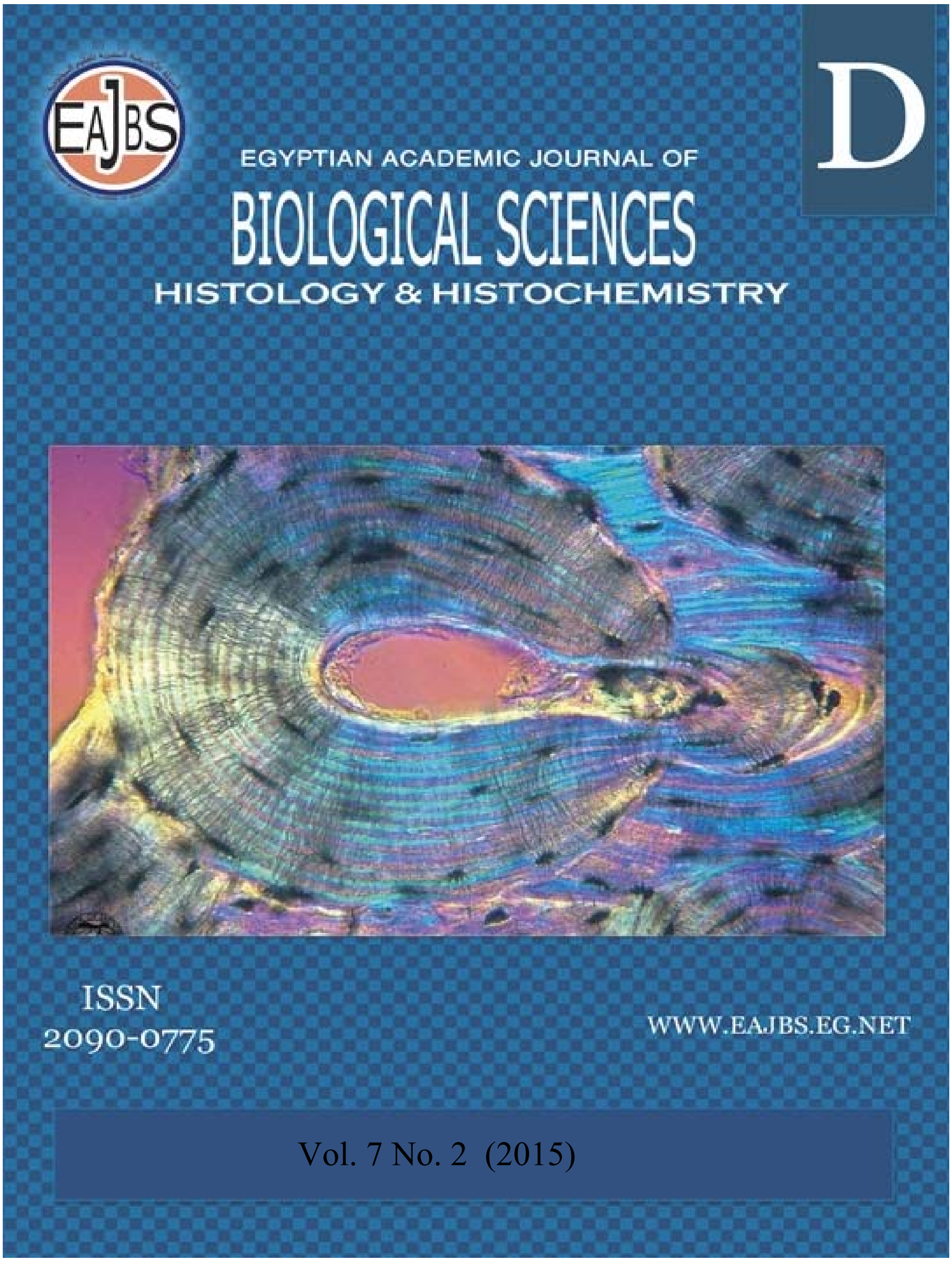

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Effects Of Soybean Meal Replacement With Dietary *Chlorella vulgaris* On Nutritional Performance And Liver Histology of Red Tilapia (*Oreochromis niloticus*)

