

Effect of replacing cod liver oil with soybean oil as dietary lipid on carcass composition, haematology and sensory properties of the Nile tilapia *Oreochromis niloticus*

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Abstract

A study was conducted to evaluate the effect of replacement of cod liver oil (CLO) by soybean oil (SO) on the carcass composition, haematology and sensory qualities of the flesh of the Nile tilapia *Oreochromis niloticus*. Four diets (30% cp) containing 6% of added CLO and/or SO were formulated. Diet 1 contained 6% of added CLO, Diet 2 had 4% CLO and 2% SO, Diet 3 had 2% CLO and 4% SO and Diet 4 had 6% SO. The diets were fed to triplicate groups of ten tilapia fingerlings (mean weight 10.06 ± 1.02 g) for 56 days. Results at the end of the trial showed that carcass quality of the Nile tilapia fed soybean oil diets was not affected. The viscerosomatic index (VSI) of fish fed experimental diets decreased with increasing percentage of soybean oil in the feed, but the hepatosomatic index (HSI) showed a trend that is the reverse of VSI. The gonadosomatic index (GSI) of fish showed variation between treatments, with the control diet having the highest value. The values of the blood parameters (i.e. Hct, Hb, Esr, Wbc, and Rbc) of fish fed different diets showed variability among treatments, but fish fed the control diet had lower values than those fed other treatments. There was no significant difference in most of the organoleptic properties of the flesh (general appearance, colour, taste, juiciness), except in the texture and aroma of fish fed Diets 1 and 4 on the one hand, and those fed Diets 2 and 3 on the other ($P < 0.05$). The results indicate that soybean oil can replace up to 100% of fish oil in diets for the Nile Tilapia *O. niloticus*, without adverse effect on organoleptic properties.

Keywords: *Oreochromis niloticus*, Soybean oil, Carcass quality, Haematology, Organoleptic properties

Introduction

Fish oil derived from capture fisheries is becoming increasingly insufficient to meet the demand in the aquafeed industry. Global fish oil production of 1.2-1.4 million tons/year (Izquierdo et al. 2005) will not be able to meet the needs in the animal and aquafeed industries. When demand outstrips supply, cost will go up following the law of demand and supply. This explains why there is growing interest in evaluating the replacement value of vegetable oils for fish oil in fish diets (Hardy et al. 2001; Rosenlund et al. 2001; Caballero et al. 2002).

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The production of soybean oil, a product of sustainable agriculture, can be increased as the need arises. Soybean is a wonderful oilseed with an increasing demand for its by-products. This has stimulated more production of this oil seed crop, reflected in the increasing acreage of its cultivation. From 57 million ha in 1990, the land used for cultivation of soybean increased to 77.1 million ha in 2002 (Casson 2003). Soybean oil has been evaluated as a replacement for fish oil in diets. Kalogeropoulos et al. (1992) evaluated soybean oil in diets for gilthead bream, *Sparus auratus*. Glencross et al. (2003) assessed soybean and canola oils as alternative lipid sources for juvenile red seabream *Pagrus auratus*.

High cost of fish oil pushes the feed cost up, and this is why efforts are being made to cut down cost of fish feed production. This can be achieved by substituting the fish oil component with cheaper but locally available alternative dietary lipid sources. This study seeks to provide information on the replacement value of soybean oil for cod liver oil in diets for the Nile tilapia. Specifically, evaluation of the effect of soybean oil on the carcass composition, haematology, and sensory properties of the Nile tilapia will be carried out.

Materials and methods

Diet preparation

Four isoproteic diets (30% crude protein) were formulated and the proximate composition determined (Table 1). The control diet (diet 1) contained 6% cod liver oil, which was replaced by soybean oil in diets 2, 3, and 4 at 33.3%, 66.7% and 100%, respectively. Each of the diets had 2% fish oil as a residual of the fish meal component of the diet. The feedstuffs were thoroughly mixed in a Hobart A-200 mixer and pelletizer (Troy, Ohio, USA) to obtain a homogenous mass. Diets were passed through a mincer with die of 0.8 mm and milled, blended, moistened, pelleted and sundried at over 30 °C for three days. The diets were well dried with moisture content reduced to below 8.5% and stored in airtight plastic containers at ambient temperature (26 °C).

