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Utilization of Different Carbohydrates at Different Dietary Protein Levels in Grass Prawn, *Penaeus monodon* Reared in Seawater

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不同飼料蛋白質含量下海水飼育草蝦對不同碳水化合物之利用性

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ABSTRACT

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To investigate the utilization of different carbohydrate sources and the possible substitution of carbohydrate for dietary protein for grass prawn, *Penaeus monodon*, reared in seawater, nine approximately isocaloric semipurified diets were prepared. Three dietary protein levels (40, 35, 30%) were achieved by substitution with three levels (20, 25, 30%) and three sources (glucose, dextrin, starch) of dietary carbohydrate. Results indicated that prawns fed starch or dextrin had significantly ($P<0.05$) better weight gain, feed efficiency ratio (FER) and protein efficiency ratio (PER) than those fed glucose. Prawn fed starch showed significantly higher ($P<0.05$) weight gain, FER and PER than those fed 30 % dextrin. Survival rates of prawn fed starch and dextrin were higher ($P<0.05$) than those fed glucose. The post-prandial blood-sugar level peaked earliest in prawns receiving glucose, followed by those fed dextrin and finally by those fed starch. Protein deposition was high in prawns fed starch, intermediate in prawns fed dextrin and low in prawns fed glucose. These data suggest that carbohydrate utilization in *P. monodon* is highest when the source is starch followed by dextrin and finally by glucose. Decreasing the dietary protein level from 40 to 30%, by increasing the starch content in the diet from 20 to 30%, did not reduce ($P> 0.05$) weight gain, FER or survival rate suggesting that starch spared some dietary protein in this species.

INTRODUCTION

In global terms penaeids are the most important and extensively cultured crustaceans and development of adequate feeds is essential for their commercial production.

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It has been reported that protein is an essential nutrient for prawns (Andrews et al., 1972; Balazs et al., 1973; Forster and Beard, 1973; Shewbart et al., 1973; Venkatarmiah et al., 1975; Alava and Lim, 1983). Protein is an expensive component in a diet and if prawns are cultured intensively, in creased feed costs may result due to higher dependency on artificial feeding. The expensive protein fraction should therefore be optimally utilized for growth rather than for maintenance of the prawn. Knowledge of the optimal level of protein and the protein-sparing effects of non-protein nutrients such as lipids or carbohydrates may be effective in reducing feed costs.

Utilization of lipid in prawn seems to be poor. For example, Andrews et al. (1972) reported that addition of 10% supplemental lipid had an adverse effect on growth and survival of *P. setiferus.* Biddle et al. (1977) suggested that total lipid content of diets of *Macrobrachium rosenbergii* should not exceed 10%. Bautista (1986) indicated that adverse growth was observed in *P. monodon* when the feed lipid content was 15% .

Carbohydrate is the most economical dietary energy source (cost/kcal). There is little information on the carbohydrate nutrition of prawns (New, 1976, 1980; Kanazawa, 1984). The types and levels of carbohydrate in the diet have been shown to affect the growth of *Penaeus japonicus* (Deshimaru and Yone, 1978; Abdel-Rahman et al., 1979), *Penaeus aztecus* (Andrews et al., 1972) and *Penaeus duorarum* (Sick and Andrews, 1973). For juvenile *P. monodon,* Pascual et al. (1983) observed a significant influence of different types and levels of carbohydrate in the diet on survival. Alava and Pascual (1987) indicated that *P. monodon* utilized trehalose and sucrose better than glucose.

The purpose of the present study was to evaluate the utilization of three different carbohydrate sources (glucose, dextrin and starch) and their possible protein-sparing effects in grass prawn, *Penaeus monodon,* reared in seawater

MATERIALS AND METHODS

Thirty-six glass aquaria (45 x 60 x 60 cm-H x L x W) were used in this study. Three rows of wooden stands, each with three layers, were constructed to contain these glass aquaria. Each aquarium was provided with continuous aeration. Seventy-five percent of the water in each aquarium was exchanged weekly to remove impurities and maintain water quality. Dissolved oxygen was monitored weekly and was > 7.5 ppm throughout the experimental period. Water temperature ranged from 27 to 29°C, pH from 6.3 to 6.5 and salinity from 32 to 34 ppt. Photoperiod was a 12-h light/dark (08.00-20.00 h) cycle.