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ABSTRACT

This study tested the potentials of dietary *Chlorella vulgaris* (CM) as a replacement for soybean meal (SBM) on the growth, nutritional performance and histology of the liver in the diet of Genetically Male Tilapia (GMT) Tilapia, *Oreochromis niloticus*. One hundred and eighty fish averaging 6.00 g were randomly stocked into 12 individual 30 -L flow-through tanks. Four isonitrogenous and isocalorific experimental diets were formulated to contain 38 % crude protein and 9 % lipids in which *Chlorella vulgaris* was used to replace soybean meal. The control was made up of fish meal (FM) which supplied 100% of the dietary protein; soybean (SBM) supplied 100% of the dietary protein in diet 2. *Chlorella vulgaris* was used to replace soybean meal at 50% and 100% in diets 3 (SCM) and 4 (CM) respectively. After the 5-wk feeding trial, the growth, nutritional performance and histology of the liver fish from each tank were assessed. Significant improvement were observed in survival, feed intake and liver histology of fish fed chlorella based diet SCM and CM, however the control had significantly better weight gain (0.79gram/day ±0.63) and feed conversion ratio (0.93 ±0.07) at the end of the 5-wk feeding trial (p>0.05). The cumulative mortality of fish fed the SCM and CM diets was significantly lower (p>0.05) than that of fish fed the FM and SBM diet at the end of the experiment. *Chlorella vulgaris* seemed to be an effective potential replacement for soybean meal for required growth, and good health of tilapia, *Oreochromis niloticus*.

INTRODUCTION

Fish (finfish and shellfish) play an important role in the world protein supplies, the global demand for fish and fish products is increasing as a result of increasing global populations (Delgado *et al*., 1999).

Furthermore there is increasing public awareness of the health benefits of consuming fish, in particular, but not exclusively due to the high levels of n-3 highly unsaturated fatty acids (HUFA) present in fish flesh (Simopoulous, 1991; FAO, 2010). The Aquaculture sector is emerging as a vibrant and significant production sector for high protein food. Capture fisheries sector has practically stopped developing since the mid-1980s, whereas aquaculture sector continued to an average annual growth globally. Global aquaculture supply of food fish has increased at three times the rate of world meat production from 1950 to 2008 and will continue to be relevant in the future as a source of protein in foods than they are today. However, the growth in aquaculture has caused major challenges; one of these challenges is the production of practical diets for the farming of fish. Increased fish farming has resulted into increased production of aqua feed which depends heavily on fishmeal as the main source of protein, because of its well-balanced nutrients (IFFO, 2008: Hussein, 2012). Therefore, it has become imperative to assess different protein sources as cheaper and appropriate replacement for fish meal as a dietary protein source in aqua feed, because of the increasing demand for fish meal and dwindling fish capture (Azaza *et al*., 2008). Soybean meal is the most widely used ingredient, among the plant protein sources considered in aquaculture diets. It is preferred as a replacement for fish meal due to its high-protein content, relatively well-balanced amino acid profile, reasonable price and steady supply. Many studies have shown that partially or totally replacing fish meal with soybean meal in fish could improve growth and reduce the cost of fish production. However, soybean meal is deficient in one or more essential amino acids contain and several compounds that may disturb the digestive process, in addition, there is evidence that soy bean induces enteritis in Atlantic salmon (NCR, 1993; Wilson *et al*., 2004, Bostock *et al*., (2012). Consideration should be given to locally available protein, lipid and carbohydrates sources, because of the economic advantages of cost reduction but not at the expense of the nutritional quality of the protein sources. Single cell proteins (SCP) are microorganisms and include the use of bacteria, yeasts, and algae, they have been considered to be a good alternative protein sources for fish meal (El-Sayed and Tacon, 1995). The high protein content and production rate of algae especially in developed, developing and under developed countries, as well as the ability to be cultured on a wide variety of substrates and waste makes it a possible replacement for fish meal in aqua feeds. Nandeesha *et al.* (1998) reported that many fish including carnivorous species can utilise algae as a part of diet formulation. Inclusion of algae in fish diets might result into better utilization of practical diets in aquaculture and improve the fish health (Mustafa *et al.,* 1994). The favourable profile of the essential amino acid and other essential nutrients in *Chlorella vulgaris* and the sustainable production method qualifies it as a potential replacement for conventional feedstuff and a main area of dietary algal research for finfish (Nandeesha *et al.,* 1998; Olivera- Novoa *et al*., 1998; Nakagawa, 2007, Palmegiano *et al.,* 2008, Liu 2010, Yp 2010). The overall resistance of fish to disease therefore depends on the nutritional status of the fish (Li *et al,* 2012); Good (2004) stated that the liver in finfish plays a central role in the regulation of lipid metabolism, where fatty acids are synthesised, esterified, and then secreted in the blood in the form of plasma lipoprotein complexes. Mumford (2007) stated that the fish liver is one of the most frequently damaged organs, it also play a primary role in metabolism, which is essential for providing fuel to the brain, muscle and other peripheral organs. Tilapia is the most adaptable and the third most commonly farmed fish after carp and salmonids, because of many cultivable qualities among which are good flesh quality and flavour, a wide tolerance of different environments, resistance to many common fish diseases, and relative ease of reproduction in captivity; more than 22 tilapia species are cultured worldwide. However, the most commercially cultured species are Nile tilapia, *Oreochromis niloticus* and blue tilapia, *Oreochromis aureus*. As a result of the growth and viability of tilapia industry, substantial increase in the global cultivation of tilapias has been recorded in the past three decades (El-Sayed 1998, Lim and Webster, 2006, Fitzsimmons, 2008 Madalla, 2008, Lupatsch, 2012). However, only a few researches have studied the potentials of dietary Chlorella as a replacement for conventional feedstuff and there is paucity of data on the effects of total replacement of fishmeal and soybean meal with dietary *Chlorella vulgaris* in the aquaculture of Tilapia. Therefore, research on the potentials of chlorella as a replacement for conventional feedstuff in fish is still highly desired. In this study, the effects of replacing soybean meal with dietary Chlorella vulgaris on the nutritional performance of Genetically Male Tilapia (GMT) Nile Tilapia, *Oreochromis niloticus* was determined using growth, nutritional performance, and histological study of the liver.

