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Effect of choline and methionine as methyl group donors on juvenile kuruma shrimp, *Marsupenaeus japonicus* Bate

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Abstract

Choline and methionine are considered as methyl-group donors; therefore, their interaction may affect the growth of the aquatic animal. Thus, a 42-day feeding trial was conducted as a 2×3 factorial design to evaluate the effect and interaction of two methyl-group donors, choline chloride (CC) and methionine (Met), on juvenile kuruma shrimp of initial size 0.91±0.04 g (mean±S.D.). Six test-diets were formulated to contain 3 levels of CC (0%, 0.06% and 0.12%) and 2 levels of Met (0% and 1.5%). Soybean protein isolate (SPI) was utilized as the main source of protein because of its limited Met content. A significant ($P<0.05$) interaction was determined between CC and Met on survival (%S), percent weight gain (%WG), specific growth rate (SGR% day^{-1}), feed efficiency ratio (FER), protein efficiency ratio (PER) and both choline and Met content of the whole body of the juvenile *Marsupenaeus japonicus*. The shrimp group with neither supplemental CC nor Met showed lower ($P<0.05$) values of the above-mentioned parameters than other shrimp groups fed with 0.06% and 0.12% supplemented CC with or without Met supplementation. The present study showed that supplementation of 0.12% CC in the diets could compensate kuruma shrimp juveniles with the needed methyl-group when fed Met-deficient diets. Also, the supplementation of Met was needed in the case of rearing kuruma shrimp juveniles on choline-deficient diets to compensate methyl group deficiency.

Keywords: Nutrition; Shrimp; Choline; Methionine; Interaction

1. Introduction

Methyl-groups have a vital importance for all terrestrial and aquatic animals. They serve in methylation reactions such as methylation of the harmful amino acid; homocysteine into the useful methionine (Met) and creatine from guanido acetic acid and phosphatidylcholine (PC) from phosphatidylethanolamine (PE) (NRC, 1993). Moreover, animals cannot synthesize methyl groups and thus need to receive them in the diets (Kidd et al., 1997).

Choline is considered to be a methyl-group donor. It is also the precursor of betaine that methylates homocystein to Met (Krebs et al., 1976). In aquatic animals, choline has been shown to play other important functions such as being a precursor of the neurotransmitter acetylcholine

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and phospholipid (PC). Choline chloride (CC) is widely used as a dietary choline source in the diets of aquatic animals. CC is metabolized into choline in the body (Bender, 1992). Signs of choline deficiency in aquatic animals included poor growth and poor feed utilization, anorexia, fatty livers, and hemorrhagic areas in kidney, liver, and intestine (Ketola, 1976; Wilson and Poe, 1988). In kuruma shrimp, the amount required from choline to maintain normal growth and survival was estimated to be 0.06% of diets when CC is used as the source of choline and 0.4% when PC of 85%-purity is used (Kanazawa et al., 1976; Michael et al., 2005).

Met is an indispensable amino acid that is required by all animals including fish and crustaceans for protein synthesis (Ketola, 1982; Teshima et al., 2002; Alam et al., 2005) also, as a sulfur source, which is needed for synthesis of other sulfur-containing compounds. One of the major functions of Met is as a methyl-group donor; this methyl group is used in methylation reactions to formulate useful compounds such as PC from PE through the S-adenosyl methionine pathway (Bender, 1992). For penaeid shrimp, *Penaeus monodon*, the requirement of Met was estimated to be less than 1.4% of diets (Liou and Yang, 1994). In kuruma shrimp, the amount required from Met to maintain normal growth and survival was found to be 1.3% of diets (Teshima et al., 2002).

As choline and Met serve as major methyl-group donors, the requirement of each can be influenced by the presence of the other in the diet (NRC, 1993; Craig and Gatlin, 1996; Wu and Davis, 2005). Kasper et al. (2000) found that Nile tilapia need choline (supplied as CC) when Met is not adequate in their diet. In case of crustaceans, however, no information is available about choline–methionine interaction therefore, because of the high economical value of the kuruma shrimp, this interaction is needed to optimize the utilization of dietary Met for protein synthesis and therefore enhance the quality, efficiency and decrease the cost of shrimp feeds.

