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Exogenous xylanase improves growth, protein digestibility and digestive enzymes activities in Nile tilapia, *Oreochromis niloticus*, fed different ratios of fish meal to sunflower meal

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Abstract

A 3 × 2 factorial experiment was designed to evaluate the effect of different ratios of fish meal (FM): sunflower meal (SFM) with or without exogenous xylanase supplementation on growth, feed utilization, digestive enzymes activities, apparent digestibility, intestinal and liver morphology and chemical composition of Nile tilapia, *Oreochromis niloticus*. Three isonitrogenous (329.80 g/kg of crude protein) and isoenergetic (18.46 MJ/kg gross energy) experimental diets were formulated as SFM_1 (FM:SFM = 2:1), SFM_2 (FM:SFM = 1:1) and SFM_3 (FM:SFM = 1:2) based on protein content. Each diet was supplemented with 0 or 0.5 g/kg of exogenous xylanase and was fed to triplicate groups of twelve fish (with initial weight, 1.31 ± 0.02 g) for 84 days. After 84 days of feeding period, the highest weight gain, specific growth rate, protein efficiency, protein productive value and the best feed conversion ratio were recorded in fish fed either SFM_1 or SFM_2 supplemented with exogenous xylanase. Whereas lowest growth performance was recorded in fish fed SFM_2 and SFM_3 un-supplemented with xylanase. The highest activities of chymotrypsin, trypsin, lipase, amylase, alkaline phosphatase and cholecystokinin were observed in fish fed SFM_1 and SFM_2 diets supplemented with xylanase. The highest ADCs of dry matter, protein, lipid and digestible energy were recorded in fish fed SFM_1 and SFM_2 diets supplemented with exogenous xylanase. Supplementation of exogenous xylanase improved muscularis mucosa thickness, height of mucosal folds and enterocytes of intestinal fish. Addition of exogenous xylanase increased the calcium and phosphorus retention. Results of this study indicated that the addition of exogenous xylanase to diet containing high inclusion level of sunflower meal improved growth, digestive enzymes, nutrient digestibility, histological morphometric of liver and intestine and nutrient retention.

KEYWORDS

digestive enzymes, fishmeal, growth, histology, sunflower meal, xylanase

1 | INTRODUCTION

In last decade, the global demand for food fish is continually expanding accordingly to the increase in population with the share of aquaculture production increasing from 29% to 38% of global fish production (FAO, 2016). However, the increased production in intensive and semi-intensive system necessitates the supply of high quantity and sustainable feed ingredients in balanced formulated diets for cultured fish. To support increasing production of aquaculture sector, there is a need for economical and sustainable feed production. Nowadays, plant protein and their by-products are a suitable strategy to increase the sustainability of aquaculture production by reducing feed costs as well as reducing the environmental adverse impact (Tacon & Metian, 2015). Several alternative plant protein sources in tilapia diets have been investigated in recent years due to the reduction in fish meal production and increasing its cost, such as cotton seed meal (El-Saidy & Gaber, 2004), okara meal (El-Saidy, 2011), fermented soybean meal (Hassaan, Soltan, & Abdel-Moez, 2015), soy protein concentrate (Ribeiro, Vidotti, Ferreira, & Gonçalves, 2016), Jatropha meal (Hassaan, Goda, & Kumar, 2017), corn protein concentrate (Khalifa, Belal, El-Tarabily, Tariq, & Kassab, 2018) and fermented sunflower meal (Hassaan et al., 2018), which were showed to be suitable as partial replacements for fish meal. However, total replacement of fish meal with plant proteins has generally resulted in decrease in fish growth performance and feed utilization. This decrease has been contributed to the presence of antinutritional factors (ANFs) or imbalance in essential amino acid.

Sunflower meal is almost exclusively employed for poultry and cattle feeding owing to its high content of highly digestible protein with an important content of essential amino acid except for lysine and sulphur amino acid (González-Pérez & Vereijken, 2007). However, in fish feeding it has been used with low inclusion level (Olvera-Novoa, Olivera-Castillo, & Martínez-Palacios, 2002; Syntayehu, Mathies, & Meyer-Burfdorff, 1996), which may be due to the presence of ANFs (Francis, Makkar, & Becker, 2001; Gatlin et al., 2007), high content of phenolic compounds (chlorogenic acid and caffeic acid) (González-Pérez & Vereijken, 2007; Prigent et al., 2003) and high fibre content (Hertrampf & Piedad-Pascual, 2012). Furthermore, the main reason for underutilization of sunflower meal is non-starch polysaccharides (NSPs) which limit the availability of nutrient (Düsterhöft, Voragen, & Engels, 1991; Sinha, Kumar, Makkar, De Boeck, & Becker, 2011). These components, NSPs, cannot be hydrolysed because fish have scare or absence of endogenous xylanase secretion in their gastrointestinal tract (Eleraky, Ibrahim, & Mahmoud, 2016). Accordingly, destruction or elimination of ANFs that inhibit nutrient utilization is compulsory for successful use of plant protein for aquafeed.

