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Impact of carbohydrate to lipid ratio and bile salts supplementation on performance, body gain and body composition of Nile tilapia fish

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Abstract
A 8-weeks feeding trial was conducted to evaluate growth performance, body composition and some serum biochemical alteration of Nile tilapia fish fed three isonitrogenous and different energy level diets containing different levels of carbohydrates (40.1%, 34.9% and 29.9%) and lipids (6.1%, 10.6% and 16.1%) with carbohydrate-to-lipids (CHO/L) ratios 6.6, 3.3 and 1.86:1 respectively, without or with bile acids (BAs) supplementation (0.5g BAs/Kg diet) to make six experimental groups. Decreasing CHO: L ratio (from 6.6 to 3.3 or 1.86: 1) in Nile tilapia diet had no significantly effect on final body weight. Moreover, decreasing CHO/L ratio increased total body gain (TBG), gain% and SGR% in Nile tilapia diet, also numerically reduced feed intake and improved FCR, PER and EEU throughout whole experimental period. On the other hand, decreased CHO: L ratio from 6.6 to 3.3:1 with BAs supplementation increased final weight, TBG, gain% and SGR%, while lower ratio had no significant effect. Body lipid increased with lowering of CHO/L ratio and BAs addition in Nile tilapia fish diet increased whole body or hepatopancreas moisture or protein contents, while significantly reduced lipids content compared with fish group fed on the same diet without BAs supplementation. Serum total protein, globulin, total cholesterol, triglycerides and HDL concentrations increased before and after experimental challenge with decreasing CHO/L ratio while both ratio or BAs addition had no significant effect on serum creatinine, uric acid, GOT, GPT, total cholesterol, triglycerides and HDL concentrations before and after experimental challenge. High lipid diet (lower CHO/L ratio) improved immune response through increasing total WBCs count, phagocytosis, lysosomal activity before and after A. hydrophila experimental challenge and consequently reduced mortality%. The obtained data indicated that the optimum CHO/L ratio in Nile tilapia diet for maximum growth performance, better immune response and health condition is 3.30:1 with BAs supplementation.

Keywords: Carbohydrate-to-lipid ratio, bile salts, growth performance, body composition, Nile tilapia fish

Introduction

Fish is an important food source in Egypt, accounting for 25.3% of the average household's protein intake. Fish consumption is 23.5 kg/person/year. Tilapia is the species most farmed in the country, which has 115,000 hectares of aquaculture ponds. The majority of the country's fish production is consumed in the country, providing the equivalent of one fish per person per week [1].

Protein considered as the most expensive nutrient in fish diets and inclusion of high dietary protein level leading to its utilization as energy source [2]. On the other hand, Omnivorous or herbivorous warm-water fish tolerate high dietary carbohydrate levels, utilizing them as a source of energy more effectively than carnivorous species. Dietary excess of carbohydrates or lipids can be stored in the form of body lipids [3]**.** Both carbohydrates and lipids spare protein use as energy sources for fish. Lipids well utilized by most fish species, but high dietary lipids may reduce growth performance and produce fatty fish [2, 4]. However, lipids deficiency in fish diets might impair growth and immune response [5]**.** It is well known that, there is no specific carbohydrate requirement for fish and required to avoid any disproportionate catabolism of proteins and lipids for the supply of energy and metabolic intermediates [6]. However, high dietary carbohydrate levels adversely deteriorate fish health through metabolic disturbances [7]. Moreover, fish physiological alterations were also observed, such as prolonged hyperglycemia

[8]. To optimize the formation of muscle tissue in fish, proteins and amino acids must not be diverted to oxidative energy metabolism and, concurrently, the levels of non-protein energy sources (lipids and carbohydrates) must be at concentrations that do not overload the liver and pancreas [3]. Therefore, suitable dietary ratio of non-protein energy sources (carbohydrate and lipid) is important for better fish growth performance and feed utilization, considering dietary protein level in fish diets [2,9,10].

In modern aquaculture production, the amount of fat in the feed is getting higher. On the one hand, an animal's body can't secrete enough bile to effectively absorb fat; on the other hand, a large amount of fat increases the burden on animals, especially the liver, resulting in hepatocytes damage, fatty liver, hepatitis and other symptoms. This will further inhibit the ability of the liver to secrete bile, leading to disorders of fat metabolism [11, 12]. As bile acids (BAs) are known to play an important role in a large number of metabolic disorders and improve nutrients utilization [13, 14]. Bile acids (BAs) are formed in the liver from cholesterol, and then, they are conjugated with taurine or glycine before excretion from the hepatocyte [15]. Bile acids have been reported to decrease lipid deposition in the liver of turbot *(Scophthalmus maximus*; [16], giant prawn *Macrobrachium rosenbergii* [17], as well as in higher vertebrates like rats [18], whereas they have also been reported to decrease body lipid deposition in *R. canadum* [19].

The carbohydrate to lipid (CHO/L) ratio in fish diets has been investigated by a number of authors, but in these previous studies, fish were fed isoenergetic diet while limited information is available about using different CHO/L ratios with different energy concentrations and no available data about bile acids supplementation with different non protein energy sources inclusion ratio. Therefore, the present study through light to evaluate the effect of different CHO/L ratios without or with bile acids supplementation on growth performance, body composition and immune response of *A. hydrophila* challenged Nile tilapia fish.

