 0 U/kg (0 g/kg), a negative control (NC) diet (high plant content), 0 U/kg (0 g/kg) and five other xylanase (U/kg) supplemented diets were formulated 600 (0.001 g/kg), 1200 (0.002 g/kg), 1800 (0.003 g/kg), 2400 (0.004 g/kg), 3000 (0.005 g/kg). The activity of xylanase was determined according to the method of Esteve-Garcia et al. (Esteve-Garcia et al., 1997) and the actual xylanase values were 75 (PC diet), 72 (NC diet), 680, 1273, 1882, 2486 and 3091 U/kg. Cottonseed meal (41 %), sunflower meal (30 %), corn gluten 60 % (57 %), rice polish (12 %), wheat (11 %) and fish meal (45 %) were added as protein sources in the diet (the crude protein composition is provided in brackets with the plant ingredients).

Individual feed ingredients were obtained from a local market. They were dried and crushed to make a fine powder of 0.05 mm. Ingredients were thoroughly mixed as per the composition of experimental diets (Table 1) and the corresponding xylanase levels were supplemented. Chromic oxide, an inert marker, was added to the experimental diets to measure nutrient digestibility. Fish oil was added gradually during mixing as a dietary lipid source. The ingredients were mixed uniformly in an electric mixer. Distilled water was added (up to 15 %) to the premix ingredients to produce an adequate level of moisture. The mixture was passed through a hand noodle maker to make feed pellets of 2 mm size. Lastly, the pellets were air dried until the moisture content remained up to 10%. Feed pellets were kept in labeled plastic bags and frozen at − 20 ℃ until they were required for feeding.

2.2. Experimental fish and feeding trial

A total of 315 mori, *Cirrhinus mrigala* juveniles were obtained from Imran Ali Hatchery, Alipur Chattha, Gujranwala, Punjab, Pakistan. They were given salt bath (5 g NaCl/kg) for removing ectoparasites. Before the trial began, juvenile mori were temporarily reared and fed basal diet (NC diet) for two weeks to acclimatize in the indoor recirculating aquaculture system (10 % daily water exchange) comprising of 21 rearing tanks (70 L water capacity each), circulation pump, mechanical filter, biological filter, UV sterilization lamp and temperature control device. Water was pumped to all tanks to maintain a speed of 1 L/min through the system. The dissolved oxygen level was maintained by continuous supplemental aeration in all rearing tanks. Fish were fasted for 24 h and weighed to determine the initial weight (8.34 $\pm$ 0.23 g) before the start of the feeding trial. After this period, 15 fish having similar weight were randomly assigned to tanks. Fish were hand-fed two times a day i.e., 09:00 and 17:00 to apparent satiation. Each diet treatment was assessed in triplicate. After active feeding, uneaten pellets were siphoned out to determine feed conversion ratio (FCR). Fecal material was siphoned off daily after two hours of feeding session for a duration of one month. Fecal samples were dried in oven, ground and pooled separately for each replicate. Parameters of water quality were maintained as follow: pH (7.2–8.5), temperature (26–31 ℃) and dissolved oxygen (5.4–7.7 mg/l). Final weight of the fish was measured after the termination of the trial.

2.3. Data collection

For sampling, the fish were starved for 24 h and euthanized by anesthesia overdose (clove oil). Data about fish number, initial body weight (IBW), final body weight (FBW), feed intake (FI), liver weight and viscera weight was used in the calculation of the following parameters,

Weight gain (WG) was calculated according to the following equation,

$$WG = FBW(g) - IBW(g)$$

FCR was calculated by the formula below,

$$FCR = \frac{FI(g)}{WG(g)}$$

Survival rate (SR) was calculated by the equation given below,

$SR(\%) = 100 \times \frac{\text{Final fish number}}{\text{Initial fish number}}$

Viscerosomatic index (VSI) and hepatosomatic index (HSI) were analyzed by the formula given below,

