EFFECT OF DIETARY LIPID LEVEL AND SOURCE ON GROWTH PERFORMANCE, BLOOD METABOLITES AND BODY COMPOSITION OF TILAPIA FISH

A. M. Orma; T. I. Mohamed & Hoda E. Ali

Dept. of Nutrition & Nutritional Deficiency Diseases, Faculty of Vet. Med., Mansoura University

ABSTRACT

The present investigation was carried out for evaluation the effects of fat source and levels in isocaloric-isonitrogenous diets on growth performance data, physiological response, body composition and healthiness of the internal organs and muscles of the Nile tilapia fingerlings. The experiment was conducted to determine the optimum fat level in Tilapia nilotica diet isocaloric (3000 Kcal DE/Kg), isonitrogenous (32% CP) diets containing 2 sources (fish oil and soybean oil) and three different levels of dietary lipid (4, 8, 10 & 12%). Nile tilapia fingerlings (224 in number of average body weight 14.23±0.91g) were divided into 7 equal groups and reared in 14 glass aquaria (2 replicates for each group) were used in the experiment. Daily feed allowance was calculated according to the biweekly body weight as 3% of mean body weight of each group). The experiment was extended for 12 weeks. Fish was weighed once every two weeks. Growth performance (body gain, feed consumption & feed conversion "FCR" were measured. At end of the experiment, 6 fish were obtained from each group for proximate chemical analysis of whole body. Also, blood samples were collected from 6 fish from each group for serum preparation for determination of total protein, albumin, total lipids, triglycerides total cholesterol. Low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoproteins (HDL) were also determined. For histopathological examination, 3 fish were taken from each group as random samples for microscopic examination.

The results revealed that feeding the diets supplemented with SBO to contain 8% total lipid did not increase body weight (BWt), body weight gain (BWG) or feed conversion ratio (FCR) compared to the control groups. Increasing the supplemented SBO to raise the total dietary lipid to 10 and 12% significantly (p<0.05) increase BW, BWG, FCR, and protein efficiency ratio (PER). Feeding the Nile tilapia fingerlings diets supplemented with FO to contain 8, 10 and 12% total dietary lipid significantly (p<0.05) increased BW, BWG, FCR, and PER. On comparing the allover effects of SBO or FO supplementation on growth performance, fish groups supplemented with FO had significantly higher BW (44.36 Vs 41.44 g) and better FCR (1.46 Vs 1.66) than the fish groups fed the SBO supplemented diets. Feeding the oil supplemented diets increased the level of fat in the whole body of fish. Increasing the dietary lipid *levels increased (p<0.05) level of fat in the whole body of fish. Supplementing the fish diets with SBO or FO significantly (p<0.05) increased the serum levels of the total lipids and total cholesterol. Also, serum level of HDL increased as well. In the fish groups fed the oil supplemented diets to contain 12% total lipids, there were some pathological changes in the liver and heart. Therefore, the present results indicated that oil (SBO and FO) supplementation of Nile tilapia fingerlings significantly improve body weight gain, feed conversion ratio and protein utilization. However, increasing the dietary lipid level than 10% resulted in pathological changes in liver, and heart and hurt the health condition and flesh quality. Furthermore, fish oil is more profitable than soybean oil in maximizing growth performance.*

INTRODUCTION

World fish oil production exceeded 1.36 x 106 t **(FAO, 2002)**. Some authors reported that more than 70 % of the production is the most reliable estimate of its use by the aquaculture feed industry **(Olsen et al., 2004)**. Unfortunately, fish oil is a generic ward which includes very different types, according to species, period of the year, latitude of catch, and chemical features **(Bimbo, 1990)**, and it is characterized by fluctuating prices, depending on the variability of the catch by the fishery industry. Fish oil has been used at higher dietary lipid source as a general practice for its protein sparing effect, but the demand for fish oil by the aquafeed industry has been predicted to exceed the available resources within the next decade **(Barlow and Pike, 1999)**. Therefore, use of different kinds of alternative lipid sources in fish nutrition should be investigated in studies. Alternative oils obviously represent more sustainable sources in most countries. **Lim et al. (2008)** demonstrated that up to 90% of dietary fish oil can be replaced by vegetable (palm) oils without compromising growth or feed utilization of some fish species such as Clarias gariepinus. Partial replacement of fish oil by certain vegetable oils has proved feasible in several species without affecting growth negatively **(Bell et al., 2003; Regost et al., 2003)**. However, the effects of vegetable oils on lipid metabolism and health status of fish remain unclear. Although high lipid diets improve growth rate, and feed and protein utilization, these may be associated with rancidity and lowered flesh quality due to lipid oxidation **(Scaife et al., 2000)**. Also providing fish with adequate energy through dietary lipids can minimize the use of expensive protein **(De Silva et al., 1991)**.

