

Optimum inclusion level of cotton seed meal as a source of protein in the diets of Nile tilapia, *Oreochromis niloticus* diet.

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Abstract

The present study was designed to investigate the effect of incorporation of cotton seed meal CSM in the diets of Nile tilapia *Oreochromis niloticus*. A set of 108 fish of *O. niloticus* L. mono-sex male fingerlings with average initial weight of 11.60 ± 0.72 g was used in this trail. Twelve fish were randomly stocked into each aquarium with three replications for each treatment and fed the experimental diet (295 g kg⁻¹ crude protein) and isocaloric (18.76 MJ kg⁻¹ gross energy). The first diet CSM1 which contained (fish meal : cotton seed meal 2:1), the second CSM2 which contained (fish meal : cotton seed meal 2:2) and the third CSM3 which contained (fish meal : cotton seed meal 1:2). Results of the present experiment indicated that, inclusion of cotton seed meal CSM had slightly significant effect on final body weight BW between groups CSM1(FM: CSM; 2:1) and CSM2 (FM:CSM; 2:2), but had no significantly effect on final BW between groups CSM1(FM: CSM; 2:1) and CSM2 (FM:CSM; 2:2). Weight gain (WG) and specific growth rate (SGR) were significantly higher in fish fed diet containing (FM: CSM 2:1) ratio, but no significant differences in WG, SGR and survival rate (SR) values of groups received the ratios of FM: CSM (2:2 and 1:2). The best feed conversion ratio (FCR), and protein productive value (PPV) were obtained by fish fed containing (FM: CSM= 2:1) followed by (FM: CSM= 2:1). There were no significant differences in protein efficiency ratio (PER). The highest apparent digestibility dry matter (DM) was obtained from fish fed diet containing (FM: CSM= 2:1) followed by (FM: CSM= 2:1), with significant ($P>0.005$) differences between FM: CSM = 2:1 and 2:2 ratio. The opposite trend was observed in apparent digestibility of protein. Meanwhile, there was no significant differences in apparent digestibility of lipid and energy.

Key words: cotton seed meal, fish meal, tilapia diets, growth and feed utilization

Introduction

The global aquaculture industry contributed 42.2% to world food fish production in 2012 and accounted for half of the world's fish for direct consumption (FAO, 2014). To maintain its current average growth rate of 8 to 10 percent per year up to 2025, the supply of nutrient and feed inputs will have to grow at a similar rate which is a tuff task (Tacon et al., 2011). In fact success of aquaculture, a reliable source of economic gain and employment generation, mainly stands on the cost of formulated fish feed which is the largest production cost for commercial aquaculture. Thus, improving feed efficiency in industrial systems is already a priority (Naylor et al., 2000). In aquaculture production, fishmeal (FM) typically is regarded as the main protein source in formulated diets due to its high level of protein, excellent amino acid profile, low carbohydrate level and high digestibility (Zhou et al., 2004). The FM prices have risen continuously in real terms in the past decades and are likely to increase further with continued growth in demand (FAO, 2010). As a result, restricted FM supplies can no longer meet the needs of the expanding fish-feed industry (Bostock et al., 2010). Moreover, the use of animal protein for feeding herbivorous fish might be unnecessary. Therefore, replacements for FM have an international

research priority (Kumar et al., 2010) and to bring down the feed cost and to develop low cost and high yield production system (Cho et al., 1994).

In view of the increasing cost of ingredients for aquafeeds, the search for cheap and nutritionally balanced ingredients as well as available for fish feed has become an urgent need for the aquaculture industry. Especially, the evaluation of the nutritional value and potential of locally available nutrient sources is an important aspect for the substitution of conventional fish feed ingredients. Plant proteins might be considered as the most viable alternative in this respect for economic fish production in most of the developing countries (Becker and Makkar, 1999). Several studies in recent past to make supplementary feeding of fish cost-effective 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.

