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Effects of Partial and Full Dietary Substitution of Fish Meal and Soybean Meal by Sunflower Meal on Growth Performance, Feed Consumption, Body Indices, Serum Chemistry and Intestine Morphology of *Oreochromis niloticus*

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Abstract

A 03-months feeding experiment was designed to study the effects of partial and full dietary substitution of fish meal and soybean meal with sunflower meal (SFM) on growth performance, feed consumption, body indices, serum chemistry and intestine morphology of *Oreochromis niloticus*. Five isonitrogenous and isocaloric dietary treatments: T1, T2, T3, T4 and T5 were prepared by supplementing SFM at 0%, 25%, 50%, 75% and 100% respectively and fed to fish (4 ± 0.07 g) in triplicates. The growth performance and feed consumption in *O. niloticus* was significantly ($p < 0.05$) improved and body indices was significantly reduced in groups fed with T1 (control), T2 and T3 however, survival rate was remained unaffected among all dietary treatments. Significantly increased whole-body protein, serum total protein and albumin content was found in groups fed with T1 (control), T2 and T3, compared to T4 and T5 groups whereas, whole-body lipid content was significantly elevated in T5 group. In contrast, serum glutamic-pyruvic transaminase (SGPT) and glutamic-oxalacetic transaminase (SGOT) activity was significantly elevated in groups fed with T4 and T5. Additionally, serum triglycerides and cholesterol level were significantly increased in group T1 compared to other groups however, glucose and creatinine were not affected by dietary treatments. Uran's pooled score for intestinal histology was significantly increased in SFM supplemented treatments as compared to control group. In conclusion, replacement of SBM with SFM up to 50% is possible without negative impacts on growth performance and health status of *O. niloticus*.

Introduction

Protein is the most expensive component of aquafeeds since it plays a critical role in providing indispensable amino acids (IAAs) to achieve optimum growth in a limited time. Conventionally, fish meal (FM) is used as a primary protein source for farmed fish due to its high palatability and premium quality of nutrient contents. However, the price of FM has been increasing continuously with its growing demand against its limited supply. Hence, the development of good quality alternative protein sources, which are cheaper and available year around is highly needed nowadays (Iqbal et al., 2021). The numbers of plant protein sources have been evaluated for aquafeeds to reduce the feed cost and to gain optimum yield, including soybean meal, corn gluten meal, lupin meal, rapeseed meal, canola meal, cottonseed meal, peanut meal, palm kernel meal, cassava leaf meal, mulberry leaves, rubber seed meal, sesame meal, coconut meal, linseed meal and aquatic plants such as *Azolla*, etc. (Maina et al., 2002; Iqbal et al., 2021).

One of the main problems associated with the supplementation of plant protein alternatives is the presence of various anti-nutritional components. Despite that, these plant protein alternatives are considered effective to reduce feed costs (Sanchez-Lozano et al., 2007). Most of the previous studies are conducted by supplementing soybean meal (SBM) or soy products (soy protein concentrate and soy protein

isolate) as a potential substitute for FM. Soybean is also extensively used for human nutrition and its meal is being used in the livestock, poultry, and swine feed industry. That is why fish farmers are facing the problem of the stagnant supply of SBM as a protein source. Due to all these predicaments, the dependence of the aquaculture industry on conventional protein sources must be reduced by some alternative local protein sources (Iqbal et al., 2021).

Pakistan is a dynamic agricultural country where multiple crops are been cultivated. Sunflower locally known as Sooraj Mukhi is the fourth largest crop worldwide, currently contributing approximately about 30% of the total domestic production of edible oil (Anjum et al., 2014). Presently, it is cultivated over an area of 397, 000 ha in Pakistan with a total annual production of 407,224 tons (GOP, 2012-13). The crude protein content of sunflower meal (SFM) ranges between 36–40%, in addition it has high methionine and tryptophan content compared to other plant protein sources (Mushtaq et al., 2020). It is also a rich source of choline, niacin, riboflavin, biotin, pantothenic acid, pyridoxine, and vitamin E (Nishino et al., 1980). Additionally, iron, zinc, magnesium, potassium, and selenium are also available in SFM at high concentrations (Sanz et al., 1994). Unlike other plant protein sources, no known antinutritional components are present in SFM, but it contains 0.48 and 1.56% quinic and chlorogenic acids, respectively (Milic et al., 1968). According to previous reports, the presence of high fiber content, phytic acid, chlorogenic acid, phenolic compounds, and relatively low availability of lysine content are the main limiting factors for the use of SFM as a protein source. Despite that, SFM has been supplemented in livestock, poultry and pig feeds (Day and Levin, 1954; Rad and Keshavarz; 1976). Additionally, various nutritional studies demonstrated that SFM has great potential as an FM replacer in aquafeeds. However, available data are limited to regarding the growth and digestibility of some aquatic species such as rainbow trout, *Oncorhynchus mykiss* (Sanz et al., 1994), redbreast tilapia, *Coptodon rendalli* (Olvera-Novoa et al., 2002), Atlantic salmon, *Salmo salar* (Gill et al., 2006), European eel, *Anguilla anguilla* (Bilguven and Baris, 2011) and mrigal, *Cirrhinus mrigala* (Hussain et al., 2014). These studies suggested that SFM had no negative impact on growth performance and nutrient utilization at low inclusion levels, but reduced growth was observed with higher concentrations of SFM (Hassaan et al., 2015; 2018). Tilapia is a group of fishes belonging to the Cichlidae family and is native to Africa and the Middle East. Currently, is the most widely cultured species known as the second most important farm cultured fish used as food worldwide (Iqbal et al., 2021). *Oreochromis niloticus* culture is gaining attention in Pakistan due to its fast growth rate, high disease resistance, and favorable market attributes (Laghari et al., 2018) whereas fish farmers are lacking the availability of diets for *O. niloticus* based on indigenous plant protein sources. Therefore, the current study aimed to evaluate the effects of partial and full dietary substitution of (imported protein sources) FM and SBM with SFM on growth performance, feed consumption, body indices, serum chemistry, and intestine morphology of *O. niloticus*.