Application of exogenous enzymes may be used to inactivate ANFs and consequently results in improved rate of digestion and nutrient absorption of plant protein for aquatic animal (Dalsgaard et al., 2012; Jiang et al., 2014; Lin, Mai, & Tan, 2007; Soltan, 2009). Exogenous xylanase is capable of disrupting plant cell wall integrity, thereby reducing molecular size characteristics of NSPs. Consequently, this enhances rapid digestion by reducing viscosity in the gut (Adeola & Cowieson, 2011). Furthermore, exogenous enzymes may alter substrate availability for specific populations of gut microbes, which promote digestion of nutrient and synthesize nutrient substance that the fish need (Jiang et al., 2014; Zhou et al., 2013). Also, the degradation of arabinoxylans, one of the crucial categories of soluble NSPs, depended on the level of xylanase supplementation (Dornez, Gebruers, Delcour, & Courtin, 2009). In addition, the application of exogenous enzymes can make the usage of lower quality plant protein ingredients to be more fixable in diet formulation (Adeoye, Jaramillo-Torres, Fox, Merrifield, & Davies, 2016). Effects of exogenous enzymes were variable and dependent on enormous factors, for instance the fish age, the quality and the type of diet (Zamini, Kanani, Esmaeili, Ramezani, & Zoriezahra, 2014). Exogenous enzymes are widely used in fish diets to increase the efficiency of nutrient of feed (Adeoye et al., 2016; Ai et al., 2007; Farhangi & Carter, 2007; Hassaan, Soltan, Agouz, & Badr, 2013; Hlophe-Ginindza, Moyo, Ngambi, & Ncube, 2016; Lin et al., 2007; Liu, Wu, Li, Duan, & Wen, 2018; Magalhães et al., 2016). Otherwise, a few studies have been investigated the effect of xylanase on growth performance and nutrient utilization. These studies indicated that growth and feed utilization were improved by xylanase supplementation in Japanese sea bass, *Lateolabrax japonicus* (Ai et al., 2007), and African catfish, *C. gariepinus* (Babalola, 2006). The digestibility of all nutrients including carbohydrates, protein and minerals was affected by exogenous enzymes (Felix & Selvaraj, 2004). Also, the activities of endogenous enzymes such as trypsin, chymotrypsin, lipase and amylase in the intestine of juvenile carp, *Cyprinus carpio*, were improved with xylanase supplementations (Adeoye et al., 2016; Jiang et al., 2014). Consequently, there is a need for further studies to establish the benefits of dietary xylanase supplementation for fish. To the authors' knowledge, no studies have been investigated the effect of exogenous xylanase on Nile tilapia fed diet containing sunflower meal. Therefore, the objective of this study was conducted to evaluate the effect of xylanase supplementation on growth, apparent digestibility and digestive enzymes activities of Nile tilapia fed diet containing different ratios of fish meal to sunflower meal.

2 | MATERIALS AND METHODS

2.1 | Experimental design and feeding trial

Nile tilapia, *O. niloticus*, were obtained from El-Kanater El-Khayria, Fish Research Station, National Institute of Oceanography and Fisheries (NIOF), Kalubiya Governorate, Egypt. Fish were acclimatized to the experimental conditions for 15 days with continuous aeration in fish nutrition laboratory of NIOF. During this period, they were fed on commercial diet (320g/kg crude protein). Prior to the commencement of the experiment, all fish were starved for 24 hr.

TABLE 1 Formulation and proximate chemical composition of experimental diets (g/kg diets) and hydrolysed essential amino acid composition (g7kg)

	SFM_1	SFM_2	SFM_3
Ingredient			
Fish meal[a]	180	150	90
Sunflower meal[b]	170	270	360
Soybean meal[c]	300	300	300
Yellow corn[d]	150	150	120
Wheat bran[e]	140	70	70
Soya oil	40	40	40
Vitamin and Mineral[f]	14.50	14.50	14.50
Vitamin C[g]	0.5	0.5	0.5
Chromic oxide[h]	5	5	5
Chemical analysis			
Dry matter	892.50	899.25	921.90
Protein	319.40	322.24	312.00
Lipid	79.40	79.20	77.30
Ash	63.50	59.00	53.80
Fibre	76.40	90.70	108.90
Nitrogen-free extract[i]	461.30	448.7	448.00
Gross energy (MJ/kg diet)[j]	18.60	18.41	18.07
Essential amino acids			
Arginine	20.8	21.2	20.9
Histidine	8.7	8.8	8.4
Lysine	18.3	17.9	16.6
Methionine	5.9	5.8	5.3
Leucine	24.2	24.4	23.5
Threonine	12.4	12.4	11.8
Phenylalanine	14.9	15.3	15.1
Valine	15.8	16.0	15.5
Total essential amino acids	121	121.8	117.1

[a]Fish meal Pakistan fish meal 580g/kg protein obtained from Plot-19, Sector, 16 Korangi Industrial Area Karachi, Pakistan. [b]Sunflower meal (300g/kg protein, 53g/kg lipid, 69g/kg ash, 215g/kg, fibre and 363g/kg carbohydrate) was supplied from Research Institute of Oil Crops, Agricultural Research Center Cairo, Egypt. [c]Egyptian soybean 440g/kg protein was supplied from Research Institute of Oil Crops, Agricultural Research Center Cairo, Egypt. [d]Yellow corn 85g/kg protein purchased from local market [e]Wheat bran 137.5g/kg protein purchased from local market [f]Vitamin and mineral mixture per kg of mixture contains 4,800 I.U. Vit A, 2,400 IU cholecalciferol (vit. D), 40 g Vit E, 8 g Vit K, 4.0 g Vit B_{12}, 4.0 g Vit B2, 6 g Vit B6, 4.0 g, pantothenic acid, 8.0 g nicotinic acid, 400 mg folic acid, 20 mg biotin, 200 gm choline, 4 g copper, 0.4 g iodine, 12 g iron, 22 g manganese, 22 g zinc, 0.04 g selenium. Folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39% Na, 61% Cl), 3,077 mg; ferrous sulphate ($FeSO_4 \cdot 7H_2O$, 20% Fe), 65mg; manganese sulphate ($MnSO_4$, 36% Mn), 89 mg; zinc sulphate ($ZnSO_4 \cdot 7H_2O$, 40% Zn), 150 mg; copper sulphate ($CuSO_4 \cdot 5H_2O$, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I). [g]Vitamin C Tecno-C, (TM, TECHNOMD) 1 Montazh street, Heliopolis, Cairo, Egypt. [h]Oxford laboratory reagent, Mumbai, India [i]Nitrogen-free extract = 100 − (crude protein +lipid + ash +fibre content). [j]Gross energy calculated using gross calorific values of 23.63, 39.52 and 17.15 kJ/g for protein, fat and carbohydrate, respectively, according to Brett, 1973.

This practice was to eliminate variation in weight due to residue feed in the gut and to prepare the gastrointestinal tract for the experimental diets, while at the same time to increase the appetite of the fish. After acclimation, the fingerlings were stocked in 18 glass aquariums (60 L) with static conditions system. Two hundred and sixteen of Nile tilapia, *O. niloticus*, with an average initial body weight (1.31 ± 0.02 g, n = 36) were used for the experiment. Three replicate aquaria were randomly distributed to each treatment at a rate of 12 fish per aquarium. The aquaria were supplied with dechlorinated tap water and were continuously supplied with compressed air for oxygen requirement. Triplicate groups of fish were fed close to apparent satiation four per day at 9:00 a.m., 11.00 a.m., 13.00 p.m. and 15.00 p.m. Fish were weighed every 2 weeks to record their growth. About one-third of the water volume in each aquarium was daily renewed with water flow-rate (4 L/min) for each aquarium after cleaning and removing the accumulated excreta. A photoperiod of 12-hr light and 12-hr dark (08:00–20:00 hr) was used with fluorescent ceiling lights supplied the illumination. Water quality was monitored every week throughout the feeding trial. Water temperature was recorded daily with a mercury thermometer suspended at 15-cm depth. pH was determined by using a pH meter (Orion pH meter, Abilene, Texas, USA), whereas dissolved oxygen (mg/L) was measured using YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). During the feeding trial, the water quality parameters averaged (±standard deviation): water temperature 27.9 ± 0.50°C; dissolved oxygen 5.85 ± 0.22; pH values 8.10 ± 0.30; and total ammonia 0.18 ± 0.01 mg/L, and all tested water quality criteria (temperature, dissolved oxygen, pH value and total ammonia) were within the acceptable limits for rearing Nile tilapia, *O. niloticus* (Boyd, 1990).

