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Intestine histology, nutrient digestibility and body composition of Nile tilapia (*Oreochromis niloticus*) fed on diets with both cotton and sunflower seed cakes

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Physiological response of Nile tilapia to diets with both sunflower (SFSC) and cotton seed cakes (CSC) at increasing proportions (10%CSC25%SFSC; 15%CSC20%SFSC; 20%CSC15%SFSC; 25%CSC10%SFSC and a control that was a commercial tilapia diet) was investigated in two trials using diets containing 25% crude protein. Trial 1 run for 120 days in 15 happas of 1 m^3 stocked with 35 fish of 5.72 g. Each treatment had three replicates. Fish were fed 4% of their body weight per day. The number and length of intestinal folds was investigated. Trial 2 was run for 60 days in 24 tanks with 60 L of water and 30 fish of 3 g. Each treatment had six replicates fed to apparent satiation. Nutrient digestibility and body composition were determined. The test diets did not negatively affect the number and length of intestinal folds as they were similar to the control. The diets with 10%CSC25%SFSC and 20%CSC15%SFSC had the most efficiently digested protein, and highest protein deposition implying that the dietary protein was effectively used. The formulations with 10%CSC25%SFSC and 20%CSC15%SFSC could be used for making Nile tilapia diets.

Key words: Plant protein, intestine histology, digestibility, nutrient retention, feed.

INTRODUCTION

Nile tilapia is a species that is popularly farmed in the tropics because it grows fast, feeds on a wide range of foodstuffs and tolerates stress (El-Sayed, 1999; Mahmoud, 2009). Plant protein sources are increasingly being used in diet of Nile tilapia because they are relatively cheaper than conventional protein sources like fish meal and soya beans (Garcia-Abiado et al., 2004; Agbo et al., 2011; Munguti et al., 2012). Sunflower and cotton seed cakes (SFSC and CSC respectively) are among the plant protein sources that have been

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demonstrated to enhance the performance of Nile tilapia (Jauncey, 1998; Tacon et al., 2009; Mbahinzireki et al., 2001; Olvera-Novoa et al., 2002; Rinchard et al., 2002). Although Nile tilapia is adapted to feeding on plant protein (Jauncey, 1998), the inclusion levels are restricted because CSC and SFSC contain high fiber content of 18-23% which could decrease palatability, food intake and nutrient digestibility thereby reducing growth (El-Sayed, 1999; Olvera-Novoa et al., 2002; Silvia et al., 2010). Moreover, fiber does not have any nutritional value in fish (Maina et al., 2002). Cotton also contains 400 to 800 mg/kg of an anti-nutritional factor called gossypol (Gatlin et al., 2007) which can impair fish growth (Luo et al., 2006; Meric et al., 2011; Renuka et al., 2005). Renuka et al. (2005) noted that cotton seed contains more gossypol (0.08%) compared to the leaf (0.05%), pod (0.04%), stem (0.03%) and root (0.02%). Tilapia can tolerate up to 0.18% free gossypol without adverse effects on growth (Robinson and Li, 1995; Evans et al., 2010). Sunflower contains low quantities of anti-nutritional factors namely: protease inhibitors, saponins, arginase inhibitor (Francis et al., 2001; Silvia et al., 2010). Heat treatment can however reduce the effects of the anti-nutritional factors (Olvera-Novoa et al., 1998; Evans et al., 2005) thereby making it possible to increase the quantities of CSC and SFSC that could be used in tilapia diets (Jauncey, 1998). Teichert-Coddington et al. (1997) noted that incorporation of up to 15% CSC in tilapia diets causes no gossypol toxicity while Jauncey (1998) and Hecht (2007) recommend a maximum inclusion of 30%. On the contrary, Agabo et al. (2011) observed that CSM could substitute upto 50% of fish meal protein in the diet of Nile tilapia without negative effects on growth and feed utilization. For SFSC, Olvera-Novoa et al. (2002) recommends the use of up to 20% sunflower in the diet of *Tilapia rendalli*. Jauncey (1998) recommends an inclusion level of SFSC of up to 25% for Nile tilapia while Maina et al. (2002) reports that it should not exceed 30% of the total crude protein. But the supply and price of cotton and sunflower fluctuates especially in developing countries thus the need to combine SFSC and CSC in the same diet to complement one another. However, there is scanty information on the physiological effects of diets containing both SFSC and CSC incorporated at different quantities. This information is vital in order to produce feed that does not cause adverse effects on Nile tilapia. Most studies conducted have evaluated CSC and SFSC independently. For instance, Garcia-Abiado et al. (2004) determined effect of diets with 0, 25, 50, 75 and 100% CSM as fish meal replacement and noted similar growth between fish fed on 0%CSM and 25-50% CSM and a decline in growth with 75-100% CSM. However, significant pathological effects on blood plasma were observed with diets formulated with 25-100% CSM. Although El-Saidy and Gaber (2003) substituted fish meal with 75 and 100% of a plant protein mixture (PPM) comprised of 25% soya bean, 25% cotton seed meal, 25% sunflower meal, and 25% linseed and noted good growth performance, the physiological effect of CSM and SFSC in the diet was not evaluated. Soltan and El-Bab (2008) also investigated effect of replacing fish meal with a PPM comprising 20% cotton seed, 20% sunflower, 20% canola, 20% seasmae, and 20% linseed meal. At 15-45% substitution of fish meal, the growth was similar to the control that contained only fish meal but all the fish fed on PPM had significant adverse pathological effects on the blood. The pathological defects intensified with increasing dietary levels of PPM whereas Aanyu et al. (2012) investigated the performance of Nile tilapia fed on diets with increasing quantities of SFSC and CSC and observed the highest absolute growth with 15% SFSC and 20% CSC, the physiological effect of the diets were not investigated. Physiological effect of diets such as histological response of the digestive system, nutrient digestibility and retention are some of the vital aspects for guiding feed manufacturers to manipulate feed formulae to improve the quality of feed.