Materials and Methods

Experimental Fish

The feeding experiment and analytical procedures were approved by the Animal Ethics Committee (AEC) of Government College University (GCU), Lahore Pakistan. *O. niloticus* at the fingerling stage were acquired from Fish Farms of the University of Veterinary and Animal Sciences (UVAS) Pattoki, Campus Lahore, and transported to Animal House at Fish Rearing Facility, GCU. To acclimatize, fish were equally distributed in two groups and stocked in outdoor concrete tanks (width 1.5 m x length 2.0m x height 2.0m) for one week. During this, fish were fed on commercial pelleted feed (crude protein; 320 g/kg) provided by *Oryza Organics*®. The shower system with a continuous flow (1 liter/30 min for 24x7) was installed at the outdoor facility to keep the water saturated with dissolved oxygen (DO).

Diets and Experimental Design

The dry fish feed ingredients were purchased from a local supplier (*Khushi Feed Company*®) in Lahore, Pakistan. These ingredients were ground in an electric mixer (Cambridge CG Grinder and mixer 502, China) to acquire the suitable size of ingredient particles (0.5mm). Fish meal (FM) and soybean meal (SBM) were used as protein source in the control treatment (T1; 0% SFM) and replaced with 25, 50, 75, and 100% SFM in T2, T3, T4, and T5 treatments, respectively (Table 2). Distilled water (20–30%) and fish oil were added to dry ingredients to prepare a dough. Thereafter, the noodles were prepared by passing this dough through an extruder (Jinan Saibainuo Machinery Co., Ltd., Model no. SYSLG30-IV Experimental Extruder). Subsequently, noodles were converted into pellets (2mm), and the moisture content of pellets was reduced up to 10% by air drying. Pellets were kept in labeled polythene airtight bags and stored at -20 °C until feeding. The proximate composition of experimental diets was analyzed as described by AOAC (2005) (Table 1). Two hundred and twenty-five specimens with a mean initial weight of 4.07 ± 0.01 (g/fish) were kept in the glass tanks (water capacity of 80 L; width 45 cm × length 60 cm × height 60 cm) at the stocking density of 15 fish/tank. Three replicated tanks were assigned randomly to each treatment (45 fish/treatment), and the fish were fed for three months.

During this time, diets were given to fish manually daily in two feeding sessions (8:00 – 16:00). After that, the uneaten diets were collected from the tanks, dried,

and stored for the calculations of feed consumption parameters. The water was renewed after cleaning and removing the excreta from each tank. Quality of water was monitored by measuring physico-chemical variables using digital instruments, such as WTW Temperature and pH meter (Model: Temp. and pH Inolab 720), Lutron dissolves oxygen meter (Model: DO-5510), and WTW electrical conductivity meter (Model; EC 3110). The ranges of physicochemical parameters and feeding procedure during the experiment are summarized in

Table 1. Formulation and proximate composition of experimental diets for *O. niloticus*

Ingredients (g/kg)	Treatments				
	T1	T2	T3	T4	T5
Fish meal[a]	250	187.5	125	62.5	-
Soybean meal	402	301.5	201	100.5	-
Sunflower meal	-	163	326	489	652
Corn gluten meal	60	60	60	60	60
Wheat flour	60	60	60	60	60
Rice polish[b]	65	65	65	65	65
Cod liver oil	90	90	90	90	90
Vitamin premix[c]	20	20	20	20	20
Mineral mix[d]	20	20	20	20	20
Binder	15	14.5	14.5	14.5	14.5
α-tocopherol acetate (mg)	0.5	0.5	0.5	0.5	0.5
Citric acid	05	05	05	05	05
Choline chloride	03	03	03	03	03
Dicalcium phosphate	9.5	9.3	7.9	7.2	6.5
Lysine (g/100g)	-	0.5	1.0	1.5	2.0
Methionine (g/100g)	-	0.2	0.4	0.6	0.8
Nutrient composition (g/kg)					
Dry matter	912.19	905.35	923.64	914.12	920.36
Crude protein	395.90	397.47	394.58	396.21	389.79
Lipid	152.60	149.82	141.97	143.27	142.28
Ash	120.10	115.54	119.63	119.97	124.41
NFE[e]	243.56	242.52	267.46	254.67	263.88
Energy (MJ/kg)	19.37	18.74	18.42	18.97	18.79

a Pakistan Fish meal Plot-19, Sector, 16 Korangi Industrial Area Karachi, Pakistan, **b** Al-Hilal Industries, Rafhan Mills and Iqbal Rice Mills Lahore, **c** *Bio Pharmachemie*-VIETNAM (100 g contains: 1,000,000 IU A, 200,000 IU D_3, 150 mg B_1, 300 mg B_2, 250 mg B_3, 150 mg B_6, 1050 mg B_{12}, 100 mg K_3, 5 mg Folic acid, 1 mg Biotin, 2.5 g Niacin, 20 g $NaHCO_3$, 1 g NaCl, 2 g KCl), **d** *Nawan Laboratories* (PVT) LTD., Animal Health Division Karachi-Pakistan (1 kg contains: 155 g Ca, 135 g P, 55 g Mg, 45 g Na, 1 g Fe, 3 g Zn, 2 g Mn, 0.6 g Cu, 10 mg Co, 40 mg I, 3 mg Se), **e** Nitrogen Free Extract (NFE) = 100 – (moisture + crude protein + lipid + ash)

Table 2. Rearing conditions and water quality parameters during the feeding of *O. niloticus* with varying levels of sunflower meal (SFM) containing diets

Parameters	Treatments				
	T1 (0% SFM)	T2 (25% SFM)	T3 (50% SFM)	T4 (75% SFM)	T5 (100% SFM)
Feeding rate (% of body mass)	3	3	3	3	3
Feeding frequency/day	2	2	2	2	2
Feeding method	Hand-fed	Hand-fed	Hand-fed	Hand-fed	Hand-fed
Water replacement/week	3	3	3	3	3
Photoperiod (Light: Dark)	12:12	12:12	12:12	12:12	12:12
Aeration time (hr.)	24	24	24	24	24
Dissolved oxygen (mg/L)	6.0±0.12	5.9±0.18	6.2±0.45	6.7±1.12	6.3±0.42
pH	7.9±1.13	7.9±1.8	7.8±0.07	8.1±0.02	8.1±0.02
Water temp. °C	29-31	30-31	28-31	30-31	28-31
Air temp. °C	32-39	32-39	32-39	32-39	32-39
Electrical conductivity C (µS/cm)	409±10	412±15	404±15	418±09	419±13
Total ammonia (mg/L)	0.06-0.09	0.05-0.09	0.06-0.08	0.07-0.10	0.05-0.08

Table 2. The natural photoperiod (12 hr. light: 12 hr. dark) was maintained throughout the feeding trial.