The amount of non-protein energy in the diet is one of the factors influencing the quantitative dietary protein requirement of a fish species **(Jauncey, 1982)**. This is because part of the dietary protein may be utilized as an energy source, if the diet is deficient in non-protein energy. Since the use of protein for energy is wasteful from nutritional, economic, and ecological points of view when compared to lipids and carbohydrates, it is worthwhile supplying as much of the required energy as possible as lipid and carbohydrate **(Peres and Oliva-Teles, 1999)**. Therefore, the present study was carried out in one experiment by two fat sources (soybean oil and fish oil) and four levels of total dietary fat to evaluate the effect of levels and sources of dietary fat on terms of growth performance (mean weight, absolute body gain & specific growth rate in wet weight), feed efficiency (feed intake, feed conversion ratio & protein efficiency ratio), and histopathological changes in different body organs of Nile tilapia fingerlings (Oreochromis niloticus).

MATERIALS AND METHODS

The present study was carried out to evaluate the effect of levels (4, 8, 10 & 12 %) and sources (soybean oil "SBO" and fish oil "FO') of dietary fat on terms of growth performance (mean weight, absolute body gain), feed efficiency (feed intake, feed conversion ratio & protein efficiency ratio), body composition and histopathological changes in body organs of Nile tilapia fingerlings (Oreochromis niloticus). 224 Nile tilapia fingerlings were equally distributed into 7 fish groups which were

Experimental fish and diets:

224 Nile tilapia fingerlings Oreochromis niloticus (Nile tilapia) monosex (males) fingerlings obtained from a commercial fish hatchery were used in the experiment. The experiment was carried out in the Fish Nutritional Laboratory, Faculty of Veterinary Medicine, Mansoura University. Fish were received and stocked in 14 glass aquaria (80cm length, 35cm width & 40 cm height), 2 aquaria per treatment. Each one was covered by net cover to prevent fish escaping and protect it against insects. The received fish was kept for two weeks before the time of beginning the experiment to adapt fish to the experimental conditions and on the formulated diets. By the end of the 3rd week of the adaptation period, all fish become completely adapted to the new environmental conditions and the experimental diets. The fish were fed the formulated isonitrogenous (32% CP) & isocaloric (3000 Kcal DE / Kg) diets containing different levels of SBO or FO (Table 1). All the formulated diets were supplemented with vitamin C (100 mg / kg) and vitamin E (200 IU / kg). The pellets were air dried in wire nets for 3 days (till obtaining well dried diets), then were broken into an appropriate size and then stored in paper bags in the refrigerator (at 4°C) during the time of use. Samples of the experimental diets were analyzed for moisture, crude protein, ether extract and ash by standard methods according to **AOAC (1995)**. The feeding experiment was extended for 12 weeks. Feed allowances (on air-dry basis) was offered for fish till satiation. Fish was fed twice daily (at 8:00 am & 5:00 pm) to minimize over feeding. The calculated feed intake was weighed every 2 weeks. Dechlorinated tap water was used to fill the aquaria. Daily cleaning for each aquarium was carried out with partial replacement of water by previously stored (for 48 hrs). The complete cleaning of the aquaria was done biweekly (of fish weighing) during the first six weeks of the experiment and then become weekly till the end of the experiment to prevent rapid pollution of water by the waste products and feed residues. Water temperature was kept between 24 to 27°C during the experimental period. Water pH values were recorded day after day by using a portable pH meter. The values were ranged from 7.25 to 8.25 during the experimental period in the different aquaria. At recording values higher than 8.25 part of the aquaria water (one third) was replaced. Continuous air flow was supplied to each aquarium by using

electric air pumps from two sources for each aquarium.