Materials and methods

Experimental fish

To achieve the purpose of the present study, a total of 270 monosex Nile tilapia *(Oreochromis niloticus)* were obtained from a private local farm in Kafrelsheikh, Egypt, with an average body weight of 20±5 g/fish. Fish were transported in a well-aerated tank to the laboratory of Animal Health Research Institute at Kafrelsheikh and then kept in glass aquaria. These aquaria were supplied with chlorine-free tap water. The aquaria were continuously aerated by an electric pump and were held at 28±2ºC. Half of the water was changed daily. Fish were acclimated for two weeks. During the acclimatization period, fish were fed on the basal diet only.

Experimental Design and Procedure

Three isonitrogenous and different energy levels diets containing different levels of carbohydrates and lipids were prepared using a pellet press with a 2 mm diameter (Table, 1). The maize grain was used as a source of carbohydrate and corn oil as the main lipid source in the diet.

The feed ingredients were thoroughly mixed, moisten with warm water (400 ml/kg) and then cold pressed and extruded to produce 2mm pellets. The diets were dried in an air convection oven set at 45^0c. After drying, the diets were stored in airtight bags prior to use.

Fish were randomly allotted into 6 equal groups (45 fish per group) of 3 aquaria (in aquaria measuring 40 x 40 x 80 cm) containing 15 fish each. Table 2 shows the applied experimental design. Fish were fed to apparent visual satiation (no eating); by hand twice a day at 9:00 and 14:00 according to [20], and extreme care was taken to assure that all supplied feed was consumed. Fish in each group were weighed at the beginning (W0) and then every two weeks weighed for a successive period of 8 weeks. and feed intake was readjusted according to the average body weight each period.

Calculation

Weight gain = Weight gain = (Final body weight- Initial body weight

Gain% = (Total gain/Initial Wt.) X100

Specific Growth Rate (SGR) = SGR = (lnWf - lnWi x100)/t (InWf = the natural logarithm of the final weight, InWi = the natural logarithm of the initial weight and t = time (days) between lnWf and lnWi).

Feed Conversion Ratio (FCR) was calculated by dividing total feed intake per aquarium by the total body weight gain per the same aquarium. [21]

Protein intake= Feed intake x protein% of the feed used/100

Protein Efficiency Ratio (PER) = Weight gain/Protein intake, was calculated according to [22].

Energy intake= Feed intake x energy content of the feed used/1000

Efficiency of energy utilization (EEU) = Energy intake/weight gain

Nutrient Retention Efficiency% = 100 X {Nutrient gain in fish body (g)/Nutrient intake (g)}

Hemato-immunological parameters

Blood samples were collected without anticoagulant from six fish of each group (two of each aquarium) at the end of the growth trial and from another six fish of each group one week after experimentally challenged. Samples sera were separated by centrifugation and stored at -20ºC until analysis. Serum total protein [23], albumin [24], Moreover serum globulin was calculated by subtract the total serum albumin from total serum protein according to [25]. GOT, GPT [26]. Creatinine and uric acid were determined according to [27], triglyceride [28], cholesterol [29], HDL and LDL [30] were estimated using commercial kits produced by Bio Diagnostic (Diagnostic and Research Reagents). Serum lysozyme activity was measured with the turbidimetric method described by [31]; using 0.2 mg / ml lyophilized Micrococcus lysodekticus as the substrate in phosphate buffer adjusted to pH 5.75. Fifty microliters of serum were added to 3 ml of bacterial suspension. The 540 nm absorbance was measured by spectrophotometer after-mixture (Ao) and after incubation for 30 min at 37 C (A). The result was expressed as one unit of lysozyme activity was defined as a reduction in absorbency of 0.001/min. (Lysozyme activity = {(A0-A)/A}. Another six samples of the blood from each group were taken on anticoagulant (sodium citrate) at the end of the growth trial and one week after experimentally challenged. Blood smears were prepared for the determination of total WBCs [32]. Additional smears were prepared for phagocytic index and activity calculation [33].

Table 1: Physical and Chemical Composition of the Basal Diet

Items	CHO/L ratio		
	6.60:1	**3.30:1**	**1.86:1**
Ingredients%			
Yellow corn	30.75	20.95	13.25
Fish meal (60% CP)	12.5	12.5	12.5
Soybean meal (44% CP)	39	41	43
Wheat flour	5	7	7
Corn gluten	7	7	7
Vegetable oil (corn oil)	3	7.8	13.5
Dicalcium phosphate (DCP)	1	1	1
Methionine	0.2	0.2	0.2
Salt	0.25	0.25	0.25
Mineral & Vitamin premix*	0.3	0.3	0.3
CMC**	1	2	2
Chemical composition			
Crude protein%	31.92	32.08	32.06
Total lipid%	6.1	10.6	16.1
Crude fiber%	4.8	5.73	5.6
Ash%	6.48	6.49	6.51
NFE%	40.1	34.9	29.9
Lysine%	1.77	1.81	1.85
Methionine%	0.89	0.89	0.88
Calcium%	1.09	1.09	1.1
Total phosphorus%	0.88	0.89	0.87
DE Kcal/Kg***	2901.7	3196.3	3571.6
P/E ratio****	11.0	10.04	8.97

*Vitamins and mineral mixture- each one Kg contains:

Vitamin A 12000000 IU, vitamin D3 2200000 IU, vitamin E 10 g, vitamin K3 2 g, vitamin B1 1 g, vitamin B2 5 g, vitamin B6 1.5 g, vitamin B12 0.01 g, vitamin C 250 g, Niacin 30 g, Biotin 0.050 g, Folic acid 1 g and Pantothenic acid 10 g and carrier to 1000 g, Manganese 60g, Copper 4 g, Zinc 50g, Iodine 1g, iron 80g, Cobalt 0.1g, Selenium 0.1g, calcium carbonate ($CaCO_3$) carrier to 3000g. **CMC= carboxy methyle cellulose. ***Digestible energy (DE) was calculated (kcal/kg) using formula based on chemical composition of feed stuffs nutrients [34]. ****P/E ratio = mg of protein/Kcal of DE [34].