MATERIALS AND METHODS

Fish Holding Facility

The feeding trial was conducted in a freshwater recirculation system in the laboratory facility of the center for sustainable aquatic research (CSAR), Swansea University. The culture tanks were made of 30 liters rectangular tanks. Water was supplied to each culture tank through the inlet pipes and the effluent water was removed from the system through the outlet pipe. Water from the inlet pipe helps in aeration and facilitates self- cleaning. Aeration is also supplied with the aid of air stones, water temperature was kept constant at 26.5-27° C using a thermostatically controlled water heater , photoperiod was 12:12h light: dark.

Fish Handling

The fingerlings used for this trial were Genetically Male Tilapia (GMT) obtained as fry from Fishgen® Ltd. Swansea. Fry were reared at the CSAR for 1 month on a commercial fish feed containing approximately 45% protein and 13% lipid until they reached the appropriate size which was approximately 6.00g . Fish were handled according to standard procedure of the home office regulations as stipulated by the Animal scientific procedures Acts of 1986, UK. 15 fish were sampled, and stored in the freezer providing an initial sample for subsequent analyses. A total number of 15 fingerlings was bulk weighed and stocked in each of the 12 tanks. The trial lasted for 37 days, during which time weighing was repeated every week for the calculation of growth. During this period fish were fed *ad libitum* manually up to four times daily but new pellets were only added when all the feed were consumed from the feeding before. Mortality was monitored by removing and recording the number of any dead fish.

Diet formulation

Four experimental diets were formulated to contain 38 % crude protein and 9 % lipids in which *Chlorella vulgaris* was used to replace fishmeal and soybean. The composition of the ingredients used in this trial is shown in Table 1. The control was made up of fish meal (FM) which supplied 100% of the dietary protein; soybean (SBM) supplied 100% of the dietary protein in diet 2.1 *Chlorella vulgaris* was used to replace soybean meal at 50% and 100% in diets 3

(SCM) and 4 (CM) respectively. Vitamins and mineral premix was omitted from the diet formulation in order to assess to the effects of dietary *Chlorella vulgaris* supplementation on the growth and health of tilapia without the complementary nutritional and health benefits of vitamins and minerals premix in the formulated diets. Dry ingredients for each diet were mixed properly before the sunflower oil was added. Then, a doughy mixture was achieved by adding hot water. This dough was extruded through a meat grinder with 2.4 mm diameter orifice plate. The resulting spaghetti-like strands were dried in an oven for 24 hrs at 45° C. The dry mixture was afterwards broken up by a kitchen blender resulting in pellets of approximately 5mm in length and 2.4mm in diameter.

Table 1: Formulation of the experimental diets (for 1kg feed each).

	Diet 1 Fishmeal (FM) (g)	Diet 2 Soybean meal (SBM) (g)	Diet 3 SB +50%algae (SCM) (g)	Diet 4 100% Chlorella Meal (CM) (g)
Fishmeal	615			
Chlorella vulgaris			290	750
Soybean meal		660	410	0
Wheat starch	330	0	40	150
DCP[1]	0	40	45	50
Sunflower oil	25	70	45	20
Alginate	30	30	30	30

[1]DCP- Dicalcium phosphate - source of phosphorus
[2] Binder

Chemical analysis

Feed and whole body of fish were sampled at the beginning and end of the trial. Before the analysis fish were blended to a homogeneous mince using a meat grinder with a 4 mm diameter orifice plate. A sub-sample of this mince from each tank was taken and stored for estimation of dry matter which was determined after drying in the oven (Gallenkamp, UK) at 105°C for 24 hrs. The remaining fish homogenate was dried in the oven and used for all subsequent analyses. Ash content was calculated by weight loss after incineration in a muffle furnace (Carbolite, UK) for 12 hrs at 550ºC.

A Parr bomb calorimeter was used to calculate gross energy content, this method measures energy content by combustion under an atmosphere of compressed oxygen with benzoic acid as a standard. The Kjeldahl technique was used to measure crude protein. In this technique, the nitrogen (N) content is determined and multiplied by a conversion factor of 6.25.