$VSI(\%) = 100 \times \frac{\text{Viscera weight(g)}}{\text{FBW(g)}}$

$HSI(\%) = 100 \times \frac{\text{Liver weight(g)}}{\text{FBW(g)}}$

For the determination of weight gain% (WG%) and specific growth rate (SGR) following formulas were used,

$$WG(\%) = 100 \times \frac{FBW(g) - IBW(g)}{IBW(g)}$$

$$SGR = 100 \times \frac{\ln(FBW, g) - \ln(IBW, g)}{Time(days)}$$

2.4. Proximate composition analysis

Feed ingredients, feed, feces and fish whole body (5 fish from each replicate) samples were analyzed for proximate composition according to the standard method of AOAC, 1995. Samples were homogenized in pestle and mortar and three sub-samples were prepared from each replicate group (n = 3 × 3). Dry matter (DM) was determined by weighing the oven dried (UNB-400, Memmert, Germany) samples. Crude protein (CP) was analyzed by micro Kjeldahl's method. For this purpose, the digestion mixture (K_2SO_4, $FeSO_4$ and $CuSO_4$) and sample were heated till color change (greenish), filtered and diluted. Afterward, distillation was carried out with NaOH. Liberated ammonia was collected in a flask containing boric acid as indicator. After titrating against sulfuric acid, nitrogen percentage was calculated. Crude fat (CF) was determined by soxhlet extraction method with petroleum ether and crude ash (CA) was determined by using muffle furnace (Ney VULCAN 3–550) at 600 ℃ for 24 h. Gross energy (GE) was determined by adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline. USA).

2.5. Digestibility calculation

Apparent digestibility coefficient (ADC) of DM, CP, CF, CA and GE of feed and stored fecal samples was assessed by following AOAC, 1995. Three sub-samples from each replicate (n = 3 × 3) were taken to determine the content of chromic oxide in feed and stored fecal samples by following the procedure described by Czarnocki et al. (Czarnocki et al., 1961). Briefly, the samples were allowed to stand overnight in nitric acid, heated to dryness afterward and digestion mixture (sodium molybdate, distilled water, sulfuric acid and perchloric acid) was subsequently added until green color changed to yellow. The colorimetric reaction was quantified spectrophotometrically at 350 nm. Values of analyzed nutrients and chromic oxide from feed and feces samples were used to determine the value of apparent digestibility coefficient (ADC) by following formula (National Research Council, 2011),

$ADC(\%) = 100 - 100 \times \frac{\text{Percent marker in diet} \times \text{Percent nutrient in feces}}{\text{Percent marker in feces} \times \text{Percent nutrient in diet}}$

2.6. Digestive enzymes assays

Analysis of amylase, protease and lipase was done by taking five fish samples from each replicate for intestinal and

Table 2
Effect of xylanase supplemented plant-based diet on growth indices of mori.

Variables	Xylanase U/kg							Pooled SE *	p-value
	PC	NC	680	1273	1882	2486	3091		
IW (g)	8.21	8.48	8.43	8.35	8.37	8.26	8.32	–	–
FW (g)	27.31^{b}	24.46^{f}	25.30^{e}	26.72^{c}	28.40^{a}	26.83^{c}	25.92^{d}	0.07	< 0.05
WG (g)	19.10^{b}	15.96^{f}	16.87^{e}	18.37^{c}	20.03^{a}	18.58^{c}	17.61^{d}	0.06	< 0.05
WG (%)	232.80^{ab}	188.40^{f}	200.30^{ef}	220.00^{d}	239.50^{a}	225.00^{bc}	211.80^{de}	2.22	< 0.05
SGR	2.00^{ab}	1.77^{e}	1.83^{de}	1.94^{bc}	2.04^{a}	1.96^{b}	1.90^{cd}	0.01	< 0.05
FI (g)	19.32^{bc}	20.56^{a}	18.91^{c}	19.90^{b}	19.91^{b}	19.44^{bc}	19.82^{b}	0.11	< 0.05
FCR	1.01^{e}	1.29^{a}	1.12^{b}	1.08^{c}	0.99^{e}	1.05^{d}	1.13^{b}	0.01	< 0.05
SR (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	–
HSI	4.94^{a}	4.54^{e}	4.65^{de}	4.77^{bc}	4.88^{ab}	4.75^{cd}	4.61^{e}	0.02	< 0.05
VSI	18.51^{a}	16.52^{c}	16.80^{bc}	17.13^{b}	18.10^{a}	17.24^{b}	16.80^{bc}	0.08	< 0.05

Means with dissimilar superscript were determined by Tukey's HSD test and are significantly different ($P < 0.05$). Mean values represent the average value of three aquaria per treatment (N = 3).

