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Effect of dietary high protein frog meal supplementation on the anti-hypercholesterolemic influenze, growth performance, feed conversion and blood serum chemistry in tilapia, *Oreochromis aureus*

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Abstract
The frog high protein meal supplementation was employed to ascertain its anti-hyper-cholesterolemic effect and growth performance on the local farmed pond tilapia, *Oreochromis aureus*. Forty healthy tilapia (0.11±0.02 kg) were randomly allotted into four groups fed with different treatments for 40 days experimental feed trials, viz. one group fed standard diet (SM control group), one group fed high cholesterol diet (CM) and two groups fed with different diets (FM, 2FM groups) containing variable strength of frog high protein meal supplement. The dosage for the two diet groups viz. FM group was fed frog protein meal strength 1g/1kg BW/d while 2FM group was fed 2g/1kg BW/d. Tilapia fed frog protein meal had elevated significantly ($P<0.5$) HDL levels by 8.4% (0.71±0.13 mmol.L^{-1}) of its initial value (0.65±0.26 mmol.L^{-1} at day 0); while lowered the total cholesterol by 37.5% (1.90±0.21 mmol.L^{-1}) of its initial value (3.04±0.15 mmol.L^{-1} at day 0) on day 40 respectively during the study period. In addition, the blood TG and LDL are lowered by 17.9% (initial value of 0.67±0.05 mmol.L^{-1} at day 0) and 9.5% (initial value of 0.73±0.03 mmol.L^{-1}) for the 2FM group respectively. This reflects the ability of the high protein frog meal supplement to function as an anti-hypercholesterolemic effect on the treated tilapia fed indigenous high protein meal. The liver-kidney markers and related biochemical enzyme indicators in the treated and untreated tilapia, viz. GGT and ALT appeared to remain stable; however, the blood BUN, creatinine and urea increased by 31.1%, 6.2% and 21.2% of their initial levels all the way throughout the experimental period. Moreover, supplementation in the tilapia had also elevated significantly ($P<0.5$) the protein levels by 13.6% and calcium by 4.2% of their initial levels on day 40 respectively. Tilapia fed frog meal gave better performance than those fed only with the standard commercial and the high cholesterol meals. There was insignificant differences between all groups in terms of their average final weights ($p>0.05$). Insignificant differences were observed in specific growth rate, feed conversion rate and weight gain. However, there were significant differences in terms of feed conversion rates between groups ($p<0.05$) and it was the highest in group 2FM (1.39±0.13) fed with frog meal and the lowest in group CM (1.98±0.10) fed high cholesterol diet. It has been shown that the best percentage specific growth rate (1.51±0.12%) and live weight gain (6.38±0.55 g) were recorded in 2FM group.

Keywords: frog meal, blood serum chemistry, lipoprotein activity, *Oreochromis sp.*

1. Introduction
The important of fish supplementary feeding has been well realized in the modern aquaculture and has been stimulated intensive research on the formulation of cheaper feeds. Many attempts were made to develop cheaper nutritive diets with local high protein animal source as the principal sources of protein. These ingredients are rich in protein and are easily available at low cost. The diets developed can be tested by comparing their performance with a standard fishmeal-based diet fed to fish.

