

Effect of fermented soybean on growth performance of Nile Tilapia (*Oreochromis niloticus*)

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Abstract: A feeding trial was conducted to evaluate the effect of replacing diet protein with fermented soybean meal (FSM) on the growth performance, feed utilization, body composition, of Nile tilapia (*Oreochromis niloticus*) fingerlings. Five isonitrogenic and isocaloric diets were prepared with levels of 0 (control), 25, 50, 75 and 100% FSM for T1, T2, T3, T4 and T5 respectively. Three hundred Nile tilapia (*Oreochromis niloticus*) fingerlings with initial weight of 12.78 ± 0.12 g were divided into five groups (20 fish per tank, Triplicate per treatment). Fingerlings Nile tilapia were hand-fed to visual satiation at two meals per day for 8 weeks (56 days). Fish were fed diets containing different levels of FSM had no significant differences regarding survival. Final and weight gain, SGR, PER and PPV were significantly higher in group of fish on T5 and T4 groups respectively. FCR was the lowest in T5 and T4 compared with the control group. Feed and protein intake was significantly highest in group of fish on T1 and T2 and lowest in T4 and T5. Body protein significantly increased with increasing level of incorporation of fermented soybean meal in the diets. These results indicate that incorporation of fermented soybean meal into fingerlings Nile tilapia diets reduced the price of one kg diet to 91.17, 78.21, 74.60 and 60.25% for the T2, T3, T4 and T5 respectively compared to the control group (100% of the price).

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) was one of the important fish species cultured in the Egypt. All illustrations from Egyptian tombs, suggest that Nile tilapia were cultured more than 3000 years ago. It is still the most widely cultured fish species in Africa, and it is an important cultured fish group in the world, after carps and salmonids. (FAO, 2009).

Soybean meal (SBM) is commonly used as an effective plant-derived protein in animal feed industry. Fermentation is a process allows microorganisms such as *Bacillus subtilis* to degrade protein macromolecules to a large extent water-soluble low molecular weight compounds (Kiers *et al.*, 2000). It was found that fermentation of soybean meal induced removal or inactivation of anti-nutritional factors (Reddy and Pierson, 1994; Lim *et al.*, 2010), improvement of the nutritional quality (Canella *et al.*, 1984), improvement of digestibility (Kiers *et al.*, 2000) and shelf life of the processed food (Skrede and Nes, 1988). Fermented fish silage and fermented soybean meal (FSM) were reported as suitable protein sources in the diets of catfish, *Claris gariepinus* (Burchell) and Nile tilapia *Oreochromis niloticus* L. (Fagbenro *et al.*, 1994). It was shown that FSM induced higher growth and feed efficiency compared to non-fermented soybean meal in diets of yellowtail *Seriola quinqueradiata* (Shimeno *et al.*, 1993a and Shiu *et al.*, 2015).

Fermented soybean meal (FSBM) is a microbial treated form of SBM, and is rich in protein (56%) and low in ANFs. Antinutritional levels in the FSBM are reducing during the enzymatic degradation and fungal fermentation, and provide many benefits against

digestion issues (Kim *et al.*, 1999; Hong *et al.*, 2004). Moreover, the protein quality can be significantly higher than that of SPC due to the low processing temperatures during the microbial process. Over the past decade, there has been a growing interest in evaluating the effects of FSBM inclusion on animal diets, but very little published information addresses the use of FSBM in fish diets (Shimeno *et al.*, 1994; Refstie *et al.*, 2005; Zhou *et al.*, 2011; Yuan *et al.*, 2012).

The aim of the present study is to investigate the effect of replacing protein of diets with fermented soybean meal at levels 25, 50, 75 and 100% on growth performance, feed utilization, body composition and economic efficiency.

MATERIALS AND METHODS

The experiments were conducted at fish Research center in Suez Canal University Ismailia- Egypt to study the effect of replacing different levels of protein with fermented soybean meal on growth performance of Nile tilapia fingerlings. The experiments were carried out through (5th October– 3rd December 2014) 56 days.