Growth performance measures:

Mean weight of fish in aquaria, absolute growth = (W2-W1) for 14 days. Feed intake was calculated as the total weight of diet offered in a given period (2 weeks) divided by the number of survival fish. Feed conversion ratio (FCR) = feed intake (g) / weight gain (g).

Chemical composition of whole body composition of fish:

At the end of the experimental period, random fish samples (5 fish / group) were collected. The taken fish samples were minced, dried at 70c for 72 hrs then analyzed for whole body chemical composition (DM, CP, EE and ash) according to **AOAC (1995)**.

Histopathological examination:

At the end of the experiment samples were taken for histopathological examination. Specimens were collected from muscles, gills, liver, kidney, spleen, intestine and heart of 6 fishes from each group (3 fish from each replicate) then preserved in 10% formalin saline, dehydrated, cleared, blocked in paraffin wax, cut into sections then stained by hematoxoline and eosin **(Carlton, 1967)**.

Statistical analysis:

Data obtained in the present work were statistically analyzed for analysis of variance (ANOVA) and least significant difference (LSD) as described by **Senedecor and Cochran (1967)**.

Discussion

Growth performance:

The growth performance (body weight development & absolute body gain) data of Nile tilapia fingerlings fed diets containing 8, 10 & 12% dietary lipids supplemented from fish oil (FO) and soybean oil (SBO) are presented in (Tables 2). The growth data showed that adding SBO to the basal experimental diet (control) to raise dietary fat level of the diet fed to the Nile tilapia fish to 8% did not increase BW or BWG throughout the 12 weeks experimental period. On the other hand, adding SBO to maintain the dietary lipid to 10 and 12% increased BW & BWG of Nile tilapia fingerlings (Tables 2). However, there was no significant difference in the increase in BW due to increasing dietary lipid. Adding FO to the basal experimental diets fed to the growing fish to contain 8 & 10 dietary lipids improved BW & BWG till the end of the experiment. In spite of this finding, increasing the dietary lipid to 12% of the diet through adding FO achieved further increase in BW & BWG (Tables 2). It had been reported that high dietary fat (14%) had protein sparing effect and improved growth rate and feed utilization in Nile tilapia fingerlings fed diets containing 25 or 30% CP (Ali, 1991). Similar results reported by Han et al. (2010) in hybrid tilapia juveniles. In contrast, it has been reported that Nile tilapia fingerlings fed a high fat diet (12% fat) had lower final body weight than fish groups fed on diets containing either 6 or 9% dietary fat (Orma, 2007).

The results of the present experiment (Table 2) showed that feeding of Nile tilapia fingerlings the diets contained different amounts of SBO to maintain dietary lipid at 8, 10 & 12% for 12 weeks generally improved the BW and BWG. Similarly, adding of FO to the basal control diets maximize the BW and BWG. Therefore, the present results confirmed that

FO is more profitable than SBO in maximizing growth performance of Nile tilapia fingerlings. **Bell et al. (2004)** reported that replacement of FO with vegetable oils up to 100% in salmon & trout diets did not markedly influence growth and FCR and that flesh lipid content was not unaffected by dietary lipid source. Thus a diet rich in vegetable oils can provide the same energy for growth as one composed purely of FO. In agreement with the present results, **Chou & Shiau (1996)** reported that weight gain was higher in growing tilapia fish fed 10 & 15% lipid diets, followed by the 5% lipid diet, then the 20% lipid diets and was lowest in fish fed the lipid-free control diet. The authors concluded that the optimal dietary lipid for maximal growth in about 12%. With these finding **(Han et al., 2010)** found that hybrid tilapia fish fed diets supplemented with SBO and containing 7.6 and 10.7% total dietary lipid had a significantly ($P<0.05$) higher body weight gain than those fed the diets containing 4.8 & 13.7% lipid.

