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Growth and physiological responses of Nile tilapia, *Oreochromis niloticus* fed dietary fermented sunflower meal inoculated with *Saccharomyces cerevisiae* and *Bacillus subtilis*

Mohamed S. Hassaan[a,*], Magdy A. Soltan[b], Eman Y. Mohammady[a], Mohamed A. Elashry[b], Ehab R. El-Haroun[c], Simon J. Davies[c]

[a] Aquaculture Division Fish Nutrition Research Laboratory, National Institute of Oceanography and Fisheries (NIOF), Egypt
[b] Animal Production Department, Faculty of Agriculture, Benha University, Egypt
[c] Fish Nutrition and Aquaculture, Department of Animal Production, Welfare and Veterinary Sciences, Harper Adams University, England, UK

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ABSTRACT

The aim of this study was to enhance the nutritional value of sunflower meal used as a feed ingredient for Nile tilapia by application of a solid-state fermentation process with *Saccharomyces cerevisiae* (YFSFM) or *Bacillus subtilis* (BFSFM). At 72 h of fermentation, the crude protein, lipid content, essential amino acid contents were increased in YFSFM and BFSFM, but fiber content, chlorogenic acid, caffeic acid, phytic acid and saponins were decreased. A feeding trial of 84 days was conducted to study the response of feeding YFSFM and BFSFM on growth, hematological and physiological responses in Nile tilapia. Seven isonitrogenous and isocaloric were prepared by the replacing fish meal protein with the SFM protein (0% control diet), YFSFM-25 (25% YFSFM), YFSFM-50 (50% YFSFM) YFSFM-75 (75% YFSFM) and BFSFM-25 (25% BFSFM), BFSFM-50 (50% BFSFM), BFSFM-50 (50% BFSFM) and BFSFM-75 (75% BFSFM) and fed twice daily. Growth performance and feed efficiency of fish fed diets with fish meal replaced by YFSFM up to 25% was not significantly different from the control group, whereas ADCs were not significantly different from control up to 50% of YFSFM. No significant ($P > 0.05$) differences were found in hemoglobin (Hb), hematocrit (Htc), red blood cell (RBCs) and white blood cells (WBCs) among different experimental diets. The control diet, YFSFM-25 and BFSFM-25 exhibited lowest value of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in Nile tilapia. The highest value of serum phosphorus and calcium content was detected in the control diet and the lowest one was recorded in YFSFM-75 and BFSFM-75. Tilapia fed diet YFSFM-75 or BFSFM-75 recorded the lowest level of cholesterol, triglycerides high-density lipoprotein (HDL-C). There was no significant difference ($P > 0.05$) in low- density lipoprotein (LDL-C) and the ratio of HDL-C/LDL-C of the serum of fish among experimental diets.

1. Introduction

The rapid world-wide expansion of aquaculture and livestock production strongly indicates that a crisis will be precipitated in the livestock and aquaculture feed industries to meet such growing demands (FAO, 2014). Aquaculture industry obviously considered a main source of economic gain and employment generation, feed is the largest production cost for commercial aquaculture which accounting ~ 60–80% with being protein is the most expensive macro-nutrient (Bolivar et al., 2006; Brown et al., 2014; Ng et al., 2013). Several alternative plant protein source ingredients have been studied in recent years due to the reduction in fish meal production and increasing cost of fish meal (Tacon and Metian, 2015). A promising source of protein is that derived from plant raw materials, including secondary materials such as oil seed meals; a residual by-product of the oil extraction industry (Shchekoldina and Aider, 2014). Among the potential plant ingredients, sunflower meal (SFM) is one of the most promising ones (Rodrigues et al., 2012). According to the Food and Agriculture Organization of the United Nations (FAO, 2012), sunflower seed is among the world's most important oilseeds. Chemically, SFM consists of about 27.8 to 37.4% crude protein (strongly affected by sunflower variety (Munguti et al., 2006)) of which, 89–99% is true protein and the other 1–11% originates from peptides, amino acid or other nitrogenous substances (Gassmann, 1983). However, SFM is lower in sulphur amino acids compared to soybean meal, but does provide an array of other amino acids, especially glutamic and aspartic acid (Shchekoldina and Aider,

* Corresponding author.
E-mail address: ms.hassaan@niof.sci.eg (M.S. Hassaan).

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2014). Economically, SFM is cheaper than soybean meal (Köprücü and Sertel, 2012). These characteristics have attracted some attention as a potential substitute for use in formulated aquaculture feeds. Sunflower seed meal has been tested with species such as *Oreochromis mossambicus* (Jackson et al., 1982), *Oreochromis. niloticus* Linnaeus (Syntayehu et al., 1996), *Oncorhynchus mykiss* Walbaum (Stickney et al., 1996), and *Anguilla anguilla* Linnaeus (Garcia-Gallego et al., 1998). These studies revealed that SFM had no adverse effect on growth performance and feed conversion at low inclusion levels, but growth reduction can occur at high inclusion levels. According to Joachim and Felicitas (2000) the high crude fiber content of SFM limits its use in aquaculture diets. The high contents of phenolic compounds, notably chlorogenic acid and caffeic acid is the main reason for limitation of SFM in the aquafeed industry as well as these compounds appreciably reducing protein solubility (González-Pérez and Vereijken, 2007). In parallel, there has been increasing interest in maintaining certain useful phenolic compounds, and even in applying them to the aquaculture feed, due to their potent antioxidant activity (Sun et al., 2017). In general, oilseed meals processed in traditional ways cannot be utilized at high levels without compromising growth and production in farmed fish. Enhancement of the nutritive value of these ingredients by processing to increase the bio-availability of nutrients, reduce or remove anti-nutritional factors and the inclusion of appropriate additives could result in oilseed meals being incorporated at higher levels in fish feeds (Soltan, 2005).Thus, different methods to reduce Anti-nutritional factors (ANFs) and fiber content have been proposed, among them solid state fermentation (SSF) which has been demonstrated to be very effective with different microorganisms on arrange of substrate materials (Hassaan et al., 2015; Kader et al., 2012; Shiu et al., 2015a; Shiu et al., 2015b; Yuan et al., 2013; Zhou et al., 2011). The major advantages for utilization of low cost plant ingredients as substrates in SSF might be its great economic feasibility and as a way of nutrient recycling (Hassaan et al., 2017). In this context, it is unclear whether phenolic components should be removed or not when SFM is fermented through a solid-state fermentation process. The aims of this study were: (1) eliminate the anti-nutritional factors, (2) improve the nutritive value of SFM by decreasing the fiber content and increase the amino acid and protein content of sunflower meal, and (3) evaluate processed non-conventional fermented sunflower meal in formulation of diets for Nile tilapia, *Oreochromis niloticus* to assess growth performance and feed utilization parameters and selected hematological and metabolic indices of health and nutritional status.

2. Materials and methods

2.1. Bacterial strains and inoculum conditions

A Strain of the yeast, *S. cerevisiae* (Fermipan®, GB ingredients, China), with a cell density of 3×10^6 cell g^{-1}., together with a Strain of *Bacillus subtilis* E20 used was obtained from the Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt for the respective fermentation pathways. An inoculum of *B. subtilis* E20 was prepared by introduction of a loop-full of stock culture of strain *B. subtilis* into a medium which contained ($g\,L^{-1}$): peptone 5.0; beef extract, 3.0 and adjusted to pH 7.0. The incubation was performed at 37 °C which is the optimal growth temperature for *B. subtilis*. After the incubation stage, the cells were harvested by centrifugation (4000 *g* for 15 min) according to Sun et al. (2011). *B. subtilis* cell counts (colony-forming units, CFU) were determined using the spread plate method Al-Harbi and Uddin (2004). The overnight grown culture (OD~0.8) was used as the inoculum for further use to achieve fermentation of the sunflower meal via a bacterial route.