Table 1. Ingredient and proximate composition of diets containing soybean oil for the Nile tilapia *O. niloticus*

Ingredients (g/100g DM)	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal (61% cp)	26	26	26	26
Blood meal (82%)	17	17	17	17
Starch	46	46	46	46
Cod liver oil	6	4	2	0
Soybean oil	0	2	4	6
Vitamin/mineral premix	3	3	3	3
Carboxyl methyl cellulose	2	2	2	2
Proximate composition				
Moisture (%)	7.85 ± 0.11	8.31 ± 0.33	6.34 ± 0.13	7.54 ± 0.10
Ash (%)	10.60 ± 0.12	12.68 ± 0.27	12.70 ± 0.30	11.13 ± 0.53
Crude protein (%)	28.65 ± 0.30	30.05 ± 0.09	29.32 ± 0.42	33.95 ± 0.20
Crude lipid (%)	8.39 ± 0.56	8.63 ± 0.50	8.16 ± 0.39	8.45 ± 0.68
Crude fibre (%)	5.42 ± 0.10	4.38 ± 0.12	2.56 ± 0.28	3.72 ± 0.17
Nitrogen free extract (%)	38.25 ± 1.51	36.10 ± 1.25	40.93 ± 1.81	35.20 ± 1.20
Gross energy (Kcal/kg)	4374.52 ± 3.45	4429.63 ± 4.02	4487.44 ± 3.22	4360.18 ± 4.10

Fish, experimental design and experimentation

Juvenile Nile tilapia, *O. niloticus* were obtained from the Ondo State Agricultural Development Programme (ADP) Fish Farm at Alagbaka, Akure, Nigeria and transported in oxygen bags to the laboratory. The fish were then acclimated to laboratory conditions and fed with a commercial fish feed (35% cp) for 14 days. After acclimation, groups of ten *O. niloticus* fingerlings (mean weight 10.06 g ± 1.02) were randomly stocked into the 12 circular plastic tanks (21 l volume) containing 15 liters of water each for growth trials. Each of the diets was fed to the fish in triplicate containers at 4% body weight twice daily (9.00-10.00 h and 16.00- 17.00 h) for 56 days. The weight of

each group of fish was taken fortnightly using a Triple Beam Balance (700 series, Ohaus Florham park, N.J. 07932, USA), and the feeding rate was adjusted accordingly.

Carcass analyses

Both at the beginning and at the end of the feeding trial, six *O. niloticus* fingerlings, randomly selected from the initial pool and two fish pooled together from each replicate (glass tank) were homogenized and further prepared for carcass analysis (AOAC 2005).

Haematological examination

The fish for haematology were anaesthetized with 150 mg/l solution of tricaine methane sulphonate (MS-222, Sigma Chemical co. St. Louis, MO, USA) (Wagner et al. 1997). Blood samples were taken with 2ml heparinized syringes and 21 swg needles from the caudal vein of a set of three *O. niloticus* fingerlings from each treatment and put separately in 2ml heparinized tubes and taken to the laboratory for determination of packed cell volume (pcv), haemoglobin (Hb), erythrocyte sedimentation rate (ESR), white blood cells (WBC), and red blood cells (RBC) using the methods of Svobodova et al. (1991).

Sensory evaluation

Sensory evaluation to assess fish quality was performed at the end of experiment. The fish fillets were assessed by a panel of four trained panelists. The samples were evaluated using a 9-point hedonic scale, with 1 being the lowest and 9 the best. Both fresh and cooked samples were assessed. Fresh samples attributes assessed were general appearance and colour, while the attributes of cooked samples assessed were texture, aroma, taste and juiciness.

Statistical Analyses

Haematological, carcass analysis and sensory evaluation data collected from the experiment were subjected to one way analysis of variance (ANOVA) test using the SPSS (Version 10.0) FOR WINDOWS ON PC, and where significant differences were indicated, means were tested using Least Significant Difference (LSD) test at the 5% level of significance (Zar 1984).

Results

Carcass composition

Carcass composition of fish at the end of the feeding trial showed that the percentage crude protein of the experimental fish in all treatments was not significantly different from the value of the initial fish. Similarly, for crude lipids, no significant differences existed in the values obtained in the initial fish and those obtained for fish fed experimental diets ($P > 0.05$) except the value for fish fed diet 1 which were significantly higher ($P < 0.05$). The viscerosomatic index (VSI) of the initial fish was not significantly different from the value for the fish fed diet 4 ($P > 0.05$) but was significantly different from the values of fish fed all the other dietary treatments ($P < 0.05$). The gonadosomatic index (GSI) was significantly different ($P < 0.05$) among the treatments, with the value for initial fish, fish fed diets 1, 3 and 4 significantly higher than those of fish fed diet 2. The hepatosomatic index (HSI) increased with increasing soybean oil substitution.

