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The Role of Ursodeoxycholic Acid on Growth Performance and Digestive Enzyme Activities of Tilapia *Oreochromis niloticus* and Kuruma Prawn *Marsupenaeus japonicus*

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Abstract

Feeding trials were conducted to test the effects of ursodeoxycholic acid (UDCA) on growth performances and digestive enzyme activities (α– amylase, lipase and protease) of juvenile tilapia *Oreochromis niloticus* and kuruma prawn *Marsupenaeus japonicus*. A UDCA and a control diet were fed to two triplicate groups of tilapia (0.47 ± 0.01 g) for 42 days and prawn (2.00 ± 0.02 g) for 75 days. The weight gain, specific growth rate, feed efficiency and feed intake of the tilapia fed the UDCA diet were significantly higher ($P < 0.05$) than that of the control diet. In the case of prawn, little influences were observed on the growth performance. The survival of the prawn fed UDCA diet was significantly higher than the group fed the control diet. To see the effects of UDCA on the digestive enzyme activities, another triplicate groups of tilapia (0.48 ± 0.01) and prawn (1.97± 0.03 g) were also fed the UDCA and control diets during the same feeding period as growth studies. Digestive organs were collected and enzyme assays were done at different sampling periods of time. No statistical differences were observed among the dietary groups for tilapia and prawn for α-amylase activities ($P > 0.05$). Significantly higher lipase activities were detected with the lapse of holding time for tilapia and prawn fed the UDCA diet as compared to the control diet. The protease activities for both tilapia and prawn fed the UDCA diet did not show any notably differences as compared to the control. The results suggest that UDCA has some influences to increase digestive enzyme activities particularly lipase of tilapia and kuruma prawn.

Introduction

Oral administration of UDCA to human affords many pharmacological effects, such as stimulation of bile secretion, enhancement of nutrient digestibility, and improvement of hepatic activities and lipid metabolism (Ward et al. 1984, Heller et al. 1990, Thompson 1996). UDCA has been used as fish

feed additives in the aquaculture industries of Japan. However, research about the effect of the dietary UDCA on fish or shrimp is still scarce. Recently, Alam et al. (2000) investigated the effect of UDCA on growth performance and digestive (α-amylase, lipase and protease) activities of the Japanese flounder juveniles *Paralichthys olivaceus* and reported that dietary UDCA trended to improve the growth performance of the flounder. Our previous investigation also demonstrated that the lipase activities of the Japanese flounder juvenile fed on diet containing UDCA were higher than those of the juveniles fed diet without UDCA. Similar findings were also observed in the case of juvenile red sea bream (*Pagrus major*) by Alam et al. 2001. Maita et al. (1996) have investigated the pharmacological effect of UDCA in the Japanese eel (*Anguila japonica*) and reported that UDCA increased digestive enzyme (amylase, lipase and protease) activities. Deshimaru et al. (1982) reported that addition of UDCA in diet improved growth of the yellowtail (*Seriola quinqueradiata*). Teshima et al. (1999), using radioactive UDCA orally administered to Japanese flounder, suggested that about 95% of UDCA was absorbed in the flounder body. They assumed that UDCA could possibly enhance the growth of fish by stimulating digestion and absorption of lipids and/or improve liver and pancreatic function.

Kuruma prawn *M. japonicus* is the top ranking prawn that is consumed by the Japanese people. A large number of pharmaceutical agents and chemicals are tested in shrimp farm to improve growth and disease resistance of the prawn. On the other hand, Nile tilapia *O. niloticus* is one of the important species that are cultured in many tropical and subtropical countries. There are many nutritional studies about tilapia and kuruma prawn, but there is none about the pharmacological effects of UDCA on the growth performance and digestive enzyme activities. In the present study, two experiments were conducted to determine (1) the effects of UDCA on growth performance of kuruma prawn and tilapia, and (2) the effects of UDCA on the digestive enzyme (α-amylase, lipase and protease) activities of juvenile tilapia and kuruma prawn at different feeding periods.

Materials and Methods

Experimental animals and diets

Juvenile tilapia were obtained from Nakamura Suisan, Fukuoka, Japan, and prawns were obtained from Mitsui Norin Kaiyosangyo Co., Kagoshima, Japan. The fish and prawns were maintained on a commercial pellet diet (Higashimaru Foods, Kagoshima, Japan) until use.

