Growth, feed utilization and body composition of tilapia (*Oreochromis* sp.) fed with cottonseed meal-based diets in a recirculating system

G.B. MBAHINZIREKI[1], K. DABROWSKI[1], K.-J. LEE[1], D. EL-SAIDY[2] & E.R. WISNER[3]

[1] *School of Natural Resources, The Ohio State University, Columbus, OH, USA; Mbahinzireki's present address, FIRI, Jinja, Uganda;* [2] *Department of Poultry Production, Menofiya University, Shebin El-Kom, Egypt;* [3] *Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH, USA*

Abstract

The suitability of cottonseed meal (CSM) as a major source of plant protein in feeds for tilapia (*Oreochromis* sp.) was tested by examining growth and feed intake, feed digestibility, liver gossypol concentrations, feed utilization, and body mineral composition. Juvenile tilapia at an initial average size of 11.8 ± 1.6 g were divided into triplicate groups per dietary treatment and offered five different formulated diets. In these feeds fish meal (FM) protein was gradually replaced by protein from CSM (0, 25, 50, 75, and 100%; diets 1, 2, 3, 4 and 5, respectively). The experiments were conducted in a recirculation system at a water temperature of 27 ± 1°C in glass aquaria for 16 weeks. Tilapia growth did not differ significantly ($P > 0.05$) with up to 50% substitution of FM with CSM. Fish meal replacement above 50% resulted in significant growth decline with time. Fish fed with 100% FM and diets including 50% CSM had significantly better daily weight gain, daily feed intake and feed efficiency ratio than those fed with 100% CSM. Fish fed with 75% CSM and above had lower concentrations of body iron, calcium and phosphorus than controls (100% FM). Concentrations of total gossypol in diets (ranging from 0.11 to 0.44% in diets 2–5) resulted in proportional increase of total gossypol in fish liver (32.3, 72.3, 99.4 and 132.1 μg g^{-1} wet weight) in groups fed with diet 2, 3, 4, and 5, respectively. We concluded that CSM can partially replace FM as a main source of protein in feed for tilapia at not more than 50%. The presence of gossypol in CSM was identified as the major limiting factor for acceptance and utilization of CSM-based diets in tilapia farming.

KEY WORDS: Body composition, cottonseed meal, fish meal, gossypol, growth, tilapia (*Oreochromis* sp.)

Received 10 February 2000, accepted 10 October 2000

Correspondence: K. Dabrowski, School of Natural Resources, The Ohio State University, 2021 Coffey Road, Columbus, OH 43210-1085. USA. E-mail: dabrowski.1@osu.edu

Introduction

Feed is the single largest expenditure in semi-intensive and intensive fish culture operations. This is because of extensive reliance on marine animal protein sources such as fish, shrimp and squid meal to meet the high dietary protein requirements of fish. These feedstuffs have high palatability and nutritional value but are expensive and not always readily available (Lim & Dominay 1990). From 1984 to 1990, world aquaculture production increased at an average rate of about 14% annually, and the total production will be dramatically increased at least by the year 2005 (Hardy 1999). However, the world fish meal production is not expected to increase further (Pauly *et al*. 2000). Therefore, in order to develop economical aquaculture systems, alternate sources of high quality proteins will have to be identified to replace high-cost fish meal. Current research is addressing the use of plant ingredients and plant protein concentrates in feeds for fish.

Several sources of plant proteins to replace the more expensive fish meal (FM) partially or completely have been experimented on various finfish. Plant proteins examined have included soya bean meal (Quartararo *et al*. 1998), cacao husks (Pouomogne *et al*. 1997) various cereals (Al-Ogaily *et al*. 1996), brewery draff (Pouomogne *et al*. 1992), napier grass (Chikafumbwa 1996) and cottonseed meal (CSM)

(Robinson *et al.* 1984a,b; El-Sayed 1990). For tilapia diets typical plant protein alternatives have included soya bean meal (Brandt 1979; Jackson *et al.* 1982) and sunflower seed meal (Jackson *et al.* 1982) among others. Results of using these plant proteins ranged from high to poor growth and survival of tilapia species.

Cottonseed meal is widely used as a protein supplement in ruminant livestock because of its high protein content and ample availability. Cottonseed meal ranks third in the world in tonnage among the vegetable protein concentrates produced (Swick & Tan 1995) and is available at a much lower cost than animal proteins. Furthermore, cottonseed is a by-product of cotton, a cash crop widely grown in the tropics where extensive tilapia culture is practised. The nutritional value of cottonseed has been evaluated for several species of fish such as chinook (*Oncorhynchus tsawytscha*) and coho salmon (*Oncorhynchus kisutch*) (Fowler 1980), tilapia (Jackson *et al.* 1982; Viola & Zohar 1984; El-Sayed 1990), and channel catfish (*Ictalurus punctatus*) (Robinson 1991; Robinson & Li 1994; Robinson & Tiersch 1995). However, the use of cottonseed meal in fish diets is limited by the presence of gossypol, a toxic component for monogastric animals. Further, a low availibility of lysine limits the nutritional value of this protein source (Jauncey & Ross 1982). Generally, it has been reported that the amount of CSM that can be included in feeds depends on the animal species, levels of free gossypol, dietary protein and available lysine (Martin 1990).

The research data in the literature on the effects of feeding CSM to tilapia is contradictory. Robinson *et al.* (1984a) reported that tilapia can tolerate gossypol acetate supplemented to purified diets up to 0.2% with no decrease in growth. Jackson *et al.* (1982) obtained good growth of tilapia (*Sarotherodon mossambicus*) when 35.2% prepressed, solvent-extracted CSM (0.03% free gossypol) was used as a substitute for 50% of FM protein. The growth rate was essentially the same as in the control group. Likewise, El-Sayed (1990) demonstrated that CSM could be used as a main dietary protein source for *Oreochromis niloticus*. Viola & Zohar (1984) found that low-gossypol cottonseed meal could be included in feeds for hybrid tilapia *O. niloticus* × *O. aureus* at the same levels as soya bean meal. In contrast, Robinson *et al.* (1984a) reported that neither glanded nor glandless cottonseed meals appeared to be as high in nutritive value for *Tilapia aurea* as soya bean and peanut meals. Ofojekwu & Ejike (1984) also reported a much lower weight gain and feed efficiency of *O. niloticus* fed with a cottonseed cake diet as compared with tilapia fed with a FM based diet.

The present study was conducted to evaluate the inclusion of graded levels of CSM on growth, feeding efficiency, protein digestibility, gossypol concentrations in the liver and survival rate. Juvenile tilapia require 34–40% protein in the diet for optimum growth (Jauncey & Ross 1982; DeSilva *et al.* 1989). We have used upper level of protein in the present study because there is no significant difference in the growth rate in the range of 35–45% protein (DeSilva *et al.* 1989) and inclusion of plant protein as replacement of animal protein will be more challenging. Feed intake was estimated by incorporating glass beads in diets followed by radiographic analysis. At the end of the experiment proximate body analysis was conducted to estimate their mineral composition because gossypol is known to form complexes with metals in monogastric animals (Martin 1990). Experiments were set up to test the hypothesis that fish in a recirculation system fed with high protein CSM-based diets would perform as well as those given FM-based diets.

