



## **International Fishmeal & Oil Manufacturers Association**

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### **FISH MEAL FOR SHRIMP - IMPORTANCE OF RAW MATERIAL FRESHNESS**

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# FISH MEAL FOR SHRIMP - IMPORTANCE OF RAW MATERIAL FRESHNESS

## Editor's Summary

The freshness of raw material used in the production of fish meal for shrimp feeds affects growth of the shrimp; fresher fish raw material results in better growth.

The effect of freshness was found to be greater with the more carnivorous species (*P. monodon*<sup>1</sup> > *P. stylirostris* > *P. vannemi*<sup>2</sup>). This is the conclusion of a series of trials at the University of Nuevo Leon in Mexico and the IFREMER Centre in Tahiti. The work is published in the Journal Aquaculture; a copy follows.

Younger shrimp (under 1g) are more sensitive to the freshness of fish used for fish meal fed to them than older shrimp. This has since been further confirmed in a trial with *P. stylirostris* at Nuevo Leon results of which were presented to the World Aquaculture Society Regional Meeting held in Tampa, Florida in 1999. A copy of this follows.

The findings above were the result of an extensive series of trials conducted in recirculating simulated seawater bio-assay system at the University of Nuevo Leon in Mexico<sup>3</sup> and in a throughflow seater system at the IFREMER Centre in Tahiti<sup>4</sup>. Shrimp were reared in tanks at both centres.

The fish meals produced from the same anchovy raw material were supplied by IFOMA. They were caught off the coast of Chile and processed at different stages of freshness. Details of this raw material and its processing are given in Research Report Number 1993-3. This report describes preliminary work with shrimp (*P. monodon*) at the University of Bangor (Wales) the results from which were in agreement with the latest data.

For all shrimp tested, raw material freshness had no effect on either feed conversion or mortality. The growth effect appears to have been caused by an effect on feed intake - shrimp consumed more feed made from fish meal produced from further fish.

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<sup>1</sup> also called 'tiger' shrimp representing around 65% of global production

<sup>2</sup> the small numeric improvement in growth with fresher raw material fish meal fed to *P. vannemi* was not statistically significant

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It is recommended that fish meal for shrimp should be prepared from fresh raw material (TVN in raw material of 30mgN/100g fish or less for anchovy; under 2000 ppm biogenic amine content in fish meal) for use in feeds designed to produce optimum growth.

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## Raw material freshness, a quality criterion for fish meal fed to shrimp

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### Abstract

Raw material freshness is an important criterion of fish-meal quality for salmonids and also for swine and broiler starter feeds, but needs to be assessed for shrimp. Three batches of anchovy meal were produced in a commercial low-temperature processing plant in Chile, from a unique source of raw fish, either fresh (FR, 12 h post capture), moderately fresh (MF, 25 h post capture) or stale (ST, 36 h post capture). Freshness was assessed through the total volatile nitrogen content in fish before process (TVN: 14, 30 and 50 mg N/100 g, respectively), and biogenic amines in fish meal (histamine 28, 1850 and 4701 mg/kg, respectively, and also with increasing content of cadaverine, putrescine and tyramine). Samples of the three fish meals were incorporated at levels of 30% or 40% into isoenergetic diets fed ad libitum to shrimp during various feeding trials. Feeding trials were conducted in Monterrey, Mexico, on *Penaeus vannamei* early juveniles (0.9 and 1.5 g initial weight) held in a synthetic seawater recirculating system, and in Tahiti on *P. vannamei* (7.6 g), *P. monodon* (2.5 g) and *P. stylirostris* (8.4 g) in a natural seawater flow-through system. Small *P. vannamei* (0.9 g) expressed significantly higher feed consumption ( $P = 0.028$ ) and percent weight gain ( $P = 0.048$ ) when fed the fresh raw material fish meal:

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growth increased by 25% compared with the moderately fresh and stale raw material treatments, but no significant differences were detected in feed conversion, nor survival. Larger *P. vannamei* (1.5 and 7.6 g) did not show any significant response. *P. monodon* had a tendency to better growth with the FR treatment (non significant,  $P = 0.109$ ). Finally, *P. stylirostris* late juveniles (8.4 g) fed the FR feed showed a highly significant increase in weight gain ( $P = 0.007$ ), but also a poorer feed conversion ( $P = 0.004$ ). A global interpretation of this set of results could be that susceptibility to raw-material spoilage would be higher in species with carnivorous tendencies (like *P. stylirostris*, known for its higher protein requirement), and also in young stages of less carnivorous species (as in the case of 0.9 g *P. vannamei*). However, raw material freshness, as indicated by TVN levels in raw material (less than 30 mg N/100 g) or by the sum of amine contents in the final product (less than 2000 mg/kg), is a quality parameter that should be considered when selecting fish meal for shrimp diets, particularly for very young juveniles and carnivorous species. © 1998 Elsevier Science B.V. All rights reserved.

**Keywords:** Nutrition; Fish meal; Dietary value; *Penaeus vannamei*; *Penaeus monodon*; *Penaeus stylirostris*

## 1. Introduction

Fish meals vary widely in their protein quality and nutrient composition depending on the freshness and type of the raw material, state of residual lipid (lipid quality) and processing temperature exposure (drying process). Increasing production of farmed aquatic species which are sensitive to these parameters has created a demand for high quality fish meals (McCallum and Higgs, 1989; Barlow and Pike, 1990; Pike et al., 1990; Hardy and Castro, 1994). 'Special products', made from very fresh fish processed at low temperature, are now marketed principally for salmonid feeds (Pedersen and Opstvedt, 1992; Romero et al., 1994). It appears important to investigate if other major farmed species, such as penaeid shrimps, are susceptible to the same quality factors.

