

DIETARY SORGHUM STARCH INFLUENCES GROWTH PERFORMANCE, APPARENT DIGESTIBILITY COEFFICIENT AND SOME HEPATIC ENZYME ACTIVITIES OF CARBOHYDRATE METABOLISM IN HYBRID RED TILAPIA (*OREOCHROMIS MOSSAMBICUS* × *O. NILOTICUS*) FINGERLINGS

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ABSTRACT: A 120-day feeding trial was conducted to investigate the effects of dietary sorghum starch on growth performance, feed utilization, apparent digestibility coefficient (ADC) and some hepatic enzyme activities regulating glycolytic and gluconeogenic metabolic pathways of fingerlings hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*) with initial body weight of 10.9 ± 0.2 g. Five isonitrogenous and isolipidic diets containing graded levels of sorghum starch (15, 20, 25, 30 and 35%) were formulated. The results demonstrated that weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER) and net protein utilization (NPU) values increased with increasing dietary sorghum starch up to 30%. Hepatosomatic index, plasma glucose, triglycerides, liver glycogen and liver lipid concentration of fish significantly increased with increasing dietary sorghum starch level. ADC of starch decreased significantly with increasing sorghum starch level over 30%. However, whole body compositions, and ADC of protein and lipid showed no significant differences. Dietary sorghum starch supplements tended to enhance gluconokinase and pyruvate kinase activities of the liver, but insignificant differences were observed in activities of hexokinase, phosphofructokinase-1, fructose-1, 6-bisphosphatase, and glucose-6-phosphatase in the liver for all dietary treatments. Based on WG and FCR results, the appropriate dietary sorghum starch supplementations of fingerlings hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*) can be incorporated up to 30% of diet.

KEYWORDS: Hybrid Red Tilapia, Growth Performance, Whole Body Composition, Digestibility Coefficient, Sorghum Starch, Hepatic Enzyme Activities.

INTRODUCTION

Carbohydrates are the most economical source of energy available in abundant quantities at low prices and have a protein-sparing effect in some low-protein diets and for binding other ingredients [1,2]. Feed supply and feed costs are amongst the greatest challenges for the development of sustainable fish farming. Therefore, the aquaculture industry is searching for feed ingredients that can be used to formulate cheap fish feed [2]. It was estimated that fish meal and fish oil contribute 75% of the protein and 35% of the energy in aquaculture feed [3]. It has been estimated that the cost of feed constitutes 74% of total costs for farm-made feeds and 92% for manufactured pellet feeds [4]. The cost of aquaculture production can be reduced by efficient feed formulation [5].

Dietary carbohydrate inclusion in several fish species appears to produce positive effects on growth and digestibility [6,7,8]. However, using the appropriate level of

carbohydrates in aquafeeds is of great importance, because if the appropriate amount of carbohydrates is not provided, this may have negative effects on nutrient utilization, growth, metabolism and health [9,10]. Several studies have reported that an increase in dietary carbohydrate content improves metabolism, and growth in tilapia [11,12,13]. Improved growth was observed in tilapia fed diets with 10-40% inclusion of starch [14,15].

In most aquaculture feeds, complex carbohydrates such as starch have been introduced as the principal source of carbohydrate due to the fact that dietary monosaccharides are rapidly absorbed [16] but poorly utilized [17]. There are still many inter-specific differences in the utilization and metabolism of dietary CHO by fish. For example, there is a strong relationship between the natural trophic level of fish species and their ability to utilize CHO, with herbivorous and omnivorous species usually better at digesting and utilizing CHO than carnivorous species [18,19,20,21]. Omnivorous fish species such as Nile tilapia and common carp, which feed at low trophic levels, can efficiently utilize high dietary levels of carbohydrates (30-50%) in comparison to the high trophic level carnivorous fish species [18,21,22,23]. In addition, digestibility of CHO by different species appears to be dependent on the complexity of the CHO as well as its dietary content, with simpler CHO being more easily digested while digestibility of CHO generally decreases as dietary content increases [20,24,25,26]. Knowledge of the optimum inclusion of dietary carbohydrate is indispensable for improving growth performance of fish and reducing the amount of nitrogen waste and diet costs [27]. However, feeding excessive dietary carbohydrates of fish may have harmful effects on growth, feed efficiency, physiological dysfunction, and fat deposition by stimulating lipogenic enzymes [28,29].