Materials and methods

Growth and feed utilization

Juvenile tilapia (*Oreochromis* sp.) averaging 11.8 ± 1.6 g (mean ± SD) were randomly distributed among 15 glass aquaria (32 L) at an initial density of 15 fish per aquarium and water temperature maintained at 27 ± 1°C. Aquaria without substrate were maintained in a semirecirculated system (10% exchange daily) which included a mechanical filter (Aquanetics System Pak, San Diego, CA, USA) and UV-sterilization lamp (ETRI, model 126 LJ, Japan). Supplemental aeration was provided to maintain dissolved oxygen levels near saturation. The water was constantly replaced in aquaria by continuous flow at a rate of 250 mL min^{-1} to provide oxygen and remove excess nitrogenous wastes. In addition, the aquaria were siphoned daily to remove faecal materials.

Five diets, each containing a different proportion of CSM (Table 1) were prepared by steam-pelleting, dried and stored at −10°C until use. Each diet was assigned randomly to the three aquaria. The fish were fed with the experimental diets for 2 weeks to acclimate them to the diets before taking growth parameters at an interval of 2 weeks. The fish were fed with their respective diets at a rate of 3% of their body weights per day, and later diminished to 1.5% towards the end of the experiment. An equal amount of diet was given 3 times a day at 11:00, 14:00 and 17:00 h for 13 days and the fish were then starved for 24 h before recording their weights. Fish in each tank were weighed

Table 1 Compositions (g kg^{-1}) and chemical analyses for five experimental diets (dry matter basis)

	Diets				
Ingredients	1 (0% CSM)	2 (25% CSM)	3 (50% CSM)	4 (75% CSM)	5 (100% CSM)
FM menhaden	200.0	150.0	100.0	50.0	0.0
FM herring	200.0	150.0	100.0	50.0	0.0
Cottonseed meal	0.0	147.0	294.2	441.1	588.0
Krill hydrolysates	50.0	50.0	50.0	50.0	50.0
Wheat middlings	280.0	212.0	144.0	75.0	6.0
Corn gluten meal	116.0	126.0	136.0	146.0	156.0
Yeast	60.0	60.0	60.0	60.0	60.0
Methionine	0.0	1.0	2.0	3.0	4.0
Lysine	0.0	2.0	4.0	6.0	8.0
Vitamin mixture[1]	5.0	5.0	5.0	5.0	5.0
Mineral mixture[1]	5.0	5.0	5.0	5.0	5.0
Vitamin C-MP[2]	0.5	0.5	0.5	0.5	0.5
Choline chloride	1.0	1.0	1.0	1.0	1.0
Menhaden fish oil	80.0	89.0	98.0	107.0	116.0
Cellulose	2.5	1.5	0.3	0.4	0.5
Proximate analysis (g kg^{-1})					
Protein	409	431	429	424	429
Lipid[3]	144	145	146	147	148
Methionine[3]	11.8	11.7	11.7	11.6	11.6
Lysine[3]	27.8	27.1	26.3	25.5	24.8
Energy (kCal kg^{-1})[3]	4180	4177	4176	4170	4163
Analysed gossypol (g kg^{-1})					
Total	0.00	2.27	4.24	7.38	9.16
(+)-isomer	0.00	1.14	1.92	3.80	5.01
(−)-isomer	0.00	1.13	2.32	3.58	4.15
Free	0.00	0.26	0.52	0.70	0.99

[1] Dabrowski *et al.* (1995).
[2] Mg-L-ascorbyl-2-phosphate (Showa Denko K.K., Tokyo, Japan).
[3] Values calculated based on the compositions of ingredients (NRC 1993).

individually and the average weight gain per aquarium was calculated. The feeding trial was conducted for 16 weeks from May 21 to September 11, 1998. Specific growth rate (SGR) and feed conversion ratio (FCR) were calculated at the end of the feeding trial.

Estimation of feed intake

The experiment was conducted to estimate feed intake at the end of the growth study using radiographic methods (Jobling *et al.* 1989; Arnesen *et al.* 1993). About 100 g of dry pellets of each diet were ground in a mortar into a powder to which 4% (w/w) of glass beads (0.4–0.6 mm diam.) were added as a marker and mixed thoroughly. Cold water was added to the mixture and stirred thoroughly to form a thick paste. This was then re-pelletted by extruding the paste through a mincer, resulting in 'spaghetti-like' strings. They were placed on aluminium foil and freeze-dried. The feeds were then broken up and sieved into convenient pellet sizes (3–5 mm) for the fish and stored at −20°C until used.

Fish feeding and radiography

Before the fish were given the marker-containing diets (week 17), they were not fed for 24 h to allow evacuation of most of the gut contents. The fish were then fed with their respective diets labelled with leaded glass beads (supplied by D.F. Houlihan, University of Aberdeen, Scotland, UK) to satiation (within about 15 min) early in the morning. Two hours after feeding, four fish were randomly picked out of each aquarium. Each fish was marked for later identification by fin clipping. Each group of fish from each tank was anaesthetized with MS-222 (Tricaine methanesulphonate) at the concentration of 100 mg L^{-1}. They were laid out one by one on a radiographic cassette. Radiographic images were obtained using an X-ray machine (Picker VTX 1050, Picker International, Cleveland, OH, USA) operating at 44 kVp, 250 mA, 1.9 mAS; and a high detail radiographic film and screen combination (ultra-vision L film/ultra-vision detail screens, Sterling Diagnostic Imaging, Inc., Newark, DE, USA). The fish were returned to their original buckets where

they recovered from anaesthesia within 5 min. They were weighed and returned to their respective tanks. The radiography was carried out at the Ohio State University Veterinary Hospital, Radiology Department. The experiments were carried out in 2 days.

Exposed radiographs were developed and the number of glass beads revealed in the gut of individual fish were counted. A standard curve describing the relationship between the amount of labelled feed (x) and the number of glass beads (y) was prepared by radiography of the weighed samples of labelled food. The number of glass beads in the gut of each fish was converted into grams of feed by the following formula:

$$y = 271.2x - 8.4 \ (r^2 = 0.965, \ n = 20).$$

Feed digestibility

Digestibility experiments commenced on the 11th week of the feeding study. The fish were not fed for 24 h before initiating this phase of the study. Early each morning, for three days, the fish were fed with their normal respective diets to satiation. Each diet contained a 0.5% chromic oxide marker which gave a green appearance to the feed pellets of all diets. Two hours after feeding the excess feed was removed and the fish transferred to circular tanks with a steep conical bottom, an apparatus designed to collect fish faeces. Three conical tanks, each holding about 50 L of water, were used. Water maintained at 28°C continually flowed in at the top and out near the bottom of the tanks at a rate of 1 L min^{-1}. Opening the tap at the end of the tank allowed faeces to sink and settle through a 5-cm drain into a 2-cm diameter, 10-cm long, settlement chamber which tapered into a 2-cm diameter, 8-cm length of PVC-tubing. The tubing into which the faeces settled was packed in ice and kept at less than 4°C to reduce bacterial action. The faeces were collected three times a day at an interval of 3 h (over 9 h) by detaching the collecting tube. Fish were prevented from stirring the faeces by a circular mesh net placed at the base of the tank. Any faeces held on the walls of the tank were siphoned into a container and filtered together with the faeces obtained in the collecting tubes to remove excess water, transferred into Petri dishes and stored below −20°C.