Fish freshness is affected by conditions and length of storage before processing. From the time of catching, fish undergo changes brought about by the action of the enzymes of the fish (autolysis) and also from the action of bacteria present on the surface of the fish and in the gut. The rate at which raw material spoils depends on the species of fish, storage time and temperature, and degree of microbiological contamination (Klausen and Lunde, 1986; Aksnes and Brekken, 1988; Haaland et al., 1990). As fish spoils, proteins are broken down to peptides, free amino acids, amines and volatile ammonia. Trimethylamine oxide is broken down to the volatile amines di- and trimethylamine. Total volatile nitrogen content (TVN) of fish is an indicator of the raw material freshness (Pike et al., 1990; Veciana, 1990). Other amines are formed from the decarboxylation of amino acids (i.e. histamine → histidine, lysine → cadaverine, arginine → putrescine, tyrosine → tyramine). These biogenic amines are nonvolatile. They also indicate fish spoilage and are thermally stable, in contrast to TVN. Thus, their levels in dried products such as fish meals are useful indicators of the raw material freshness. Nevertheless, because most of the biogenic amines go with the fish solubles in the production of fish meals, the value obtained can be affected by the amount of fish solubles returned to the final product (Pike and Hardy, 1997). However, describing the

breakdown of proteins via free amino acids to amines and ammonia is a simple view, and there may be other materials produced such as endotoxins for which TVN and/or biogenic amines are only indicators.

The evaluation of different fish meals by these chemical parameters has been paralleled with biological assays by several authors for different species. For example, Huisman et al. (1992) found that performance of chickens was not negatively affected by dietary inclusion of 12% of LBA (low biogenic amine) or MBA (medium biogenic amine) fish meals; nevertheless, a level of 12% of HBA (high biogenic amine) fish meal (3750 mg/kg histamine) significantly depressed both feed intake and weight gain, without affecting the feed conversion ratios. Similarly, rainbow trout performance was not affected by dietary biogenic amines supplementation, at levels commonly found in fish meals. Putrescine inclusion in practical diets up to 4000 mg/kg had remarkably little effect on feed intake, growth or feed conversion, which were significantly depressed only at the very high level of 13300 mg/kg (Cowey and Cho, 1992). In a trial on rainbow trout (Fairgrieve et al., 1994), supplementation with histamine alone (2000 mg/kg) or combined with putrescine and cadaverine (500 mg/kg each) had no depressing effect on the same performance parameters; no signs of acute toxicity or mortality occurred in 16 weeks study, except an apparently inoffensive stomach distension syndrome.

Several feeding trials with salmonids have used fish meal made from fish of different freshness. In a trial with fresh or stale sand eel meal (TVN 30 or 130 mg N/100 g fish), trout were fed at two different levels; the fresh raw material fish meal gave an improvement in the daily weight gain (1.44% vs. 1.34%) at the higher feeding level (Jensen, 1986). In Vancouver, Canada, two fish meals were produced on a pilot scale from frozen Pacific herring, which was thawed and stored at 15°C for 5 days (TVN 20 and 113 mg N/100 g raw fish; histamine + putrescine + cadaverine + tyramine = 848 and 3092 mg/kg, respectively) (Anderson et al., 1997); raw material freshness was found to give a higher daily growth rate (0.95% vs. 0.85%), but lower (unexpected) apparent digestibility figures both for the organic matter (90.3% vs. 93.9%) and crude protein (86.0% vs. 91.9%).

The International Fishmeal and Oil Manufacturers Association (IFOMA) has undertaken two trials to produce and assess fish meals made from the same raw material at different freshness, obtaining two different sets of fish meals from European herring (TVN 22, 62 and 143 mg N/100 g fish; storage temperature 0–7°C), or South American anchovy (14, 30 and 50 mg N/100 g fish; storage temperature 20–28°C). The sum of histamine, cadaverine, putrescine and tyramine contents were 390, 2070 and 3860 mg/kg, or 114, 3384 and 7873 mg/kg, respectively, for fresh, moderately fresh and stale herring or anchovy.

Samples of these herring or anchovy meals were tested on salmon smolts (91 and 135 g initial weights respectively), resulting in differences in the specific daily growth rates obtained with the fresh vs. stale material: 1.75% vs. 1.2% and 1.7% vs. 1.6%, respectively (Pike, 1996).

The IFOMA anchovy fish meals were also fed to *Penaeus monodon* at the University of Bangor, Wales (UK). In a first trial on shrimp held in individual compartments, the meal made with fresh fish gave growth rates around 10% higher than those with stale

fish, although difference did not reach significance (Jones, 1992, unpublished data). In further trials, using a change-over design with each animal acting as its own control, the stale fish meal showed the lower growth, but there was an indication of better growth with moderately fresh than fresh fish meal (Jones, op. cit.; Pike, 1996; Pike and Hardy, 1997). It was decided to repeat the trials with larger number of shrimp, using groups of shrimp housed together.

The following experiments were performed to measure the effects of raw material freshness of the samples of South American anchovy meals collected by IFOMA, on the performance of *Penaeus vannamei*, *P. monodon* and *P. stylirostris* juveniles, in terms of growth, feed consumption, feed conversion ratio and survival.