Processing CHO by cooking-extrusion has been found to increase digestibility of CHO in most fish species, largely by breaking down the molecular structure of starch and increasing the degree of gelatinization [24]. Digestion is thought to be the primary limiting step in the utilization of starch for growth [20]. The inability of fish to utilize digested CHO is reflected in reduced growth, inferior feed conversion ratio (FCR) and lower protein retention efficiency. Excessive dietary CHO may also decrease the palatability of feeds leading to reduced feed intake. However, fish which can tolerate and utilize high levels of CHO in their aqua-feeds allow feed manufacturers much greater flexibility to explore reductions in dietary protein and lipid content of complete feeds and therefore feed cost.

The digestion and absorption of nutrients are mostly dependent on enzyme activities involved in breakdown and assimilation of food [30]. Therefore, analysis of enzyme activities is a convenient and reliable technique that can provide comprehensive information relating to digestive physiology and nutritional conditions in the fish [31]. Digestible efficiency of digestible and non-digestible carbohydrates varies in herbivorous and carnivorous fish species [26,32]. The herbivorous fish species can utilize part of the non-starch carbohydrates in their diet due to symbiosis with the gut microbiota. However, most fish species are unable to utilize non-starch carbohydrates properly because they lack the adequate gut microbiota in their gut [26].

The optimal dietary carbohydrate level of hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*) has not been reported yet. Therefore, this study was designed to evaluate the effect of dietary sorghum starch on the growth, feed utilization, body composition, apparent digestibility, and hepatic enzyme activities of carbohydrate metabolism on fingerlings hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*).

MATERIALS AND METHODS

Experimental Diets

Five isonitrogenous and isolipidic experimental diets were formulated varying only in their dietary sorghum starch level (15, 20, 25.30 and 35%) (Table1). The dietary 30% protein and 10% lipid level was selected because it is recommended to cover the requirements of this specie [20]. Fish meal and casein were used as protein sources where, fish oil and sunflower oil were used as lipid sources. Chromic oxide (Cr_2O_3) was used as an inert marker to determine apparent digestibility of nutrients. Ingredients were ground into fine powder through a 150- μm mesh before pelleting and an appropriate amount of water was added to produce stiff dough. The dough was pelleted using California pelleting machine with 2mm diameter.

Table 1. Ingredients and proximate composition of the experimental diets (% DM basis).

Ingredients	Sorghum level				
	15%	20%	25%	30%	35%
Fish meal	10	10	10	10	10
Poultry- by product meal	15	15	15	15	15
Casein ¹ 26	15	15	15	15	15
Sorghum starch ²	15	20	25	30	35
Microcrystalline cellulose	34	29	24	19	14
Oil mix ³	6	6	6	6	6
Choline chloride	0.5	0.5	0.5	0.5	0.5
Ascorbyl-2-monophosphate	0.4	0.4	0.4	0.4	0.4
Vitamin. mineral mix ⁴	2.0	2.0	2.0	2.0	2.0
Yttrium oxide	0.1	0.1	0.1	0.1	0.1
Sodium alginate	2.0	2.0	2.0	2.0	2.0
Proximate analysis					
Dry matter	91.2	91.4	91.5	91.6	91.4
Crude protein	30.0	30.4	30.8	31.2	31.6
Crude lipid	10.2	10.1	10.2	10.1	10.1
Starch	15.0	19.9	25.1	30.2	34.9
Crude fiber	4.5	4.6	4.8	5.0	5.2
Ash	12.6	12.2	12.4	12.5	12.4
Tannin ⁵	0.25	0.3	0.32	0.36	0.42
Gross energy(MJ kg ⁻¹ diet) ⁶	13.86	14.77	15.82	16.77	17.7
ME (MJ kg ⁻¹ diet) ⁷	11.51	12.26	13.14	13.92	14.7