2.2. Solid state fermentation of de-oiled sunflower seed meal

A commercially de-oiled sunflower meal (SFM) was purchased from a local company (Kafr-elsheikh, Egypt) and ground to a particle size (< 500 μm) by a screen diameter. The substrate of SFM was subsequently divided into two portions (three replicates for each portion). The first portion was performed by a method of Yabaya et al. (2009). Each replicate, 2 kg SFM was inculcated with 60.5 mg of commercial dry yeast, *S. cerevisiae*, with a cell density of 3×10^6 cell g^{-1} (Fermipan®, GB ingredients, china) and 1.1 L of distilled water (50% moisture) were homogenized for 15 min. Each replicate was subjected to treatment for 24 and 48, 72 h in a 10-l glass bowl covered with aluminum foil and incubated at 40 °C which is the optimal growth temperature for *S. cerevisiae*. The second portion was performed by the method of Joshi et al. (2011). Each replicate, 2 kg SFM samples were inoculated with 600 ml of *B. subtilis* inoculum, followed by incubation at 37 °C and 65% relative humidity in a humidity controlled biochemical oxygen demand (BOD) for the last period 24, 48 and 72 h. After fermentation at each period, the fermented seed meal with *S. cerevisiae* and *B. subtilis* was dried (105 °C) for 3 h to arrest the microbial growth. The yeast fermented sunflower meal (YFSFM) and *Bacillus* fermented sunflower meal (BFSFM) were dried to constant weight at 70 °C. At the beginning (0 h) and after 24, 48 and 72 h of fermentation, 10 g of YFSFM and BFSFM was sampled to analyze the ANFs and chemical composition. Crude protein lipids, ash and fiber were estimated following the methods of the AOAC (1990).

2.3. Determination of anti-nutritional factors (ANF's)

Chlorogenic acid and caffeic acid in de-oiled SFM, YFSFM and BFSBM were detected as described by Ky et al. (1997). Phytic acid content was determined in de-oiled SFM, YFSFM and BFSBM using a spectrophotometric procedure according to the method of Vaintraub and Lapteva (1988). Saponin contents were determined using a spectrophotometric method as described by Hiai et al. (1976).

2.4. Determination of amino acid composition

The amino acid compositions of de-oiled SFM, YFSM and BFSM were determined using an automated amino acid analyzer after hydrolyzing the samples with 6 M HCl at 110 °C for 24 h (Bassler and Buchholz, 1993). Sulphur-containing amino acids were oxidized using performic acid before the acid hydrolysis.

2.5. Experimental diets

Seven isonitrogenous (295 $g\,kg^{-1}$ crude protein) and isocaloric (18.76 $MJ\,kg^{-1}$ gross energy) experimental diets were formulated and the proximate chemical composition of the experimental diets is presented in Table 6. The control diet contained an inclusion level of 180 g FM Kg^{-1} diet. This level was chosen as the sacrificial protein since fish meal has a high Biological Value (BV) and was deemed a reliable balanced protein for the optimum assessment of the SFM and the fermented SFM products. In the first three diets, FM protein was replaced with YFSFM at levels of 25% (YFSFM-25), 50% (YFSFM-50) and 75% (YFSFM-75), respectively. In last three diets, FM protein was replaced with BFSFM at levels of 25% (BFSFM-25), 50% (BFSFM-50) and 75% (BFSFM-75), respectively. Also, each of the diets contained 5 $g\,kg^{-1}$ chromic oxide (Cr_2O_3) as the reference inert marker to determine the nutrient digestibility of experimental diets. All dry ingredients of the fish meal, soybean meal, yellow corn and wheat bran were blended for 5 mins and thoroughly mixed with soybean oil. The ingredients were mixed well and made into dry pellets using a laboratory pellet mill

Table 1
Formulation and proximate composition of the experimental diets (g100 g^{-1} diet).

	Experimental diets						
	Control	YFSM-25	YFSM-50	YFSM-75	BFSM-25	BFSM-50	BFSM-75
Fish meal	18.00	13.50	9.00	4.50	13.50	9.00	4.50
Soybean meal	35.00	35.00	35.00	35.00	35.00	35.00	35.00
YFSFM†	0	5.20	10.44	15.66	0	0	0
BFSFM‡	0	0	0	0	5.40	10.60	15.80
Yellow corn	31.00	31.00	31.00	31.00	31.00	31.00	31.00
Wheat bran	8.50	7.80	7.06	6.34	7.60	6.90	6.20
Soybean oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin and Minerals[a]	2.70	2.70	2.70	2.70	2.70	2.70	2.70
Vitamin C	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Proximate analysis							
Dry matter	90.01	89.81	89.70	89.29	89.89	89.90	89.80
Crude protein	29.85	29.70	29.60	29.50	29.62	29.50	29.40
Ether extract	7.50	7.24	7.00	6.79	7.24	7.00	6.79
Ash	6.02	5.91	5.86	5.82	5.91	5.86	5.82
Fiber content	5.1	4.9	5	5.1	4.9	5.39	5.3
NFE[b]	51.53	52.25	52.54	52.79	52.33	52.25	52.69
Gross energy (MJ kg^{-1})[c]	18.85	18.84	18.77	18.71	18.84	18.70	18.67
Chlorogenic acid (mg kg^{-1})	–	0.370	0.743	1.111	0.406	0.806	1.19
Caffeic acid (mg kg^{-1})	–	0.00172	0.00345	0.00517	0.00211	0.00413	0.00616

[a] Vitamin and minerals kg^{-1} of mixture contains: 4800 I.U. Vit A, 2400 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), 3077 mg; ferrous sulfate ($FeSO_4.7H_2O$, 20% Fe), 65mg; manganese sulfate ($MnSO_4$, 36% Mn), 89 mg; zinc sulfate ($ZnSO_4.7H_2O$, 40% Zn), 150 mg; copper sulfate ($CuSO_4.5H_2O$, 25% Cu), 28 mg; potassium iodide (KI, 24% K, 76% I).

[b] NFE (Nitrogen free extract) = 100 - (crude protein + lipid + ash + fiber content).

[c] Gross energy calculated using gross calorific values of 23.63, 39.52 and 17.15 kJ g^{-1} for protein, fat and carbohydrate, respectively according to (Brett, 1973).
YFSFM† = Yeast fermented sunflower meal BFSFM‡ = Bacillus fermented sunflower meal.

(California Pellet Mill, San Francisco, CA, USA). The pellets (2-mm die) were dried for 4 h at 60 °C and stored at −20 °C until use (Table 1).