## 2. Materials and methods

### 2.1. Experimental fish meals

Anchovy meals were produced from fresh fish (FR, 12 h post capture) or after two periods of storage at 20°–28°C to give moderately fresh (MF, 25 h post capture) and stale raw material (ST, 36 h post capture). Anchovy were caught in a similar location within a 12-h period, off the coast of Chile. Processing was run in a commercial facility by low-temperature drying (80°C) using two dryers in sequence: the first, a steam dryer,

Table 1  
Composition of the experimental fish meals (as fed)

Experimental fish meals <sup>a</sup>	FR	MF	ST
<i>Proximal analysis</i>			
Moisture (%)	9.6	10.9	11.1
Ash (%)	14.5	15.3	15.4
Crude fat (%)	7.7	7.4	9.4
Crude protein (%)	69.6	67.7	65.8
<i>Raw material freshness parameters</i>			
TVN in raw material (mg N/100 g)	14	30	50
TVN in soluble concentrate (mg N/100 g)	106	190	239
Soluble N in fish meal (% of total N)	21.3	24.9	27.3
Soluble protein in fish meal (% of crude protein)	5.4	6.5	7.1
Histamine (mg/kg)	28	1850	4701
Cadaverine (mg/kg)	51	803	1599
Putrescine (mg/kg)	35	446	916
Tyramine (mg/kg)	—	285	657
Total amines	114	3384	7873
True protein digestibility in mink (%)	91.4	89.7	89.8

<sup>a</sup>FR: fish meal made from fresh raw material.

MF: fish meal made from moderately fresh raw material.

ST: fish meal made from stale raw material.

followed by an indirect hot-air dryer. Before the experimental fish meals were taken, the process was allowed to reach equilibrium, such that the solubles being returned were coming from the fish going through the plant. The fish meal for trial purposes was selected when the production line had been running the particular batch of raw material for at least 30 min. During preparation, an anti-oxidant (ethoxyquin) was added to give 100 mg/kg residual content at time of shipping.

Total volatile nitrogen (TVN) was analysed by AOAC method 920-03 (magnesium oxide method; Helrich, 1990) at the processing plant in the raw material and solubles concentrate. Percentage of soluble protein in the final product was determined at FCB/UANL; fish meal aliquots were incubated at 40°C for 1 h in a saline solution (0.15 M NaCl; Crooker et al., 1978), filtrated, and the filtrate analysed for total nitrogen (Kjeldahl, Tecator, 1987) and protein (Bradford, 1976). Biogenic amines content (Seiler and Knödgen, 1985) and digestibility in mink (Skede et al., 1980) in the final product were analysed at the Torry Research Station, UK. Each of the three fish meals was found to have a true protein digestibility in mink of around 90% (Table 1).

## 2.2. Experimental diets

Three isoenergetic diets containing 30% fish meal were formulated at the Universidad Autónoma de Nuevo León (FCB/UANL), according to penaeid shrimp nutritional requirements (Tacon, 1989; Akiyama et al., 1991), modifying the soybean paste and wheat meal levels, maintaining other ingredients constant. At the Centre Océanologique du Pacifique (IFREMER/COP), the experimental fish meals were incorporated at a 40% dietary level, with all other ingredients being constant (Table 2).

Dry feedstuffs were finely ground, weighed and thoroughly homogenized in a dry-feed mixer for 10 min. Vitamins were blended with a portion of the dry ingredients, lipids and lecithin were combined before mixing with dry materials. Water (25% to 40% by weight) was added, and the ingredients thoroughly kneaded to wet all particles and to form a stiff dough, which was extruded with a Hobart meat grinder through a die with 2-mm holes. The spaghetti-like strands were dried in an electrical oven (50°C, 15 h) and broken into pellets about 3 mm long.

Proximate analysis of each experimental diet was conducted to verify the expected chemical composition (Table 2). In the diets including 30% fish meal, crude protein ranged from 37.1% to 38.7% of dry matter, lipids from 6.0% to 6.8%, and calculated gross energy content was equal for the three diets: 4.6 kcal/g (Table 2). In the 40% dietary fish meal diets, also including higher levels of shrimp meal and fish oil, crude protein ranged from 51% to 53%, and crude lipid from 12.5% to 13%, with a gross energy content of 4.7–4.8 kcal/g.

Stability of the diets in water was evaluated by measuring the loss of dry matter after 1 h immersion in seawater (35‰, 28°C). Feed samples were placed in galvanized wire netting baskets, which moved underwater in a gentle circular movement in a vertical plane (5 rpm, 2 cm radius) (Aquacop, 1978). Each sample was run in triplicate. In both diets sets (made at FCB/UANL or IFREMER/COP), the loss of dry matter was higher for the stale material diet than for the fresh and moderately fresh material diets (Table 2).