Two types of diets, a UDCA diet and a control diet (without UDCA) were formulated. URSO-5% (Tokyo Mitsubishi Seiyaku Co., Ltd., formerly Tokyo Tanabe Co., Ltd., Japan) containing 0.025% of UDCA was used as a source of UDCA. The basal diets were formulated in consideration of recent information on the nutritional requirements of the tilapia and prawn. To prepare diets, all dry ingredients were mixed thoroughly using a food mixer

(Model KSMS, Kitchen Aid Inc., St. Josheph, Michigan, USA). Blended oil and lecithin were added to the mixture. About 30% of water was added to the diet ingredients to facilitate pelleting using a meat chopper (Royal, Japan; type 22VR-1500). After pelleting, diets were dried at 70°C for 30 min in a constant temperature oven (DK 400, Yamato Scientific Co., Ltd., Japan) and stored at –30°C until used. The pellet sizes were 750 to 1000 μm for tilapia and 1.5 mm for prawn. The pellets of prawn were steamed at 100°C for 10 min in a cylindrical steamer. Diets of the prawn were further dried in an oven at 70°C. Diets were stored in freezer at 30°C until used. The composition of diets and proximate analyses for tilapia and prawn are shown in tables 1 and 2.

Experimental feeding protocol for tilapia

Two separate studies were designed to evaluate the growth performance and digestive enzyme activities of tilapia by feeding the diets with and without UDCA. In growth studies, triplicate groups of the juveniles were fed the two test diets in six 18 l plastic tanks. Thirty juveniles were stocked randomly (0.47 ± 0.01 g in initial body weight) in each tank and fed the respective test diet by hand at 8% of body weight three times a day: (0730, 1400 and 1930 h). Known quantities of feed were given and unconsumed feeds were removed 30 min after feeding, pooled, and oven-dried, and feed intake was quantified. Fecal matters were removed by siphoning from the bottom of the tank 1 h before giving the feed. The water exchange rate for the system in each tank was 0.30 $l \cdot min^{-1}$ and lighting was provided using a 12 h light:12 h dark regime. Continuous aeration was provided by an electric blower and air stones. Sampling was done every two weeks and the tanks were cleaned during sampling and filled with fresh water again. All tanks were covered with plastic lids to prevent fish from jumping out of the tanks. The water temperature during the study period was 28 to 29°C. The fish were fed the experimental diets for 42 days.

To analyze the digestive enzyme activities of the juveniles in different feeding periods, another two triplicate groups of tilapia (0.48 ± 0.01 g initial weight) were fed the UDCA and the control diet in six tanks (tank size, the same as that used for the growth study) for 42 days. Thirty tilapia were placed in each tank. The water management, feeding, lighting, and cleaning of tanks were similar to those used for the growth study. Sampling was done every two weeks.

Experimental feeding protocol for prawn

To conduct growth studies, two triplicate groups of prawns, 2.00 ± 0.02 g initial body weight, were fed the two test diets (with or without UDCA) in six tanks (75 l capacity, filled with 60 l sea water) for 75 days. The experimental tanks were equipped with a circulating system by filtration through a sand bed supported by a false bottom. Twenty prawns were stocked randomly in each tank and fed by hand with their respective diets at 4% of

body weight at 1630 h every day. Known quantities of feed were given and fecal matter and uneaten diets were removed by siphoning from the bottom of the tank every morning. The water flow of tanks was 0.1 $l \cdot min^{-1}$ and continuous aeration was provided. Each tank was covered by a plastic lid to minimize disturbance and to prevent prawn from jumping out. Lighting was provided using a 12 h light:12 h dark regime. Sampling was done at 10, 20, 30, 45 and 75 days of feeding periods, and the tanks were cleaned thor-

Table 1. Composition of the test diets (%) for tilapia.

