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Effects of Lecithin on Cholesterol Digestibility in the Prawn, *Artemesia longinaris* (Crustacea, Penaeidae)

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Abstract

Cholesterol and dietary phospholipids are important to the growth and survival of crustaceans. Soy lecithin enhances cholesterol solubilization while phosphatidylcholine transfers cholesterol from the hepatopancreas to the hemolymph. To study the influence of dietary lecithin on digestibility and transport of cholesterol to the hemolymph in *Artemesia longinaris*, diets with different levels of supplemental cholesterol (0.5, 1, 1.5, and 2%) and soy lecithin (0, 0.5, 1, and 2%) were fed to prawns for two weeks. The diet containing the lowest level of cholesterol (0.5%) and lecithin (1%) resulted in the lowest apparent digestibility of cholesterol. The diets containing 2% cholesterol and 1% or 2% lecithin resulted in the highest hemolymph cholesterol contents. There was a linear relationship ($r = 0.832$; $p<0.01$) between hemolymph cholesterol and the amount of lecithin in the diet. There were no significant differences between treatments in cholesterol contents in the hepatopancreas. In a second 6-week experiment, diets containing 1.5% cholesterol significantly improved weight gains, regardless of the lecithin content. Survival rates did not differ among treatments. Hence, the efficacy of cholesterol does not seem to be related to dietary inclusion of lecithin.

Introduction

Sterols are essential nutrients for prawns because crustaceans are unable to synthesize these compounds (Zandee, 1966; Teshima and Kanazawa, 1971). Feeding experiments have demonstrated the necessity of dietary sterols for growth and survival (Castell et al., 1975; Petriella et al. 1984). Essential components such as molting hormones, sex hormones, bile acids, and vitamin D are synthesized from cholesterol. Cholesterol is also a membrane component and functions in absorption and transport of fatty acids (Akiyama et al., 1991). Cholesterol is obtained directly from the diet or via the meta-

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bolic conversion of other dietary sterols. Meals and invertebrate marine oils are excellent sources of cholesterol (Akiyama, 1992).

Phospholipids are cell membrane components that maintain their fluidity and flexibility, and play a role in lipid digestion and absorption (Akiyama et al., 1991). Dietary phospholipids apparently assist in the mobilization of cholesterol and triglycerides from the hepatopancreas to the hemolymph (D'Abramo et al., 1982). Dietary phospholipids have positive effects on weight gain and survival, in both juveniles and shrimp larvae (Piedad-Pascual, 1986; Chen and Jenn, 1991; Gonzalez-Felix et al., 2002).

The commercially valuable prawn, *Artemesia longinaris*, is distributed along the coastal waters of Argentina, Uruguay, and Brazil from 22˚30'S to 43˚20'S (Boschi and Scelzo, 1968). The greatest abundance of this species occurs in Mar del Plata and Rawson, Argentina. The aims of this work were to determine the effects of soy lecithin, a phospholipid, on cholesterol digestibility and the transport of cholesterol to the hemolymph in *A. longinaris* and to study the effects of different combinations of dietary cholesterol and lecithin on growth and survival.

Materials and Methods

In the first experiment, a 2-week trial was conducted to study the effect of soy lecithin on cholesterol digestibility. Six isoproteic feeds were prepared from basal diet E with a proximal composition of 56.2% protein, 6.9% lipids, and 15.55% ash (Fenucci et al., 1983). Chromic oxide was added as an inert marker to measure apparent digestibility. The feeds contained 0.5, 1.0, 1.5, or 2.0% cholesterol and 0, 0.5, 1.0, or 2.0% soy lecithin and were prepared by the cold extrusion method (Fenucci, 1981; Table1).

Prawns (1.35-1.45 g) were randomly distributed into 150-l glass aquaria with undergravel filters at a density of 5 prawns/aquarium and the six diets were tested in triplicate. Prawns were acclimated for seven days during which they were fed the experimental feeds *ad libitum* daily. Water ranged 19-22°C and salinity was 33 ppt. After seven days of acclimation, prawns were fed the experimental diets for another two weeks.