The histology of the gastro-intestinal tract is influenced by the type of feed eaten by the fish (El-Bakary and El-Gammal, 2010; Delashoub et al., 2010). The intestine plays a vital role in absorbing nutrients that are used for growth (Rodrigues et al., 2009). Harmful effects on the intestinal anatomy can decrease its efficiency in nutrient absorption and fish growth (Mahmoud, 2009; Delashoub et al., 2010; Rašković et al., 2011). Hence, histological changes in the intestine can give insights on the performance of the fish when fed on a specific diet (Hu et al., 2007; Rašković et al., 2011). Besides, apparent digestibility coefficient measurements indicate the extent to which the nutrients in a diet are digested and made bio-available for the fish (Koprucu and Ozdemir, 2005; Jimoh et al., 2010). When feed with a good nutritional composition cannot be efficiently digested then fish performance is likely to be poor (Deganp and Yehuda, 1999; Cook et al., 2000). Knowledge of the body composition of the fish as influenced by the type of diet eaten is also vital (Maina et al., 2003; Rust, 2003). Digested nutrients are first used for maintenance of body systems and the balances are deposited in the body (Jobling, 1994). Excess protein is used for growth while excess fat leads to fatty fish. Moreover, fatty fish are not preferred by consumers (Jauncey, 1998). The objective of this study was therefore to determine the effect of diets with both cotton and sunflower on the histology of the fore intestine, nutrient digestion, and retention by Nile tilapia. This information will guide feed producers as they manipulate feed formulae to improve feed quality.

MATERIALS AND METHODS

An experiment comprising two feeding trials was conducted to investigate the effect of diets with cotton and sunflower seed cakes

Table 1. Proximate composition of feed ingredients used for formulating the test diets for trial 1 and 2.