Ingredient	UDCA diet	Control diet
Brown fish meal	54.0	54.0
α-Starch	24.0	24.0
L-Methionine	0.5	0.5
L-Tryptophan	0.5	0.5
Vitamin mix [1]	4.0	4.0
Mineral mix [2]	5.0	5.0
Soybean lecithin [3]	5.4	5.4
Pollack liver oil [4]	3.6	3.6
α-cellulose	2.5	3.0
URSO-5% [5]	0.5	0.0
Proximate composition (%)		
Moisture	7.8	7.0
Crude protein	43.3	42.4
Crude lipid	10.8	9.6
Crude ash	10.7	9.6

[1](mg/100 g dry diet): ρ-amino benzoic acid, 96.40; biotin, 1.43; inositol, 968.20; nicotinic acid, 193.61; ca-pantothenate, 67.78; pyridoxine-HCl, 11.54; riboflovin, 48.40; thiamin-HCl, 14.53; menadione, 11.54; ß-carotin, 11.54; α-tocopherol, 24.21; cyanocoalamine, 96.82; calciferol, 0.02; Na-ascorbate, 2.43; folic acid, 3.63 and cholin chloride, 1979.08.
[2](mg/100 g dry diet): NaCl, 50.00; $MgSO_4 \cdot 7H_2O$, 750.00; $NaH_2PO_4 \cdot 2H_2O$, 1200.00; KH_2PO_4, 1600.00; $Ca(H_2PO_4)_2 \cdot 2H_2O$, 1000.00; Fe-citrate, 125.00; Ca-lactate, 175.00; $AlCl_3 \cdot 6H_2O$, 0.90; $ZnSO_4 \cdot 7H_2O$, 17.90; $CuCl_2$, 0.50; $MnSO_4 \cdot 4H_2O$, 4.00; KI, 0.80 and $CoCl_2$, 5.00 and cellulose 87.90.
[3]Kanto Chemical Co., Inc., Tokyo, Japan.
[4]Feed oil ?, Riken Vitamin, Tokyo, Japan.
[5]Containing 0.025% UDCA (Tokyo Mitsubishi Seiyaku Co., Ltd. Formerly Tokyo Tanabe Co., Ltd., Japan).

Table 2. Composition of the test diets (%) for prawn.

Ingredient	UDCA diet	Control diet
Casein	15.00	15.00
Squid meal	20.00	20.00
Krill meal	20.00	20.00
Albumin	5.00	5.00
Sucrose	5.00	5.00
Dextrin	4.37	4.37
α-Starch	5.00	5.00
Feed oil ika [1]	5.00	5.00
Soybean lecithin [2]	3.00	3.00
Cholesterol	0.50	0.50
Lysine-HCl	1.20	1.20
Arginine-HCl	2.75	2.75
Glocosamine	0.80	0.80
Na-succinate	0.30	0.30
Na-citrate	0.30	0.30
Vitamin mix [3]	2.00	2.00
Mineral mix [4]	6.00	6.00
Attractants [5]	2.20	2.20
α-Cellulose	1.08	1.58
URSO-5%[6]	0.50	0.00
Proximate composition (%)		
Moisture	5.56	5.85
Crude protein	52.92	53.91
Crude lipid	12.31	11.70
Crude ash	10.08	10.51

[1]Feed oil ika, Riken Vitamin, Tokyo, Japan.
[2]Kanto Chemical Co., Inc., Tokyo, Japan.
[3](mg/100g dry diet): ρ-amino benzoic acid, 15.8; biotin, 0.63; inositol, 632.00; niacine, 63.2; Ca-pantothenate, 94.8; pyridoxine-HCl, 18.96; riboflovin, 12.64; thiamin-HCl, 15.00; menadione, 6.34; vitamin A-palmitate, 30.34; a-tocopherol, 31.6; cyanocoalamine, 0.13; calciferol, 1.88; vitamin C (Phosphitan), 136.07; folic acid, 1.26 and cholin chloride, 948.00.
[4](g/100 g dry diet): $MgSO_4 \cdot 7H_2O$, 2.134; $NaH_2PO_4 \cdot 2H_2O$, 0.554; KH_2PO_4, 1.403; $Ca_3(PO_4)_2$, 1.909.
[5](g/100 dry diet): taurine 0.5, betaine 0.5 and inosine-5'-monophosphate 0.1, proline 0.3, alanine 0.3, glutathione 0.1 and Na-glutamate 0.4.
[6]Containing 0.025% UDCA (Tokyo Mitsubishi Seiyaku Co., Ltd. Formerly Tokyo Tanabe Co., Ltd., Japan).

oughly during sampling and filled with seawater again. The water temperature, salinity and pH were measured daily. The mean values of water temperature, salinity and pH value during the experimental period were about 23°C ± 1.9, 34.5 ± 1.5 ppt and 7.8 ± 1.8, respectively. These values were thought to be sufficient for suitable environmental conditions for the prawn *M. japonicus* (Shigueno et al. 1975).