P. monodon juveniles (0.54 ± 0.02 g) were obtained from a commercial hatchery and acclimated to laboratory conditions for 2 weeks in a plastic tank (74 x 95 x 45 cm-W x L x H). During this period they received commercial feed.

Three dietary protein levels (40, 35 and 30%) were achieved by substitution with three levels (20, 25 or 30%) of carbohydrate. At each carbohydrate level three carbohydrate sources, namely glucose, dextrin and corn starch (Sigma Co., USA), were used. There were a total of nine treatments in this study. All the diets were analyzed for moisture, protein, ether extract, crude fiber and ash by standard AOAC methods (AOAC, 1980). The formulation and proximate composition of each diet are presented in Table 1.

The diets were prepared by thoroughly mixing the dry ingredients with oil and then adding water until a stiff dough resulted. This was then passed through a mincer with a die and the resulting spaghetti-like strings were dried at 28°C using an electrical fan. After drying, the diets were broken up and sieved into convenient pellet size and stored at - 20°C. Each diet was fed to four aquaria of prawn. At the beginning of the experiment, each aquarium was stocked with 26 prawns with an average weight of 0.54 ± 0.02 g. The duration of the study was 8 weeks. Prawns were fed their respective diets at a rate of 10% of body weight/day for the first 4 weeks and 6% of body weight/day for the last 4 weeks. This amount was close to the maximum daily ration for *P. monodon* according to the feed consumption during the acclimation period of the study. The daily ration was subdivided into two equal feedings per day at 08.00 and 17.00 h. Each group of prawn was weighed biweekly and the amount of diet fed was adjusted accordingly.

Diet performance was evaluated by calculation of percentage weight (Wt) gain, feed efficiency ratio (FER) and protein efficiency ratio (PER). The apparent digestibilities of protein and dry matter were determined using diets containing 0.5% chromic oxide as an indicator (Maynard and Loosli, 1969). In each case, the feces were collected and pooled for all prawns fed one of the diets. The fecal material was freeze-dried and a sample ashed at 550°C for chromium analysis (Gehrke et al., 1950). The apparent digestibility was calculated after Maynard and Loosli(1969).

Protein deposition was determined and calculated according to the method of Wilson and Poe (1987). Carcass analysis was performed according to AOAC methods (AOAC, 1980)

Protein deposition (%)

$$= \left(\frac{\text{Carcass protein (final)} - \text{Carcass protein (initial) (g)}}{\text{Total protein fed (g)}} \right) \times 100$$

At the end of the feeding trial, prawns were starved for 24 h before sampling the hemolymph. Prawns were bent over and a disposable syringe was used to collect the hemolymph from the conjunction between the head and the body. Two or three prawns were taken randomly from each tank and the hemolymph was sampled and pooled to

Table I Formulation and proximate composition of nine diets

Ingredient (%)	40 % Protein			35 % Protein			30 %Protein		
	1	2	3	4	5	6	7	8	9
Casein	29.54	29.54	29.54	23.74	23.74	23.74	17.94	17.94	17.94
Glucose	20.00	-	-	25.00	-	-	30.00	-	-
Dextrin	-	20.00	-	-	25.00	-	-	30.00	-
Corn starch	-	-	20.00	-	-	25.00	-	-	30.00
Cellulose	3.96	3.96	3.96	4.76	4.76	4.76	5.56	5.56	5.56
Common ingredieuts[1]	46.50	46.50	46.50	46.50	46.50	46.50	46.50	46.50	46.50
Moisture	9.43	10.09	10.11	9.82	10.51	10.23	12.05	12.09	12.12
Crude protein	40.78	40.46	40.82	35.66	35.29	34.45	30.84	29.88	30.79
Ether extract	9.53	8.31	8.68	9.05	8.24	8.83	7.78	7.60	8.06
Crude fiber	4.31	4.53	4.55	5.48	5.10	5.01	5.34	5.94	5.15
Ash	9.69	9.10	8.91	9.32	8.89	8.97	8.98	8.73	8.19
N-free extract	26.26	27.51	26.93	30.67	31.97	32.51	35.01	35.76	35.69

