EFFECT OF CARBOHYDRATE TO LIPID RATIOS WITH OR WITHOUT THIAMIN SUPPLEMENTATION ON GROWTH AND IMMUNITY IN OREOCHROMIS NILOTICUS

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ABSTRACT

The purpose of the work was carried out to determine the effect of carbohydrate: lipid (CHO:L) ratios on growth performance, body composition, immune response , protection against Aeromonas hydrophila and to explore the relationship between thiamin, carbohydrate and lipid utilization of O. niloticus. Three iso-nitrogenous (crude protein, 34%); iso-caloric (3400 ME Kcal/Kg) experimental diets, with varying CHO:L ratios (1.51 – 6.16) were fed to 6 groups (20 fish /group) with average body weight 9 grams. Fish of groups 1 and 2 were fed on diet 1 (containing 1.51 CHO: L) without or with 30 mg thiamin/Kg diet respectively. Fish of groups 3 and 4 were fed on diet 2 (containing 2.93 CHO: L) without or with 30 mg thiamin/Kg diet respectively. Fish of groups 5 and 6 were fed on diet 3 (containing 6.16 CHO: L) without or with 30 mg thiamin/Kg diet respectively.

At the end of the experimental period (20 weeks) no significant differences were observed in growth rate, feed conversion, protein efficiency ratio and condition factor of O. niloticus fed on diets with CHO:L ratios ranging from (1.51 – 6.16). While the highest performance was observed in fish groups which were fed on high CHO and low lipid diet without or with thiamin supplementation (groups 5 and 6 respectively). It was clear that O. niloticus can efficiently utilize carbohydrates and lipids as energy sources in accordance with their respective metabolic fuel values and the dietary requirement of thiamin has been correlated with the carbohydrate level in the diet. The average dressing % did not differ significantly between fish groups. Changes in body composition were not appreciable with respect to moisture and protein content of the carcass. Increasing the dietary lipid level resulted in an increase in the carcass lipid content. Blood picture and both the phagocytic activity and index were improved by decreasing the lipid content in the used diet. Also, the total protein and globulin were decreased while the cholesterol content was increased in all fish groups which fed on high lipid diet. Moreover the changes in ALP(Alkaline phosphatase) were significantly lower in fish groups fed on low lipid diet and serum transaminase (GOT and GPT) were not significantly differ between various fish groups. Finally, the protection of the fish was examined by using the virulent strain of Aeromonas hydrophila and recorded the antibody titer which showed a significant decrease in all groups which fed on high lipids as well as the survival rate was reduced also.

It was concluded that O. niloticus were able to store significant quantities of lipids in the carcass, but were not able to utilize this energy source to improve growth or food utilization efficiency, at least in diets containing adequate levels of protein, and the dietary thiamin requirement has been correlated with CHO level in the diet. While the high lipid content of the used diet reduced the immune response and protection level against diseases of O. niloticus.

INTRODUCTION

Nowadays aquaculture is considered as an important source of fish production for meeting the world's increasing demand for protein. A concurrent objective of many nutritionists is to obtain economically faster growth of a given animal species.

In a successful fish husbandry practice, consideration is generally given to the dietary protein content to produce optimal fish growth. Equally important is the inclusion of appropriate levels of non-protein energy sources in the diet that determines the efficiency of protein utilization (Steffens, 1981 and Wilson and Halver, 1986). Carbohydrates and lipids are the major non-protein energy sources in fish diets. Compared to dietary lipids, carbohydrates are relatively inexpensive and act as a readily available source of energy to many fish species.

In fish culture, nutrition obviously plays an important role in the maintenance of a healthy and marketable product. In warm water fish, dietary carbohydrate utilization is considerably high, and incorporation of this nutrient may add beneficial effects to the pelleting quality of the diet and fish growth (NRC, 1983 and Wilson, 1994). High levels of dietary lipid may creat problems in the pelleting quality of the diet (Jauncey, 1982) as well as may reduce fish growth and produce fatty fish (Garling and Wilson, 1977 and Hanley, 1991).

Any imbalance in non-protein energy sources and/or their inclusion levels may have a direct affect on growth, conversion efficiencies, nutrient retention and body composition. It is thus imperative to determine the optimum dietary CHO:L ratio, that produces the best growth, feed conversion and improved nutrient retention, body composition as well as immune defence mechanism. Ealier studies in this respect have been reported for channel catfish (Garling and Wilson, 1977) and Tilapia zilli (El-Sayed and Garling 1988 & Erfanullah, 1998).

The primary and best understood role of thiamin (vitamin B_1) is within intermediary metabolism, although this vitamin is also associated with neural transmission. The coenzyme from thiamin is thiamin pyrophosphate (TPP). Thiamin plays a role in controlling carbohydrate metabolism as a coenzyme for oxidative decarboxylation of pyruvic acid and α -ketoglutorate and for transketolation in the pentose phosphate shunt (Bender, 1992). Consequently, thiamin plays a role in the metabolism of fish and hence deficiency may result in a metabolic dysfunctions (Halver, 1989). The thiamin requirements of many aquatic species has been reported by Halver (1989) and Woodward (1994).

Many studies have focused on growth rate of O.niloticus as a function of dietary lipid (Viola and Arieli, 1983; Schwarz et. al., 1988; Viola et. al ., 1988 and Hanley, 1991). Differences in immune response and disease resistance as a function of dietary lipid source have been reported by Fracalossi and Lovell, (1994) who demonstrated significantly higher antibody titers to E. ictaturi, 2 weeks after immunization in Catfish fed menhaden oil versus corn oil, linseed oil or mixed oils. We initiated this study to determine if dietary lipid levels and thiamin supplementation, will affect disease resistance and hematological parameters in O. niloticus. Total erythrocyte and leucocyte counts were used as indicators of hemopoesis and thiamin status (NRC, 1993).