Samples collection and chemical analysis

Samples from different experimental diets were collected and a total of 42 fish were sampled for chemical analysis (Six fish before distributing the fish to groups at the beginning of the growth trial and 6 fish from each group at the end of the experiment ″2 fish from each aquarium" were collected for whole body composition), also hepatopancreas samples from 36 fish were collected, 6 fish from each group at the end of the experiment (2 fish from each aquarium). All feed, whole fish and hepatopancreas samples were stored at -4°C until analysis. Dry matter (DM) contents of feed samples were determined by oven-drying overnight at 105ºC [35]. Ash contents of feed samples were determined by incineration overnight at 550ºC. Crude protein (CP) in feed samples was determined using the Kjeldahl method as described by [36]. Ether extract (EE) was determined according to the technique of [37], modified by [38].

The challenge test

At the end of growth performance trial (8 weeks from beginning), apparently healthy and active fish from each group (nearly 120 fish, about 20 fish from each group) were challenged with the pathogen *Aeromonas hydrophila* (which was kindly obtained from the Dept. of Bacteriology, Animal Health Research Institute, Kafrelsheikh, Egypt) at 0.2 ml/ fish dose of 24 hrs. The bacterial broth (3 x 10^8 cells/ml) was injected intraperitoneal (IP) according to the technique of [39]. Fish were kept under observation for two weeks where the clinical signs and mortality rates were recorded according to [40].

Statistical analysis

Statistical analysis was made using Analysis of Variance (ANOVA) two-way analysis of variance for study the effect of different treatment groups on the different studied variables studied that includes (body weight performance characters, hematological and biochemical) variables using [41].

Table 2: Experimental Design Outline

Group No.	Dietary CHO/L ratio			Bile acids (BAs)* supplementation (0.5g/Kg diet)
	6.60:1	**3.30:1**	**1.86:1**	
1	+	--	--	--
2	--	+	--	--
3	--	--	+	--
4	+	--	--	+
5	--	+	--	+
6	--	--	+	+

*The BAs (Shang dong Longchang Animal Health Product Co., Ltd, Jinan, China) mainly contained hyodesoxycholic acid, chenodeoxycholic acid and hyocholic acid at a level of 699.2, 189.2 and 77.5 g/kg BAs, respectively, estimated by HPLC.

Results

Growth performance

Decreasing CHO: L ratio (from 6.6 to 3.3 or 1.86: 1) in Nile tilapia diet had no effect on final body weight (table 3). Moreover, decreasing CHO: L ratio from 6.6 to 3.3:1 significantly increased total body gain (TBG), gain% and SGR% by about 17.9%, 17.9% and 23.6% respectively, while more CHO: L ratio reduction (1.86:1) had no effect on the previous mentioned parameters. Decreasing CHO: L ratio (from 6.6 to 3.3 or 1.86: 1) in Nile tilapia diet significantly improved FCR throughout whole experimental period.

On the other hand, decreased CHO: L ratio from 6.6 to 3.3:1 with BAs supplementation significantly increased TBG, gain% and SGR%, while lower ratio had no significant effect. Moreover, BAs supplementation non significantly decreased FCR and EEU and increased PER in fish fed on diet CHO: L ratio from 6.6:1. BAs supplementation also significantly decreased EEU in fish fed on diet with CHO: L ratio 3.3:1.

Table 3: Growth performance and feed efficiency parameters of Nile tilapia fish as affected by various CHO/L ratio without or with bile acids supplementation. (No. of sample = 45)

Items	CHO/L ratio	Bile acids (BAs) supplementation (0.5g/Kg diet)	
		Without BAs	With BAs
Initial weight (g/fish)	6.60:1	25.06±1.59^{ay}	25.44±2.03^{ax}
	3.30:1	25.06±2.28^{ax}	25.50±1.67^{ax}
	1.86:1	25.25±1.69^{ax}	25.06±2.56^{ax}
Final weight (g/fish)	6.60:1	38.47±2.36^{ax}	41.25±2.42^{ax}
	3.30:1	40.87±2.72^{ax}	43.29±2.36^{ax}
	1.86:1	39.23±1.56^{ax}	40.93±2.51^{ax}
Total body gain (TBG g/fish)	6.60:1	13.40±1.14^{abx}	15.81±1.10^{bx}
	3.30:1	15.80±0.99^{ax}	17.79±0.98^{ax}
	1.86:1	13.98±0.49^{bx}	15.87±1.11^{bx}
Gain%	6.60:1	53.48±2.79^{by}	62.16±3.36^{bx}
	3.30:1	63.06±5.24^{ay}	69.75±1.71^{ax}
	1.86:1	55.37±2.06^{by}	63.31±2.36a^{bx}
SGR%	6.60:1	0.332±0.01^{bx}	0.375±0.02^{bx}
	3.30:1	0.379±0.02^{ax}	0.410±0.01^{ax}
	1.86:1	0.342±0.01^{bx}	0.380±0.01^{bx}
Total Feed intake (TFI g/fish)	6.60:1	36.1	33.2
	3.30:1	34.9	32.1
	1.86:1	32.8	30.9
Feed conversion ratio (FCR)	6.60:1	2.69±0.11^{ax}	2.10±0.11^{ay}
	3.30:1	2.21±0.12^{bx}	1.80±0.12^{ax}
	1.86:1	2.35±0.11^{abx}	1.95±0.12^{ax}
Protein efficiency ratio (PER)	6.60:1	1.16±0.02^{ax}	1.30±0.04^{ay}
	3.30:1	1.24±0.03^{ax}	1.49±0.03^{ax}
	1.86:1	1.15±0.02^{ax}	1.39±0.02^{ax}
Efficiency of energy utilization (EEU)	6.60	7.81±0.34^{ax}	6.09±0.31^{ay}
	3.30	6.40±0.30^{ax}	5.24±0.28^{ay}
	1.86	6.51±0.32^{ax}	5.65±0.36^{ax}

