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Effects of dietary carbohydrate level on growth and body composition of juvenile tilapia, *Oreochromis niloticus* × *O. aureus*

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Abstract

A growth trial was conducted to feed juvenile tilapia (initial weight, 9.1 ± 0.1 g), *Oreochromis niloticus* × *O. aureus*, isonitrogenous diets for 8 weeks. Six diets were formulated containing 29% crude protein from casein and gelatin, 10% crude fat from soybean oil and refined soybean lecithin and varying levels of corn starch ranging from 6% to 46% at increments of 8%, with corresponding energy to protein (E/P) ratios of 35.6, 37.9, 40.2, 42.5, 44.8 and 47.1 kJ g^{-1}. Weight gain (WG), specific growth rate, feed efficiency ratio and protein efficiency ratio were significantly higher in fish fed diets with starch ≥ 22% (or E/P ratio ≥ 40.2 kJ g^{-1}) than in fish fed diets with 6% or 14% starch (or E/P ratio of 35.6 or 37.9 kJ g^{-1}). No further improvement was measured when dietary starch content increased beyond 22%. Body protein retention showed the same general pattern as WG, and was highest in fish fed the 22% starch diet. Body composition was significantly affected by dietary starch level. Fish fed diets with starch ≥ 30% had significantly higher lipid content than fish fed diets with 6% or 14% starch. Ash content was negatively correlated with starch inclusion level, but moisture and protein contents did not show discernible trends among treatments. Results indicate that hybrid tilapia can utilize 46% dietary starch without growth retardation, while 22% starch in feed for juvenile tilapia containing 29% protein and 10% lipid, or an E/P ratio of 37.9 kJ g^{-1} is optimal.

Keywords: *Oreochromis niloticus* × *O. aureus*, tilapia, carbohydrate, utilization, starch, E/P ratio

Introduction

Carbohydrate utilization by fish varies greatly among species (Cowey, Adron & Brown 1975; Garling & Wilson 1977; Furuichi & Yone 1980; Hilton & Atkinson 1982; Berger & Halver 1987; Ellis & Reigh 1991; Nemaipour, Brown & Gatlin 1992). Generally speaking, warmwater fish utilize digestible carbohydrate more readily than do marine or coldwater species (Wilson 1994). Anderson and colleagues (1984) reported that the growth rates of Nile tilapia improved with increasing carbohydrate levels from 0% to 40%. Shiau and Lin (1993) reported a high weight gain (WG) value of 896.8% in hybrid tilapia fed diet with 40% starch. A level of 29% protein and 10% lipid in diet was considered to be adequate for growth of hybrid tilapia reared in fresh water according to previous studies (El-Sayed & Garling 1988; Chou & Shiau 1996). It has also been observed that tilapia had a higher availability of starch than that of glucose (Shiau & Chuang 1995). In these previous studies, fish were fed isoenergetic diets in which protein, lipid and carbohydrate were interchanged at ratios commensurate with their physiological fuel values to obtain equal energy contents. In the experiment described in this paper, to study the utilization of carbohydrate by juvenile tilapia, a design was used such that fish were fed diets containing the same protein and lipid content but different energy concentrations that arose solely from different carbohydrate inclusion levels.

Materials and methods

Diet preparation

Six purified and isonitrogenous diets with different levels of energy were formulated. Casein and gelatin

Table 1 Composition of experimental diets (% dry matter)