Growth performance and nutritional utilisation

Growth performance and nutritional utilisation were determined according to the following parameters:

Daily weight gain (g/fish/day) = (final BW (g) - initial BW (g)) / days

Feed conversion ratio (FCR) = feed consumed (g)/ (final BW (g) - initial BW (g))

Specific growth rate (%) = 100 × (ln[final body weight]- ln[initial body weight])/no. of days.

Histology of the liver of experimental fish

Preparation of fixatives and Dissection of Tilapia

Two tilapia were randomly removed per tank, six per treatment, for histological study, MS-222, tricaine methane sulphonate was used for euthanizing fish. After complete desensitization, and death of the fish, the liver was removed by dissection. Liver tissues was fixed in Davidson's fixative**,** tissue were removed from the Davidsons' fixative by 24hrs then rinsed and put into

10% ethanol until processing. Longitudinal section of tissue was cut and mounted on glass slide. The tissues were embedded in paraffin, and two 4-µm sections of the front tentacle area were cut with a rotary microtome (Leica RM2235, Nussloch, Germany). These sections was stained with Haematoxylin and Eosin (H&E) and mounted. After cover slipping the tissues were stored at room temperature before examination under the Olympus BX 41 light microscope (Japan). Tissue sections were compared after examination under the microscope, for significant differences in the morphology of the tissues.

Statistical analyses

All data collected during the trial were tested for normality using Levene's homogeneity of variance test and subjected to one way analysis of variance (ANOVA). Differences between mean of treatments were considered significant at $P < 0.05$ using Tukey post hoc tests. All analyses were performed using SPSS software version 13 (SPSS Inc.).

RESULTS

Nutritional analyses and Growth performance of Tilapia

Table 2 showed the proximate composition of the experimental diets, this result confirmed the intended crude protein (37.8 ± 0.53) and energy content (18.45 ± 0.28 g) per kg feed. ^{a,b,c} values in each row with the same superscript are not significantly different (p>0.05) by using ANOVA Post Hoc (Tukey test).

Table 2: Analysed composition of the experimental feeds (as fed basis).

	Dry Matter (%)	Crude protein (%)	Ash (%)	Gross energy (kJ/g)
Diet1	92.26	38.30	12.90	18.05
Diet2	92.52	38.30	11.44	18.67
Diet3	91.67	37.69	10.66	18.49
Diet4	91.61	37.20	10.40	18.58

Figure 1 showed the growth of tilapia over the time period of the study. Difference in growth could be observed after the second week, fish fed FM had a progressive growth throughout the period of the experiment. The body weight of tilapia fed SBM was lowest at the end of the trial than the other three groups. By the final day (37), tilapia fed FM showed the best body weight followed closely by SBM.

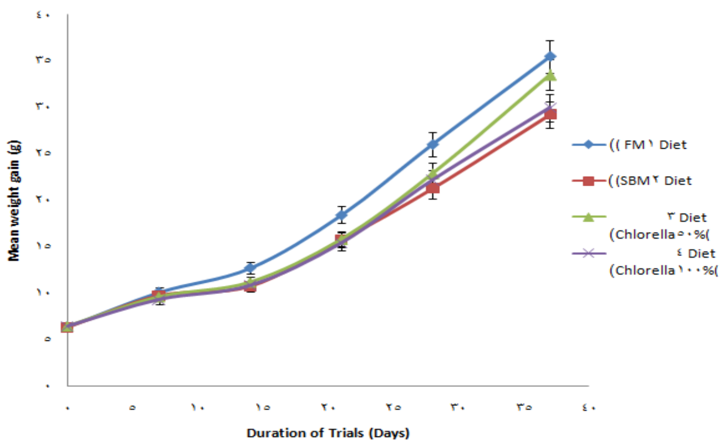

Fig. 1: Growth of O. *niloticus* fed the four experimental diets over a period of 37 days (mean values ± SD, n = 3)

Growth performance and nutrient utilisation of *O. niloticus* fed four experimental diets

The growth performance of tilapia fed the four experimental diets is shown in Table 3. Significantly highest weight gain of 29.11 ± 0.63 was obtained by tilapia fed FM by tilapia fed SCM whereas fish feed SBM and CM had significantly lowest growth performance of 22.87 ± 0.25 and 23.56 ± 0.70 respectively SCM (P< 0.05).

Table 3: Growth performance and nutrient utilisation of *O. niloticus* fed four experimental diets (mean ± SD, n=3).