The aim of the present work was to determine the effect of carbohydrate : lipid ratios (CHO:L ratios) on growth, body composition, immune responses, protection against ***Aeromonas hydrophila*** and to explore the relationship between thiamin, carbohydrate and lipid utilization of O. niloticus fed iso-nitrogenous and iso-caloric diets.

MATERIALS AND METHODS

Culture technique:
A total of 120 apparently healthy Oreochromis niloticus (9 grams average body weight) were collected from the River Nile for this work. Fish were kept in glass aquaria, each of 150 liters capacity for 4 week accommodation period, after which fish were allotted in 6 glass aquaria (20 per group)) on the basis of an equal average body weight. The water quality remained nearly stable for a pH of 7.6 and temperature of 26± 2 ^{0}C throughout the experimental period. A natural light was available, providing nearly 12 hour light/day. Each aquarium was provided with an automatic aeration and was partially cleaned daily and completely every 4 days.

Diets and experimental design:
Fish meal, soybean meal, ground yellow corn, corn starch, wheat bran, sunflower oil, α - cellulose, carboxy methyl cellulose, mineral mixture and vitamin mixture were used to formulate balanced three experimental diets that provide the nutrients required and recommended by the NRC (1993). The ingredient composition and chemical analysis of the experimental diets are shown in table 1. The three experimental diets were iso-nitrogenous and similar in terms of metabolizable energy (ME), while differing with respect to carbohydrate and lipid content. The ratio of ME derived by O. niloticus from equivalent amount of corn starch and oil is 4:9 (Jauncey and Ross, 1982). Thus using supplemental sunflower oil and corn starch as sources of non-protein energy. Three experimental diets of three levels of sunflower oil were formulated and designated as HL, ML and LL for the high, medium and low levels of oil respectively. Of the six groups of fish there were fed on the aforementioned diets after being supplemented with thiamin at the rate of 30 mg/Kg, half of the recommended requirement suggested by Jauncey and Ross (1982).

The experimental design can be summerized as follows:

Group	Diet No.	Thiamin supplementation (mg/kg diet)
1	1 (HL)	---
2	1	30
3	2 (ML)	---
4	2	30
5	3 (LL)	---
6	3	30

The diets were pelleted into size No. 2 (1.2 mm diameter), suitable for fish size (Meske, 1985), and the fish in all groups were fed at a rate of 3% of fish biomass per day, divided into 3 meals throughout the experimental period. The experimental period extended for 20 weeks.

Table (1): Ingredient composition and chemical analysis of the used diets:

	DIETS		
	1 (HL)	2 (ML)	3 (LL)
Ingredients %:			
Fish meal (72%)	35.0	35.0	35.0
Soybean oil meal (44%)	14.0	14.0	14.0
Yellow corn	15.5	15.5	15.5
Wheat bran	10.0	10.0	10.0
Corn starch	0.0	9.0	18.0
Sunflower oil	10.0	6.0	2.0
A-Cellulose	10.0	5.0	0.0
CMC [1]	2.0	2.0	2.0
Vit. Mix [2]	1.0	1.0	1.0
Min. Mix . [3]	2.0	2.0	2.0
Thiamin in starch [4]	0.5	0.5	0.5
Chemical analysis:			
Moisture%	11.08	11.12	11.70
Crude protein%	34.02	34.29	34.01
Ether extract%	14.06	10.01	6.07
Crude fiber%	14.21	9.93	5.22
Ash%	5.43	5.35	5.61
NFE [5]	21.2	29.30	37.39
ME Kcal /Kg [6]	3465.17	3414.54	3348.18
P/E ratio [7]	98.18	100.42	101.58
CHO: L ratio	1.51	2.93	6.16

1) **Binder:** Sodium carboxy methyl cellulose (high viscosity) according to Jauncey and Ross (1982), Murai et al. (1986) and Shiau et al. (1988).

2) **Each 1 Kg of the vit. mix. contains**: Vit. A 2200000 IU; Vit. K 792 mg; Vit. D_3 396000 IU; Vit. E; 660 IU; Vit. B_2 3 g; Vit. B_{12}, 2.200 mg, nicotinamide 13.3 g; calcium pantothenate, 4.8 g.

3) **Each 1 Kg of the mineral mix. contains:** magnesium sulphate 255g, sodium chloride 120 g, potassium chloride 100g, iron sulphate 50g, zinc sulphate 11g, manganese sulphate 5.075 g, copper sulphate 1.57g, cobalt chloride 0.808 g, potassium iodate 0.5005 g, chromic chloride 0.25 g.
4) **Thiamin in starch:** represents the space within the formulation allocated to the thiamin supplementation.
5) **Nitrogen free extract** (NFE) calculated by difference.
6) **Metabolizable energy** was calculated using a value of 4.5 Kcal/g protein, 8.51 Kcal/g fat and 3.48 Kcal/g CHO according to Jauncey and Ross (1982).
7) **P/E ratio** calculated as mg of protein/ Kcal ME according to Jauncey and Ross (1982) and Alexis et al. (1985).

Measurements:
Body weight and length of fish in different aquaria were carried out biweekly throughout the experimental period. Body weight gain, specific growth rate {SGR), food conversion ratio {FCR), protein efficiency ratio {PER) and condition factor (K) were calculated.

Body composition analysis:
Representative fish samples were randomly chosen, 10 fish from the total at the start and 5 from each group at the end, weighed and ground where triple pooled samples, of 5 g, were taken and kept frozen till analysis were performed using the standard AOAC (1985) methods.

Slaughter value:
Another 5 fish from each aquarium were used to determine the dressing percentage. At the time of slaughter, the liver weight with respect to empty fish weight was calculated and the heptosomatic index determined as outlined by Morris et al. (1995).

Blood analysis:
At the end of the 8^{th}, 14^{th} and 20^{th} weeks during the experimental period approximately 1.5 ml blood sample were collected from the different groups via the caudal vessel from 3 fish using disposable tuberculin syringe.