2.6. Experimental fish and culture technique

O. niloticus were obtained from a private farm (El-Fyum Governorate, Egypt). Tilapia were acclimated to the experimental conditions for two weeks at the laboratory of the Faculty of Agriculture, Benha University, Egypt. During the acclimation period, fish were fed a control diet (30% crude protein) at a rate of 3% of biomass, which was presented in equal rations at 09:00 am and 3:00 pm daily for 2 weeks to adapt tilapia the artificial diet and conditions of the trial. After the acclimatization, the experimental fish were distributed randomly into the experimental plastic tanks (0.5 m^3 for each) representing the seven treatments studied. 420 *O. niloticus* L fingerlings with an average initial weight of 10.30 ± 0.122 g were used in this trial. Twenty fish were randomly stocked into each tank with three replicates for each treatment. De-chlorinated public utility water was supplied to each aquarium housed within an artificially illuminated room. About one-third of water volume in each tank was daily replaced by aerated fresh water after removing the accumulated excreta. A photoperiod of 12-h light, 12-h dark (08:00–20:00 h) was used and fluorescent ceiling lights supplied the illumination. During the 84-days experimental period, fish were hand-fed with the respective diet to apparent satiation twice daily at 09:00 am and 3:00 pm. Thirty minutes after the feeding, uneaten feed were removed by siphoning, and then dried and weighed. Feed intake was calculated by the difference between them and expressed as the total feed intake in 84 days per fish. Water temperature and water quality parameters were monitored during the feeding experiment. Water temperature was recorded daily at 13.00 pm using a mercury thermometer. Dissolved oxygen (DO) was measured at 07.00 am using YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). Total ammonia was measured twice weekly using a DREL, 2000 spectrophotometer (Hash Company, Loveland, CO, USA). pH was measured in the morning by using a pH meter (Orion pH meter, Abilene, Texas, USA). Water temperature ranged from 26.9 to 28.3 °C; dissolved oxygen (DO) ranged between 5.85 and 6.20 mg L^{-1}; pH values ranged between 8.10 and 8.20 and total ammonia ranged from 0.17 to 0.19 mg L^{-1} for the different treatments during the entire experimental period of the study. All tested water quality criteria (temperature, pH value, DO and total ammonia) were within the acceptable limits for rearing Nile tilapia *O. niloticus* fingerlings (Boyd, 1990).

2.7. Calculation of growth indices

Growth performance and feed utilization were measured by using the following equations: Condition factor (K) = K = (W/L^3) × 100, Where W is weight of fish in grams and L = total length of fish in “cm”; Weight gain (WG) = final weight (g) – initial weight (g); Specific growth rate (SGR) = $\frac{LnW2 - LnW1}{t} x100$, Where Ln is the natural log; W_1 = initial body weight; W_2 = the final body weight in grams and t = period in days; Feed conversion ratio (FCR) = Feed ingested (g)/ Weight gain (g); Protein efficiency ratio (PER) = Weight gain (g)/ Protein ingested (g); protein productive value (PPV) = (retained protein/protein intake) × 100.

2.8. Digestibility study

After two-months from the experiment start, feces were collected from each pond once daily every morning prior to feeding for a one-month period. The feces were collected on filter paper for drying as described by El-Saidy and Gaber (2002). The collected fecal samples for 10 days were pooled and freeze-dried prior to analyze. The chemical analysis was conducted according to AOAC (1990). Chromic oxide was employed as a suitable marker since it was a well validated method having been used in many previous fish studies successfully. In the current situation it was deemed more appropriate and cost effective

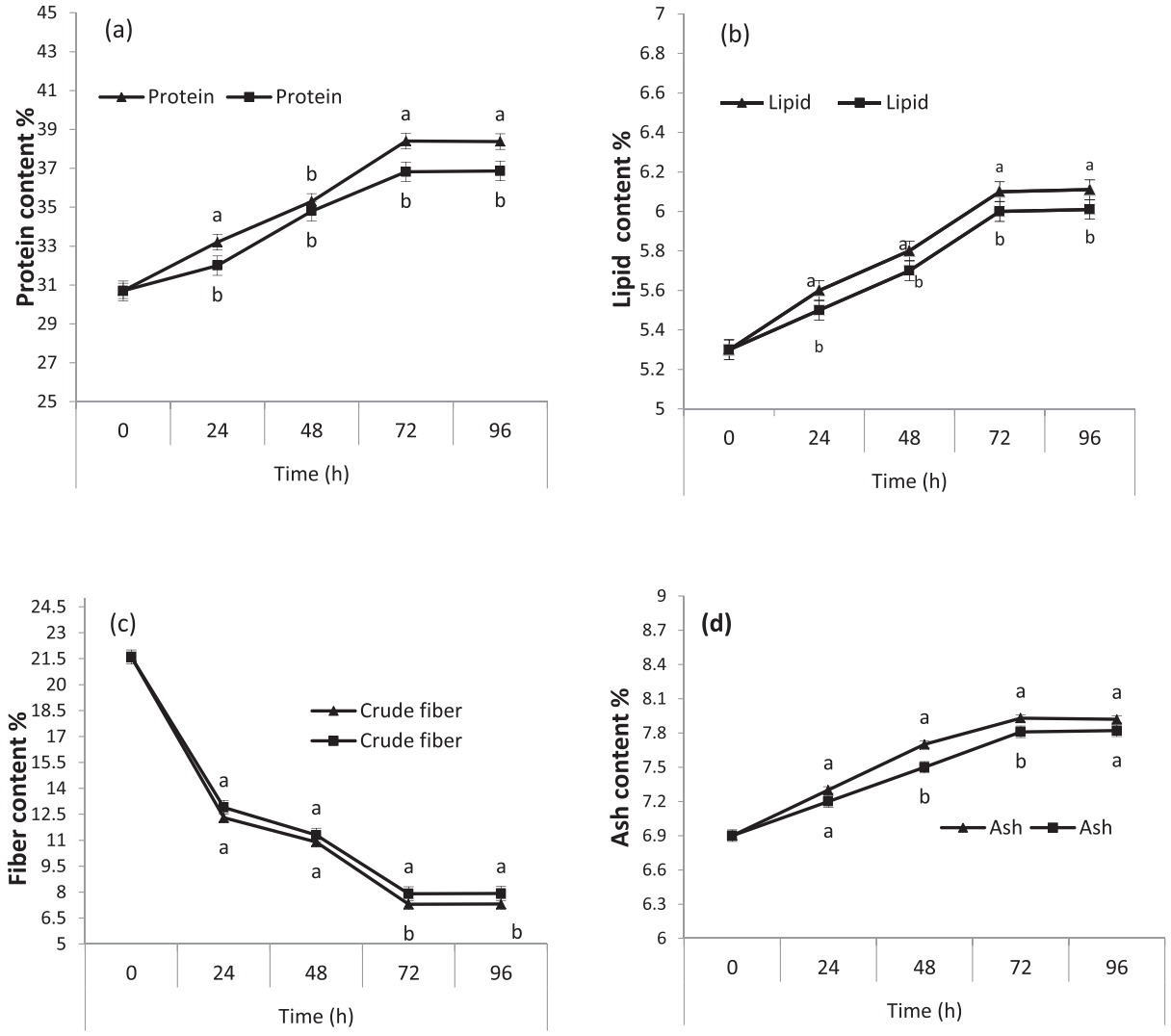

Fig. 1. Changes in protein content (a), lipid content (b), fiber content (c) and ash content (d) of de-oiled RSFM during SSF with *S. cerevisiae* or *B. subtilis* at different fermentation periods. Data (mean ± SE) with different letters at the same time significantly differ among treatments ($p < 0.05$).

compared to other techniques using yttrium oxide as a digestibility marker although similar relative data for digestibility coefficients can be expected (Davies and Gouveia, 2006). Chromic oxide was determined according to the procedure described by Furukawk (1966). Apparent nutrient digestibility was calculated using the equations of Schneider et al. (2004) as following: $ADC_{\text{dietary nutrient}} = 1 - ((\text{marker}_{\text{diet}})/(\text{marker}_{\text{feces}}) \times (\text{nutrient}_{\text{feces}})/(\text{nutrient}_{\text{diet}}))$.