The Experiment

Fermentation of soybean meal

Fermentation of soybean meal was applied as the preparation process of the Meju with some modification (Figure 1). Soybean meal was finely ground, soaked with water at a ratio of 1:3 for 40 min and Cooked for 2 h at 100° C. After cooling at 28°C and left for air 4 days at 25°C. The fermented soybean meal (F-SBM) was directly incorporated into the F-SBM diet. The composition of fermented and soybean meal are presented in Table (1).

Table (1): Fermented soybean and soybean meal composition .

Ingredient	Crude protein	Crude fat	Crude fiber	Ash
Fermented soybean meal	50	0.8	9.3	6.20
Soybean meal	44	1.1	7.3	6.3

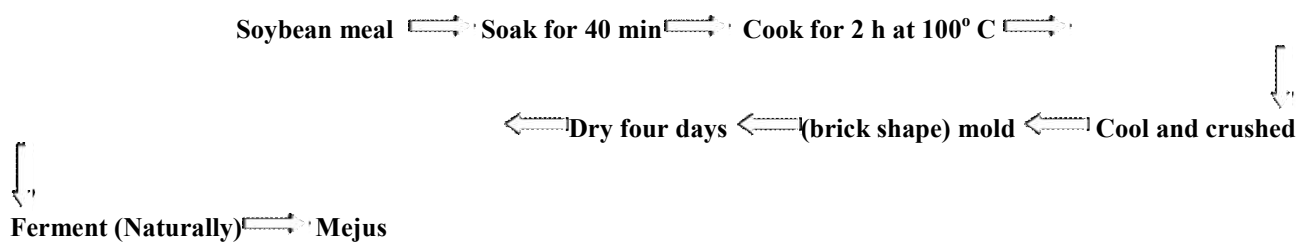


Figure (1). Fermentation processes for Meju and soybean meal (F-SBM).

Experimental diets

Five isonitrogenous and isocaloric diets were formulated from practical ingredients (Table 2) where the control diet and the other four diets protein content was replaced by 25, 50, 75 and 100% with fermented soybean meal. The composition and proximate analysis of the experimental diets are presented in Table (2). The fish were hand-fed to apparent satiation within half an hour, twice/day (11 am and 3pm) for 56 days. The experimental diets were formulated to contain almost 30% crude protein. The experimental diets were prepared by individually weighing of each component and by thoroughly mixing the mineral and vitamins with corn. This mixture was added to the components together with oil. Water was added until the mixture became suitable for making granules. The wet mixture was passed through CBM granule machine with 2mm diameter. The produced pellets were dried at room temperature and kept frozen until experiment start.

Experimental unit

Fish were stocked in 15 plastic tank (60 litre) randomly divided into five equal experimental groups (20 fish each treatment, three replicate/treatment) and tanks supported with air blowers. Photoperiod was 12h light/ 12h dark. Water temperature was maintained at $(28 \pm 0.12^\circ\text{C})$. The tank water was exchanged with fresh water 2-3 time/week. Tanks were washed and changed with fresh water every 4 weeks.

Experimental fish

Nile tilapia fingerlings used in this study with average initial weight of $12.78 \pm 0.12\text{g}$ were obtained from Fish Research Center, Suez Canal University, Ismailia, Egypt. Fish were homogenous in body weights and apparently healthy, unhealthy fish were removed from tanks, and replace with others healthy. Fish were acclimated to laboratory conditions for 2 weeks before beginning.

Table (2): Experimental diet composition (%)

Ingredient	Diet1 (T1)	Diet2 (T2)	Diet3 (T3)	Diet4 (T4)	Diet5 (T5)
Fish meal	40	40	40	40	40
FSBM	-	127.5	255	382.5	510
SBM	510	382.5	255	127.5	-
Yellow corn	30	150	310	330	330
Wheat brain	160	100	20	10	10
Rice brain	160	100	20	10	10
Vegetable oil	60	60	60	60	60
Vitamin Mix1	10	10	10	10	10
Mineral Mix2	10	10	10	10	10
C.M.C3	20	20	20	20	20
TOTAL	1000	1000	1000	1000	1000
Chemical Composition (%)					
Moisture	7.2	7.9	8.1	8.2	7.3
Crude protein	30.04	30.08	30.05	30.53	30.3
Crude fat	8.94	8.92	8.66	8.68	8.64
Crude fiber	6.46	5.62	5.42	5.49	5.75
Ash	4.24	4.42	4.32	4.18	4.21
NFE	43.12	43.06	43.45	42.92	43.80
Growth Energy	431.43	431.22	430.19	430.92	432.86
Kcal/Kg					
P/E	69.63	69.75	69.85	70.84	69.99