Calculations

The mean initial weight for each replicate was recorded at the start of the experiment, and an increase in weight was measured bi-weekly. The physiological condition of the fish was checked and the record of fish mortality was maintained, whereas the final weight and survival rate were recorded at the termination of feeding trial. Growth performance and feed consumption parameters were calculated using the following standard equations:

WG (Weight gain, g) = Final weight (g) – Initial weight (g)

WG% (Weight gain percentage) = (Final weight – Initial weight) x 100/ (Initial weight)

SR (Survival rate) = Final number of fish x 100 / Initial number of fish

FI (Feed intake, g/fish) = Feed given (g) – Uneaten feed (g)

FCR (Feed conversion ratio) = Dry feed intake (g) / Wet weight gain (g)

PER (Protein efficiency ratio) = Wet weight gain (g) / Dry protein intake (g)

Three fish were randomly chosen from each tank (*n* = 3; 9 fish/treatment), weighed individually, and viscera and liver samples were extracted to calculate the following body indices.

VSI (Viscerosomatic index) = 100 x Weight of viscera (g)/ Total weight of body (g)

HSI (Hepatosomatic index) = 100 x Weight of liver (g)/Total weight of body (g)

Sample Collection

Four fish specimens were randomly collected from each tank (*n* = 4; 12 fish/ treatment) and oven-dried for the whole-body proximate analysis. Another batch of five fish was taken from each tank (15 fish/treatment), anesthetized with amino-benzoic acid (120 mg/L), and blood was collected in plain glass tubes from the caudal vessels complex. After centrifugation at 6000 rpm for 10 minutes at 4°C, the serum was separated to analyze the serum chemistry. The intestine sections (approximately 1 cm) were excised from three fish in each replicated tank (9 fish/treatment) and stored in 10% neutral buffered formalin solution for histological studies.

Chemical Analyses

The proximate composition of experimental diets and fish whole-body was performed as described by AOAC (2005). The samples were oven-dried for 6 h at 105^{o}C to determine the moisture content (Memmert UN30). Nitrogen (%) was determined to measure the crude protein content (N_2 x 6.25) through the micro Kjeldhal technique (Hanon K1100F micro- Kjeldhal auto-analyzer). The petroleum ether extraction method was used to determine lipids content (Soxtec HT2 1045 System). Ash content was determined by burning the samples at high temperatures (650 ^{o}C) in an electric muffle furnace (Eyela-TMF 3100). Gross energy was determined using a bomb calorimeter, calculating the heat of combustion. The serum glutamic-pyruvic transaminase (GPT), glutamic-oxalacetic transaminase (GOT), total protein (TP), albumin (Alb), cholesterol (CHO), triglycerides (TG), and high-density lipoproteins-cholesterol (HDL-C), glucose (Glu), and creatinine (CR) content were assayed using commercially available kits (Human Diagnostics World Wide®).

Histological Evaluation

H & E Slides Preparation

The intestine tissues were kept in formalin solution for 24 hrs. After the fixation process, a graded ethanol series was used for dehydration. Thereafter, tissues were placed in xylene and embedded in paraffin wax. Each tissue was sliced into longitudinal sections having a thickness of 5 µm (Microtome THERMO WTO/PAT/E/008) and stained in hematoxylin and eosin (H&E). The intestine sections were deparaffinized in xylene and rehydrated using a graded ethanol series. The dehydrated sections were blotted once again and cleared in two changes of xylene for the durations of 10 and 15 min. The sections were mounted in Distyrene Plasticizer Xylene (DPX) and were examined under a light microscope (Leica Microsystem Wetzier GmbH, Germany).

Semi-Quantitative Scoring System

A previously designed scoring system known as Uran's score system was set to evaluate the morphological changes and degree of severity in histological responses of *O. niloticus* intestine fed with varying inclusion percentages of SFM including (i) thickness of connective tissues between mucosal and submucosal layer (ii) length of mucosal folds (iii) width of lamina propria and (iv) presence of vacuoles in the folds. Each response was ranked according to the Uran's score, where 1 was for the normal appearance of the intestine, 2 indicated the slight morphological changes, 3 showed clear changes in the intestine, 4 represented damaged morphology of the intestine, and 5 exhibited severely damaged and potentially lethal condition. The

mean (n = 3) of these four histological responses were recorded and pooled with Uran's score for each intestine sample (Refstie et al., 2010).

Statistics Analysis

All data were presented as mean ± standard error of mean (SEM). The normality of data and homogeneity of variance were verified by Welch and Brown-Forsythe tests, respectively. Thereafter, data were subjected to a one-way analysis of variance (ANOVA), and Tukey's Honestly Significance (THS) test was applied as a post-hoc test to determine the significance among the means of different treatments. The histological data were subjected to a non-parametric (Kruskal-Wallis) test, and the difference among the treatments was considered significant at $P < 0.05$. SPSS (version IBM 22.0) statistical software for Windows program (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis.

Results

Effects of Sunflower Meal Supplementation on Growth Performance of *O. niloticus*

The dietary supplementation of SFM at varying inclusion percentages showed a significant effect on the growth performance of *O. niloticus* (Table 3). The growth performance was significantly decreased as the dietary supplementation level of SFM increased. Among SFM supplemented groups, fish fed T2 and T3 diets showed higher FW, WG, and WG %, which did not significantly differ from the fish fed with T1 diet. However, significantly lower growth performance was exhibited by the fish fed with T5 diet as compared to control and other SFM supplemented fish ($P < 0.05$). Throughout the feeding experiment, all fish were in good condition and their survival rate did not significantly ($P > 0.05$) differ among the treatments (Table 3).

Effects of Sunflower Meal Supplementation on Feed Consumption of *O. niloticus*

The feed consumption parameters of *O. niloticus* fed at varying inclusion percentages of SFM showed significant differences among the treatments (Table 3). FI and PER significantly increased, whereas FCR significantly ($P < 0.05$) decreased in the fish fed with T1, T2, and T3 diets. However, FI and PER decreased and FCR increased in the fish fed with T4 and T5 diets.