Dietary and faecal samples were freeze-dried and analysed in triplicate per treatment for total nitrogen and other elements at the Research-Extension Analytical Laboratory, Wooster. The faecal matter was digested with perchloric acid and chromic oxide was analysed using the method described by Scott (1978) in the same laboratory. The apparent digestibility (AD) of a given nutrient was calculated using the following formula (Spyridakis *et al.* 1989):

AD (%) = 100 – {100 (% Cr_2O_3 diet/% Cr_2O_3 faeces) (% nutrient faeces/% nutrient diet)}.

Analysis of gossypol in diet and liver

Total and free gossypol concentration in liver tissue (6 fish per treatment) and diets were determined according to the method described by Kim & Calhoun (1995) with some modifications. Wet liver tissue was used for (+)-and (−)-isomers of gossypol, because the detection of gossypol in freeze-dried liver sample was lower than in wet sample from the preliminary analysis which was done to compare the two processing methods. The liver and dry diets were weighed and 10 volumes of complexing reagent added to obtain the 2-amino-1-propanol derivatives of (+)-and (−) isomers of gossypol. The complexing reagent consisted of 2% 2-amino-1-propanol and 10% glacial acetic acid in N, N-dimethyl-formamide. The samples in complexing reagent were homogenized on ice for 10 s for liver and 30 s for diets, heated at 95°C for 30 min, cooled on ice, and then centrifuged at 1,500 × g for 5 min. After centrifugation an aliquot of the supernatant was taken, diluted with mobile phase to make desirable concentrations, and centrifuged again to prevent the syringe filter (0.45 μm) from clogging, and then the supernatant was filtered with the syringe filter before injection to high performance liquid chromatography (HPLC). The sample volume used for injection was 20 μL. The HPLC system consisted of Beckman 506 A with a C-18 reverse phase column (Shodex, Japan) packed with octadecyl-bonded porous silica gel (5 μm), and a UV detector (Programmable detector module 166, Beckman, USA). The mobile phase was made of 80% acetonitrile and 20% 10 mM KH_2PO_4 adjusted to pH 3.0 with H_3PO_4. The retention time for (+) and (−)-isomer of gossypol were 2.1 and 3.4 min, respectively, with flow rate of 1.8 mL min^{-1} at 254 nm. The standard gossypol (+)-and (−)-isomers were provided by Dr Quezia B. Cass, Departamento de Química, Universidade Federal de São Carlos, São Carlos, Brazil (Cass *et al.* 1999). Recovery rate for extraction of gossypol enantiomers in liver was 92.4%, and detection level was 1.0 ng gossypol/20 μL of injection volume with a signal-to-noise ratio of 3.

Chemical body composition of fish

Three fish samples from the killed fish (nine per diet) were freeze-dried and ground into powder for proximate chemical analysis at the Ohio State University Research-Extension

Analytical Laboratory, Wooster, Ohio. Minerals were determined in the whole-body dry matter. Samples were prepared for analysis using a dry-wet ashing procedure (AOAC 1995). Mineral composition of fish whole-body ash was measured by the inductively coupled plasma (ICP) emission spectrophotometry method with the use of an ARI-3560 Spectrometer (Applied Research Labs, Valencie, CA, USA) according to Watson & Isaac (1990).

Statistical analysis

The results were reported as the mean ± standard deviation (SD). The mean values were compared using Student's *t*-test, and the differences at $P < 0.05$ were considered significant. A one-way ANOVA was performed after transformation of the data into arcsin square root in order to demonstrate the significance of differences among treatments. Means were compared with the Newman–Keuls multiple range test (Sokal & Rohlf 1981) at the 0.05 probability level.

Results

Growth and feed utilization

Gossypol concentration in the diets increased with higher proportions of CSM inclusion in the diets (Table 1). Tilapia growth did not differ ($P > 0.05$) with up to 50% substitution with cottonseed meal (CSM) in the diet given over a period of 16 weeks (Fig. 1a). Fish meal replacement at 75 and 100% resulted in a significant decline in growth rates after the 12th week. Fish which had 100% CSM in their diet had the lowest average weight gain, less than half of the weight gained by fish given diets 1–3 (Fig. 1b). Growth rates and FCR for tilapia varied depending on experimental diets (Table 2). Fish fed with the control diet (100% FM) and diets including 25 and 50% CSM had significantly better daily weight gain, feed efficiency ratio, and SGR ($P < 0.05$) than those fed with 100% CSM. Furthermore, fish fed with 100% CSM (diet 5) exhibited 11% of cumulative mortality (Table 2).

Feed intake

Figure 2 shows three examples of radiographs used to calculate the number of ingested glass beads by the fish. The feed intake values were calculated from a calibration curve showing the relationship between the number of glass beads and feed pellet weights (Fig. 3a). The average feed intake by the fish given varying levels of CSM-based diets is shown in Fig. 3b. Fish fed control diet consumed more compared with fish fed the CSM-containing diets, least being fish fed with diets 4 and 5. Fish fed with diet 3 consumed more than

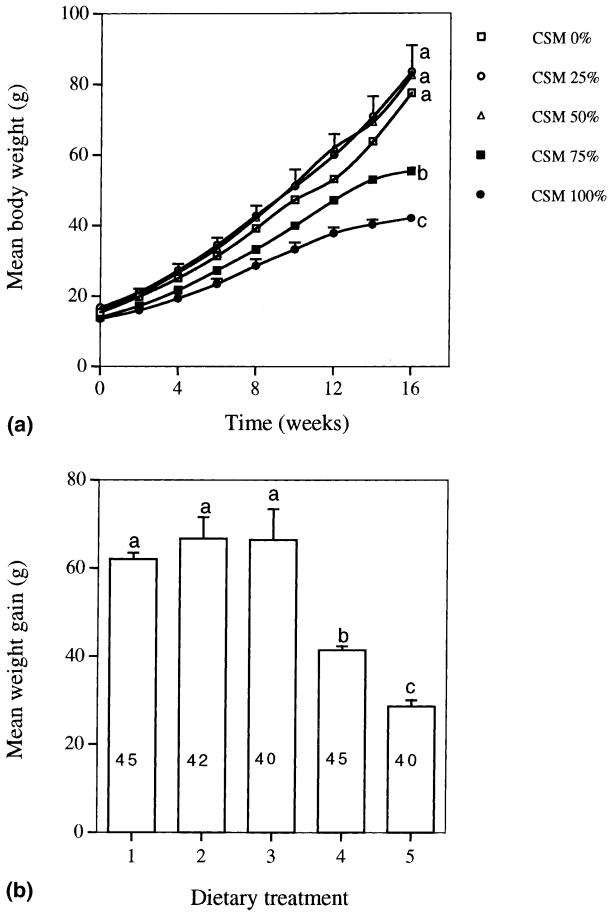

Figure 1 Growth (a) and weight gain (b) of tilapia after 16 weeks of feeding graded levels of CSM. Sample size of fish is shown on each histogram. Different letters indicate that the means are significantly different from each other ($P < 0.05$).

Table 2 Performance of tilapia (*Oreochromis* sp.) fed with diets containing graded levels of cottonseed meal[1]

	Diets				
	1	2	3	4	5
Initial body wt. (g)	15.4	16.8	16.1	14.1	14.2
Final body wt. (g)	77.5^a	84.1^a	82.5^a	55.4^b	42.1^c
Wt. gain (g day^{-1})	0.54^a	0.59^a	0.58^a	0.36^b	0.25^c
SGR[2]	1.4^a	1.4^a	1.4^a	1.2^b	1.0^c
FCR[3]	1.59^a	1.46^a	1.48^a	2.04^b	2.28^c
Survival	100	100	100	100	89

[1] Values are means of three replicate groups; values in the same row with different superscripts are significantly different ($P < 0.05$).
[2] Specific growth rate (%) = (ln final wt − ln initial wt) × 100 days.
[3] Feed conversion ratio = g dry diet fed/g fish weight gain.