To conduct enzyme study for prawn, another two triplicate groups of the prawns (1.97 ± 0.03 g initial weight) were fed the UDCA and the control diets in six tanks for 75 days. Fifty prawns were placed in each 500 l tank (filled with 300 l sea water). The water exchange rate in this case was 0.6 $l \cdot min^{-1}$. Feeding, lighting, and cleaning of tanks were the same as those for the growth study. The water quality parameters (temperature, salinity and pH) of the enzyme study tanks were more or less the same compared to the growth studies.

Collection of digestive organs and preparation of crude enzyme extracts

Digestive organs (esophagus, stomach, and intestine up to anus) of tilapia were collected every two weeks from 10 juveniles of each tank in the morning 16 hours after final feeding. Digestive organs were pooled out, cut into small pieces and washed with 0.25 M sucrose solution after discarding the intestinal contents. Then digestive organs were blotted dry to remove the solution and weighed. On the other hand, hepatopancreas samples of prawn were also collected from 10 prawn at 10, 20, 30, 45 and 75 days of feeding in the morning 16 hours after the last feeding. The hepatopancreas was washed with 0.25 M sucrose solution, blotted dry and weighed. Collected digestive organs from the juveniles and the hepatopancreas from the prawn were immediately stored in –85°C and as soon as possible enzyme assay was done.

Wet digestive organs plus same volume of 0.25 M sucrose solution were added in a 10 ml test tube and homogenized with Polytron homogenizer (KINEMATICA, GmbH LITTAU, Switzerland). The homogenate was centrifuged at 9000 x g for 30 min with a high speed refrigerated micro-centrifuge (MX-160, Tomy, Japan). The supernatant was diluted to an adequate volume with 0.25 M sucrose solution (if necessary) and was used as crude enzyme solution.

Assay of enzyme activity

The α–amylase activity (EC 3.2.1.1) of tilapia was assayed using a Mammalian α-amylase Test Kit (Wako Pure Chemical Industries, Ltd., Japan) and starch as a substrate. The assay was carried out by incubating 0.02 ml of the enzyme extract with 1 ml of reaction reagent (substrate and phosphate buffer solution, pH 7.1) in a 10 ml test tube at 37°C for 7.5 min. The incubation was terminated with the adding of 1 ml Color reagent (0.01N iodine) and 5 ml distilled water and absorbance of the reaction mixer was

recorded using spectrophotometer (Shimadzu, UV-120-02) at 660 nm. Duplicate assays were done for the sample collected from each tank. One unit is the amount of amylase activity that catalyzes the substrate in 30 min·mg protein in the extract (Unit·mg^{-1} protein).

The α-amylase activity for the prawn was determined using a Test Kit from Boehringer Mannheim (Cat. No. 882763, Mannheim, Germany). The α-amylase activities were measured with 4, 6-ethylidine(G_7)-*p*-nitrophenyl (G_1)-a,D-maltoheptaoside (ethylidene-G_7PNP) as the substrate. The assay was carried out by incubating 0.05 ml of the enzyme extract with 2 ml of reaction reagent (substrate and HEPES buffer solution, pH 7.1) in a 10 ml test tube at 37°C for 2 minutes, and then the change in absorbance of the reaction solution was recorded by spectrophotometer (Shimadzu, UV-120-02) at 450 nm. Duplicate assays were done for the sample pooled from each tank. The specific activity for α-amylase was expressed as μ moles of substrate hydrolyzed per min per mg protein in the extract (Unit·mg^{-1} protein).

Lipase activities (EC 3.1.1.3) of tilapia were measured by using Mammalian Lipase Kit (Boehringer Mannheim Cat. No. MPR 1 159697, Mannheim, Germany). Triolein was used as a substrate to determine the lipase activity. Crude enzyme solution of 0.1 ml was added to the reagent solution (substrate and Tris buffer solution, pH 9.2) at 37°C and the change in absorbance of the reaction solution was recorded at 340 nm. The absorbance change of a supplied standard was also determined for each sampling assayed period by running the standard instead of the sample and was used for the calculation of lipase activity. Duplicate assays were done for the sample pooled from each tank and the specific activities were expressed as μ moles of substrate hydrolysed per min per mg of protein in the extract (U·mg^{-1} protein).