Values are means ± standard error. Mean values with different letters at the same column (a - c letters) or row (x – z letters) differ significantly at (P<0.05).

Body composition and nutrient retention

Table 4: Proximate chemical composition in tissues of Nile tilapia fish as affected by various CHO/L ratio without or with bile acids supplementation. (No. of sample = 6)

Items	CHO/L ratio	Initial body composition	Final body composition	
			Bile acids (BAs) supplementation (0.5g/Kg diet)	
			Without BAs	With BAs
Whole body				
Moisture%	6.60:1	75.7	76.53±0.41^{ax}	72.99±0.76^{ay}
	3.30:1		73.19±0.47^{bx}	73.64±0.58^{ax}
	1.86:1		72.67±0.49^{bx}	73.32±0.55^{ax}
Crude protein (CP%)	6.60:1	16.26	16.61±0.19^{ay}	18.41±0.72^{ax}
	3.30:1		16.02±0.47^{ax}	16.82±0.35^{bx}
	1.86:1		16.24±0.24^{ay}	17.22±0.47^{bx}
Lipid%	6.60:1	3.07	4.12±0.13^{bx}	4.01±0.15^{ax}
	3.30:1		5.56±0.18^{ax}	4.63±0.22^{aby}
	1.86:1		5.75±0.21^{ax}	4.39±0.06^{by}
Ash%	6.60:1	4.32	4.04±0.11^{bx}	3.92±0.22^{bx}
	3.30:1		4.55±0.21^{ax}	4.49±0.19^{ax}
	1.86:1		4.68±0.35^{ax}	4.65±0.18^{ax}
CHO%	6.60:1	0.65	0.78±0.31^{ax}	0.48±0.18^{ax}
	3.30:1		0.68±0.11^{ax}	0.42±0.12^{ax}
	1.86:1		0.66±0.09^{ax}	0.41±0.11^{ax}
Hepatopancreas				
Moisture%	6.60:1		69.47±1.06^{ay}	71.77±1.29^{ax}
	3.30:1		66.73±0.56^{by}	69.50±0.78^{bx}
	1.86:1		64.80±0.52^{cy}	68.10±0.69^{cx}
Crude protein (CP%)	6.60:1		15.37±0.36^{ax}	15.00±1.11^{ax}
	3.30:1		14.93±0.63^{ax}	14.80±0.49^{ax}
	1.86:1		14.97±0.52^{ax}	15.00±0.94^{ax}
Lipid%	6.60:1		9.13±0.35^{cx}	7.00±0.79^{cy}
	3.30:1		11.40±0.37^{bx}	9.00±0.52^{by}
	1.86:1		12.67±0.65^{ax}	9.83±0.78^{ay}

Values are means ± standard error. Mean values with different letters at the same column (a - c letters) or row (x – z letters) differ significantly at (P<0.05).

Moisture percent of whole fish body and hepatopancreas tissues significantly decreased while, lipid percentage of whole body and hepatopancreas significantly increased with CHO/L ratio reduction (table 4) from 6.6 to 3.3 or 1.86:1. On the other hand, decreasing CHO: L ratio (from 6.6 to 3.3 or 1.86: 1) in Nile tilapia diet increased hepatopancreas lipid percentage. BAs inclusion in Nile tilapia fish diet significantly increased whole body and hepatopancreas moisture content, while significantly reduced lipids content compared with fish group fed on the same diet without BAs supplementation. Protein and energy retention efficiency% significantly increased with CHO/L ratio reduction in Nile tilapia fish diet (table 5) and the highest protein, lipid and energy retention efficiency% was obtained by fish group fed on diet containing 3.3:1 CHO/L ratio. BAs inclusion in Nile tilapia diet with high (6.6:1), medium (3.3:1) or low (1.86:1) CHO/L ratio increased body protein% by about 90.4%, 7.7% and 34.7% respectively compared with fish group fed on the same diet without BAs supplementation. However, BAs addition with high CHO/L ratio significantly increased lipid and energy retention, while BAs with medium or low ratio significantly decreased lipid compared with fish group fed on the same diet without BAs supplementation.