2.9. Proximate composition

At the initiation and termination of the trial a random sample of five individual fish were sampled from each aquarium, then oven-dried 105 °C for 24 h, ground, and stored at − 20 °C for subsequent analysis. Proximate analysis was conducted on de-oiled SFM, YFSFM and BFSFM diets and fish samples. Dry matter, crude protein, crude lipid and ash contents were all determined by the standard (AOAC, 1990). Dry matter was determined after drying the samples in an oven (105 °C) for 24 h. Crude protein was determined by micro-Kjeldhal method, N × 6.25(using Kjeltech auto analyzer, Model 1030, Tecator, Höganäs, Sweden) (method number 984.13) and crude fat by Soxhlet extraction with diethyl ether (40–60 °C) (method number 920.39). Ash was estimated by incineration at 550 °C for 12 h (method number 942.05). Crude fiber content of de-oiled SFM, YFSFM and BFSFM was determined using the method of Van Soest et al. (1991). Nitrogen-free extract (NFE) was calculated as [100- (Crude protein + Ether extract + Fiber + Ash)].

2.10. Hematological and biochemical blood indices

At the end of the experiment blood samples were collected from the caudal vein of all treatments fish and were divided into two portions. The first portion was collected with anticoagulant 10% ethylenediaminetetraacetate (EDTA) to determine the hematocrit (Htc), hemoglobin (Hb), erythrocyte counts (RBCs) and total count of white blood cells (WBCs) according to standard methods described by Rawling et al. (2009). The second portion of the blood sample was allowed to clot overnight at 4 °C and then was centrifuged at 3000 rpm for 10 min. The non-hemolysed serum was collected and stored at − 20 °C until use.

Levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) according to the method described by Reitman and Frankel (1957) and serum creatinine were measured by the calorimetric method and enzymatic determination as described by Henry et al. (1974). Total serum protein and albumin were determined according to Henry (1964) and Wotton and Freeman (1974), respectively. However, the total serum globulin was calculated by subtracting the total serum

albumin from the total serum protein according to Coles (1974). Serum phosphorus, calcium, cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL−C) and low-density lipoprotein cholesterol (LDL-C) were measured spectrophotometrically using commercial kits produced by Pasteur labs (Egyptian American Co. for Laboratory Services, Egypt).

2.11. Statistical analysis

A probability of 0.05 was utilized to account for the statistical difference between the means. Before the analysis, percentage data were normalized by arcsine-transformation. All data are presented as means ± (SE). Growth, hematology and blood chemistry data were analyzed using one-way ANOVA, followed by Duncan's multiple range tests which were used to compare differences among individual means, with statistical software SAS ANOVA procedure (Statistical Analysis System, 1993). Regression analysis was performed with graphic software Sigma Plot version 8 (SPSS Inc. Chicago, IL, USA).

3. Results

3.1. Nutritional value of de-oiled sunflower after solid state fermentation

The duration of the solid-state fermentation (SSF) technique statistically affected the nutritional value of de-oiled SFM after SSF with *S. cerevisiae* (YFSFM) or *B. subtilis* E20 (BFSFM). The results in the study are shown in (Fig. 1: a-d) and indicated that at 72 h of solid-state fermentation (SSF) by *S. cerevisiae*, crude protein (38.4%), crude lipid (6.1%) and ash content (7.9%) was significantly ($P < 0.05$) higher and crude fiber was significantly lower than BFSFM and de-oiled SFM. Changes in crude protein, crude lipid, fiber and ash contents were steady in both YFSFM and BFSFM after 72 h.

An increase in the total hydrolyzed amino acid content of 15.6% and 7.1% was found in YFSFM and BFSFM, respectively, compared with de-oiled SFM (Table 3). The content of essential amino acid in YFSFM and BFSFM increased by 19.0% and 10.3%, respectively. Furthermore, the content of non-essential amino acid increased by 12.1% and 4.7% in YFSFM and BFSFM, respectively. There were no significant differences ($P > 0.05$) in the protein levels due to diets being iso-nitrogenous in their conception.

3.2. Degradation in anti-nutritional factors of de-oiled sunflower after solid state fermentation

After SSF by *S. cerevisiae* or/and *B. subtilis*, phenolic compounds represented by chlorogenic and caffeic acid significantly reduced from 490.50 and 2.52 mg kg^{-1} in raw de-oiled SFM to 71.12 and 0.33 mg kg^{-1} in YFSFM and 75.25 mg kg^{-1} and 0.39 mg kg^{-1} in BFSFM, respectively Table 3. The same trend was observed for phytic acid and saponins where the SSF also significantly ($P < 0.05$) decreased it from 17.25 mg kg^{-1}, and 0.678 g100 g^{-1} in de-oiled SFM to 1.78 mg kg^{-1} and 200 mg kg^{-1} in YFSFM and 1.91 mg kg^{-1}, and 220 mg kg^{-1} in BFSFM, respectively Table 2.

3.3. Growth performance

There were differences in the growth of tilapia receiving the experimental diets, and lower weight gain was observed in BFSFM Fig. 2. Table 4 showed that FBW, WG, SGR, PER and PPV of Nile tilapia fed diet with 75% replacement of FM protein (BFSFM-75) was significantly ($P < 0.05$) lower compared with that of Nile tilapia fed the control diet and other experimental diets. The highest FBW, WG, SGR, PER and PPV and the best FCR were recorded by fish fed the control diet and YFSFM-25 with no significant difference ($P > 0.05$). In general, growth performance of fish fed diet containing YFSFM was higher than those fed diet contained BFSFM in all replacement levels. Data of WG for tilapia fed different levels of YFSFM and BFSFM fitted a liner model (Fig. 3)

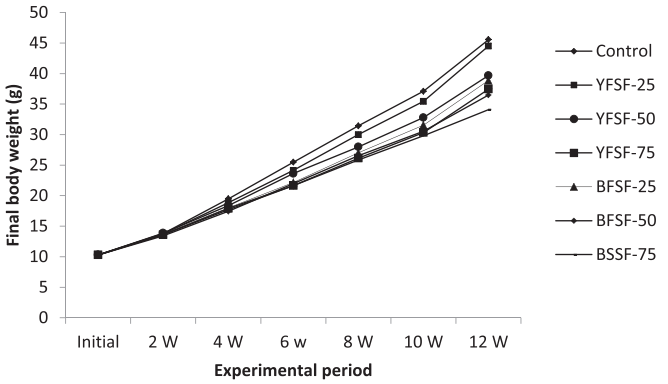

Fig. 2. Changes in mean live weight of tilapia at each bi-weekly period over the 12-week trial for each diet.

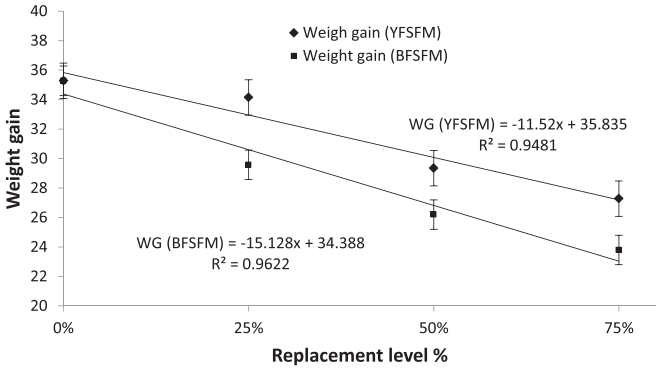

Fig. 3. Effect of replacement of fish meal by YFSFM and BFSFM on weight gain of Nile tilapia.