	Diet 1	Diet 2	Diet 3	Diet 4
Initial (g)	6.40 ± 0.04^{a}	6.35 ± 0.01^{a}	6.42 ± 0.03^{a}	6.42 ± 0.03^{a}
Final (g)	35.51 ± 0.66^{c}	29.23 ± 0.25^{a}	33.57 ± 0.49^{b}	29.98 ± 0.68^{a}
Weight gain g/day	0.79 ± 0.63^{c}	0.62 ± 0.25^{a}	0.73 ± 0.49^{b}	0.64 ± 0.70^{a}
Feed consumed g/fish/day	0.73 ± 0.07^{a}	0.74 ± 0.03^{a}	1.00 ± 0.01^{b}	0.95 ± 0.02^{b}
FCR	0.93 ± 0.07^{a}	1.20 ± 0.01^{b}	1.36 ± 0.08^{b}	1.49 ± 0.07^{c}
SGR	4.63 ± 0.04^{c}	4.13 ± 0.25^{a}	4.47 ± 0.35^{b}	4.17 ± 0.70^{a}
PER	41.33 ± 3.21^{b}	33.33 ± 1.15^{a}	30.33 ± 1.53^{a}	28.00 ± 2.65^{a}
EER	37.33 ± 3.06^{c}	26.00 ± 0.00^{b}	23.00 ± 1.00^{ab}	21.00 ± 1.15^{a}
Survival (%)	60.00 ± 6.67^{a}	60.00 ± 0.00^{a}	82.22 ± 3.65^{b}	77.78 ± 10.18^{b}

^{a,b,c} values in each row with the same superscript are not significantly different (p>0.05) by using ANOVA Post Hoc(Tukey test).

Table 4: Analysed composition of tilapia fed the experimental diets (% wet weight basis mean ± SD, n=3).

	Dry matter %	Protein %	Ash %	Energy kJ/g
Initial	25.78	14.18	3.20	6.52
Diet1	26.16 ± 0.16^{b}	14.59 ± 0.35^{a}	3.71 ± 0.06^{a}	6.27 ± 0.06^{c}
Diet2	26.14 ± 0.18^{b}	15.60 ± 0.34^{ab}	3.94 ± 0.07^{b}	6.00 ± 0.06^{b}
Diet3	25.23 ± 0.06^{a}	15.12 ± 0.15^{ab}	3.83 ± 0.04^{ab}	5.56 ± 0.06^{a}
Diet4	25.23 ± 0.12^{a}	15.20 ± 0.63^{b}	3.75 ± 0.32^{a}	5.64 ± 0.12^{a}

^{a,b,c} values in each column with the same superscript are not significantly different (p>0.05) by using ANOVA Post Hoc (Tukey test).

The best feed conversion ratio (FCR) was recorded in FM which was 0.93 ±0.07 and highest in CM with 1.49 ±0.07 there were significant differences (P<0.05) in the feed conversion ratio in all the dietary treatments except diet SBM and SCM. Mortality was recorded in all the dietary treatments, the highest survival was recorded in SCM and CM diets.

Whole body composition of tilapia

Tilapia fed SCM and CM obtained intermediate values between FM and SCM regarding energy content , the lowest value of dry matter content was found in tilapia fed FM whereas highest dry matter, were found in fish fed the fish meal feed SBM and CM. All the fish in this study had increased percentage of protein and ash over the initial percentage, however lower percentages were recorded for energy compared to initial content. The ash content were similar for all treatments, with the exception of tilapia fed FM where the lowest ash content of 14.4 ± 0.22 fish were recorded (P<0.05) (Table 4).

Liver

In the livers of the fish fed the experimental diets, distortion of the cellular architecture of the hepatocytes and occasional breakdown of cells were seen in fish fed FM and SBM as shown in Figures 2 and 3, respectively. Histopathological changes were also noticed in the liver of fish fed FM and SBM, the cord-like parenchymal structure of the liver was lost, induced steatosis in the hepatocytes, Figure 2 and Figure 3. (circles), which showed highly

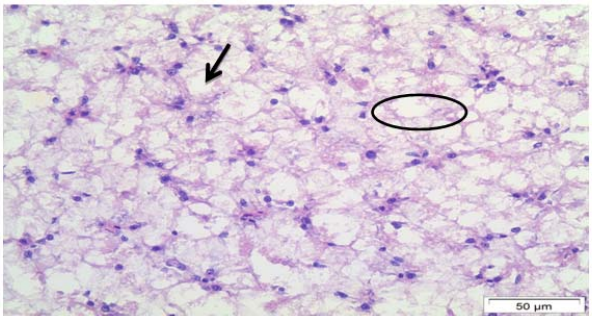

Fig. 2: Liver of fish fed FM showing some variability in fat distribution resulting in swollen and loss of hepatocyte.