Protein is the main constituent of the fish body; as such, a generous dietary supply is needed for rapid growth. Furthermore, protein is more expensive than carbohydrate or fat therefore the amount of protein in the diet should be limited to that which is needed for growth and tissue repair while the energy should come from the cheaper sources. As for the protein level in fish diets, fish required a higher percentage of protein in their diet than do warm blooded animals. For example, the optimum level of protein in practical diets for warm water food fish is 30 to 36%, whereas in poultry diets the practical level is 16-22% (NRC, 1993) [17]. Fish are among the most efficient of animals in converting food energy to body tissue, partly because they require less than 10% of the energy for maintenance required by birds or mammals of the same size. Fish foods, often expressed as a percentage of dry matter, seem to be high in protein content; thus what appear to be a high requirement for protein is really a low requirement for energy (Gatlin *et al*., 1986) [11]. Proteins and lipids in food are important energy sources for many species of commercially raised fish; however the value of dietary carbohydrates varies especially among species (NRC, 1993) [17]. Demands for energy are influenced by physical activity, water temperature, body size and stress. Protein and essential amino acid requirements have been primarily studied in juvenile fish viz. channel catfish declined as the fish approached maturity (Page & Andrews, 1973) [19]. Studies have shown that hypercholesterolemia state could contribute significantly to the development of atherosclerosis and coronary heart disease. Hypercholesterolemia is a state of elevated serum cholesterol which results from the overproduction and under-utilization of low density lipoprotein. One of the earliest reports on the reduction of blood serum cholesterol by high plant protein in alga, *Spirulina* was carried on rats by Devi and Venkataranam (1983) [7]. Kato *et al*., (1984) [16] carried out feeding experiments in rats, and reported that the elevation in total

cholesterol (TC), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) and phospholipids in serum caused by cholesterol feeding was reduced when the high cholesterol diet was supplemented with 16% *Spirulina*. The vast majority of fish that we ate daily was gravely devastated by adulteration of aqua-feeds or fish feeds with unnecessary growth hormones and antibiotics. To replace or supplement the feed with quality nutritive food sources preferably from animal origins rich in protein, minerals and vitamins is of upmost importance.

The determination of plasma blood chemistry in terms of the total cholesterol, triglyceride and high density lipoprotein is important to evaluate the health status of the fish liver and kidney. Iwata *et al*., (1990) [15] reported that high protein cyanobacteria had an inhibitory effect on the elevation in plasma cholesterol, triglyceride, and phospholipid in the fructose induced rats. In the present study, we carried out an experimental research on the effect of dietary supplementation of the locally farmed frog (*Rana catesbeiana*) for its high protein source on the growth, feed conversion and plasma lipoprotein activity in tilapia cultured for human consumption**.**

2. Material and Methods

The proximate analysis of crude protein, fiber, lipid, fat, moisture and phytonutrient were carried out in the nutrition laboratory of the Faculty of Veterinary Medicine. Blood chemistry and other related pathophysiological tests were carried out in the path-lab facilities of both the private and the University. The mature fully grown tilapia, *Oreochromis aureus* obtained from the local fish ponds, were kept in aquariums in the animal room controlled at 22± 1^0C temperature and 55± 5% humidity under lighting at intervals of 12 h.

The fish were divided into 4 groups of 8 fish (body weight, 0.12 ±0.02 kg). The control group (SD) was fed on the standard commercial fish diet. The other experimental groups were fed on high cholesterol diet (CD), frog protein meal (FD) and high frog protein meal (2FM). Tilapia under experiment were fed twice daily with these diets. Body weight was recorded once a week, and food intake was weighed at every other day during experimental period. The crude protein, ash and moisture contents were determined following AOAC (1975) procedures. The fish were subjected to intravenous injection of heparin at the dose of 200 U/100g body weight through the dorsal left cervical vein under anaesthesia with Numbutal after fasting for 12 h. Plasma total cholesterol, HDL- cholesterol, triacylglycerol, and HDL were determined by enzymatic methods using assay kits (Takayama *et al*., 1977) [27]. Statistical analysis was performed by ANOVA while the significant difference between the different diet groups was done with the t-test.