Table 2

Formulation and proximate analysis of the experimental diets

Experimental diets <sup>a</sup>	FCB/UANL diets			IFREMER/COP diets		
	FR	MF	ST	FR	MF	ST
<i>Ingredients (% as fed)</i>						
FR raw materials (fish meal)	30	—	—	40	—	—
MF raw material (fish meal)	—	30	—	—	40	—
ST raw material (fish meal)	—	—	30	—	—	40
Soy paste (48%, 5 TOI/mg)	11	13	14	15	15	15
Wheat flour	38	36	34	10.5	10.5	10.5
Shrimp meal (Chile)	6	6	6	12	12	12
Corn gluten	8	8	8	—	—	—
Wheat gluten	—	—	—	10	10	10
Soybean lecithin	2.8	2.8	2.8	3	3	3
Fish oil <sup>b</sup>	1.7	1.7	1.7	5	5	5
Vitamin mixture <sup>c</sup>	1	1	1	1.9	1.9	1.9
Binder (Maxibon)	0.6	0.6	0.6	—	—	—
CaHPO <sub>4</sub>	—	—	—	2	2	2
Oyster shells	—	—	—	1	1	1
<i>Proximate analysis (% dry matter)</i>						
Crude protein	37.1	38.7	38.6	53.6	51.0	51.4
Crude fat	6.8	6.1	6.0	12.5	12.6	13.0
Ash	8.0	8.2	8.3	14.9	15.7	15.2
Crude fiber	2.4	2.4	2.4	4.5	4.7	4.7
NFE (by difference)	45.8	44.8	44.7	14.5	16.0	15.7
Gross energy (kcal/g) <sup>d</sup>	4.6	4.6	4.6	4.8	4.7	4.8
<i>Leaching</i>						
Loss of dry matter (%)	19	17	26	2.6	4.1	5.5

<sup>a</sup> Experimental diets including fish meals made from: FR = fresh raw material; MF = moderately fresh raw material; ST = stale raw material.

<sup>b</sup> Sardine oil (Guaymas, Sonora, México) in FCB/UANL diets, menhaden oil (USA) in IFREMER/COP diets.

<sup>c</sup> FCB/UANL: Vitamin and mineral solution: potassium 250 mg; ascorbic acid 200 mg; riboflavin 100 mg; calcium 10 mg; DL- $\alpha$ -tocopherol acetate 100 mg; cyanocobalamine 100 mg; zinc 100 mg; biotin 50 mg; manganese 0.5 mg; chlorine 50 mg; sodium 25 mg; retinil acetate 25 mg; thiamin 100 mg; nicotinic acid 100 mg; cholecalciferol 50 mg; pyridoxine chloride 100 mg; magnesium 10 mg; copper 25 mg; pantothenic acid 100 mg; selenium 50 mg; carbohydrate sufficient quantity to 12 g. IFREMER/COP: Vit. Premix Rovimix No. 1693, Roche, France.

<sup>d</sup> Protein 5.6, lipid 9.5 and carbohydrate 4.1 kcal/g (Tacon, 1989).

### 2.3. Feeding trials

Feeding trials were run simultaneously in Monterrey, Mexico, at FCB/UANL, on *P. vannamei* early juveniles (0.9 and 1.5 g initial weight) held in a synthetic seawater recirculating bioassay system, and in Tahiti, French Polynesia, at IFREMER/COP, on *P. vannamei* (7.6 g), *P. monodon* (2.5 g) and *P. stylirostris* (8.4 g) held in a natural seawater flow-through system. Among the variations in the conditions between the different trials, the most important probably was the stocking densities, ranging from 83 to 20 shrimp/m<sup>2</sup> (Table 3).

Table 3  
Feeding trials experimental conditions

Place	Trial				
	I	II	III	IV	V
	Monterrey FCB/UANL	Monterrey FCB/UANL	Tahiti IFREMER/COP	Tahiti IFREMER/COP	Tahiti IFREMER/COP
Species	<i>P. vannamei</i>	<i>P. vannamei</i>	<i>P. vannamei</i>	<i>P. monodon</i>	<i>P. stylirostris</i>
Initial weight (g)	0.9	1.5	7.6	2.5	8.4
Duration (days)	14	28	30	30	30
Shrimp/tank	15	8	10	10	10
Stocking density (shrimp/m <sup>2</sup> )	83	44	20	20	20
Repetitions/diet	4 experimental tanks per diet		5 experimental tanks per diet		
Experimental tanks	60 cm × 30 cm × 35 cm, glass fiber		70 cm × 70 cm × 45 cm, glass fiber		
Area, water volume	0.18 m <sup>2</sup> , 60-l tanks		0.5 m <sup>2</sup> , 225-l tanks		
Reference	Ricque et al., 1993		Aquacop, 1977		
Water quality	synthetic (Fritz chemicals)		natural coral lagoon water, sand filtered		
Temperature	26–30°C		27.5°C		
Salinity	33–36 g l <sup>-1</sup>		35 g l <sup>-1</sup>		
Max. values (μmol l <sup>-1</sup> )	[NH <sub>3</sub> ] = 6, [NO <sub>2</sub> <sup>-</sup> ] = 4, [NO <sub>3</sub> <sup>-</sup> ] = 323		[NH <sub>3</sub> ] = 1, [NO <sub>2</sub> <sup>-</sup> ] = 0.1, [NO <sub>3</sub> <sup>-</sup> ] = 0.4		
	Water quality variations affected all tanks at the same time; water was exchanged in tanks of both systems by a continuous in-flow				

Table 4

*P. vannamei* (0.9 g initial weight) feeding trial results: Trial I (means of four replicate values)

Treatments	Fresh	Medium fresh	Stale	ANOVA significance level
Initial weight (g)	0.937 ± 0.003	0.935 ± 0.005	0.937 ± 0.005	
Final weight (g)	1.53 <sup>b</sup> ± 0.07	1.44 <sup>a</sup> ± 0.08	1.37 <sup>a</sup> ± 0.05	0.0455 * S.
Weight gain (%)	63 <sup>b</sup> ± 8	54 <sup>a</sup> ± 9	47 <sup>a</sup> ± 5	0.0477 * S.
Feed consumption (g)	1.75 <sup>b</sup> ± 0.13	1.50 <sup>a</sup> ± 0.11	1.55 <sup>a</sup> ± 0.08	0.0275 * S.
Feed conversion	3.0 ± 0.5	3.0 ± 0.4	3.5 ± 0.4	0.2280 *
Survival (%)	95 ± 3	85 ± 18	90 ± 4	0.4402 *

\* Probability given by a one-way ANOVA; S. is significant.