*Pooled SE = $\sqrt{MSE/r}$

IW= Initial weight, FW= Final weight, WG= weight gain, SGR= Specific growth rate, FI= Feed intake, FCR= Feed conversion ratio, SR= Survival rate, HSI= Hepatosomatic index, VSI= Viscerosomatic index hepatopancreatic tissue collection. Tissue samples were homogenized separately in sucrose solution and centrifuged at 5000 g for enzyme assay. The supernatant was collected for further testing. The enzyme assays were performed by taking three sub-samples from each replicate ($n = 3 \times 3$). Dinitrosalicylic acid method (Rick and Stegbauer, 1974) was used to determine amylase activity by using 2 % (w/v) starch solution as a substrate. Casein digestion method (Kunitz, 1947) was used for determining protease activity by using 1 % casein as a substrate. Lipase kit (Lipase-LQ, Spinreact, Spain) was used for determining lipase activity.

2.7. Statistical analysis

The data collected from the replicate (03 replicates per treatment) of all samples was analyzed by one-way analysis of variance (ANOVA) under CRD (completely randomized design) through CoStat statistical software package (Version 6.303, PMB 320, Monterey, CA, 93940 USA). For comparison of means, Tukey's Honestly Significant Difference (HSD) test was used. Differences were considered significant at $P < 0.05$.

3. Results

No mortalities were observed throughout the feeding trial (Table 2). The effects of xylanase supplemented plant-based diet on final weight (FW), WG, WG% and SGR% in mori are shown in Table 2. Supplementation of xylanase significantly ($P < 0.05$) improved FW, WG, WG% and SGR in comparison to NC diet containing no supplemented xylanase. As the level of xylanase increased from 72 to 1882 U/kg in diet, a gradual increase in FW, WG, WG% and SGR was observed. However, further increase in xylanase concentration from 2486 to 3091 U/kg decreased the FW, WG, WG% and SGR. The mean values recorded at 1882 U/kg xylanase level for all growth parameters were statistically higher or equal to PC diet (high fish meal content). The optimum value of xylanase supplementation was recorded as 1948 U/kg after subjecting the data (WG% vs xylanase levels U/kg) to quadratic regression analysis. The equation $Y_{WG\%} = 179.9 + 0.05064\,x - 0.000013\,x^2$ gives the relationship between WG% and xylanase levels U/kg. Data for FI and FCR is also provided in Table 2. Xylanase supplementation significantly improved the FI and FCR values in comparison to NC diet. The best FCR was observed at 1882 U/kg level of xylanase which was non-significant to PC diet (high fish meal content).

Values of HSI and VSI of juvenile mori fed experimental diets with graded xylanase supplementation are shown in Table 2. HSI significantly ($P < 0.05$) improved as the level of xylanase was increased. The HSI value recorded at 1882 U/kg showed statistical similarity to PC diet (high fish meal content). In comparison, the value recorded at 680 and 3091 U/kg showed a non-significant ($P > 0.05$) impact of xylanase supplementation and was similar to NC diet (high plant content). Also, VSI showed a similar trend where it significantly ($P < 0.05$) increased from 72 to 1882 U/kg of xylanase and decreased with further increase (2486–3091 U/kg) in xylanase supplementation. Furthermore, highest value of VSI was recorded at 1882 U/kg xylanase which was non-significant to the PC diet.

The effect on proximate analysis of whole body of juvenile mori fed graded xylanase levels is recorded in Table 3. Dietary xylanase significantly ($P < 0.05$) improved the whole body DM, CP, CF, CA and GE in comparison to NC diet. However, the value of CA recorded at 3091 U/kg of xylanase was non-significant to NC diet ($P > 0.05$). Overall, an increasing trend was observed, where the increase in xylanase supplementation level increased the values of whole body parameters, peaking at 1882 U/kg of xylanase which was non-significant ($P > 0.05$) to PC diet. However, a decline was noted with further increase in xylanase levels (2486–3091 U/kg).

