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PROTEIN - SPARING EFFECT BY CARBOHYDRATE IN DIETS OF RABBITFISH, *SIGANUS RIVULATUS*

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ABSTRACT

A feeding trail was carried out to study the effect of varying ratios of dietary carbohydrate to protein (CHO/CP) on growth, feed conversion, protein and energy efficiency utilization, body composition and cost-benefit analysis of marine rabbitfish, *Siganus rivulatus*, fingerlings. Ten net cages, each holding 0.5 m^3 with dimensions of 1x 1x 0.5 m were used to stock 10 fingerlings with an average of 1.1 g/fish initial weight. Two sub-optimum protein levels (35 and 25 % CP), each having two varying CHO/CP ratios (0.6; 0.9 and 1.6; 1.9, respectively), were compared with diet containing 44% CP that recorded as optimum level for *S. rivulatus* species by Shalaby (1998). Corn starch and α-cellulose were used instead of fish meal in the isocaloric experimental diets. Fish were fed the experimental diets at rate of 6% of biomass daily, six days a week for 84 days. Fish were weighed every two week intervals and feed amounts were adjusted on the basis of the new fish weight.

Results revealed that no significant differences (P<0.05) were observed in growth and feed conversion between varying CHO/CP ratios. Although protein sparing effect occurred at two sub-optimum (35 and 25% CP) protein levels, the maximum sparing of protein was found when fish were fed 1.9 CHO/CP ratio in diet containing lower protein (26% CP) and higher carbohydrate (50.13% CHO) levels based on feed conversion and protein efficiency ratio. PER and PPV% improved as CHO/CP ratio increased. Higher PER values were obtained with diet of 1.9 CHO/CP ratio. Results show that a ratio between 0.6-0.9 CHO/CP in diets containing 35% could be recommended to spare about 7-8% protein by carbohydrate. However, 22% carbohydrate could spare about 18% protein when *S.*

rivulatus fed low dietary protein levels. In addition, cost-benefit analysis indicated that protein-sparing effect of 1.9 CHO/CP ratio at low protein level was economically superior to 0.6-1.6 CHO/CP ratios.

INTRODUCTION

Protein requirements of rabbitfish have been studied for *Siganus rivulatus* (Shalaby, 1998) and *S. guttatus* (Parazo, 1989 and 1990). The above studies demonstrated that higher protein levels were needed for optimum growth of rabbitfish. Parazo (1990) found that SGR increased as dietary protein percentage increased from 25 to 45% in diets of *S. guttatus*. A more recent study by Shalaby (1998) indicated that the growth curve didn't reach plateau upto 45% CP in diets of *S. rivulatus*.

Sparing of protein level by using non protein energy sources such as lipid and carbohydrate may be useful in reduction of fish feed cost (Cho and Kaushik, 1990; Higgs *et al.* 1992; Catacutan and Coloso, 1996; Seenappa *et al.*,1995). However, utilization of carbohydrate as a protein-sparing energy sources has required less attention than lipid in fish diets (Takeuchi *et al.*, 1979; Watanabe *et al.* 1987; Wilson, 1994). Poor growth response was found for lipid by *S. rivulatus* (Shalaby, 1989) due to the weak lipolytic enzymes activity of this species (Von- Westerhagen, 1974). Thus, the dietary carbohydrate may serve as the most economical source of energy instead of protein in rabbitfish diets

. The present study therefore, was undertaken to evaluate of the effect of different dietary carbohydrate to protein ratio (as a protein replacer for energy) on the growth, conversion efficiencies, body composition and cost-benefit analysis of rabbitfish *S. rivulatus*.

MATERIALS AND METHODS

Experimental facilities

This work was carried out at the Mariculture Research Center, Faculty of Environmental Agricultural Sciences, Suez Canal University, El-Arish, North-Sinai, Egypt. Ten net cages with dimensions of 0.5x1.0x1.0m were used in duplicates. Each cage was provided with pieces of lead surrounding the bottom to prevent floating and a wooden frame on the top for supporting.