Table 2. Carcass composition, VSI, GSI and HSI of *O. niloticus* (DM basis) fed diets containing soybean oil for 56 days, mean \pm SEM of three replicates

	Moisture	Crude protein	Crude lipid	Ash	VSI	GSI	HSI
Initial	6.28 \pm 0.21 ^a	64.90 \pm 1.00 ^a	6.01 \pm 0.64 ^a	5.37 \pm 0.49 ^a	9.61 \pm 2.23 ^b	4.50 \pm 0.87 ^b	1.02 \pm 0.65 ^b
Diet 1	7.01 \pm 0.50 ^a	63.51 \pm 1.23 ^a	7.72 \pm 0.31 ^b	4.84 \pm 0.08 ^a	12.30 \pm 1.67 ^c	6.83 \pm 0.25 ^c	0.39 \pm 0.28 ^a
Diet 2	7.14 \pm 0.08 ^a	63.06 \pm 1.36 ^a	5.82 \pm 0.14 ^a	4.90 \pm 0.12 ^a	8.96 \pm 2.54 ^a	2.85 \pm 1.44 ^a	0.89 \pm 0.15 ^b
Diet 3	7.73 \pm 0.37 ^a	63.86 \pm 0.58 ^a	5.68 \pm 0.39 ^a	4.81 \pm 0.27 ^a	11.64 \pm 1.67 ^c	4.21 \pm 0.99 ^b	1.56 \pm 0.33 ^c
Diet 4	6.88 \pm 0.08 ^a	64.25 \pm 0.22 ^a	6.02 \pm 0.03 ^a	3.90 \pm 0.17 ^a	10.28 \pm 1.99 ^b	5.67 \pm 0.23 ^{b,c}	1.22 \pm 0.43 ^{b,c}

* Means with same superscript are not significantly different.

Table 3. Haematology of *O. niloticus* fed diets containing soybean oil for 56 days (mean \pm SEM)

	HCT (%)	HB (g d/l)	ESR (mm/hr)	WBC ($\times 10^3/\mu\text{l}$)	RBC ($\times 10^6/\mu\text{l}$)	MCH (pg)	MCHC (g/l)	MCV (fl)
Initial	21.67 \pm 0.88 ^{c,d*}	7.12 \pm 0.38 ^c	10.33 \pm 0.33 ^b	5.23 \pm 0.23 ^c	2.13 \pm 0.12 ^c	33.60 \pm 0.05 ^a	33.27 \pm 29.13	102.43 \pm 0.84
Diet 1	17.67 \pm 0.33 ^{a,b}	6.20 \pm 0.06 ^b	8.33 \pm 0.88 ^{a,b}	3.93 \pm 0.08 ^a	1.57 \pm 0.03 ^{a,b}	39.63 \pm 0.12 ^b	35.13 \pm 9.94	112.77 \pm 0.03
Diet 2	19.33 \pm 0.88 ^{b,c}	5.50 \pm 0.06 ^b	8.67 \pm 0.67 ^{a,b}	4.00 \pm 0.06 ^b	1.80 \pm 0.06 ^b	36.13 \pm 0.08 ^{a,b}	33.80 \pm 16.41	107.73 \pm 0.67
Diet 3	16.33 \pm 0.33 ^a	5.37 \pm 0.08 ^a	10.00 \pm 0.58 ^b	4.27 \pm 0.03 ^b	1.43 \pm 0.09 ^a	37.80 \pm 0.29 ^{a,b}	32.90 \pm 4.51	115.03 \pm 0.89
Diet 4	22.67 \pm 1.20 ^d	7.33 \pm 0.08 ^c	7.33 \pm 0.88 ^a	4.77 \pm 0.03 ^b	2.10 \pm 0.06 ^c	34.97 \pm 0.12 ^{a,b}	32.57 \pm 20.17	108.77 \pm 0.41

* Means with same superscript are not significantly different.