Research animals and experimental set-up

Forty healthy local tilapia, *Oreochromis aureus* were randomly allotted into four groups fed with different treatments for 40 days experimental feed trials, viz. one group fed standard diet (SM control group), one group fed high cholesterol diet (CD) and two groups fed with different diets (FM, 2FM groups) containing variable strength of frog protein meal supplement. The frog protein meal is derived from the bull frog (*Rana catesbeiana*) which is commercially farmed locally for its meat production. Tilapia was divided into 4 groups; tilapia fed standard diet (SD), high cholesterol diet (CD) and treated diets comprised of FM (1g/1kg body weight/d) and 2FM (2g/1 kg BW/d). The nutrient composition, including protein, carbohydrate, fiber and lipid of the standard diet (SM), cholesterol diet (CD) and treated groups (FM, 2FM) were analyzed accordingly by method of AOAC (1997) [3].

Laboratory set-up and biochemical pathophysiological tests

For the blood analysis, 3 ml blood was withdrawn from the fish caudal vein using a needle with the size of 21 G; the blood samples were centrifuged at 5000 rpm for 10 min before being subjected onto automatic analyzer Roche Coba Mira (Thermo Fisher Scientific, USA) and Hitachi 902 Machine for serum cholesterol and kidney-liver analysis specifically total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG), high density lipoprotein (HDL), aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transferase (GGT), creatinine (Cr), blood urea nitrogen (BUN) and minerals (Ca, P). All blood samples were collected at day 0 (pre-treatment), 20, 30 and 40 (post-treatment).

Statistical analysis

The blood results were determined for statistical significance (p=0.05) using one-way ANOVA by SPSS statistical software (SPSS 16.0 for windows) and Tukey's test was used for pairwise comparison of the mean values.

Ethical guideline

The ethical guidelines on handling the experimental animals were in observance with the standard operational procedure of animal ethics which were verified by the committee of the Faculty of Veterinary Medicine, University Malaysia Kelantan (UMK) and the Kelantan Veterinary Services Department.

3. Results & Discussion

Growth performance in tilapia

The proximate composition of feed material used in the experiment as shown in Table 1 which showed that all values of the tilapia feed composition changed significantly, especially protein and lipid contents in the experiment diets. Butter has been added to the CM feed meal materials; this has increased most of the chemical compositions, especially lipid and ash. Precisely, it is noted that the high cholesterol diet lowered the ash content by almost 14.3% and 15.4% loss compared to SM diet and FM diet respectively. In actuality, butter is composed of the high saturated fat which has been suggested to be the agent responsible to trigger hypercholesterolemic event in human (Wardlaw & Snook, 1990; Almendingen *et al*., 1995; Engel & Thostrup, 2015) [28, 4, 10] as well as in animal (Snowmya & Ananthi, 2011; Zare *et al*., 2012) [25]. Together with carbohydrate and protein, high content of fat or lipid is related to weight gain. All the three feed meal components have different energy component viz. fat having 9 kcal/g while protein and carbohydrate with 4 kcal/g each respectively. Fat has been suggested to be the most efficient fattening factor in weight gain (Swinburn *et al*., 2004) [26]. According to Schwartz *et al*., (1992) the feed that content higher fat might promote the weight gain because it owns a lower thermic effect compare to carbohydrate or protein.

Table 1: The proximate composition of the tilapia feeding experimental diets

Composition (%)	SM	CM	FM	2FM
Crude carbs	21.4±0.61a	14.8±1.04b	15.1±1.16c	14.7±0.74°
Crude protein	13.2±0.81a	11.5±0.88b	37.1±1.51c	58.3±2.2113d
Crude lipid	10.7±0.61a	19.1±0.72b	8.01±0.78°	7.97±0.61c
Crude fiber	7.19±0.74a	6.95±0.74b	7.01±0.63c	6.97±0.86c
Ash	6.09±0.34a	5.15±0.24b	6.01±0.43ac	5.97±0.36ac

SM standard diet group; CM cholesterol diet group; FM frog protein single strength diet group (1g/1kg BW/d); 2FM frog protein double strength diet group (2g/1kg BW/d) diet group; Values (mean ±SE) with different superscripts in the same row are significantly different at the 5% level, N number of animals in the group=8; BW body weight; carbs is carbohydrate.