Table 5: Nutrient retention efficiency% of Nile tilapia fish as affected by various CHO/L ratio without or with bile acids supplementation. (No. of sample = 6)

Items	CHO/L ratio	Final body composition	
		Bile acids (BAs) supplementation (0.5g/Kg diet)	
		Without BAs	With BAs
CP retention efficiency%	6.60:1	17.14±0.65[cy]	32.64±2.76[ax]
	3.30:1	28.47±1.76[ax]	30.65±1.01[ax]
	1.86:1	22.41±0.82[by]	30.18±1.94[ax]
Lipid retention efficiency%	6.60:1	37.41±2.35[ay]	61.70±3.00[ax]
	3.30:1	40.77±2.02[ax]	36.01±2.76[by]
	1.86:1	28.18±0.24[bx]	20.81±0.48[cy]
Energy retention efficiency%	6.60:1	15.68±0.74[by]	27.51±0.71[ax]
	3.30:1	27.35±0.73[ax]	26.70±1.19[ax]
	1.86:1	25.05±0.71[ax]	24.90±0.87[ax]

Values are means ± standard error. Mean values with different letters at the same column (a - c letters) or row (x – z letters) differ significantly at (*P*<0.05).

Blood serum biochemical change

Serum total protein and albumin concentrations significantly increased before and after experimental challenge (table, 6) with decrease CHO/L ratio from (from 6.6 to 3.3 or 1.86:1) in Nile tilapia fish diet. The highest serum total protein and globulin concentrations were obtained by fish group fed on medium ratio (3.30:1) containing diet compared with other CHO/L ratios. Addition of BAs in highest CHO/L ratio (6.60:1) containing diet significantly increased serum total protein and albumin concentrations before and after challenge, while BAs supplementation in medium and low CHO/L ratios significantly increased serum albumin before challenge compared with fish group at the same period and fed on the same diet without BAs supplementation.

Table 6: Blood serum protein profile, liver and kidney functions related parameters of Nile tilapia fish as affected by various CHO/L ratio without or with bile acids supplementation. (No. of sample = 6)

Items	CHO/L ratio	Before A. hydrophila experimental challenge		After A. hydrophila experimental challenge	
		Bile acids (BAs) supplementation (0.5g/Kg diet)			
		Without BAs	With BAs	Without BAs	With BAs
Total protein (g/dl)	6.60:1	6.28±0.17[by]	6.82±0.03[ax]	6.16±0.17[by]	6.58±0.02[bx]
	3.30:1	6.77±0.03[ax]	6.88±0.05[ax]	6.52±0.05[ax]	6.66±0.01[ax]
	1.86:1	6.60±0.22[abx]	6.78±0.12[ax]	6.37±0.22[aby]	6.63±0.02[abx]
Albumin (g/dl)	6.60:1	5.09±0.04[by]	5.53±0.07[ax]	5.03±0.04[by]	5.39±0.16[ax]
	3.30:1	5.32±0.05[ay]	5.58±0.04[ax]	5.16±0.08[ay]	5.52±0.03[ax]
	1.86:1	5.35±0.03[ay]	5.53±0.06[ax]	5.18±0.07[ay]	5.50±0.07[ax]
Globulin (g/dl)	6.60:1	1.19±0.21[bx]	1.28±0.08[ax]	1.12±0.15[ax]	1.20±0.03[ax]
	3.30:1	1.45±0.03[ax]	1.30±0.07[ax]	1.36±0.08[ax]	1.15±0.02[ax]
	1.86:1	1.25±0.23[abx]	1.25±0.07[ax]	1.19±0.07[ax]	1.13±0.03[ax]
Creatinine (mg/dl)	6.60:1	0.29±0.07[ax]	0.21±0.01[ax]	0.32±0.04[ax]	0.27±0.03[ax]
	3.30:1	0.28±0.07[ax]	0.30±0.29[ax]	0.29±0.03[ax]	0.38±0.07[ax]
	1.86:1	0.36±0.14[ax]	0.36±0.14[ax]	0.31±0.06[ax]	0.24±0.03[ax]
Uric acid (mg/dl)	6.60:1	5.67±0.04[by]	5.84±0.03[ax]	5.97±0.05[bx]	6.00±0.04[ax]
	3.30:1	5.80±0.07[ax]	5.69±0.05[bx]	6.05±0.03[ax]	5.96±0.01[ay]
	1.86:1	5.77±0.06[ax]	5.70±0.04[abx]	5.99±0.06[abx]	5.97±0.05[ax]
GOT (U/L)	6.60:1	33.00±3.09[abx]	35.67±1.18[ax]	35.00±3.57[ax]	40.33±5.09[abx]
	3.30:1	36.67±2.41[ax]	34.33±4.74ax	42.00±6.62[ax]	43.67±3.76[ax]
	1.86:1	31.33±2.78[bx]	26.67±1.78[bx]	35.00±4.09[ax]	30.67±4.89[bx]
GPT (U/L)	6.60:1	16.67±2.43[ax]	19.33±1.65[ax]	18.67±4.25[ax]	20.00±3.09[ax]
	3.30:1	19.00±5.89[ax]	16.67±3.75[ax]	20.00±3.67[ax]	18.33±1.98[ax]
	1.86:1	15.00±4.09[ax]	17.00±5.05[ax]	17.33±5.34[ax]	16.00±3.89[ax]

Values are means ± standard error. Mean values with different letters at the same column (a - c letters) or row of each period (x – z letters) differ significantly at (*P*<0.05).

Serum lipid profile

Dietary CHO/L ratio reduction (from 6.6 to 3.3:1) in Nile tilapia fish diet significantly increased blood serum triglycerides and HDL concentrations (table 7) after A. hydrophila experimental challenge.