The data for ADCs of DM, CP, CF, CA and GE is provided in Table 4. In comparison to the NC diet, supplementation of exogenous xylanase at all graded levels significantly ($P < 0.05$) improved the ADCs of DM, CP, CF, CA and GE. A subsequent increase was observed in the values of ADCs for DM, CP, CF and GE with an increase in exogenous xylanase level, reaching to a maximum value at 1882 U/kg, which was non-significant to PC diet. However, a decline was observed in these parameters with further increase in xylanase level from 2486 U/kg to 3091 U/kg. Moreover, the value of CA for 1273 U/kg of xylanase was non-significant in comparison to PC diet.

Marked differences were noted in the digestive enzyme (amylase, protease and lipase) activities of hepatopancreatic and intestinal tissues of mori fed with graded xylanase levels (Table 5). Activity of hepatopancreatic amylase showed an increasing trend with

Table 3
Effect of xylanase supplemented plant-based diet on whole body proximate composition of mori (n = 5).

Variables	Xylanase U/kg							Pooled SE *	p-value
	PC	NC	680	1273	1882	2486	3091		
DM	89.87[a]	88.94[e]	89.16[d]	89.33[c]	89.87[a]	89.63[b]	89.33[c]	0.02	< 0.05
CP	17.43[a]	16.86[d]	17.02[c]	17.24[b]	17.47[a]	17.26[b]	17.07[c]	0.01	< 0.05
CF	4.94[a]	4.17[e]	4.28[d]	4.65[b]	4.93[a]	4.53[c]	4.30[d]	0.02	< 0.05
CA	4.88[a]	4.23[d]	4.42[c]	4.50[b]	4.93[a]	4.52[b]	4.25[d]	0.01	< 0.05
GE (Kcal/g)	584.00[a]	540.00[f]	551.00[d]	563.00[b]	583.00[a]	556.00[c]	552.00[e]	1.43	< 0.05

Means with dissimilar superscript were determined by Tukey's HSD test and are significantly different ($P < 0.05$). Mean values represent the average value of three aquaria per treatment (N = 3).

*Pooled SE = $\sqrt{MSE/r}$

DM= Dry matter, CP= Crude protein, CF= Crude fat, CA= Crude ash, GE= Gross energy

Table 4
Effect of xylanase supplemented plant-based diet on ADC (%) of mori.

Variables	Xylanase U/kg							Pooled SE *	p-value
	PC	NC	680	1273	1882	2486	3091		
DM	84.87^{a}	63.18^{d}	67.82^{c}	73.32^{b}	83.77^{a}	74.36^{b}	69.10^{c}	0.25	< 0.05
CP	84.60^{a}	75.82^{d}	78.04^{c}	81.17^{b}	84.58^{a}	80.96^{b}	78.57^{c}	0.19	< 0.05
CF	81.31^{a}	59.75^{e}	72.76^{c}	76.68^{b}	81.50^{a}	72.73^{c}	69.61^{d}	0.40	< 0.05
CA	77.19^{a}	55.83^{d}	66.09^{bc}	71.97^{ab}	76.71^{a}	71.64^{ab}	65.37^{c}	1.11	< 0.05
GE	80.24^{a}	60.14 ^{f}	70.28^{d}	74.32^{b}	80.47^{a}	72.45^{c}	68.54^{e}	0.56	< 0.05

