

# Response of blue shrimp *Litopenaeus stylirostris* (Pérez-Farfante & Kensley, 1997) to dietary cadaverine supplementation

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## Abstract

Recent studies on salmon and shrimp have shown that reduced feed intake and growth caused by the consumption of low-quality fish meals, manufactured from spoiled fish, were not due to the presence of biogenic amines. Moreover, an improvement in weight gain was seen in blue shrimp fed a diet supplemented with cadaverine plus histamine. It was not clear, however, if this effect was due to the consumption of histamine or cadaverine. The objective of the current experiment was to investigate the effect of dietary cadaverine supplementation on growth parameters and various amine concentrations in tissues of the blue shrimp *Litopenaeus stylirostris*. Six experimental diets were supplemented with cadaverine at 0, 500, 1100, 2300, 3500 and 4600 mg kg<sup>-1</sup> and tested in a feeding trial lasting 28 days. Feed consumption, feed conversion ratio, survival and weight gain were not affected by the concentration of dietary cadaverine. The dietary supplementation of cadaverine, however, resulted in a linear increase in cadaverine concentration in shrimp tissues, especially in the hepatopancreas. It was concluded that dietary cadaverine does not have any effect on growth and feed intake of shrimp. Growth promotion, as reported previously in shrimp fed a diet supplemented with histamine plus cadaverine, was probably due to histamine or a combined effect of histamine plus cadaverine but not due to dietary cadaverine alone. It seems that shrimp have a limited ability to metabolize cadaverine, which then accumulates intact in shrimp tissues.

**Keywords:** penaeid shrimp, dietary cadaverine, raw material freshness, fish meal

## Introduction

Biogenic amines are used as a quality criterion in the selection of fish meals, used for commercial feeds, as their concentration in fish is an indicator of spoilage. Fish meals from warm water species such as anchovy and sardines generally have a higher concentration of histamine, while fish meals from cold water species like herring tend to display higher cadaverine concentrations (Pike & Hardy 1997). Aksnes and Mundheim (1997) observed a linear negative correlation between cadaverine content in herring meals and the growth rate, feed efficiency ratio and final body weight of Atlantic halibut *Hippoglossus hippoglossus* fed these herring meals, while Ricque, Cruz-Suárez, Abdo-de la Parra and Pike (1998) observed decreased shrimp feed consumption and reduced growth in *Litopenaeus stylirostris* and *Penaeus monodon* fed anchovy fish meals containing high concentrations of biogenic amines. Tapia-Salazar, Ricque-Marie, Cruz-Suarez and Pike (1998), using an experimental design and experimental herring meals, developed by the Norwegian Herring Oil and Meal Industry Research Institute (Opstvedt, Mundheim, Nygård, Aase & Pike 2000), confirmed that small *L. stylirostris* were especially sensitive to herring fish meals made from stale raw material and containing high levels of biogenic amines, with the effects including mortality.

Cadaverine is typically synthesized in prokaryotic cells by decarboxylation of lysine (ten Brink, Damink, Joosten & Huis in 't Veld 1990) as eukaryotic cells lack lysine decarboxylase (Louisot 1983) and this compound can be found in spoiled fish as the parent polyamine and probably as its polymers (spermidine and spermine analogues) or conjugates (e.g. *N*-(3-aminopropyl)cadaverine and *NN'*-bis(3-aminopropyl)cadaverine (Hölttä & Pohjanpelto 1983; Seiler 1992). Spermidine and spermine are present naturally in animal and plant cells (Seidel & Scemama 1997) and play a role in anabolic metabolism such as DNA, RNA and protein synthesis, cellular growth and membrane stabilization (Seiler 1992). It appears that cadaverine and its higher polyamine analogues can partly replace the normal polymers (putrescine, spermidine and spermine) in the maintenance of cellular growth, in cells depleted of polyamines (Hölttä & Pohjanpelto 1983).