Feed consumption of the fish groups fed the SBO supplemented diets (8, 10 & 12% fat) at the end of the experimental period (12 weeks) was nearly the same (Table 2). The significant differences in feed intake between the fish groups during the experimental periods maybe due to the activity and differences in BW of tilapia fish. Also, the results showed that supplementing the fish diet with FO to maintain 10% dietary lipid decreased the feed consumption and improved the FCR. Generally, supplementing the basal diet with FO to increase the dietary lipids to 8, 10 or 12% of the fish diets markedly improved FCR compared to the control diet (6% fat). Chou & Shiau (1996) reported that FCR tilapia were better ($P<0.05$) for the fish groups fed diets with 10-15% lipid. Similarly, Han et al. (2010) concluded that FCR was better for the fish groups supplemented with fish oil at 5.55 & 8.5% of the diet. However, **Tidwell et al. (2007)** concluded that there were no significant differences in terms of WG, FCR and survival percentage between groups of sea bass fish fed diets supplemented with oil (at 5% of diet) of different sources included (fish oil, corn oil, sunflower oil, linseed oil & fungal oil).

Effect of fat source and levels on whole body composition:

The results revealed that there was some effects for feeding the isocaloric (3000 Kcal DE/Kg) diet containing different levels of fat (6, 8, 10, & 12%) on dry matter percentages. The lowest value (25.85%) was reported to the fish group fed the diet containing 8% SBO, while the highest value (27.36%) was for the fish group fed the 8% FO compared to the control group. Data for crude protein content show some increase in the fish groups fed the diets supplemented with FO compared with the control fish (18.43 vs. 17.51). Likewise, there were no marked differences in the ash content. Oppositely, feeding the fish groups the SBO containing diets (8, 10, & 12% fat) significantly increased the percentage of fat content. Nevertheless, this finding did not occur in the fish groups fed the FO containing diets (Table 3). The existing results indicated that no definite relationship between dietary fat & whole body protein, & ash content. These outcomes are in agreement with that of **Orma (2007)**. However, **Han et al. (2010)** reported that increasing dietary lipid levels significantly ($P<0.05$) increased the lipid content of whole body. However, body moisture, ash and crude protein contents were not significantly affected by dietary lipid levels.

Effect of feeding Nile tilapia fingerlings SBO & FO at different levels on serum metabolites:

Feeding the growing Nile tilapia fish diets containing high levels of dietary fat (10 & 12%) through adding SBO Leads to significant (P<0.5) increase in the levels of serum total protein and globulin compared to the fish groups fed the basal control diet (contained 6% fat) or the group fed the diet supplemented with SBO to raise dietary fat to 8% (Table 4). However, statistical analysis of the data showed that feeding the SBO supplemented diets did not affect the serum concentration of albumin.

The present results showed that fish fed the FO supplemented diets (8, 10, & 12 dietary fat) has significant (P < 0.05) higher levels of serum total protein (Table 5). Also, the result revealed that albumin /globulin ratio (A/G) were mostly significant (p<0.05) lower in the fish groups fed the SBO or FO supplemented diets (except the fish group fed the 10 % FO diet) compared with the fish group fed the basal control diet. The lower values of A/G ratio reported in the present study for the fish groups fed the SBO & FO supplemented diet indicated that the oil supplementation improves the immune status of fish as serum Globulin levels represent the specific immune fraction. Comparable values of serum concentration of total protein Albumin & globulin were reported for Nile tilapia fingerlings fed diet contained 32% CP & 3000 Kcal DE/ Kg and 6% fat, **(Orma, 2007)**. Serum biochemical studies of healthy fish fed basal recommended diets are relatively scarce and makes interpretation of fish serum biochemical parameters is very difficult. Serum concentration of total lipids, triglycerides, total cholesterol, HDL, LDL and VLDL of Nile tilapia fingerlings feed diets containing different levels of dietary fat supplemented from SBO or FO are displayed in (Table 5). The results showed that feeding the oil supplemented diets significantly (P<0.05) increased the concentration of serum total lipids. The highest values were reported for the fish groups fed the 12% lipid containing diets either supplied from SBO or FO.

The results showed also that there were corresponding increase in the levels of serum triglycerides to the increase in levels of serum total lipids and the highest concentrations was reported for the fish groups fed the diet containing 12% dietary lipid. Similarly, feeding the high-FO-diet increased the level of serum cholesterol compared to the control group. But it is of worth to observe that supplementing the diet with SBO to contain 8 or 10% dietary lipid did not increase the serum cholesterol level (Table 4).