Effects of Sunflower Meal Supplementation on Body Indices and Whole-Body Proximate Composition of *O. niloticus*

The varying inclusion percentages of SFM significantly influenced the body indices of *O. niloticus* (Figure 1). A significant increase in HSI was observed in the fish fed with T5 diet. Lower HSI values were recorded in the fish that received T1 and T2 diets ($P < 0.05$). Similarly, VSI was found to be increase in the fish fed with T4 and T5 diets, whereas the lowest value was measured in the fish fed with T1 diet as compared to other treatments ($P < 0.05$). Furthermore, the whole-body proximate composition of *O. niloticus* fed at varying inclusion percentages of SFM significantly differed among treatments (Figure 2). The DM content of *O. niloticus* significantly ($P < 0.05$) increased in the fish fed with T1, T2, T3, and T4 diets compared to fish fed T5 diet. TP content was significantly ($P < 0.05$) increased in fish fed with T1, T2, and T3 as compared to the fish fed with T4 and T5 diets. Inversely, TL content decreased in the fish fed with T1, T2, and T3 diets. However, no significant ($P > 0.05$) variation was found for ash content among all the treatments.

Effects of Sunflower Meal Supplementation on Serum GPT and GOT Activity of *O. niloticus*

The activity of serum GPT and GOT in *O. niloticus* fed at varying inclusion percentages of SFM showed significant differences (Figure 3). A significant decrease in GPT and GOT activity was observed in the fish fed with T1, T2 and T3 diets. Whereas, significantly increased activity was measured in the fish fed with T4 and T5 diets ($P < 0.05$).

Effects of Sunflower Meal Supplementation on Serum Chemistry of *O. niloticus*

Inclusion percentages of SFM to diet significantly affected the serum chemistry of *O. niloticus*. A significant ($P < 0.05$) enhancement was observed in serum TP and albumin (Alb) contents of the fish fed with T1, T2, and T3 diets compared to other dietary treatments (Figure 4). Additionally, serum CHO and TG contents significantly ($P < 0.05$) elevated in the group receiving control diet compared to SFM-supplemented groups. Furthermore, serum HDL-C content significantly ($P < 0.05$) increased in the fish fed with T1, T2, and T3 diets compared to the groups receiving T4 and T5 diets. Serum Glu and CR contents showed no significant ($P > 0.05$) variations among the treatments (Table 4).

Effects of Sunflower Meal Supplementation on Intestine Morphology of *O. niloticus*

The semi-quantitative score of intestine morphology of *O. niloticus* fed at varying inclusion percentages of SFM is presented in Table 5. The Uran's pooled score of four histological traits significantly ($P < 0.05$) decreased in the fish fed with T1 diet compared to SFM-supplemented groups. The groups fed with T1, T2 and T3 diets showed a significant ($P < 0.05$) reduction in the thickness variation of connective tissues between mucosal and submucosal layers. However, the length of mucosal fold significantly ($P < 0.05$) decreased in the fish

fed with T1 and T2 diets compared to other treatments. Similarly, fish fed with T1, T2, and T3 exhibited reduced lamina propria width, while vacuolization significantly ($P < 0.05$) increased in the fish fed with T3, T4, and T5 diets (Figure 5).

Discussion

The present study showed the possibility of fish meal (FM) and soybean meal (SBM) replacement with the sunflower meal (SFM) as a dietary protein source for the diets of *O. niloticus* up to 50% which is comparable with the previous reports (Dayal et al., 2011; Jackson et al., 1982; Stickney et al., 1996; Olvera-Novoa et al., 2002). This improved growth of *O. niloticus* up to 50% SFM inclusion might be due to the improved feed intake. Additionally, it could be assumed that fish with improved growth performance was not suffering from any type of malnutrition or was not underfed during our study. However, a decline in growth performance beyond the 50% inclusion of SFM in the current study, might be due to various types of anti-nutritional factors (ANFs), such as saponins, tannins, protease inhibitors, arginase inhibitors, etc. present in SFM. Additionally, reports suggested that SFM has a large quantity of non-starch polysaccharides (NSPs) and lignin. In fish, both

Table 3. Growth performance and feed consumption of *O. niloticus* fed diets with varying levels of sunflower meal (SFM)

Parameters	Treatments					
	T1 (0% SFM)	T2 (25% SFM)	T3 (50% SFM)	T4 (75% SFM)	T5 (100% SFM)	P value
Initial weight (g)	4.69±0.51	4.69±0.42	4.70±0.52	4.72±0.74	4.72±0.64	NS
Final weight (g)	22.02±2.23^a	21.97±1.96^a	21.42±2.28^a	12.52±2.74^b	7.43±2.03^c	0.000*
Weight gain (g)	17.33±2.7^a	17.27±1.67^a	16.72±2.58^a	7.80±2.49^b	2.71±2.36^c	0.009*
Weight gain %	369.50±6.97^a	367.93±2.49^a	355.74±5.49^a	165.04±3.04^b	57.53±4.37^c	0.000*
Survival %	100±00	100±00	100±00	99.33±1.15	100±00	NS
Feed intake (g/fish)	21.72±2.01^a	21.61±3.18^a	20.48±3.07^a	14.21±1.13^b	6.01±4.02^c	0.000*
Feed conversion ratio	1.24±0.08^a	1.25±0.31^a	1.25±0.01^a	1.82±0.03^b	2.21±0.39^c	0.000*
Protein efficiency ratio	2.03±0.28^a	2.01±0.32^a	2.01±0.30^a	1.38±0.28^b	1.15±0.13^c	0.000*

All the data were presented as mean ($n = 3$) ± SEM and values of different treatments within the same row having different superscripts are significantly different at $P < 0.05$, NS = not significant.