[1]Fish meal, 10; squid meal, 10; cod-liver oil, 4; corn oil, 4; cholesterol, 1; mineral mix[2], 2; vitamin mix[2], 2; carboxymethylcelluose(CMC), 3; chromic oxide, 0.5%.
[2]Mineral mix and vitamin mix, according to Alava and Pascual(1987).

represent 0 h. Each test diet was fed thereafter to each tank and two or three prawns were taken randomly at 1, 3, 5, 7 and 10 h after feeding for hemolymph collection. The hemolymph sample was immediately centrifuged at 13,000 rpm for 5 min (HBI microcentrifuge, USA). Supernatant (serum) was taken for the sugar analysis using a blood sugar determination kit (Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan).

All data were analyzed using analysis of variance (SPSS/PC program). Multiple comparisons among means were made with the Duncan new multiple-range test (Puri and Mullen, 1980).

RESULTS

Weight gain, feed efficiency ratio (FER), protein efficiency ratio (PER) and survival rate of *P. monodon* fed the test diets are shown in Table 2. Prawns receiving one of the

Table 2 Means and s.d. for weight gain, feed efficiency ratio (FER) protein efficiency ratio (PER) and survival rate[1]

Diet code	Carbohydrate source	Initial weight (g)	Final weight (g)	Weight gain (%)	FER	PER	Survival rate (%)
40%	Protein						
1	20 % Glucose	0.51 ±0.04	1.57 ±0.26	207.52 ±36.74[d]	0.38 ±0.04[b]	0.71 ±0.13[e]	55.77 ±4.17[c]
2	20 % Dextrin	0.59 ±0.11	2.73 ±0.27	370.99 ±49.90[ab]	0.47 ±0.02[cd]	1.05 ±0.05[bc]	65.39 ±4.89[b]
3	20 % Starch	0.57 ±0.07	2.86 ±0.44	408.17 ±61.87[ab]	0.50 ±0.05[de]	1.11 ±0.11[b]	64.42 ±5.81[b]
35%	Protein						
4	25 % Glucose	0.51 ±0.04	1.69 ±0.33	232.38 ±55.38[d]	0.35 ±0.06[b]	0.91 ±0.19[cd]	47.29 ±4.91[c]
5	25 % Dextrin	0.53 ±0.06	2.27 ±0.21	328.99 ±24.59[bc]	0.44 ±0.03[cd]	1.16 ±0.09[b]	75.00 ±8.11[ab]
6	25 % Starch	0.56 ±0.08	2.73 ±0.56	388.71 ±76.91[ab]	0.48 ±0.05[de]	1.25 ±0.15[b]	80.81 ±6.23[a]
30%	Protein						
7	30 % Glucose	0.49 ±0.06	1.23 ±0.09	152.44 ±46.20[e]	0.26 ±0.04[a]	0.76 ±0.15[de]	55.94 ±5.21[c]
8	30 % Dextrin	0.56 ±0.08	2.09 ±0.37	272.68 ±35.59[cd]	0.40 ±0.02[bc]	1.20 ±0.07[b]	71.55 ±7.31[ab]
9	30 % Starch	0.58 ±0.05	2.80 ±0.22	387.36 ±69.57[a]	0.54 ±0.03[e]	1.51 ±0.13[a]	74.36 ±6.32[ab]

[1]Values in a column having the same superscripts are not significantly different ($P>0.05$)

three cornstarch-containing diets (diets 3, 6 and 9) gained significantly (P< 0.05) more weight than prawns receiving the three glucose-containing diets (diets 1, 4 and 7). Prawns receiving the 20- and 25%-dextrin diets (diets 2 and 5) also gained significantly (P<0.05) more weight than prawns receiving the three glucose-containing diets (diets 1, 4, 7). Prawns receiving the 30%-dextrin diet (diet 8) gained more weight (P <0.05) than prawns receiving the 30% glucose diet (diet 7). The weight gains of prawns receiving the starch diet were not significantly (P>0.05) higher than those of prawns receiving the dextrin diets with the exception of the diet with the 30% carbohydrate level. At this level, prawns gained more weight (P<0.05) when receiving the starch diet (diet 9) than when receiving the dextrin diet (diet 8).

