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Increase in the plant protein ratio in the diet of Nile tilapia, *Oreochromis niloticus*, using *Saccharomyces cerevisiae*-fermented sunflower meal as a replacement.

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Abstract

This study aimed to improve the nutritional value and utilization of sunflower meal (SFM) used as feed ingredient for tilapia by an approach of solid-state fermentation with *Saccharomyces cerevisiae*. The protein content and lipid content were increased, while, phytic acid and tripsin inhibitor were decreased during solid state fermentation process. A feeding trial was conducted to investigate the effect of yeast fermented sunflower meal (YFSFM) on the growth, feed utilization parameters of Nile tilapia *Oreochromis niloticus.* Dietary fish meal was replaced with yeast fermented sunflower meal (YFSFM) in four isonitrogenous (295 g/kg crude protein and isocaloric 19.5 MJ/ kg gross energy) diets. Fish meal was replaced with YFSFM in the experimental diets in four levels 0, 25, 50 or 75% to formulate four experimental diets (control diet), YFSFM-25, YFSFM-50 and YFSFM-75, respectively. Each diet was fed to triplicate groups of fish with an initial weight 10.30 ±0.12 g. The experiment was conducted for for 84 days. The highest final body weight (FBW), weight gain (WG), specific growth rate (SGR) and the best condition factor (K) were obtained with control diet and YFSFM-25. The lowest growth response was obtained with YFSFM-75. Generally, a positive protein substitution effect was observed at 25% substitution levels YFSFM-25. The highest feed intake (FI) g/fish and the best feed conversion ratio (FCR) and protein efficiency ratio (PER) were recorded for fish fed control diet and YFSFM-25, with values statistically different from the other treatments. Fish fed different levels of YFSFM did not have a significant impact on dry matter, lipid, crude protein and ash contents of the fish.

Introduction

During the last years several studies have been directed to substitute the high cost fish meal with less expensive protein sources. This aspect of feed development research is centered on the search for inexpensive, readily available and nutritious protein sources that can supply all the nutritional needs of the fish. One obvious approach involves the greater utilization of ingredients of plant origin. Moreover, the use of animal protein for feeding herbivorous fish might be unnecessary. Owing to this, the use alternative perennially available plant proteins with low seasonal nutritional quality variability are considered an important approach in fish nutrition.

Oilseeds and their byproducts usually constitute a major source of dietary protein within aquafeeds for warm water herbivorous/omnivorous fish species (Lim and Dominy 1991; Tacon 1993). Sunflower seed meal (SFM) has been used in fish feeding (Sintayehu *et al*., 1996; Abou Zead *et al*., 2008). These studies showed positive at low inclusion levels, but growth reduction at high levels which may be due to anti-nutritional factors and high fiber content in SFM. In general, oilseed meals processed in traditional ways cannot be utilized at high levels without compromising growth and production. However, the inclusion of plant based proteins in aquafeeds provides a number of problems which include the occurrence of anti-nutritional factors (ANFs), reduced digestibility, issues of palatability and limitations of certain essential amino acids (Oliva-Teles and Gonçalves, 2001; Soltan 2005 a&b; Gatlin *et al*., 2007). 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 (Wee 1991).

Heat-labile anti-nutritional factors like proteinase inhibitors and agglutinating lectins are largely deactivated by the toasting step when producing solvent extracted soybean meal (Maenz *et al*., 1999). However, several of the anti-nutritional factors are heat stable. Solid-state fermentation (SSF) is defined as any fermentation process performed on a non-soluble material that acts both as physical support and a source of nutrients in the absence of free flowing liquid (Pandey, 2001). SSF has a long history of the production of traditional foods using different organisms. It was reported that in SSF, the production of metabolites, such as enzymes, antibiotics etc. are higher than that in the submerged fermentation (Holker and Lenz 2005). Therefore, the aim of this study was to determine the effect of

SFM fermented by *Saccharomyces cerevisiae* on anti-nutritional factors, nutritive values, growth performance, feed utilization of Nile tilapia, *Oreochromis niloticus*.