The protein content in the FM and 2FM meals is raised to almost double to quadruple that of SM group respectively. The other feed components of the tilapia fed frog meal viz. crude carbohydrate and fiber did not showed much variation in values to those of CM group. The protein content in the FM (37%) and 2FM (58%) groups were 2.6 times and 4 times higher than that of the SM group respectively. In the case of lipid component in the feed, there is significant decrease of lipid component for FM and 2FM with a drop in values of 2 to 5 times lower than that of the CM group. However, the percentage composition of crude fiber in all the fish diets did not show much variation.

There were no significant different among all groups ($p<0.05$) in term of growth parameters; however, the high protein fed frog meal groups had increased their weight gain by 17.5% (6.24±0.53g) and 20.1% (6.38±0.01g) of their initial values for the FM and 2FM respectively (Table 2). The survival percentage indicators were significantly affected by the dietary treatments ($p<0.05$) and its value was the highest in the FM and 2FM groups. The values for final weight, weight gain and specific growth rate were observed to increase especially the FM and 2FM groups. Kamalaveni *et al*., (2009) and Salehi-Farsani *et al*. (2014) recorded that catfish fed high protein content cyanobacteria, *Arthrospira* sp resulted in comparatively better food conversion factor (FCR) than if fed with normal commercial diet. FCR is characterized as the amount of feed given and consumed per unit of weight gain. It is noted that high FCR value in high cholesterol fed the catfish can be related to the bad quality feed, lack of natural resources, poor growth condition and overfeeding (Coche & Muir, 1998). The three branched chain amino acids in *Arthrospira* sp viz. leucine, valine and isoleucine (Gunsett, 1984) [13] are responsible for the increase in body weight in fish and poultries (Rodehutscord *et al*., 1997; Ahmed & Khan, 2006; Peganova & Eder, 1997, Gershwin & Belay, 2008) [23, 1, 20, 12].

Table 2: Growth performance and survival rate of tilapia fed with experimental diets for 40 d of treatment with diets (SM, CM, FM and 2FM)

Growth performance	SM	CM	FM	2FM
Initial weight (g)	95.2±0.87a	95.8±0.59	99.9±0.5lab	102.9±0.82a
Final weight (g)	112.9±1.16a	113.2±1.73ab	116.1+1.72c	119.1±1.21°
Weight gain (g)	5.38±0.31a	5.41±0.41b	6.24±0.53C	6.38±0.55°
SGR (%)	1.31±0.19	1.29±0.16b	1.49±0.1P	1.51±0.12d
FCR	1.93±0.21a	1.98±0.13b	I.41±0.14c	1.39±0.13d
Survival (%)	87.5±8.2 P	85.0±7.21b	90.1+7.2P	89.6±8.2PI

SM standard diet group; CM cholesterol diet group; FM frog protein single strength diet group (1g/1kg BW/d); 2FM frog protein double strength diet group (2g/1kg BW/d) diet group; Values (mean ±SE) with different superscripts in the same row are significantly different at the 5% level, N number of animals in the group=8.

The present study showed that the use of frog meal source did provide better growth as well as the health status of their blood chemistry when compared to the group fed commercial or high cholesterol feed meals. James *et al*., (2006) [26] reported that the specific growth rate SGR, feed intake and mean body weight in the red swordfish (*Xiphophorus helleri*) increased with increasing level of the high protein alga source such as the *Spirulina*. Similarly, Jaime Ceballous *et al*., (2005) reported the effect of high protein alga meal inclusion in micro-diet for white shrimp, *Litopenaeus schmitti* larvae and found that the survival rate was around 80% for all treatments when compared to the control.