Means with dissimilar superscript were determined by Tukey's HSD test and are significantly different (P < 0.05). Mean values represent the average value of three aquaria per treatment (N = 3).
DM= Dry matter, CP= Crude protein, CF= Crude fat, CA= Crude ash, GE= Gross energy
*Pooled SE = $\sqrt{MSE/r}$

increase in xylanase up to 1882 U/kg level and decreased afterward. Also, its activity at 1882 U/kg level of xylanase was non-significant (P > 0.05) with PC diet. Furthermore, the value recorded at 3091 U/kg of xylanase (highest supplemented level) was non-significant (P > 0.05) in comparison to NC diet (high plant content diet). A similar trend was observed in intestinal amylase activity. However, all recorded values for intestinal amylase were significant (P < 0.05) compared to NC diet. In case of hepatopancreatic and intestinal protease, graded xylanase supplementation significantly (P < 0.05) improved all values in comparison to NC diet. Increase in xylanase level (up to 1882 U/kg) increased the activity of protease in both tissues, but a decline (at 2486 and 3091 U/kg xylanase levels) was observed after 1882 U/kg xylanase level. Moreover, the activities of protease recorded at 1882 U/kg and PC diet were varied non-significantly from each other. Activity of hepatopancreatic and intestinal lipase also showed a similar increasing trend up to 1882 U/kg xylanase level and declined thereafter Fig. 1.

4. Discussion

According to the present study, xylanase supplementation significantly improved the growth performance of mori up to 1882 U/kg. At this level, values of FBW and WG were higher (P < 0.05) than PC diet (high fish meal content) while WG% and SGR were varying non-significantly from PC diet. This depicts that 1882 U/kg xylanase level had a performance comparable to diet with high fish meal content (PC diet). In comparison, the fish fed NC diet (high plant content) showed a significantly lower growth performance due to a relatively higher content of NSPs (Halver, 2003). The NSPs in plant meal combine with water and cause intestinal mucosal thickening that may limit the availability of nutrients. So, fewer nutrients are available for digestion, which ultimately reduces the growth performance (Sinha et al., 2011). Supplementation of exogenous xylanase improves the digestibility of plant diet thus improving the fish growth performance. Similar results regarding growth parameters (FBW, SGR and WG%) were reported by Jin et al. (2020) in grass carp (*Ctenopharyngodon idella*) fed 1254 U/kg xylanase supplemented diet (Nanning Doing-Higher Bio-Tech., Ltd.). Nile tilapia (*Oreochromis niloticus*) fed diet supplemented with 3750 U/kg xylanase (Huvepharma, Antwerp, Belgium) showed highest FBW, WG and SGR compared to control (Hassaan et al., 2019). Similarly, Luo et al. (2020) reported a significant effect of 1200 U/kg xylanase level (Sunhy Biology Co., Ltd. Wuhan, Hubei, China) in large yellow croaker (*Larimichthys crocea*). Furthermore, Jiang et al. (2014) observed the highest FBW, WG% and SGR in Jian carp (*Cyprinus carpio* var. Jian) fed 1480 U/kg xylanase (Huvepharma, Antwerp, Belgium) supplemented diet compared to other experimental diets. In a study conducted by Babalola (2006), catfish (*Clarias gariepinus*) showed highest growth performance when fed a combination of *Moina* and microdiet supplemented with 0.1 g/kg of xylanase (Nutrase xyla derived from *Bacillus subtilis*; Nutrex N.V. Co. Ltd). Moreover, Maas et al. (2018) reported that 4000 U/kg of xylanase (Danisco

Table 5
Effect of xylanase supplemented plant-based diet on activities of hepatopancreatic and intestinal enzymes (amylase, protease and lipase) (n = 5).

Variables	Xylanase U/kg							Pooled SE *	p-value
	PC	NC	680	1273	1882	2486	3091		
Hepatopancreatic tissue									
Amylase (U/g)	1052.00^{a}	910.00^{d}	958.00^{c}	992.00^{b}	1069.00^{a}	984.00^{b}	930.00^{d}	4.10	< 0.05
Protease (U/g)	4.57^{a}	3.96^{e}	4.27^{c}	4.47^{b}	4.63^{a}	4.25^{c}	4.05^{d}	0.01	< 0.05
Lipase (U/g)	952.00^{a}	825.00^{d}	860.00^{c}	910.00^{b}	967.00^{a}	918.00^{b}	845.00 ^{cd}	3.83	< 0.05
Intestinal tissue									
Amylase (U/g)	783.00^{a}	627.00^{d}	666.00^{c}	730.00^{b}	796.00^{a}	731.00^{b}	679.00^{c}	4.04	< 0.05
Protease (U/g)	4.57^{a}	3.65^{d}	3.88^{c}	4.15^{b}	4.60^{a}	4.22^{b}	3.95^{c}	0.02	< 0.05
Lipase (U/g)	818.00^{a}	733.00^{d}	766.00^{c}	797.00^{b}	827.00^{a}	791.00^{b}	754.00^{c}	3.11	< 0.05