The contradiction between apparent association of the presence of biogenic amines at relatively acceptable dietary levels with detrimental effects on fish and crustaceans, and natural function of these substances in cell metabolism, was finally solved by Opstvedt and colleagues (2000), who demonstrated for Atlantic salmon smolts that adverse effects of herring meals, made from spoiled fish, were not due to the consumption of biogenic amines, but probably to other factors, such as reduced palatability, reduced essential amino acid content or the induced formation of as yet undescribed toxic compounds. Moreover, Tapia-Salazar and colleagues (1998) observed increased feed intake and improved weight gain in shrimp fed diets including fish meal made from fresh herring, but supplemented with cadaverine and histamine. The experimental design, however, did not allow separation of the effects of cadaverine and histamine.

The aim of the present experiment was to investigate the effect of dietary cadaverine supplementation on growth and tissue amine concentrations on small pacific blue shrimp *L. stylirostris*.

## Materials and methods

### Experimental diets

A control diet (Table 1) was formulated and supplemented with cadaverine dihydrochloride (1,5-diaminopentane, SIGMA CHEMICAL, St Louis, MO, USA) to give a total of 6 diets corresponding to free amine concentrations of 0, 500, 1100, 2300, 3500 and 4600 mg kg<sup>-1</sup>. The experimental diets were ana-

**Table 1** Control diet composition

Ingredients	%
Fish meal*	29.20
Soybean meal†	36.10
Wheat flour	23.10
Vitamin mixture‡	0.25
Mineral mixture§	0.25
Cholesterol¶	0.14
Alginate acid	3.00
Sodium hexametaphosphate**	1.00
Ethoxyquin††	0.02
Soybean lecithin F-100‡‡	2.50
Fish oil§§	3.93
Flavorpack¶¶	0.50

\*FUNDACION CHILE, Santiago, Chile: moisture 7.2%, protein 70.5%, lipid 8.4%, ash 14%, sand 0.1%, chlorides 2.3%, free fatty acids 4.6%, histamine 545 mg kg<sup>-1</sup>, total volatile nitrogen 105 mg N 100 g<sup>-1</sup>.

†46% protein. Proteinas Naturales S.A. de CV., Monterrey, Nuevo Leon, México.

‡INVE BELGIE, Baasrode, Belgium: ascorbic acid 60 000 ppm, ascorbyl polyphosphate 60 000 ppm, vitamin A 4000 IU g<sup>-1</sup>, vitamin K<sub>3</sub> 16 000 ppm, vitamin B<sub>1</sub> 24 000 ppm, vitamin D<sub>3</sub> 3200 IU g<sup>-1</sup>, vitamin B<sub>2</sub> 16 000 ppm, vitamin E 60 000 ppm, calcium-D-pantothenate. 30 000 ppm, vitamin K 400 ppm, vitamin B<sub>6</sub> 30 000 ppm, niacine 20 000 ppm, vitamin B<sub>12</sub> 80 ppm, folic acid 4000 ppm, dry matter 98%, crude ash 32.7%.

§INVE BELGIE: Zn 40 000 ppm, Cu 20 000 ppm, Fe 1 ppm, Se 100 ppm, I 2000 ppm, Co 2000 ppm, Mn 16 000 ppm, dry matter 91.86 %, crude protein. 4.17%, crude fat 0.36%, crude ash 27.2%, crude fiber 0.21%.

¶SIGMA CHEMICAL, St Louis, MO, USA.

|| Sigma-Aldrich, St Louis, MO, USA. Cat. 18094-7.

\*\*Sigma-Aldrich. Cat. 30555-3.

††DRESEN QUIMICA, México D.F., México.

‡‡CENTRAL SOYA, Ft Wayne, IN, USA.

§§INUAL-TEPUAL, Santiago, Chile.

¶¶INVE BELGIE.

lysed to determine moisture (method 920.36; AOAC 1990), protein (Kjeldahl method; Tecator 1987), lipid (Soxhlet method; Tecator 1983), ash (method 942.05; AOAC 1990), fibre (method 962.09; AOAC 1990) and carbohydrate (nitrogen-free extract (NFE), calculated by difference) content. Diet stability was analysed according to Aquacop (1978) by immersing 5-g diet samples in sea water (31 °C, salinity 34 g L<sup>-1</sup>) for 1 h with six replicates for each diet. Dietary concentrations of cadaverine, histamine, tyramine, putrescine, spermidine and spermine were determined by high-performance liquid chromatography (HPLC) before and after leaching at INUAL-TEPUAL laboratory (Santiago, Chile) according to Seiler and Knödgen (1978).