1 each Kg vitamin & mineral mixture premix contained Vitamin A, 4.8 million IU, D3, 0.8 million IU;

E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg ,

2. Each Kg minerals Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.

3. Carboxyl methyl cellulose

4. Nitrogen Free Extract = $100 - (\% \text{Protein} + \% \text{Fat} + \% \text{Fiber} + \% \text{Ash})$.

5. Gross Energy based on protein (5.65 Kcal/g), fat (9.45 Kcal/g) and carbohydrate (4.11 Kcal/g). According to (NRC, 1993).

6. Protein Energy Ratio. (mg protein / Kcal / GE)

Experimental Practices

Samples of fish

At the start and the end of the main experimental period (56 day), 5 fish were randomly taken from each experimental group. Fish were analyzed for chemical analysis of the whole body by using standard methods AOAC, 1995.

The tested diets were analyzed for crude protein (CP%) ether extract (EE%), Crude fiber (CF%), ash (%) and moisture according to the procedures described by standard AOAC methods (1995). The nitrogen free-extract (NFE%) was calculated by differences.

Acclimation and Weighing Procedure

Prior to initiation of each experiment, fish underwent a week conditioning, during which they readily adjusted to a commercial diet. At the beginning of the experiment, fish were individually weighed every two weeks. Fish weighed on a UWE MJW-300 balance to the nearest 0.01g. Fish were weighed individually at the beginning and end of feeding trial, and bulk-weighed fortnightly in between.

Fish Feeding

Fingerlings Nile tilapia was fed to satiation two times daily (10:00 am, 3:00 pm) and body weight were recorded every two weeks. After a stipulated period of feeding (20 to 30 min.), unconsumed feed, if any was collected on a fine mesh sieve, dried weighed and subtracted from food offered. Fish were deprived of food on the day of weighing and tanks were thoroughly scrubbed and rinsed with water. Each treatment had three replicates and was fed according to the experimental protocol.

Growth performance parameters:

The growth performance parameters are calculated according to the following equations:

Average Weight Gain (AWG):-

(AWG) = Average final weight (g) – Average initial weight (g)

Average Daily Gain (ADG):-

(ADG) = [Average final weight (g) – Average initial weight (g)] / time (days)

Specific Growth Rate (SGR %/day):-

(SGR % / day) = $100 \left[\frac{\ln Wt1 - \ln Wt0}{T} \right]$

Where: - Ln: normal log Wt 0: initial weight (g).

Wt 1: final weight (g) T: time of days.

Feed and protein utilization parameters:

Feed and protein utilization parameters are calculated according to the following equations:-

Feed Conversion Ratio (FCR):-

FCR = Total feed consumption/ weight gain.

Feed efficiency (FE):-

FE = weight gain/ Total feed consumption

Protein efficiency ratio (PER):-

PER = body weight gain (g)/ protein intake (g).

Protein production value (PPV, %):-

PPV (%) = $100 \left[\frac{\text{Retained protein (g)}}{\text{protein intake}} \right]$.

Survival rate (SR, %):

SR = $\frac{N_i}{N_0} \times 100$

Water quality parameters:

Water temperature were recorded daily by Mercury thermometer where the average range of temperature $28 \pm$

0.12°C, and dissolved oxygen by metteler Toledo, model 128.s/No1242 where the average range of dissolved oxygen was above 7 ± 0.064 mg/l, ammonia measured every two days by Hanna instruments where the average range of total ammonia was 0.009-0.012 mg/l. Other water quality parameters including pH was measured every two days by pH meter (Extech pH / temp pen model pH 60) where the average range of pH was in 7.5 ± 0.7 during the experiment.