Table 2
Anti-nutritional factors and level in sunflower meal, yeast fermented sunflower meal and *B. subtilis* fermented sunflower meal.

Items	RSFM[a]	YFSFM[b]	BFSFM[c]	*P* values
Chlorogenic acid (mg kg^{-1})	450.90	71.12	75.25	0.012
Caffeic acid (mg kg^{-1})	2.52	0.33	0.39	0.001
Phytic acid (mg kg^{-1})	17.25	1.78	1.91	0.041
Saponins (mg kg^{-1})	6780	200	220	0.002

[a] (SFM), De-oiled sunflower meal.
[b] (YFSFM), yeast fermented sunflower meal.
[c] (BFSFM), *Bacillus subtilis* fermented sunflower meal.

and the regression equations of WG were $WG_{YFSFM} = -11.52\times + 35.83$, $R^2 = 0.948$; $WG_{BFSFM} = -15.12\times + 34.38$, $R^2 = 0.962$. The highest WG was recorded by fish fed the control diet followed by YFSFM-25.

3.4. Apparent digestibility of experimental diets

Results of the apparent digestibility coefficient of dry matter, protein lipid and energy, are shown in Table 5. The statistical analysis indicated that the highest ADC of dry matter, protein, lipid and energy were recorded by fish fed control, YFSFM-25 diets and YFSFM-50 with insignificant ($P > 0.05$) differences between means. No significant differences were found in dry matter, protein, lipid and energy of fish fed YFSFM-75, BFSFM-25, BFSFM-50 and BFSFM-75.

3.5. Proximate analysis of whole body fish

The proximate composition of whole fish fed various experimental

Table 3
Hydrolyzed amino acid composition of De-oiled sunflower meal, yeast fermented sunflower meal and *B. subtilis* fermented sunflower meal.

Amino acid (%)	SFM	YFSFM	BFSFM
Essential amino acids			
Arginine	2.02	2.95	2.73
Histidine	0.84	0.98	0.85
Lysine	1.19	1.35	1.21
Methionine	0.52	0.61	0.56
Leucine	1.93	2.19	2.06
Isoleucine	1.23	1.42	1.29
Threonine	1.05	1.20	1.12
Phenylalanine	1.59	1.74	1.66
Valine	1.52	1.71	1.63
Total essential amino acids	11.89	14.15	13.11
None-essential amino acid			
Glutamate	5.81	6.46	6.21
Aspartate	2.76	3.27	3.17
Serine	1.27	1.31	1.29
Glycine	1.69	1.95	1.78
Alanine	1.40	1.56	1.38
Tyrosine	1.22	1.18	0.87
Cystine	0.47	0.77	0.65
Proline	1.45	1.52	1.47
Total non-essential amino acids	16.07	18.02	16.82
Total amino acid	27.96	32.06	23.74

diets is presented in Table 6. It was detected that dry matter and protein content in the whole body of fish tended to decrease with increasing dietary YFSFM or BFSFM and was significantly lower in fish fed BFSFM-75. No significant difference of whole body lipid content was observed between fish fed fermented SFM and fish fed the control diet. The whole-body ash content was increased significantly ($P < 0.05$) with increasing the dietary level of YFSFM or BFSFM. The highest whole-body ash content was recorded by fish fed BFSFM-75.

3.6. Hematological indices

Results of Table 7 showed that, no significant ($P > 0.05$) differences were found in hemoglobin (Hb), hematocrit (Htc), red blood cell (RBCs) and white blood cells (WBCs) among the various experimental diets.

3.7. Serum biochemical indices

There were significant differences in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels among various experimental diets (Table 8). The control diet, YFSFM-25 and BFSFM-25 exhibited lowest value of ALT and AST of Nile tilapia. Inclusion of fermented SFM showed an overall significant increase in serum of ALT and AST activity. Highest value of ALT and AST was detected in the YFSFM- 75 and BFSFM-75 (Table 8).

The contents of phosphorus and calcium in blood are presented in Table 8. The highest value of serum phosphorus and calcium content was detected in control diet and the lowest one was recorded in YFSFM-75 and BFSFM-75. It was mentioned that no significant differences ($P > 0.05$) were found in phosphorus between YFSF-50 and BFSFM-50. Cholesterol and triglycerides content of Nile tilapia was decreased significantly ($P < 0.05$) with increase the level of replacement of FM with either YFSFM or BFSFM Table 8. The control diet exhibited highest values of cholesterol and triglycerides. The lowest values of cholesterol and triglycerides were detected in the YFSFM- 75 and BFSFM-75. Highest high-density lipoprotein (HDL−C) of serum was observed in fish fed control diet, which is no statistically different ($P > 0.05$) to all fermented SFM, except YFSFM-75 and BFSFM-75, wherein lowest HDL−C. There was no significant difference ($P > 0.05$) in low- density lipoprotein (LDL-C) and the ratio of HDL-C / LDL-C of the serum of fish among experimental diets Table 8.

4. Discussion

The results of this study revealed that the chemical composition of sunflower meal (SFM) improved after both SSF processes by *S. cerevisiae* or *B. subtilis*, at 72 h, and all nutritional parameters measured were significantly higher in YFSFM than BFSFM treatments. The notable increment in crude protein content of SFM in the present study may be associated with increases in the overall content of available amino acids in YFSFM and BFSFM after hydrolysis, and could also be attributed to the addition of microbial protein during the biological process of fermentation or other cellular components (Belewu and Sam, 2010; Belewu et al., 2011; Broerse and Visser, 1996; Hassaan et al., 2015; Shiu et al., 2015a). Similarly, Teng et al. (2012) reported that crude protein content was increased in soybean meal by fermentation with *Bacillus subtilis* or *Aspergillus oryzae*, where the micro-organisms have been long considered as a good source of enzymes, resulting in protein breakdown in fermented soybean meal. In accordance with current results reported by Hong et al. (2004), the absence of high molecular weight polypeptides in fermented soybean meal may be attributable to the degradation of polypeptide chains by the proteolytic enzymes from *Lactobacillus. plantarum* Lp6. On the contrary, crude protein and lipid content in fermented soybean meal were not affected by fermentation process with *Bacillus spp* (Yamamoto et al., 2004). In the present study, total hydrolyzed, essential and amino acid content in YFSFM and BFSFM were significantly higher than native de-oiled SFM. Furthermore, methionine and lysine in YFSFM was increased by (17.3%, 13.4%), respectively than that found in BFSFM (7.7%, 1.7%), respectively. These results may be due to the high production of the cell mass of yeast and consequently the production of protein within the yeast population (Hassaan et al., 2015). Thus, selecting a bacterial species for fermentation process that can produce methionine is needed for the aqua-feed industry (Kumar and Gomes, 2005). *B. subtilis* which used in treated soybean produces very high proteolytic activity with subsequent increases in free hydrophobic amino acids (Sarkar et al., 1997).