Histopathological findings due to the high fat (SBO & FO) diets:

Microscopic examination of the prepared specimens of kidney, spleen, and intestine of the fish groups fed the experimental diets for 12 weeks showed no histopathological changes and liver sample showed apparently normal histopathological structure (Fig.1). The liver samples from the fish groups fed the diet containing 10% dietary fat (supplemented with SBO) (Fig. 2) showed moderate fatty changes less than those observed in the fish groups fed 12% containing diets either supplemented with SBO or FO. Also, liver showed an increased amount of adipose tissue surrounding the portal area & fat cells rupture forming fat cyst in the fish group fed 12% dietary fat from FO (Fig. 4). Furthermore, liver confirmed that feeding the diet supplemented with FO to contain 12% fat caused severe fatty change represented by clear vacuoles replacing the hepatic cytoplasm & pushing the nucleus to one side (Fig. 4). With this concept, heart of the fish from the group fed 12% fat-diet supplied from SBO showing increase intermuscular adipose tissue besides hyaline degeneration of some cardiac muscles (Fig. 3). **(Piper et al., 1982)** reported that high levels of dietary fat & to lesser degree excess protein & carbohydrates caused fatty infiltration in fish liver.

In agreement with the pervious results, **Ronald et al. (2004)** indicated that high fat (10 & 14%) & high protein (35, 40, & 45%) diets induced severe fatty change in the liver of Nile tilapia fingerlings and severity of fatty infiltration declined with lowering dietary fat & protein levels. Pathological changes in liver & heart due to feeding Nile tilapia fingerlings high fat diets (9 & 12%) had been reported **(Orma, 2007)** and the severity of pathological changes were less in the fish fed 9% fat-diet. The present results indicate that increasing the dietary fat level in the diet of Nile tilapia fingerlings than 10% resulted in pathological changes in the liver & heart, and may hurt the health condition and flesh quality. In agreement with the present findings, it has been concluded that the inclusion of excessive dietary lipids may have undesirable effects upon growth and body composition **(Riche, 2008 and Borges et al., 2009)**. Therefore, an appropriate dietary lipid level is critical for maximal growth & flesh quality of Nile tilapia Based upon the present findings, it could be concluded that for Nile tilapia fingerlings optimal dietary level of supplemental soybean oil or fish oil is to compete the total dietary lipid to 10% for maximal growth.

Table 1. Ingredients percentages of the experimental isocaloric, isonitrogenous diets containing different levels of fat.

	Dietary treatments			
Dietary fat %	**6**	**8**	**10**	**12**
Ingredients				
Ingredient				
Fish meal	20	20.3	20.7	21
Corn, yellow	14	12	9.9	12.2
Soybean meal	10.5	10	10	18
Wheat bran	24	24.2	18	4
Corn gluten	12	12	12	7.3
Rice bran	15	10	9.5	10.3
Soybean or fish oil	-	2	4	6
Berseem H meal	1.5	6.5	13	17
Sodium chloride	0.5	0.5	0.5	0.5
Min. & vit. premix**	0.3	0.3	0.3	0.3
Gelatin	1.0	1.0	1.0	1.0
Antitoxin	0.2	0.2	0.2	0.2
Dicalcium phosphate	-	0.45	0.65	1.0
Lime stone	0.9	0.4	0.1	-
Methionine	0.2	0.2	0.2	0.2
Crude protein %	32.11	32.11	32.11	32.11
DE (Kcal / kg)*	3003	3006	3000	3005
EE %	5.79	7.86	9.83	11.69
Crude fibers	4.21	4.21	4.21	4.21
Calcium%	0.90	0.92	0.95	0.94
Phosphorus%	0.65	0.61	0.59	0.60

*DE (kcal/kg) values are calculated from the feed composition tables, nutrient requirement of fish (NRC 1993)

**calculated values

**Trace minerals & vitamins premixes were prepared to cover the levels of the microminerals & vitamins for tilapia fish as recommended by the NRC (1993). Vitamins premix (IU or mg/kg diet); vit.A 15000, Vit.D3 3000, vit.E 20, vit.k3 2,vit.B1 2, vit.B2 5, vit.B6 1.5,VIT.B12 0.02, Pantothenic acid 10, folic acid 1, biotin 0.15, niacid 30. Mineral mixture(mg/kg diet); Fe 40, Mn 80, Cu 4, Zn 50, I 0.5, Co 0.2& Se 0.2.