Haematological indices

There was a significant difference in all the haematological parameters measured both among the dietary treatments and between the control and dietary treatments ($P < 0.05$) (Table 3). Apart from fish fed diet 4 whose Hct and Hb did not follow the observed trend, fish fed other dietary treatments showed a common trend, namely the higher the soybean oil substitution level, the lower the Hb and Hct. There was no significant difference between the initial (pre-treatment) values of Hct, Hb, Esr, Wbc and Rbc and the same values for fish fed diet 4 across all the treatment groups, similarly there was no significant difference in the erythrocyte sedimentation rate ($P > 0.05$), white blood cells and red blood cells of fish fed diets 2 and 3. Fish fed diet 3 showed the lowest values for Hct, Hb, and Rbc. MCH values for fish fed diets 2 and 3 were significantly higher than the values of the same parameter in fish fed other diet types ($P < 0.05$). MCHC values were not significantly different between all the treatments. MCV values were significantly different between treatment groups, with values decreasing with increasing soybean oil level in the diet, the exception being fish fed diet 1 which had a significantly lower value than fish fed all other diets.

Sensory evaluation

Fish fed different diets did not present any significant difference in the mean score for either the attributes of the raw fish (general appearance and colour) or attributes of the cooked fish (texture, aroma, taste and juiciness) ($P > 0.05$) (Table 4). Not only there was no significant difference between sensory attributes of fish fed different dietary treatments, but even the overall mean score between treatments was not significantly different.

Table 4. Sensory evaluation of *O. niloticus* fed diets containing soybean oil for 56 days (mean \pm SEM)

Diets	General appearance	Colour	Texture	Aroma	Taste	Juiciness	Overall mean for attributes
Diet 1	7.25 \pm 0.48 ^{a,b*}	7.50 \pm 0.29	6.00 \pm 1.68	7.00 \pm 0.71	7.25 \pm 0.48	7.00 \pm 0.71	7.00 \pm 0.73
Diet 2	7.25 \pm 0.48 ^{a,b}	6.00 \pm 0.41	7.25 \pm 0.75	7.25 \pm 0.48	8.00 \pm 0.71	6.25 \pm 0.25	7.00 \pm 0.51
Diet 3	6.25 \pm 0.63 ^a	6.25 \pm 0.48	7.50 \pm 0.50	7.50 \pm 0.50	7.75 \pm 0.63	7.25 \pm 0.48	7.08 \pm 0.54
Diet 4	8.00 \pm 0.41 ^b	7.25 \pm 0.63	7.25 \pm 0.85	5.75 \pm 0.85	6.50 \pm 0.50	7.25 \pm 0.48	7.00 \pm 0.62

* Means with same superscript are not significantly different.

Discussion

Carcass composition

The protein content of the carcass composition was not significantly different between treatments and that of the initial fish. The lipid content of the initial fish was not significantly different from that of fish fed experimental diets, except fish fed diet 1 (the control) which gave significantly higher crude lipid level. The ash content did present any significant difference between treatment groups. The VSI and GSI of fish fed different diets showed significant difference between groups, but the pattern of difference is similar for both parameters.

The HSI of the initial fish were not significantly different from those fed diets 2 and 4 but differed significantly from those of fish fed diets 1 and 3. In general, the HSI of fish increased with increasing soybean oil level in the diet. Piedecausa et al. (2006) obtained higher HSI values for sharp-snout seabream fed soybean oil than those fed fish oil or linseed oil. In contrast, previous studies did not record significant HSI differences on other fish species when vegetable oils were used, including Atlantic salmon (Rosenlund et al. 2001; Menoyo et al. 2003; Bendiksen et al. 2003; Ng et al. 2004), turbot (Regost et al. 2003), and European sea bass (Mourete et al. 2005).

Haematological indices

Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health status of fish (Hrubec et al. 2000). Results of analysis of the haematological parameters of *O. niloticus* in this study showed significant difference between the treatment values and the values of all the blood parameters. The initial fish have higher blood parameter values (though not necessarily significant between values obtained with some treatments), than those of fish fed treatment diets. Variability of Hct of fish fed different diets did not follow any clearly discernible trend.