Significant reduction in the fiber content has been observed in the present study due to the fermentative action SSF by *S. cerevisiae* or *B.*

Table 4
Growth performance of Nile tilapia as affected by replacement of fish meal by yeast fermented sunflower meal and *B. subtilis* fermented sunflower meal.

Items	Experimental diets							P values
	Control	YFSFM-25	YFSFM-50	YFSFM-75	BFSFM-25	BFSFM-50	BFSFM-75	
Initial body weight (g fish^{-1})	10.28 ± 0.12	10.35 ± 0.52	10.33 ± 0.33	10.33 ± 0.25	10.30 ± 0.31	10.28 ± 0.19	10.28 ± 0.25	0.999
Final body weight (g fish^{-1})	45.55 ± 1.12^{a}	44.50 ± 1.20^{a}	39.68 ± 1.32^{b}	37.60 ± 1.14^{d}	39.88 ± 1.31^{b}	36.48 ± 1.11^{d}	34.08 ± 1.25^{e}	0.002
Weight gain (g fish^{-1})	35.28 ± 1.11^{a}	34.15 ± 1.31^{a}	29.35 ± 1.10^{b}	27.28 ± 1.04^{c}	29.58 ± 1.14^{b}	26.20 ± 1.10^{c}	23.80 ± 1.17^{d}	0.0001
Specific growth rate (%, day^{-1})	1.78 ± 0.16^{a}	1.74 ± 0.13^{a}	1.61 ± 0.13^{b}	1.54 ± 0.11^{c}	1.61 ± 0.19^{b}	1.51 ± 0.11^{c}	1.43 ± 0.15^{d}	0.031
Feed intake (g fish^{-1})	52.41 ± 1.25^{a}	50.59 ± 1.85^{b}	48.62 ± 1.82^{c}	46.26 ± 1.58^{d}	47.06 ± 1.52^{d}	46.09 ± 1.88^{d}	45.66 ± 1.23^{e}	0.001
Feed conversion ratio	1.49 ± 0.05^{e}	1.48 ± 0.09^{e}	1.66 ± 0.03^{d}	1.69 ± 0.04^{c}	1.65 ± 0.03^{d}	1.76 ± 0.05^{b}	1.92 ± 0.04^{a}	0.001
Protein efficiency ratio	2.32 ± 0.05^{a}	2.33 ± 0.06^{a}	2.08 ± 0.02^{b}	2.03 ± 0.02^{c}	2.09 ± 0.07^{b}	1.95 ± 0.04^{d}	1.79 ± 0.06^{e}	0.002

Values are mean ± SE. values in within same row sharing the same superscript are not significantly different ($P > 0.05$).

Table 5
Apparent digestibility coefficient (ADC, %) of Nile tilapia as affected by replacement of fish meal by yeast fermented sunflower meal or *B. subtilis* fermented sunflower meal.

Items (%)	Experimental diets							
	Control	YFSF-25	YFSF-50	YFSF-75	BFSFM-25	BFSFM-50	BFSFM-75	P values
Dry matter	94.57 ± 1.52^{a}	94.49 ± 1.45^{a}	94.31 ± 1.55^{a}	92.93 ± 1.95^{b}	92.72 ± 2.02^{b}	93.81 ± 1.85^{b}	93.18 ± 1.82^{b}	0.024
Protein	89.37 ± 2.13^{a}	89.09 ± 1.85^{a}	89.04 ± 1.98^{a}	87.25 ± 2.01^{c}	87.59 ± 2.40^{bc}	87.76 ± 2.31^{b}	83.95 ± 2.02^{d}	0.021
Lipid	92.70 ± 1.55^{a}	91.95 ± 1.76^{a}	92.53 ± 1.52^{a}	91.46 ± 1.23^{c}	91.75 ± 1.85^{b}	91.80 ± 1.78^{b}	91.79 ± 1.89^{b}	0.019
Digestible energy	87.69 ± 1.93^{a}	87.37 ± 1.85^{a}	86.94 ± 1.95^{a}	85.73 ± 1.79^{cd}	84.97 ± 1.87^{e}	85.54 ± 1.90^{d}	83.95 ± 1.79^{f}	0.031

Values are mean ± SE. values in within same row sharing the same superscript are not significantly different ($P > 0.05$).

subtilis on None Starch Polysaccharides (NSP's) and other complex carbohydrate structures within the matrix of the sunflower meal. This is likely to be due to the combined secretion of various enzymes degrading the fiber during the SSF process. Our results are in line with those of Hassaan et al. (2017) who reported that, a decrease in fiber content of fermented *Jatropha curcas* by *B. licheniformis* and *B. pumilus* which worked synergistically due to various enzymes secreted during the fermentation process. In the present study, SSF by *S. cerevisiae* or *B. subtilis* had the most profound effect in reducing the content of the ANFs in SFM (Table, 2). It is most likely that these results may also be due to the secretion of various enzymes during the fermentation process. In this context, Broerse and Visser (1996) suggested that the enzymes could have contributed to the detoxification of all the main classes of ANFs present in native Jatropha seed meal. Also, Hassaan et al. (2015 and 2017) found that ANFs significantly decreased in soybean meal and Jatropha seed meal after SSF treatments. Additionally, phytate, lectin, trypsin inhibitors and saponin levels were markedly decreased by SSF using different fungi species (Belewu and Sam, 2010). Also, Ramachandran and Ray (2008) reported a significant reduction was found in phytic acid, tannic acid and trypsin inhibitor contents in the plant materials following the SSF process. From this finding, we can say that fermentation of sunflower meal, not only improves the physiochemical quality but also the nutritional quality of the product as a feed ingredient.

To the best of our knowledge, this investigation is the first attempt to assess the effect of using fermented sunflower meal for Nile tilapia, by the application of SSF process. The results of this study indicated that it is possible to substitute at least 25% of FM by YFSFM protein in Nile tilapia diets with no significant differences ($P > 0.05$) on growth performance and feed utilization. The superiority of YFSFM in all replacement levels (25%, 50% and 75%) compared with BFSFM may be due to, an increase in the level of some essential and non-essential amino acids in YFSFM diets which are the main factor responsible for increasing the palatability of fish food and consequently increase feed intake (Barnes et al., 2006). Also, yeast is a good source of some bioactive components such as nucleotides, glucans, vitamins, arabinoxylan and mannan oligosaccharides that have good nutritional and functional properties in fish (Oliva-Teles and Gonçalves, 2001). Fermentation techniques have advantages for higher inclusion of plant proteins instead of fish meal through inactivation of component Anti-Nutritional Factors, ANFs (Reddy and Pierson, 1994) as well as an increased range of low molecular weight proteins and peptides with potentially higher digestibility (Kader et al., 2012). It was previously shown that growth and feed utilization efficiencies of rohu (*Labeo rohita* Hamilton) fed fermented sesame seed meal diets up to 400 g kg^{-1} were superior than those fed raw oilseed meal diets (Mukhopadhyay and Ray, 1999). Also, Yuan et al. (2013) reported that the highest growth for Chinese sucker, *Myxocyprinus asiaticus* was found in the control diet, and with up to 35% replacement of fishmeal by a yeast fermented soybean meal. In the current study, the FCR in the high inclusion level (75%) of YFSFM and BFSFM was significantly lower than that for the other diets, which may be due to an imbalance in amino acids and lower protein digestibility of SFM at this inclusion level. In this context, Garcia-Gallego et al. (1998) observed an essential amino acid reduction when 50% sunflower meal was included in diets for European eel *Anguilla anguilla* (Linnaeus), and these authors consider that this could be the main, but perhaps not the only factor that affects fish performance when using alternative proteins with different quantity and quality of the essential amino acid for fish. In addition, plant protein-rich diets have a reduction in the intake of dietary essential nutrients and digestible energy (Gomes et al., 1995). It will be worth evaluating the possible further beneficial effects of supplementary amino acid on Nile tilapia in the case of using fermented sunflower meal at higher inclusions. In the current study, high concentration of ANFs such as chlorogenic and caffeic acid in high inclusion (75%) of YFSFM or BFSFM may be resulted in a decreased activity of digestive enzyme, and this could be the reason for the growth depression. Anti -nutritional factors (ANFs) inhibit the activity of protein digesting enzymes and forms complexes with minerals, thereby causes reduction in growth and feed utilization (Kumar et al., 2010; Shamna et al., 2015). Another reason for the reduction of feed efficiency is other ANFs and detrimental metabolites may be produce during the fermentation process (Zhou et al., 2011).