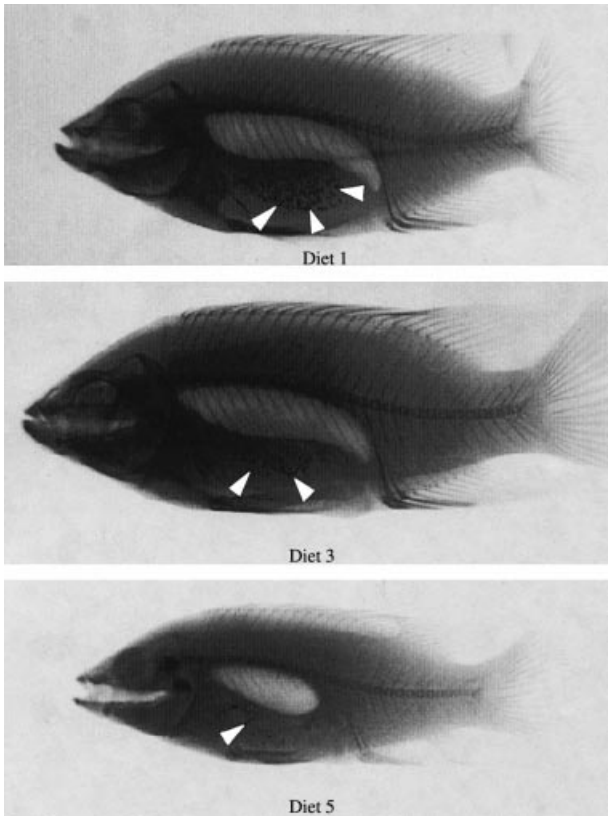

Figure 2 Radiographs showing feed intake of diets 1, 3 and 5 estimated by the number of glass beads (arrows) in the gut.

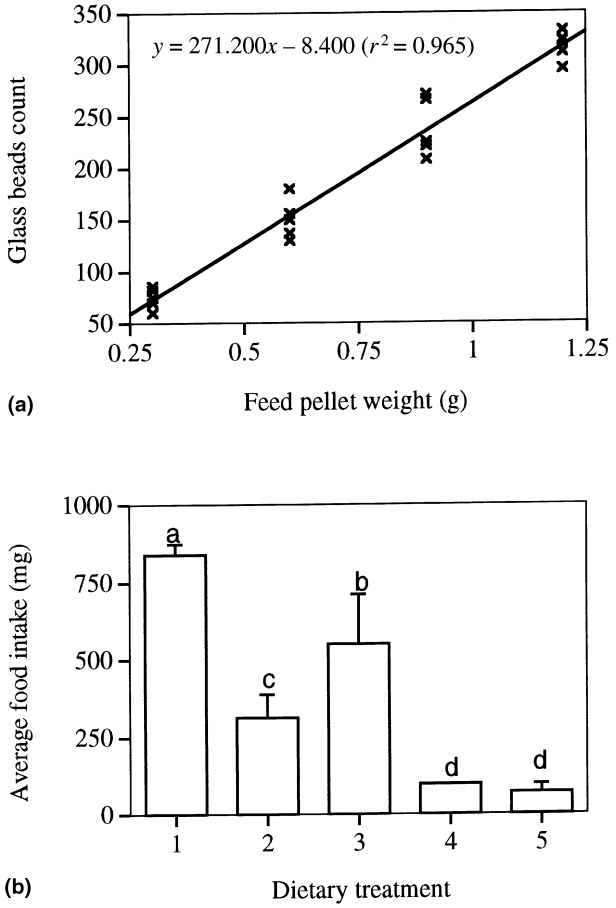

Figure 3 Calibration curve showing the relationship between the number of glass beads and feed pellet weight (a). Mean food intake by tilapia is shown in (b) (calculated from the glass beads counted in their guts, $n = 12$ fish/diet). Different letters indicate that the means are significantly different from each other ($P < 0.05$).

those fed with diet 2; however, we had no explanation for this result. It was also observed that fish fed with diets 4 and 5 frequently rejected diets after tasting.

Digestibility of feeds

Table 3 shows values of apparent digestibility of protein and absorption of minerals. Apparent digestibility of protein was high (above 80%) and at about the same level for diets 1–4 while it was significantly lower in diet 5. Minerals such as phosphorus, magnesium and copper were absorbed in moderate amounts, being the least in diets 3–5 (Table 3). However, copper was more significantly absorbed by fish given diets 4 and 5 compared with the other three test diets. Potassium and sodium were absorbed by fish in fairly high amounts (ranging between 86.1 and 98.1%) with little variation among the diets. The other minerals whose apparent absorbability was determined included iron, calcium, zinc, manganese and aluminium (not presented). All their values were highly variable and frequently negative, showing very little absorption of the minerals. Fish fed with diet 5 showed the lowest absorption of the minerals.

Gossypol concentrations in diets and liver

The proportion of two isomers (−) and (+) gossypol in the diets was approximately 50:50. The results of gossypol concentration in liver after 16 weeks of feeding are shown in Table 4. Total and each (+) and (−)-gossypol isomer concentrations of liver increased significantly ($P < 0.05$) as the CSM inclusion increased in the diets. The proportion of (−)-isomer to total gossypol was less than 30% in liver while the proportion was 50:50 in diet (Table 1). There was a linear relationship ($r^2 = 0.99$, $P = 0.001$) between liver gossypol and dietary gossypol. The colour of liver from fish fed with

Table 3 Apparent digestibility of protein and absorption of minerals (%) in dry cottonseed meal-based diets determined from fecal material of tilapia[1]

	Diets				
Nutrients	1	2	3	4	5
Protein	88.7 ± 0.8^{a}	86.6 ± 1.8^{a}	81.3 ± 1.4^{a}	82.2 ± 0.8^{a}	69.8 ± 0.6^{b}
Phosphorus	36.7 ± 0.6^{a}	30.2 ± 1.3^{a}	22.4 ± 7.5^{b}	27.6 ± 1.6^{b}	11.2 ± 5.4^{c}
Magnesium	61.9 ± 0.2^{a}	53.9 ± 8.1^{a}	43.7 ± 6.4^{b}	49.8 ± 2.7^{b}	44.0 ± 8.7^{b}
Copper	24.8 ± 9.1^{a}	10.6 ± 9.5^{b}	6.2 ± 3.0^{c}	28.1 ± 9.9^{a}	33.1 ± 1.9^{a}
Potassium	98.1 ± 0.2^{a}	96.8 ± 0.5^{a}	95.1 ± 0.6^{a}	95.1 ± 0.5^{a}	93.9 ± 1.7^{a}
Sodium	93.9 ± 0.4^{a}	86.2 ± 1.6^{a}	86.1 ± 3.6^{a}	88.7 ± 1.8^{a}	86.9 ± 4.5^{a}

[1]Means of triplicate groups; Values ± SD in the same row with different superscripts are significantly different ($P < 0.05$).