A portion of blood was collected containing anticoagulant (0.1 ml of 4% sodium citrate solution / 1ml blood) for the determination of phagocytic activity and index according to Kawahara et al. (1991). Total red blood corpuscles (RBCs) and total leukocytic count (WBCs) were determined by a haemocytometer according to Miller and Seward (1971). Haemoglobin (Hb) was determined by Sahli's method followed by Lucky (1977). Leishman's stain was used for staining blood films for differential leukocytic count according to Schalm (1986).

The rest of blood samples was used for serum separation by centrifugation of blood at 3000 rpm for 15 minutes and kept at –20 °C until assayed. Serum creatinine and cholesterol were determined according to Bartel (1971) and Schettler et al. (1975), respectively, while serum alkaline phosphatase was estimated according to modified method of Kind and king (1954). Serum transaminases (glutamin-pyruvic transaminase , GPT, and glutamin-oxalacetic transaminase, GOT) were determined according to

Reitman and Frankel (1957). Serum glucose, total protein and albumin were determined according to Trinder (1969), Doumas et al. (1981) and Reinhold (1953) respectively. While serum globulin was calculated as the difference between total protein and albumin (Coles, 1974). Moreover albumin/globulin ratio (A/G) was calculated.

Antibody production and fish challenge:

A virulent strain of *A. hydrophila* was inactivated by formaline according to Sakai et al. (1984). The inactivated *A.hydrophila* was tested for safety and sterility according to Anderson et al. (1970), and mixed with an equal volume of sterile saline (Badran, 1990). The bacterial number was adjusted at Macfarland's tube No. 2 (6 X 10^8 cells/ ml). Equal volume of the formalin-inactivated bacterial suspension was mixed with incomplete Freund's adjuvant and dose of 0.2 ml was incoulated intraperitoneally into fish. Seven days post-injection with inactivated bacteria and weekly intervals throughout 10 weeks, 2 fish were taken from each group for blood collection from the caudal vessels and used for serum collection. The antibody titer against *A. hydrophila* was detected by micro-agglutination test after preparation of stained antigen according to Eurell et al. (1979) and Collins et al. (1976) respectively. After the antibody titration, the survival fish were intramuscularly challenged with 0.1 ml /fish containing 9 X 10^7 cell of the virulent *A. hydrophila* (some strain used for antibody production). Daily morbidity and mortality were recorded. Reisolution of infected bacteria was done in case of dead fish for varifying the specificity of death.

Statistical analysis:

Statistical analysis of the obtained data was performed using Statistical Analysis System (SAS, 1987).

RESULTS AND DISCUSSION

Body development and feed conversion efficiency:

Data concerning the body weight development of fish in various aquaria throughout the experiment are illustrated in Fig. 1, while the average values related to the body weight and food conversion are presented in table 2. At the end of the experiment (20 week), the highest average final fish weight was recorded in group 6 (31.92 g), followed by those of group 5 (30.81 g), group 1 (29.44 g), group 4 (28.53 g), group 2 (27.84 g) and finally group 3 (27.67 g).

Statistical analysis of the obtained data indicated no significant difference between the different groups at the start of the experiment, while at the end, the analysis of variance of the obtained data demonstrated a non-significant ($P>0.05$) increase in the average body weight of fish groups which were fed on low lipid diet (diet No. 3) without or with thiamin supplementation (groups 5 and 6 respectively) when compared with fish groups fed on medium or high lipid diets. The above findings agree with those of Viola and Arieli (1983) who reported that, dietary oil supplementation does not produce gains in growth and food utilization of tilapia, and with those of Viola et al. (1988) who also found that no difference in the final weights of tilapia receiving basal and oil supplementing diets.

The obtained average values of total and weekly body weight gain, body weight gain % relative to the initial weight, SGR, FCR, PER, increase in body length and K factor for various fish groups (table 2) indicated the improved performance of fish in group 6 compared to the other groups. The results of the present study showed that dietary CHO:L ratios which produced non-significant effects (P>0.05) on fish performance in this study (1.51 - 6.16) was wider than that found for channel catfish. Garling and Wilson (1977) fed channel catfish on iso-nitrogenous, iso-caloric diet containing different lipid to carbohydrate ratios ranging from 0.0 to 31.50, and found that dextrin could replace lipids in the diet based on physiological fuel values (2.25 : 1) at CHO:L ranging from 4.5 to 0.45 without significant effects on fish performance. Higher levels of lipids or CHO beyond this range reduced weight gain and protein retention.

Recently, feeding iso-nitrogenous and iso-caloric semipurified diets with varying CHO:L ratios (0.02 to 43.0 g:g), in walking catfish, maximum weight gain, SGR, FCR and PER were observed in fish fed a 27% CHO and 8% lipid diet corresponding to a CHO:L ratio of 3.38, while fish either the lowest (0.02) or highest (43.0) CHO:L ratios tended to produce lower growth performance (Erfaunllah, 1998). It appear, therefore that O.niloticus can utilize higher levels of starch than catfish.

The present data are in harmony with the findings of Teshima and Kanazawa (1986) who noted an increased growth of O. niloticus due to an increase in CHO level in diets containing proper protein and lipid levels, and El-Sayed and Garling (1988) who found that growth rates of T. zilli, were improved with increasing CHO levels in the diets from 12 to 36.8%. While Morris and Davies (1996) found that growth rates of gilthead seabream, were lowered with higher levels of CHO in the diets. It would appear therefore that O. niloticus can utilize CHO (starch) as efficienctly as T.zilli and carp and more efficiently than catfish, red seabream and yellow tail.

In the present experiment, at medium and high levels of oil with thiamin supplementation (groups 4 and 2 respectively) indicated a non-significant changes in fish growth performance, while thiamin supplementation improved fish performance at high CHO and low lipid diet (group 6), this indicats that thiamin plays a role in controlling CHO metabolism (NRC, 1993). Moreover Aoe et al., 1969 found that the dietary requirement of thiamin has been correlated with the CHO level of the diet.