- 1- Casein: crude protein, 93.1%; crude lipid, 1.5% (Gannanzhou Kerui Dairy Products Development Co., Ltd., Gansu, China).
- 2- International Starch Institute, Denmark.
- 3- Oil mixture: fish oil and sunflower oil were mixed as a ratio of 1:1.
- 4- Vitamin,mineral premix (g/kg of mixture):L-ascorbic acid monophosphate, 120.0;L- α -tocopherylacetate,20.0;thiamin hydrochloride,4.0;riboflavin,9.0;pyridoxine hydrochloride,4.0;niacin,36.0;D-pantothenic acid hemicalcium salt,14.5;myoinositol,40.0;D-biotin,0.3;folic acid,0.8;menadione,0.2;retinyl acetate,1.0;cholecalciferol,0.05;cyanocobalamin,0.01;MgSO₄·7H₂O,80.0;NaH₂PO₄·2H₂O,370.0;KCl,130.0;FeSO₄·7H₂O,40.0;ZnSO₄·7H₂O,20.0;Ca-lactate,356.5;CuSO₄,0.2;AlCl₃·6H₂O,0.15;Na₂Se₂O₃,0.01;MnSO₄·H₂O, 2.0; CoCl₂·6H₂O, 1.0.
- 5- Tannin = percent tannin on a catechin equivalent basis.
- 6- Gross energy (MJ Kg⁻¹ diet) was calculated by using the following calorific values:23.9, 39.8 and 17.6 KJ g⁻¹ diet for protein, ether extract and nitrogen free extract, respectively[33].
- 7- The metabolizable energy(MJ Kg⁻¹ diet) of the experimental diets were calculated as 18.9, 35.7 and 14.7 KJ g⁻¹ diet for protein, lipid and nitrogen free extract, respectively [34].

Fish and Experimental Design

Hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*) fingerlings were obtained from the Kilo 21 hatchery belonging to General Authority for fish resource development, Alexandria road, Egypt. Fish were acclimated to the system and fed with the experimental diet twice daily for 2 weeks before the trial. After 24 hour of starvation, 1500 fish (initial body weight=10.9±0.2g) were randomly selected from the acclimatized fish and allocated into 15 circular cement pound 2m³ in equal number (n=100). During the experiment, fish were hand-fed the experimental diets to apparent satiation twice daily (10:00 p.m. and 4:00 a.m.) and weighting every two weeks to adjust the amount of feed consumption. The system contained two water pumps and upstream sandy filter units at a point between the water source (Lake Qaroun) and tanks. Each pump was drowning the water to the storage tanks and forced it through polyvinyl chloride (PVC) tubes into the rearing tanks in open system. The experimental period lasted 120 days after start. Physicochemical characteristics of water tanks were examined every week according to [35].

Sample Collection and Chemical Analysis

Before the experiment, 20 fish from the same population were randomly selected for determination of initial whole-body proximate composition. At the end of the feeding trial, fish were starved for 24 hours prior to sampling. Fish in each tank were weighed and counted for information on growth, feed efficiency, and survival. Twenty fish from each tank were randomly selected and anesthetized with tricaine methanesulfonate (MS-222, 50 mg/L) for individual weight measurements. Blood

was collected from the caudal vein of individuals using 2.5-mL sterile syringes. Plasma samples were collected after centrifugation at 3000g for 20min at 4 °C and stored at -80 °C prior to biochemical analysis. Then, the fish were quickly dissected for organ and tissue sampling. Liver and dorsal muscles were stored at -80 °C immediately before further analysis. Finally, twenty fish per tank were randomly collected for determination of final whole-body proximate composition. After the sample collection described above, the remaining fish were fed with the same diets after adding 0.5% chromic oxide (Cr_2O_3) to determine the apparent digestibility coefficients (ADCs) for dry matter, crude protein, crude lipid, and starch. Fecal collection was conducted 5–6 h after the first meal at 10:00 p.m. Fish from each replicate were anesthetized with MS-222 (50mg/L) and manually stripped of feces by applying gentle pressure in the anal area according to the method described by [36]. Feces were collected once a fortnight until sufficient dried feces had been collected for analysis. Pooled feces from each replicate were freeze-dried and stored at -20 °C until analysis of nutrient contents. Analyses of ingredients, diets, fecal samples, whole-body, and muscle composition were made following the usual procedures [37]: dry matter was determined by drying samples in an oven at 105 °C until constant weight; crude protein was determined by measuring nitrogen ($\text{N} \times 6.25$) after acid digestion using the Kjeldahl method; crude lipid was determined by petroleum ether extraction using the Soxhlet method; ash was determined by incineration in a muffle furnace at 550 °C for 16 h; starch was determined using an enzymatic method as described by [25]. Tannin content of sorghum starch was determined using a modified version of [38], vanillin-HCl assay. One gram of sorghum starch was placed in a 50ml conical flask and 50ml of analytical grade methanol was added. The flask was covered with a cork stopper, shaken thoroughly every few minutes for 2 hours and then left to stand at room temperature for an additional 20h. Two ml of 2% vanillin: 4% HCl were added to one of the test tube and 5ml of 4% HCl to other. The differences in two optical densities (the 4% HCl acting as the blank) was read on a Beckman spectrophotometer at 500nm, then compared to catchin standard curve. Diets and feces chromic oxide were determined using an inductively coupled plasma-atomic emission spectrophotometer (IRIS Advantage [HR], Thermo Jarrell Ash, Woburn, MA, USA) after perchloric acid digestion, triplicate analyses were conducted for each sample.