Cottonseed meal (CSM), a high protein by-product of the cottonseed processing industry, has been studied in numerous fish species, *Sarotherodon mossambicus* (Jackson et al., 1982) and *Oreochromis niloticus* (Yue and Zhou 2008; Soltan et al., 2011a&b), *Ictalurus punctatus* (Robinson and

Tiersch 1995), and *Oncorhynchus mykiss* (Lee et al., 2006). These studies showed positive results at low inclusion levels, but growth reduction at high inclusion levels. Among the factors which limit incorporation of these ingredients into aquafeeds are (1) amino-acid imbalance and presence of anti-nutritional factors such as gossypol (Li and Robinson 2006). In general, oilseed meals processed in traditional ways cannot be utilized at high levels without compromising growth and production. 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). Therefore, the present study examined the possibility of utilizing the optimum CSM, as source of protein for Nile tilapia.

Materials and Methods

Experimental diets

Three 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 these experimental diets are presented in Table (1). The first diet CSM1 which contained (fish meal : cotton seed meal 2:1), the second CSM2 which contained (fish meal protein : cotton seed meal protein 2:2) and the third CSM3 which contained (fish meal protein : cotton seed meal protein 1:2). All dry ingredients of the fish meal, cotton seed meal, soybean meal, yellow corn and wheat bran were blended for 5 mins and thoroughly mixed with soybean oil. Also, each of the diet contained 5 g kg⁻¹ chromic oxide (Cr₂O₃) as a marker for nutrient digestibility measurements. 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.

Experimental fish and culture technique

O. niloticus were obtained from private farm (Kafer elsheekh Governorate, Egypt). Fish were acclimated to the experimental conditions for two weeks at the laboratory of fish at Faculty of Agriculture, Benha Univ. During the acclimation period, fish were fed a control diet (30 % 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 glass aquaria (40×30×60 cm for each) representing the three treatments studied. A set of 108 fish of *O. niloticus* L. mono-sex male fingerlings average initial weight of 11.60 ± 0.0.72 g was used in this trail. Twelve fish

were randomly stocked into each aquarium 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 90-days experimental period, triplicate groups of tilapia were hand-fed with the respective diet to apparent satiation twice daily at 09:00 am and 3:00 pm.

Water quality

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). A pH was measured in morning by using a pH meter (Orion pH meter, Abilene, Texas, USA). 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, 1990). 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). Apparent nutrient digestibility was calculated using the equations of (Schneider et al., 2004) as following: $ADC_{\text{dietary nutrient}} = 1 - ((\text{marker}_{\text{diet}})/(\text{marker}_{\text{feces}}) \times (\text{nutrient}_{\text{feces}})/(\text{nutrient}_{\text{diet}}))$.

Growth performance and feed utilization Indices

Records of live body weight (g) and body length (cm) of fish were measured in all fish for each tank and registered every 14 days during the experimental period. Growth performance and feed utilization were measured by using the following equations:

Condition factor (K) = $(W/L^3) \times 100$; where: W = 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) = $\frac{\text{Ln}W_2 - \text{Ln}W_1}{t} \times 100$

Where: Ln = the natural log; W₁ = first fish weight; W₂ = the following fish weight in grams and t = period in days.

Feed conversion ratio (FCR) = Feed intake (g)/Weight gain (g);

Protein efficiency ratio (PER) = Weight gain (g)/Protein ingested (g);

Protein productive value (PPV) % = (protein gain (g) / protein intake (g)) × 100.

Proximate composition

At the initiation and 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. At the end of each experiment, two fish were randomly sampled from each aquarium and subjected to the chemical analysis of whole fish body. Moisture, dry matter (DM), ether extract (EE), crude protein (CP), crude fiber (CF) and ash content of diets and fish were determined according to the methods described in **AOAC (1995)**: dry matter after drying in an oven at 105°C until constant weight; ash content by incineration in a muffle furnace at 600°C for 12 hrs; crude protein ($\text{N} \times 6.25$) by the kjeldhal method after acid digestion; and ether extract by petroleum ether ($60\text{-}80^{\circ}\text{C}$) extraction.