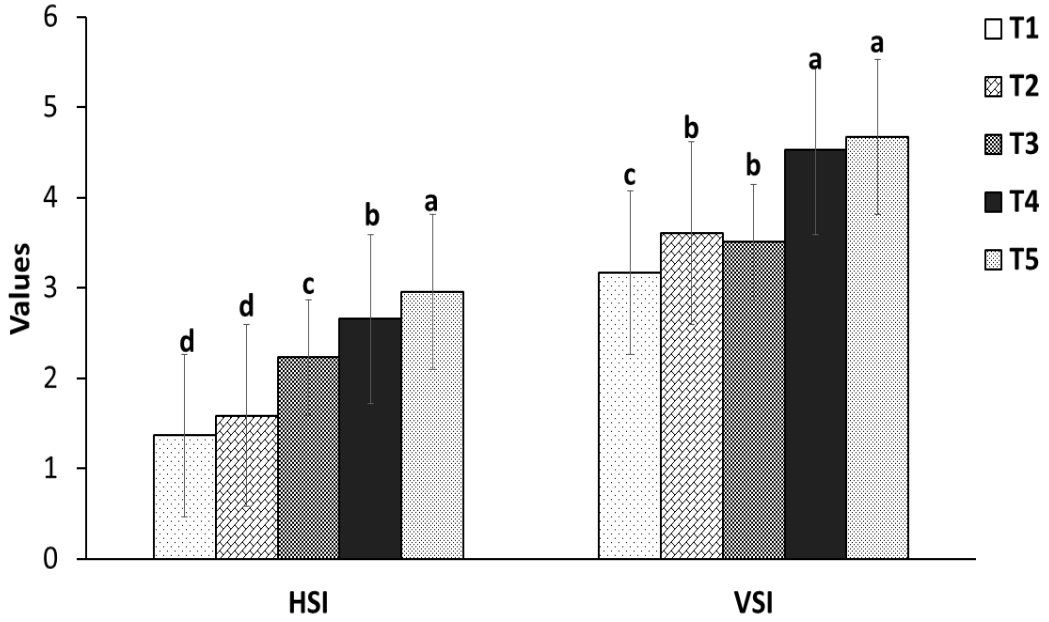

Figure 1. The hepatosomatic index (HSI) and viscerosomatic index (VSI) of *O. niloticus* fed diets with varying levels of sunflower meal (SFM). T1, T2, T3, T4, and T5 diets were supplemented with 0, 25, 50, 75, and 100% of SFM, respectively. Data are presented as the mean value of three replicates ($n = 3$) ± SEM. The graph bars with different superscripts are significantly differ at $P < 0.05$.

ANFs and NSPs, and lignin may cause negative effects by absorbing the bile acids and hindering the activity of digestive (especially proteolytic) enzymes, resulting in lower nutrient absorption and poor growth performance (Tacon et al., 1984; Francis et al., 2001). Therefore, reduced growth performance at high inclusion percentages of SFM might be associated with the obstruction in the action of digestive enzymes in the gastrointestinal tract (GIT) by these anti-nutrient factors (Lin et al., 2010). Conversely, few studies demonstrated that higher percentages of SFM inclusion to the diet had no significant effect on the growth performance of Atlantic salmon and sharp snout sea bream (Gill et al., 2006; Nogales Mérida et al., 2011). The results of nutritional studies regarding the growth response depend on several factors e.g., variations in the quality of SFM crop, cultivation process, SFM-preparing technique, difference in fish species and interaction of dietary protein source with other nutrients present in diet (Iqbal et al., 2021). Hence, one of the above-mentioned factors might be responsible for these disparities concerning the growth performance. No significant variation was observed in the survival rate of fish in the present study which is in agreement with the findings of Olvera-Novoa et al. (2002), who demonstrated that the substitution of FM and SBM by SFM had no negative effects on the survival rate of tilapia, *O. rendalli*.

Table 4. Serum chemistry of *O. niloticus* fed diets with varying levels of sunflower meal (SFM)

Parameters	Treatments					
	T1 (0% SFM)	T2 (25% SFM)	T3 (50% SFM)	T4 (75% SFM)	T5 (100% SFM)	P value
Cholesterol (mg/dL)	8.69±0.91[a]	6.36±0.47[b]	6.12±0.05[b]	6.49±0.24[b]	6.91±1.04[b]	0.000*
Triglycerides (mg/dL)	4.04±0.80[a]	1.49±0.86[b]	1.47±0.02[b]	1.26±0.02[b]	1.19±0.13[b]	0.000*
Glucose (mg/dL)	50.40±3.01	48.10±2.48	49.52±4.80	47.27±3.10	49.25±3.12	NS
HDL-C (mmol/L)[a]	1.09±0.13[a]	1.07±0.10[a]	1.08±0.17[a]	0.57±0.12[b]	0.59±0.14[b]	0.009*
Creatinine (µM/L)	41.91±2.13	39.89±2.05	41.75±2.18	42.06±2.13	41.75±2.91	NS

a HDL-C = high-density lipoprotein-cholesterol. Data were presented as mean (*n* = 3) ± SEM and values of different treatments within the same row having different superscripts are statistically different at P < 0.05, NS = not significant.