Trial 1 Ingredient	Dry matter	Crude protein	Crude lipid	Crude fibre	Ash
Cotton seed cake	91.88	33.26	6.92	22.56	21.19
Sunflower seed cake	91.61	30.84	13.25	14.46	22.43
Soy beans	90.70	35.84	17.26	1.53	16.59
Blood meal	90.19	72.00	0.52	0.0	11.50
Maize bran	88.59	7.27	5.35	0.0	10.50
Wheat pollard	91.05	14.34	4.55	6.65	15.24
Wheat flour	88.26	10.84	1.97	0.0	9.17
Trial 2 Ingredient					
Cotton seed cake	92.81	34.69	0.84	17.96	6.49
Sunflower seed cake	93.38	24.33	14.76	31.71	4.32
Soy beans	90.76	32.29	15.32	13.91	8.87
Blood meal	88.42	76.84	0.77	0.92	7.02
Maize bran	88.13	10.06	10.55	2.68	2.98
Wheat pollard	90.30	14.65	4.86	8.78	3.53

on: i) the histology of the intestine (trial 1) and 2) nutrient digestibility and body composition of Nile tilapia (trial 2).

Experimental diets, fish and feeding

The experimental diets were formulated using sunflower and cotton seed cakes, wheat pollard, maize bran, soya meal, blood meal, sunflower oil, wheat flour, vitamin and mineral premix, salt. To inactivate anti-nutritional factors, soya beans and cotton seed cake were heat treated at 40-50°C for 10 min. Ingredients were ground and sieved using a 0.2 mm mesh size sieve. The proximate composition of ingredients used in trial 1 and 2 are provided in Tables 1 and 2.

For each trial, 4 experimental diets containing 25% crude protein were formulated with varying proportions of cotton (CSC) and sunflower seed cakes (SFSC) as the main protein sources using WinPas software. The diets include 10%CSC25%SFSC; 15% CSC20%SFSC; 20%CSC15%SFSC; 25%CSC10%SFSC. The control for trial 1 was a commercial tilapia diet with 25% crude protein from Ugachick Poultry Breeders Ltd in Uganda. Table 2 shows the formulae for trial 1 and 2. An inert marker (chromic oxide) was incorporated into the diets for trial 2 to enable determination of the apparent digestibility of the nutrients. The proximate composition of the formulated diets is provided in Table 3.

Nile tilapia fingerlings were obtained from the Aquaculture Research and Development Centre, conditioned for 2 weeks and size graded to obtain fish with no significant differences in initial weights between treatments. The fish in trial 1 were fed on their respective diets three times a day between 9 - 10 am, 12 noon - 1 pm and 4 - 5 pm at a feeding rate of 4% of the live body weight per day. In trial 2, fish were fed to apparent satiation twice a day from 9:00-10:00 am and 4:00-5:00 pm.

Experimental facilities and design

Trial 1 was run for a period of 120 days in a pond with a water surface area of 1,450 M^2 (44 x 33 m) each. The water depth at the inlet side was 1 and 1.5 m at the outlet side. Fifteen (15) happas were placed in one pond. Fifteen happas were used because the five different diets (four formulated diets and a commercial feed) represented a treatment and each treatment was replicated three times. Treatments were assigned using a randomized complete block design. Each happa measured 1x1x1 m (length, width and height) and had a mesh size of 1 mm. The happas were supported using wooden poles and 70 cm depth of the happas was submerged in the pond water. The happas were installed in 3 rows and each row had 5 happas with each treatment represented in each row. The happas within the same row were installed 3 m away from each other while the happas at the end of each row were installed 8 m away from the pond dyke. The distance between the rows was 10 m. At the inlet side, the first row of happas was installed 10 m away from the rear pond dyke while at the outlet side; the last row of happas was installed 11 m away from the hind pond dyke. A 2 inch net was placed on top of each happa to prevent birds from preying on the fish. The happas were each stocked with 35 Nile tilapia fingerlings of 5.7 g. In order to minimise clogging of the happas with organic matter, they were cleaned after every three days using a brush.

Trial 2 was carried out in a flow through culture system for a period of 60 days in 24 plastic tanks each with a water volume of 60 L. Each tank had 30 fish of 3 g, aerated using an air stone and covered with a gill net mesh of 1 inch to control the stocked fish from jumping out. The treatments were randomly distributed.