	Diet no. (% starch)					
	1 (6)	2 (14)	3 (22)	4 (30)	5 (38)	6 (46)
Ingredients						
Casein	25.76	25.76	25.76	25.76	25.76	25.76
Gelatin	6.44	6.44	6.44	6.44	6.44	6.44
Soybean oil	6	6	6	6	6	6
Corn starch	6	14	22	30	38	46
Lecithin	4.12	4.12	4.12	4.12	4.12	4.12
Vitamin premix*	2	2	2	2	2	2
Mineral premix†	5	5	5	5	5	5
Ascorbyl phosphate ester	0.1	0.1	0.1	0.1	0.1	0.1
Sodium alginate	2	2	2	2	2	2
Cellulose	42.57	34.57	26.57	18.57	10.57	2.57
Yttrium oxide	0.01	0.01	0.01	0.01	0.01	0.01
Proximate analysis						
Moisture	6.47	9.79	10.30	9.53	8.48	8.86
Crude protein	30.42	31.00	30.38	31.21	30.67	31.65
Crude lipid	5.57	6.44	6.14	6.56	7.00	7.38
Ash	4.22	4.34	4.38	4.38	4.38	4.20
ME‡ (MJ kg^{-1} feed)	10.3	11.0	11.7	12.3	13.0	13.7
E/P ratio (kJ g^{-1} protein)	35.6	37.9	40.2	42.5	44.8	47.1

*Vitamin premix contained (g kg^{-1} premix) thiamin, 5; riboflavin, 5; pyridoxine, 4; nicotinic acid, 20; calcium pantothenate, 10; biotin, 0.6; folic acid, 1.5; inositol, 200; α-tocopherol, 40; retinal, 5; cyanocobalamin, 0.01; menadione, 4; cholecalciferol, 4.8, cellulose, 700.09.
†Mineral premix contained (g kg^{-1} premix) calcium biphosphate, 122.87; calcium lactate, 474.22; sodium biphosphate, 42.03; potassium sulphate dibasic, 163.83; ferrous sulphate, 10.78; ferric citrate, 38.26; magnesium sulfatesulphate, 44.19; zinc sulfatesulphate, 4.74; manganese sulfatesulphate, 0.33; copper sulfatesulphate, 0.22; cobalt chloride, 0.43; potassium iodide, 0.02; sodium chloride, 32.33; and potassium chloride, 65.75.
‡Metabolized energy, physiological values calculated for protein, lipid and carbohydrate were 20.9, 37.6 and 8.4 kJ g^{-1}, respectively, Wee and Shu (1989).

supplied 29% dietary protein and soybean oil and lecithin provided 10% dietary lipid. The differences in energy content were created by adding corn starch at 8% increments from 6% to 46% in the six diets, which resulted in correspondingly increasing energy to protein (E/P) ratios from 35.6 to 47.1 kJ g^{-1}. The composition and proximate analysis (AOAC 1984) of diets are presented in Table 1. To calculate estimated metabolizable energy for tilapia, the biological fuel values used for protein, lipid and carbohydrate were 20.9, 37.6 and 8.4 kJ g^{-1} respectively (Wee & Shu 1989).

Dry ingredients were ground and mixed with a Hobart mixer. Oil and water were then added to form a fine dough that was extruded through a feed mill with a 2.5 mm die, and the noodles were pelleted to appropriate size. After air-drying, feeds were stored at −20 °C until use. Yttrium oxide (Y_2O_3) was added to each diet at 0.01% as an indicator to determine protein digestibility (PD).

Experimental procedure

Juvenile tilapia, *Oreochromis niloticus* × *O. aureus*, were obtained from a local hatchery. They were reared in fibreglass aquaria and fed a commercial feed containing 30% crude protein and 5.8% crude fat for 2 weeks. After the acclimation period, 30 fishes were weighed in bulk (mean initial weight, 9.1 ± 0.1 g) and stocked in each of 18 aquaria (300 L). Water in the indoor recirculating system was filtered and aerated at a flow rate of 2 L min^{-1} in each aquarium. Each diet was fed randomly to three replicate groups of fish at 4–5% of body weight per day and fed in two equal parts at 09:00 and 18:00 hours. Fish were bulk weighed every 2 weeks, and the feed amounts were adjusted accordingly. A diurnal cycle of 12 h light/12 h dark was used and the feeding trial was carried out for 8 weeks. During the experiment, dissolved oxygen, ammonia and temperature were 7.37 ± 0.28, 0.11 ± 0.01 mg L^{-1} and 26.6 ± 1.8 °C respectively.