Statistical analysis

Statistical analysis of the obtained data was analyzed according to **SAS (1996)**. Differences between means were tested for significance according to Duncan's multiple rang test as described by **Duncan (1955)**.

Results

Growth performance

Results presented in Table (2) showed that the differences in fish initial body weight among different treatments were insignificant indicating that the experimental groups at the start of the experiment were randomly distributed. The data cleared that inclusion of cotton seed meal CSM had slightly significant effect on final BW between groups CSM1(FM: CSM; 2:1) and CSM2 (FM:CSM; 2:2). Similar results were obtained in body length except between groups CSM2 (FM: CSM 2:2) and CSM3 (FM:CSM 1:2) which showed insignificant differences. Condition factor (K) factor increased from 1.78 to 2.57 and 2.75 respectively, with significant differences between K values (Table 2).

Results of Table 3 showed that the different ratios of FM: CSM had significantly effect on WG, SGR and SR for tilapia. The value of WG and SGR were higher in fish fed diet containing (FM: CSM 2:1) ratio, but no significant differences ($P < 0.05$) in WG, SGR and SR values of groups received ratios of FM: CSM (2:2 and 1:2).

Feed intake and feed utilization

Results of feed intake (FI), feed conversion ratio (FCR), protein efficacy ratio (PER) and protein productive value (PPV) as affected by FM: CSM ratios are presented in (Table, 4). As described in this table, feed intake during the whole period of the experiment was 41.42, 39.93 and 39.82 for fish groups fed diet containing different ratios of FM: CSM respectively, with insignificant ($P > 0.005$) differences between these means. The best FCR and PPV was obtained by fish fed containing (FM:CSM= 2:1) followed by (FM:CSM= 2:2). There was no significant differences in PER.

Digestibility

Results of apparent digestibility as affected by different ratios of FM: CSM are presented in Table 5. As described in this table, the highest DM was obtained from fish fed diet containing (FM:CSM = 1:2) followed by (FM:CSM= 2:2), with significant ($P > 0.005$) differences between FM: CSM = 2:1 and each of 2:2 and 1:2 ratio. The opposite trend was observed in protein. Meanwhile, there was no significant differences ($P > 0.05$) in apparent digestibility of lipid and energy.

Proximate analysis of whole body fish

Proximate analysis of tilapia as affected by FM: CSM ratios, exogenous enzyme protease and their interaction is presented in Table 6. The effect of FM: CSM ratios in diets, it is shown that fish fed the first ratio 2:2 FM: CSM had the highest protein and energy content and the lowest ($p < 0.05$) lipid content while the opposite trend was observed for fish fed the diet with the third ratio (1:2) of FM: CSM) or dry matter and protein contents.

Discussion

Growth performance and feed utilization

Cotton seed meal is considered the most cost effective nutritive plant protein by product source, the most commonly used and the most abundant plant by-products as well as has been tested in numerous fish species, including *Sarotherodon mossambicus* (**Jackson et al., 1982**) and *Oreochromis niloticus* (**Yue and Zhou 2008; Soltan et al., 2011a&b**), *Ictalurus punctatus* (**Robinson and Tiersch 1995**). The obtained results for all parameters of growth performance in the present study agree with those obtained by **Garcia-Abiado et al., (2004)**, who reported that Nile tilapia fed 25-50% CSM protein as a replacer of FM showed similar body weight as the control while fish fed 75 and 100% CSM protein replacement showed a significant decline in body weight. Also, **Cai et al., (2011)** observed that dietary inclusion of CSM up to a concentration of 400 g kg^{-1} diet don't affected growth performance and feed utilization of crucian carp (*Carassius auratus gibelio* ♀ × *Cyprinus carpio* ♂). Moreover, it has no effects on growth for rainbow trout fed dietary CSM supplementation up to 588 g kg^{-1} diet or complete substitution of FM protein (**Lee et al., 2006**). No significant differences in survival, growth and feed efficiency were observed in fish fed diets replacing up to 100% FM by CSM for Black Sea Bass, *Centropristis striata* and up to 75% FM by CSM in flounder, *Paralichthys lethostigma* (**Anderson et al., 2016**).