Data collection

Samples for histological examination of the fore intestine were collected at the end of the trial 1. The fore intestine was selected because it is where most of the nutrient absorption takes place (Rust, 2003). Three fish were randomly picked from each happa and those from the same treatment pooled together. The abdomen was opened and the digestive system was carefully dissected to cut out the intestine. Two (2) cm sections were cut from the anterior part of the intestine. The anterior intestine was defined as the portion that is three quarters the total length of the intestine from the stomach and posterior intestine as the remaining portion which

Table 2. Proportions (%) of the ingredients used for making diets for trial 1 and 2

Trial 1 ingredient	10%CSC 25%SFSC	15%CSC20%SFSC	20%CSC15%SFSC	25%CSC10%SFSC
Sunflower seed cake	25	20	15	10
Cottonseed cake	10	15	20	25
Soybean meal	10	10	10	10
Blood meal	7	7	7	7
Wheat pollard	34.1	34.1	34.1	34.1
Maize bran	7	7	7	7
Sunflower oil	2	2	2	2
Vitamin and mineral premix	2	2	2	2
Wheat flour	2.5	2.5	2.5	2.5
Salt	0.4	0.4	0.4	0.4
Trial 2 Ingredient				
Sunflower seed cake	25	20	15	10
Cottonseed cake	10	15	20	25
Soybean meal	2.5	2	3	3
Blood meal	10	10	10	9
Wheat pollard	42	43.1	40.1	41.1
Maize bran	3.6	3	5	5
Sunflower oil	1.5	1.5	1.5	1.5
Vitamin and mineral premix	2	2	2	2
Wheat flour	2	2	2	2
Salt	0.4	0.4	0.4	0.4
Chromium oxide	0.5	0.5	0.5	0.5

Vitamin and mineral premix contained: Vitamin A, 7,000,000 I.U; Vitamin D3, 2,000,000 I.U; Vitamin E, 10,000 mg; Vitamin K3 STAB, 200 mg; vitamin B1, 300 mg; Vitamin B2, 800 mg; vitamin B6, 400 mg; vitamin B12, 2 mg; niacin, 3,000 mg; pantoth acid, 1,000 mg; folic acid, 100 mg; biotin, 75 mg; choline, 35,000 mg; manganese, 6,000 mg; iron, 4,000 mg; zinc, 5,000 mg; copper, 800 mg; cobalt, 30 mg; iodine, 100 mg; selenium 1%, 20 mg; antioxidant, 20,000 mg; olaquindox 10%, 20,000 mg; salox 12%, 50,000 mg; ronozyme p, 5,000 mg; ronozyme g2, 12,000 mg; carophyl yellow, 2,500 mg; carophyl red, 500 mg

Table 3. Analyzed proximate composition (%) of the diets fed to Nile tilapia during trial 1 and 2.

Trial 1	Diet				
	10%CSC25%SFSC	15%CSC20%SFSC	20%CSC15%SFSC	25%CSC10%SFSC	Control
Crude protein	25.23	25.26	24.68	25.22	24.52
Crude lipid	7.52	8.26	8.03	7.51	5.95
Nitrogen free extract	48.42	45.53	50.97	45.13	49.85
Crude fibre	1.88	2.15	1.93	3.64	2.35
Ash	16.96	18.69	14.39	18.50	17.33
Trial 2					
Crude protein	25.81	24.89	25.71	25.89	
Crude lipid	7.48	4.38	3.38	2.37	
Nitrogen free extract	49.23	51.30	51.51	53.65	
Crude fibre	11.41	12.74	12.85	11.60	

extended distally from the anterior intestine to the anus. All tissues were fixed in Bouin's fixative and embedded in paraffin wax. Sections of 5 µm were cut and stained with Harris hematoxylin and eosin (H&E).