### Feeding trial

A feeding trial was carried out for 28 days in synthetic sea water. Ten blue shrimp *L. stylirostris* (50–108 mg initial body weight) were weighed and stocked into 10-L fibreglass tanks. There were five replicate tanks for each diet. Approximately 40% of water was changed daily and the water quality of the added water remained constant at 28 °C; salinity 35 g L<sup>-1</sup>; pH 8.1; dissolved oxygen = 4.9 mg L<sup>-1</sup>; total ammonia-N = 0.66 mg L<sup>-1</sup>; nitrite-N = 1.75 mg L<sup>-1</sup>; nitrate-N = 98.5 mg L<sup>-1</sup>; phosphate = 0.50 mg L<sup>-1</sup>.

The daily feed ration was calculated initially as 10% the biomass of each tank and the amount was then adjusted according to feed consumption of each tank to minimize the amount of uneaten feed. Pellets were not crumbled as this might produce debris and possibly change the composition of the ingested diet. The spaghetti-like strands of 1.6-mm diameter were cut into small sections of 2–3 mm to allow handling by the small shrimp and to avoid competition between individuals. The shrimp were fed two times per day at 12:00 hours and again at 17:00 hours, each time feeding 50% of the total daily ration. Uneaten feed was determined visually the next morning (07:00 hours) before cleaning the tank. Uneaten feed ranged between 5% and 10% of the total feed offered daily.

Weight gain, feed consumption, feed conversion ratio and survival were calculated for each tank as follows: %weight gain = [(final weight – initial weight)/ initial weight] × 100; individual feed consumption (g) =  $\sum_{i=1}^{28}$  [(feed given on day *i* – uneaten feed)/number of shrimp on day *i*]; feed conversion ratio = individual feed consumption (g)/individual mean increase in weight (g); survival rate (%) = [(final number of shrimp/initial number of shrimp) × 100].

### Determination of polyamine concentrations in shrimp tissues

At the end of the feeding trial, shrimp were left unfed for 24 h to clear out the intestinal tract. The animals of each tank were then pooled and taken as one sample. The hepatopancreas was removed from each shrimp and each pooled sample of hepatopancreas and remaining shrimp freeze dried, vacuum packed and stored at –80 °C until analysed. Histamine, cadaverine, putrescine, spermidine and spermine were analysed for the shrimp hepatopancreases and remaining body tissues by HPLC according to Tapia-Salazar, Smith and Harris (2000).

### Statistical analysis

A random block design was used and the growth parameters of the treatments tested were compared by orthogonal contrasts (Zar 1974) using SAS software (1987). Differences were significant when a linear fit or the quadratic model showed differences of  $P \leq 0.05$ . Differences in cadaverine concentration in the tissues of the shrimp was compared statistically, employing Duncan's multiple comparison of the means.

## Results

### Experimental diets

Chemical composition of the experimental diets was found to be: moisture 8.7 ± .3%, protein 40.5 ± .5%, lipid 9.3 ± .3%, ash 8.4 ± .2%, fibre 1.7 ± .1% and NFE 31.5 ± .4%. Loss of dry matter after 1 h immersion in sea water was 5.5 ± 0.3%. Table 2 shows the amine concentrations found in the diets before and after leaching. Cadaverine concentration in the experimental diets approximated the expected values and varied after leaching from 18% to 31% of the initial value.

### Feeding trial

The results of the feeding trial are shown in Table 3. Feed consumption, weight gain, feed conversion ratio and survival were not affected by dietary cadaverine concentration. Feed intake varied from 0.61 to 0.70 g shrimp<sup>-1</sup> 28 days<sup>-1</sup>. Dietary cadaverine supplementation slightly decreased weight gain compared with the control, but not at a significant level. Feed conversion ratio ranged from 1.9 to 2.2. Survival for all the treatments was 90% or higher.