2.2 | Diet formulation and preparation

Three isonitrogenous (329.80 g/kg of crude protein) and isoenergetic (18.46 MJ/kg gross energy) experimental diets were formulated as SFM_1 (fishmeal [FM]: sunflower meal [SFM] ratio = 2:1), SFM_2 (FM: SFM ratio = 1:1) and SFM_3 (FM: SFM ratio = 1:2) based on protein content. Each diet was supplemented with 0 and 0.5 g/kg of exogenous enzyme xylanase (product, Huvepharma, Antwerp, Belgium). The xylanase (7,500 U/g) was added to the diets to provide two concentrations of 0 (0.0 g/kg) and 3,750 (0.5 g/kg) U xylanase/kg diet. The activity of xylanase in the test diets was estimated according to Esteve-Garcia, Brufau, Perez-Vendrell, Miquel, and Duven (1997), and the actual xylanase levels of the experimental diets were 3,723 U/kg diet. The proximate chemical composition of the experimental diets is presented in Table 1. The ingredients were blended in a feed mixer using a homogenous mixture (Hobart Corporation, Troy, OH, USA) grinder and thoroughly mixed with soybean oil. Distilled water was added to the premixed ingredients and homogenized until a dough-like paste was formed. The dough was passed through pelleting hand-noodle maker. The moist pellets were immediately fan-dried at room temperature and after dried kept in cellophane bags and cooled at −4°C until use.

TABLE 2 Antinutritional factors of sunflower meal and soybean meal and experimental diets

	Antinutritional factor (ANFs)		
	Phytic acid (g/kg)	Chlorogenic acid (IU/mg protein)	Trypsin inhibitor (IU/mg protein)
Ingredient			
Sunflower meal	36.30	1,660	920
Soybean meal	56.00	Not determined	1,690
Diets			
SFM_1	11.09	87.62	169.23
SFM_2	13.12	136.5	198.52
SFM_3	15.89	181.3	221.70

2.3 | Determination of antinutritional factors and amino acids

Phytic acid, chlorogenic acid and trypsin inhibitor were estimated in sunflower meal and experimental diets after preparation (Table 2). Phytic acid content was determined using a spectrophotometric procedure by the method of Vaintraub and Lapteva (1988). The colour was measured at 830 nm against a blank. The results were calculated as mg phytic acid g/kg dry sample using standard phytic acid. Chlorogenic acid in samples and diets was detected as described by Ky, Noirot, and Hamon (1997), and results are expressed as IU/mg protein of dry sample. Trypsin inhibitor content was estimated according to the method of Smith, Van Megen, Twaalfhoven, and Hitchcock (1980), and results are expressed as IU/mg protein of dry sample. The samples of diets for amino acid analysis were grounded followed by digestion using 10 ml 6N HCl solution at 110°C for 24 hr. Amino acids were separated using high-performance liquid chromatography (HPLC; Shimadzu Corp., Tokyo, Japan) following the method showed by Kader, Koshio, Ishikawa, Yokoyama, and Bulbul (2010).

2.4 | Growth indices

Initial body weight (g) (IBW) and final body weight (g) (FBW) of individual fish were recorded for all fish/each aquarium at the initiation and the termination of the experiment. Weight gain (WG) was calculated as follows: WG = FBW (g)–IBW (g); condition factor (K) was calculated using the following formula: $K = (W/L^3) \times 100$, where W = weight of fish in grams and L = total length of fish in cm; specific growth rate (SGR) =100× (Ln W2–Ln W1)/T, where Ln = natural log, W1 = initial body weight, W2 = final body weight and T = study period (84 days); feed conversion ratio (FCR) was calculated according to the equation: FCR = feed intake (g)/weight gain (g); protein efficiency ratio (PER) = weight gain (g)/protein ingested (g); protein productive value (PPV) = (protein gain [g]/protein intake [g]) × 100; relative intestine length (RIL), hepatosomatic index (HSI) and spleen index (SI) were calculated using the following equations: RIL = intestine length (cm)/whole-body weight (g); HSI (%) = 100× (liver weight [g]/whole-body weight [g]) and SI (%) = 100 × (spleen weight [g]/whole-body weight [g]). Calcium retention value (CRV) = 100× (fish calcium gain [g]/calcium intake [g]). Phosphorus retention value (PRV) = 100 × (fish phosphorus gain [g]/phosphorus intake [g]).

2.5 | Determination of digestive enzymes activity and cholecystokinin content

Samples of intestine from four fish in each aquarium of treatments were immediately homogenized in 10 volumes (w/v) of ice-cold physiological saline solution and centrifuged at 5,000 *g* for 15 min at 4°C; then, the supernatant was stored for endogenous enzymes activity analysis (Furné et al., 2008). Chymotrypsin activity was estimated by using the method of Hummel (1959) with N-benzoyl-L-tyrosine ethyl ester (BTEE) as substrate at 254 nm. 0.2 ml diluted sample solution was added to 6 ml of 0.0005 M BTEE in Tris buffer (10.55 g $CaCl_2$. $2H_2O$ dissolved in 250 ml 0.2 M Tris [hydroxymethyl] aminomethane, adjusted to pH 7.8, diluted to 1 L, and 432 ml methanol added). Also, trypsin activity was measured by using methods of Hummel (1959) with Na-p-toluenesulfonyl-L-arginine methyl ester (TAME) as substrate at 247 nm. 0.2 ml diluted sample solution was added to 6 ml of 0.00104 M TAME in Tris buffer (1.47 g $CaCl_2$. $2H_2O$ dissolved in 200 ml 0.2 M Tris [hydroxymethyl] aminomethane diluted to 1 L, pH 8.1). Lipase activity was determined as described by Zamani, Hajimoradloo, Madani, and Farhangi (2009), and titration method was detailed by using olive oil-gum. Amylase activity was estimated according to Bernfeld (1951) at 540 nm, and starch was used as the substrate. One ml of diluted sample was incubated for 3 min with 1% starch (1 g soluble starch and 0.035 g NaCl in 100 ml 0.02 M Na3PO4, pH 6.9). After 3 min, the reaction was stopped by the addition of 2 ml 3,5-dinitrosalicylic acid reagent. The solution was then heated for 5 min in boiling water and then cooled with 20 ml distilled water added. Intestinal alkaline phosphatase (IAKP) activity was determined by the methods of Wahlefeld, Holz, and Bergmeyer (1974) with 4-nitrophenyl phosphate as substrate at 405 nm. The content of cholecystokinin (CCK) in intestine of fish was determined by the method of Maton, Selden, and Chadwick (1984).