Statistical analysis

The data obtained in this study were analyzed by one-way ANOVA procedure of Statistical Analysis System (SAS, 1998). Means were compared by Duncan's new multiple range test Duncan (1955).

Economical Evaluation:

The cost of feed to raise unit biomass of fish was estimated by a simple economic analysis. The estimation was based on local retail sale market price of all the dietary ingredients at the time of the study (Eid and Mohamed, 2008).

Cost/kg diet (LE) = Cost per Kg diet L.E.

Consumed feed to produce 1kg fish (kg) =

Feed intake per fish per period/ final weight per fish Kg/Kg

Feed cost per kg fresh fish (LE) = Step 1X step 2

Relative % of feed cost/ kg fish = Respective figures for step 3/ highest figure in this step

Feed cost/1Kg gain (LE) = Feed intake per Kg gain X step 1

Relative % of feed cost of Kg gain =

Respective figures for step 5/ highest figure in this step

RESULTS AND DISCUSSION

Growth performance

Averages of initial weights, final body weights (g) and the weight gain (g/ fish) are presented in Table 3. As presented in this table averages of initial weights had ranged between 12.62 to 12.91 g with insignificant differences ($p > 0.05$) among the experimental groups. The group of fish on T4 and T5 had significantly highest ($P < 0.05$) FBW, weight gain, SGR while the lowest values of FBW were recorded in group of fish in T1. Therefore, several practical ways have been suggested to improve utilization of plant proteins including; blending Jackson *et al* (1982) and fermentation Kader *et al* (2012). Day and Gonzalez (2000). So, the improved growth performance of fish is attributed to a number of factors including improved palatability, digestibility and reduced exposure to anti-nutritional factors. These results are in agreement with Hammed (2012) for fingerlings African catfish, *Clarias gariepinus*. And, also Khalafalla (2013) when was used Digeston-1 (fermented soybean meal) for Nile tilapia (*Oreochromis niloticus*) fingerlings at level (0.5% and 0.7%). Table (2) showed that treatment applied had no effect on survival rate throughout the experimental period. These results are in agreement with Hammed (2012) found no significant difference ($P > 0.05$) in mortality for *Clarias gariepinus* fingerlings. These results indicate that increase the levels of fermented soy bean meal (FSBM) improved growth performance of Nile tilapia fingerlings. This may be improving its

nutritional quality though its effect on the anti-nutritional factors present in the soy bean meal. These results may lead us to recommend the replacement of fermented soy bean meal on diets of Nile tilapia fingerlings at 100% levels.

Effects on feed utilization

Results of nutrient utilization in terms of feed conversion ratio (FCR): protein Efficiency ratio (PER) and protein productive value (PPV) are illustrated in Table (3). It was found a significantly ($P<0.05$) different among experimental groups. The highest value of feed and protein intake was recorded in group of fish fed diet T1 and T2 followed by T3 T4 and T5 respectively. The best value of feed conversion ratio was recorded for T5 (1.34) and T4 (1.43) while the lowest feed conversion ratio value was recorded for T1 (2.12) followed by T2 (1.90) and T3 (1.6) respectively. The same trend was observed in PER T5 (2.48) and T4 (2.32) and PPV were T5 (53.27) and T4 (46.88). Jiao *et al.* (1992) found that the fermentation leads to bio-modification of the substrate. Modification can also be done by treating the soybean products directly with enzymes. These results are in agreement with the finding of Hammed (2012) for *Clarias gariepinus* fingerlings. Also, Khalafalla (2013) reported that when used digestion-1 (fermented soybean meal) for Nile tilapia (*Oreochromis niloticus*) fingerlings at level (0.5% and 0.7%). These results may indicate that fermented soy bean meal could replace up to 100% by soy bean meal in growing tilapia diets

without any adverse effect on feed utilization parameters FCR or PER, As presented in Table (3).