Enzyme activities

Liver samples were homogenized in four volumes of ice-cold 100mM Tris-HCl buffer containing 0.1mM EDTA and 0.1% Triton X-100 (v/v), pH 7.8. Homogenates were centrifuged (Kubota model 6900, Kubota Corporation, Tokyo, Japan) at 30 000 g at 4°C for 30 min and the resultant supernatants divided in aliquots and stored at -80°C for further enzyme assays. All enzyme activities were performed at 25°C and absorbance read at 340nm in a micro plate reader (ELx808TM, Bio-Tek Instruments, USA). Hexokinase (HK; EC 2.7.1.1) and glucokinase (GK; EC 2.7.1.2) activities were measured as described by [23] using a reaction mixture containing 50 mM imidazole-HCl buffer (pH 7.4), 2.5 mM ATP, 5 mM MgCl_2 , 0.4 mM NADP, 2 units mL⁻¹ G6PDH and 1 mM (HK) or 100 mM (GK) glucose. Pyruvate kinase (PK; EC 2.7.1.40) activity was measured according to [39] with a reaction mixture consisting of 50 mM imidazole-HCl buffer (pH 7.4), 5 mM MgCl_2 , 100 mM KCl, 0.15 mM NADH, 1 mM ADP, 2 units mL⁻¹ LDH and

2 mM PEP. Fructose 1,6-bisphosphatase (FBPase; EC 3.1.3.11) activity was measured as described by [40] using a reaction mixture consisting of 50 mM imidazole–HCl buffer (pH 7.4), 5 mM MgCl₂, 12 mM 2-mercaptoethanol, 0.5 mM NADP, 2 units mL⁻¹ G6PDH, 2 units mL⁻¹ PGI and 0.5 mM fructose 1,6-bisphosphate. Glucose 6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) activity was measured as described by [41,42], using a reaction mixture containing 50 mM imidazole–HCl buffer (pH 7.4), 5 mM MgCl₂, 2 mM NADP and 1 mM glucose-6-phosphate. The reaction mixture contained 50 mM imidazole–HCl buffer (pH 7.4), 5 mM MgCl₂, 0.4 mM NADP and 2 mM L-malate. Protein concentration in liver crude extracts was determined at 600 nm according to the [43] method using bovine serum albumin as a standard. All enzyme activities were expressed as per mg of hepatic soluble activity was defined as the amount of enzyme that catalyzed the hydrolysis of 1 μmol of substrate per minute at assay temperature. Plasma glucose and triacylglycerol concentration were determined using commercial kits from Enzyline, Biomerieux, Linda-A-Velha, Portugal (ALAT/ GPT, ref. 63313; ASAT/GOT, ref. 63213). Liver and muscle glycogen concentration were determined at 620 nm using the anthrone reagent method [44].

Growth performance

The following growth performance parameters were calculated as follows:

- Specific growth rate (SGR%) = $100 \times (\ln \text{ final weight} - \ln \text{ initial weight}) / 120$.
- Condition factor (CF g/cm⁻³) = $(\text{wet weight}) / (\text{total length}^3) \times 100$.
- Feed conversion (FCR) = $(\text{feed given per fish}) / (\text{weight gain per fish})$.
- Protein efficiency ratio (PER) = $(\text{weight gain per fish}) / (\text{protein intake per fish})$.
- Net protein Utilization (NPU%) = $100 \times (\text{Final body protein} - \text{initial body protein}) / \text{protein intake}$.
- Hepatosomatic index (HIS %) = $(\text{liver weight}) / (\text{fish weight}) \times 100$.
- ADC of dry matter (%) = $100 - (100 \times \% \text{Cr}_2\text{O}_3 \text{ in diet} / \% \text{Cr}_2\text{O}_3 \text{ in feces})$
- ADC of nutrients (%) = $100 - (100 \times \% \text{nutrient in feces} / \% \text{nutrient in diet} \times \% \text{Cr}_2\text{O}_3 \text{ in diet} / \% \text{Cr}_2\text{O}_3 \text{ in feces})$.