Since no lipase activity was detected using the serum lipase kit for the prawn, the lipase activity of the prawn was measured by using trioline/agar emulsion screening (Lawrence et al. 1967). The assay was carried out by incubating 0.25 ml of crude enzyme extract with 0.25 ml of substrate solution (trioline emulsified in Tris buffer with gum arabic, pH 8.0) in a 10 ml test tube at 30°C for 1 hour with continuous agitation. After 1 hour, reaction was terminated by adding 30 μl 5M HCl, mixed with a touch mixer and lipids were extracted using diethyl ether. A standard lipase (from porcine pancreas containing 24% protein, 54U·mg^{-1} protein, Nacalai Tesque, Inc., Japan) solution was prepared by dissolving in Tris buffer (pH 8.0) and lipids were extracted by running the standard lipase instead of the crude enzyme extract. All extracted lipids were subjected to analysis of lipid classes by thin-layer chromatography-flame-ionization detector (TLC-FID) system, IATROSCAN TH10 (Iatron Lab. Inc., Japan) as described by Koshio et al. (1992). Lipase activities for the prawn were determined from the digestion product free fatty acid (FFA) *in vitro* hydrolysis of triolein by the digestive lipase at pH 8.0 compared with the digestion product FFA by the standard lipase. Specific activities for the lipase were expressed as the liberation of FFA in μ moles per min for 1 mg soluble protein-N from the trioline emulsion.

Protease activities of both tilapia and prawn were measured following Konagaya's method (1980). Casein 3% solution was used as a substrate. The assay was carried out by incubating 0.5 ml of enzyme extract, 0.5 ml of substrate solution and 1.5 ml of 1/15 M phosphate buffer in a 10 ml test tube at 37°C for 2 hours. Then 2.5 ml of 5% TCA (trichloroacetic acid) solution was added to stop the reaction. The mixture were filtered and 1 ml of filtrate, 5 ml of 0.55 M Na_2CO_3 and 1 ml of Folin reagent solution were mixed and color were determined by spectrophotometer at 660 nm. Blank was used as distilled water instead of sample solution. Protease activities were expressed as the difference of the absorption at 660 nm between sample and blank. The specific activities were defined as the absorbance value of the Folin-CIOCALTEU color at 660 nm for 1 mg soluble protein of the TCA filtrate (Unit·mg^{-1} protein).

Other chemical analysis

Crude protein and lipid contents of the test diets and whole body were determined by using Kjeldhal and Bligh and Dyer (1959) methods, respectively. Ash and moisture contents were determined according to AOAC (1990) method. The amount of soluble protein in the extracts was determined following the Lowry (1951) method using casein as a standard.

Statistical analysis

Significant effects of diets on the growth, digestive enzyme and other biochemical parameters were evaluated by one-way ANOVA (Super-ANOVA package Abacus Concepts, California, USA). Significant differences between the means were tested by Tukey Kramer Test (Kramer 1956). Probabilities of $P < 0.05$ were considered significant.

Results

Effect on growth performance of tilapia and prawn

Results of the growth performance of tilapia (after 42 days) and prawn (after 75 days of dietary treatments) are presented in table 3. Results showed that the mean body weight gain for the tilapia fed UDCA diet was significantly higher (1206%) than those of the control group (956%). Similar higher specific growth rate, feed conversion efficiency (FCE) and feed intake were observed for the juvenile tilapia fed the UDCA diet than to the control diet. There were no significant influences ($P > 0.05$) among the dietary groups on the survival rate of tilapia. On the other hand, a little higher weight gain was observed for the prawn fed the UDCA diet than the control diet after 75 days of feeding trial, but no statistical differences were detected. The survival rate for the prawn fed the UDCA diet was significantly higher than those of the control groups. No significant differences were observed among the dietary treatments for feed intake and FCE of the prawn.