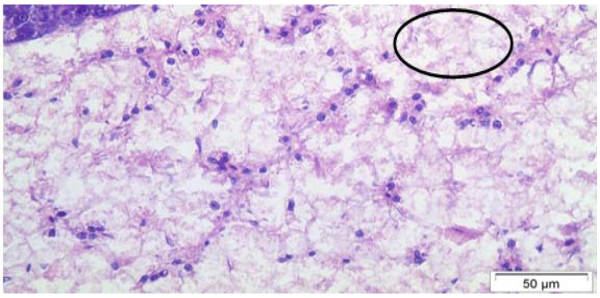

Fig. 3: Histopathological changes in liver of fish fed SBM showing that the cord-like parenchymal structure of the liver was lost, hepatocytes (circles) showed highly vacuolized cytoplasm and deformed nuclei because of lipid accumulation, vacoulation in the hepatocytes and sinusoid congestion.

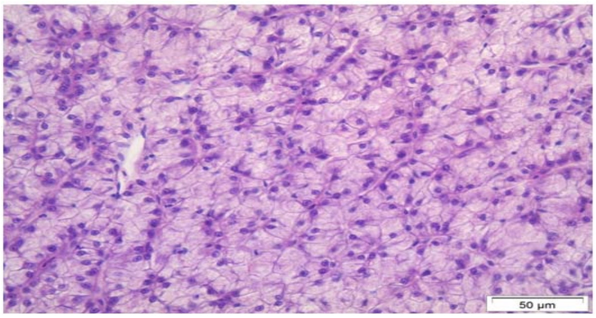

Fig. 4: Liver with normal hepatocytes morphology in fish fed SCM

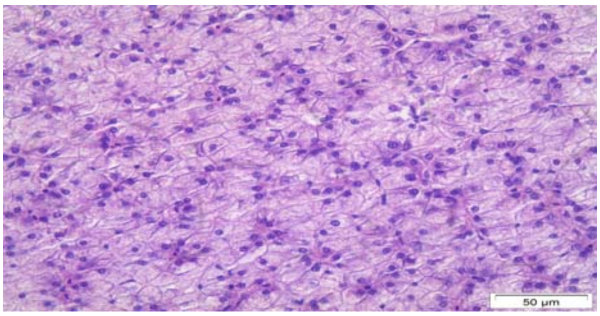

Fig. 5: Liver with normal hepatocytes morphology in fish fed CM

vacuolized cytoplasm and deformed nuclei because of lipid accumulation, vacoulation in the hepatocytes Figures 2 and 3 (arrow),sinusoid congestion and blood congestion in the hepatic vein. However fish fed SCM and CM did not show any histopathological changes in the liver, the histology of the liver of fish in these groups showed normal hepatocytes and cytoplasmic organelles as shown in Figures 4 and 5.

DISCUSSION

Growth performance and nutrient utilisation of *O. niloticus* fed four experimental diets

Fish fed FM showed the best growth and nutritional performance in the present study in term of weight gain and feed conversion ratiodespite the fact that vitamin and mineral premix was not added to the feed. This result agreed with major findings in fish nutrition, which stated that fish meal is still the major protein source in aquaculture feeds, because it is very palatable, highly digestible and rich in essential amino acids, which closely matches the amino acid requirement for fish and also a good source of fatty acids, energy and minerals, it has no antinutrients (Tacon 1993, El-Sayed 2006, Ogunji, 2004, Lim and Webster 2006, Gatlin *et al.,* 2007). There were significant differences (P<0.05) between the growth performance in term of weight gain and specific growth rate of fish fed FM and the other diets. Moreover, the growth performance in this study was consistent with the findings of Blake and Lupatsch (2012) who reported that growth performance and feed efficiency of *O. niloticus* was best when fed the fish meal diet and gradually decreased with increasing chlorella inclusion at the expense of fishmeal. Hussein *et al* (2012) also recorded differences in Nile Tilapia; *Oreochromis niloticus* fed different levels of corn gluten meal replacement using biofuel algae or spirulina. The highest weight gain and specific growth rate in the current study was recorded for fish fed FM followed by SCM , while the lowest was found in fish fed diet SBM and CM . Barrow *et al* (2008) found that vitamin premix did significantly increase survival and feed intake in *Oncorhynchus mykiss*, but positively affected the weight gain and feed conversion ratio, FCR. Miles and Chapman (2012) stated that fishmeal is considered to be a moderately rich source of vitamins of the B-complex especially cobalamine (B12), niacin, choline, pantothenic acid, and riboflavin; however the content of fat-soluble vitamins in fishmeal is relatively low because of their removal during extraction of the oil.