Experimental fish

Experimental fish were collected from Mediterranean Sea Coast at El-Arish, Egypt. Caught fish were transported in a 50-L plastic bucket to the rectangular fiberglass tank filled with fresh seawater. Fish were acclimated gradually to the new conditions of the rearing water tank, to minimize the stress on fish sudden change of water conditions, prior to stocking in he experimental cages. Fish spent one week in the rearing tank, then they were graduated according to the weight. Ten fish in the same weight (1.1 g/fish as an average initial weight) were selected and randomly distributed into each experimental cage. Fish were fed a control diet for two weeks and during this period healthy fish of the same weight replaced dead fish.

Experimental design

Four different ratios of carbohydrate to protein (0.6, 0.9, 1.6 and 1.9 CHO/ CP) were prepared at two suboptimum protein levels (35 and 25% CP) to compare with diet containing 44% CP that recorded as optimum level for *S. rivulatus* by Shalaby (1998).

Experimental diets

Ingredient composition of the experimental diets is presented in Table (1). Fishmeal were replaced by corn starch while α-cellulose was used to keep the diets isocaloric. Cholesterol and vitamin and mineral premixes were the same as those used by Parazo (1990) for *S. guttatus*. The experimental diets were prepared by mixing dry ingredients with water. Then the mixtures were pelleted using a meat mincer with a 1-mm diameter. The pellets were air dried and stored at 20 °C until use. Fish were fed the experimental diets three times daily (900, 1200 and 1500), six days a week at a rate of 6% of their biomass. Feed amounts were adjusted biweekly interval on the basis of the new fish biomass. The feeding experiment lasted 84 days.

Experimental conditions

Fish were reared in fresh seawater. Salinity, temperature, pH, dissolved oxygen and photoperiod values were 34±2 ppt, 28±2 °C, 8.5±0.2, 7 ppm and 12 hours, respectively. Water exchange rate was 30% daily of the total volume of rearing water.

Analytical methods

Fish samples were taken at the start and end of the experiment to determine body composition. Chemical analysis of feed and fish were carried out according to the methods described by A.O.A.C. (1990) for crude protein, lipid, crude fiber, ash, dry matter and carbohydrate. The gross energy contents of the experimental diets and fish samples were calculated by using factors of 5.65, 9.45 and 4.2 Kcal/g of protein , lipid and carbohydrate, respectively (NRC, 1993).

Statistical analysis

Analysis of variance was carried out according to Snedecor and Cochran (1982) using a completely randomized design (CRD). Differences were subjected to Duncan's (1955) Multiple Range-test at a level of significance of ($P>0.05$).

RESULTS

Results of the present study indicate that no significant differences ($P<0.05$) were found in SGR and FCR when rabbitfish, *S. rivulatus* fingerlings were fed varying dietary ratios of carbohydrate to protein of 0.6 and 0.9, also between 1.6 and 1.9 CHO/CP ratios (Table2). However, growth performance and feed conversion ratio were slightly declined at ratios of 1.6 and 1.9 CHO/CP (diets 4 and 5, respectively). Protein sparing effect has occurred at the two sub optimum protein levels (35 & 25% CP). However, the maximum sparing effect of protein was found with diet (5) that contained lower protein level (26% CP) and higher carbohydrate (50.13% CP) based on feed conversion and protein efficiency ratios. No significant differences ($P<0.05$) were observed in feed intake and FCR among all test groups of fish and control diet. On the other hand, PER increased as CHO/CP ratio increased. it recorded 1.4, 1.50, 1.66 and 1.80 for fish fed diets contained 0.6, 0.9, 1.6 and 1.9 CHO/CP ratio, respectively. All fish fed test diets were more efficient to utilize protein than fish fed control diet that recorded PER of 1.28. The same trend was found for PPV%. No significant differences ($P<0.05$) were observed in energy retention efficiency between fish fed diets 1, 2 and 3. However, the low energy retention values were recorded when fish fed diets (4) and (5).