Table 4 Average gossypol concentrations (μg g^{-1} wet weight) in liver of tilapia fed with five experimental diets with different levels of CSM for 16 weeks[1]

	Gossypol			
Diets	(+)-isomer	(−)-isomer	Total	(−)-isomer/total (%)
Control (1)	1.3 ± 0.6^{e}	–	1.3 ± 0.6^{e}	–
CSM25 (2)	22.9 ± 6.0^{d}	9.4 ± 4.3^{d}	32.3 ± 10.3^{d}	28.3 ± 4.0^{a}
CSM50 (3)	51.3 ± 8.3^{c}	21.0 ± 3.4^{c}	72.3 ± 11.6^{c}	29.1 ± 0.9^{a}
CSM75 (4)	72.3 ± 5.8^{b}	27.1 ± 2.0^{b}	99.4 ± 5.9^{b}	27.3 ± 2.2^{a}
CSM100 (5)	95.9 ± 14.2^{a}	36.2 ± 1.5^{a}	132.1 ± 15.7^{a}	27.6 ± 2.2^{a}

[1]Means of six individual fish; Values (mean ± SD) in the same column with different superscript are significantly different ($P < 0.05$).

diets 4 and 5 was more yellow as a result of high levels of gossypol than those of fish fed with diets 1–3.

Body mineral composition

The mineral composition of the whole fish body varied with test diets (Fig. 4). The concentration of zinc in fish body gradually decreased with the increase of CSM proportion, while its level in the diets was the same. The mineral elements in fish body which showed a decline in concentrations as the amount of CSM was increased included calcium, phosphorus, magnesium and iron (Fig. 4). Dietary calcium, phosphorus and iron followed a similar trend. However, dietary magnesium increased as the CSM level increased. Copper and manganese concentrations in fish showed significant differences and peaked in diet 3. The protein level of the whole fish body was not affected significantly by the difference in the composition of feeds.

Discussion

This study demonstrates that up to 50% CSM could be used to replace fish meal as a protein source in the diet of tilapia without affecting the overall growth performance of fish. Beyond that level growth was depressed and the fish population experienced mortality when fed 100% CSM diet. Wu *et al*. (1996) indicated that a 40% protein diet for tilapia achieved the best feed conversion ratio and is suitable to examine alternative protein sources. The SGR and FCR of fish in the present experiment was directly affected by dietary protein source in fish fed with CSM-based diets. The results of the present experiments also indicate that tilapia cannot be raised successfully by feeding diets formulated on CSM alone as a source of protein. The findings are consistent with those reported by other investigators. Ofojekwu & Ejike (1984) reported that CSM could not be used as a sole protein source for *O. niloticus* because they exhibited poor growth, food conversion and specific growth rate. Robinson *et al*. (1984a) also observed that glanded CSM had limited utilization in tilapia and resulted in poor growth and decreased feed conversion. Similar results were reported by Robinson *et al*. (1984b) for channel catfish fed glanded CSM. These findings contradicted the earlier report (Jackson *et al*. 1982) that tilapia grew well on CSM-based protein, even at 100% level of inclusion. Fowler (1980) found that CSM was efficiently utilized as a partial replacement (34%) for FM protein in diets for chinook and coho salmon.

The results of the decreased digestibility confirmed earlier observations that diets 4 and 5 were less utilized for growth in the second phase of the study, after the 12th week (Fig. 1). At the end of the 16th week trial feed intake was low in both diets and the least eaten was diet 5 where CSM was 100%. The level of CSM in diets 4 and 5 rendered them so unpalatable that it suppressed the appetite of the fish. The effect of poor appetite

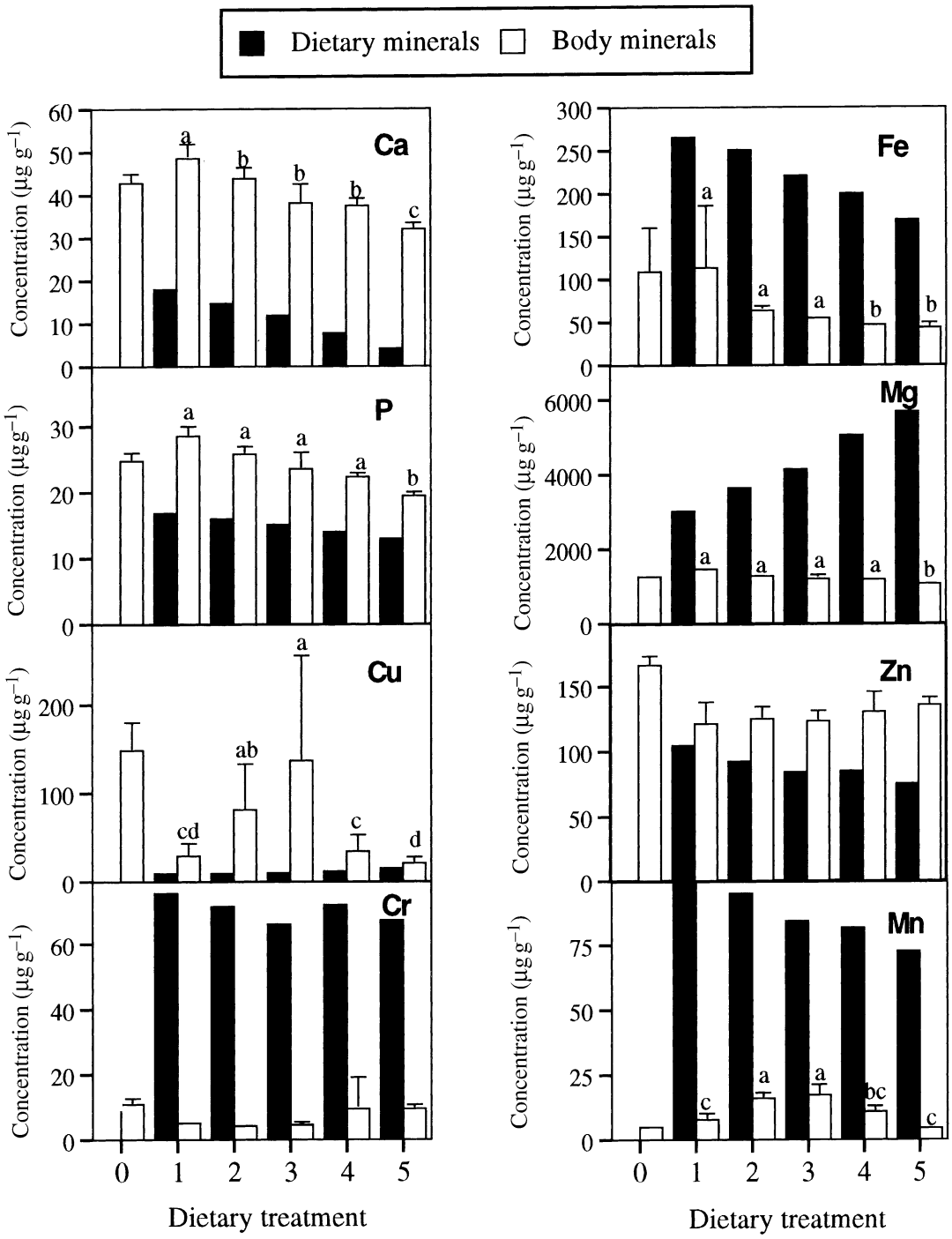

Figure 4 Mineral concentrations of Ca, P, Fe, Mg, Cu, Cr, Zn and Mn in test diets and fish body before (0) and after feeding for 16 weeks. The sample size was nine fish per diet. Different letters indicate that the means are significantly different from each other ($P < 0.05$).

became worse after 12 weeks of feeding when retarded growth was clearly noticed in fish fed with these two diets. The fish fed with diets 2 and 3 had significantly lower feed intake than the control. This study is further proof that the glass bead-radiographic bioassay is a useful method for assessing total feed intake by individual fish.