Table 3 showed increase in the values of tilapia total protein (TP) and calcium (Ca); while the albumin remained stable during the 40 day feed trial study period. The change in the level of TP and Ca had insignificant influence the tilapia blood chemistry. There was a significant increase in the blood protein levels in the frog meal groups with values of 13.6% (initial value 54.4±5.80 $g.L^{-1}$) and 25.1% (initial value 39.1±3.40 $g.L^{-1}$) increment on day 40 for the FM and 2FM groups respectively; however the SM group did not showed any significant change. For the CM group, there was a decrease of total protein from 17.8±1.03$g.L^{-1}$ to 15.1±1.93$g.L^{-1}$ indicating a 15.1% drop in the protein.

Table 3: Values of total protein (TP), Albumin, Gamma GT and Calcium (Ca) in tilapia on 0, 15, 30 and 40 d of treatment with diets (SM, CM, FM and 2FM)

Time (d)		SM	CM	FM	2FM
Total Protein (g.L-1)	0	12.6±1.08ab	17.8±1.03 ab	54.4±4.78ab	39.1±1.88ab
	15	11.8±1.19a	17.1±1.16b	56.4±4.78ab	42.2±6.13c
	30	13.2±1.12ab	18.6±1.19b	60.9±4.78ab	43.6±6.14ab
	40	12.1±2.17a	15.1±1.93b	61.8±4.78ab	48.9±6.48c
Albumin (g.L-1)	0	10.6±1.18ab	12.8±1.13ab	31.7±3.14ab	30.4±3.18ab
	15	10.8±1.29a	11.1±1.16b	32.2±3.13c	31.5±3.16d
	30	10.2±1.12ab	12.6±1.19b	33.6±3.14ab	30.9±3.82c
	40	10.1±2.17a	11.8±2.13b	32.9±3.18c	31.8±3.78d
Ca (mtnol.L-1)	0	1.30±0.23a	1.49±0.27b	2.35±0.41bc	2.60±0.26d
	15	1.35±0.31ab	1.48±0.11b	2.39±0.13c	2.89±0.24c
	30	1.33±0.27a	1.49±0.17b	2.38±0.11°d	2.80±0.28d
	40	1.31±0.22a	1.48±0.15b	2.27±0.12ed	2.71±0.23d

SM standard diet group; CM cholesterol diet group; FM frog protein single strength diet group (1g/1kg BW/d); 2FM frog protein double strength diet group (2g/1kg BW/d) diet group; Values (mean ±SE) with different superscripts in the same row are significantly different at the 5% level, N number of animals in the group=8.

Furthermore, the albumin levels also did not showed any noticeable change in all experimental groups (SM, CM, FM, 2FM) during the study period. Interestingly, the level of blood calcium did increase with values of 3.4% (initial value 2.27±0.12 mmol.L^{-1}) and 4.2% (initial value 2.71±0.23 mmol.L^{-1}) on day 40 for the FM and 2FM groups respectively; however the SM and CM groups did not showed any significant change. It is noted in both case, the percentage increase in blood protein and calcium is insignificant with increase fed in frog meal.

Studies on the effect of high protein supplement on tilapia are scare and rare; thus, comparison has to be made on other animals. Fish are among the most efficient of animals in converting food energy to body tissue, partly because they require less than 10% of the energy for maintenance required by birds or mammals of the same size. As a consequence, protein efficiencies (protein gain/ protein fed) of 45% have been seen in catfish fed diets containing 11.4 Kcal of digestible energy (DE) per gram of protein (Gatlin *et al*., 1986) [11]. Optimum DE to protein ratios that have been determined for fish are twice dietary protein for terrestrial farm animals (Smith, 1980; Lovell, 1998) [24]. The National Research Council (NRC, 1993) [17] has published optimum ratios for weight gain and the relationship of digestible protein to digestible energy of several species of fish.