Feed efeciency ratios (FER) and protein efficiency ratios (PER) generally followed the same patterns as weight gain. Generally speaking, both FER and PER values fell into three clusters, high in prawns fed starch diets, intermediate in prawns fed dextrin diets and low in prawns fed glucose diets.

Prawns fed starch and dextrin had a significantly higher (P< 0.05) survival rate than prawns fed glucose.

Blood-sugar levels of prawns fed the test diets are shown in Fig. 1. At the 20%-carbohydrate level, blood sugar of prawns fed glucose did not rise to a peak where as for prawns fed dextrin and starch, blood-sugar levels peaked at 3 and 5 h, respectively,after the meal. At the 25%-carbohydrate level, blood-sugar levels of prawns peaked at 1, 3 and 3 h after the meal when glucose, dextrin, and starch were fed, respectively. At the 30%-carbohydrate level peaks were observed 1, 3 and 5 h after the meal in prawns fed glucose, dextrin, and starch, respectively.

Apparent protein digestibility, dry matter digestibility and body protein deposition are shown in Table 3. Both protein and dry matter digestibilities in prawns fed glucose were low compared to those in prawns fed starch and

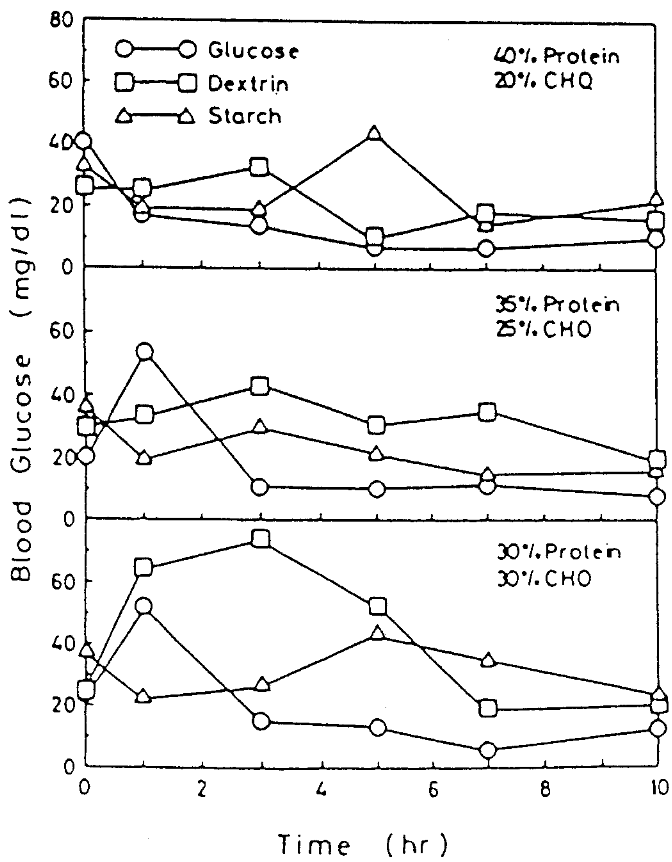

Fig. 1. Blood suger level of *P. Monodon* at various times after feeding the test diets.