The values for digestibility of proteins and absorption of mineral elements brought out several important observa-

tions. The minerals are critical in skeleton formation, regulation of acid-base equilibrium and as cofactors for biologically important compounds such as hormones and enzymes (Watanabe *et al.* 1997). Mineral deficiencies can cause biochemical, structural and functional pathologies like those found in tilapia which were subjected to the test diets with CSM higher than 50% protein. According to Watanabe *et al.* (1997), the biological availability of a mineral varies depending on the feedstuffs and the composition of the diet. Several factors influencing bioavailability of mineral include their level, form, particle size, digestibility and their interactions in diet which may be either synergistic or antagonistic, physiological and pathological conditions of the fish concerned. In the present study, the concentrations of minerals in the test diets met the requirement levels for tilapia (NRC 1993).

The data for chemical composition of the fish body in response to dietary levels could be divided into four groups. The first group refers to elements for which fish body composition did not show any marked change with the graded level of CSM (e.g. zinc). Sun & Jeng (1998) reported that zinc was evenly distributed throughout the body tissues and organs of tilapia (*O. mossambicus*) but more concentrated in the gut tissue of the common carp (*Cyprinus carpio*). The authors did not explain why zinc was concentrated and retained in the fish body in comparison with other minerals. They suggested that zinc might be associated with membrane proteins, a property which could make the element more stable in the fish body. It is well established in some monogastric animals that zinc bioavailability can be reduced by dietary calcium and phytate (Lo *et al.* 1981) which is present in several feedstuffs of plant origin. Phytic acid present in CSM (63.1% of total P; Richie & Brown 1996) was expected to strongly chelate divalent minerals, including zinc, to form insoluble phytates in the intestinal lumen, thereby lowering zinc availability. McClain & Gatlin (1988) found that bioavailability of dietary zinc to blue tilapia (*O. aureus*) was significantly reduced by dietary phytate and recommended that to satisfy the dietary zinc requirement of tilapia, relatively high levels of supplemental zinc would be required in practical diets to overcome the binding capacity of phytate.

The second group was composed of minerals such as calcium, phosphorus, magnesium, and iron whose concentrations in the fish body fell progressively as the level of CSM increased (Fig. 4). The low content of these minerals could be related in part to their low amount absorbed by the fish from the diets as noted earlier. It is therefore clear that the amount of minerals in the diets, in case of elevation of CSM, need to be increased to prevent its influence on the final concentration of minerals in the fish body. However, Skonberg *et al.* (1997) noted that whole body calcium, phosphorus and magnesium concentrations in salmonid fish were responsive to graded levels of their dietary concentrations. This is probably also true for tilapia, given the fact that fish derived these elements mostly from the diet. Phosphorus is required in rather high levels by fish in comparison with other mineral elements (Robinson *et al.* 1987). Fish can absorb dissolved calcium from the surrounding water mostly through their gills. This may be the reason why the mineral was more concentrated in the body tissues of the tilapia compared with what was available in their diets (Fig. 4).

Unlike calcium, however, the primary source of phosphorus for fish is of dietary origin and its requirements range from 0.5 to 0.8% of dry diet for various aquatic species (Wilson *et al.* 1982). While imbalances in the dietary Ca:P ratio can lead to malabsorption of one of the elements in terrestrial vertebrates, the dietary Ca:P ratio does not impair growth or tissue mineral concentrations in fish reared in freshwater as long as dietary phosphorus is adequate and calcium is present in the rearing water (Wilson *et al.* 1982). This observation was supported by the direct relationship that existed between the amount of phosphorus found in the fish body and the amount of the mineral available in the fish diets. Watanabe *et al.* (1980) found that the availability of phosphorus in fish meal for tilapia was low compared with that for rainbow trout (*Oncorhynchus mykiss*) and chum salmon (*Oncorhynchus keta*). The differences in the availability of phosphorus to salmonids and tilapia were attributed to the limited secretion of gastric juices by tilapia. Feedstuffs that originate from plant seeds contain phosphorus primarily as the calcium-magnesium salt of phytic acid known as phytin. Phytin phosphorus is unavailable to animals with simple stomachs because they lack the enzyme phytase in the gastrointestinal tract (NRC 1993). Phytic acid also forms insoluble salts with many minerals in the digestive tract. Hence, the availability of phosphorus in most plant products is low, for example, that of soya bean meal in catfish is between 29 and 54% (Wilson *et al.* 1982).

Iron concentration in tilapia fed with 100% CSM diet declined to only half of that in fish fed fish meal-based diet (Fig. 4). Pellett *et al.* (1990) reported the conversion of iron from a soya diet to haemoglobin iron in rats to lie between 40 and 47% and even lower (29–34%) from a cottonseed source. An antagonistic relationship between dietary iron and whole body manganese, but not body zinc, was observed in Atlantic salmon (*Salmo salar*) by Andersen *et al.* (1996). This relationship seemed to hold true for tilapia fed diets 1–3.

Therefore, we can conclude that the decreasing dietary iron could improve the absorption of manganese through competition for the same binding sites.

Copper and manganese which constituted the fourth group, showed peak concentrations in fish fed with diet 3 (50% CSM) and lower levels in fish fed the rest of the diets. It was not clear why this level of CSM favoured fairly high concentrations of copper and manganese in the bodies of fish fed with diet 3. It was, however, noted that whereas the quantity of dietary copper increased with the increase of the proportion of CSM, dietary manganese correspondingly decreased. The requirement for copper depends to a great extent on the physiological state of the fish, the copper concentration in the water, and the levels of zinc, iron, cadmium and molybdenum, which are metabolic antagonists of the mineral. Copper and zinc may compete for binding sites on proteins responsible for mineral absorption and/synthesis of metalloenzymes (Watanabe *et al.* 1997). Berntssen *et al.* (1999) showed that dietary copper concentrations of 500 mg Cu kg^{-1} and above caused toxic responses in Atlantic salmon fry as concluded from the antagonistic interaction with selenium. In the present study with tilapia dietary levels of copper were well below that level. The highest concentration was about 15 μg g^{-1} dry diet in diet 5. The concentrations of manganese in the diets ranged between 72 mg kg^{-1} for diet 5 and 100 mg kg^{-1} for diet 1, far above the normal FM range of 4–38 mg kg^{-1}, depending on the fish species (Watanabe *et al.* 1997). In general, the diets contained most of the required mineral nutrients in adequate, though varied amounts.