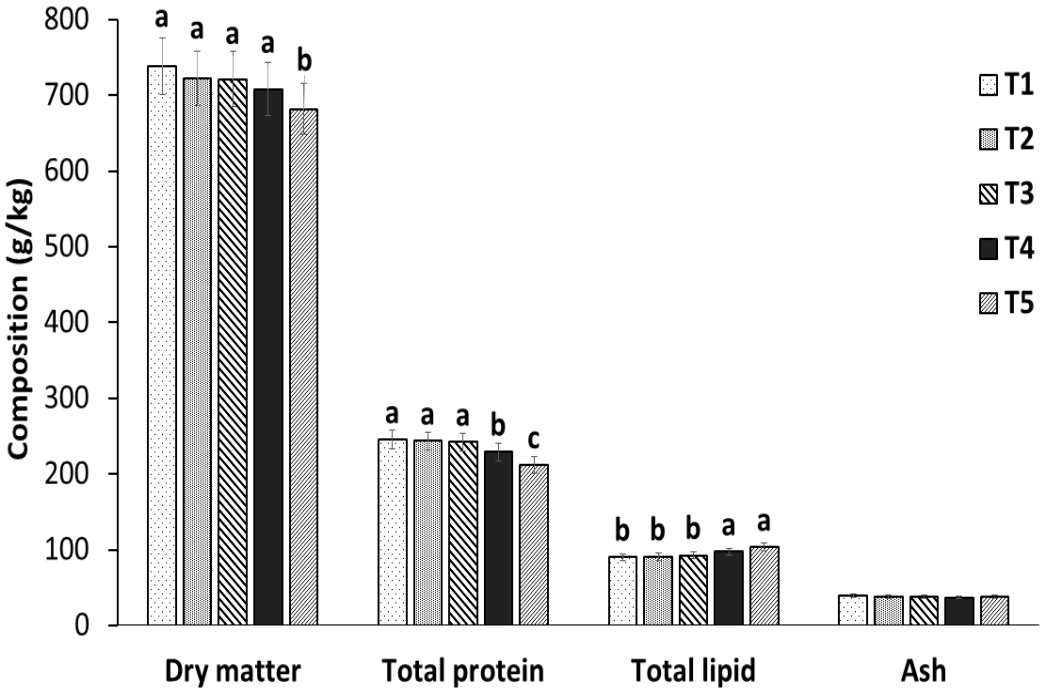

Figure 2. The whole-body dry matter, total protein, total lipid, and ash content (g/kg) of *O. niloticus* fed diets with varying levels of sunflower meal (SFM). T1, T2, T3, T4, and T5 diets were supplemented with 0, 25, 50, 75, and 100% of SFM, respectively. Data are presented as the mean value of three replicates (*n* = 3) ± SEM. The graph bars with different superscripts are significantly differ at P < 0.05.

In our current study, improved feed consumption parameters up to 50% inclusion percentage of SFM is following the previous reports in tilapia, Japanese flounder (*Paralichthys olivaceus*), Ussuri catfish (*Pelteobagrus ussuriensis*), freshwater shrimp (*Macrobrachium rosenbergii*), and Atlantic salmon (Sanz et al., 1994; Ye et al., 2011; Mugo-Bundi et al., 2013; Wang et al., 2015). The increased feed consumption of fish up to 50% SFM inclusion level in the present study indicated that the fish fed with diet 50% SBM content of which replaced by SFM were not suffering from the deficiency of dietary essential amino acids and fatty acid (Zhou et al., 2005). In addition, past studies have suggested that low dietary fiber content could decrease

Table 5. The semi-quantitative score of *O. niloticus* intestine fed diets with varying levels of sunflower meal (SFM)

Parameters	Treatments					P value
	T1 (0% SFM)	T2 (25% SFM)	T3 (50% SFM)	T4 (75% SFM)	T5 (100% SFM)	
Mucosal and submucosal layer	1.3^{bc}	1.3^{bc}	1.0^{c}	1.7^{b}	3.0^{a}	0.000*
Length of mucosal fold	1.0^{d}	1.3^{d}	2.6^{c}	3.7^{b}	4.6^{a}	0.000*
Width of lamina propria	1.0^{b}	1.0^{b}	1.6^{b}	4.0^{a}	4.3^{a}	0.000*
Vacuolization	1.0^{c}	1.3^{c}	3.0^{b}	4.3^{a}	4.7^{a}	0.000*
Uran pooled score	1.08^{d}	1.25^{cd}	2.08^{c}	3.41^{b}	4.16^{a}	0.000*

Data were presented as mean (n = 3) ± SEM and values of different treatments within the same row having different superscripts are statistically different at P < 0.05.

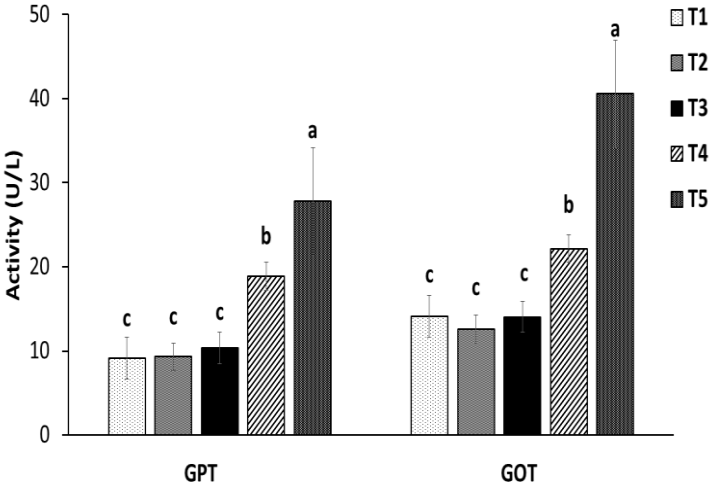

Figure 3. The serum glutamic-pyruvic transaminase (GPT) and glutamic-oxalacetic transaminase (GOT) activity (U/L) of *O. niloticus* fed diets with varying levels of sunflower meal (SFM). T1, T2, T3, T4, and T5 diets were supplemented with 0, 25, 50, 75, and 100% of SFM, respectively. Data are presented as the mean value of three replicates (n = 3) ± SEM. The graph bars with different superscripts are significantly differ at P < 0.05.

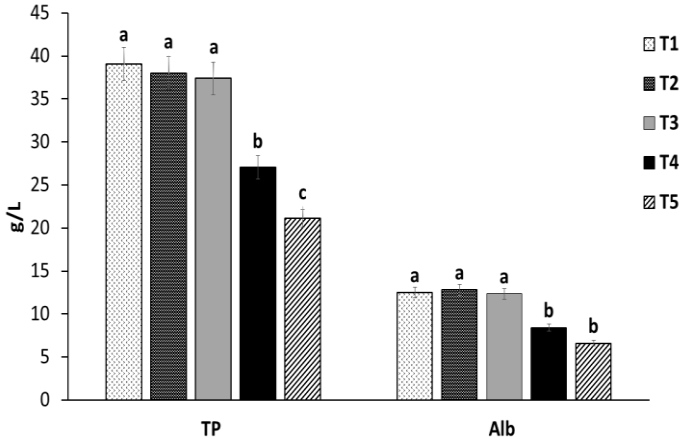

Figure 4. The serum total protein (TP) and albumin (Alb) content (g/L) of *O. niloticus* fed diets with varying levels of sunflower meal (SFM). T1, T2, T3, T4, and T5 diets were supplemented with 0, 25, 50, 75, and 100% of SFM, respectively. Data are presented as the mean value of three replicates (n = 3) ± SEM. The graph bars with different superscripts are significantly differ at P < 0.05.