2.6 | Digestibility measurements

The apparent digestibility coefficients (ADCs) of different experimental diets were measured using chromic oxide (Cr_2O_3) as an

TABLE 3 Growth response and feed utilization of Nile tilapia fed experimental diets for 84 days

		Growth performance				Feed utilization			Morphometric index		
Variables	Xylanase U/kg	IBW (g/fish)	FBW (g/fish)	WG (g/fish)	SGR (%/day)	FCR	PER	PPV[c] (%)	RIL	HSI	SI
Individual treatment means[a]											
SFM_1 (2:1)	0	1.30	15.86^{ab}	14.55^{ab}	3.32^{a}	1.44^{b}	2.30^{c}	57.60^{b}	0.92^{d}	3.30	0.022^{c}
SFM_1 (2:1)	3,750	1.31	16.31^{a}	14.99^{a}	3.36^{a}	1.18^{d}	2.61^{a}	65.44^{a}	0.86^{d}	3.36	0.021^{c}
SFM_2 (1:1)	0	1.32	13.36^{c}	12.05^{c}	3.09^{b}	1.78^{a}	1.86^{d}	46.64^{d}	1.25^{b}	3.56	0.028^{b}
SFM_2 (1:1)	3,750	1.32	15.90^{a}	14.59^{a}	3.33^{a}	1.33^{c}	2.49^{a}	62.35^{a}	0.96^{c}	3.24	0.022^{c}
SFM_3 (1:2)	0	1.31	12.94^{d}	11.63^{d}	3.05^{b}	1.74^{ab}	1.92^{d}	49.50^{c}	1.48^{a}	3.54	0.034^{a}
SFM_3 (1:2)	3,750	1.30	14.95^{b}	13.27^{b}	3.21^{ab}	1.44^{b}	2.35^{b}	58.95^{b}	1.17^{b}	3.23	0.026^{b}
Pooled SE		0.02	0.73	0.62	0.41	0.12	0.16	5.63	0.23	0.13	0.001
Means of the main effect[b]											
SFM_1 (2:1)		1.31	16.09^{p}	14.78^{p}	3.34^{p}	1.36^{q}	2.46^{p}	61.52^{p}	0.88^{r}	3.40	0.021^{r}
SFM_2 (1:1)		1.31	14.63^{q}	13.32^{q}	3.21^{q}	1.56^{p}	2.18^{q}	54.49^{q}	1.10^{q}	3.38	0.025^{q}
SFM_3 (1:2)		1.30	13.77^{q}	12.46^{q}	3.13^{q}	1.57^{p}	2.13^{q}	53.45^{q}	1.32^{p}	3.33	0.029^{p}
	0	1.32	14.05^{y}	12.74^{y}	3.15^{y}	1.66^{x}	2.03^{y}	50.73^{y}	1.21^{x}	3.47	0.028^{x}
	3,750	1.31	15.60^{x}	14.29^{x}	3.30^{x}	1.34^{y}	2.48^{x}	62.24^{x}	0.99^{y}	3.27	0.022^{y}
ANOVA (p-value)											
FM: SFM		0.806	0.002	0.005	0.004	0.042	0.012	0.013	0.001	0.880	0.008
Xylanase		0.578	0.001	0.001	0.001	0.002	0.001	0.001	0.002	0.142	0.004
FM: SFM × Xylanase		0.096	0.013	0.001	0.021	0.001	0.012	0.001	0.011	0.001	0.021

Note. FBW: final body weight; FCR: feed conversion ratio; HSI: hepatosomatic index; IBW: initial body weight; PER: protein efficiency ratio; PPV: protein productive value; RIL: relative intestine length; SGR: specific growth rate; SI: spleen index; WG: weight gain.

[a]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $p < 0.05$). Means followed by the same letter are not significantly different. [b]Main effect means followed by the same letter are not significantly different at $p < 0.05$ by Duncan multiple range test; p, q and r for FM:SFM ratios and x and y for xylanase levels. [c]Crude protein of initial fish sample was (523 g/kg crude protein, dry matter).

external marker at a level of 5 g/kg diet. After 2-month feeding of experimental diets, faeces were collected from each aquarium once daily in the morning for one-month period. All aquaria were cleaned each morning before feeding. After a 2-hr feeding time, all leftover feed was removed from each aquarium. The collection was done manually by siphoning the faecal matter and straining through a fine-meshed net (Baruah et al., 2007). Faecal matter collection from each aquarium was pooled and dried in Petri dishes at 60°C. Chromic oxide was determined according to the procedure described by Furukawa (1966). Briefly, the sample of faecal matter was digested by nitric acid and subsequent oxidation of chromic oxide with perchloric acid (70%). After an orange colour formed, the colorimetric reaction was quantitated spectrophotometrically at 350 nm against distilled water using a spectrophotometer (Uvikon 810; NorthStar Scientific, Bedfordshire, UK). Chromic oxide (%) = [weight of chromic oxide/sample weight (mg)] × 100%. Apparent digestibility coefficient (ADC) of nutrient was calculated using the equations of Schneider et al. (2004) as follows:

$$\text{ADC dietary nutrient} = 1 - \left[\frac{(\text{marker}_{\text{diet}})}{(\text{marker}_{\text{faeces}})}\right] \times \frac{(\text{nutrient}_{\text{faeces}})}{(\text{nutrient}_{\text{diet}})}$$