Feces were collected daily with a hand net, washed with distilled water, frozen, and dried at 45°C. Hemolymph samples were taken by syringe from all prawns in the intermolt stage, from the base of the fourth pereiopod. The hepatopancreas was dissected and frozen at -20°C for subsequent laboratory analysis. Molt cycle stages were determined by observation of the uropod setae development (Petriella, 1984). Cholesterol (Aikel, 1977) and chromium oxide (Fenucci, 1981) in the feces and diets were analyzed. Cholesterol was quantified as mg/ml in the hemolymph and as a percentage of the dry weight of the hepatopancreas. Apparent cholesterol digestibility (Ap Dig) was calculated according to the formula (Fenucci, 1981): Ap Dig (%) = 1 - (%Cr_2O_3 in diet/100% Cr_2O_3 in feces) x (% cholesterol in feces/% cholesterol in diet) x 100.

A second 45-day experiment was carried out to study the combined effect of cholesterol and soy lecithin on growth and survival. Four isoproteic test feeds were prepared from the standard rations used in the first experiment, with all combinations of soy lecithin and cholesterol at levels of 0 and 1.5%.

Seven prawns (1.39-1.50 g initial wt) were stocked in under-gravel filter aquaria. Each diet was tested in triplicate. The prawns were fed daily, initially *ad libitum* and then according to their requirements. Uneaten food, dead prawns, and exuviae were removed daily before feeding. Individuals were weighed to the nearest 0.01 g prior to stocking and at the end of the experiment. To prevent mortality, they were not weighed during the trial.

Data were analyzed using Bartlett's test to determine homoscedasticity of variances, factorial ANOVA, and chi square (Sokal and Rohlf, 1979).

Results

Cholesterol contents in the diets, hemolymph, and hepatopancreas are given in Table 2. Cholesterol levels in the hemolymph were significantly higher in prawns fed C_2L_2 or C_2L_1 (p<0.01). Cholesterol levels in the hepatopan-

Table 1. Composition (%) of diets with different levels of lecithin and cholesterol, fed to prawns (*Artemesia longinaris*) of 1.35-1.45 g for two weeks.

	Diet name					
	$C_{0.5}L_1$	$C_{1.5}L_0$	$C_2L_{0.5}$	C_2L_1	C_2L_2	C_1L_1
Cholesterol	0.5	1.5	2	2	2	1
Lecithin	1	0	0.5	1	2	1
Wheat bran	20.95	20.95	19.95	19.45	18.45	20.45
Mussel meal	30					
Squid meal	15					
Soybean meal	5					
Fish meal	20					
Sodium hexametaphosphate	1					
Alginate	2					
Fish soluble	2					
Fish oil	1					
Chromium oxide	1					
Vitamin mix	0.55					

Table 2. Cholesterol in diets, hemolymph, and hepatopancreas. Cholesterol digestibility (%) and digested cholesterol in 100 g of diet.

Diet	*In diets (%)*	*In hemolymph (mg/ml)*	*In hepatopancreas (%)*	*Cholesterol digestibility (%)*	*Digested cholesterol (g per 100 g diet)*
$C_{0.5}L_1$	0.83	0.166±0.0290	0.774±0.0757	59.55±2.192**	0.49±0.018
C_1L_1	1.48	0.122±0.0262	0.554±0.0413	85.32±0.990	0.71±0.008
$C_{1.5}L_0$	1.96	0.139±0.0133	0.799±0.0870	84.28±0.445	1.65±0.008
$C_2L_{0.5}$	2.46	0.115±0.0171	1.083±0.1287	84.90±0.714	2.08±0.021
C_2L_1	2.50	0.207±0.0265*	0.740±0.0865	80.48±1.520	2.01±0.038
C_2L_2	2.38	0.235±0.0122*	1.362±0.1983	79.17±0.898	1.88±0.028