Koumi *et al.* (2009) stated that inclusion of 50 and 100% soy protein in diet decreased the growth of *O. niloticus*, compared with the fishmeal control, this decrease of fish growth can be due to the quantity and the quality of soy protein in diet. Goda *et al.* (2007) reported that soybean meal could fully substitute fish meal without a significant reduction in tilapia growth if the diet contained sub-optimal (28%) levels of protein. Growth reductions were observed when soybean meal substituted fish meal at 32% protein level (Koumi *et al.* 2009). The result in this study is also corroborated by Abdelghany, (2003) which recorded that the lowest feed efficiency and growth performances were shown in the fish fed diet containing soybean as a complete substitute for protein supplied by fish meal in diets for growth of red tilapia, *Oreochromis niloticus* × *O. mossambicus,* fingerlings. These reports clearly demonstrate that substituting animal protein with plant protein reduces the growth of tilapia (Spinelli *et al.*, 1983, Shiau *et al*. 1988, Lim and Dominy 1989, Liener, 1994, Khajepour and Hosseini 2012). In contrast, it has been demonstrated that dietary fishmeal levels can be considerably reduced without any adverse consequence in terms of somatic growth or nutrient utilization in *Oreochromis karongae* (Nyirenda*et al*., 2000), Milkfish *Chanos chanos* (Shiau *et al.,* 1988) and red tilapia, *Oreochromis niloticus* × *O. Mossambicus* (Abdelghany, 2003).

There was a consistent increase in the weight gain of fish fed SCM, which contained partial replacement of the soy bean with chlorella at 50 % of the protein source, from week three to the end of the experiment as shown in figure 3.. Growth and nutritional performance in term of weight gain (WG) and feed conversion ratio (FCR), this agreed with other works on red sea bream, rainbow trout, Japanese flounder and *Girella punctata* using chlorella at different supplemental levels also recorded improved weight gained and feed efficiency (Yone*et al.* 1986; Mustafa *et al*. 1995). However, dietary chlorella supplementation did not improve the growth and nutritional performance in ayu, *Plecoglossus alrivelis,* (Nematipour *et al.* 1988, Nakagawa *et al.* 1997). Kim *et al* (2002) summarised that dietary Chlorella supplementation rates in fish are species specific.

CM which had significantly reduced growth and nutrient utilisation in term of weight gain (WG) and, specific growth rate (SGR) and feed conversion ratio (FCR), this agreed with the work of Husseini *et al.* (2012) where higher amount of algae (100%) significantly depressed growth and feed utilization in terms of WG, SGR and FCR in *O. niloticus*. Blake and Lupatsch (2012) reported that the reduced growth performance of tilapia fed chlorella based diet and the inability of the fish to utilize the algal feeds effectively as the fishmeal feed was most mainly due to the low digestibility of Chlorella. Moreover, Tartiel *et al.* (2008) stated that chlorella has low levels of sulfur-amino acids and has a high percentage of unsaturated fatty acid. Low methionine levels in the plant protein diets (soybean, corn gluten, maize, Spirulina, and chlorella) were expected to result in poor growth, for all animals. Therefore ingredients that contain no or low amount of methionine would consequentially produce reduce growth. (Lovell 1989; Cowey and Walton, 1992).

Nutritional analyses and Whole body composition of tilapia

There were dietary effects and influences on the body composition of experimental fish in this study. Table 4 showed that fish fed SBM, SCM and CM exhibited higher ash content than those fed diet 1 with fish meal inclusion, Koumi *et al* (2009) reported that the carcass ash content increased with highest level of ash and fish meal content in diet *O. niloticus*. Fish meal has high levels of minerals including phosphorus associated with the bone fraction which were highly available and retained for *O. niloticus*. In this study the diets containing 100% soybean meal as protein source were not efficiently utilised for growth of *O. niloticus* this was supported by the work of Abdelghany (2003) and Koumi *et al.* (2009) which reported that the lipid and energy contents in fish carcass increased with dietary lipid and soy protein level, these studies also suggested that there were increased lipogenesis with increasing levels of lipid and energy contents in fish carcass as observed in FM and SBM which had the highest energy levels in this study.

Feed intake and palatability

The best feed intake was recorded in fish fed SCM similar to this result Blake and Lupatsch (2012) found that feed intake was higher in three algal feeds with 30, 60 and 100% Chlorella included at the expense of fishmeal. Furthermore, Hussein *et al.*, (2012) recorded that the best feed intake in algae based diets may have been due to increased fish appetites; high acceptability for alga based diet among cultured tilapia was due to their higher palatability and preference of the fish to take it as their potential food. However, too much feeding does not necessarily result in higher growth, beyond a certain level excessive feeding has no influence on the growth, the actual feed intake, which is the amount of feed that the fish is physically able to consume will produce the feeding level at which the fish grows best and the FCR is minimal (De Silva and Anderson 1998, Lim and Webster, 2006). Fish in treatment 1 in this study produced the best growth despite having lower feed intake than fish fed diet SCM and CM which may be related to the balanced amino acid profile of the fish meal based diet.