Means with dissimilar superscript were determined by Tukey's HSD test and are significantly different (P < 0.05). Mean values represent the average value of three aquaria per treatment (N = 3).
*Pooled SE = $\sqrt{MSE/r}$

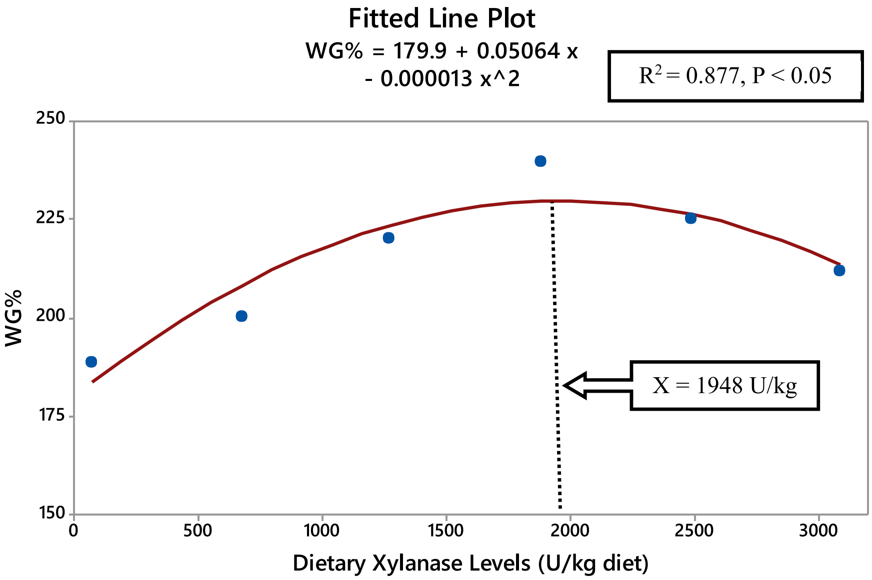

Fig. 1. Quadratic regression analysis of weight gain% (WG%) for juvenile mori fed plant-based diet supplemented with graded levels of xylanase for 60 days. Each point in the graph represents the mean of triplicate diet treatment of mori with 15 fish per group. Optimal xylanase supplementation level was calculated to be 1948 U/kg.

Animal Nutrition, Marlborough, UK) in combination with 1000 FTU/kg of phytase (Buttiauxella sp. Danisco Animal Nutrition, Marlborough, UK) improved the growth performance of Nile tilapia (*Oreochromis niloticus*). In another recent study Maas et al. (2021) reported that 6000 U/kg of xylanase (Danisco®, DuPont Animal Nutrition) and 1000 FTU/kg of phytase (Axtra® PHY, *Buttiauxella* sp., DuPont Animal Nutrition) mixture improved the growth performance and SGR of Nile tilapia (*Oreochromis niloticus*) to a greater extent in comparison to the fish fed probiotics (Enviva® PRO 202 GT 60 mg kg feed providing 150,000 CFU/g feed) supplemented diet. The combination of phytate hydrolytic effect of phytase and the xylan hydrolytic effect of xylanase might be more effective in improving the growth performance. Similar studies with xylanase and phytase mixture for other fish species will be worth evaluating which may lead towards effective feed formulation.