2.7 | Histological techniques

On 84 days of the feeding trial, the livers and intestine mid-sections were excised from five fish randomly obtained from each treatment. The liver and intestine tissues of the experimental fish were dissected and fixed in Bouin's fluid for 24 hr. The fixed tissues were dehydrated in graded ethanol series, cleaned in xylene and embedded in paraffin wax (congealing point 58–60°C). The longitudinal and transverse sections each of 6 μm thickness were stained in haematoxylin and eosin. The sections were deparaffinized in xylene each and rehydrated using a graded ethanol series. The dehydrated sections were blotted once again and cleared in two changes of xylene with the first change of 10 minutes duration and second change of 15 minutes duration. Further blotted, the sections were mounted in DPX (Distyrene Plasticizer Xylene) and were examined under light microscope (Nikon E600, Tokyo, Japan). The histological measurements were estimated according to Wassef, Wahbi, Saqr, and Saleh (2016). All measurements of liver and intestine structures were taken using Multi Scan Base v. 8.08 (computer scanning system Ltd., Warsaw, Poland).

2.8 | Chemical composition

Five individual fish were sampled from each aquarium, euthanized with overdose of tricaine methanesulfonate (1 g/L), oven-dried (JSON-100, Gongju, Korea) at 70°C until constant weight and calculating weight loss and stored in polyethylene bags at −20°C for subsequent analysis. Chemical composition was analysed on experimental diets, faeces and fish samples according to AOAC (1990). Crude protein was determined by micro-Kjeldahl method, $N \times 6.25$ (using Kjeltec auto analyzer, Model 1,030, Tecator, Höganäs, Sweden). Lipid content was determined by Soxhlet extraction with diethyl ether (40–60°C). Ash was determined using Barnstead/Thermolyne Benchtop 47900 (Thermo Scientific, Massachusetts, United States) by incineration at 550°C for 12 hr. Fibre content of the experimental diets was determined using the method described by Van Soest, Robertson, and Lewis (1991). Nitrogen-free extract was computed by taking the sum of values for crude protein, crude lipid, crude fibre and ash and by subtracting this sum from 100. Phosphorus in the diet, initial and final whole-body, and faeces were analysed by the same method, molybdovanadate method (AOAC, 1990). Calcium in diets was analysed by atomic absorption spectrophotometer (AAS; Hitachi Z-2300, Tokyo, Japan).

2.9 | Statistical analysis

All the data were analysed by ANOVA using SAS ANOVA procedure (SAS, version 6.03, Soft Inc., Tusla, OK, USA, SAS, 1993). One-way analysis of variance (one-way ANOVA) was used to determine whether there was significant variation among the treatments. When overall differences were found, differences between means were tested by Duncan (1955) new multiple range test. Two-way ANOVA was used for analysing the individual effects of fish meal protein: sunflower meal ratios and xylanase enzyme. All differences were considered significant at $p < 0.05$, and the results are presented as means with standard error of the mean. The data were arc-sin-transformed prior to analysis (Zar, 1984); however, data are presented untransformed to facilitate the comparisons.

3 | RESULTS

3.1 | Growth performance, feed utilization and somatic indices

The effects of different ratios of SFM_1, SFM_2 and SFM_3 or exogenous xylanase and their interactions in tilapia diets on growth performance and feed utilization are shown in Table 3. Growth performance and feed utilization parameters were significantly affected with different ratios of SFM_1, SFM_2 and SFM_3 and exogenous xylanase and their interaction. Growth performance and feed utilization for fish fed SFM_3 and un-supplemented with xylanase were lower than fish fed SFM_3 and supplemented with xylanase (3,750 U/kg). The highest FBW, WG, SGR, PER, PPV and the best FCR were found in fish fed SFM_1 or SFM_2 and supplemented with (3,750 U/kg) xylanase with no significant differences ($p > 0.05$) between means. No significant ($p > 0.05$) difference was detected in hepatosomatic index of fish among treatments. Relative intestine length and spleen index of fish were affected significantly ($p < 0.05$) by either different ratios of SFM_1, SFM_2 and SFM_3 or xylanase enzyme. The higher significant ($p < 0.05$) relative intestine length and spleen index were recorded in SFM_3 without xylanase supplementation than other diets. Correlation analysis showed that length of intestine was negatively correlated to body weight ($r = -0.872$, $p < 0.024$).

TABLE 4 Endogenous enzymes activity and cholecystokinin content of Nile tilapia fed experimental diets for 84 days

		Endogenous digestive enzymes (U/g tissue)					
Variables	Xylanase U/kg	Chymotrypsin	Trypsin	Lipase	Amylase	Alkaline phosphatase	CCK ng/g
Individual treatment means[a]							
SFM_1 (2:1)	0	5.56^{b}	0.78^{c}	913^{c}	712^{c}	31.58^{c}	38.25^{b}
SFM_1 (2:1)	3,750	7.05^{a}	1.32^{a}	1,200^{a}	915^{a}	43.27^{a}	46.25^{a}
SFM_2 (1:1)	0	4.56^{c}	0.71^{c}	902^{d}	694^{d}	26.69^{d}	35.98^{c}
SFM_2 (1:1)	3,750	6.89^{a}	1.28^{a}	1,191^{a}	901^{a}	41.23^{a}	44.89^{a}
SFM_3 (1:2)	0	4.05^{c}	0.62^{d}	879^{d}	643^{e}	22.78^{d}	30.25^{d}
SFM_3 (1:2)	3,750	6.36^{b}	0.98^{b}	1,185^{b}	880^{b}	39.81^{b}	40.12^{b}
Pooled SE		1.15	0.02	15.04	13.52	2.56	3.14
Means of the main effect[b]							
SFM_1 (2:1)		6.31^{p}	1.05^{p}	1,057^{p}	813.5^{p}	37.43^{p}	42.25^{p}
SFM_2 (1:1)		5.73^{q}	1.00^{q}	1,047^{q}	797.5^{q}	33.96^{q}	40.44^{p}
SFM_3 (1:2)		5.21^{q}	0.80^{r}	1,033^{q}	761.5^{r}	31.30^{r}	35.19^{q}
	0	4.72^{y}	0.70^{y}	898^{y}	683.0^{y}	27.02^{y}	34.83^{y}
	3,750	6.77^{x}	1.19^{x}	1,192^{x}	898.7^{x}	41.44^{x}	43.75^{x}
ANOVA (p-value)							
FM: SFM		0.001	0.002	0.007	0.011	0.001	0.012
Xylanase		0.002	0.001	0.001	0.012	0.001	0.002
FM: SFM × Xylanase		0.001	0.002	0.013	0.001	0.015	0.001

Note. CCK: Cholecystokinin content.