* significantly different at $p<0.01$
** significantly different at $p<0.05$

creas did not differ significantly ($p>0.05$) between diets. The diet with the lowest cholesterol digestibility was diet $C_{0.5}L_1$, which contained the smallest percentage of cholesterol. There were no significant differences in apparent digestibility among the other diets ($p>0.05$). Cholesterol digestibility was adjusted to a second order polynomial curve (Fig. 1). There was a significant correlation ($r = 0.832$; $p<0.01$) between cholesterol in the hemolymph and

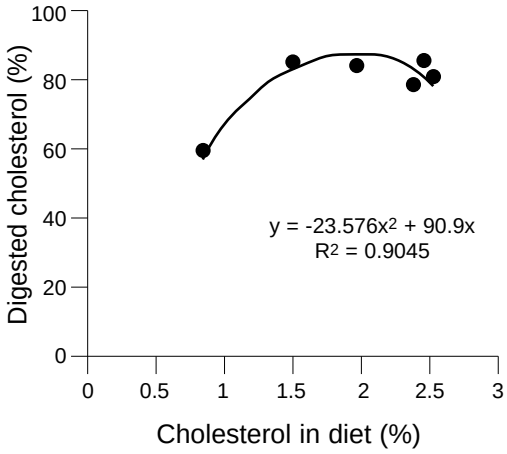

Fig. 1. Relationship between apparent digestibility of cholesterol in prawns (*Artemesia longinaris*) and the percent of cholesterol in the diet.

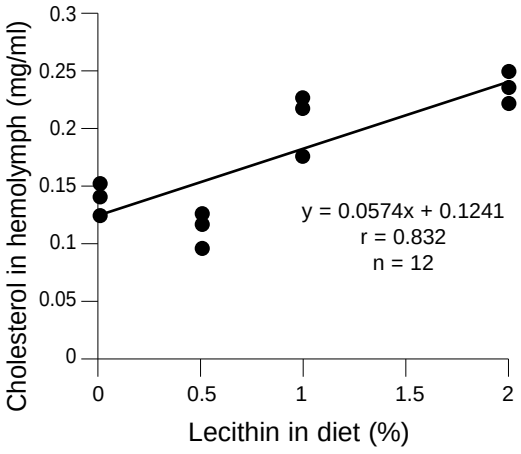

Fig. 2. Relationship between cholesterol in the hemolymph of prawns (*Artemesia longinaris*) and the percent of supplemental lecithin in the diet.

total lecithin in the diet (Fig. 2).

Cholesterol supplementation significantly improved growth ($p<0.01$) while lecithin supplementation did not (Table 3). Survival was not affected by the addition of cholesterol or soy lecithin ($p>0.05$). There was no significant ($p>0.05$) interaction between dietary lecithin and cholesterol on growth or survival.

Discussion

The present work shows that, in *A. longinaris*, the cholesterol concentration in the hemolymph is related to dietary lecithin. Phospholipids play an important role in the metabolism of crustacea. In the hemolymph of *Marsupenaeus japonicus*, the transport of dietary lipids is carried out mainly by phospholipids associated with high density lipoproteins (Kanazawa, 1981). Crustacean hemolymph has a lower enzymatic activity than mammalian blood (Mankura et al., 1980). In mammals, dietary cholesterol enters the circulatory system after being esterified by the action of lecithin-cholesterolacyltransferase which catalyzes the transference of an acyl group from the β-position of phosphatidylcholine to the β-hidroxy group of cholesterol (Teshima, 1997).

Phosphatidilcholine is the active component of soy lecithin in purified diets (D'Abramo et al., 1981). In *Homarus* sp., the lack of a dietary source of phosphatidilcholine apparently results in a phosphatidilcholine deficiency in the hemolymph that limits the effective cholesterol transport from the hepatopancreas to the hemolymph (D'Abramo et al., 1982). The beneficial effect of dietary phospholipids in *M. japonicus* might be due to better mobilization of lipids from the hepatopancreas or gut to the hemolymph, enhancing lipid deposition in tissues and increasing energy available for growth (Kontara et al., 1998). The absence of dietary soy lecithin can cause failure of juvenile lobsters to extricate themselves from old exoskeletons during molt (Conklin et al., 1980) while a lack of phosphatidilcholine can cause death during molt in *Homarus* sp. (D'Abramo et al., 1982).