Xylanase supplementation at the level of 3091 U/kg, in the present study, showed poor growth performance compared to 1882 U/kg. Similarly, Jin et al. (2020) reported poor growth performance in grass carp (*Ctenopharyngodon idella*) fed 3076 U/kg of xylanase (Nanning Doing-Higher Bio-Tech., Ltd.) supplemented diet in comparison to 1254 U/kg of xylanase. Moreover, similar result was also reported in Jian carp (*Cyprinus carpio* var. Jian) fed 2470 U/kg of xylanase (Huvepharma, Antwerp, Belgium) in comparison to 1480 U/kg xylanase (Jiang et al., 2014). Lower growth performance at higher levels of xylanase supplementation may be attributed to the excess xylooligosaccharide production in fish gut (Chapla et al., 2012). The excess xylooligosaccharides retain more moisture in gastrointestinal tract, which leads to diarrhea (Gupta et al., 2016) ultimately reducing the growth performance. Poor growth performance was observed in crucian carp (*C. auratus gibelio*) fed diet supplemented with overdose of xylooligosaccharides (Xu et al., 2009). However, Dalsgaard et al. (2012) observed a non-significant effect of xylanase supplementation (XYL, RONOZYME® WX (L), DSM Nutritional Products, Saint-Louis Cedex, France) in rainbow trout (*Oncorhynchus mykiss*) which may be due to the carnivorous feeding nature of the fish and short trial duration i.e. 17–19 days.

The NSP utilization in aquatic species is less efficient (Saputra et al., 2016). In the present study, highest FI and FCR were observed in the NC diet, depicting that the fish fed unsupplemented high plant content diet had poor ability to convert the diet ingredients to their desired output hence increasing the feed intake. In our study, a significant ($P < 0.05$) decrease in FI and FCR was observed against xylanase supplementation. Supplementation of exogenous xylanase might improve the feed efficiency leading to an improved feed utilization. Similar to our findings, Jin et al. (2020) reported the highest feed efficiency in grass carp (*Ctenopharyngodon idella*) fed 1254 U/kg of xylanase supplemented diet (Nanning Doing-Higher Bio-Tech., Ltd.) compared to other experimental diets. Xylanase supplementation at the level of 3750 U/kg (Huvepharma, Antwerp, Belgium) improved the feed utilization in Nile tilapia (*Oreochromis niloticus*) (Hassaan et al., 2019). Also, Jian carp (*Cyprinus carpio* var. Jian) fed 1070 and 1480 U/kg xylanase supplemented diet (Huvepharma, Antwerp, Belgium) showed highest feed utilization (Jiang et al., 2014). Cheng et al. (2018) noted the highest feed efficiency in red tilapia (*Oreochromis* sp.) fed 1500 EPU/kg of xylanase (Hostazym® X-xylanase, Huvepharma, Antwerp, Belgium). However, Luo et al. (2020) reported a non-significant effect of xylanase supplementation (Sunhy Biology Co., Ltd. Wuhan, Hubei, China) on feed efficiency of large yellow croaker (*Larimichthys crocea*) which might be related to the carnivorous feeding nature of this fish.

Values of organ indices i.e. HSI and VSI are correlated with nutritional status, body growth and physiology of fish (Lee et al., 2002). According to the present study, xylanase supplementation positively affected ($p < 0.05$) the HSI and VSI values in mori. This result shows that xylanase supplementation may support the normal growth and physiology of mori juveniles. Jiang et al. (2014) reported a similar significant positive effect regarding the body indices of Jian carp (*Cyprinus carpio* var. Jian) fed graded xylanase (Huvepharma, Antwerp, Belgium) levels (650, 1070, 1480, 1810 and 2470 U/kg). However, Luo et al. (2020) noted a non-significant improvement in HSI and VSI values in large yellow croaker (*Larimichthys crocea*) fed xylanase supplemented (Sunhy Biology Co., Ltd. Wuhan, Hubei, China) diet. This non-significant response may be attributed to the fact that this fish is carnivorous and the addition of plant-based feed ingredients supplemented with xylanase might not have any effect on fish body indices.