Immune response

It was observed that decreasing dietary CHO/L ratio (from 6.60:1 to 3.30 or 1.86:1) significantly increased phagocytic activity & index (table, 8) before and after A. hydrophila experimental challenge, however medium CHO/L ratio (3.30:1) containing diet exhibited the highest response of the above mentioned items. Moreover, BAs supplementation with different CHO/L ratios non significantly ($P \geq 0.05$) improved total WBCs counts, phagocytic activity & index and lysozyme activity before and after A. hydrophila experimental challenge compared with fish group fed on the same diet without BAs supplementation.

Table 7: Blood lipids profile of Nile tilapia fish as affected by various CHO/L ratio without or with bile acids supplementation. (No. of sample = 6)

Items	CHO/L ratio	Before A. hydrophila experimental challenge		After A. hydrophila experimental challenge	
		Bile acids (BAs) supplementation (0.5g/Kg diet)			
		Without BAs	With BAs	Without BAs	With BAs
Total cholesterol (mg/dl)	6.60:1	184.53±5.08^{ax}	187.13±7.52^{ax}	187.67±7.06^{ax}	190.57±7.47^{ax}
	3.30:1	186.10±6.95^{ax}	188.03±6.95^{ax}	189.57±6.76^{ax}	190.70±6.71^{ax}
	1.86:1	188.17±5.96^{ax}	192.60±1.00^{ax}	191.07±6.29^{ax}	198.37±1.78^{ax}
Triglycerides (mg/dl)	6.60:1	193.50±2.89^{ax}	194.83±1.88a^{bx}	188.30±4.53^{by}	196.33±1.75^{ax}
	3.30:1	194.17±1.53^{ax}	193.50±1.59^{bx}	197.13±1.95^{ax}	196.47±1.59^{ax}
	1.86:1	195.37±1.09^{ax}	196.63±1.35^{ax}	195.10±0.29^{ax}	197.37±0.47^{ax}
HDL (mg/dl)	6.60:1	49.70±2.00^{ax}	49.83±0.54^{ax}	46.30±1.90^{bx}	45.70±1.02^{bx}
	3.30:1	52.30±0.76^{ax}	50.83±1.82^{ax}	49.47±1.05^{ax}	50.07±0.53^{ax}
	1.86:1	49.73±0.49^{ax}	50.70±0.53^{ax}	47.43±1.29^{abx}	48.00±1.25^{abx}
LDL (mg/dl)	6.60:1	96.03±6.94^{ax}	98.33±7.70^{ax}	103.71±7.96^{ax}	105.60±8.35^{abx}
	3.30:1	94.96±7.02^{ax}	98.50±7.41^{ax}	100.67±7.48^{ax}	101.34±6.59^{bx}
	1.86:1	99.33±5.94^{ax}	102.57±1.12^{ax}	104.61±6.78^{ax}	110.89±3.06^{ax}

Values are means ± standard error. Mean values with different letters at the same column (a - c letters) or row of each period (x – z letters) differ significantly at ($P<0.05$).

Health condition and survival rate

Table (9) shows that the lowest mortality% (5%) after infection belonged to Nile tilapia fish group fed diet containing medium CHO/L ratio (3.30:1) with BAs supplementation, followed by fish group fed on medium ratio without BAs addition (10.5%). The majority of fishes died without exhibiting any clinical signs. Others showed petechial hemorrhages widely distributed on different parts of the external body surface. They also showed loss of scales and skin ulceration. Internally, ascites, hemorrhagic liver, enlarged spleen and gall bladder were commonly detected (See Fig. 1). The severity of clinical signs and post-mortem lesions differed following the order of fish group fed on lowest ratio (1.86:1) with BAs addition, followed by group fed on highest ratio (6.60:1) without or with BAs addition.

Table 8: Immunological response (TWBCs, Phagocytosis and lysozyme activity) of Nile tilapia fish as affected by various CHO/L ratio without or with bile acids supplementation. (No. of sample = 6)

Items	CHO/L ratio	Before A. hydrophila experimental challenge		After A. hydrophila experimental challenge	
		Bile acids (BAs) supplementation (0.5g/Kg diet)			
		Without BAs	With BAs	Without BAs	With BAs
Total WBCs (x10^3/mm^3)	6.60:1	27.56±3.08^{ax}	28.09±5.08^{ax}	29.77±1.55^{ax}	30.65±4.05^{ax}
	3.30:1	29.45±4.34^{ax}	30.34±3.09^{ax}	32.09±5.05^{ax}	33.67±3.56^{ax}
	1.86:1	29.09±1.65^{ax}	29.75±2.95^{ax}	32.11±3.76^{ax}	32.97±2.24^{ax}
Phagocytic activity (%)	6.60:1	34.20±2.41^{bx}	34.27±0.94^{bx}	44.77±0.82^{abx}	44.27±0.42^{ax}
	3.30:1	38.83±2.71^{ax}	36.73±1.71^{ax}	46.00±1.54^{ax}	44.27±0.76^{ax}
	1.86:1	36.53±2.32^{abx}	37.37±1.53^{ax}	42.00±0.93^{bx}	43.10±0.56^{bx}
Phagocytic index	6.60:1	1.17±0.04^{abx}	1.11±0.02^{bx}	1.11±0.05by	1.19±0.02ax
	3.30:1	1.24±0.03^{ax}	1.20±0.05^{ax}	1.17±0.03bx	1.20±0.04ax
	1.86:1	1.15±0.02^{bx}	1.13±0.01^{bx}	1.26±0.10ax	1.19±0.03ay
Lysozyme activity	6.60:1	0.12±0.01^{ax}	0.11±0.01^{ax}	0.21±0.01^{ax}	0.21±0.01^{ax}
	3.30:1	0.14±0.001^{ax}	0.14±0.01^{ax}	0.21±0.02^{ax}	0.21±0.01^{ax}
	1.86:1	0.12±0.01^{ax}	0.11±0.01^{ax}	0.19±0.01^{ax}	0.20±0.01^{ax}

Values are means ± standard error. Mean values with different letters at the same column (a - c letters) or row of each period (x – z letters) differ significantly at ($P<0.05$).