Determination of nutrient digestibility might be an important step in evaluating the potentiality of an ingredient for use in diet formulation that sustain fish growth by providing appropriate amounts of available nutrients as well as reducing waste (Stone et al., 2003). In the present study, apparent digestibility coefficient of dry matter, protein, lipid and energy digestibility were higher in the replacement of FM protein by YFSFM up to 50%. The reduction of ADC of dry matter, protein, lipid and energy in other levels of fermented SFM may be associated with feed efficiency, thereby displaying a decrease in the

Table 6
Proximate analysis of Nile tilapia as affected by replacement of fish meal by yeast fermented sunflower meal and *B. subtilis* fermented sunflower meal.

Items (g kg^{-1})	Experimental diets							
	Control	YFSF-25	YFSF-50	YFSF-75	BFSF-25	BFSF-50	BFSF-75	P values
Dry matter	268.50 ± 0.52^{a}	258.50 ± 0.61^{b}	255.50 ± 0.48^{b}	254.00 ± 0.56^{b}	255.00 ± 0.55^{b}	250.00 ± 0.65^{c}	245.50 ± 0.55^{e}	0.012
Protein	653.10 ± 1.12^{a}	652.10 ± 1.04^{a}	645.00 ± 1.02^{b}	637.51 ± 1.11^{c}	631.50 ± 1.14^{c}	625.50 ± 1.04^{c}	617.50 ± 1.17^{e}	0.011
Lipid	175.00 ± 0.23	175.50 ± 0.44	176.10 ± 0.52	176.00 ± 0.56	176.40 ± 0.57	175.50 ± 0.66	176.50 ± 0.47	0.021
Ash	151.50 ± 0.71^{e}	150.50 ± 0.56^{e}	153.00 ± 0.45^{d}	154.50 ± 0.56^{c}	158.50 ± 0.56^{b}	158.00 ± 0.56^{b}	160.50 ± 0.52^{a}	0.011

Values are mean ± SE. values in within same row sharing the same superscript are not significantly different ($P > 0.05$).

Table 7
Hemoglobin, hematocrit, red blood cell count (RBCs) and white blood cell count (WBCs) of Nile tilapia as affected by replacement of fish meal by yeast fermented sunflower meal and *B. subtilis* fermented sunflower meal.

Items	Experimental diets							P values
	Control	YFSF-25	YFSF-50	YFSF-75	BFSF-25	BFSF-50	BFSF-75	
Hemoglobin (g dl^{-1})	10.95 ± 0.65	11.10 ± 0.58	10.60 ± 0.74	10.25 ± 0.72	11.15 ± 0.71	10.95 ± 0.63	10.75 ± 0.58	0.873
Hematocrit (%)	15.90 ± 0.82	16.80 ± 0.94	16.06 ± 0.93	15.80 ± 0.81	16.25 ± 0.90	15.95 ± 0.85	15.75 ± 0.92	0.816
RBCs $\times 10^6$ cmm^{-1}[a]	3.25 ± 0.09	3.15 ± 0.10	3.10 ± 0.09	3.30 ± 0.01	3.15 ± 0.08	3.20 ± 0.11	2.10 ± 0.08	0.550
WBCs $\times 10^3$ cmm^{-1}[b]	12.55 ± 0.88	12.25 ± 0.69	12.95 ± 0.95	11.00 ± 0.66	11.6 ± 0.63	10.95 ± 0.82	11.95 ± 0.56	0.160

Values are mean ± SE. values in within same row sharing the same superscript are not significantly different ($P > 0.05$).
[a] RBCs, red blood cell count.
[b] WBCs, white blood cell count.

growth performance. Decreased protein digestibility could be caused by imbalanced essential amino acid content in fermented soybean as well as retention of nutrients within plant cellulosic material (Zhou et al., 2011). Reduction of ADC of dry matter and energy digestibility in the present study may be due to cellulose indigestibility in plant protein (Eusebio et al., 2004). Similarly, apparent digestibility of nutrients significantly decreased with increasing dietary fermented soybean meal level in diets of Black Sea Bream, *Acanthopagrus schlegelii* (Zhou et al., 2011). Collectively all these factors mentioned would help to explain the superior performance of Nile tilapia fed the control diet based on fishmeal as the primary protein source. Although fishmeal would not be included at such high levels in practical diets for this species, it does provide an excellent reference protein with a balanced amino acid profile providing the optimum 'ideal protein' for nutritional comparisons for other protein sources. However, substitution of fishmeal protein basis in experimental diets assumes equal digestibility of protein and amino acids and this can lead to bias when an ingredient with lower digestibility of protein is compared at higher inclusions to the reference fishmeal source or control diet as in the current trial. A more elaborate study is thus warranted to evaluate the SSF products using digestible crude protein and digestible amino acid data for more reliable 'balanced' formulations to compare results with a fishmeal reference control diet. In terms of chemical body composition profile of tilapia, there were significant changes in whole-body chemical composition of Nile tilapia with increasing dietary inclusion of YFSFM or BFSFM. The lowering in protein content in the tilapia fed high inclusion level of fermented SFM may be related to lowering dietary essential amino acid, such as lysine and methionine in diets, which have induced protein catabolism rather than anabolism (Lim et al., 2004). Similar trend was detected by (Hassaan et al., 2017; Hassaan et al., 2015; Saha and Ghosh, 2013). Whole body lipid content in the present study was not affected by increasing level of YFSFM or BFSFM. and may be associated to chlorogenic acid content thus reducing lipid deposition in whole body fish (Sun et al., 2017). This result agree with earlier reports (Lim et al., 2004; Zhou et al., 2011). By contrast, other researchers reported significantly increased lipid content with increasing level of plant protein in diets (Hassaan et al., 2015; Kumar et al., 2011), and the inconsistent reports noted above were probably due to the species of fish, diet composition, environment and feeding period, etc. Ash content increased in the present study with increasing dietary level of YFSFM and BFSFM, indicating sufficient amounts of dietary minerals in diets and its absorption. Similarly, (Kumar et al., 2011; Zhang et al., 2012) showed the same trend of ash contents in whole body fish.