Condition factor of fish is essentially a measure of relative muscle to bone growth and the differing growth responses of these tissues to diet treatment may be reflected by changes in condition factor (**Ostrowski and Garling, 1988**). In an earlier study, **Robinson and Daniels (1987)** investigated CSM as a partial or complete substitute for soybean meal in

catfish diets. They reported that fish fed a diet containing CSM to totally replace of all soybean meal had similar condition factor compared to fish fed a soybean meal. **Yue and Zhou (2008)** indicated that up to 60% of SBM could be replaced by CSM without adverse effect on growth performance of hybrid tilapia fed the diet highest in CSM had a significantly lower protein efficiency ratio and a significantly higher feed conversion ratio than fish fed the other diets. High survival rate was observed in all dietary treatments, and no significant ($P>0.05$) difference among treatments was observed. A 64-day growth trial estimated the ability of three different sources of CSM cotton variety/mutant, two transgenic Ultra-low Gossypol Cottonseed lines, a non-transgenic parental control and a commercial variety, instead of 355 g kg⁻¹ fishmeal in a diet containing 350 g kg⁻¹ crude protein. Juvenile *Litopenaeus vannamei*. No significant ($P>0.05$) differences were found in terms of final weight, survival and feed conversion ratio among treatment **Richardson et al., (2016)**.

Digestibility

Cheng and Hardy (2002) revealed that rainbow trout fed CSM diets at the 5% or 10% inclusion rates were not significantly different compared to fish fed the fish meal control diet in terms of ADC ($P>0.05$), indicating that CSM could be used at the 10% inclusion rate in rainbow trout feed formulations without adverse effect on growth performance and feed utilization. No significant differences in ADC was observed in fish fed diets replacing up to 100% FM by CSM for Black Sea Bass, *Centropristis striata* and up to 75% FM by CSM in flounder, *Paralichthys lethostigma* **Anderson et al., (2016)**. The apparent digestibility coefficients (ADC) of dry matter significantly decreased with the increase of dietary CSM level, whereas the ADC of lipid was not affected by dietary treatment for juvenile hybrid tilapia, *Oreochromis niloticus* × *O. aureus* **Yue and**

Zhou (2008). Similar results were showed in Nile tilapia (**El-Saidy and Gaber, 2004**), and rainbow trout (**Luo et al., 2006**). However, the ADC of protein for diets containing CSM with two level 15 and 100 g kg⁻¹ was significantly lower than that of the control diet. In contrast, the ADCs of protein for tilapia (*Oreochromis* sp.) decreased when the dietary CSM level increased (**Mbahinzireki et al., 2001**).

Proximate analysis of whole body fish

Chemical analysis at the end of a feeding trial is frequently used to determine the influence of feed on fish composition. According to **Hepher (1990)**, endogenous factors (size, sex and stage of life cycle) and exogenous factors (diet composition, feeding frequency, temperature etc.) affect the body composition of fish. It should be noted that within endogenous factors, the composition of the feed is only the factor, which could have influenced the chemical composition of fish, as other endogenous factors were maintained uniform during the study. As CSM in the diet increased protein content of whole fish significantly reduced protein content compared to the control group while the opposite trend was also observed for fat content where the increasing levels of CSM in the diets not significantly increased in fat content of the whole fish. **Yildirim et al., (2003)** supplemented juvenile channel catfish diets with 0, 300, 600, 900, 1200 and 1500 mg gossypol from gossypol-acetic acid and fish fed the experimental diets to satiation twice daily for 12 weeks. They found that Body ash did not differ in fish fed diets containing 0 to 900 mg gossypol/kg but was significantly higher in fish fed higher levels of gossypol. No significant differences in body composition was observed in fish fed diets replacing up to 100% FM by CSM for Black Sea Bass, *Centropristis striata* and up to 75% FM by CSM in flounder, *Paralichthys lethostigma* **Anderson et al., (2016)**.