Statistical Analyses

The results are presented as means ± SE of three replications. All data were subjected to one-way analysis of variance and tested. One way Analysis of Variance (ANOVA) was applied to test the effect of different sorghum starch levels on various growth parameters, nutrient utilization, chemical composition and hepatic enzyme activity of experimental fish according to [45]. Duncan Multiple Range test was used to detect the significant differences between the means of treatments [46]. All analysis were performed using SAS (version 6, 2004 SAS Institute, Cary, NC, USA) [47].

RESULTS

Water physico-chemical characteristics (Table 2) revealed that water temperature, salinity, pH, dissolved oxygen and unionized ammonia are within the optimum ranges for rearing red tilapia. Similar physico-chemical condition was observed in all tanks of the present study as presented in (Table 2).

Table 2: An averages of water physicochemical characteristics parameters during experimental period.

Parameters	Sorghum level				
	15	20	25	30	35
Temperature °C	29.4±0.3	29.5±0.4	29.3±0.2	29.4±0.4	29.5±0.3
Salinity (g/l)	32.2±1.2	32.1±1.1	32.2±1.2	32.1±1.1	32.1±1.2
pH	7.8±0.3	7.6±0.4	7.7±0.2	7.6±0.4	7.6±0.2
Dis.Oxy ¹ . (mg/l)	7.2±0.1	7.1±0.2	7.2±0.4	7.1±0.2	7.0±0.4
Uni.am ² . (mg/l)	0.03±0.01	0.03±0.01	0.04±0.01	0.03±0.01	0.04±0.01

1- Dissolved oxygen.

2- Unionized ammonia.

After 120-days growth trial, survival rate of red hybrid tilapia (*Oreochromis mossambicus*×*O. niloticus*) was not affected by dietary starch levels (Table 3). As presented in the same table, averages of initial weights ranged between 10.85 to 10.96 g/fish with insignificant differences among the dietary groups indicating the random distribution of the experimental fish among treatment groups. Fish in all dietary treatments survived well during the trial (97%), indicating that the tested diets had no effects on red hybrid tilapia survival rates, thus all mortalities were due to accidental factors during the samples collection every two weeks to adjust the feed amounts. Significant differences in weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), feed conversion ratio (FCR), net protein utilization (NPU) and hepatosomatic index (HSI) were observed among dietary treatments ($P<0.05$). Fish fed with 30% sorghum starch diet had significantly higher WG, SGR, and PER, and lower FCR than those fish fed with diets containing 15, 20, 25 and 35% sorghum starch ($P<0.05$). NPU significantly increased with dietary sorghum starch level from 15 to 30% and then decreased with 35% level ($P<0.05$). Fish fed with diets containing 25-30% sorghum starch had significantly higher HSI values than fish fed with 15 and 35% sorghum starch diet ($P<0.05$).

Table 3. Growth performance mean values (Mean ±S.E. n=3) of red tilapia fed on different experimental diets for 120 days.

Parameters	Sorghum level				
	15	20	25	30	35
Initial weight (g/fish)	10.85 ^a ±0.32	10.91 ^a ±0.32	10.96 ^a ±0.32	10.94 ^a ±0.32	10.88±0.32
Final weight (g/fish)	61.6 ^b ±2.47	64.4 ^b ±1.8	78.5 ^a ±1.6	81.0 ^a ±2.4	50.4 ^c ±2.2
Total gain (g/fish)	50.75 ^b ±1.4	53.49 ^b ±1.8	67.54 ^a ±2.2	70.06 ^a ±1.6	39.52 ^c ±1.8
Average gain (g/fish/d)	0.42 ^b ±0.1	0.44 ^b ±0.1	0.53 ^a ±0.1	0.58 ^a ±0.1	0.32 ^c ±0.1
Specific growth rate	1.74 ^b ±0.1	1.78 ^b ±0.2	1.97 ^a ±0.12	2.0 ^a ±0.2	1.27 ^c ±0.1