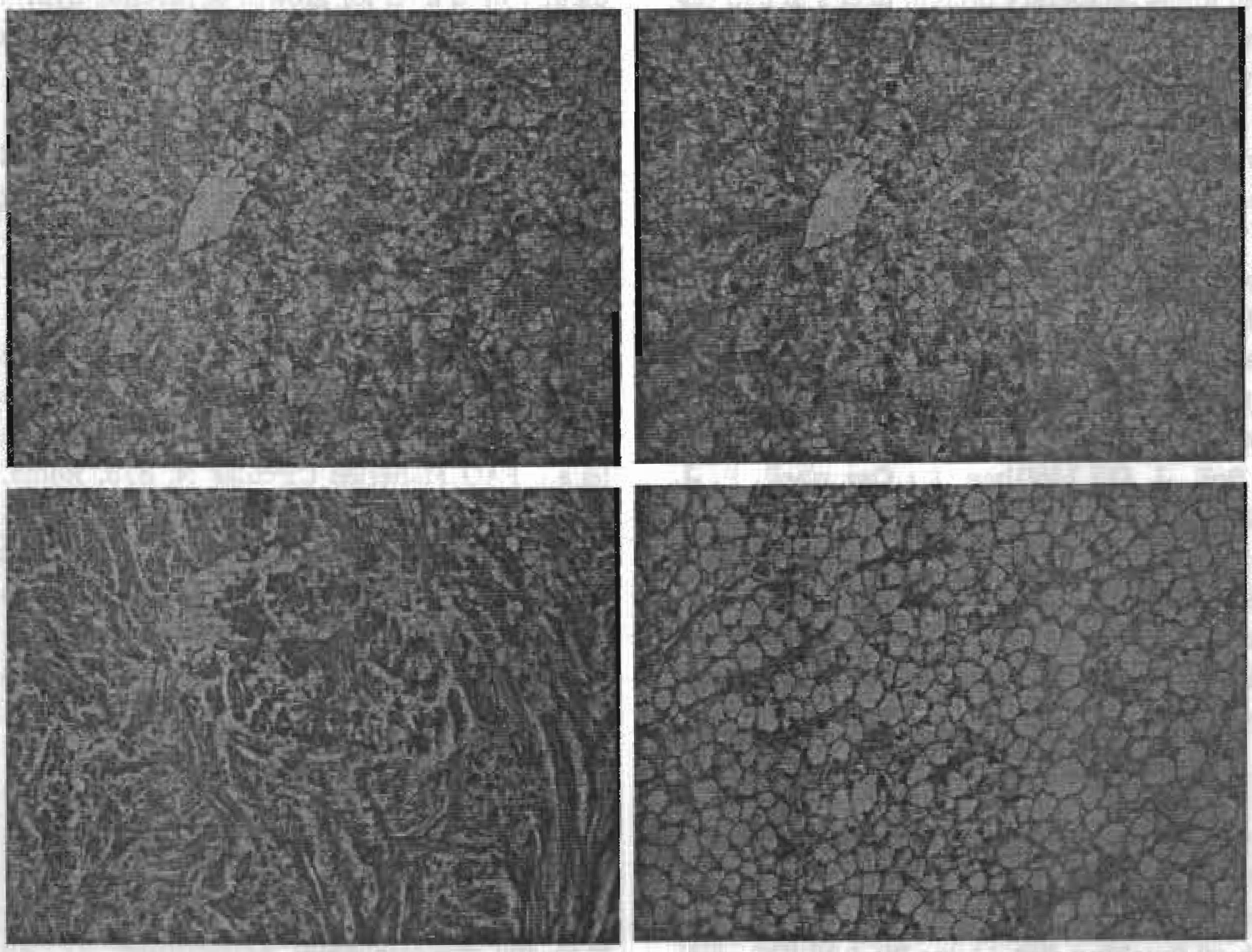

Fig (1): The liver showed apparently normal histological structure. H&E., x 40.

Fig (2): The liver of tilapia (Gp 3) showed moderate increase in fat content (fatty changes) less than those observed in gp 7 and 4. H&E., x20.

Fig (3): Heart of fish group 4 showing increase intramuscular adipose tissue besides hyaline degeneration of some cardiac muscles. H&E., x20.