The protein and essential amino acid requirements, expressed as a percentage of air dry weight diet, decline as fish approach maturity. As examples, channel catfish weighing 14 to 100g require 35% protein, while channel catfish weighing 114 to 500g require 25% protein (Page & Andrews, 1973) [19]. Similar differences with age have been described for salmonids, common carps and tilapia (Wilson & Halver, 1986) [11]. Studies had shown that amino acid deficiencies generally impaired growth; a deficiency of methionine also leads to cataracts in Atlantic salmon, rainbow trout and lake trout (Poston *et al*., 1977) [21]. Tryptophan deficiency has also been associated with cataracts in rainbow trout (Poston & Rumsey, 1983; Walton *et al*., 1984) [22] and leads to scoliosis (lateral curvature of the spine) in rainbow trout (Walton *et al*., 1984), sockeye salmon (wilson & Halver, 1986) [11] and chum salmon (Akiyama *et al*., 1986).

Table 4" showed the values of blood total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG) and high density lipoprotein HDL for a study period of 40 day of treatments imposed on the tilapia fed on various diets. Values of plasma cholesterol remained unchanged and stable in control group throughout the observation period; however, TC values decreased significantly ($p<0.5$) to 27.8% and 37.5% for FM and 2FM respectively until the end of experiment. The cholesterol rich diet (CM) induced a 60% (initial value 1.82±0.11 mmol.L^{-1}) fold increase in plasma cholesterol at the end of 40th day. At the end of the study period, total cholesterol in plasma from fish fed with cholesterol rich diet (CM) was significant higher ($p<0.05$) by more than 2 folds compared with the control group. Typical plasma cholesterol concentration for fish in the CM group during the study period is in the range of 1.82 - 2.91 mmol.L^{-1} and the values encountered during the study period are within the upper range of the normal range.

Table 4: Values of total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG) and HDL-cholesterol in the tilapia on 0, 15, 30 and 40 d of treatment with diets (SM, CM, FM and 2FM)

Time (d)	SM	CM	FM	2FM
TC (mmo1.L-1)				
0	2.71±0.27a	2.83±0.11b	3.04±0.151x	2.33±0.31k
15	2.69±0.3P	2.51±0.41b	2.79±0.32C	2.19±-0.328
30	2.70±0.461	2.31±0.32b	2.33±0.37C	2.05±0.32d
40	2.68±0.361	2.01±0.33b	1.90±0.211x	1.68±0.39bc
TG (nuno1.L-1)				
0	0.49-10.021	0.21±0.03k	0.59±0.01C	0.67±0.05d
15	0.47±0.075	0.49±-0.06"	0.51±0.02Ix	0.59±0.02k
30	0.48±0.015	0.56-1-0.03"	0.49±0.05Ix	0.56-1-0.09d
40	0.49±0.245	0.59±0.08k	0.48±0.09c8	0.55±0.01d
LDL (mmol.L-1)				
0	0.69±0.025	0.38±0.03k	0.38±0.01bc	0.73±0.03d
15	0.71±0.075	0.62±-0.03b	0.27±0.026c	0.65±0.02d
30	0.68±0.015	0.55±0.03b	0.19±0.066c	0.63±0.09d
40	0.71±0.045	0.69±0.08b	0.17±0.096c	0.66-1-0.07k
HDL (mmol.L-1)				
0	1.66±0.131	2.31±-0.17th	1.98±0.12k	0.65±0.26k
15	1.65±0.1P	1.51±-0.11"	2.01±0.23k	0.61±0.14bc
30	1.67±0.17a	0.96-1-0.27"	2.08±0.1Pd	0.70±0.18d
40	1.64±0.22a	0.85±-0.15"	2.15±0.12cd	0.69±0.13d

SM standard diet group; CM cholesterol diet group; FM frog protein single strength diet group (1g/1kg BW/d); 2FM frog protein double strength diet group (2g/1kg BW/d) diet group; Values (mean ±SE) with different superscripts in the same row are significantly different at the 5% level, N number of animals in the group=8.