Condition factor	1.8 ^b ±0.2	1.9 ^b ±0.3	2.32 ^a ±0.3	2.4 ^a ±0.1	1.5 ^c ±0.2
(g/cm ⁻³)					
Survival rate %	97	97	97	97	97
Feed consumed (g/fish)*	90.0	92.0	98.0	100.0	110
Feed conversion ratio	1.8 ^b ±0.3	1.71 ^b ±0.1	1.42 ^a ±0.3	1.42 ^a ±0.2	2.78 ^c ±0.1
PER	1.87 ^b ±0.2	1.85 ^b ±0.1	2.23 ^a ±0.2	2.24 ^a ±0.1	1.13 ^c ±0.2
NPU (%)	30.66 ^b ±2.4	29.77 ^b ±2.8	35.77 ^a ±3.2	36.16 ^a ±2.8	17.94 ^c ±1.8
HSI (%)	1.84 ^b ±0.1	1.76 ^b ±0.4	2.20 ^a ±0.2	2.32 ^a ±0.4	2.75 ^c ±0.2

Means in the same row with different superscript letters are significantly different (P<0.05).

*Non-consumed portion of food was collected, dried and deducted from total given ration.

No significant differences were observed in whole body dry matter, crude protein, crude lipid and ash among dietary treatments (Table 4).

Table 4: Carcass analysis of red tilapia fed on the experimental diets, %w/w basis (Mean ±S.E n=3).

Items	Sorghum starch level					
	Initial	15	20	25	30	35
Dry matter	27.3±1.4	27.5 ^a ±1.6	27.5 ^a ±1.8	27.2 ^a ±1.5	27.1 ^a ±1.4	27.4±1.6
Protein	16.8±1.2	16.4 ^a ±1.4	16.2 ^a ±1.2	16.1 ^a ±1.5	16.2 ^a ±1.2	16.0±1.2
Lipid	5.0±1.2	5.2 ^a ±1.1	5.4 ^a ±1.2	5.7 ^a ±1.1	5.9 ^a ±1.2	6.4±1.1
Ash	5.5±1.1	5.7 ^a ±1.2	5.7 ^a ±1.2	5.5 ^a ±1.1	5.6 ^a ±1.2	5.8±1.2

Means in the same row with different super script letters are significantly different (P<0.05).

The ADCs of dry matter increased from 70% to 80% with dietary sorghum starch levels increasing. Fish fed diets containing 30 and 35% sorghum starch were significantly higher than fish fed with 15% sorghum starch diet P<0.05 (Table 5). ADCs of starch were significantly lower when dietary sorghum starch level is more than 30% compared with fish fed with the other sorghum starch diet (P<0.05). On the other hand, the ADCs of crude protein and crude lipid were not significantly different among dietary treatments (P>0.05).

Table 5: Apparent digestibility coefficient (ADCs) of the experimental diets (Mean ± S.E n=3).

ADCs%	Sorghum starch level				
	15	20	25	30	35
Dry mater	70±2.4	71±1.2	74±1.6	76±1.5	80±1.2
Crude protein	91.5±2.2	91.6±1.8	91.4±2.5	91.6±2.4	91.2±1.8
Crude lipid	89.2±1.8	89.0±1.5	89.4±1.6	89.5±1.5	89.1±1.4
Starch	88.6±2.1	86.4±2.4	84.5±2.4	85.2±2.2	80.4±2.4

Means in the same row with different superscript letters are significantly different (P<0.05).

As it is demonstrated in (Table 6), significantly higher plasma glucose and triglyceride concentrations were obtained in fish fed diets containing 30 and 35%

sorghum starch than those fish fed with 15,20 and 25% sorghum starch diets ($P<0.05$). However, lower liver glycogen concentrations were observed in fish fed with 15% sorghum starch diet compared with fish fed the other diets ($P<0.05$). Fish fed with 35% sorghum starch diet showed higher liver lipid concentrations than fish fed with the other diets ($P<0.05$).

Table 6. Plasma glucose and triglycerides, liver glycogen and lipid concentrations of red tilapia fed different dietary sorghum starch levels (Mean \pm S.E $n=3$).

Parameters	Sorghum starch level				
	15	20	25	30	35
Plasma glucose	3.12 \pm 0.14	3.18 \pm 0.18	3.45 \pm 0.15	3.52 \pm 0.12	3.86 \pm 0.14
Plasma triglycerides	4.25 \pm 0.16	4.32 \pm 0.14	4.66 \pm 0.18	4.72 \pm 0.16	4.98 \pm 0.11
Liver glycogen	26.5 \pm 1.12	27.12 \pm 1.15	32.52 \pm 1.22	32.62 \pm 1.44	34.44 \pm 1.22
Liver lipid	322.12 \pm 1.11	322.84 \pm 1.16	333.65 \pm 1.34	334.12 \pm 1.42	342.15 \pm 1.56

Means in the same row with different super script letters are significantly different ($P<0.05$).