Cost-benefit analysis (incident cost and profit index) of the test diets indicated that protein sparing effect by carbohydrate of 1.9 CHO/CP ratio at low protein level (26% CP) was economically superior, compared with 0.6 and 0.9 CHO/CP ratios at 35% protein level. Incident cost was decreased as CHO/CP ratio increased. On contrast, the profit index greatly improved forward. Data in Table (2) show the effect of varying CHO/CP ratio on body composition. Results indicated that no significant differences ($P<0.05$) were noted in protein and lipid contents of fish among all fish groups. However, ash content were significantly ($P<0.05$) higher with diets having lower CHO/CP ratios. Ash content was decreased as CHO/CP ratio increased.

DISCUSSION

Results of the present study indicate that no differences were found in final body weight, gain and SGR %/day when rabbitfish, *S. rivulatus* fingerlings were fed varying dietary ratios of carbohydrate to protein of 0.6, and 0.9 CHO/CP ratios. However, growth performance and FCR were slightly declined at higher ratios of 0.6 and 0.9 CHO/CP than control diet (diet 1). SGR was the same for fish fed diets contained 36 -37% CP (diets 2 and 3 respectively) and fish fed control diet that contained 44% protein level (diet 1). A ratio between 0.6- 0.9 CHO/CP in diets containing about 35% protein level may be used to spare about 7-8% protein by carbohydrate in diets of *S. rivulatus*. Protein sparing effect generally occurs at sub optimum dietary protein levels, it was noticed with tilapia at levels of 28 and 24% protein compared with the optimum level of 32% CP (Shiau and Peng, 1991). Jafri (1995) found that protein sparing effect by carbohydrate was not as pronounced as at sub optimum dietary protein levels 35 -30% CP when fish fed the optimum protein level (40% CP) in diets of *Labeo rohita*. Such protein sparing action by carbohydrate has been noted for other species e.g. catfish (Garling and Wilson, 1976), rainbow trout (Pieper and Pfefer, 1980), European eel (Hidalgo et al., 1993), *Catla catla* (Seenpappa and Devaraj, 1995) and sunshine bass (Hutchins *et al.,* 1998).

In the present study, maximum sparing of protein occurred when rabbitfish fed diet (5) containing higher carbohydrate level (1.9 times more than protein). It is suggesting that 22% carbohydrate could spare about 18% protein when *S. rivulatus* fed low dietary protein based on PER results.In this respect, Parazo (1990) postulated that rabbitfishes are capable to utilize dietary carbohydrate to the extent of sparing protein for growth. The better growth for *S. javus* was obtained with diet contained 35-46 % carbohydrate instead of 29 or 56% protein (Basyari & Tanaka, 1989). At the same time, histochemical studies on the intestinal bulb of *S. rivulatus* showed a strong activity of ß-glucuronidase which is responsible for carbohydrate digestion beside the moderate proteolytic enzymes activity & lower activities of lipo-enzymes (Lundberg & Liphin, 1979).

In the present study, PER increased as CHO/CP ratio increased. Higher PER values were obtained with diet contained low protein and higher carbohydrate levels (diet 5). However, the lower value of PER was found with diet containing 44% CP level. The same trend was noted for PPV%. However, the present study confirmed the negative relationship between dietary protein level and PER of rabbitfish that recorded for *S. guttatus* (Parazo, 1990) and *S. rivulatus* (Shalaby, 1998; El-Dakar, 1999). Moreover, it also has been reported for other species such as carp (Khan and Jafri, 1991; Santiago and Ryes, 1991), European eel (Degani, 1987; Hidalgo *et al.*, 1993), *L. rohita* (Jafri, 1995). Siddiqui *et al.* (1988) suggested that more protein is diverted into catabolic pathways as its proportion in the diet increased that led to waste of excessive amount of protein, either unassimilated or used unnecessarily as energy source, demonstrating a reduction of PER and PPV%.