Slaughter values:

The dressing percentage, viscerosomatic index (VSI) and liver characteristics of the fish in different experimental groups are shown in table 3. In the presnt work, the average dressing percentage did not differ significantly among fish groups, and ranged between 87.36 – 89.73. These values are similar to the normal values recorded by El-Katcha (1996). Thiamin supplementation in different diets leads to a significant increase in VSI and hepatosomatic index especially with decreasing fat level in the diet.

Body composition:

Regarding the proximate body composition of fish in the different groups, the data expressed on fresh as well as dry matter basis are presented in table 4. The high lipid or low lipid diet produced no appreciable changes in the moisture and protein content of the carcass, increased in the carcass fat of fish which fed on high lipid diet without or with thiamin supplementation (groups 1 and 2 respectively). This may indicate that when dietary lipid was supplied in excess a proportion of this lipid was deposited as fats. This is in agreement with the results of tilapia zilli (El-Sayed and Garling, 1988), tilapia nilotica and carp (Viola et al., 1988) and tilapia nilotica (Hanley , 1991).

Nutrient retention data presented in table 5, indicated highest retention of crude protein in fish of groups 5 and 6 which were fed on diet containing low lipid content without or with thiamin supplementation respectively. While the highest fat retention was observed in fish groups which fed on high lipid diet (groups 1 and 2), followed by those fed on medium lipid diet (groups 3 and 4).

Blood picture:

From (table 6) one can notice that there was a significant improvement in RBCs, WBCs counts and Hb content with decreasing oil and increasing CHO content in the diet, while non- significant improvement of those blood picture was observed with thiamin (30 mg/Kg diet) supplementation . The data indicated an increase in the neutrophil and lymphocyte % in blood of fish fed on the diet low lipid and high CHO (diet No. 3) without or with thiamin supplementation (groups 5 and 6, respectively).

Phagocytosis:

Effect of CHO:L ratios without or with thiamin supplementation on phagocytosis of different experimental groups are presented in table 7. The results revealed an increase in phagocytic activity and index in fish fed on low lipid diet after 8, 14 or 20 week of feeding (groups 5 and 6) than the value obtained in fish fed medium or high lipid diet (groups 1 – 4). The results were parallel to the improvement of blood picture of fish groups fed on low lipid diet (table 6), and are in agreement with those obtained by Li and Lovell (1985).

Blood chemistry:

The serum creatinine, cholesterol, ALP, SGPT, SGOT, glucose, total protein, albumin and globulin concentration are summarized in table 8. The present results revealed that there were significant decrease in cholesterol concentration in fish group fed on high CHO:L ratio (groups 5 and 6) than those of fish groups fed on high lipid diet (groups 1 and 2), while not significantly, different with those fed on medium lipid content (groups 3 and 4). The dietary treatment had no effect on serum creatinine.

The data revealed that fish groups which received diet with medium and low lipid content with or without thiamin supplementation (groups 3 – 6, respectively) had a significant decrease in ALP, and non-significant decrease in serum transaminase concentrations. It is well known that the enzymes are intracellular, being located in mitochondria, cytoplasm or both, consequently, circulating levels increase only following liver damage (Doxey, 1971).

Moreover the serum glucose concentration was significantly increased in fish groups which were fed on diets containing low lipid and high CHO (Groups 5 and 6). Also thiamin supplementation (group 6) significantly increase glucose concentration compared with the fish group fed the same diet without thiamin supplementation (group 5). The role of dietary CHO and the contribution of glucose to the total energy requirement of fish remains unclear (NRC, 1993), while in this study it can be mentioned that the increase of CHO level in the Nile tilapia diet leads to increase of tricarboxylic acid cycle, pentose phosphate shunt, gluconeogensis and glycogen synthesis enzymes and thus increases the serum glucose concentration.

In the present investigation, the results showed an increase in total protein and globulin fraction in low lipid and higher CHO treated fish (groups 5 and 6), followed by medium lipid treated groups (groups 3 and 4) with a lowering albumin/globulin ratio. These results could be attributed to the stimulatory effect of polyunsaturated fatty acids (EFA) on synthesis of imunoglobulin, especially with normal levels of polyunsaturated fatty acids. Higher levels decreased the serum immunoglobulin production. These data are supported by Kanazawa et al. (1980) who demonstrated that T.zilli require about 1% dietary linoleic or arachidonic acids for maximum performance. Since diet No. 3 contained 6% lipid this may indicate that EFA for fish can be met by satisfying this percent, while the higher lipid levels in fish diets may reduce fish performance (Garling and Wilson, 1977).

Antibody production and fish challenge:

Table (9) presented the results of haemoagglutination test to *A. hydrophila* bacteria. Statistical analysis of the data revealed a significant increase of haemoagglutination titer in fish groups fed on low lipid diet without or with thiamin supplementation (groups 5 and 6, respectively), compared with fish groups which fed on high lipid diets (groups 1 and 2)

High antibody titer was obtained after 7 weeks post-injection of bacterin, and thiamin supplementation had no role on the antibody production of different fish groups. The production of antibody appeared to be dependent upon sufficient EFA as required, but was inhibited by excess EFA intake. These results were parallel with that observed in broiler chicks by Friedman and Sklan (1995) who found that optimal antibody production appeared to be dependent upon sufficient polyunsaturated fatty acids as required, but was inhibited by excess polyunsaturated fatty acids intake.

In table 10, *A. hydrophila*_ produced 85, 85, 30, 40 , 10 and 15% mortalities in experimentally infected O. niloticus fish withis 7 days post-infection in the six groups respectively. The mortality percentage was correlated with the antibody production, as the highest protection level was observed in. fish groups which were fed on low lipid diets followed by the medium one.