Whole body composition is an important marker for understanding the habitat quality, feeding conditions of the species (Wicker and Johnson, 1987) and determines the bioenergetics (Van Pelt et al., 1997). In this study, xylanase supplementation significantly affected the whole body contents of DM, CP, CF, CA and GE. The improvement in whole body proximate composition of mori may be attributed to the improved nutrient digestibility as was observed in this study. Higher nutrient availability might result in their higher deposition in fish body. Jiang et al. (2014) observed the highest body protein content at 1480 U/kg xylanase (Huvepharma, Antwerp, Belgium) in Jian carp (*Cyprinus carpio* var. Jian). However, rest of the whole body parameters were non-significant in the same study (Jiang et al., 2014). Moreover, studies performed on large yellow croaker (*Larimichthys crocea*) (Luo et al., 2020), Nile tilapia (*Oreochromis niloticus*) (Hassaan et al., 2019) and red tilapia (*Oreochromis* sp.) (Cheng et al., 2018) showed that xylanase supplementation did not significantly affect the whole body composition. The disagreement in the results might be attributed to the difference in feed production processes which may impact the overall feed composition and the activity of xylanase.

The NSPs in plant-based diet combine with water and increase the gut viscosity. This increased viscosity traps the nutrients and decreases their digestibility. However, supplementation of xylanase eliminates these effects by increasing xylan breakdown (Adeola and Cowieson, 2011) thus, improving the nutrient digestibility. According to the findings of this study, xylanase supplementation significantly improved the ADC of DM, CP, CA, CF and GE in comparison to the NC diet. Moreover, at optimal level, the performance of xylanase was comparable to PC diet (higher fish meal content). Our result was in agreement with the result reported by Hassaan et al. (2019) who observed a significant effect of xylanase supplementation at the level of 3750 U/kg (Huvepharma, Antwerp, Belgium) on ADC of DM, CP, crude lipid and digestible energy in Nile tilapia (*Oreochromis niloticus*).

Exogenous xylanase supplementation may enhance the activity of endogenous enzymes. As the activity of endogenous enzymes improve, so does the rate of digestion and absorption (Wen et al., 2009). In this study, supplementation of xylanase significantly improved the activity of amylase, protease and lipase. Highest activities of these digestive enzymes were expressed at the level of 1882 U/kg of xylanase which was similar to the PC diet. This result was in agreement with the previous studies. Hassaan et al. (2019) observed a significant effect of 3750 U/kg of xylanase (Huvepharma, Antwerp, Belgium) on intestinal digestive enzyme activities (chymotrypsin, trypsin, amylase and lipase) of Nile tilapia (*Oreochromis niloticus*). Similar effects were observed in the hepatopancreatic and intestinal digestive enzymes (chymotrypsin, trypsin, amylase and lipase) of Jian carp (*Cyprinus carpio* var. Jian) fed 1070 and 1480 U/kg of xylanase (Huvepharma, Antwerp, Belgium) (Jiang et al., 2014). Luo et al. (2020) also observed a significant effect of xylanase (Sunhy Biology Co., Ltd. Wuhan, Hubei, China) on the activities of stomach pepsin and intestinal lipase of large yellow croaker (*Larimichthys crocea*). Due to the scarcity of available literature, further investigations are required to understand the underlying mechanisms and pathways through which exogenous xylanase may improve the endogenous enzyme activities in both herbivorous and carnivorous fishes.

5. Conclusion

The results of this experimental study conclude that mori fed xylanase supplemented plant-based diet had a better ability to utilize the plant ingredients in comparison to mori fed unsupplemented diet (NC diet). The highest values of growth performance, nutrient digestibility and digestive enzyme activities were found at the level of 1882 U/kg supplemented xylanase. Performances of those fish were not significantly different from the performance of fish fed PC diet, which contained high levels of fish meal. Moreover, quadratic regression analysis showed that 1948 U/kg of xylanase was the optimum value for the supplementation of xylanase in plant-based diet for mori juveniles. Higher xylanase supplementation levels (2486 and 3091 U/kg) resulted in lower growth performance, indicating that higher levels of supplementation are unnecessary.

CRediT authorship contribution statement

Hafsa Nadeem: Trial conduction, manuscript preparation, manuscript revision, **Syed Zakir Hussain Shah**: Supervision of whole study, manuscript revision, **Mahroze Fatima**: Experimental design, statistical analysis, **Maryam**: Sample analysis, manuscript revision, **Muhammad Mudassar Shahzad**: Sample analysis.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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