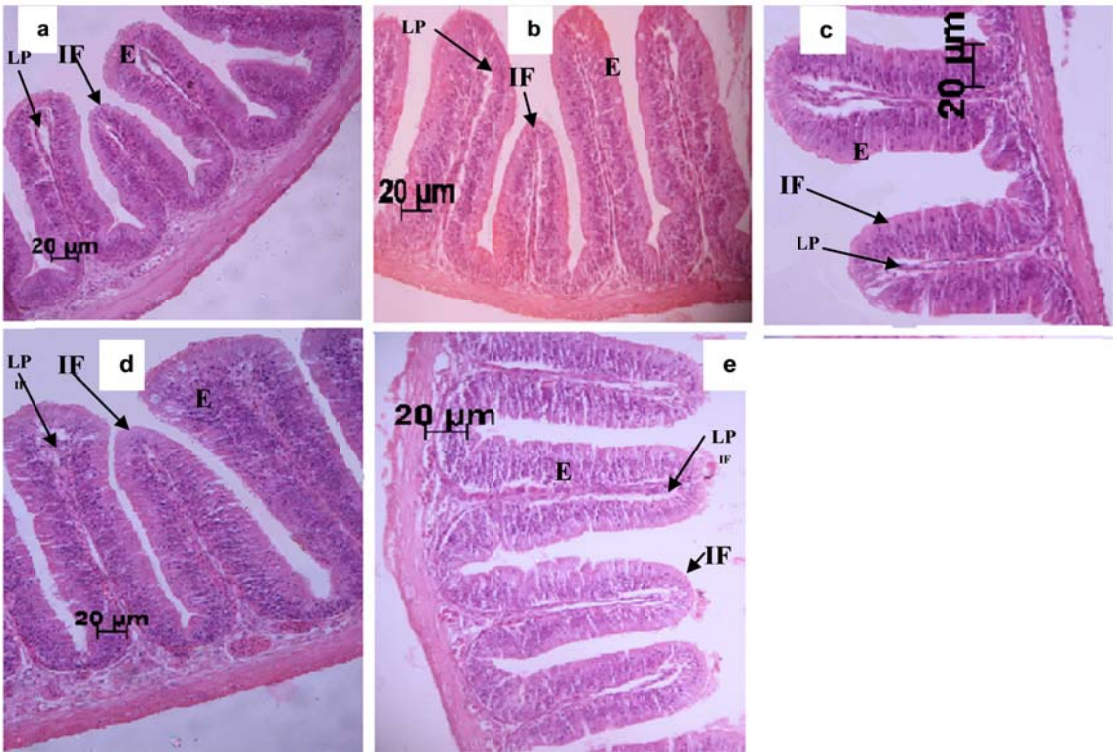

Figure 1. Light micrograph of the anterior part of the intestine of Nile tilapia. The intestine is arranged into intestinal folds (IF) with an epithelial sheet (E) covering the folds and it bears a lamina propria (LP) of loose connective tissue. **A.** 10%CSC25%SFSC; **b.** 15%CSC20%SFSC; **c.** 20%CSC15%SFSC; **d.** 25%CSC10%SFSC; **e.** Control diet. Magnification × 200.

At the beginning of trial 2, a total of 20 fish were removed from the pool of fish and 5 fish were removed from each happa at the end of the trial for determining the initial and final body composition of the fish from different treatments. The fish were frozen at -20°C until they were analysed.

After two weeks of the trial 2, faecal samples were collected daily from each tank by siphoning the bottom of the tank. Faecal collection started one hour after fish feeding and stopped after 3 h. The samples were dried in an oven at 40°C for 12 h. Samples from each tank were pooled together, ground and homogenized before proximate analysis.

Laboratory analysis

Proximate composition (crude protein, crude lipid, crude fibre, ash, moisture, Nitrogen free extract) of the fish and diets was determined based on the method described by the AOAC (1990). Crude protein was measured by determining the nitrogen content of the ingredient using the micro-Kjeldahl method and calculating the crude protein level by multiplying the nitrogen content by 6.25. Crude lipid was determined by ether extraction method using soxhlet apparatus. Ash content was measured by placing a sample of known weight in a furnace of 470-550°C for 3 h and the remaining weight was considered the ash. Moisture content was measured by placing a sample of known weight in an oven set at 105-110°C until the sample attained a constant weight. The lost weight from the sample was considered the moisture content and the remaining weight dry matter.