### Polyamine concentrations in shrimp tissues

Cadaverine concentration in shrimp tissues increased significantly with dietary cadaverine supplementation (Table 4, Fig. 1). A linear increase in cadaverine concentration, with increasing supplementation level, was found in the hepatopancreas of the shrimp where most of the dietary cadaverine was stored. Cadaverine also displayed a linear increase in the remaining body tissues, but to a much lesser extent. Spermidine and spermine concentrations in shrimp tissues were not affected by the consumption of cadaverine (Table 4), while histamine and putrescine concentrations were below the detection limit of 50 pmol mL<sup>-1</sup>. Different letters in the same column of Table 4 and the same graph of Fig. 1 indicate different homogenous sub-sets.

**Table 2** Amine concentrations of the experimental diets (mg free amine kg<sup>-1</sup>)

Dietary cadaverine supplement	Cadaverine		Tyramine		Histamine		Putrescine		Spermidine		Spermine	
	b	a	b	a	b	a	b	a	b	a	b	a
0	115	<50	30	<50	380	173	32	<50	125	<50	25	<5
500	664	136	120	<50	993	170	12	<50	119	<50	<5	<5
1100	1251	222	12	<50	0	50	405	<50	118	<50	<5	<5
2300	2470	619	149	<50	430	<50	30	<50	125	<50	25	<5
3500	3819	808	15	<50	386	<50	–	<50	119	<50	<5	<5
4600	4931	1547	5	60	394	52	12	<50	168	<50	<5	<5

b, before leaching; a, after leaching.

**Table 3** Final weight, feed consumption, weight gain, feed conversion ratio and survival of shrimp after 28 days of feeding

Dietary cadaverine supplement (mg kg <sup>-1</sup> )	Final weight (g)*	Feed consumption (g)†	Weight gain (%)†	Feed conversion ratio†	Survival (%)†
0	0.43	0.70	471	2.0	99
500	0.41	0.64	433	1.9	96
1100	0.38	0.61	398	2.1	90
2300	0.43	0.67	464	1.9	92
3500	0.38	0.66	405	2.2	92
4600	0.42	0.68	450	2.0	93
Pooled SD	0.07	0.06	98	0.4	7
Significance (probability)					
Linear model	0.90	0.76	0.80	0.74	0.29
Quadratic model	0.51	0.18	0.50	0.78	0.15

\**n* = 50 animals.

†*n* = five tanks.

SD, standard deviation.

**Table 4** Amine content in shrimp tissues (µg of free amine per g of dry pooled sample)

Dietary cadaverine supplement (mg kg <sup>-1</sup> )	Whole body			Hepatopancreas		
	C*	Spd	Spm	C*	Spd	Spm
0	8a	15	39	58x	500	269
500	8a	19	45	105x	398	249
1100	9a	16	46	154x	431	246
2300	13a	14	42	307y	432	248
3500	18b	18	45	331y	453	243
4600	25b	12	39	506y	440	261
Pooled SD	5.6	4.1	13.1	102	92	49
Significance (probability)						
Linear model	0.0001	0.1011	0.7840	0.0001	0.8245	0.8546
Quadratic model	0.2146	0.2387	0.1661	0.8678	0.4400	0.3985

\*Different letters in the same column indicate different homogenous sub-sets as calculated using Duncan's multiple comparison of the means.

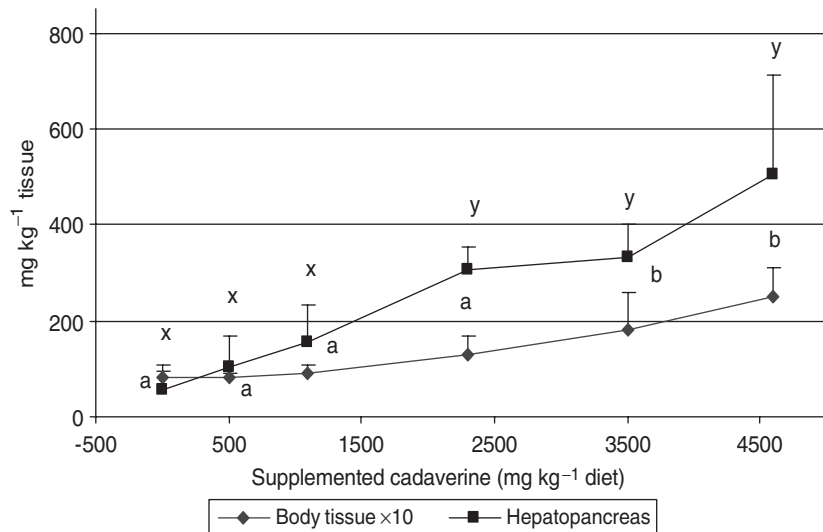
C, cadaverine; Spd, spermidine; Spm, spermine.