Table 3 Apparent digestibility coefficients (%) and protein deposition (%)[1]

Diet code	Carbohydrate source	Protein digestibility	Dry matter digestibility	Protein deposition
40% Protein				
1	20% Glucose	78.77 ± 2.66^{b}	60.82 ± 3.20^{c}	12.09 ± 2.43^{f}
2	20% Dextrin	83.78 ± 2.78^{ab}	68.01 ± 6.02^{ab}	18.14 ± 1.21^{cd}
3	20% Starch	86.30 ± 2.27^{a}	72.68 ± 2.22^{a}	20.33 ± 0.98^{bc}
35% Protein				
4	25% Glucose	78.07 ± 1.24^{b}	65.99 ± 2.66^{b}	16.13 ± 3.13^{de}
5	25% Dextrin	83.83 ± 2.65^{ab}	67.62 ± 4.09^{ab}	20.44 ± 0.74^{bc}
6	25% Starch	81.46 ± 3.42^{ab}	67.42 ± 1.38^{ab}	23.22 ± 2.48^{b}
30% Protein				
7	30% Glucose	77.60 ± 3.69^{b}	64.85 ± 2.88^{b}	13.17 ± 3.00^{ef}
8	30% Dextrin	80.55 ± 8.63^{ab}	70.94 ± 3.70^{ab}	20.90 ± 1.42^{bc}
9	30% Starch	79.85 ± 3.73^{ab}	66.84 ± 5.07^{ab}	28.69 ± 2.91^{a}

[1]See note 1, Table 2.

dextrin. Signicificant differences ($P<0.05$) existed between the 20%-starch diet (diet 3) and the 20%-glucose diet (diet 1). Body protein deposition was high in prawns fed starch (diets 3, 6, 9), intermediate in prawns fed dextrin (diets 2, 5, 8) and low in prawns fed glucose, (diets 1, 4, 7). Differences between the low group and the intermediate or the high groups were significant ($P<0.05$). Prawns fed 30% starch deposited significantly ($P<0.05$) more protein than prawns fed 30% dextrin.

DISCUSSION

After 8 weeks, of prawns fed the three test carbohydrates (glucose, dextrin and starch), those fed starch showed the best weight gain followed by those fed dextrin and finally by those fed glucose. Poor utilization of glucose by P. *monodon* was found in the present study, in general agreenment with Alava and Pascual (1987). This poor ability to utilize glucose has also been reported for other penaeid species such as *P. japonicus* (Kitabayshi et al., 1971;. Deshimaru and Yone, 1978; Abdel-Rahman et al., 1979), *P. setiferus* (Andrews et al., 1972) and *P. duorarum* (Sick and Andrews, 1973).

The mechanism of the poor utilization of glucose is not yet fully understood. Abdel-Rahman et al. (1979) reported that serum glucose levels in *P. japonicus* increased rapidly after administration of glucose and remained at high levels for 24 h. Whereas, when the diet contained disaccharides and polysaccharides, serum glucose was found to increase to a maximal level at 3 h and then to decrease to a low level. These authors suggested that dietary glucose was quickly absorbed from alimentary canal and released into the hemolymph, resulting in a physiologically abnormal elevation of serum glucose concentration, impairing its utilization as an energy source. In the present study, it was found that serum glucose levels in *P. monodon*, fed the glucose-containing diets, peaked prior to those of prawns fed the dextrin or starch diets. The reason why the blood-sugar level of prawns fed the 20%-glucose diet did not rise to a peak (Fig. 1) is not known. One possible explanation is that the blood-sugar level peaked within 1 h after the meal.

Another possible factor, that may also relate to the poor growth performance of prawns fed glucose-containing diets, is the inhibition of amino acid absorption in the intestine by the presence of glucose (Alvarado and Robinson, 1979). This was not measured in the present study, but Hokazono et al. (1979) reported that the presence of 10 m*M* glucose reduced the uptake of L-lysine from 26.64 to 12.34% in the midintestine and from 23.24 to 5.4% in the posterior intestine of the rainbow trout.

It is interesting to note that as the dietary carbohydrate level in our experiment increased from 20 to 30%, and dietary protein levels from 40 to 30%, weight gains were not affected ($P>0.05$) when starch was used as the carbohydrate source. In contrast, weight gains,were reduced ($P<0.05$) when dextrin or glucose were used as carbohydrate sources.