Material and Methods

O. niloticus were obtained from private farm (Elfyum Governorate, Egypt). Fish were acclimated to the experimental conditions for two weeks at the laboratory of fish at Faculty of Agriculture, Benha University. During the acclimation period, fish were fed a control diet (29.99 % crude protein) at a rate of 3% of biomass, which provided of equal rations at 09:00 am and 3:00 pm for 2 weeks to adapt the artificial diet and conditions of the trail. After the acclimatization, the experimental fish were distributed randomly into the experimental plastic tanks (0.5 m^3 for each) representing the four treatments studied. A set of 240 fish of *O. niloticus* L. mono-sex male fingerlings average initial weight of (10.30 ±0. 122 g) were used in this trail. Twenty fish were randomly stocked into each tank with three replications 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. During the 84-days experimental period, tilapia 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 weighted. Feed intake was calculated by the difference between them and expressed as the total feed intake.

Water temperature was recorded daily at 1.00 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). A pH was estimated on morning by using a pH meter (Orion pH meter, Abilene, Texas, USA). Water temperature ranged from 27.20 to 29.25°C; dissolved oxygen (DO) ranged between 5.32 and 6.81 mg/l; pH values ranged between 8.04 and 8.30 and total ammonia ranged from 0.18 to 0.2 mg/l for the different treatments during the entire experimental period (90 days) of the study. All tested water quality criteria (temperature, pH value, DO and total ammonia) were suitable and within the acceptable limits for rearing Nile tilapia *O. niloticus* fingerlings (Boyd, 1979). These positive findings in water quality criteria related with good growth performance since there were no mortalities among all treatments. A photoperiod of 12-h light, 12-h dark (08:00–20:00 h) was used fluorescent ceiling lights supplied the illumination.

A commercially de-oiled sunflowers meal (SFM) was purchased from a local company (Kafr-elsheikh, Egypt) and ground to a particle size (<500 μm) by screen diameter. The fermentation process was performed with three replicate by a modification method of Hassaan *et al*., (2015). Each replicate, 2 kg CSFM, 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 in a Hobart food mixer for 15 minutes. This provided a yeast density of 1×10^3 cell g^{-1} meal. Each replicate was conducted for 12, 24, 48 hours in a 10 liters glass jar covered with aluminum foil and incubated at 40°C which is the optimal growth temperature for *S. cerevisiae.* After fermentation, the fermented seed meal was dried (105 ^oC) for 3 hours to stop the microbial growth. The yeast fermented sunflower meal (YFSFM) was dried to constant weight at 70°C. In the beginning (0 hours) and after 12, 24, 48 and 72 hours of fermentation, 10 g of YFSFM was sampled to analyze the anti-nutritional factors and chemical composition. Crude protein lipids, ash and fiber were determined following the methods of the AOAC (1995) (Table 1).

Phytic acid concentration was determined in de-oiled SFM and YFSFM using a spectrophotometric procedure according to the method of Vaintraub and Lapteva (1988). The color was measured at 830 nm against a blank. Results were calculated as mg phytic acid/100 g dry sample using standard phytic acid. Trypsin inhibitor activity (TIA) was determined according to the method of Smith *et al*. (1980). Results were expressed by mg trypsin inhibited per g of dry sample.

Four isonitrogenous (297 g/kg crude protein) and isocaloric (19.6 MJ/ kg gross energy) experimental diets were formulated and the proximate chemical composition of the experimental diets is presented in (Table 2). The first is the control diet which contained 18 g FM/100 g diet (control diet). In the other three diets, FM protein was replaced with YFSBM at levels of 25% (D2), 50% (D3) and 75% (D4), respectively. All dry ingredients of the fish meal, soybean meal, yellow corn and wheat bran were blended for 5 min and thoroughly mixed with soybean oil. The ingredients were mixed well and made into dry pellets using a laboratory pellet mill (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.

At termination of the trail 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, diets and fish samples. Moisture, total lipids, crude protein and ash contents were all determined by the standard (AOAC, 1995). Dry matter was determined after drying the samples in an oven (105°C) for 24 h. Ash by incineration at 550°C for 12 h. Crude protein was determined by micro-Kjeldhal method, N×6.25 and crude lipid by Soxhlet extraction with diethyl ether (40–60° C). Crude fiber content of de-oiled SFM and YFSFM was determined using the method of Van Soest *et al*. (1991). Nitrogen-free extract was computed by taking the sum of values for crude protein, crude lipid, crude fiber, ash and moisture then subtracting this sum from 100.