Survival

Mortality was first recorded after the second week of the experiment and gradually increased till the end of the experiment. Death in the tanks did not occur suddenly, there was usually a gradual withdrawal from feeding, inactivity, with fish fed FM and SBM showing scorbutic – like deficiency symptoms (Dabrowski, 2001). Soliman *et al.* (1994) recorded that vitamin and mineral premix is important in aquaculture fish feeds. Survival was significantly different and mortality was recorded in all the treatments. However, fish fed Chlorella based diet in treatment 3 and 4 showed higher survival rate than fish fed FM and SBM. Vitamin and mineral deficiency were the suspected cause for this decrease in survival. Dietary vitamin and mineral deficiency resulted in increased mortality in fish and the inclusion has been reported to be potentially beneficial in increasing and reducing oxidative damage to tissues (Dabrowski, 2001, Khan and Zhera 2012). The survival trend agreed with the study of Hussein *et al.* (2012) where Spirulina improved the survival in the diet of Tilapia when fed ad libitum. Hirahashi *et al.* (2002) suggested that Chlorella have immune potentiating abilities similar to Spirulina. Kim *et al.* (2002) also reported higher survival rate with increasing level of Chlorella in the diet of Juvenile Japanese Flounder, *Paralichthys olivaceus* with the inclusion of vitamin and mineral premix.

Liver

One of the major significant differences between fish fed chlorella based diets and other dietary treatments in this study were the changes in histological appearance of the tissues mainly in the liver. Various histopathological changes were noticed in the liver of fish fed diet FM and SBM which were not noticed in fish fed the chlorella based diets in treatment 3 and 4, these changes were as follows: the cord-like parenchymal structures of the liver were lost, induced steatosis in the hepatocytes. These changes were observed by Bobadilla *et al* (2005) when fish meal was replaced by plant protein source, which in some cases led to steatosis, with some aggregates of macrophages surrounding pancreatic acini. Fish fed SCM and CM did not show any histopathological changes in the liver, the histology of the liver of fish in these groups showed normal hepatocytes and cytoplasmic organelles. The positive influence of chlorella on the liver of fish in this study is supported by the report of Nakagawa (2007) on the effects of algae on fish physiology which stated micro-algae generally enhanced a synergistic effect of algae and certain vitamins, Chlorella-extract distinctly improved tolerance of hypoxic conditions and liver function, this may be as a result of some internal barrier to disease. In commercial aquaculture diets, the contributions of vitamins from ingredients are not normally considered. However, they could make a significant contribution to fish performance, hepatocytes integrity seemed to be a more sensitive measurement of vitamin status than growth, and nutritional performance in health of rainbow trout, *Oncorhynchus mykiss* fed vitamin premix un-supplemented diet (Barrow *et al*, 2008). In this study, there were significant differences in hepatocytes for fish fed the diets with chlorella without vitamin premix compared to those fed diets with FM or SBM without vitamin and mineral premix supplementation. Fish fed chlorella based diets had hepatocytes with normal morphology, unlike fish fed diet without chlorella which had disorganised and decreased number of hepatocytes as shown in Fig 2 to 3. Vitamins C (ascorbic acid, AA) and E (tocopherols) are strong antioxidants, these two vitamins have been extensively studied in fish nutrition, they affect affects growth and non-specific immune responses which play important roles in growth and immunity of fish (Lim and Shiau, 2005). Puerto *et al* (2010) stated that the antioxidant activity of naturally occurring dietary antioxidants is one explanation for their effectiveness at preventing histopathologies in the liver of *O. niloticus*, these vitamins seemed to provide some protective, anti-oxidant, effect on the liver of fish fed chlorella based diet in this study therefore increasing survival of fish , this was also reported by Barrow *et al.* (2008) that the presence of vitamins C and E increased survival in rainbow trout. Vitamin and mineral deficiency may be partly responsible for the histopathological changes noticed in the liver of fish in the present study. Baudin *et al.* (1989) stated that histopathological examinations were carried out on the liver of seabass fed ascorbic acid deficient feed, and lipid vacuolisation was particularly significant, simultaneously with degeneration phenomena (nucleolus swelling, chromatin margination), ceroid deposits and granulomatous hypertyrosinaemia was directly related to vitamin deficiency. Amar and Lavilla-Pitogo (2004) stated that deposits of lipids in the liver can be directly traced and be used to diagnose vitamin and mineral deficiency signs in fish. Most compounds absorbed by the intestine pass through the liver, which enables it to regulate the level of many metabolites in the blood. The liver is therefore necessary for survival of vertebrates including fish, the various functions of the liver are carried out by the liver cells or hepatocytes and the destruction of these cells can lead to liver failure and death of fish this is shown by the pattern of mortality in this study.

CONCLUSION

This study provided data suggesting that *Chlorella vulgaris* can be considered as a potential feed component for replacing fish meal and soy bean for required growth, and good health of tilapia, *Oreochromis niloticus*.

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