Because of carbohydrate is the cheapest energy sources, it may saves reduce the feed cost specially when used instead of expensive dietary item such as protein. In the present study increasing of CHO/CP ratio resulted in a decrease of incident cost and an increase of profit index. These results are confirmed by findings of results Watanabe *et al.* (1987); Shiau and Peng (1993); Jafry (1995). Diet (5) that containing 1.9 CHO/CP at low protein level was superior in redaction feed cost and elevation of profit index than all other diets. Therefore, it is recommended to use 1.9 CHO/CP on the commercial scales for rabbitfish farms.

Protein and lipid content of the fish didn't affect by varying CHO/CP ratio. However, ash content had significantly ($P<0.05$) higher at diets having lower CHO/CP ratios. Ash content was decreased as CHO/CP ratio increased. This reduction may be due to decrease amount of fish meal in their diets. Similar results were obtained by (Shalaby, 1998).

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Table (1). Ingredient and proximate analysis of the experimental diets

Item	Experimental diets				
	1 control	2	3	4	5
Grams per 100 g					
Fish meal	55	40	40	25	25
Soybean meal	15	15	15	15	15
Corn starch	10	10	20	30	40
α-cellulose	10	25	15	20	10
Sunflower oil	2.5	2.5	2.5	2.5	2.5
Fish oil	2.5	2.5	2.5	2.5	2.5
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Vitamin C	0.50	0.50	0.50	0.50	0.50
Cholesterol	0.50	0.50	0.50	0.50	0.50
Sodium chloride	0.50	0.50	0.50	0.50	0.50
Vitamin premix[1]	1.00	1.00	1.00	1.00	1.00
Mineral permix[2]	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100
Proximate analysis %					
Moisture	6.01	4.44	5.10	6.13	5.70
Crude protein	44.19	37.18	35.99	25.98	26.17
Lipid	8.98	8.10	8.40	8.01	7.30
Crude fiber	11.00	26.00	16.00	20.00	11.00
Nitrogen free extract	27.85	22.60	32.42	5.04	50.13
Ash	7.98	6.12	7.19	40.97	5.40
Gross energy *Kcal/100g*	452	382	420	395	428
Digestible energy *Kcal/100g*	369	312	349	340	371
CHO: CP ratio		0.62	0.90	1.60	1.92
Cost per Kg diet[3]	2.20	1.50	1.55	1.10	1.10

1-vitamin premix contained 12000IU, 2000000IU, 10g, 2g, 1g, 4g, 1.5g, 10g, 20g, 10g, 1g, 50 mg and 500 mg of vitamin A, D_3, E, K, B_1, B_2; B_6, B_{12}, nicotinic acid, pantothonic acid, folic acid, biotin and colin, respectively.

2-Minerals premix contained 13.4, 33.4, 3.2, 13.4, 23.2, 8.4, 0.6, 4.2, 0.6, 0.6, 0.6 and 897.4 of calcium dihydrogen phosphate, calcium lactate, ferric citrate, magnesium sulfate, dipotassium phosphate, sodiumhydrogen phosphate, sodium dihydrogen phosphate, aluminum chloride, zinc sulfate, cupper sulfate, manganese sulfate, copalte chloride and wheat, respectively.

3- Costs were as common commercial feeds in local markets. Prices in Egyptian pounds (LE): LE 1.00 =US$=0.24, basis on 2001 exchange prices.

Table (2). Growth, feed and nutrient efficiencies utilization and cost-benefit analysis of rabbitfish fed varying carbohydrate to protein ratio (mean±SE)*