Table 9: Survival and Mortality Rates of Nile Tilapia as affected by various CHO/L ratio without or with bile acids supplementation.

		Bile acids (BAs) supplementation (0.5g/Kg diet)	
		Without BAs	With BAs
Total No.	6.60:1	20	20
	3.30:1	19	20
	1.86:1	20	19
Dead No.	6.60:1	3	3
	3.30:1	2	1
	1.86:1	3	3
Survival%	6.60:1	85	85
	3.30:1	89.5	95
	1.86:1	85	84.2
Mortality%	6.60:1	15	15
	3.30:1	10.5	5
	1.86:1	15	15.8

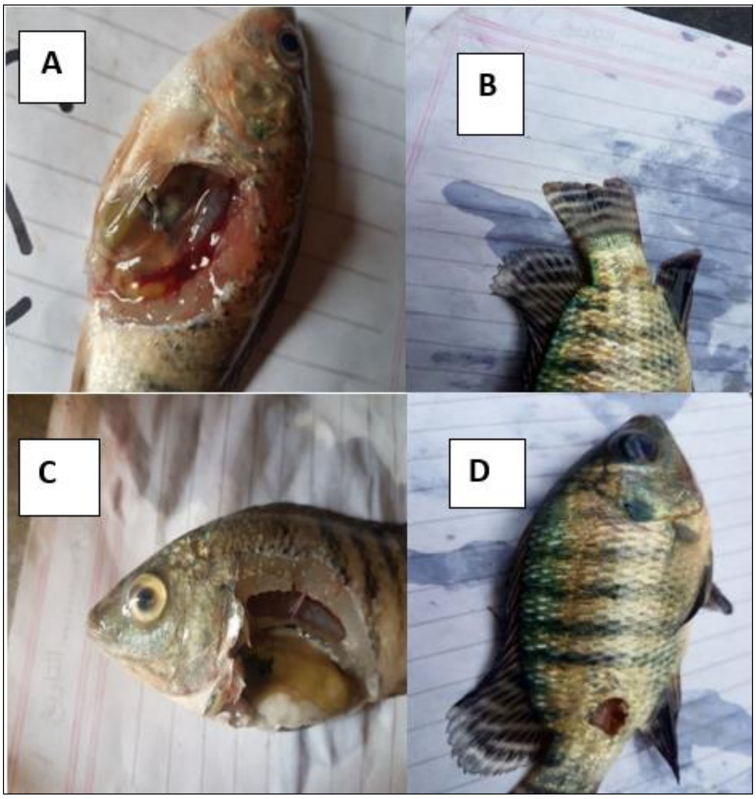

Fig 1: Enlarged spleen and gall bladder & ascites (A), loss of scales (B) protruded eye (C) and skin ulcer (D) of Nile Tilapia fish after *A. hydrophila* challenge.

Discussion

Growth performance

Compared to control group (fed on diet containing 6.6:1 CHO/L ratio) with other groups fed on lower CHO/L ratio resulted in the statistically same growth rate. According to the experimental feed formulation, the differences in feed composition between these three groups were the carbohydrate and lipid content. Thus, it considers as a very good approach to clarify that both carbohydrates and lipids were equally acceptable by Nile tilapia fish within the levels used on an energy equivalent basis in this experiment. As far as the carbohydrate and lipid levels in fish diets are concerned, our data are supported by [42] who reported that Nile tilapia fingerlings give its optimal growth performance with 30–40% proteins, 12–15% lipids and 30–40% digestible carbohydrates containing diets. Also, [2] observed nearly similar performance of Nile tilapia fish over a wide range of CHO/L ratios (4.95–0.94) and suggested that Nile tilapia can possibly utilize dietary lipids as an energy source more efficiently than carbohydrates. The present data neither support wide range of CHO/L ratios in the diets for Nile tilapia fish nor confirm that dietary lipids are more efficiently utilized as an energy source than carbohydrates. Slightly lower feed intake with lowering CHO/L ratio in Nile tilapia diet may be related to higher energy content in low ratio containing diets. However, lowest CHO/L ratio (1.86:1) starting to non-significantly deteriorate FCR or PER compared to medium ratio (3.3:1) which indicated that poor utilization of dietary nutrients when lipids level in the diets increase than 16%. Similarly, the efficiency of protein and energy utilization decreased when the CHO/L ratio in the diets dropped to lower than 1.86:1. These results indicate that Nile tilapia fish is capable of best utilizing lipids up to a level of 16% with a minimum level of carbohydrates (29.5%) in their diets. Lowering the carbohydrate level beyond this limit with a simultaneous increase in lipid level, even on an energy equivalent basis, not only affected their growth performance but also the overall efficiency of energy and protein utilization [2]. Fish fed low lipid high carbohydrate diets might metabolize less protein to meet their energy needs than fish fed high lipid low carbohydrate diets, resulting in higher dietary protein retention in tissues as supported by [9].