Fig (4): Liver (Gp: 7) showing severe fatty changes represented by clear vacuoles replacing the hepatic cytoplasm and pushing the nucleus to one side.

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الملخص العربى

تأثير مستوى ومصدر الدهون في العليقة على معدلات النمو ومكونات مصل الدم والتركيب الكيميائى للجسم في سمك البلطى

أ.د/ عبد الهادى محمد عرمه أ.د/ طارق ابراهيم محمد ط.ب/ هدى السيد علي

قسم التغذية وأمراض سوء التغذية - كلية الطب البيطري - جامعة المنصورة

أجريت هذه الدراسة لتقييم إضافة زيت السمك أو زيت الصويا بنسب مختلفة لرفع نسبة الدهون في العليقة الضابطة من 6 إلى 10 -8 و 12% - استخدمت علائق تجريبية تحتوي على 32% بروتين خام و 3000 كيلو كالورى طاقة هضم/كجم علف. أجريت التجربة على مجموعة من أسماك البلطي النيلي النامية عددها 224 وزنها 14.23جم قسمت في 7 مجموعات متساوية في الوزن. واستخدمت الأحواض الزجاجية (عدد 14) بواقع حوضين لكل مجموعة (16 سمكة بالحوض) وأجريت التجربة لمدة 12 أسبوع. تم قياس الوزن كل أسبوعين. العلف المستهلك يوميا. نسبة تحويل العلف. كفاءة تحويل البروتين طول فترة التجربة. في نهاية التجربة أخذت عينات من دم الأسماك (عدد 5 من كل مجموعة) لفصل مصل الدم وقياس كل من البروتين الكلي ,الألبيومين ,الدهون الكلية ,الكوليستيرول الكلي, الجليسريدات الثلاثية الليبوبروتينات منخفضة الكثافة ,والليبوبروتينات عالية الكثافة . كما أخذت عينات من 5 اسماك من كل مجموعه لفحص التركيب النسيجى للاعضاء الداخليه وبيان التغيرات الهستوباثولوجية في الأسماك في كل المجموعات.

وأدت التجربة إلى النتائج التالية :

- إضافة زيت فول الصويا لرفع نسبة الليبيدات الكلية في العليقة إلي 8 % لم ي يؤثر على معدلات النمو ومعامل تحويل العلف أو كفاءة استخدام البروتين بالمقارنة بالمجموعة الضابطة. إضافة زيت فول الصويا للعليقة لرفع نسبة الليبيدات الى 10و 12% أدت إلى زيادة معدلات النمو ومعامل تحويل العلف. إضافة زيت السمك إلى العليقة أدى إلى زيادة معدلات النمو وتحويل العلف وكفاءة استخدام البروتين بزيادة نسبة الليبيدات في العليقة طرديا من 10-8 إلى .12% زيادة الدهون في العليقة بإضافة زيت فول الصويا أو زيت السمك أدت إلى زيادة نسبة الدهن في جسم السمك طرديا بزيادة نسبة الزيت المضاف ودون تأثير ملحوظ في نسبة البروتين والأملاح المعدنية في الجسم مع زيادة قليله في المادة الجافة .
- إضافة زيت فول الصويا أو السمك إلى العليقة أدى إلى زيادة نسبة الدهون الكلية - الجليسريرات الثلاثية - الكوليستيرول الكلي

في مصل دم السمك مع انخفاض نسبة الليبوبروتينات منخفضة الكثافة - أما نسبة الليبوبروتينات عالية الكثافة فقد زادت نسبتها في مصل الدم بإضافة كل من زيت فول الصويا أو زيت السمك للعليقة .

- إضافة زيت السمك أو فول الصويا إلى العليقة ليرفع نسبة الليبيدات إلى 12% أدى إلى حدوث تغييرات مرضية في نسيج كل من كبد وقلب السمك في نهاية مدة التجربة (12أسبوع).

وخلصت النتائج إلى أن إضافة زيت السمك أو زيت الصويا أدى إلى زيادة معدلات النمو وتحويل العلف وكفاءة استخدام البروتين وأن نتائج زيت السمك أفضل من زيت الصويا مع عدم زيادة نسبة الدهون في العليقة عن . 10%