<sup>a,b</sup> Different letters indicate different homogeneous subsets as defined by a Scheffe multiple range procedure at 5% risk.

For the feeding trials at FCB/UANL, *P. vannamei* juveniles were pregrown from hatchery-raised postlarvae (wild broodstock from Nayarit, México) at the La Marina facility on the Mexican Gulf Coast, transported to Monterrey and held in a 500-l tank in the bioassay hall (Ricque et al., 1993). Shrimp were selected in the shortest size class possible and distributed to obtain a similar-size frequencies pattern in the twelve 60-l tanks. They were weighed to ± 1 mg, after being blotted on a wet cloth. Dead shrimp were replaced during the first three days after weighing. Shrimp were fed ad libitum twice a day at 1000 and 1800 h (about 10% of the biomass daily); feed ration was adjusted in each tank to allow a minimum amount of uneaten feed to remain until next feeding. Feed refusals were visually estimated, recorded and cleaned just before next feeding.

For the feeding trials at IFREMER/COP, shrimp from the three species (*P. vannamei*, *P. monodon* and *P. stylirostris*) were bred in captivity (Aquacop, 1977). Postlarvae were seeded in earthen ponds (100–300 m<sup>2</sup>) and grown up to the size suitable for the experiment. A cast net was thrown several times in the pond until about 600–700 animals were caught and transferred to the bioassay room for the randomization, which proceeded immediately after 1–2 h acclimation. Shrimp were blotted, weighed with a Mettler electronic scale to ± 10 mg, and distributed in 5-l pots to dispatch them to the replicated tanks with the minimum stress. Animals were kept under observation for 4 days after allocation, dead ones being replaced. After this period, animals added to keep the stocking density constant during the course of the trial were not taken into account for final assessment of mortality rate. Shrimp were fed ad libitum

Table 5

*P. vannamei* (1.5 g initial weight) feeding trial results: Trial II (means of four replicate values)

Treatments	Fresh	Medium fresh	Stale	ANOVA significance level
Initial weight (g)	1.52 ± 0.03	1.56 ± 0.01	1.52 ± 0.02	
Final weight (g)	3.44 ± 0.10	3.28 ± 0.32	3.39 ± 0.33	
Weight gain (%)	126 ± 4	112 ± 21	122 ± 22	0.5495 <sup>a</sup>
Feed consumption (g)	6.48 ± 0.62	5.41 ± 0.21	6.09 ± 1.43	0.2993 <sup>a</sup>
Feed conversion	3.22 ± 0.14	3.12 ± 0.21	3.23 ± 0.21	0.9074 <sup>a</sup>
Survival (%)	100 ± 0	91 ± 12	94 ± 7	0.2955 <sup>a</sup>

<sup>a</sup> Probability given by a one-way ANOVA.

Table 6

*P. vannamei* (7.6 g initial weight) feeding trial results: Trial III (means of five replicate values)

Treatments	Fresh	Medium fresh	Stale	ANOVA significance level
Initial weight (g)	7.62 ± 1.55	7.62 ± 1.58	7.60 ± 1.56	0.9997 <sup>a</sup>
Final weight (g)	10.68 ± 1.71	10.64 ± 2.00	10.46 ± 1.83	0.818 <sup>b</sup>
Weight gain (%)	41 ± 8	41 ± 12	38 ± 6	0.796 <sup>b</sup>
Feed conversion	2.3 ± 0.4	2.0 ± 0.5	1.9 ± 0.3	0.432 <sup>b</sup>
Survival (%)	86 ± 11	90 ± 7	90 ± 10	0.7564 <sup>a</sup>

<sup>a</sup>Probability given by a one-way ANOVA.<sup>b</sup>Probability given by an ANOVA taking the initial weight as a covariate.

two or three times a day (about 5.5% of the biomass daily). Surplus feed percentage was estimated each morning.

Growth, feed conversion ratios, and survival for each tank were estimated by the following parameters: %weight gain = [(final mean weight – initial mean weight)/initial mean weight] × 100; feed conversion ratio = [individual feed intake/weight gain]; survival rate = [final no. organisms/initial no. organisms] × 100.

The individual feed intake was estimated from feed consumption and shrimp number per tank; daily estimates in a particular tank were summed over the trial duration: individual feed intake =  $\sum_{i=1}^{28} [(\text{tank feed ration on day } i - \text{feed refusals on day } i) / \text{number of shrimp present in the tank on day } i]$ .

The statistical sample for each treatment was constituted from the four (FCB/UANL) or five (IFREMER/COP) values obtained from the replicate tanks. Differences among means of the shrimp performance parameters were assessed by an analysis of variance; in the case of trials performed in Tahiti, the initial weights were used as covariates to adjust final weights, since the differences between tanks were appreciable at the beginning of the experiment (Zar, 1974).

### 3. Results

In Trial I, feed consumption and growth were significantly improved for the fresh material treatment compared with the moderately fresh and stale material diets (Table 4).