Supplementation of BAs in Nile tilapia fish diet containing different CHO: L ratio improved growth performance and feed efficiency parameters compared with fish group fed on the same CHO:L ratio containing diet without BAs supplementation. This improvement may be related to increasing pancreatic lipase secretion and improve intestinal absorption by BA inclusion in tilapia fish diet [43], also [44] reported that BA might enhance lipase activity and improving lipid digestion in shrimp. On the other hand, bile acids addition improves lipid emulsifications as well as lipid transport [45]**.** The obtained data are in harmony with [46] stated that BAs addition in 7% lipid containing diet improved grass carp fish growth performance and feed efficiency parameters. Growth performance response was higher in other previous study than obtained in the present trial as reported by [47]**,** this difference may have related to the authors used plant based diet and in the present study Nile tilapia fish fed on moderate fish meal containing diets. This explain insufficient synthesis of BAs or bile salts may occur in fish fed plant-based feed and be responsible for poor utilization of lipid and depressed growth of fish [48]**.** However, can be concluded that BAs addition in Nile tilapia fish diet can improve growth performance and feed efficiency parameters in both plant and fish meal containing diets [16, 49]**.**

Body composition and nutrient retention efficiency

Generally, fish chemical body composition data have a high positive correlation with growth performance. Both the endogenous and exogenous factors operate simultaneously to influence the body composition of fish [2, 50]. Dietary energy sources and level for fish plays an important role in determining body lipid deposition, the dietary lipid content is regarded as the most important factor influencing carcass lipid in fish [50, 51]. In the present study, increase in dietary lipid (decreasing CHO/L ratio) in Nile tilapia diet significantly increased whole body and hepatopancreas lipids contents, while slightly reduced moisture and crude protein contents. So that using lipids as energy source for Nile tilapia associated with high body and liver fat storage [2]. Our data are in agreement with those obtained by [9,10] concluded that higher lipids inclusion and reduction of CHO/L ratio in rainbow trout or catfish diets increased body lipid reserve compared to high CHO/L ratio fed group. However, in comparative studies, there exist many differences such as diet formulation and composition, feeding rate and strategy, fish size and age, water quality and culture system [52]. In contrast BAs supplementation with different CHO/L ratio containing diets reduced whole body or hepatopancreas lipid contents and increased moisture% compared with fish group fed on the same diet without BAs addition. Similar findings were reported in *C. auratus gibelio* [53], *S. maximus* [54], and *R. canadum* [19], which indicated that BA simulated lipid catabolism in *C. idella.*

The higher body protein retention efficiency% and lower lipid retention efficiency% with decreasing CHO/L ratio in Nile tilapia diets suggested that carbohydrates might have been converted into lipids and then accumulated in Nile tilapia fish body and more dietary lipids were used for energy in these fish, decreasing lipid retention efficiency and sparing more protein for fish growth. Thus, the dietary carbohydrate and lipid concentrations played a vital role in nutrient retention, despite the interactions identified between diets [55].

Blood serum biochemical change

Feed composition for fish nutrition does not only affect the growth of fish but also the physiological conditions, such as plasma or serum biochemical parameters [56]. Higher serum total protein and globulin concentrations with decreasing of dietary CHO/L ratio (low CHO and high lipid) indicating that high energy diet sparing more protein for growth and biological body processes. The obtained data supported by [57] reported that high dietary lipid increased serum protein and globulin levels of Nile tilapia broodstocks mainly during winter season. Moreover, dietary CHO/L ratio without or with BAs supplementation had no adverse effect on serum creatinine, uric acid, GOT and GPT concentrations [58] stated that CHO/L ratio had no significant effect on serum GOT and GPT of rainbow trout, also [47] reported that BAs addition non significantly increased serum GOT and GPT activity of Nile tilapia fish.

In the present study, slightly lower serum triglycerides, cholesterol and HDL concentrations with increasing CHO/L ratio in Nile tilapia fish diet. This data is in agreement with those obtained by [59] concluded that TG, CHO, and HDL values of fish fed diets with CHO: L ratios higher than 4.91 were significantly lower than those of the other groups ($P<0.05$). BAs supplementation numerically increased serum lipids compared with fish group fed on the same diet without Bas addition, which supported by [47] reported that serum cholesterol and HDL increased with BAS addition in tilapia diet. Opposite results have been observed in grass carp [49] and in turbot [16]. These inconsistent results may be related to the different experimental conditions including species, nature of test the diets or other culture conditions used for different studies.

Immune response and health condition

Recently, the relationship between nutrition and immunology disease resistance in homeotherms has received considerable attention [60]. It has been suggested that the minimal daily requirements which have been set for brood fish may not be adequate for optimal functioning of the immune system. In the present study the highest immune response of Nile tilapia fish (highest WBCs count, phagocytic activity and lysosomal activity) was obtained by fish fed on medium CHO/L ratio (contain 34.9 CHO and 10.6 lipid) indicating that 10% dietary lipids% seemed to be adequate for optimal functioning of immunoglobulin. The obtained data are in contrast with [57] reported that 3% lipid considered adequate for maximum immune response of bloodstocks Nile tilapia. The difference may be related to fish size and environmental conditions. Higher immune response of fish group fed on medium ratio reflected on lowering mortality% after A. hydrophila infection. [61] stated that cumulative mortality following experimental challenge with *S. iniae* was not affected by dietary lipid sources.

Conclusion

The results of the present study indicating that the optimum CHO/L ratio in Nile tilapia diet for maximum growth performance, feed efficiency parameters, higher nutrient utilization efficiency%, better immune response and health condition is 3.30:1. Moreover, BAs supplementation improve the mentioned parameters.

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