Weight gain, specific growth rate (SGR), feed efficiency ratio (FER), protein efficiency ratio (PER), protein retention (PR), PD, hepatosomatic index (HSI) and intraperitoneal fat ratio (IPF) were calculated after the experiment as follows:

$$WG = 100 \times (W_f - W_i)/W_i$$
$$SGR = 100 \times (\text{Ln}\, W_f - \text{Ln}\, W_i)/d$$
$$FER = 100 \times \text{wet weight gain (g)/dry feed fed (g)}$$
$$PER = \text{wet weight gain (g)/total protein intake (g)}$$
$$PR = 100 \times (W_f \times P_f - W_i \times P_i)/\text{total protein intake} \times \text{number of fish per tank}$$
$$PD = 100 - 100 \times (Y_d \times P_{fe}/Y_{fe} \times P_d)$$
$$HSI = 100 \times \text{liver weight (g)/body weight (g)}$$
$$IPF = 100 \times \text{IPF weight(g)/body weight(g)}$$

where W_f and W_i are the mean final body weight and the mean initial body weight, d is the experimental duration in days, P_f and P_i are the final body protein content and the initial body protein content, Y_d and Y_{fe} are the Yttrium contents in diet and feces and P_d and P_{fe} are protein contents in diet and a feces respectively.

Proximate analyses of diets and fish carcasses were conducted after the growth trial. Moisture was determined gravimetrically in an oven at 110 °C. Crude protein was determined using the Kjeldahl method using a Kjeltec 1030 Auto-analyser (Tecator AB, Höganäs, Sweden). Feed crude fat was determined using an acid hydrolysis method, and carcasses fat using the Soxhlet extraction method using a Tecator Soxtem HT6 (Tecator AB). Ash content was determined by combustion in a muffle furnace at 550 °C.

Statistical analyses

Data were analysed by one-way analysis of variance (ANOVA), and multiple comparisons among means were made with Duncan's multiple range test (Duncan 1955) using SPSS11.0 software. The significance level was at $P<0.05$.

Results

Weight gain, SGR, FER, PER and survival rate are shown in Table 2. Fish fed diets 3–6 containing ≥ 22% starch had significantly more WG than those fed diets 1 and 2 with 6% and 14% starch. SGR, FER and PER showed the same pattern among dietary treatments as that of WG. Survival rates were not affected by experimental treatments.

The values of biological parameters are shown in Table 3. Hepatosomatic index of fish fed diet 4 with 30% starch were significantly higher than values for fish fed diets 1 and 2 with 6% and 14% starch. Intraperitoneal fat ratios of fish fed diets 4–6 were significantly higher than those of fish fed diets 1 and 2. The values of PD were all above 90% and were not significantly different except that PD values were significantly higher for diet 4 than for diet 1. As was observed for WG, fish fed diets with ≥ 22% starch had higher PR than those fed diets with 6% and 14% starch.

Body composition values are shown in Table 4. Moisture, crude protein, crude fat and ash were significantly affected by experimental treatments. Significant differences of moisture content appeared between fish fed diet 2 and fish fed diet 5, and the former was higher than the latter. The crude protein content of fish fed diet 2 was significantly lower than that of fish fed diets 1, 5 and 6. Generally, lipid content showed an increasing trend with increasing corn starch and was significantly higher in fish fed diets 3–6 than in fish fed diet 2. In contrast, ash content showed a decreasing trend with increasing corn starch level, except for diet 6. It was the highest in fish fed diet 1, intermediate in fish fed diets 3 and 4 and lowest in fish fed diets 5 and 6.