The present values showed that the frog meal diet group 2FM showed a decrease of plasma total cholesterol of 1.90 ± 0.21 mmol.L^{-1} at day 40 from 3.04±0.29 mmol.L^{-1} at day 0; while those of HDL increase to 0.71 ± 0.13 mmol.L^{-1} from 0.65±0.26 mmol.L^{-1} respectively. There was a slight decrease in the TG value from 0.67±0.05 mmol.L^{-1} to 0.55±0.01 mmol.L^{-1} while those of LDL also decrease from 0.73±0.03 mmol.L^{-1} to 0.66±0.07 mmol.L^{-1} on day 40. Thus the high protein meal from the frog diet was able to reflect as an anti-hyperlipidermic agent which is capable of preventing the high blood cholesterol induced by the high cholesterol diet.

The detailed summarized result of the kidney-liver was shown in Table 5. The liver-kidney markers and related biochemical enzyme indicators in tilapia fed frog meal supplement such as BUN, creatinine and urea showed significant decrease in their blood values; while conversely, those of the uric acid showed significant increase in value especially the tilapia fed frog protein diet. In actuality, uric acid which is derived from purine can be found in the feed that is the causing agent for gout in human (Allard, *et al.*, 1994). Table 5 showed comparatively that blood uric acid increase significantly by 49.6% (initial value 389.4±56.8 µmol.L^{-1}) and 25.4% (initial value 322.7±33.4 µmol.L^{-1}) on day 40 for the 2FM and FM treated groups respectively. In the case of BUN, creatinine and urea, their values also showed significant decrease by 31.1% (1.97±0.18 mmol.L^{-1}), 6.2% (29.8±1.11 mmol.L^{-1}) and 21.2% (0.85±0.05 mmol.L^{-1}) of their initial values on day 40 for the FM and 2FM groups respectively.

Table 5: Values of Blood Urea Nitrogen (BUN), Creatinine, urea and uric acid in the tilapia on 0, 15, 30 and 40 d of treatment with diets (SM, CM, FM and 2FM)

Time (d)	SM	CM	FM	2FM
BUN urea (mg.dL4)				
0	2.004.15•	1.79:4-).18b	3.014.17'	2.86±0.90'
15	1.984.11•	1.77=0.15'	2.56th0.17'	2.250.13"
30	2.020.18'	1.814.14'	2.014.16'	2.06E0.184
40	1.900.16'	1.3 0.11`	1.98?=0.12'	1.974.18'
Creatinine	)			
0	29.3+.1.971	26.1.61th	29.9±1.91'	31.8a-1.73'
15	29.7-11.61'	26.6±1.381	29.5-11.66'	31.9-11.68'
30	28.911.36•	26.1.02`	28.7-11.08'	29.1-11.01'
40	29.911.03'	26.8-11.121'	29.1-11.18'	29.8-11.11'
Urea (romol.L4)				
0	0.714.07'	0.90=0.03`	1.104.06'	1.084.04'
15	0.754.01•	0.374.08`	0.914.06'	0.994.01'
30	0.714.06•	0.866±0.01`	0.8910.07'	0.844.06'
40	0.704.01•	0.90=0.02`	0.80*0.05'	0.854.05'
Uric acid (prima')				
0	319.6e.35.8"'	386.8:-12- 1.3th	389.4±56.8th	322.7-133.4'
15	310.8345.9'	385.1-146.e	397.36.6'	402.33.3'
30	312.1-1-31.2'	386.041.9	469.9-141.4'	424.66±40.4th
40	317.1-131.7'	386.8±31.3'	488.8340.8'	482.9440.8"

SM standard diet group; CM cholesterol diet group; FM frog protein single strength diet group (1g/1kg BW/d); 2FM frog protein double strength diet group (2g/1kg BW/d) diet group; Values (mean ±SE) with different superscripts in the same row are significantly different at the 5% level, N number of animals in the group=8.