Data were statistically analyzed by ANOVA using SAS ANOVA procedure (Statistical Analysis System 2004). The data were submitted to one ways classification variance analysis. Duncan's multiple range test was used to compare differences between treatment means when significant F values were observed (Duncan 1955), at ($P < 0.05$) level.

Results and Discussion

Chemical composition and anti-nutritional factors of de-oiled SFM and YFSFM are presented in (Table 1). Results showed that YFSFM using SSF with *S. cerevisiae* significantly increased crude protein, lipid and ash content. Moreover, SSF with *S. cerevisiae* significantly decreased fiber content of YFSFM. While the phytic acid and trypsin inhibitor in YFSFM was significantly decreased than de-oiled SFM. The significant increase in crude protein content of YFSFM may be due to the elevation in the level of amino acids during the process of fermentation, and this is in agreement with the findings of Hassaan *et al*. (2015). The decrease in crude fiber content of YFSFM, compared with RSFM in this study may be due to the secretion of various enzymes during solid state fermentation, which degraded crude fiber and complex polysaccharides, as previously reported by Belewu *et al*. (2011). The anti-nutritional factors such as phytic acid and trypsin inhibitor were decreased in de-oiled SFM after SSF by *S. cerevisiae* in the current study and these results are compatible with Hassaan *et al*. (2015). Other studies have used other bacteria and fungi species to reduce anti-nutritional factors (Jacqueline and Visser, 1996; Shiu *et al*., 2013).

After 84 days feeding period, the highest final body weight (FBW), weight gain (WG), specific growth rate (SGR) and the best condition factor (K) were obtained with control diet (D1) and D2 (25% of fish meal was replaced with YFSFM). The lowest growth response was obtained with D4. Generally, a positive protein substitution effect was observed at 25% substitution levels as shown in(table 3). D1 and D2 showed the highest feed intake (FI), and the best feed conversion ratio (FCR) and protein efficiency ratio (PER) with values statistically different from the other treatments (D3 or D4) as shown in (table 4). The reduction of growth and feed utilization observed in higher inclusions, 50% and 75%, of YFSFM, in the present study, may be due to anti-nutritional factors, lower digestibility of YFSFM protein and amino acid imbalance and other anti-nutritional factor introduced during the fermentation process. The noticed results agree with recent study Hassaan *et al*. (2015) who showed that up to 50% FM in the diets for Nile tilapia could be replaced by fermented soybean meal by *S. cerevisiae* in order to enhance the growth performance and the optimal performance at 37.4% inclusion. Also, Yuan *et al.* (2013) showed that the replacement of fish meal protein up to 350 g/ kg with YFSFM produced growth rate similar to that produced by fish meal-based diets for the juvenile Chinese sucker, *Myxocyprinus asiaticus*. On the other hand, substitution of brewer's waste instead of fishmeal, up to 50%, did not have any significant difference on growth rate and feed utilization of Nile tilapia (Zerai *et al*., 2008). Gause and Trushenski (2011) showed that higher growth performance for sunshine bass was observed by replacing of FM with bio-ethanol yeast at substitution levels, 27–41%. The improvement of Nile tilapia growth, by replacing of FM with YFSFM in the present study, may be due to the increase of amino acids in submitted fermented yeast. Furthermore, *S. cerevisiae* has been considered as responsible for increasing the palatability of fish food items (Barnes *et al*., 2006).

According to the body analysis composition data (Table 5) at the end of the experiment, Fish fed different levels of YFSFM did not have a significant ($P > 0.05$) impact on dry matter, lipid contents, crude protein and ash content of the fish.

Conclusions and Outlook

The feeding trial results show that it is possible to replace animal protein in tilapia fingerlings diets with fermented sunflower seed meal, with optimum growth response at a 25% substitution level. These results also show a reduction in growth and feeding efficiency as plant protein content increased beyond 25%.