The present study suggested that the addition of oil to fish diets did not improve the growth and food conversion of O. niloticus. Furthermore, the addition of oil may result in the production of fatty fish, a situation which could be undesirable, as the

incidence of high level of fat in the flesh of farmed fish may have deleterious effects on the flavor, consistency and storage life of finished product. Also, it was concluded that the dietary thiamin required has been correlated with CHO level in the diet. Moreover the addition of oil to the fish diet in a high level leading to decrease the immune bacterial infection.

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Table (2): Average values of performance parameters of fish in the differentgroups

Parameters	GROUPS					
	1	2	3	4	5	6
Initial Wt. (g)	8.28± 0.35 [a]	8.32± 0.29 [a]	8.58± 0.38 [a]	8.61± 0.43 [a]	8.88± 0.35 [a]	8.65± 0.48 [a]
Final Wt. (g)	29.44± 1.75 [a]	27.84± 2.12 [a]	27.67± 1.49 [a]	28.53± 2.09 [a]	30.81± 1.59 [a]	31.92± 1.94 [a]
Total body gain (g)	21.19± 1.51 [ab]	19.51± 1.81 [ab]	19.09± 1.19 [b]	19.93± 1.71 [ab]	21.9± 1.42 [ab]	23.27± 1.23 [a]
Weekly body gain (g)	1.06±	0.98	0.95	1.0	1.10	1.16
Weight gain % [1)]	255.92	234.50	222.49	231.36	246.95	269.02
SGR % [2)]	0.876± 0.03 [ab]	0.838± 0.03 [b]	0.828± 0.02 [b]	0.824± 0.03 [b]	0.87± 0.03 [ab]	0.93± 0.02 [a]
FCR % [3)]	3.71± 0.32 [a]	3.79± 0.34 [a]	3.75± 0.05 [a]	4.13± 0.67 [a]	3.93± 0.68 [a]	2.94± 0.21 [a]
PER % [4)]	0.87± 0.06 [a]	0.90± 0.08 [a]	0.82± 0.05 [a]	0.83± 0.07 [a]	0.80± 0.05 V	1.02± 0.07 [a]
Initial body length (cm)	7.33± 0.14 [a]	7.44± 0.14 [a]	7.35± 0.17 [a]	7.62± 0.15 [a]	7.38± 0.13 [a]	7.34± 0.19 [a]
Final body length (cm)	12.14± 0.21 [ab]	11.79± 0.35 [b]	11.59± 0.35 [b]	12.09± 0.33 [ab]	12.1± 0.28 [ab]	12.53± 0.31 [a]
Increase in length (cm)	4.81	4.35	4.24	4.47	4.74	5.19
K final [5)]	1.65± 0.04 [b]	1.70± 0.05 [ab]	1.78± 0.05 [a]	1.61± 0.04 [b]	1.73± 0.04 [ab]	1.62± 0.04 [b]

Means ± standard error.

Means with different letters at the same row differ significantly at $P< 0.05$.

1) Weight gain percent relative to the initial body weight.

2) Specific growth rate {SGR = 100 (Log_e W_f – Log_e W_i / T)} wher W_f is the final weight(g)}, W_i,is the initial weight (g), and T the time between weighings in days.

3) Food conversion ratio {FCR = (Feed fed (g) / Wet weight gain, g)}.

4) Protein efficiency ratio {PER = Weight gain (g)/ Protein consumed (g)}

5) Condition factor {K = 100 (W/L^3) where W is the fish weight (g), L is the length in cm}

Table (3): Dressing percentage and liver characteristics in the differentexperimental groups

Parameters	GROUPS					
	1	2	3	4	5	6
Dressing %	89.73± 0.64 [a]	87.36± 0.43 [a]	88.34± 0.75 [a]	88.39± 0.72 [a]	87.69± 0.86 [a]	87.80± 1.31 [a]
VSI [1)]	11.45± 0.78 [b]	14.79± 0.58 [a]	13.21± 0.97 [ab]	12.89± 0.92 [b]	14.05± 1.11 [a]	15.26± 0.58 [a]
Liver weight (g)	0.47± 0.12 [a]	0.40± 0.15 [a]	0.27± 0.03 [b]	0.40± 0.06 [a]	0.27± 0.03 [b]	0.43± 0.03 [a]
HSI [2)]	1.33± 0.37 [a]	1.44± 0.33 [a]	1.06± 0.25 [b]	1.43± 0.07 [a]	0.83± 0.06 [b]	1.35± 0.15 [a]

Means ± standard error 1)Viscerosomatic index 2) Hepatosomatic index

Table (4): Proximate body composition of fish in the different groups at the startand end of the experimental period

Composition %	Initial	Final					
		1	2	3	4	5	6
%on fresh basis							
Dry matter (DM)	25.94	28.83	28.98	28.91	28.61	28.37	27.78
Moisture	74.06	71.17	71.02	71.09	71.39	71.63	72.27
Crude protein	16.99	16.32	16.52	16.92	16.78	17.19	16.95
Ether extract	3.98	6.01	6.19	5.83	5.52	4.99	4.89
% on DM basis							
Crude protein	65.50	56.61	57.00	58.53	58.65	60.59	61.02
Ether extract	15.34	20.85	21.36	20.17	19.29	17.59	17.60

Table (5): Nutrient retention in fish of different experimental groups

Nutrient retained	Groups					
	1	2	3	4	5	6
Dry matter (DM)						
DM retained (g)	6.34	5.91	5.77	5.93	6.44	6.63
DM increased % of initial	294.88	273.61	258.74	265.92	280.0	295.98
Crude protein (CP)						
CP retained (g)	3.39	3.19	3.22	3.33	3.79	3.93
CP increased % of initial	240.43	226.24	220.55	228.08	250.9	267.35
Ether extract (EE)						
EE retained (g)	1.44	1.39	1.27	1.23	1.19	1.22
EE increased % of initial	436.36	421.21	373.53	361.76	340.0	358.82