Table 7

*P. monodon* (2.5 g initial weight) feeding trial results: Trial IV (means of five replicate values)

Treatments	Fresh	Medium fresh	Stale	ANOVA significance level
Initial weight (g)	2.52 ± 0.30	2.50 ± 0.27	2.48 ± 0.29	0.9762 *
Final weight (g)	5.84 ± 0.38	5.60 ± 0.82	5.28 ± 0.58	0.135 * *
Weight gain (%)	133 <sup>b</sup> ± 17	123 <sup>ab</sup> ± 11	113 <sup>a</sup> ± 13	0.109 * *
Feed conversion	3.6 ± 0.6	4.2 ± 0.8	4.1 ± 0.6	0.436 * *
Survival (%)	98 ± 4	96 ± 5	96 ± 5	0.7828 *

\*Probability given by a one-way ANOVA.

\* \*Probability given by an ANOVA taking the initial weight as a covariate.

<sup>a,b</sup>Different letters indicate different homogeneous subsets as defined by a Duncan multiple range procedure ( $P = 0.05$ ).

Table 8  
*P. stylirostris* (8.4 g initial weight) feeding trial results: Trial V (means of five replicate values)

Treatments	Fresh	Medium fresh	Stale	ANOVA significance level
Initial weight (g)	8.40 ± 0.47	8.40 ± 0.47	8.42 ± 0.48	0.9971 *
Final weight (g)	15.08 <sup>b</sup> ± 0.89	14.38 <sup>ab</sup> ± 0.65	14.00 <sup>a</sup> ± 0.60	0.010 ** Hi.S.
Weight gain (%)	80 <sup>b</sup> ± 2	71 <sup>a</sup> ± 8	66 <sup>a</sup> ± 7	0.007 ** Hi.S.
Feed conversion	4.3 <sup>b</sup> ± 0.4	3.8 <sup>a</sup> ± 0.2	3.6 <sup>a</sup> ± 0.2	0.004 ** Hi.S.
Survival (%)	100 ± 0	100 ± 0	100 ± 0	

\* Probability given by a one-way ANOVA

\*\* Probability given by an ANOVA taking the initial weight as a covariate; Hi.S. is highly significant

<sup>a,b</sup> Different letters indicate different homogeneous subsets as defined by a Duncan multiple range procedure ( $P = 0.05$ )

Weight gain was increased by 25%, when compared with the mean for moderately fresh and stale material treatments, and consumption by 15%. Survival and feed conversion ratios were not significantly affected; however, feed conversion ratio was higher with the stale material treatment, and survival mean value was higher with the fresh material diet (95%) than with the moderately fresh and stale material diets (85% and 90%, respectively).

In Trials II and III (using larger *P. vannamei* juveniles), although weight gains were satisfactory (taking into account they were performed in a controlled environment without natural productivity), no clear tendency nor significant differences appeared in any performance parameter (Tables 5 and 6).

In Trial IV on *P. monodon*, there was a tendency for better growth and feed conversion ratio (lower) with the fresh raw material treatment (Table 7), and this difference approached statistical significance ( $P = 0.109$ ): growth improved by 9% and 16% if compared with moderately fresh or stale raw material treatments. Survival was unaffected ( $P = 0.78$ ).

In Trial V, *P. stylirostris* showed markedly improved growth with the fresh raw material treatment, up 21% if compared to the stale raw material treatment. Poorer feed conversion (Table 8) indicates that growth improvement was relatively less than the feed consumption increase. Survival was 100% in all treatments.

#### 4. Discussion

The experimental fish meals tested in this study are unique in that they were produced commercially in such a way that only the freshness of the raw material varied. In contrast, raw material in the work of Anderson et al. (1997) was frozen, thawed and allowed to deteriorate in the laboratory. This may have resulted in the development of a bacterial population which may not have been representative of the microbial changes in a factory fish-storage facility. Another attribute of the fish-meal samples in the present work was that their raw material TVN values ranged from 14 to 50 mg N/100 g, indicating a very fresh raw material, even in the so-called 'stale raw material fish meal'.

Standard F.A.Q. (fair average quality) fish meals in Chile can exceed these values; moreover, fish meals with raw material TVN content lower than 50 mg N/100 g are

classified as 'special quality' and marketed at higher price (Castro-Campos, 1995, personal communication; Chamberlain, 1995). Whether this can be justified will depend on the improvement in performance resulting from the use of these special fish meals.

TVN values lower than 50 mg N/100 g would also be considered as representative of raw material used in 'special' fish meals in Scandinavia, for example, to give low amines in the fish meal (histamine + cadaverine + putrescine + tyramine not exceeding 2000 mg/kg). In the experimental fish meals of the present work, the sum of these biogenic amines ranged from 114 to 7873 mg/kg (Table 1), reaching higher values than the Scandinavian 'specials'. However, with fish caught in South America (anchovy, sardines and mackerel, for example), for a given TVN the amine content appears to be higher than with fish in Northern Europe; this may be a result of different species and higher temperatures than in the fishing area in the former case (Pike and Hardy, 1997).

It is probable that a great part of these amines and TVN were extracted from the fish bulk together with the solubles, water and oil, and returned with the concentrated solubles to the press-cake during the drying process, as it occurs in a normal full fish meal manufacturing process; in the present work, special care was taken to ensure that concentrated solubles were returned to the press cake from which they were removed. Indeed, as the fish storage time increased for the different experimental fish meals, there was a similar increase of the TVN content in the raw material and solubles concentrate, and, also, of the total soluble nitrogen, soluble protein and biogenic amines contents in the final products. From the soluble nitrogen present in fish meal (21% to 27% of total nitrogen), only a small part was in form of true soluble protein (5.4% to 7.1% of the total crude protein ( $N \times 6.25$ )), representing the same proportion of about 25% of total soluble nitrogen for the three experimental fish meals (Table 1). This tends to prove that no aleatory variations were introduced during the process.