Hematological parameters often give important information for physiological responses and nutritional status affecting fish species (Nrc, 2011). The present study showed that there were no significant differences ($P > 0.05$) in Hb, Htc, RBCs and WBCs of fish fed with different inclusion level of YFSM or BFSFM may be associated with decrease antinutritional factors binding iron and amine group of amino acids which in turn lowers their availability in the blood and increases the erythrocytes (Soltan, 2005). It may be speculated that the increased in hematological indices of fish fed YFSFM-25 and BFSFM-25 diets may assist oxygen binding efficiency for tilapia fed these diets. Accordingly, the increased number of RBC multiplies the concentration of hemoglobin ultimately resulting in a higher capacity for oxygen carrying in fish. These results agree with that obtained with Heidarieh et al. (2013) who reported no significant differences ($P > 0.05$) in hematological parameters of rainbow trout (*Oncorhynchus mykiss*) following supplementation with 5 g kg^{-1} fermented *S. cerevisiae*. Also, Kim et al. (2009) concluded that Htc and Hb of parrot fish (*Oplegnathus fasciatus*) were not affected by the dietary fermented soybean meal.

An inclusion of fermented SFM showed overall significant increase in serum of ALT and AST activity. In line with our results, Hassaan et al. (2015) showed that ALT and AST were increased in Nile tilapia fed high inclusion level of fermented soybean meal diet. On the other hand, decreased GOT and GPT activities were found in muscle and liver of

Table 8
Serum alanine aminotransferase, aspartate aminotransferase, calcium, phosphorus, cholesterol, triglyceride, high and low density of lipoprotein cholesterol levels in Nile tilapia as affected by replacement of fish meal by yeast fermented sunflower meal and *B. subtilis* fermented sunflower meal.

Items	Experimental diets							P values
	Control	YFSF-25	YFSF-50	YFSF-75	BFSF-25	BFSF-50	BFSF-75	
Alanine aminotransferase UL^{-1}	43.00 ± 1.31[d]	43.80 ± 1.96[d]	45.50 ± 1.65[c]	48.00 ± 1.51[a]	44.50 ± 1.25[d]	47.00 ± 1.18[c]	49.50 ± 1.16[a]	0.032
Alanine aminotransferase UL^{-1}	13.50 ± 0.56[c]	13.90 ± 0.87[c]	16.50 ± 0.58[b]	18.50 ± 0.84[a]	14.90 ± 0.45[c]	17.50 ± 0.57[b]	19.00 ± 0.56[a]	0.012
Calcium mg dl^{-1}	9.65 ± 0.10[a]	9.35 ± 0.40[a]	7.2 ± 0.27[c]	6.6 ± 0.23[e]	9.22 ± 0.15[a]	7.85 ± 0.35[c]	6.8 ± 0.20[e]	0.043
Phosphorus mg dl^{-1}	3.75 ± 0.21[a]	3.25 ± 0.18[a]	2.75 ± 0.22[c]	2.40 ± 0.25[c]	3.19 ± 0.21[a]	2.65 ± 0.18[c]	2.25 ± 0.10[e]	0.048
Cholesterol mmol L^{-1}	4.60 ± 0.23[a]	3.90 ± 0.11[b]	3.75 ± 0.56[b]	3.36 ± 0.21[c]	3.85 ± 0.62[b]	3.70 ± 0.15[b]	3.30 ± 0.23[c]	0.038
Triglyceride mmol L^{-1}	5.00 ± 0.62[a]	4.02 ± 0.36[b]	4.00 ± 0.62[b]	3.15 ± 0.72[c]	4.15 ± 0.12[b]	3.95 ± .15[b]	3.10 ± 0.33[c]	0.020
(HDL–C) mmol L^{-1}†	2.12 ± 0.56 [a]	2.00 ± 0.15 [a]	2.11 ± 0.41 [a]	1.79 ± 0.15[b]	2.10 ± 0.25[a]	2.01 ± 0.09 [a]	1.80 ± 0.12[b]	0.048
LDL-C mmol L^{-1}‡	0.36 ± 0.03	0.34 ± 0.1	0.35 ± 0.04	0.34 ± 0.02	0.33 ± 0.07	0.34 ± 0.01	0.34 ± 0.05	0.410
HDL-C/ HDL-C	5.88 ± 0.20	5.85 ± 0.35	6.02 ± 0.25	5.30 ± 0.30	5.82 ± 0.15	5.92 ± 0.25	5.30 ± 0.15	0.331

Values are mean ± SE. values in within same row sharing the same superscript are not significantly different ($P > 0.05$).
†HDL-C, High density of lipoprotein cholesterol.
‡ LDL-C, Low density of lipoprotein cholesterol.

Labeo rohita fingerlings fed Jatropha protein concentrate containing diets compared to one containing fermented Jatropha protein concentrate (Shamna et al., 2015). These contradictory results might be due to the fermentation methods, species of microorganisms and growth conditions as these may influence the levels of the active components present in the final products incorporated into diets (Heidarieh et al., 2013). Higher serum phosphorus and calcium was determined in fish fed diets control followed by YFSF-25 and BFSFM-25 in this study which suggests that mineral utilization was enhanced in these diets. The current results are in compliance with Jahanbakhshi et al. (2012) who reported that the highest serum phosphorus value observed in fish fed control diet and the lowest was observed in fish fed 480 g kg^{-1} plant protein (sesame oil cake and corn gluten). The few data that have been published on the effect of the fermented SFM on serum phosphorus and calcium are not always consistent in the scientific literature.

Tissue triglyceride profiles are usually used as an indicator reflecting lipid metabolism (Ma et al., 2016) in animal studies. Lipoprotein content and profile can be a measure of the degree of promotion of reversed cholesterol and metabolism where High Density Lipoprotein (HDL−C) aids in removal of cholesterol from the periphery for delivery to the liver and excretion into the bile (Norata et al., 2006) and LDL-C is the major cholesterol carrier in circulation and its physiological function is to convey cholesterol to the cells (Deng et al., 2010). Thus, HDL-C/LDL-C is usually related to reflect the transport of cholesterol, and the higher ratio indicates higher lipid content in fish liver (Yun et al., 2011). In this study cholesterol and triglyceride content of Nile tilapia was decreased significantly with increase the level of replacement of FM with either YFSFM or BFSFM, also lower content of HDL-C was detected with YFSFM-75 and BFSFM-75 may be related to hypocholesterolemic effects of high chlorogenic acid in SFM (Shu-Yuan et al., 2009). Amount of LDL-C and HDL-C/LDL-C have no significant difference ($P > 0.05$) in this study. However, there are no suitable research references in the literature to date to reliably assess the importance of these parameters in fish, particularly in relation to plant ingredient substitution on lipid metabolism in warm water species like tilapia.

In conclusion, this investigation has provided good evidence for the overall benefits associated with the processing of plant materials in this case sunflower seed meal using a simple fermentation strategy. These are basic methods with the promise of greatly enhancing the nutritional quality and value of plant by-products in practical fish diets. Although the study was conducted on a laboratory scale with the ingredient processed under controlled conditions, there is scope for larger scale production to generate sufficient volumes to utilize in the aquafeed industry. Indeed, similar solid-state fermentation SSF based ingredients are being used successfully as functional feed additives and supplements to enhance digestion and gut health in fish and available from the commercial sector using various cereals and bran as substrates. It should be an additional goal to develop new types of SSF plant ingredients with a focus on improved overall dietary utilization at various stages of fish production. These will need to take into account extrusion technologies where the higher temperatures encountered can modify and reduce enzyme activities and effects on any beneficial bioactive components that may be thermo-labile. It is evident that further research and development is needed in these areas to fully appraise these products as viable dietary supplements for fish and especially for warm water omnivorous species like tilapia.

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