There was no interaction between dietary lecithin and cholesterol on growth and survival. In the shrimp, *Fenneropenaeus penicillatus*, diets supplemented with phosphatidylcholine or cholesterol significantly improved weight gain but the interaction between the phosphatidylcholine and the cholesterol did not cause a significant effect on growth, food conversion, or survival (Chen and Jenn, 1991). Likewise, there were no significant

Table 3. Mean weight and survival in prawns (*Artemesia longinaris*; 1.39-1.50 g) fed diets containing different amounts of cholesterol and lecithin for 45 days.

Diet	*Mean wt*			*Survival*
	Initial (g)	*Final (g)*	*Change (%)*	*(%)*
C_0L_0	1.39±0.348	1.73±0.318[a]	24.46	90.48
$C_0L_{1.5}$	1.40±0.288	1.75±0.273[a]	25.00	95.24
$C_{1.5}L_0$	1.39±0.263	1.96±0.366[b]	41.01	100.00
$C_{1.5}L_{1.5}$	1.42±0.233	1.98±0.276[b]	39.44	90.48

Different superscripts in a column indicate significant differences (p<0.01).

effects from the interaction of lecithin and cholesterol on growth and survival of *Penaeus monodon* zoea, mysis, and postlarvae (Paibulkichakul et al., 1998). On the other hand, there was an interaction between dietary cholesterol and phospholipids that affects growth in *L. vannamei* postlarvae (Emery, 1987, in Castille et al., 2004).

There was no significant effect of dietary lecithin on survival in *A. longinaris*. Likewise, there were no significant effects of lecithin on growth and survival of *H. americanus* fed different combinations of lecithin and cholesterol (Kean et al., 1985) or on final weight, percentage weight gain, or growth rate of juvenile *Cherax quadricarinatus* fed diets containing increasing percentages of soy lecithin for 10 weeks (Thompson et al., 2003). The highest weight gain, survival, and feed efficiency in *Fenneropenaeus merguiensis* were obtained in the group fed diets containing 1-2% lecithin but there was no advantage to adding cholesterol (Thongrod and Boonyaratpalin, 1998).

On the other hand, the best apparent digestibility of cholesterol was obtained with the diet containing 2.46% cholesterol. The optimum percentage of dietary cholesterol varies. Kanazawa et al. (1971) reported that 0.5% dietary cholesterol promotes good growth in *M. japonicus*, whereas Deshimaru and Kuroki (1974) obtained the best growth with 2.1% dietary cholesterol. Kanazawa (2001) determined that the dietary cholesterol requirements in crustaceans ranges 0.1-2.0% of the dry weight of the diet. Better survival was obtained in *A. longinaris* fed a defatted diet with added cholesterol than a diet lacking cholesterol (Petriella et al., 1984). In the same species, best growth and survival was obtained with a diet containing 2.5% cholesterol while optimum digestibility was obtained with 2.3% cholesterol (Martinez Romero et al., 1991).

Nutrient requirements vary with the size of the prawn. The assimilation pattern of proteins in small juvenile *Litopenaeus stylirostris* (1-4 g) differs from that in large animals while a positive linear relation was determined between carbohydrate assimilation and mean weight in juvenile *L. setiferus* (Fenucci, 1981). The cholesterol requirement in juvenile *L. vannamei* was estimated to be 0.35% in the absence of supplemental phospholipids but only 0.14% and 0.13%, in diets containing 1.5% and 3% phospholipids, respectively. When 5% phospholipids were provided, 0.05% dietary cholesterol was needed for optimal growth (Gong et al., 2000). Indeed, recommended cholesterol levels in commercial shrimp feeds vary 0.40-0.25% in animals of 0.5-40.0 g, i.e., a higher level of cholesterol is necessary for smaller *L. vannamei* (Akiyama et al., 1991). A change in the digestion ability with the size of the shrimp was suggested as the explanation.

While the function of phospholipids in cho-

lesterol transport is well known, further studies are needed to determine possible effects of interactions between the two compounds on the growth and survival of different sized prawns.

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