Table (6): Blood picture of fish in different experimental groups

Items	Groups					
	1	2	3	4	5	6
Hb (g/100 ml)	4.94± 2.07 ^{c}	5.77± 2.21 ^{c}	10.83± 0.27 ^{b}	11.29± 0.15 ^{ab}	11.89± 0.26 ^{a}	12.6± 0.25 ^{a}
RBCs(X 10^3/cm ^{3})	0.93± 0.34 ^{b}	1.02± 0.21 ^{b}	1.37± 0.11 ^{a}	1.45± 0.08 ^{ab}	1.6± 0.09 ^{a}	1.65± 0.15 ^{a}
WBCs(X 10^3/cm ^{3})	20.09± 2.66 ^{d}	22.83± 4.30 ^{c}	35.0± 0.71 ^{b}	36.47± 0.70 ^{b}	42.09± 0.7 ^{a}	43.55± 0.66 ^{a}
Neutrophil (%)	27.43± 2.03 ^{a}	27.57± 1.94 ^{a}	21.0± 1.70 ^{b}	20.56± 1.98 ^{b}	16.58± 2.15 ^{c}	18.57± 1.92 ^{c}
Eosinophil (%)	29.57± 3.88 ^{a}	26.14± 4.15 ^{a}	24.29± 3.62 ^{ab}	23.86± 3.33 ^{bc}	20.14± 2.78 ^{c}	20.14± 3.73 ^{c}
Monocyte (%)	1.14± 0.67 ^{c}	2.00± 0.90 ^{a}	2.00± 0.44 ^{a}	1.86± 0.34 ^{ab}	1.57± 0.37 ^{b}	1.29± 0.29 ^{c}
Lymphocyte (%)	38.0± 4.07 ^{c}	40.29± 3.26 ^{c}	49.00± 4.71 ^{b}	50.43± 4.22 ^{b}	59.0± 3.63 ^{a}	57.86± 2.19 ^{a}
Basophil (%)	3.86± 0.80 ^{ab}	4.00± 1.25 ^{a}	3.71± 1.02 ^{b}	3.29± 1.02 ^{bc}	2.71± 0.89 ^{c}	2.14± 0.55 ^{c}

Means ± standard error

Means with differe letters at the same row differ significantly at P <0.05.

Table (7): Effect of CHO: L ratios and thiamin supplementation on phagocytosis of different fish groups

Phagocytosis	Groups					
	1	2	3	4	5	6
Activity						
After 8 weeks	17.3	18.0	23.67	25.67	42.67	32.0
After 14 week	14.52	15.0	17.26	17.86	18.56	19.45
After 20 week	15.0	12.5	21.0	21.0	20.5	21.5
Index						
After 8 week	6.2	6.8	7.5	7.6	8.85	8.8
After 14 week	4.4	4.6	6.0	6.2	7.36	8.15
After 20 week	6.8	7.3	8.55	8.25	8.2	8.30

Table (8): Effect of CHO:L ratios and thiamin supplementation on some blood parameters of the different fish groups

Items (Period/week)		Groups 1	2	3	4	5	6
Creatinine (mg%)	(14)	0.58	0.60	0.60	0.62	0.72	0.73
	(20)	0.78± 0.04 ^{a}	0.93± 0.13 ^{a}	0.78± 0.11 ^{a}	0.60± 0.02 ^{a}	0.90± 0.02 ^{a}	0.83± 0.09 ^{a}
Cholesterol (mg%)	(14)	231.67	221.37	190.0	198.0	189.17	166.67
	(20)	240.0± 17.8 ^{a}	237.5± 11.09 ^{a}	207.5± 8.5 ^{ab}	212.5± 5.95 ^{ab}	198.8± 7.18 ^{b}	191.3±8. 26 ^{b}
ALP (g/dl)	(14)	14.67	15.33	17.67	14.2	14.5	13.33
	(20)	14.25± 1.95 ^{ab}	12.25± 0.48 ^{b}	17.5± 1.04 ^{a}	15.5± 1.6 ^{ab}	7.50± 0.86 ^{c}	8.0± 1.0 ^{c}
SGPT (I.U./L)	(14)	74.17	72.5	47.0	46.67	51.5	50.5
	(20)	87.25± 5.85 ^{a}	87.75± 1.10 ^{a}	46.25± 1.25 ^{c}	56.0± 2.61 ^{bc}	60.5± 4.5 ^{b}	56.0± 4.7 ^{bc}
SGOT (I.U./L)	(14)	72.0	69.5	69.0	69.17	64.33	66.33
	(20)	60.25± 2.72 ^{ab}	64.0± 2.61 ^{ab}	65.25± 1.7 ^{a}	55.25± 5.63 ^{b}	63.0± 1.73 ^{ab}	64.5± 1.3 ^{ab}
Glucose (mg%)	(14)	57.83	64.83	71.16	74.5	73.33	84.5
	(20)	71.5± 1.19 ^{c}	75.25± 4.01 ^{c}	76.5± 1.6 ^{c}	77.5± 2.4 ^{c}	97.75± 1.3 b	110.3±3. 4 ^{a}
Total protein (g/dl)	(14)	9.22	4.95	5.38	5.75	6.02	6.02
	(20)	3.22± 0.25 ^{c}	3.63± 0.29 ^{c}	5.65± 0.25 ^{b}	6.18± 0.01 ^{b}	7.38± 0.27 ^{a}	6.90± 0.41 ^{a}
Albumin (g/dl)	(14)	3.45	2.72	3.35	3.62	2.47	2.39
	(20)	2.63± 0.25 ^{c}	2.48± 0.23 ^{c}	3.03b± 0.01 ^{c}	2.80± 0.02 ^{bc}	3.55± 0.25 ^{a}	3.53± 0.3 ^{ab}
Globulin (g/dl)	(14)	0.77	2.23	2.03	2.13	3.55	3.63
	(20)	0.80± 0.24 ^{c}	0.09± 0.01 ^{c}	2.63± 0.29 ^{b}	3.38± 0.28 ^{ab}	3.83± 0.25 ^{a}	3.38± 0.5 ^{ab}
A/G ratio	(14)	4.48	1.22	1.65	1.70	0.69	0.66
	(20)	3.29± 0.29 ^{a}	2.76± 0.19 ^{a}	1.15± 0.14 ^{b}	0.83± 0.13 ^{b}	0.93± 0.19 ^{b}	1.04± 0.16 ^{b}

Means ± standard error

Means with different letters at the same row differ significantly at P< 0.05.