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Table 1: Chemical composition and anti-nutritional factors of raw sunflower meal and yeast fermented sunflower meal

Items	Raw SFM[1]	YFSFM[2]
Crude protein g/100 g	30.70	38.40
Crude lipid g/100 g	5.30	6.10
Crude fiber g/100 g	21.60	7.30
Nitrogen free extract* g/100 g	35.50	40.40
Ash g/100 g	6.90	7.80
Phytate g/100 g sample	0.363	0.131
Tripsin inhibitor (IU/mg protein)	1660	1030

[1](SFM), sunflower meal [2](YFSFM), yeast fermented sunflower meal

*Nitrogen-free extract = 100−(crude protein + crude lipid + ash + fiber).

Table 2: Formulation and proximate composition of the experiment diets (g/ 100 g diet)

	Experimental diets			
	control	YFSFM-25	YFSFM-50	YFSFM-75
Fish meal	18	13.5	9	4.5
Soybean meal	35	35	35	35
Fermented soybean meal	0	5.2	10.44	15.66
Yellow corn	31	31	31	31
Wheat bran	8.5	7.8	7.06	6.34
soybean oil	4	4	4	4
Vitamins andMinerals[1]	2.7	2.7	2.7	2.7
Vitamin C	0.3	0.3	0.3	0.3
Chromic oxide	0.5	0.5	0.5	0.5
Proximate analysis				
Dry matter	90.01	89.81	89.61	89.29
Crude protein	29.85	29.7	29.60	29.50
Ether extract	7.50	7.24	7.00	6.79
Ash	6.02	5.91	5.86	5.82
Gross energy (MJ kg^{-1})[2]	19.73	19.68	19.62	19.58

[1]Vitamin and mineral mixture 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), [2]Gross energy calculated using gross calorific values of 23.63, 39.52 and 17.15 kj/g for protein, fat and carbohydrate, respectively according to Brett (1973).

Table 3: Effect of replacement of fish meal by yeast fermented sunflower meal on growth performance of Nile tilapia

Parameters	Experimental diets					
	Control	YFSFM-25	YFSFM-50	YFSFM-75	± SE	P values
IBW	10.28	10.35	10.33	10.33	0.122	0.99
IBL	8.76	8.65	8.73	8.78	0.087	0.82
Final body weight	45.55^{a}	44.50^{a}	39.68^{b}	37.60^{c}	0.179	0.01
Final body length	13.24	12.72	12.14	11.67	0.060	0.01
Condition factor	1.52^{c}	1.66^{b}	1.65^{b}	1.72^{a}	0.021	0.01
Weight gain	35.28^{a}	34.15^{a}	29.35^{b}	27.28^{c}	0.227	0.01
Specific growth rate	1.78^{a}	1.74^{a}	1.61^{b}	1.54^{c}	0.015	0.01

Means in the same row with different superscript letters were significantly different ($p < 0.05$).

Table 4: Effect of replacement of fish meal by yeast fermented sunflower meal on feed utilization of Nile tilapia

Parameters	Experimental diets					
	Control	YFSFM-25	YFSFM-50	YFSFM-75	± SE	P values
Feed intake	52.41^{a}	50.59^{b}	48.62^{c}	46.26^{e}	0.14	0.01
Feed conversion ratio	1.49^{e}	1.48^{e}	1.66^{d}	1.69^{c}	0.01	0.01
Protein efficiency ratio	2.32^{a}	2.33^{a}	2.08^{b}	2.03^{c}	0.01	0.01

Means in the same row with different superscript letters were significantly different ($p < 0.05$).

Table 5: Effect of replacement of fish meal by yeast fermented sunflower meal on chemical composition of Nile tilapia

Parameters	Experimental diets					
	Control	YFSF-25	YFSF-50	YFSF-75	± SE	P values
Dry matter	26.7	26.3	25.6	25.7	0.33	0.68
Crude protein	65.5	65.2	64.3	64.1	0.14	0.66
Crude lipid	17.2	17.3	17.5	17.1	0.65	0.59
Ash	15.2	15.00	15.2	14.9	0.32	0.11

Means in the same row with different superscript letters were significantly different ($p < 0.05$).