The use of CSM in fish feed is limited because of gossypol toxicity (El-Sayed 1990). Besides being toxic to some fishes, gossypol may render lysine, an essential amino acid, unavailable (Jauncey & Ross 1982). However, this action was diminished as the diets with CSM were supplemented with the recommended amount of synthetic lysine (Table 1). The response of fish to gossypol is species specific. For instance, rainbow trout fed with a CSM-based diet containing 0.03% free gossypol, exhibited poor growth and high mortality (Herman 1970). Robinson *et al.* (1984a) argued that gossypol was not responsible for the adverse effect of CSM on growth of *T. aureus*. They showed that 0.2% of gossypol acetate added to casein-based diet which did not affect growth rate of tilapia. The values of free gossypol determined in the test diets (Table 1) were high enough to affect the health and growth rates of tilapia. Free gossypol is reported to be a membrane-active agent with cytocidal properties and the ability to inhibit membrane-bound enzymes, causing haemolytic anaemia at high concentrations (Makinde *et al.* 1997). Indeed in the accompanying studies on tilapia haematology, we observed the levels of haematocrit and haemoglobin to be between 36 ± 0.5% for diet 1 and 9.3 ± 1.2% for diet 5 and, 8.7 ± 0.2 g dL^{-1} for diet 1 and 1.7 ± 0.1 g dL^{-1} for diet 5, respectively (Mbahinzireki 1999). Analysis of gossypol in liver of tilapia revealed its high concentration in that organ (Table 4). Roehm *et al.* (1967) reported that the amount of total and free gossypol were the highest in liver than any other tissues determined in rainbow trout. Skutches *et al.* (1974), while studying the effect of dietary free gossypol on blood components in swine and rats, suggested that free gossypol bound the iron from the liver of those animals, thus reducing the liver iron concentration. After this complexing action of free gossypol and iron, the levels of hemoglobin and hematocrit in the animals went down. The authors postulated further that there was a possibility that gossypol could interfere with the intestinal absorption of iron. Skutches *et al.* (1974) also found that CSM-based diets containing 0.02–0.03% free gossypol caused a high incidence of death in growing pigs. Some of the clinical signs of chronic gossypol toxicity seen in a variety of animals as compiled by Berardi & Goldblatt (1980) include loss of appetite, weakness, emaciation and weight loss, among others. The present study showed that these signs were observed among tilapia fed with diets containing a high level of gossypol derived from CSM.

Plant protein sources as alternatives to fish meal are comparatively inexpensive and abundant, but the use may be limited by the presence of a number of antinutritional factors. These include various protease inhibitors, phytic acid, tannins, a high fibre content and natural toxicants such as gossypol, erucic acid, glucosinolates and isothiocyanates (Davies *et al.* 1990). In fact, besides gossypol, cottonseed also contains cyclopropenoid fatty acids, namely, malvalic and sterculic acids, which have been shown to have detrimental effects on salmonid fish (Hendericks *et al.* 1980). The authors also reported that the fatty acids greatly increased the incidence and severity of liver cancer in rainbow trout.

Conclusion

Cottonseed meal can only partially replace FM as a source of protein in compound feed at a limited amount of no more than 50% for tilapia raised in recirculation systems. This is in contrast with the animal by-products which were shown to completely and effectively replace FM as protein sources and other nutrients in tilapia diets as reported elsewhere (Mbahinzireki 1999).

Acknowledgements

We would like to thank the graduate students and staff of the aquaculture programme of the School of Natural Resources at The Ohio State University and Piketon Research and Extension Center. Improvements of the manuscript suggested by Dr P. Fuerst, Dr D. Culver and Dr D. Johnson are highly appreciated. The funds were provided by the North Central Regional Aquaculture Center, U.S.D.A., East Lansing, MI ('Tilapia' Project 96–38500–2631). The salaries were partly provided by state and federal funds awarded to the Ohio Agriculture Research and Development Center, Wooster, Ohio, and by the International Development Association of the World Bank loan to Uganda.

References

Al-Ogaily, S.M., Al-Asgah, N.A. & Ali, A. (1996) Effect of feeding different grain sources on the growth performance and body composition of tilapia, *Oreochromis niloticus* (L.). *Aquaculture Res*., **27,** 523–529.

Andersen, F., Maage, A. & Julshamn, K. (1996) An estimation of dietary iron requirement of Atlantic salmon, *Salmo salar* L. parr. *Aquacult Nutr*., **2,** 41–47.

AOAC (Association of Official Analytical Chemists) (1995) *Official Methods of Analysis*, 16th edn. AOAC, Arlington, VA.

Arnesen, A.M., Jorgensen, E.H. & Jobling, M. (1993) Feed intake, growth and osmoregulation in Arctic charr, *Savelinus alpinus* (L), following abrupt transfer from freshwater to more saline water. *Aquaculture*, **114,** 327–338.

Berardi, L.C. & Goldblatt, L.A. (1980) Gossypol. In: *Toxic Constituents of Plant Foodstuffs* (Liener I.E. ed.), 2nd edn, pp. 183–237. Academic Press, New York, NY, USA.

Berntssen, M.H.G., Lundebye, A. & Maage, A. (1999) Effects of elevated dietary copper concentrations on growth, feed utilization and nutritional status of Atlantic salmon (*Salmo salar* L.) fry. *Aquaculture*, **174,** 167–181.

Brandt, T.M. (1979) Use of heat-treated full-fat soybeans in channel catfish and golden shiner feeds. *Texas Fish Farming Conference*, January, (Cited by Jauncey and Ross, 1982).

Cass, Q.B., Bassi, A. & Marlin, S.A. (1999) First direct resolution of gossypol enantiomers on a chiral high-performance liquid chromatography phase. *Chirality*, **11,** 46–49.

Chikafumbwa, F.J.K. (1996) The use of napier grass (*Pennisetum purpureum*) and maize (*Zea mays*) bran as low-cost tilapia aquaculture inputs. *Aquaculture*, **146,** 101–107.

Dabrowski, K., Ciereszko, R.E., Blom, J.H. & Ottobre, J.S. (1995) Relationship between vitamin C and plasma testosterone in female rainbow trout, *Oncorhynchus mykiss*. *Fish Physiol. Biochem*., **14,** 409–414.

Davies, S.J., McConnell, S. & Bateson, R.I. (1990) Potential of rapeseed meal as an alternative protein source in complete diets for tilapia (*Oreochromis mossambicus* Peters). *Aquaculture*, **87,** 145–154.

DeSilva, S.S., Gunasekera, R.M. & Atapattu, D. (1989) The dietary protein requirements of young tilapia and an evaluation of the least cost dietary protein levels. *Aquaculture*, **80,** 271–284.

El-Sayed, A.M. (1990) Long-term evaluation of cottonseed meal as a protein source for nile tilapia *Oreochromis niloticus* (Linn.). *Aquaculture*, **84,** 315–320.

Fowler, L.G. (1980) Substitution of soy bean and cottonseed products for fish meal in diets fed to chinook and coho salmon. *Prog. Fish Cult*., **42,** 87–91.

Hardy, R.W. (1999) Collaborative opportunities between fish nutrition and other disciplines in aquaculture: an overview. *Aquaculture*, **177,** 217–230.

Hendericks, J.D., Sinnhuber, R.O., Loveland, P.M. & Nixon, J.E. (1980) Hepatocarcinogenicity of glandless cottonseeds and cottonseed oil to rainbow trout (*Salmo gairdnerii*). *Science*, **208,** 309–311.

Herman, R.L. (1970) Effects of gossypol on rainbow trout, *Salmo gairdnerii* Richardson. *J. Fish Biol*., **2,** 293–304.

Jackson, A.J., Capper, B.S. & Matty, A.J. (1982) Evaluation of plant proteins in complete diets for tilapia *Sarotherodon mossambicus*. *Aquaculture*, **27,** 97–109.

Jauncey, K. & Ross, B. (1982) *A Guide to Tilapia feeds and Feeding*. Inst. Aquaculture, University of Stirling, Scotland, p. 111.