In the present experiment, the high frog protein supplement has decreased significantly the AST level with the value of 13.3% (initial value 300.9±21.1 $U.L^{-1}$) and 29.4% (initial value 357.1±31.3 $U.L^{-1}$) on day 40 in the treated FM and 2FM groups respectively while the CM and SM groups have their AST level being stabilized throughout the experimental period (Table 6). However, all ALT values obtained in all groups (SM, CM, FM, 2FM) are within the fish normal range (250 -370 $U.L^{-1}$). The experiment carried out by El-Sabagh *et al*., (2014) on young lambs resulted in the drop of AST level by 13%. In this present experiment, the ALT and GGT levels in both CM and SM groups showed very little variation in their blood values during the study period.

Table 6: Values of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and GGT in the tilapia on 0, 15, 30 and 40 d of treatment with diets (SM, CM, AF and 2FM)

Time (d)	SM	CM	FM	2FM
AST (U.I.,-1)				
0	278.3±28.7ab	264.5±31.95b	300.9±21.1C	357.1±31.30
15	279.7±31.1a	261.6±20.6a	290.5±21.60	351.9±31.80
30	281.9±31.6a	266.0±21.2b	275.9±21.8C	276.1±30.1d
40	280.9±29.3a	268.8±20.1b	260.4±30.8C	252.2+-31.35
ALT (U.I.:1)				
0	6.80±0.67a	7.40±0.30b	7.90±0.61a	5.68±0.41a
15	6.98±0.71a	7.56±0.41b	8.1±0.81C	5.51±0.91a
30	6.91±0.86a	7.71+0.8 lb	8.00±0.77a	5.41±0.66d
40	6.82±0.91a	7.72±0.92b	8.01±0.99	5.52±0.85d
Gamma GT (Uhl)				
0	212±2.19	28.1±1.78b	27.0±2.07a	23.5±2.10d
15	21.6±2.91a	29.8±2.69	29.8±2.87c	23.5±2.73d
30	193±2.785	28.5±2.64b	28.7±336c	22.0±2A 8d
40	21.1±1.065	29.6±2.61b	28.3±2.12c	23.3±2.08d

SM standard diet group; CM cholesterol diet group; FM frog protein single strength diet group (1g/1kg BW/d); 2FM frog protein double strength diet group (2g/1kg BW/d) diet group; Values (mean ±SE) with different superscripts in the same row are significantly different at the 5% level, N number of animals in the group=8.

The AST, ALT and GGT of the hepatic serum function also specialized in signaling the problems of the liver whereby these enzymes are released into the blood as a result of cellular impairment or injury. ALT served as the most specific indicator of hepatic injury; however GGT functions as a sensitive marker but not as specific as ALT (Al-Sultan, 2008) [5].

4. Conclusion

Until this present time, studies on the effectiveness of the high protein frog meal and pre-clinical practice on the local tilapia seem to be scarce. This also reflects the ability of the high frog protein meal supplement to function as an anti-hypercholesterolemic agent in tilapia. It also provided the betterment in the fish health, blood chemistry and growth performance. The frog meal had increased the HDL level and lowered the cholesterol as well as stabilized the GGT and ALT blood level without indicating any adverse effects towards kidney-liver function. As a consequence, frog protein feed production and requisition have been on the increase since the proliferation of its usage as supplement products which are free from toxic chemicals. In view of the facts that the present aqua-feeds or fish feeds available locally or imported from abroad have been dreadfully adulterated with toxic growth promoters such as hormones and antibiotics, we need to replace or supplement the existing feeds with safe, natural and healthy feed derived especially from locally animal or plant materials. In the case of frog protein meal of local source should contain beneficial materials such as, protein, essential amino acids, polyunsaturated fatty acids, vitamin and mineral contents. Using the fish tilapia as a model test animal, the present study showed the usage of high protein frog diet inclusion at the recommended level for tilapia meal can have the positive effects on the growth, feed conversion and plasma lipoprotein lipase activity in tilapia.

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