Whole body composition

Average of whole body chemical compositions including crude protein (CP) ether extracts (EE), ash, nitrogen free extract (NFE) are presented in Table (4). Average of DM contents in tilapia bodies were found to be (23.83, 22.70, 22.63, 22.40 and 22.25) for T1, T2, T3, T4 and T5 respectively. It was found no significantly ($P<0.05$) different among experimental groups. The same trend was observed with ether extracts (EE) and Ash content contents in dry matter of tilapia whole bodies. Hong *et al.* (2004) showed that after fermentation with *Aspergillus oryzae* GB-107, the trypsin inhibitor in soy meal was reduced from 2.70 mg/g to 0.42 mg/g.. The trypsin inhibitor may be degraded or modified during fermentation and lose its activity binding to trypsin. These results are in agreement with the finding of Khalafalla (2013) who used digestion-1 (fermented soybean meal) for Nile tilapia (*Oreochromis niloticus*) at level (0.5% and 0.7%). Liyan Chen (2013) found that dry matter content kept decreasing during fermentation, in parallel with the trend of protein increase. Average of CP was found to be a significantly ($P<0.05$) different among experimental groups. The highest value of crude protein contents was recorded in T5 (58.40%) while the lowest value of crude protein contents was recorded in T1 (56.49). These results in agreement with the finding of Khalafalla (2013) for Nile tilapia (*Oreochromis niloticus*).

Table (3): Growth Performance and feed utilization by Nile tilapia fingerlings fed experimental diets.

ITEM	Diet1 (T1)	Diet2 (T2)	Diet3 (T3)	Diet4 (T4)	Diet5 (T5)
Average initial body weight (IBW) g	12.62±0.10	12.57±0.12	12.89±0.13	12.91±0.15	12.91±0.14
Average final body weight (FBW) g	30.12 ^c ±0.26	31.80 ^c ±0.24	34.31 ^b ±0.23	36.39 ^a ±0.25	37.16 ^a ±0.20
Average body weight gain (g)	17.50 ^c ±0.23	19.23 ^c ±0.22	21.42 ^b ±0.21	23.48 ^a ±0.24	24.25 ^a ±0.22
SGR % day	1.56 ^c ±0.10	1.65 ^c ±0.01	1.74 ^b ±0.02	1.84 ^a ±0.03	1.88 ^a ±0.04
Survival rate %	100%	100%	100%	100%	100%
Feed intake(g)/ fish	37.1 ^a ± 0.15	36.53 ^a ±.01	34.27 ^b ±0.22	33.72 ^c ±0.13	32.55 ^c ±0.02
CP intake (g)/fish	11.13 ^a ±0.20	10.95 ^a ±0.15	10.28 ^b ±0.13	10.11 ^c ±0.14	9.76 ^c ± 0.21
FCR(g/g)	2.12 ^a ± 0.01	1.90 ^b ± 0.02	1.60 ^c ± 0.10	1.43 ^d ± 0.22	1.34 ^c ± 0.21
PER(g/g)	1.57 ^c ± 0.14	1.75 ^d ± 0.10	2.08 ^c ± 0.12	2.32 ^b ± 0.13	2.48 ^a ± 0.15
PPV (%)	29.55 ^c ±0.12	30.95 ^d ±0.13c	44.35 ^c ±0.13c	46.88 ^b ±0.10	53.27 ^a ±0.12

Means in the same row having different superscript letters are significantly different ($P<0.05$).

Table (4): Chemical composition of the whole body of Nile tilapia fed experimental diet (%DM).

Treatment	Initial	T1	T2	T3	T4	T5
Dry matter	25.80	23.83±0.01	22.70±0.02	22.63±0.22	22.40±0.21	22.25±0.20
Crude protein	53.15	56.49 ^{cb} ±0.23	56.50 ^{cb} ±0.26	57.76 ^b ±0.24	57.94 ^b ±0.20	58.40 ^a ±0.25
Ether extract	15.98	18.90±0.10	18.78±0.12	18.69±0.13	18.60±0.15	18.73±0.14
Ash	21.26	23.98±0.10	23.88±0.01	23.66±0.02	23.59±0.03	23.55±0.04

Means in the same row having different superscript letters are significantly different (P<0.05).