## Discussion

### Feeding trial

The feeding of low dietary concentrations of polyamines has been shown to promote the growth of

chicks and shrimp (Smith 1990; Tapia-Salazar *et al.* 1998). Sousadias and Smith (1995) and Smith, Morigridge and Sousadias (1996), however, observed that these positive effects decline with increasing molecular weight and cationic charge of the polyamine



**Figure 1** Cadaverine concentration in body tissues (amplified  $\times 10$ ) and hepatopancreas ( $\mu\text{g}$  of free amine  $\text{g}^{-1}$  of dry sample).

molecule. Some studies with rainbow trout *Oncorhynchus mykiss* (Fairgrieve, Myers, Hardy & Dong 1994) and Atlantic salmon *Salmo salar* L. (Opstvedt *et al.* 2000) indicated that the supplementation of biogenic amines did not improve or impair growth. Intestinal damage and reduced feed intake have been observed when feeding putrescine or histamine to rainbow trout *Oncorhynchus mykiss* (Watanabe, Takeuchi, Satoh, Toyana & Okusumi 1987; Cowey & Cho 1992; Fairgrieve *et al.* 1994; Fairgrieve, Dong & Hardy 1998). Histological changes in the hepatopancreas or other tissues were not examined in this study and, though no negative effect was seen for the parameters tested, it is possible that damage to tissues might have occurred if the feeding trial had been continued longer. A chemoattractant effect has been reported in *Macrobrachium rosenbergii* (Mendoza, Montemayor & Verde 1997) and *L. stylirostris* (Tapia-Salazar *et al.* 1998) when diets supplemented with cadaverine or histamine plus cadaverine were fed. The effects on feed consumption and growth in the current experiment are similar to previous observations on fish, but are in contrast to results reported by Mendoza and colleagues (1997) and Tapia-Salazar and colleagues (1998). The increased feed consumption and weight gain observed in an earlier study on shrimp fed histamine plus cadaverine was probably due to histamine supplementation or a combined effect of histamine plus cadaverine, but it was not due to cadaverine alone. Mortalities have been reported in chicks fed high levels of histamine and spermine (Brugh 1984; Brugh & Wilson 1986; Sousadias 1991). In the current experiment, dietary cadaverine supplementation did not cause mortality of blue shrimp.

### Polyamine concentrations in shrimp tissues

Little information is available on cadaverine concentrations in animal tissues. Heningsson and Heningsson (1983) reported that oxidative products of cadaverine might be of importance in various physiological processes and patho-physiological status in pregnant rats, while Smith, Fleming and Seddon (1996) observed a reduction in feed intake and weight gain in chicks fed supplemental cadaverine, although chick tissue cadaverine concentration was not affected by dietary cadaverine. In the current experiment, a linear increase in shrimp tissue cadaverine concentrations was seen with increasing dietary supplementation, but no difference in final weight of shrimp was observed. This would mean that shrimp cannot metabolize cadaverine or can only do so to a small extent. Storing of cadaverine possibly has an energy cost which might be reflected in poor growth, but more information is required to confirm this. It has also been reported that cadaverine can serve as a substrate in the formation of putrescine and spermidine. This reaction, however, proceeds at only 5% of the rate of conventional pathways (Morgan 1998). In the current experiment, spermidine and spermine concentrations in shrimp tissues were not affected by dietary cadaverine supplementation.

### Conclusions

Dietary cadaverine does not have nutritional value for shrimp, nor does it have any negative effect on growth, survival or feed consumption. Growth promotion that had been observed in shrimp fed

supplemental histamine plus cadaverine was probably due to histamine consumption or a combined effect of histamine plus cadaverine, but not dietary cadaverine alone. It appears that shrimp cannot metabolize cadaverine, or can only metabolize it to a small extent and that this compound is stored in shrimp tissues, primarily in the hepatopancreas, as the parent compound.

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