[a]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $p < 0.05$). Means followed by the same letter are not significantly different. [b]Main effect means followed by the same letter are not significantly different at $p < 0.05$ by Duncan multiple range test; p, q and r for FM:SFM ratios and x and y for xylanase levels

3.2 | The activity of endogenous enzymes and cholecystokinin

Data for the endogenous enzyme, chymotrypsin, trypsin, lipase, amylase and alkaline phosphatase, and cholecystokinin content are reported in Table 4. Different ratios of SFM_1, SFM_2 and SFM_3 had significant ($p < 0.05$) effect on activity of these endogenous enzymes. Lower activities of chymotrypsin, trypsin, lipase, amylase and alkaline phosphatase, and cholecystokinin content were detected in SFM_3 without xylanase supplementation. The activities of chymotrypsin, trypsin, lipase, amylase, alkaline phosphatase and cholecystokinin content were significantly higher in fish fed SFM_1, SFM_2 and SFM_3 supplemented with xylanase enzyme than fish fed the similar diets without addition of xylanase.

3.3 | Apparent digestibility coefficients

Data of ADCs of dry matter (DM), crude protein (CP), lipid and digestible energy (DE) are presented in Table 5. The ADC of DM, CP, lipid and DE energy was significantly ($p < 0.05$) lower in SFM_3 un-supplemented with exogenous xylanase. Diets supplemented with exogenous xylanase increased the apparent ADCs of dry matter (DM), crude protein (CP), lipid and energy. However, the highest ADCs of DM, CP, lipid and DE were recorded in SFM_1 and SFM_2 supplemented with exogenous xylanase.

3.4 | Histological morphometric

The histological morphometric of liver and intestine is shown in Table 6. No significant differences were found in the size of hepatocyte and nuclei of fish among treatments. The muscularis mucosa thickness, height of mucosal folds and enterocytes were significantly lower in fish fed SFM_3 and un-supplemented with exogenous xylanase but adding of xylanase increased these indices. Either SFM_1 or SFM_2 supplemented with exogenous xylanase recorded the highest muscularis mucosa thickness, height of mucosal folds and enterocytes of intestinal fish. No significant ($p > 0.05$) difference was found in size of nuclei of intestinal fish.

3.5 | Chemical composition of whole fish, calcium and phosphorus retention value

Table 7 shows the proximate analysis of chemical composition, calcium retention value (CRV) and phosphorus retention value (PRV) of *O. niloticus* fed different ratios of SFM_1, SFM_2 and SFM_3, and xylanase. No differences ($p > 0.05$) among treatments were detected for

TABLE 5 Apparent digestibility coefficient (%) of Nile tilapia fed experimental diets for 84 days

Variables	Xylanase U/kg	Apparent digestibility coefficient (%)			
		Dry matter	Crude protein	Crude lipid	Digestible energy
Individual treatment means[a]					
SFM_1 (2:1)	0	87.31[a]	88.92[b]	88.19[b]	76.13[b]
SFM_1 (2:1)	3,750	88.19[a]	91.58[a]	91.12[a]	78.19[a]
SFM_2 (1:1)	0	85.98[b]	86.19[c]	87.19[b]	74.87[c]
SFM_2 (1:1)	3,750	87.09[a]	89.95[a]	90.13[a]	77.82[a]
SFM_3 (1:2)	0	83.01[c]	84.78[d]	86.78[c]	73.19[d]
SFM_3 (1:2)	3,750	84.19[c]	86.89[c]	88.13[b]	74.80[c]
Pooled SE		1.67	1.80	1.03	1.04
Means of the main effect[b]					
SFM_1 (2:1)		87.75[p]	90.25[p]	89.66[p]	77.16[p]
SFM_2 (1:1)		86.53[q]	88.07[q]	88.66[q]	76.35[q]
SFM_3 (1:2)		83.60[r]	85.84[r]	87.46[q]	74.00[r]
	0	85.43[y]	86.63[y]	87.39[y]	74.73[y]
	3,750	86.49[x]	89.47[x]	89.79[x]	76.94[x]
ANOVA (p-value)					
FM: SFM		0.001	0.013	0.024	0.011
Xylanase		0.002	0.012	0.021	0.011
FM: SFM × Xylanase		0.001	0.001	0.021	0.012

[a]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $p < 0.05$). Means followed by the same letter are not significantly different. [b]Main effect means followed by the same letter are not significantly different at $p < 0.05$ by Duncan multiple range test; p, q and r for FM:SFM ratios and x and y for xylanase levels.

dry matter, protein, lipid and ash content of whole fish. Fish fed diets supplemented with exogenous xylanase contained more calcium and phosphorus than fish fed the similar diets without addition of xylanase also, the same trend was observed in CRV and PRV, and the highest value was detected in SFM_1 supplemented with exogenous xylanase.

4 | DISCUSSION

The present study showed that fish fed diet containing high level of sunflower meal (SFM_3, 360 g/kg diet) and un-supplemented with exogenous xylanase had significantly lower growth performance and nutrient utilization, which may be related to high fibre content (108.9g/kg) and ANFs (15.89 g/kg phytic acid, 181.30 IU/mg chlorogenic acid and 221.70 IU/mg trypsin inhibitor, Table 2). Use of plant-derived materials such as SFM in fish feed ingredients is limited due to the presence of a wide variety of ANFs and NSPs (Halver, 2002). Often substitution of fish meal protein by sunflower meal protein did not compromise growth of Nile tilapia (Maina et al., 2002; Olvera-Novoa et al., 2002; Sintayehu, Mathies, Meyer-Burgdorff, Rosenow, & Günther, 1996). However, in the present study, the addition of 3,750 U/kg xylanase improved the WG and FCR in fish either SFM_1 or SFM_2. Similar results reported that xylanase supplementation enhanced growth performance of fish (Ai et al., 2007; Babalola, 2006; Hlophe-Ginindza et al., 2016; Jiang et al., 2014; Lin et al., 2007). The improvement in growth and nutrient utilization of fish fed diet supplemented with xylanase or complex with other enzymes could be attributed to the degradation of NSPs to the level that the viscosity property of these fractions is largely reduced (Jiang et al., 2014). Also, the suppression of nutrient utilization by exogenous enzymes addition due to its role in eliminating the ANFs effect (Farhangi & Carter, 2007; Lin et al., 2007; Soltan, 2009). In the present study, HSI did not vary significantly ($p > 0.05$) among the treatments. The same trend was found when *Labeo rohita* fed xylanase and phytase supplementation in the de-oiled rice bran-based diet (Ranjan, Sahu, Deo, & Kumar, 2018). In contrast, Jiang et al. (2014) obtained the maximum values of HSI at 1,480 U/kg diet of xylanase ($p < 0.05$) for juvenile Jian carp, *Cyprinus carpio*.