Broadly, the Hb of fish fed different diets increased (with the exception of fish fed diet 3) with increasing level of soybean oil inclusion in the diet. Perruzzi et al. (2005) obtained Hb values of 5.28 ± 0.31 and 4.60 ± 0.32 for diploid and triploid sea bass respectively produced by sub-optimal pressure treatments and held in communal environments under standard rearing conditions. Haemoglobin (Hb) content of fish fed different diet types in this study (range 5.37 ± 0.08 to 7.73 ± 0.08) were higher than those obtained by Peruzzi et al. (2005) and those obtained by Subhadra et al. (2006) for the largemouth bass with diets containing canola oil, chicken oil and Menhaden fish oil, which ranged between 3.7-3.9 g/dl. There was no significant difference in the erythrocyte sedimentation rate (Esr) of fish fed different treatments ($P > 0.05$), except that fish fed diet 4 had significantly lower value than those fed all other diets. There was also no significant difference in the white blood cells (Wbc) count of fish fed different diets except those fed 4 which had a significantly higher mean value. The mean RBC values obtained in this study showed variability between treatments, but the trend is similar to that described for Hb.

The mean cell haemoglobin (MCH) of fish fed different diets was significantly different between treatments, and the broad trend is that it decreases with increasing level of soybean oil substitution in the diet. There was no significant difference between mean cell volume (MCV) and the mean cell haemoglobin concentration between treatments values for fish fed experimental diets for both parameters were higher than the pre-treatment value. Lane (1979) cited by Lie et al.(1989), reported that an increase in MCH and MCHC values reflect a preserving mechanism in rainbow trout activated at reduced water temperatures. There was no temperature variation in this study, hence no significant increase relative to the initial MCH and MCHC values were observed ($P > 0.05$). The mean cell haemoglobin concentration (MCHC) values obtained in this study (32.57 ± 20.17 – 35.13 ± 9.94) are slightly higher than the range of 22-29 provided as reference interval for this species (Hrubec et al. 2000). The difference may be due to the high degree of variability of different values for a given treatment, as reflected in the high standard deviation of the values. Mean cell volume (MCV) values ranging from 102.43 ± 0.084 for initial fish to 115.03 ± 0.89 fall close to the reference interval of 115–183 for this species (Hrubec et al. 2000). MCV values higher than the normal range is an indication of macrocytosis and a value smaller than the normal range is indicative of microcytosis (Etim et al. 1999).

Sensory evaluation

Sensory evaluation of fish is an important index in its overall assessment. This is because of eating quality is an important determinant of the overall impression of a food (Rasekh et al. 1970). A food tasting poorly is unlikely to enjoy future patronage. The sensory quality of fish is determined by its composition (Robb et al. 2002). Kestin et al. (1995) showed that muscle lipid level significantly affected the eating quality of rainbow trout.

General appearance and colour were assessed on fresh samples whereas texture, aroma, taste and juiciness were assessed on cooked samples. There was no significant difference between treatments for all the attributes assessed by the panelists ($P > 0.05$). Consequently, the mean overall score ranged from 7.33 ± 0.48 to 7.73 ± 0.50 on a scale of 9.00. Increasing levels of dietary oil result in higher muscle lipid levels in the fish (Robb et al. 1997), but because the lipid level in this study was controlled, it resulted in product quality with little or no variation in eating quality. The overall assessment showed that there was no significant difference between fish fed diets containing different levels of soybean oil substitution. Fauconneau and Laroche (1996) opined that quality definition of cultured fish is focused on its nutritional and sensory aspects. The water holding capacity of fish flesh is generally high compared to meat (Fauconneau et al. 1995), a characteristic generally associated with greater juiciness of the flesh (Paredes and Baker 1987). A mean rating of about 7 points on a 9 point scale for juiciness obtained in this study confirms that the flesh of this species, like most fish species, is very juicy. It has been proposed that composition of lipid in the diet could significantly affect either positively or negatively catfish flavour (Gibson et al. 1977; Dupree et al. 1979). Hence the use of soybean oil in feeding the Nile tilapia does result in product with good eating quality.

Conclusion

This study shows that fish (cod liver) oil can be replaced with soybean oil in diets for the Nile tilapia without any negative effect on carcass quality. While the VSI of fish fed experimental diets decreased with increasing percentage of soybean oil in the diet, the reverse was the case with HSI which showed increase with increasing level of soybean oil content in the feed. The GSI of fish showed variation between treatments, with the control diet having the highest value. The values of the blood parameters (Hct, Hb, Esr, Wbc, and Rbc) of fish different diets showed variability between treatments. Therefore substituting one-third of the dietary lipid requirement of Nile tilapia with soybean oil makes economic sense.

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