Effects on digestive enzyme activities

The effects of UDCA on digestive enzyme activities (α-amylase, lipase and protease) of the juveniles are shown in figure 1. Feeding of dietary UDCA for 14, 28 and 42 days did not show any significant influences on the α-amylase and protease activities of the tilapia as compared to the juveniles fed without UDCA diet. The activities of lipase for the fish fed test diets for 42 days were not determined owing to an accident. However, the activities of lipase for the fish fed UDCA diet after 14 and 28 days were significantly high ($P < 0.05$) as compared to those of the control. The influences of UDCA on digestive enzyme activities of the prawn are presented in figure 2. Results showed that the activities of α-amylase were a little higher at each sampling period in the group fed UDCA diet, but no statistical differences were observed for the prawn fed the test diets with and without UDCA. The prawn fed the UDCA diet showed significantly higher lipase activities at 45 and 75 days of feeding as compared to the control. The activities of the protease for the prawn fed with or without UDCA diet did not show any statistical difference ($P > 0.05$) at 20, 30, 45 and 75 days of feeding.

Discussion

Information regarding the effects of UDCA on fish is limited. The present growth study for the tilapia showed that the addition of UDCA to the diet improved body weight gain significantly. This is in agreement with the findings for yellowtail (Dashimaru et al. 1982). The improved growth may be due to the increase in feed intake by the oral administration of UDCA. On the other hand, dietary UDCA did not improve the weight gain of the prawn significantly. Similar findings were also observed for the juvenile Japanese flounder fed the diet with or without UDCA in our previous study (Alam et al. 2000).

Table 3. Final body weight (FBW), weight gain (WG), feed conversion efficiency (FCE), feed intake (FI), survival rate (SR), specific growth rate (SGR) of juvenile tilapia and prawn fed the test diets.

Parameters	Tilapia		Prawn	
	UDCA	Control	UDCA	Control
FBW	6.14 ± 0.25a	5.03 ± 0.03b	5.18 ± 0.88a	4.98 ± 0.91a
WG (%)	1206 ± 38.3a	956.9 ± 9.0b	158.9 ± 2.55a	151.7 ± 5.00a
SGR	6.13 ± 0.15a	5.66 ± 0.58b	1.26 ± 0.04a	1.21 ± 0.02a
SR	93.4 ± 4.74a	91.1 ± 1.19a	88.3 ± 4.41a	65.0 ± 5.00b
FCE	1.50 ± 0.01a	1.42 ± 0.02b	0.41 ± 0.01a	0.39 ± 0.01a
FI	4.11 ± 0.15a	3.43 ± 0.02b	7.68 ± 0.78a	7.51 ± 0.98a

Specific growth rate = [ln (mean final weight) - ln (mean initial weight)/feeding period] x100.
Feed conversion efficiency = weight gain (g)/total feed intake in dry basis (g).
FI=g/fish/42 days for tilapia and g/prawn/75 days for prawn
Values are means ± S.E of three replicate groups.
Means with different letters differ significantly ($P< 0.05$).

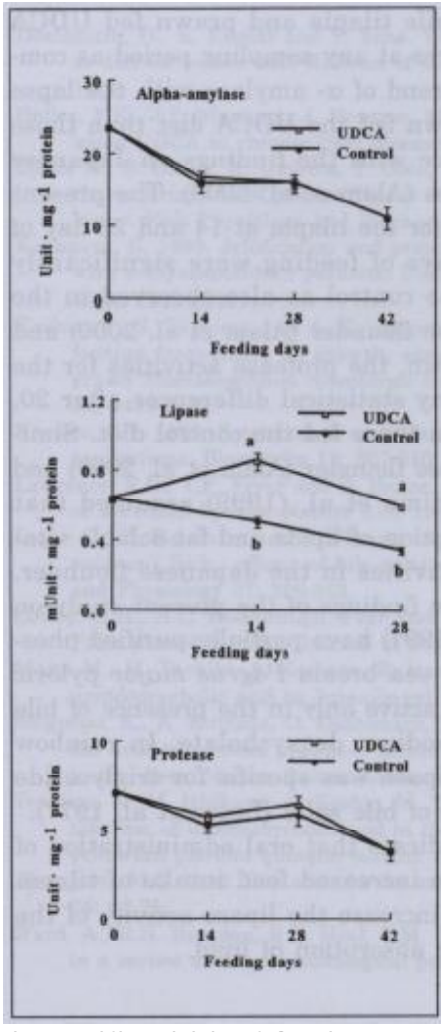

Fig. 1. Specific activities of digestive enzymes (α–amylase, lipase and protease) of the tilapia fed the UDCA- and control diets. Values are means ± SE of six determinations.