The growth improvements obtained with the fresh raw material treatment during Trial I (*P. vannamei* early juveniles), IV (*P. monodon*) and V (*P. stylirostris*) (Fig. 1) are in line with results obtained on salmonids; moreover, they demonstrate a relatively high sensitivity to raw-material freshness. For swine (Castro-Campos, 1990) and salmonids (Watanabe et al., 1987; Pike et al., 1990), higher biogenic amine contents or TVN levels (TVN higher than 80 and 90 mg N/100 g, respectively) were necessary to affect significantly the growth rate and the feed efficiency. The same set of experimental anchovy generated only small differences in salmonid growth: 1.7% vs. 1.6% daily specific growth rates (a 6% improvement) obtained with the fresh vs. stale material on 135 g salmon smolts (Pike, 1996). However, the set of herring meals (TVN ranging from 22 to 143 mg N/100 g fish, sum of biogenic amines from 390 to 3860 mg/kg), tested on 91 g salmon smolts, resulted in differences of 0.5% in the daily specific growth rates obtained with the fresh or stale material (1.7% vs. 1.2%, respectively, a 29% improvement) (Pike, 1996).

Although growth improvement was significant for *P. vannamei* early juveniles (0.9 g) in Trial I, the lack of response in Trials II and III suggests that larger *P. vannamei* shrimp juveniles (1.5 and 7.6 g) were less susceptible to the freshness of raw material. This different sensitivity could be explained by lower nutritional requirements and metabolic rate due to their larger size (Tacon, 1989; Akiyama et al., 1991), or because the shrimp were more stressed in the first bioassay because at higher density; under

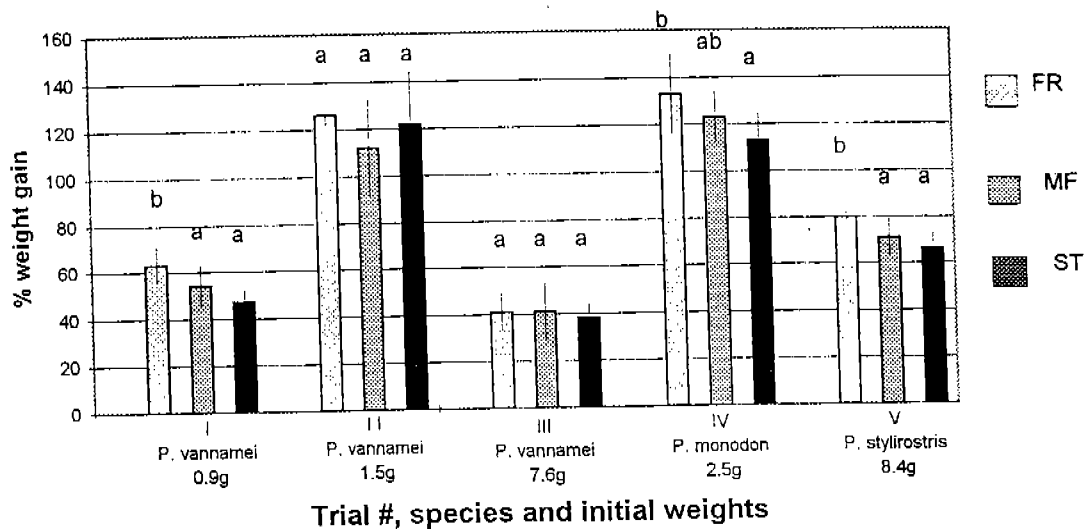


Fig. 1. Growth of different species with fish meals made from fresh (FR), moderately fresh (MF) or stale (ST) raw material (different letters indicate different homogeneous subsets as defined by Duncan's procedure,  $P = 0.05$ ).

stressful conditions, they might be more susceptible to the differences on the fish meal quality. However, *P. vannamei* juveniles appeared to be less susceptible to raw material freshness than *P. stylirostris* or *P. monodon* juveniles of the same size, since the growth improvement obtained with *P. stylirostris* was highly significant ( $P = 0.007$ ), although less significant with *P. monodon* ( $P = 0.10$ ). These species were already classified the same way in a comparative study on their growth response to the squid protein fraction (Cruz-Suarez et al., 1987).

Obtaining lower feed consumption with the meal from moderately fresh and stale fish was contrary to what was expected, taking into account the attractant effect of some soluble molecules like free amino acids (Cruz-Suarez and Guillaume, 1983) and certain amines, e.g. cadaverine or putrescine (Montemayor-Leal, 1995). However, it is known that in young pigs or chicken, high doses of biogenic amines can switch the consumption and growth improvement effect to an adverse effect (Huisman et al., 1992). Note that the lower stability of the stale material diets (Table 2) possibly increased artificially the consumption estimate (low stability feed can break up and pass through the tank false-bottom mesh), explaining the higher feed conversion ratio for the stale material treatment in Trials I and V, as an artefact. The lower stability of these diets could be related to the bacterial hydrolysis during raw fish storage, reducing native proteins into smaller, more soluble, components.