Table 2 Growth performance of tilapia fed varying levels of starch diets for 8 weeks*

Diet no. (% starch)	Weight gain (%)	SGR	FER	PER	Survival (%)
1 (6)	235.43 ± 17.07^a	2.16 ± 0.09^a	60.26 ± 3.93^a	1.94 ± 0.12^a	100.0
2 (14)	243.66 ± 3.36^a	2.20 ± 0.02^a	61.37 ± 1.70^a	1.92 ± 0.03^a	98.9
3 (22)	301.44 ± 5.53^b	2.48 ± 0.02^b	71.56 ± 1.55^b	2.23 ± 0.02^b	98.9
4 (30)	314.93 ± 7.59^b	2.54 ± 0.03^b	71.20 ± 2.14^b	2.24 ± 0.04^b	98.9
5 (38)	299.85 ± 10.28^b	2.47 ± 0.05^b	67.88 ± 1.97^b	2.14 ± 0.06^b	100.0
6 (46)	319.93 ± 20.28^b	2.56 ± 0.09^b	70.39 ± 2.91^b	2.22 ± 0.09^b	98.9

*Different superscripts within each column indicate significant ($P<0.05$) differences between fish fed different diets.
SGR, specific growth rate; FER, feed efficiency ratio; PER, protein efficiency ratio.

Table 3 Biological parameters of tilapia fed varying levels of starch diets for 8 weeks*

Diet no. (% starch)	HSI (%)	IPF (%)	PR (%)	PD (%)
1 (6)	1.96 ± 0.24^{a}	2.17 ± 0.27^{a}	36.38 ± 2.28^{a}	90.45 ± 0.93^{a}
2 (14)	1.97 ± 0.15^{a}	2.44 ± 0.40^{a}	34.72 ± 0.10^{a}	90.84 ± 1.11^{ab}
3 (22)	2.40 ± 0.40^{ab}	2.83 ± 0.35^{ab}	42.02 ± 1.78^{b}	91.89 ± 0.57^{ab}
4 (30)	2.43 ± 0.19^{b}	3.27 ± 0.62^{b}	41.16 ± 0.76^{b}	92.96 ± 0.46^{b}
5 (38)	2.29 ± 0.07^{ab}	3.13 ± 0.14^{b}	40.71 ± 2.05^{b}	91.54 ± 2.24^{ab}
6 (46)	2.39 ± 0.21^{ab}	3.27 ± 0.09^{b}	39.84 ± 0.63^{b}	92.70 ± 1.04^{ab}

*Different superscripts within each column indicate significant ($P < 0.05$) differences between fish fed different diets.
HSI, hepatosomatic index; IPF, intraperitoneal fat ratio; PR, protein retention; PD, protein digestibility.

Table 4 Body composition of tilapia fed varying levels of starch diets for 8 weeks (% wet weight)*

Diet no. (% starch)	Moisture	Protein	Lipid	Ash
1 (6)	70.16 ± 1.16^{ab}	17.45 ± 0.08^{b}	8.02 ± 0.86^{ab}	4.12 ± 0.36^{c}
2 (14)	71.44 ± 1.29^{b}	16.83 ± 0.31^{a}	7.67 ± 0.99^{a}	3.95 ± 0.07^{bc}
3 (22)	69.75 ± 0.79^{ab}	17.14 ± 0.14^{ab}	9.41 ± 0.82^{b}	3.70 ± 0.09^{b}
4 (30)	69.74 ± 1.45^{ab}	17.33 ± 0.37^{ab}	9.58 ± 1.55^{b}	3.65 ± 0.15^{b}
5 (38)	69.25 ± 0.07^{a}	17.61 ± 0.36^{b}	9.69 ± 0.33^{b}	3.21 ± 0.19^{a}
6 (46)	69.61 ± 0.20^{ab}	17.24 ± 0.28^{b}	9.71 ± 0.46^{b}	3.32 ± 0.03^{a}

*Different superscripts within each column indicate significant ($P < 0.05$) differences between fish fed different diets.