Among the three carbohydrates tested, starch provided prawns with higher FER values (0.48-0.54), dextrin ranked second (0.40-0.47) and glucose last (0.26-0.38) (Table 2). It should also be noted that FER values did not decline when the dietary protein level decreased from 40 to 30% and starch was used as the carbohydrate source. However, lower FER values were observed at higher carbohydrate levels when glucose or dextrin were used as carbohydrate sources.

PER values were highest in prawns fed the starch diets (1.11- 1.51), followed by the dextrin-fed prawns (1.05-1.20) and lowest in prawns fed the glucose diets(0.71-0.91) (Table 2). Several dietary factors may affect PER. First, part of the dietary protein may be catabolized for energy use when dietary carbohydrate, is not properly utilized. For instance, the poor ability of *P. japonicus* to utilize glucose lowered PER values as the dietary glucose level increased; PER values of 0.6, 0.3 and 0.2 were noted as the dietary glucose level increased from 5 to 10, then to 20%, respectively (Abdel-Rahman et al., 1979). Second, different protein sources with different protein quality may result in different PER values. Hajra et al. (1988) studied six different protein sources at a constant dietary protein level (46 %) in *P. monodon* and obtained various PER values. Finally, the presence of some nutrient in the diet may influence PER. For instance, PER of 1.55 and 0.82 were obtained for *P. japonicus* with and without 0.5% cholesterol added to the diet, respectively (Teshima and Kanazawa, 1986).

In the present study, the protein sources and nutrient contents of all the test diets were kept approximately identical, the only difference was the source of carbohydrate. Thus the changes in PER values obtained in the present study may be attributed only to the differences in carbohydrate utilization in *P. monodon.*

Another important parameter which provides information on the nutriidonal value of a feedstuff is the digestibility. Akiyama et al. (1989) reported that soy protein had a higher protein digestibility than soybean meal for *P. vannamei* and suggest that the carbohydrate component of soybean meal may have decreased protein digestibility. The present study further indicates that glucose in the diet may have caused lower digestibility values.

We found that starch as a carbohydrate source was utilized to a greater extent by *P. monodon,* and that it had a better protein-sparing effect in the diet than dextrin or glucose. The findings of the present study may provide important information about crustacean nutrition especially for experiments designed to determine optimal dietary protein levels. In such studies, purified synthetic test diets are prepared by varying the protein levels. Glucose or dextrin are often included in the test diets to serve as carbohydrate or protein replacing energy sources. Based on the results of the present study, it is reasonable to suggest that the optimal dietary protein level for prawn would be lower if starch were chosen as the carbohydrate ingredient instead of glucose or dextrin.

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不同飼料蛋白質含量下海水飼育草蝦對不同碳水化合物之利用性

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摘　　要

以九種等熱量的純化飼料餵食飼養於海水的草蝦，以測定草蝦對不同來源之碳水化合物的利用性；以及以碳水化合物來取代飼料中蛋白質的可能性。三種飼料蛋白質含量(40、35和30％)下分別以三種不同比例(20、25和30％)及來源(葡萄糖、糊精和澱粉)之碳水化合物取代。實驗結果顯示，當草蝦餵以澱粉或糊精時較餵食葡萄糖組有顯著(p＜0.05)較佳的增重百分率、飼料轉換率(FCR)和蛋白質利用效率(FER)。當草蝦餵以澱粉時，其增重百分率、飼料轉換率和蛋白質利用效率皆顯著(P＜0.05)較餵以30％ 糊精組為佳。存活率則以餵以澱粉和糊精組顯著(P＜0.05)較餵以葡萄糖組為佳。進食後的血糖變化則以葡萄糖最早出現高峰，隨後則為糊精組，最後則為澱粉組。蛋白質蓄積則以餵食澱粉組為最高，糊精次之，以餵食葡萄糖組最差。這些結果顯示草蝦對不同碳水化合物的利用以澱粉最佳、糊精次之，葡萄糖組則最差。當飼料蛋白質從40％ 降至30％ 時而將飼料澱粉含量由20％ 升至30％ ，對草蝦之增重百分率、飼料轉換率和存活率並無影響，因此草蝦飼料中澱粉具有節約蛋白質的效用。