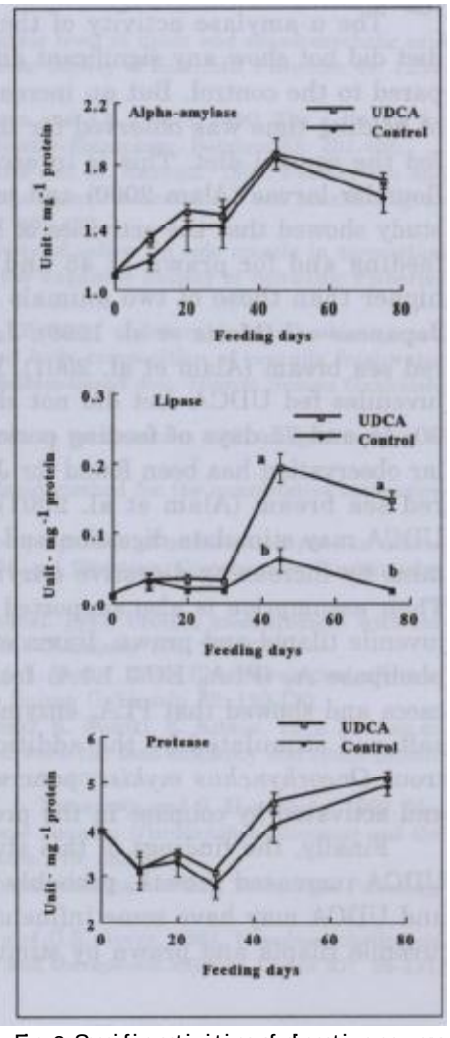

Fig. 2. Specific activities of digestive enzymes (α-amylase, lipase and protease) of prawn fed the UDCA-and control diets. Values are means ± SE of six determinations.

Maita et al. (1996) showed that UDCA increased the digestive enzyme activities (amylase, lipase and protease) of Japanese eel, but they did not report any effects of UDCA on the growth performance of eel. The survival rate for the tilapia fed diet with or without UDCA did not show any statistical differences, which was also observed for the Japanese flounder in our previous study (Alam et al. 2000). A significant higher survival was observed for the prawn fed the UDCA diet than those fed the diet without UDCA. The present results indicate a positive effect of UDCA on the growth performance of the tilapia while less effective on the prawn. Teshima et al. (1999) reported the faster turnover of UDCA in the yellowtail than in the Japanese flounder and suggested that optimum dietary levels of UDCA may vary with fish species.

The α-amylase activity of the juvenile tilapia and prawn fed UDCA diet did not show any significant differences at any sampling period as compared to the control. But an increasing trend of α- amylase with the lapse of holding time was observed for the prawn fed the UDCA diet than those fed the control diet. This is in accordance with the findings on Japanese flounder larvae (Alam 2000) and juveniles (Alam et al. 2000). The present study showed that the activities of lipase for the tilapia at 14 and 28 day of feeding and for prawn at 45 and 75 days of feeding were significantly higher than those of two animals fed the control as also observed in the Japanese eel (Maita et al. 1996), Japanese flounder (Alam et al. 2000) and red sea bream (Alam et al. 2001). In prawn, the protease activities for the juveniles fed UDCA diet did not show any statistical differences after 20, 30, 45 and 75 days of feeding period than those fed the control diet. Similar observation has been found for Japanese flounder (Alam et al. 2000) and red sea bream (Alam et al. 2001). Teshima et al. (1999) assumed that UDCA may stimulate digestion and absorption of lipids and fat soluble vitamins by increasing digestive enzyme activities in the Japanese flounder. Their assumption is also supported by the findings of the present study on juvenile tilapia and prawn. Iijima et al. (1997) have partially purified phospholipase A_2 (PLA_2 EC.3.1.1.4) from red sea bream *Pagrus major* pyloric caeca and showed that PLA_2 enzyme was active only in the presence of bile salt and stimulated by the addition of sodium deoxycholate. In rainbow trout *Oncorhynchus mykiss*, pancreatic lipase was specific for triglyceride and activated by colipase in the presence of bile salts (Leger et al. 1977).

Finally, the findings of this study indicate that oral administration of UDCA increased growth, probably due to increased feed intake of tilapia and UDCA may have some influences to increase the lipase activity of the juvenile tilapia and prawn by stimulating absorption of lipid.

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