As presented in (Table 7), activities of GK and PK in liver were significantly affected by dietary sorghum starch levels. GK and PK activities were significantly higher and positively correlated with dietary sorghum starch levels from 25-35 compared with fish fed with 15% sorghum starch diet ($P<0.05$). Insignificant differences were detected in activities of HK, PFK-1,FBPase, and G6Pase in liver among dietary treatments ($P>0.05$).

Table 7. Activities of glucolytic and gluconeogenic enzymes in liver of hybrid red tilapia fed different dietary sorghum starch levels (Mean \pm S.E $n=3$).

Parameters	Sorghum starch level				
	15	20	25	30	35
HK	2.98 \pm 0.18	3.12 \pm 0.14	3.22 \pm 0.12	3.26 \pm 0.15	3.52 \pm 0.16
GK	24.16 \pm 1.1	24.24 \pm 1.4	26.15 \pm 1.2	26.12 \pm 1.5	29.46 \pm 1.8
PK	26.12 \pm 1.4	26.22 \pm 1.2	29.14 \pm 1.6	29.45 \pm 1.4	34.16 \pm 1.2
PFK-1	1.14 \pm 0.16	1.8 \pm 0.14	1.21 \pm 0.11	1.25 \pm 0.12	1.22 \pm 0.15
FBPase	15.45 \pm 1.6	16.12 \pm 1.1	16.22 \pm 1.2	16.66 \pm 1.4	16.44 \pm 1.1
G6Pase	11.14 \pm 1.2	11.22 \pm 1.4	11.16 \pm 1.2	12.18 \pm 1.1	12.46 \pm 1.5

Means in the same row with different super script letters are significantly different($P<0.05$).

HK=hexokinase,GK=gluconokinase,PK=pyruvate kinase,PFK-1=6-phosphofructo-1-kinase,FBPase=fructose-1,6-bisphosphatase,G6Pase= glucose-6-phosphatase.

DISCUSSION

In this study, the hybrid red tilapia was growing well under physico-chemical characteristics of water tanks. This is important finding will improve the extension of red tilapia culture under scarce and restricted of fresh water supply to fish farms. These results are in agree with the previous results reported by [48,49], where, this specie can tolerance to high salinity conduction.

The carbohydrate utilization varies greatly among fish species, where the appropriate dietary carbohydrate can improve growth and feed efficiency of fish [21,50]. The present study showed that WG, SGR and CF of hybrid red tilapia significantly increased with increasing dietary sorghum starch level from 15 to 30%, while FCR had a contrary tendency. In the same trend, PER and NPU showed a significance increase up to 30% sorghum starch level. These results are agree with the results of [51] in red tilapia, [52] in Nile tilapia, [53] in sea bream. However, in this study, fish fed diets with high sorghum starch contents (>30%) showed a decreased growth and feed utilization compared with those fed diets containing 30% or lower sorghum starch levels. Similar findings were reported in sea bream, [53] and rainbow trout, *Oncorhynchus mykiss* [54] and gibel carp, *Carassius auratus* [28]. In (Table 1) it's clear that the maximum level of tannin was 0.42% in 35% sorghum starch diet and this value less than 0.59 % in sea bream diet, which had no negative effects on growth performance [53]. In the present trial, the depressed in growth performance in 35% sorghum starch diet, maybe due to the decreased in digestibility coefficient of nutrients in this diet (Table 5).

Enlargement of liver size and glycogen concentration was increased with elevated levels of dietary carbohydrate in several fish [29]. Absorbed carbohydrate that is not used for energy usually accumulated in the liver of fish both as lipid and as glycogen after being converted [54]. This study showed that the value of HSI was increased with dietary sorghum starch levels. In the same manner, [29] reported that HSI, liver lipid, and glycogen concentrations increased with increasing in dietary wheat starch level, and demonstrated that grass carp, *Ctenopharyngodon idella*, had a very high capacity of transforming absorbed starch into tissue lipids. For instance, plasma glucose, triglycerides, liver glycogen and lipid concentration also significantly increased with increasing dietary sorghum starch levels. These results found that excess dietary carbohydrate was deposited as lipid and glycogen in hybrid red tilapia, similar to those observed in European sea bass, *Dicentrarchus labrax* [55]. In the present trial, whole-body lipid content of hybrid red tilapia was positively related to dietary sorghum starch levels and reflected with different response of this specie to glucose metabolic of starch. These results were in agreement with the previous results in several fish species [50,52,56]. However, [27] reported that the body lipid content of gibel carp was stable as the dietary starch level increased from 24 to 28% and decreased as the starch level increased from 28 to 40%. This study showed that the dry matter, protein and ash contents of whole body and muscle were not affected by dietary sorghum starch levels, which agreed with the findings in European sea bass [23] , gilthead sea bream, *Sparus aurata*[23,53] and silver barb [57].