In terms of feed-conversion ratios, no clear tendency was observed among the different trials. This could be expected, since the spoilage of raw material had a minor effect on the digestibility in mink, which dropped from 91.4% to 89.7%. Indeed, raw material freshness per se has been found to have only a slight effect on true digestibility of fish meal fed to mink, when these fish meals were dried at low temperature (80°C) (Pike et al., 1990). However, the differences in growth during Trials I, IV and V

correlate with the true protein digestibility values in mink (Table 2). Mink digestibility has proved to be the best indicator of 'premium' quality for salmonids, correlating well with apparent digestibility in vivo (Skede et al., 1980; Mundheim and Opstvedt, 1989, in Pike et al., 1990; Pedersen and Opstvedt, 1992). Indeed, Romero et al. (1994) obtained a very high correlation coefficient between these two parameters ( $r^2 = 0.99$ ); in contrast, chemical parameters like ash, salt, cadaverine or total biogenic amines, as measured in a variety of fish meals samples, never reached  $r^2$  values higher than 0.64 with the in vivo digestibility in salmon.

In the case of shrimp, it appears that best results obtained with fresh raw material could be simply reported as a new argument to sustain the idea that native (non-denaturated) protein sources tend to be more favorable to shrimp growth than those which have faced a processing operation like enzymatic hydrolysis, since it has been observed that ingredients such as fish protein concentrates produced by enzymatic action, tend to have a negative effect on growth of *P. stylirostris* (Aquacop, 1990, pers. com., Aquacop et al., 1995) or *P. vannamei* (Cruz-Suarez et al., 1995).

## 5. Conclusion

Small *P. vannamei* juveniles (less than 1 g), held at high density (83/m<sup>2</sup>) in a recirculating system, were sensitive to small variations in terms of raw material freshness in a set of high digestibility fish meals: a slight increase in the raw material TVN level (14 to 30 mg N/100 g), but also a substantial increase in histamine (28 to 1850 mg/kg) and other amines concentration, were related to a significant decrease in the growth on a short period of time (14 days). Raising the raw material TVN up to 50 mg/100 g, and histamine concentration to 4700 mg/kg (total amines over 7500 mg/kg), further diminished the growth, although not significantly ( $P > 0.05$ ).

This effect could not be reproduced on larger *P. vannamei* shrimp (1.5 g or 7.6 g) at lower density (44/m<sup>2</sup> or 20/m<sup>2</sup>), possibly due to a lower sensitivity of older shrimp to the fish meal quality, and less stressful density. These results also suggest a rather low sensitivity of *P. vannamei* if compared with the high susceptibility of still larger *P. stylirostris* (8.4 g), and medium susceptibility of rather small *P. monodon* (2.5 g), held at the same density (20/m<sup>2</sup>).

However, the results of bioassays I, IV and V highlight the raw material freshness as a quality parameter for fish meals to be used in shrimp feeds, in particular for *P. stylirostris* and probably for young juveniles of most penaeid species.

This kind of study could be repeated using fish meals differing on a wider range in their raw material TVN values (from 14 to 100 mg N/100 g and over), to address to the large variations eventually found in commercial products in terms of raw material freshness.

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## EFFECT OF RAW MATERIAL DETERIORATION ON THE NUTRITIONAL VALUE OF ANCHOVY MEALS FOR VERY SMALL *Penaeus stylirostris* JUVENILES.

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Raw material deterioration has been shown as an important quality parameter when selecting fish meals to be fed not only to salmonids, but also to penaeid shrimp, due to its negative effect on growth. In shrimp, this effect seems dependant on consumer species, with *P. stylirostris* being especially sensible, and size, with small sized *P. vannamei* (down to 0.9g) being also more susceptible. Moreover, there is a suspicion about reduced survival when very small *P. vannamei* (around 0.1g) are fed fish meals containing high levels of biogenic amines, i.e. fish meals made from deteriorated raw material. The present study aims to determine the response of very small *P. stylirostris* to experimental anchovy meals that have been previously manufactured for IFOMA in a Chilean low temperature processing facility from the same shoal but with different storage times: 12 h (fresh = FR), 24 h (medium fresh = MF), and 36 h post capture (stale = ST).

The experimental fish meals were incorporated at 30.3 to 32.0% levels in practical shrimp diets; each experimental fish meal contributed with the 50% of the total dietary crude protein. A commercial diet was used as a external control. In a growth trial, the experimental diets were evaluated in small shrimp juveniles *P. stylirostris* "Super shrimp" (72 mg average initial weight) during 36 days. An *in vivo* digestibility trial was run on *P. vannamei* (average initial weight 250, 400 and 550 mg respectively for 3 replicate groups) to evaluate the effect of raw material deterioration on protein and dry matter apparent digestibility of the fish meals and experimental diets.

Feed consumption, percent weight gain and biomass were significantly affected by the raw material deterioration, while feed conversion ratio and survival were not. Shrimp fed the FR meal had a growth 33 and 25.7% higher respectively than the shrimp fed the MF and ST fish meal. Shrimp fed the FR meal consumed more feed (.82 g) than the shrimp fed the MF and ST meal (.65 g and .65 g respectively). In contrast, the protein and dry matter digestibility increased with the fish deterioration (from 83 to 104% and 75 to 87% respectively for fish meal, and from 87 to 91 and 81 to 84% respectively for the experimental diets).

The absence of difference in feed conversion ratios, and better *in vivo* digestibilities for the ST meal indicates that raw material deterioration do not affect negatively the quality of fish protein for shrimp. The growth fall seems rather due to lower feed consumption, while toxicity seems low in the present case, since no significant effect on survival has been observed.

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