Table (9): Antibody titers (Log_{10}) of O. niloticus fish in different groups infectedwith *A. hydrophila* bacteria

Time post-infection (week)	GROUPS					
	1	2	3	4	5	6
1	2.0 ± 0.0 ^{c}	2.0± 0.0 ^{c}	3.5 ± 0.3 ^{b}	3.5 ± 0.5 ^{b}	4.0 ± 0.0 ^{ab}	4.5 ± 0.5 ^{a}
2	3.5 ± 0.5 ^{ac}	3.0 ± 0.0 ^{c}	4.0 ± 0.0 ^{ab}	4.5 ± 0.5 ^{a}	4.5 ± 0.5 ^{a}	4.5 ± 0.5 ^{a}
3	3.5 ± 0.5 ^{d}	4.0 ± 0.0 ^{cd}	5.5 ± 0.5 ^{ab}	5.0 ± 0.58 ^{bc}	6.0 ± 0.0 ^{ab}	6.5 ± 0.5 ^{a}
4	3.5 ± 0.5 ^{c}	3.5 ± 0.5 ^{c}	5.5 ± 0.5 ^{b}	5.5 ± 0.5 ^{b}	7.0 ± 0.0 ^{a}	7.5 ± 0.5 ^{a}
5	5.5 ± 0.5 ^{c}	4.5 ± 0.5 ^{c}	7.0 ± 0.0 ^{b}	7.0 ± 0.5 ^{b}	8.5 ± 0.5 ^{a}	9.0 ± 0.0 ^{a}
6	5.5 ± 0.5 ^{c}	6.0 ± 0.0 ^{c}	8.0 ± 0.0 ^{b}	7.5 ± 0.5 ^{b}	9.0 ± 0.0 ^{a}	9.0 ± 0.0 ^{a}
7	6.0 ± 0.0 ^{c}	5.5 ± 0.5 ^{c}	8.5 ± 0.5 ^{b}	9.0 ± 0.0 ^{b}	10.0 ± 0.0 ^{a}	9.5 ± 0.5 ^{a}
8	7.0 ± 0.0 ^{c}	7.5 ± 0.5 ^{c}	9.0 ± 0.0 ^{b}	8.5 ± 0.5 ^{b}	10.0 ± 0.0 ^{a}	10.0 ± 0.0 ^{a}
9	8.0 ± 0.0 ^{c}	8.5 ± 0.5 ^{b}	10.0 ± 0.0 ^{a}	9.5 ± 0.5 ^{a}	9.5 ± 0.5 ^{a}	10.0 ± 0.0 ^{a}
10	7.0 ± 0.0 ^{c}	7.5 ± 0.5 ^{c}	9.5 ± 0.5 ^{a}	10.0 ± 0.0 ^{a}	9.0 ± 0.0 ^{a}	10.0 ± 0.0 ^{a}

Means ± standard error

Means with different letters at the same row differ significantly at $P< 0.05$.

Table (10): Mortality percentage of different fish groups during 7 days post challenge

Days post- challenge	GROUPS					
	1	2	3	4	5	6
1	10	5	--	--	--	--
2	15	15	5	5	--	--
3	20	20	10	20	--	5
4	20	25	10	15	5	10
5	20	20	5	--	5	--
6	--	--	--	--	--	--
7	--	--	--	--	--	--
Total	85	85	30	40	10	15