Item	Experimental diets				
	1 control	2 0.6 CHO:CP	3 0.9 CHO:CP	4 1.6 CHO:CP	5 1.9 CHO:CP
Initial Wt $g.fish^{-1}$	1.10 ± 0.00^{a}	1.10 ± 0.00^{a}	1.10 ± 0.00^{a}	1.10 ± 0.00^{a}	1.10 ± 0.00^{a}
Final Wt $g.fish^{-1}$	29.72 ± 0.20^{a}	28.59 ± 0.02^{a}	28.58 ± 0.12^{a}	26.56 ± 0.09^{b}	26.94 ± 0.06^{b}
Gain $g.fish^{-1}$	28.62 ± 0.27^{a}	27.49 ± 0.02^{a}	27.48 ± 0.12^{a}	25.46 ± 0.09^{b}	25.84 ± 0.06^{b}
SGR^{1} $\%.d^{-1}$	3.93 ± 0.01^{a}	3.88 ± 0.01^{a}	3.88 ± 0.01^{a}	3.79 ± 0.01^{ab}	3.81 ± 0.01^{ab}
Feed intake $g.fish^{-1}$	58.95 ± 2.86^{a}	55.34 ± 0.01^{a}	53.71 ± 0.0^{a}	63.06 ± 1.87^{a}	55.49 ± 3.70^{a}
FCR^{2}	1.89 ± 0.08^{a}	2.02 ± 0.01^{a}	1.96 ± 0.01^{a}	2.48 ± 0.07^{a}	2.27 ± 0.14^{a}
PER^{3}	1.28 ± 0.03^{e}	1.40 ± 0.01^{d}	1.50 ± 0.01^{c}	1.66 ± 0.05^{b}	1.80 ± 0.06^{a}
PPV^{4} %	22.85 ± 0.62^{e}	24.51 ± 0.61^{d}	26.98 ± 0.42^{c}	30.21 ± 0.05^{b}	31.03 ± 0.65^{a}
ER^{5} %	25.02 ± 0.75^{a}	25.58 ± 0.78^{a}	26.36 ± 0.80^{a}	22.60 ± 0.19^{a}	22.67 ± 0.89^{a}
IC^{6}	4.15 ± 0.02^{c}	3.03 ± 0.79^{b}	3.03 ± 0.05^{b}	2.72 ± 0.07^{a}	2.49 ± 0.32^{a}
PI^{7}	1.69 ± 0.02^{c}	2.32 ± 0.39^{b}	2.32 ± 0.05^{b}	2.58 ± 0.07^{a}	2.82 ± 0.09^{a}

- Values in the row having a common superscript letter are not significantly ($P>0.05$).

1- *Specific growth rate = 100 (Ln final weight-Ln Initial weight) / days*
2- *Feed conversion ratio = dry matter intake / gain*
3- *Protein efficiency ratio = weight gain / protein intake*
4- *Productive protein value = 100 (protein gain/protein intake)*
5- *Energy retention = 100 (gross energy gain / gross energy intake)*
6- *Incident cost = feed cost consumed / Kg fish produced*
7- *Profit index = value of fish crop /cost of feed consumed, 1 Kg fresh fish equals 7 LE*

Table (3). Body composition of rabbitfish fed on varying carbohydrate to protein ratio (mean±SE)*

Diets No.	Dry matter %	% on the DM basis		
		Crude protein^{ns}	Crude lipid^{ns}	Ash
Initial fish	21.00	65.02	11.40	22.60
1 (control)	32.23 ± 0.34^{b}	54.90 ± 0.19	32.63 ± 0.15	13.53 ± 0.01^{a}
2 (0.6 CHO:CP)	30.74 ± 0.16^{c}	56.65 ± 0.86	29.70 ± 1.20	13.60 ± 0.01^{c}
3 (0.9 CHO:CP)	32.63 ± 0.52^{b}	54.73 ± 0.46	32.53 ± 1.09	12.75 ± 0.12^{c}
4 (1.6 CHO: CP)	33.49 ± 0.40^{a}	53.90 ± 0.80	31.93 ± 0.06	13.05 ± 0.04^{a}
5 1.9 CHO: CP)	32.80 ± 0.16^{b}	52.98 ± 0.34	33.41 ± 0.43	12.50 ± 0.06^{b}

- Values in the column having a common superscript letter are not significantly ($P>0.05$).

ns means not significant at level of 0.05