the passage time in the intestine, consequently providing more contact time with the absorptive epithelium in the gastrointestinal tract. This delayed passage time of intestinal content that positively influencing the digestion and absorption of nutrients that might be another plausible explanation for the increased feed consumption (El-Sayed and Gaber, 1997). Whereas, high fiber content due to increased inclusion percentages of SFM in diet may adversely affect the taste of feed, so fish may not accept the feed, resultantly suffering from depressed feed consumption and utilization (Santiago and Lovell, 1988). This deprived feed consumption beyond the 50% inclusion percentage of SFM is similar to the previous data published for Nile tilapia and European eel (Garcia-Gallego e al.,1998; El-Sayed, 1999). Former studies have reported the presence of phytate or phytic acid also known as myo-inositol 1-6- hexaphosphate in SFM that stores the phosphorous (up to 80%) and builds the insoluble complexes with other nutrients (such as proteins, carbohydrates, lipids, minerals, and vitamins) in the intestine that could not be available to fish (NRC, 1993). Furthermore, previous reports recommended the occurrence of two different varieties of NSPs (13-16%) based on their solubility in SFM; one is soluble and the other one is in-soluble (Erdman,1979; Dusterhoft et al., 1992). Among these, soluble NSPs, such as beta-glucans, mannans, galactans, xyloglucan, and fructan, are more detrimental to the fish. The full mechanisms of these NSPs have not been elucidated yet. However, a few past experiments revealed that NSPs produce gum-like masses after trapping the water in the intestine and subsequently, enhance the viscosity of intestinal content thus interrupting the nutrient absorption in the intestine (Refstie et al., 2000; Becker et al., 2001).

The body indices showed increased value after the 50% inclusion percentages of SFM in the present study, which is similar to the previous studies conducted with rainbow trout and Arctic charr (Brauge et al., 1995; Gelineau et al., 2001; Smith et al., 2018). According to the previous findings, high dietary fat intake may play a significant role on the fat deposition of liver and other visceral organs in the fish body (Rocha et al., 1994). Additionally, the increased production of lipogenic enzymes (such as ATP-citrate lyase, acetyl-coenzyme A carboxylase, fatty acid synthase, etc.) might be another plausible explanation for the increased liver fat. Since these enzymes are mainly considered to be responsible for converting glucose into triglycerides in the body. On the other, high synthesis rates of lipogenic enzymes may interfere with the other adipose metabolic pathways by hindering the transport of nutrients (especially lipids and proteins) in the animal body (Kaushik et al., 1995).

In the present study, whole-body proximate composition e.g., dry matter and total protein of *O. niloticus* showed significant differences among dietary treatments that are in line with previous findings in redbreast tilapia and sharp snout sea bream (Olvera-Novoa et al., 2002; Nogales Mérida et al., 2011). The protein synthesis in the body, as well as protein deposition rate in muscles, is mainly influenced by dietary protein sources (Tacon et al., 1984; Hardy,

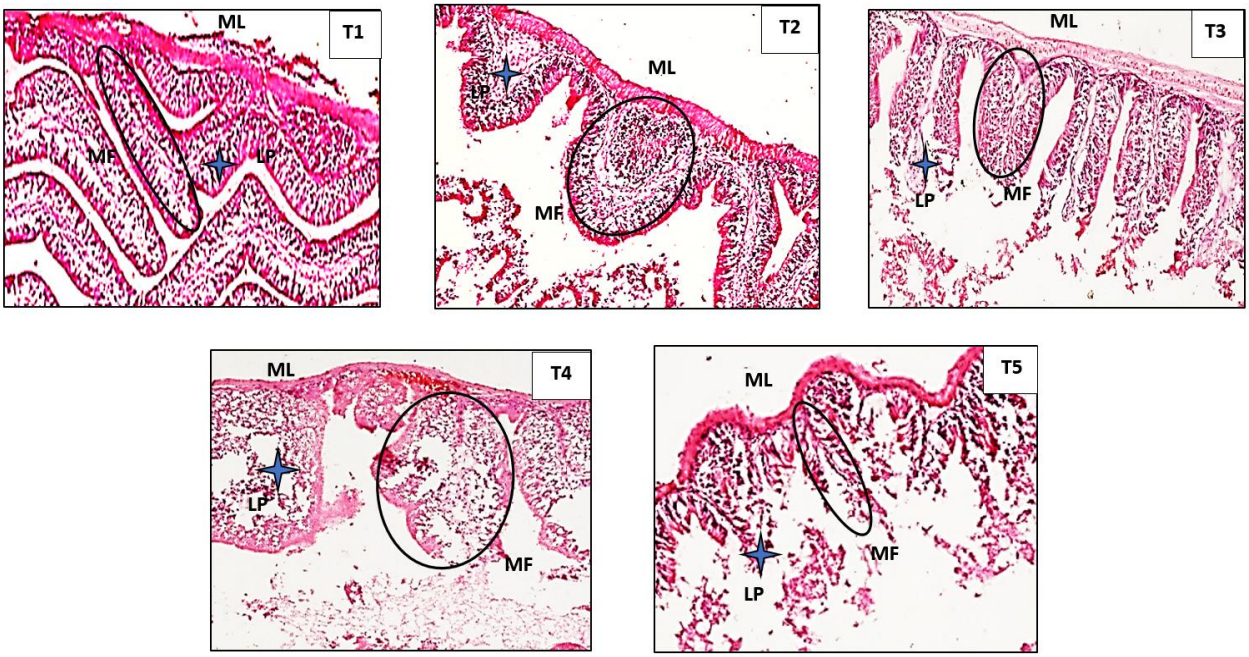

Figure 5. The longitudinal section (40 X) of the distal intestine of Nile tilapia fed diets with varying levels of sunflower meal (SFM). T1, T2, T3, T4, and T5 diets were supplemented with 0, 25, 50, 75, and 100% of SFM, respectively. ML = mucosal layer, LP = lamina propria, MF = Mucosal fold.

2010). The intake of inferior quality protein, low dietary energy, phosphorous, and suboptimal levels of amino acids (methionine and lysine) might be the possible cause of this response (Rahmdel et al., 2018). In contrast, whole-body lipids were significantly increased with increasing the inclusion percentage of SFM in the present study, which is similar to the observations made in redbreast tilapia (Olvera-Novoa et al., 2002). The feeds prepared with the high SFM primarily supply inadequate dietary essential amino acids. Hence, these feeds may interfere with the metabolic pathways by altering the catabolic and anabolic functions, consequently causing fat deposition in the body (Rahmdel et al., 2018). Whereas, studies conducted with redbreast tilapia and gilthead sea bream have shown no significant effects of SFM on body fat content (Gill et al., 2006; Sanchez-Lozano et al., 2007). The plausible explanation for the observed differences in the fat deposition might be variation in the dietary lipid's sources, rearing regimes, fish species, and age (Nogales Mérida et al., 2011). The present study displayed a non-significant difference in the ash content among treatments, which is in line with the reports of Sanchez-Lozano et al. (2007).