Gelatinization of starch can enhance its digestibility compared to the low digestibility of native starch [55]. The present results indicated that sorghum starch

was very well digested by hybrid red tilapia (ADC of starch $\geq 85.2\%$) when their levels were not more than 30%. However, ADC of starch reduced significantly when dietary sorghum starch level was up to 30%, which is similar to the results of sea bream [53], cobia, *Rachycentron canadum* [36], and large yellow croaker, *Pseudosciaena crocea* [56]. The progressive enhancement in apparent dry matter digestibility concomitant with increasing dietary sorghum starch level is in line with findings in grass carp [29] and it may be explained that higher dietary cellulose level caused the lowered apparent dry matter digestibility. Dietary starch level is found to highly influence digestibility of other nutrients, especially lipid [56,58]. The decrease in protein digestibility with increasing dietary carbohydrate level was reported in white sea bream, *Diplodus sargus* [59] and large yellow croaker [56]. However, the apparent protein and lipid digestibility in this trial were not affected by dietary sorghum starch levels, in agreement with the reported results in hybrid tilapia [60], sea bream [53], cod, *Gadus morhua* [25,61] and Atlantic salmon, *Salmo salar* [5].

Carbohydrates are metabolized by glycolysis or the pentose phosphate pathway, leading to generation of energy transfer molecules in fish [62,63]. Also, dietary carbohydrates could depress the increase rate of amino acid metabolism and utilization by gluconeogenic pathways in salmon fish [64]. Hepatic gluconeogenesis is an important metabolic pathway in fish; science available scientific data on its regulation and effects by dietary carbohydrate is relatively scarce and somewhat discordant. There is a few studies on the key hepatic glycolytic (HK, GK, PFK-1, and PK) and gluconeogenic (G6Pase and FBPase) enzymes involved in glucose metabolic pathway in red tilapia. GK catalyzes the phosphorylation of glucose to glucose-6-phosphate and G6Pase hydrolyzes the glucose-6-phosphate to glucose, the two key enzymes also catalyzes the hepatic glucose/glucose-6-phosphate cycle and both play a major role in glucose homeostasis [65]. Our results showed that GK activities in liver were positively correlated with dietary sorghum starch levels, confirming that this enzyme could be regulated by dietary carbohydrates in hybrid red tilapia, as previously observed in rainbow trout [37], European sea bass [23] and gilthead sea bream [66]. On the other hand, HK activity was not affected by dietary sorghum starch levels, similar to the findings in rainbow trout, European sea bass, and gilthead sea bream [23, 53, 67]. In the same manner, our data showed that the G6Pase activity was not affected by dietary sorghum starch levels in hybrid red tilapia. Similar findings were reported in rainbow trout, gilthead sea bream and European sea bass suggesting that G6Pase gene expression and activity were also unaffected by dietary carbohydrate levels and sources [68,69,70]. PK is a key glycolytic enzyme that catalyzes the last step in glycolysis, the conversion of phosphoenolpyruvate to pyruvate [71]. The present results showed that PK activities in liver were positively correlated with dietary sorghum starch levels, comparable to those observed in rainbow trout [39] and European sea bass [23]. In this study, the dietary sorghum starch levels did not affect PFK-1 and FBPase activities in liver, as it was similar to those reported in other fish species [39,71,72,73], where these enzymes activities are not regulated by dietary carbohydrates. This finding was reported in rainbow trout, where FBPase and G6Pase activities were regulated by dietary protein levels and their activities were significantly higher with fish fed on 68% protein diet rather than 48% protein diet [74].

In conclusion, our data suggest that the dietary sorghum starch level can be incorporated up to 30% of diet, without negative effects on growth performance, nutrients utilization, digestibility coefficients, body composition and hepatic enzyme activities of carbohydrate metabolism in hybrid red tilapia fingerlings. The present results encourage the use of slain water as alternative to the limited sources of fresh water in fish culture.

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