The present study was designed to evaluate the interactive effect of CC and Met on the various biological and biochemical performances of juvenile kuruma shrimp.

2. Materials and methods

2.1. Experimental design and test diets

A two-way layout feeding experiment was conducted to clarify the effect and interaction of CC and Met on the performances of juvenile kuruma shrimp, *Marsupenaeus japonicus*. Six semi-purified diets (Table 1) were formulated to contain 3 levels of CC (Nacalai Tesque Inc., Kyoto, Japan) (0%, 0.06%, and 0.12%) and 2 levels of Met (0% and 1.5%). As Met is the most limiting amino acid in soybean proteins (Akiyama et al., 1991), the protein sources used in the test diets were soybean protein isolates (SPI) as 45% of the total ingredients and 10% vitamin-free casein.. The test diets were prepared according to Teshima et al. (1986). Prior to diet preparation, both CC and Met were coated with carboxymethyl cellulose (CMC) as single layer coating (Alam et al., 2004a,b) to prevent leaching into water. The mixtures of vitamins and minerals were prepared according to Teshima et al. (2001) (Table 1). Briefly, the fat-soluble vitamins and cholesterol, were dissolved in the squid liver oil, and mixed well with other dry ingredients with a professional mixer (Kitchen Aid—

Table 1
Ingredients, and proximate composition of the test diets

Ingredient (g/100 g)	Test diets					
	1	2	3	4	5	6
Soybean protein isolates (SPI)	45.0	45.0	45.0	45.0	45.0	45.0
Casein (vitamin-free)	10.0	10.0	10.0	10.0	10.0	10.0
Amino acid mixture[a]	6.3	6.3	6.3	6.3	6.3	6.3
Pollack liver oil	9.0	9.0	9.0	9.0	9.0	9.0
Sucrose	5.0	5.0	5.0	5.0	5.0	5.0
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mix[b]	0.9	0.9	0.9	0.9	0.9	0.9
Mineral mix[c]	8.6	8.6	8.6	8.6	8.6	8.6
α-Starch	4.5	4.5	4.5	4.5	4.5	4.5
α-Cellulose	1.9	1.8	1.7	0.4	0.3	0.2
CMC	5.0	5.0	5.0	5.0	5.0	5.0
Attractants[d]	2.4	2.4	2.4	2.4	2.4	2.4
Soybean lecithin (SBL)	1.0	1.0	1.0	1.0	1.0	1.0
Choline chloride (CC)	0.0	0.06	0.12	0.0	0.06	0.12
L-Methionine (Met)	0.0	0.0	0.0	1.5	1.5	1.5
Proximate composition (%)						
Moisture	15.5	14.7	15.2	14.8	15.1	15.2
Crude protein	55.3	55.9	55.6	56.1	55.8	56.3
Crude lipid	10.2	10.6	10.8	11.1	9.9	10.2
Ash	4.5	4.7	5.1	4.4	5.2	5.0
Met	0.28	0.26	0.30	1.67	1.66	1.64
Choline	0.03	0.08	0.14	0.04	0.07	0.14

[a] Trp (1.4), Arg (3.9) and Lys (1.0).
[b] Vitamin mix., mg/100 g dry diet; *p*-amino benzoic acid (9.48); D-Biotin (0.38); Inositol (379.20); Niacin (37.92); Ca-pantothenate (56.88); Pyridoxine-HCl (11.38); Riboflavin (7.58); Thiamin-HCl (3.79); L-ascorbyl-2-phosphate Mg (APM) (296.00); Folic acid (0.76); Cyanocobalamine (0.08); Menadione (3.80), Vitamin A-palmitate (17.85); α-tocopherol (18.96); Calciferol (1.14).
[c] Mineral mix. g/100 g diet; K_2PO_4 (2.011); $Ca_3(PO_4)_2$ (2.736); Mg SO_47H_2O (3.058); $NaH_2PO_42H_2O$ (0.795).
[d] Attractants, g/kg diet; sodium citrate (3); sodium succinate (3); glucosamine HCl (8); Taurine (3); IMP (1); Glutathione (1); Glycine (2).