Regardless of xylanase supplementation, the present results indicated that the relative length of intestine was higher in fish fed SFM_3 than the other diets. Increasing the length of the Nile tilapia intestine may be attributed to the increasing activity of digestion to achieve a digestive balance and fit the increase in the ratios of SFM protein content. Buddington, Krogdahl, and Bakke-McKellep (1997) reported that omnivorous fish require longer time to digest plant protein than the animal protein-based diets. However, in the present study, the length of fish intestine decreased with xylanase

TABLE 6 Histological morphometric of liver and gut (μm) of Nile tilapia fed experimental diets for 84 days

Variables	Xylanase U/kg	Muscularis mucosa thickness	Height of mucosal folds	Height of enterocytes	Size of nuclei	Size of hepatocyte	Size of nuclei
Individual treatment means[a]							
SFM_1 (2:1)	0	57.94[a]	251.78[a]	25.11[a]	9.36	9.53	6.35
SFM_1 (2:1)	3,750	60.13[a]	269.69[a]	26.91[a]	9.13	9.02	6.86
SFM_2 (1:1)	0	53.25[b]	232.16[b]	23.75[b]	8.59	9.56	7.91
SFM_2 (1:1)	3,750	59.77[a]	239.68[b]	25.71[a]	8.38	10.76	6.19
SFM_3 (1:2)	0	50.50[c]	215.83[c]	23.69[b]	9.17	10.03	6.03
SFM_3 (1:2)	3,750	56.91[c]	232.76[b]	25.94[a]	8.68	9.87	6.38
Pooled SE		1.63	10.57	0.92	0.45	0.58	1.12
Means of the main effect[b]							
SFM_1 (2:1)		59.04[p]	260.74[p]	26.01[p]	9.25	9.28	6.60
SFM_2 (1:1)		56.51[q]	235.92[r]	24.73[q]	8.49	10.16	7.05
SFM_3 (1:2)		53.07r	244.28[q]	24.82[q]	8.93	9.95	6.21
	0	53.90[y]	233.26[y]	24.18[y]	9.04[x]	9.70	6.76
	3,750	58.94[x]	247.36[x]	26.19[x]	8.73[y]	9.90	6.44
ANOVA (*p*-value)							
FM: SFM		0.0001	0.0001	0.052	0.133	0.547	0.440
Xylanase		0.042	0.045	0.027	0.255	0.922	0.592
FM: SFM × Xylanase		0.001	0.012	0.101	0.307	0.411	0.315

[a]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $p < 0.05$). Means followed by the same letter are not significantly different. [b]Main effect means followed by the same letter are not significantly different at $p < 0.05$ by Duncan multiple range test; p, q and r for FM:SFM ratios and x and y for xylanase levels.

supplementation. This decrease may be due to increase in the activity of digestion in intestine with xylanase supplementation.

Endogenous enzymes play functions in digesting nutrients for fish, directly enhance the digestive ability (Adeoye et al., 2016; Wen, Zhou, Feng, Jiang, & Liu, 2009). The present study showed that the high level of sunflower meal SFM_3 and un-supplemented with xylanase recorded the lowest activity of all digestive enzymes. This decreased was attributed to the presence of antinutritional factors, high levels of fibre and NSP in SFM which reduced the digestion (Hassaan et al., 2017; Refstie, Storebakken, & Roem, 1998). On the other hand, addition of 3,750 U/kg xylanase increased the activities of chymotrypsin, trypsin, lipase, amylase and alkaline phosphatase in the intestine of *O. niloticus*. Several authors reported that exogenous enzymes increased the activity of endogenous enzymes (Hlophe-Ginindza et al., 2016; Jiang et al., 2014; Lin et al., 2007; Wei, Xiu-Mei, & Li-Bo, 2010; Xu, Wang, Li, & Lin, 2009). This improvement in the activity of endogenous enzymes might be attributed to the role of xylanase in the degradation products of arabinoxylans, hydrolyse cell wall components in the plant material; thereby, it reduced the molecular size characteristics of NSPs content of the plant materials. Consequently, this finding might be related to the role of the exogenous xylanase in releasing the bound nutrients and promoting rapid digestion in Nile tilapia by reducing the digesta viscosity in the gut of fish. Jiang et al. (2014) reported that the high viscosity might hinder the interaction between the enzymes with their substrate. In this context, the increase in the digesta viscosity produced laminar flow, which reduce the rate of digestion and movement of nutrients from the lumen of the mucosal epithelium (Macagno, Christensen, & Lee, 1982).

The secretion of digestive enzymes is controlled by the hormone (Singh & Webster, 1978). Cholecystokinin content (CCK) is an important gastrointestinal hormone in fish and it has a crucial role in stimulating the secretion of trypsin and chymotrypsin from the pancreas (Einarsson, Davies, & Talbot, 1997). The present study showed that CCK content in intestine was improved by xylanase supplementation; also, the higher content of intestinal CCK was paralleled with higher activities of trypsin and chymotrypsin. The improved activity of CCK may be due to the stimulation of released products from xylanase hydrolysis, subsequently enhanced the activity of trypsin and chymotrypsin (Jiang et al., 2014).