Photomicrographs were taken using a light microscope (Carl Zeiss) with an Axio-Vision 2.05 image analysis system. Samples from the anterior intestine of the fish were compared between treatments. The intestinal sections were evaluated according to criteria described by Dimitroglou et al. (2010) which include among others: intestinal fold length, width, and number.

Data analysis

The effect of the experimental treatments on the digestibility of dry matter, crude protein, fat, ash and fiber in the faeces; composition of protein, fat and ash in fish carcass; and the number, length and width of intestinal fold was compared using SPSS one way analysis of Variance (ANOVA). Differences between treatments means were determined using Tukey's multiple comparison test. Significant differences were considered at $p<0.05$.

RESULTS

Histology of the anterior intestine

Figure 1 shows histology images of the fore intestine of Nile tilapia fed on the experimental diets. All the diets had similar morphology of the intestinal folds. However, fish fed on 20%CSC15%SFSC had the highest number and

Table 4. Number, length and width of intestinal folds of the fore intestine of Nile tilapia fed on different diets with increasing quantities of cotton and sunflower seed cakes.

Parameter	10%CSC 25%SFSC	15%CSC 20%SFSC	20%CSC 15%SFSC	25%CSC 10%SFSC	Control	P value
Number of intestinal folds	46.00±4.00	37.70±5.86	48.00±2.00	44.50±0.50	42.00±5.00	NS
Length of intestinal folds (µm)	27.18±4.35	29.86±9.38	31.45±4.79	29.87±5.12	30.44±4.00	NS
Width of intestinal folds (µm)	11.08±1.85^{a}	13.38±3.87^{b}	13.92±2.49^{b}	13.45±3.75^{b}	14.82±2.41^{b}	0.000

Values on the same row with different superscripts (a, b, c, d) are significantly different (P<0.05). NS is no significant difference.

Table 5. Initial and final body composition of the Nile tilapia fed on diets containing increasing quantities of cotton and sunflower seed cakes.

Parameter	Diet				
Initial body composition (%):					
Ash	15.26				
Crude protein	59.02				
Fat	11.57				
Final body composition (%):					
	10%CSC 25%SFSC	**15%CSC 20%SFSC**	**20%CSC 15%SFSC**	**25%CSC 10%SFSC**	**P value**
Ash	12.44±0.27^{b}	11.38±0.14^{a}	12.20±0.11^{b}	12.42±0.27^{b}	0.001
Crude protein	59.91±0.75^{b}	57.25±0.14^{a}	59.79±0.40^{b}	56.33±0.60^{a}	0.000
Fat	18.67±0.27^{a}	26.27±1.03^{c}	22.03±0.18^{b}	23.45±0.78^{b}	0.000

Table 6. Apparent digestibility coefficients of the nutrients in diets containing increasing quantities of cotton and sunflower seed cake fed to Nile tilapia.

Parameter	10%CSC 25%SFSC	15%CSC 20%SFSC	20%CSC 15%SFSC	25%CSC 10%SFSC	P value
Dry matter	97.45±0.01	97.59±0.02	97.61±0.01	97.44±0.05	NS
Crude protein	99.60±0.47^{b}	98.36±0.04^{a}	98.34±0.02^{a}	98.46±0.01^{a}	0.000
Crude fat	99.78±0.25^{c}	98.93±0.01^{b}	99.06±0.02^{b}	98.22±0.03^{a}	0.000
Crude ash	98.46±1.77	97.15±0.31	97.82±0.17	97.59±0.20	NS
Crude fibre	96.93±3.50	94.81±0.09	95.74±0.23	95.77±0.10	NS

length of intestinal folds although not significantly different from the other treatments (Table 4). The width of the intestinal folds was significantly lower with fish fed on 10%CSC25%SFSC.

Body composition

There was a reduction in the amount of ash in the fish after feeding on the experimental diets (Table 5). Higher protein content was obtained with diets containing 10%CSC25%SFSC and 20%CSC15%SFSC. Fish fed on 15%CSC20%SFSC had a significantly lower amount of ash but higher fat content (P<0.05) compared to the other treatments. The lowest fat content was obtained with fish fed 10%CSC25%SFSC.