Table 4. Feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV) for *O. niloticus* fed experimental diets for 90 days.

Items	FI g/fish	FCR	PER	PPV
FM : CSM (2:1)	41.42	1.29 b	2.59	64.80 a
FM : CSM (2:2)	39.93	1.32 b	2.53	63.43 a
FM : CSM (1:2)	39.82	1.37 a	2.44	61.03 b
Stander error	0.721	0.044	0.078	2.16

Means followed by different letters in the same column are significantly different ($P<0.05$).

Table 5. Apparent digestibility of dry matter (DM), protein, lipid and energy for *O. niloticus* fed experimental diets for 90 days.

Items	DM	protein	Lipid	Energy
FM : CSM (2:1)	93.09 b	89.75 a	91.03	85.81
FM : CSM (2:2)	93.85 a	88.05 b	91.11	84.85
FM : CSM (1:2)	93.87 a	86.87 b	90.36	84.91
Stander error	0.560	0.348	0.354	0.529

Means followed by different letters in the same column are significantly different ($P<0.05$).

Table 6. Chemical composition for *O. niloticus* fed experimental diets for 90 days.

Items	DM	protein	Lipid	Ash
FM : CSM (2:1)	26.56a	59.70a	20.41a	13.33b
FM : CSM (2:2)	25.33b	60.52a	18.50b	15.17a
FM : CSM (1:2)	25.21b	58.37b	20.52a	14.96a
Stander error	1.57	1.87	1.25	0.87

Means followed by different letters in the same column are significantly different (P<0.05).

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النسبة المثلى لإدخال كسب بذرة القطن كمصدر للبروتين في علائق أسماك البلطي النيلي

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أجريت هذه التجربة لدراسة تأثير إدخال كسب القطن في علائق سمك البلطي النيلي وحيد الجنس ولذلك استخدمت 108 سمكة بلطي بمتوسط وزن 0.72 ± 11.60 جرام تم توزيعها على على تسعة أحواض بواقع 12 سمكة للحوض (3 معاملات ، 3 مكررات لكل معاملة) تم تغذيتها على علائق متساوية في محتواها من البروتين (295 جرام بروتين/كجم علف) ومتساوية في محتواها من الطاقة (18.76 ميجا جول/كجم علف طاقة كلية) وقد احتوت العليقة الأولى والثانية والثالثة على مسحوق السمك وكسب القطن بنسبة 2 : 1 ، 2:2 ، 1 : 2 على التوالي. وأستمرت التجربة 90 يوماً وفي نهاية التجربة أظهرت النتائج أن إختلاف نسب مسحوق السمك وكسب القطن المختلفة قد أثرت معنوياً على وزن الجسم والزيادة في وزن الجسم ومعدل النمو والحيوية كما اثرت نسب مصادر البروتين المختلفة (مسحوق السمك وكسب القطن) تأثيراً معنوياً على كمية الغذاء الماكول والإستفادة من الغذاء حيث اظهرت النتائج ان افضل معدل تحويل غذائي وكذلك الإستفادة من بروتين الغذاء قد تحقق بواسطة الأسماك التي تغذت على العليقة الأولى والتي أحتوت على مسحوق السمك وكسب القطن بنسبة 2 : 1 كما أظهرت نتائج التحليل الكيمياءى للأسماك هذه المجموعة أنها أحتوت اعلى نسبة من المادة الجافة واقل نسبة من البروتين في أجسامها.