The protein enzymes are frequently analyzed to evaluate the protein metabolism and health status of fish (Adhikari et al., 2004). In the present study, GOT and GPT activity increased significantly after the 50% inclusion percentage of SFM. This result is similar to the reports of Hassaan et al. (2015; 2018) with Nile tilapia fed with fermented SFM. On the contrary, decreased GOT and GPT activities were measured in the muscles of rohu, *Labeo rohita*, fed with Jatropha protein concentrate (Shamna et al., 2015). These variations in the response indicated the effect of different diet formulations on the metabolism. Additionally, different dietary protein sources could affect the protein enzyme activity differently (Jahanbakhshi et al., 2013). Likewise, enhanced protein enzyme activity in the current study suggested that high inclusion percentages of SFM could cause damage to the liver and thus, may promote stress in fish (Ngo et al., 2016).

Moreover, serum TP and Alb were significantly improved up to 50% inclusion percentage of SFM. Similar to this, increased plasma protein and albumin were observed in common carp and juvenile turbot (Kumar et al., 2010; Nagel et al., 2012). The reduced serum protein in fish fed with high inclusion percentages of SFM could be attributed to the deprived protein intake, protein synthesis of amino acids, and nutrient digestibility (Bransden et al., 2001; Zhou et al., 2005). Whereas, some reports suggested no significant effect of SFM inclusion on plasma protein levels of gilthead seabream, European seabass, and common carp (Peres et al., 2014; Rahmdel et al., 2018; Diogenes et al., 2019). In the present study, serum CHO and TG contents was significantly elevated in control. Whereas, HDL-C was significantly enhanced up to 50% of SFM replacement, similar to the previous findings observed in rainbow trout, gilthead seabream, and common carp (Kaushik et al., 1995; Sitja-Bobadilla et al., 2005; Kumar et al., 2010). The past studies revealed that SFM has various types of anti-cholesterol factors known as phytosterols, however, which are not available in animal proteins. During digestion, these phytosterols undergo various mechanisms to reduce cholesterol, thus, improving the health status of fish (Wester, 2000). Furthermore, protein catabolic metabolites are largely present in the liver which are the main constituents of cholesterol (Baghchi et al., 1963). Thus, diets containing a high quantity of FM may enhance cholesterol levels in the body which could explain the high levels of serum cholesterol in fish fed with control diet in the present study. Serum TG content is mainly used to mirror the lipid metabolism in animal studies (Ma et al., 2016). Increased plasma TG level has been reported by Dias et al. (2005) and Hassaan et al. (2018) in European seabass and Nile tilapia, respectively. The unavailability of essential amino acids in diets with high inclusion percentages of SFM may boost the process of lipogenesis and reduces the beta-oxidation of fatty acids. Therefore, liver dysfunction might be a significant factor for high TG content in the control group (Baghchi et al., 1963). In the present study, no significant difference was observed in serum Glu and CR contents among the treatments. In contrast, increased plasma glucose was found in Atlantic salmon, common carp, and Asian seabass (Bransden et al., 2001; Kumar et al., 2010; Rahmdel et al., 2018; Ngo et al., 2016). This contradiction in the results might be linked to the difference in rearing regimes, feeding habits, and fish species. Moreover, the non-significant variation in CR content among treatments in the current study indicates a normal renal function in fish.

In the current study, the degree of variation in the morphology of the distal intestine of *O. niloticus* was significantly increased by increasing the inclusion percentages of SFM. Adequate literature concerning the effect of dietary SFM inclusion on the intestine histology of tilapia-like species is not available so far. However, some studies reported significant pathomorphological changes in the distal intestine of carp species and Atlantic salmon (Van den Ingh et al.,1991; Baeverfjord and Krogdahl,1996; Krogdahl et al., 2003; Urán et al., 2008; Refstie et al., 2010). In addition, inflammation, vacuolization, and cell infiltration in distal intestine of rainbow trout, Atlantic salmon, and Arctic charr were reported by Refstie et al. (2000) and Smith et al. (2018) with some other plant proteins. It has already been established that the fish are highly sensitive to both – nutrient and anti-nutrient constituents of feed or anti-nutrients present in SFM (NRC, 1993; Hasan et al., 1997; Mukhopadhyay and Ray, 1999). The former observations have assessed the effect of various dietary formulations on the morphological changes and pathological conditions, such as enteritis in fish that might be due to some ANFs, such as trypsin inhibitor, chlorogenic acid, or phytic acid (Refstie et al., 2010). On

the other side, decreased feed consumption at high inclusion percentages of SFM in this study is well parallel to the digestive disturbances and poor gut health in fish (Bakke-McKellep et al., 2000; Glencross et al., 2007). On contrary, Overland et al. (2009) and Colburn et al. (2012) found no significant difference in intestine morphology of the Atlantic salmon and Atlantic cod fed with pea protein concentrate and soy protein concentrate. The difference in plant material, crop quality, oil extraction technique, inclusion percentage, and process of feed preparation could be responsible for these morphological changes in the intestine.

In conclusion, SFM could replace SBM and FM in the diets for *O. niloticus* up to 50% without compromising its productivity, body indices, whole-body proximate composition, protein enzyme activity, and serum chemistry. Meanwhile, higher inclusion percentages of SFM could show adverse effects on intestine histology. Hence, we suggest that SFM could be included up to 326 g/kg in diets of *O. niloticus*.

Ethical Statement

The experimental work and analytical procedures were approved by the Ethics Committee of Government College University Lahore, Pakistan.

Funding Information

Not applicable

Author Contribution

Maryam Iqbal: Conceptualization, Methodology, Data Curation, Formal Analysis, Writing -review and editing; Atif Yaqub: Supervision, Investigation, Visualization, review and editing; Writing -review and editing; Muhammad Ayub: Resources and Analysis.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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