Digestibility of nutrient

There was no significant difference in the digestibility of dry matter, ash and fibre between treatments. Crude protein and fat were more efficiently digested by fish fed on 10%CSC25%SFSC and 20%CSC15%SFSC (Table 6).

DISCUSSION

Besides assessing the impact of a diet on growth and feed utilization efficiency in fish, it is also vital to know the mechanisms responsible for the observed performance. These include among others nutrient digestibility, absorption in the digestive system and retention in the body.

The efficiency at which the diets ingested are digested largely determines growth performance (Deganp and Yehuda, 1999; Guillaume and Choubert, 1999). This is because the nutritive value of food depends not only on its nutrient content but also on the capacity of the animal to digest and absorb the nutrients (Cook et al., 2000; Rust, 2003).

In this study, the apparent digestibility of crude protein, fat, ash and fibre for all the experimental diets ranged between 94 and 99% despite the high fibre content in SFSC and CSM (Table 1). This high level of nutrient digestibility is attributed to the fact that the feed was cooked before pelleting making it easier for the fish to digest (Jauncey, 1998; Engin and Ozkan, 2008). Deganp and Yehuda (1999) also found high digestibility values for protein in sunflower seed meal (78%), in rapeseed meal (86%) and in cottonseed meal (79%). In this study, 10%CSC25%SFSC had the most efficiently digested crude protein, fat, ash and fibre suggesting that the diet had more bio-available nutrients for the fish (Rust, 2003).

The efficiency at which digested nutrients are absorbed can be assessed using the histology of the intestine because it is the main site for nutrient absorption (Rodrigues et al., 2009). Negative effects on the anatomy of the intestine reduce the efficiency of its performance (Hu et al., 2007; Rašković et al., 2011). Borgeson et al. (2006) noted a decrease in intestinal fold length (villi) with decreasing fish meal and increasing plant protein in the diet of Nile tilapia and this corresponded with reduced growth.

In this study, the diets caused no significant negative changes in the histology of the intestine of Nile tilapia in terms of the number and length of intestinal folds when compared to the control diet that contained fish meal. An increase in the number, length and width of intestinal folds is associated with an increase in the surface area for absorption of nutrients vital for fish growth (Delashoub et al., 2010; Dimitroglou et al., 2010; El-Bakary and El-Gammal, 2010). The diet with 20%CSC15%SFSC had the highest absolute number, length and width of intestinal folds implying that the nutrients in this diet could have been more efficiently absorbed by the fish.

Nutrients absorbed in the digestive system are first used for maintenance of body functions and the surplus is retained in the body. Excess protein is deposited in the body for growth while excess energy is stored as fat (Jobling, 1994; Cook et al., 2000). This study observed a higher crude protein deposition and lower fat content in the carcass of fish fed 10%CSC25%SFSC and 20%CSC15%SFSC.

The lowest crude protein retention and highest fat deposit was obtained with fish fed 15%CSC20%SFSC. The results suggest that the diets with 10%CSC25%SFSC and 20%CSC15%SFSC were the most efficiently assimilated. Deposition of protein in fish is known to result into fish growth. The higher the protein deposition, the higher the weight gained (Sveier et al., 2000; Lupatsch et al., 2003). This observation is in agreement with findings by Aanyu et al. (2012) where the diet with 20%CSC15%SFSC had the best growth performance although it was not significantly different from the diet with 10%CSC25%SFSC while the diet with 15%CSC20%SFSC had a significantly lower growth.

In conclusion, the test diets did not negatively affect the number and length of intestinal folds as they were similar to the control. The diets with 10%CSC25%SFSC and 20%CSC15%SFSC had the most efficiently digested protein, and highest protein deposition implying that dietary protein was effectively used. Feed formulations with 10%CSC25%SFSC and 20%CSC15%SFSC could be used for making Nile tilapia diets. Future research should analyze Nile tilapia diets formulated based on the amino and fatty acid profiles of cotton and sunflower seed cakes. In this study, the feed was formulated based on the proximate composition of the ingredients. More factors relating to the intestine morphology also need to be assessed since the intestine is the key site for nutrient absorption.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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