Economic Evaluation

Results of Economic Evaluation including feed costs of one kg gain in weight and its ratio to that of the control group are presented in Table (5). As presented in this Table cost of one kg of the diet for fermented soy bean meal in T1, T2, T3, T4 and T5 were 4.44, 4.52, 4.60, 4.68 and 4.77 LE, respectively. Costs of one kg gain in weight were 9.41, 8.58, 7.36, 7.02 and 5.67 LE for T1, T2, T3, T4 and T5 respectively. These results indicate

that incorporation of fermented soybean meal into tilapia diets reduced the price of one kg diet to 91.17, 78.21, 74.60 and 60.25% for the T2, T3, T4 and T5 respectively compared to the control group (100% of the price). These results are in agreement with the finding of Hammed (2012) for *Clarias gariepinus* fingerlings and also of Khalafalla (2013) who used digestion-1 (fermented soybean meal) for Nile tilapia (*Oreochromis niloticus*) at level (0.5% and 0.7%).

Table (5): Effect of different dietary fermented soy bean meal levels on the economic efficiency of Nile Tilapia (*Oreochromis niloticus*).

Treatment	Feed cost/Kg diet (LE)	Feed conversion ratio	Feed cost/Kg fresh fish(LE)	Relative feed cost/Kg fresh fish (%)
T1	4.44	2.12	9.41	100.000
T2	4.52	1.9	8.58	91.17
T3	4.60	1.6	7.36	78.21
T4	4.68	1.5	7.02	74.60
T5	4.77	1.4	5.67	60.25

CONCLUSION

This study indicated that fermented soybean meal can be used to replace up to 100% of protein for optimum growth Performance, feed utilization and cost efficiency in the diet of fingerlings of Nile Tilapia (*Oreochromis niloticus*).

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تأثير مسحوق الصويا المخمر على أداء النمو لسماك البلطي النيلي

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أجريت تجربة تغذية لتقييم تأثير استبدال بروتين العليقة باستخدام كسب فول الصويا المتخمرة (FSM) على أداء النمو، والاستفادة من الغذاء ومكونات الجسم، في إصبعيات البلطي النيلي. تم استخدام (٥) خمس علائق متساوية في البروتين (٣٠%) والطاقة (٤٣١,٣٢) كيلو كالورى/ ١٠٠ جرام وكان متوسط وزن السمكة 12.78 ± 1.2 ، جرام تقريبا وقسمت الأسماك إلى ٥ معاملات (٣ مكررات في كل معاملة). وكانت نسب إحلل البروتين الموجود في العليقة بكسب فول الصويا المتخمّر (٠ و ٢٥ و ٥٠ و ٧٥ و ١٠٠%). حيث غذيت الأسماك في كل معاملة لدرجة الشبع مرتين في اليوم. لمدة ٨ أسابيع. وقد وجد أن هناك فروق غير معنوية في نسبة الإعاشة والرماد والدهن في المعاملات التجريبية المختلفة. وقد وجد أن أعلى استهلاك للغذاء والبروتين الموجود به في المعاملة الأولى والثانية. وكانت أفضل معامل تحويل غذائي في المعاملات الخامسة والرابعة. وكانت أعلى معامل استفادة من البروتين والكفاءة الإنتاجية للبروتين في المعاملة الخامسة والرابعة. ارتفاع بروتين الجسم بشكل ملحوظ مع ارتفاع مستوى التأسيس وجبة فول الصويا المتخمرة في النظام الغذائي. وتشير هذه النتائج إلى أن إدراج وجبة فول الصويا المتخمرة في النظام الغذائي للبلطي عمل على تخفيض سعر واحد كيلو غرام من الطعام إلى (٩١.١٧، ٧٨.٢١، ٧٤.٦٠ و ٦٠.٢٥%) للمعاملات T2، T3، T4 و T5 على التوالي مقارنة مع مجموعة التحكم (١٠٠%). ويستنتج من هذه الدراسة أن إدراج الصويا المخمرة بنسبة ١٠٠% في المعاملة الخامسة كان أفضل من حيث النمو والاستفادة الغذائية والتحليل الاقتصادي وذلك تحت هذه الظروف التجريبية.