Jobling, M., Baardvik, B.M. & Jorgensen, E.H. (1989) Investigation of food-growth relationships of Arctic charr, *Salvelinus alpinus* (L.) using radiography. *Aquaculture*, **81,** 367–372.

Kim, H.L. & Calhoun, M.C. (1995) Determination of gossypol in plasma and tissues of animals. *Proceedings and of 86th AOCS Annual Meeting*, San Antonio, Texas, (Abstract B) 487.

Lim, C. & Dominay, W. (1990) Evaluation of soybean meal as a replacement for marine animal protein in diets for shrimp *Penaeus vannamei*. *Aquaculture*, **87,** 53–63.

Lo, G.S., Settle, S.L., Steinke, F.H. & Hopkins, D.T. (1981) Effect of phytate: zinc molar ratio and isolated soybean protein on zinc bioavailability. *J. Nutr*. **111,** 2223–2235.

Makinde, M.O., Akingbemi, B.T. & Aire, T.A. (1997) Gossypol-induced erythrocyte fragility in the rat is ameliorated by ethanol intake. *South Afri. J. Sci*., **93,** 141–143.

Martin, S.D. (1990) Gossypol effects in animal feeding can be controlled. *Feedstuffs*, **62,** 14–17.

Mbahinzireki, G.B. (1999) Tilapia, *Oreochromis* sp. (L.): Sex reversal and performance on alternative sources of protein. Dissertation, The Ohio State University, Ohio.

McClain, W.R. & Gatlin, D.M. III (1988) Dietary zinc requirement of *Oreochromis aureus* and effects of dietary calcium and phytate on zinc bioavailability. *J. World Aquacult. Soc*., **19,** 103–108.

NRC (National Research Council) (1993) *Nutrient Requirements of Fish*. National Academy Press, Washington, D.C., USA.

Ofojekwu, P.C. & Ejike, C. (1984) Growth response and food utilization in the tropical cichlid, *Oreochromis niloticus* (Linn) fed on cottonseed-based diets. *Aquaculture*, **42,** 27–36.

Pauly, D., Christensen, V., Froese, R. & Palomares, M.L. (2000) Fishing down aquatic food webs. *Amer. Sci*., **88,** 46–51.

Pellett, L.J., Schnepf, M.I., Johnson, P.E. & Dimarco, N.M. (1990) A comparative study of iron bioavailability in rats from soybean and cottonseed protein isolate diets. *J. Food Quality*, **13,** 419–433.

Pouomogne, V., Rouger, Y. & Kaushik, S.J. (1992) Nitrogen and energy utilization by *Oreochromis mossambicus* fingerlings fed diets containing graded levels of brewery draffs. *J. Aqua. Trop*., **7,** 225–237.

Pouomogne, V., Takam, G. & Pouemegne, J. (1997) A preliminary evaluation of cacao husks in practical diets for juvenile Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, **156,** 211–219.

Quartararo, N., Allan, G.L. & Bell, J.D. (1998) Replacement of fish meal in diets for Australian snapper, *Pagrus auratus*. *Aquaculture*, **166,** 279–295.

Richie, M. & Brown, P.B. (1996) Availability of phosphorus from feed stuffs fed to rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **142,** 269–282.

Robinson, E.H., Rawles, S.D., Oldenburg, P.W. & Stickney, R.R. (1984a) Effects of feeding glandless or glanded cottonseed products and gossypol to *Tilapia aurea*. *Aquaculture*, **38,** 145–154.

Robinson, E.H. (1991) Improvement of cottonseed meal protein with supplemental lysine in feeds for channel catfish. *J. Appl. Aquacul.*, **1,** 1–14.

Robinson, E.H. & Li, M.H. (1994) Use of plant proteins in catfish feeds: replacement of soybean meal with cottonseed meal and replacement of fish meal with soybean meal and cottonseed meal. *J. World Aquacult. Soc.*, **25,** 271–276.

Robinson, E.H. & Tiersch, T.R. (1995) Effects of long-term feeding of cottonseed meal on growth, testis development and sperm mortality of male channel catfish *Ictalurus punctatus* broodfish. *J. World Aquacult. Soc.*, **26,** 426–431.

Robinson, E.H., LaBomascus, D., Brown, P.B. & Linton, T.L. (1987) Dietary calcium and phosphorus requirements of *Oreochromis aureus* reared in calcium-free water. *Aquaculture*, **64,** 267–276.

Robinson, E.H., Rawles, S.D., Oldenburg, P.W. & Stickney, R.R. (1984b) Evaluation of glanded and glandless cottonseed products in catfish diets. *Prog. Fish Cult.*, **46,** 92–97.

Roehm, J.N., Lee, D.J. & Sinnhuber, R.O. (1967) Accumulation and elimination of dietary gossypol in the organs of rainbow trout. *J. Nutr.*, **92,** 425–428.

Scott, K. (1978) Cause and control of losses of chromium during nitrite perchloric acid oxidation of aquatic sediments. *Analyst*, **103,** 754.

Skonberg, D.I., Yogev, L., Hardy, R.W. & Dong, F.M. (1997) Metabolic response to dietary phosphorus intake in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **157,** 11–24.

Skutches, C.L., Herman, D.L. & Smith, F.H. (1974) Effect of dietary free gossypol on blood components and tissue iron in swine and rats. *J. Nutr.*, **104,** 415–422.

Sokal, R.R. & Rohlf, F.J. (1981) *Biometry*. Freeman, New York.

Spyridakis, P., Metailler, R., Gabaudan, J. & Riaza, A. (1989) Studies on nutrient digestibility in European Sea Bass (*Dicentrarchus labrax*). 1. Methodological aspects concerning feces collection. *Aquaculture*, **77,** 61–70.

Sun, L.T. & Jeng, S.S. (1998) Comparative zinc concentrations in tissues of Common Carp and other aquatic organisms. *Zool. Studies*, **37,** 184–190.

Swick, R.A. & Tan, P.H. (1995) Considerations in using common Asian protein meals. American Soybean Association Technical Bulletin MITA (P) no. 083/12/94, Singapore.

Viola, S. & Zohar, G. (1984) Nutrition studies with market size hybrids of tilapia *Oreochromis* in intensive culture. *Bamidgeh*, **36,** 3–15.

Watanabe, T., Kiron, V. & Satoh, S. (1997) Trace minerals in fish nutrition. *Aquaculture*, **151,** 185–207.

Watanabe, T., Murakami, A., Tacheuchi, L., Nose, T. & Ogino, C. (1980) Requirement of chum salmon held in fresh water for dietary phosphorus. *Bull. Japan. Soc. Sci. Fish.*, **46,** 361–367.

Watson, M.A. & Isaac, R.A. (1990) Analytical instruments for soil and plant analysis. In: *Soil Testing and Plant Analysis*, , 3rd edn, pp. 691–740. SSSA Book series, no. 3, Soil Science Society of America, Madison, WI, USA.

Wilson, R.P., Robinson, E.H., Gutlin,D.M. III & Poe, W.E. (1982) Dietary phosphorus requirement of channel catfish. *J. Nutr.*, **112,** 1197–1202.

Wu, Y.V., Rosati, R.R. & Brown, P.B. (1996) Effect of diets containing various levels of protein and ethanol coproducts from corn on growth of tilapia fry. *J. Agric. Food Chem.*, **44,** 1491–1493.