Discussion

In the present study, WG, SGR, FER and PER all showed significant responses to dietary starch levels. When fed diets containing corn starch from 22% to 46%, fish had significantly higher WG, SGR, FER and PER than those fed diets containing 6% or 14% starch. Nile tilapia had higher growth rates when fed diets containing increasing carbohydrate levels from 0% to 40% (Anderson, Jackson, Matty & Capper 1984). *Tilapia zillii* showed no growth retardation when fed diets with carbohydrate level up to 36.8% (El-Sayed & Garling 1988). Shiau and Chuang (1995) reported that tilapia (*O. niloticus* × *O. aureus*) fed diet containing 44% starch (33.7% protein, 9.2% crude fat) for 8 weeks had WG, FCR, PER and protein deposition of 426.74%, 1.49%, 2.03% and 35.67%, respectively, compared with those of 319.93%, 1.42 (FER: 64.52%), 2.22% and 39.84% (diet 6, similar to Shiau & Chuang) in this study. The difference in WG may be because of the initial average body weight of 9 g in this study compared with 0.80 g in the study of Shiau and Chuang (1995).

Dietary carbohydrates are mainly used as a source of energy, so inclusion levels should be determined based on the protein and the energy content in feed. It is well known that excessive energy content for a moderate level of protein leads to fat deposition and reduces feed consumption (Jauncey & Ross 1982; NRC 1983), while at inadequate energy levels protein will be used as an energy source (Cowey & Sargent 1979) and ammonia excretion increases (Cho & Kaushik 1985). In this study, the design of varying levels of carbohydrate compensated with cellulose led to varying E/P ratios. The results denoting an optimal carbohydrate level for tilapia could also be concluded from an optimal E/P ratio at fixed experimental protein and lipid contents. When fed diets with 6% and 14% cornstarch, fish utilized a higher proportion of protein not for deposition in their body but as an energy source. While dietary starch content increased to a level of 22% or more, improved growth performance appeared to suggest that starch was utilized as energy by tilapia and thus spared protein for growth because the increase in energy content arose solely from the increase in carbohydrate content. In other words, the E/P ratios of 35.6 and 37.9 kJ g^{-1} were not high enough in diets with 6% and 14% starch for tilapia to attain better growth. Similar results were obtained in other studies. El-Sayed and Teshima (1992) fed Nile tilapia with diets containing varying protein and energy content, and found that at each protein level, fish growth improved with increasing dietary energy content. In this study, no further improvements of WG, SGR, FER and PER were seen when dietary starch level increased from 22% to 46%, which indicated that diets containing > 22% starch could not be utilized to produce better growth at a level of 29% protein and 10% lipid. This was in accordance with the observation that WG was not significantly different in juvenile white sturgeon fed diets with same protein and lipid levels and varying D-glucose levels more than 14% (Fynn-Aikins, Hung, Liu & Li 1992). The values of PR followed a pattern similar to WG, but a decreasing trend appeared among the last four treatments. This also demonstrates that feed with a starch level exceeding 22% does not yield better growth.

Hepatosomatic index values showed a significant difference between diet 4 and diet 1 or 2. Nemaipour and colleagues (1992) reported that HSI of hybrid striped bass was not significantly affected by dietary carbohydrate, and a similar result was seen in red drum (Serrano, Nematipour & Gatlin 1992). However, different results were observed in sturgeon (Fynn-Aikins *et al*. 1992), rainbow trout (Hilton & Atkinson 1982) and red drum (Daniels & Robinson 1986). In the present study, starch inclusion level had no discernible trend in HSI. Significantly higher IPF ratio appeared in fish fed diets with $\geq$ 30% starch than in those fed 6% and 14% starch diets, apparently caused by increasing levels of dietary starch transferred to lipid and accumulated in the intraperitoneal cavity of the fish.

Body composition results showed considerable responses to dietary starch levels. It was difficult to observe definite trends in moisture and protein in this study, but lipid contents were positively related and ash content was negatively related to starch concentration. Similar results were obtained with channel catfish, *Ictalurus punctatus* (Garling & Wilson 1976), *O. aureus* (Winfree & Stickney 1981), rainbow trout, *Salmo gairdneri* (LeGrow & Beamish 1986) and Nile tilapia, *O. niloticus* (El-Sayed & Teshima 1992).

Based on the results in this study, up to 46% corn starch in feed could be utilized to support rapid growth by juvenile tilapia. Within this range, a carbohydrate level of 22% starch was found to be optimal to meet the energy requirement.

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