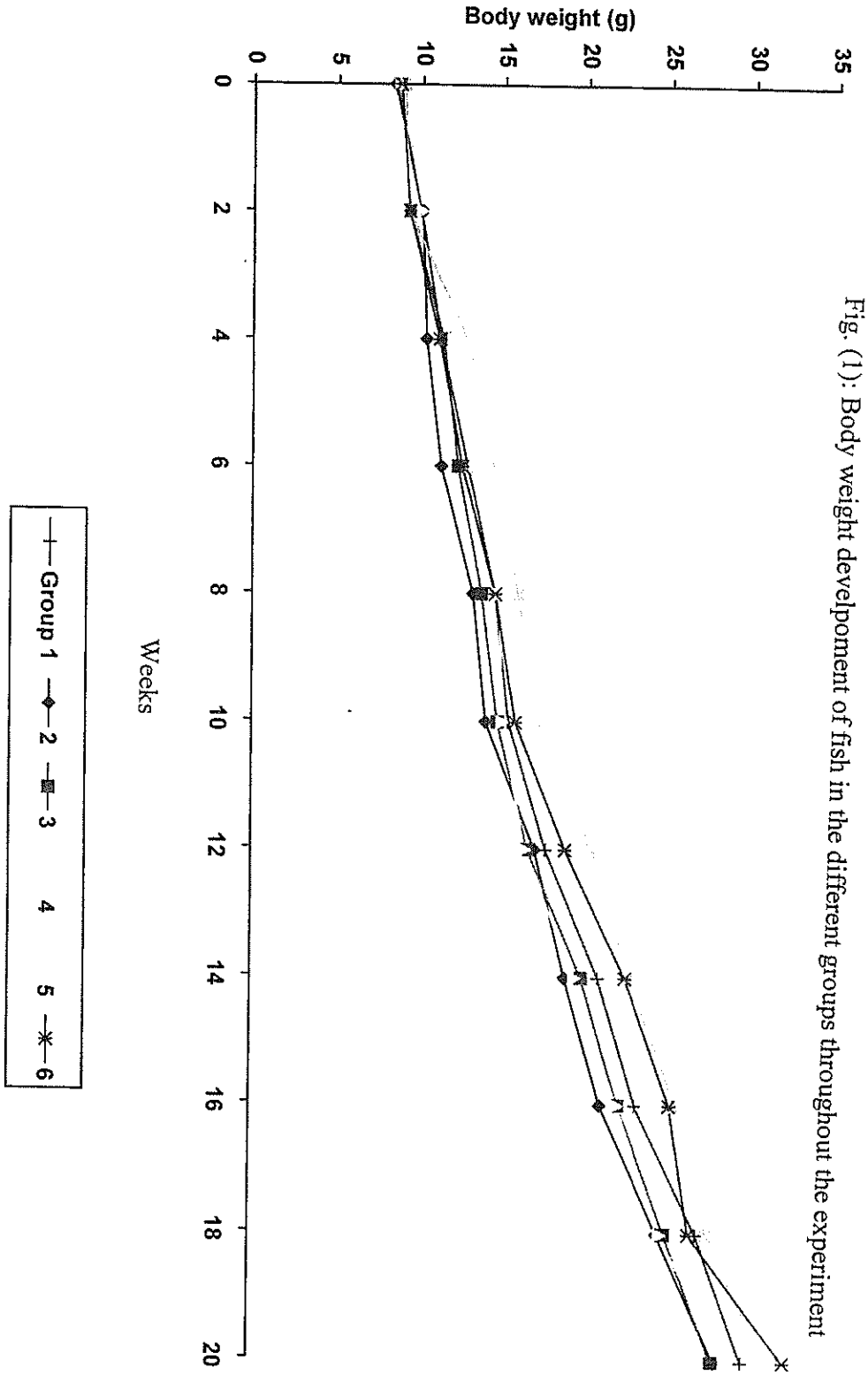

Fig. (1): Body weight development of fish in the different groups throughout the experiment

الملخص العربى

تاثير نسبة الكربوهيدرات الى الدهون باضافة او بدون اضافة الثيامين على الاداء والاستجابة المناعية فى البلطى النيلى

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استهدف هذا العمل دراسة تاثير نسب الكربوهيدرات الى الدهون فى علائق البلطى النيلى على الاداء والتركيب الكيميائى للجسم, الاستجابة المناعية, الحماية ضد *Aeromonas hydrophila* وفحص العلاقة بين نسبة الثيامين فى العليقة ومعدل استهلاك الكربوهيدرات والدهون للبلطى النيلى. ولهذا الغرض تم تركيب ثلاثة علائق تحتوى على ٣٤% بروتين خام , ٣٤٠٠ ك كلورى/ كجم عليقة, ولكنها فى العليقة الاولى تحتوى على ١,٥١ كنسبة بين الكربوهيدرهت والدهون , والثانية تحتوى على ٢,٩٣ والثالثة على ٦,١٦ . وقد اجريت التجربة على ١٢٠ سمكة بلطى نيلى متوسط وزنها ٩ جرام تقريبا قسمت عشوائيا الى ستة مجاميع , غذيت المجموعة الاولى والثانية على العليقة الاولى بدون اضافة او باضافة ٣٠ مجم ثيامين/كجم عليقة على التوالى, وغذيت المجموعة الثالثة والرابعةعلى العليقة الثانية بدون اضافة او باضافة ٣٠ مجم ثيامين/كجم عليقة على التوالى, غذيت المجموعة الخامسة والسادسة على العليقة الثالثة بدون اضافة او باضافة ٣٠ مجم ثيامين/كجم عليقة على التوالى,

و قد اتضح ان النسب المختلفة بين الكربوهيدرات الى الدهون (١,٥١ - ٦,١٦) بدون اضافة او باضافة ٣٠ مجم ثيامين/كجم عليقة لم تؤثر معنويا على معدل النمو, معامل التحويل الغذائى , معدل استهلاك البروتين فى البلطى النيلى ولكن لوحظ ان اعلى معدل اداء فى الاسماك التى غذيت على العليقة الثالثة (المجموعة الخامسة والسادسة) واتضح ايضا ان احتياج البلطى النيلى للثيامين مرتبط بنسبة الكربوهيدرات فى العليقة.

– لوحظ ان النسب المختلفة بين الكربوهيدرات والدهون واضافة الثيامين لم تؤثر معنويا على نسبة التصافى للاسماك ولم تؤدى الى اختلاف ملحوظ فى محتوى الرطوبة والبروتين فى الجسم ولكنها ادت الى زيادة كبيرة فى محتوى الجسم من الدهون مع زيادة نسبة الدهن فى العليقة. وقد لوحظ تحسن واضح فى صورة الدم و Phagocytic activity and index مع زيادة النسبة بين الكربوهيدرات والدهون وكذلك ادت الى زيادة فى نسبة سيرم البروتين والجلوبيولين ونقص فى نسبة الكلوستيرول وبعض الانزيميات وفى النهاية كانت مقاومة الاسماك عالية ضد الامراض عندما غذيت على عليقة بها نسب اقل من الدهون.

–من الدراسة يستنتج ان البلطى النيلى يستطيع ان يخزن كمية معنوية من الدهون فى الجسم ولكن لا يستطيع استهلاكها كمصدر للطاقة لتحسين معدل النمو على الاقل فى العلائق التى تحتوى على مستويات كافية من البروتين , وان احتياج الثيامين مرتبط بنسبة الكربوهيدرات فى العلائق وان اضافة نسبة مرتفعة من الدهون يقلل من الاستجابة المناعية والحماية ضد الامراض المعدية فى البلطى النيلى.