In the present study, the lowest values of muscularis mucosa thickness, height of mucosal folds and enterocytes of intestine were recorded in fish fed SFM_3, whereas its values were enhanced by the addition of xylanase enzyme. Similarly, Jiang et al. (2014) indicated that xylanase supplementation increased absorptive ability of juvenile Jian carp, *Cyprinus carpio*. The improvement of intestinal morphology in the current study was related to increasing rates of digestion and absorption of nutrient for fish fed diet supplemented

TABLE 7 Proximate and mineral composition (g/kg wet tissue), calcium and phosphorus retention value of Nile tilapia fed diet fed experimental diets for 84 days

Variables	Xylanase U/kg	Dry matter	Crude protein	Total lipid	Ash	Calcium	Phosphorus	CRV	PRV
Individual treatment means[a]									
SFM_1 (2:1)	0	272.6	153.1	51.6	38.5	2.1^{a}	1.6^{a}	69.1^{b}	47.3^{a}
SFM_1 (2:1)	3,750	261.3	146.7	49.4	39.1	2.1^{a}	1.6^{a}	72.1^{a}	50.6^{a}
SFM_2 (1:1)	0	251.2	138.9	47.2	35.2	1.7^{d}	1.4^{b}	65.6^{c}	43.8^{b}
SFM_2 (1:1)	3,750	256.7	141.3	51.1	35.7	2.1^{a}	1.5^{a}	69.3^{b}	45.2^{b}
SFM_3 (1:2)	0	255.0	142.3	52.8	33.5	1.8^{d}	1.3^{c}	62.7^{d}	39.2^{c}
SFM_3 (1:2)	3,750	259.1	142.5	49.55	35.4	1.8^{c}	1.4^{b}	68.1^{b}	42.9^{b}
Pooled SE		0.96	0.25	0.57	0.46	0.42	0.39	0.79	0.59
Means of the main effect[b]									
SFM_1 (2:1)		267.0	149.9	50.5	38.9	2.1^{p}	1.6^{q}	70.6^{p}	48.9^{p}
SFM_2 (1:1)		254.0	140.1	49.2	35.5	1.9^{q}	1.5^{q}	67.4^{q}	44.5^{q}
SFM_3 (1:2)		257.1	142.4	51.2	34.5	1.8^{q}	1.7^{p}	65.4^{r}	41.0^{r}
	0	259.6	144.7	50.5	35.8	1.8^{y}	1.6^{y}	65.8^{y}	43.4^{y}
	3,750	259.0	143.5	50.0	36.7	2.0^{x}	1.5^{x}	69.8^{x}	46.2^{x}
ANOVA (*p*-value)									
FM: SFM		0.563	0.611	0.231	0.113	0.032	0.041	0.032	0.012
Xylanase		0.121	0.035	0.657	0.322	0.031	0.012	0.012	0.004
FM: SFM × Xylanase		0.221	0.354	0.092	0.123	0.012	0.051	0.011	0.001

Note. CRV: Calcium retention value; PRV: phosphorus retention value.

[a]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $p < 0.05$). Means followed by the same letter are not significantly different. [b]Main effect means followed by the same letter are not significantly different at $p < 0.05$ by Duncan multiple range test; p, q and r for FM:SFM ratios and x and y for xylanase levels.

with enzymes (Adeoye et al., 2016). Digestion, absorption and intestinal barrier function, and the functional of mucosa were affected by the thickness and fluidity of the intestinal brush-border membrane (Einarsson et al., 1997). The enterocytes of intestine are covered with a mucus layer, which is secreted by goblet cells throughout the gastrointestinal tract (Johansson et al., 2011). The function of the muscularis mucosa is to promote intestine peristalsis; a thinner muscularis decreased the rate of peristalsis, and the time of feed remaining in the small intestine is increased, thus increasing the time for absorption of nutrients (Liu et al., 2018).

Our results indicating that intestinal morphology was associated with the apparent digestibility results. The improvement of intestinal morphology in this study enhanced the rate of digestion and assimilation of Nile tilapia. The present results indicated that the apparent digestibility coefficient (ADC) of dry matter, crude protein, lipid and energy was enhanced by xylanase supplementation. Also, this results are confirmed by many authors (Kumar, Sahu, Pal, Choudhury, & Mukherjee, 2006; Lin et al., 2007) which found that nutrient digestibility enhanced by enzyme supplementation. The beneficial effects of exogenous non-starch polysaccharides (NSPase) may be directly related to NSPs hydroxylation, which improves nutrients digestibility (Adeola & Cowieson, 2011). Also, NSP-degrading enzymes (e.g., cellulase, xylanase) were capable of disrupting plant cell wall integrity and this subsequently enhances digestion by reducing viscosity in the gut (Bedford & Cowieson, 2012) which concurred with the present results. Similarly, Ogunkoya, Page, Adewolu, and Bureau (2006) found that exogenous enzyme supplementation to the soybean meal-based diet improves the apparent digestibility coefficient of dry matter, protein, lipid and carbohydrate in *Oncorhynchus mykiss*. Also, Lin et al. (2007) reported that supplementing a mixture of enzymes containing neutral protease, b-glucanase and xylanase to a plant-based diet promoted the secretion of endogenous protease and amylase in the Nile tilapia which in turn enhanced the protein digestibility of *Oreochromis mossambicus*.

In our results, neither different ratios of FM:SFM nor xylanase enzyme had a significant effect on the whole-body dry matter, lipid, ash, calcium and phosphorus among all treatments ($p > 0.05$). Our findings are consistent with the results of Adeoye et al. (2016) who found significant difference ($p < 0.05$) in dry matter when tilapia fed formulated diet with exogenous enzymes (Protease, lipase and carbohydrases). Similarly, Yildirim and Turan (2010) detected that no significant difference in the whole-body composition except crude protein of *Clarius gariepinus* fed with exogenous enzyme supplemented diet. In the present study, whole-body protein and lipid were significantly higher in fish fed SFM_1 and SFM_2 supplemented with xylanase enzyme, respectively. Thus, due to increasing the

availability of non-protein energy in the enzyme supplemented diets to allow more dietary protein and energy for growth and higher nutrient digestibility as detected previously in *Labeo rohita* supplemented with cocktail enzymes (phytase and xylanase) by Ranjan et al. (2018). Our finding is also in accordance with the findings of Magalhães et al. (2016) who observed higher whole-body protein and lipid in *Diplodus sargus* and *Clarias gariepinus* when fed with the diet supplemented with exogenous multi-enzymes complex (containing phytase, xylanase, cellulase and carbohydrases).

5 | CONCLUSION

Results of the present study revealed that Nile tilapia has a limited ability to utilize sunflower meal as a protein source with high inclusion level (360 g/kg diet), but addition of exogenous xylanase to diet containing high inclusion level of sunflower meal improved growth, digestive enzymes nutrient digestibility, histological morphometric of liver and intestine and nutrient retention.

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