



**International Fishmeal & Oil
Manufacturers Association**

**FEEDING TRIALS WITH VARIOUS
QUALITIES OF FISH MEAL WITH
SHRIMP**

**A Progress Report on the European
Union Project**

RESEARCH REPORT NUMBER: 1994-4

December 1994

STRICTLY CONFIDENTIAL

FEEDING TRIALS WITH VARIOUS QUALITIES OF FISH MEAL WITH SHRIMP

A Progress Report on the European Union Project

Project participants: University of Nuevo Leon, Monterrey, Mexico
 IFREMER, Tahiti
 IFOMA

by

I.H. PIKE

S U M M A R Y

A shrimp project financed by the European Union is currently being carried out jointly by IFOMA, the University of Nuevo Leon in Mexico and IFREMER the French Fisheries/Aquaculture research organisation in Tahiti. The project is designed to provide information about the quality of fish meal required to optimise growth in tropical shrimp. It is intended to provide the fish meal producer with guidelines for the production of fish meal for shrimp, which in turn should improve productivity and reduce costs of production for shrimp farms.

Joint work in Mexico and Tahiti has indicated that fish raw material should be fresh at the time of producing fish meal. Fish meals causing gizzard erosion in poultry have been shown to have deleterious effects on shrimp. From this work, undertaken in Mexico, it is recommended that fish meals for shrimp should be checked first in chicks to ensure they do not have gizzard erosion producing tendencies.

Early results of work to study digestibility of protein in fish meals fed to shrimp suggest differences found in salmon, indicated by mink tests, may be reflected in shrimp.

BACKGROUND

The rapid growth in the farming of shrimp by intensive and semi-intensive systems has led to a large demand for feed. This in turn has led to a new rapidly growing market for fish meal. With an estimated 700 thousand tonnes (TT) production of farmed shrimp last year, around 400 TT was estimated to be raised intensively/semi-intensively - that is, manufactured feed was used. With an overall feed requirement of around two tons for each ton of shrimp production, world shrimp feed production was estimated to be

around 800TT. The fish meal content of this is believed to have been around 275TT.

In the absence of any quality requirements for fish meal for shrimp, the Association began a research programme to investigate this question around five years ago. Initial work was done by the University of Bangor in Wales. Subsequently contact was made with Dr. Elizabeth Cruz of the University of Nuevo Leon in Mexico, a leading expert on shrimp nutrition. In conjunction with this group, and Dr. Gerard Cuzon of the French Fisheries/Aquaculture research organisation IFREMER group based in Tahiti, the Association succeeded in getting funds from the European Union (150T ECU) to fund a three year study into quality requirements for fish meal used in feeds for tropical shrimp. I am co-ordinating this project which will be undertaken jointly by the University of Nuevo Leon in Mexico and IFREMER in Tahiti.

THE EU PROJECT

The objectives of the EU shrimp project are as follows:-

1. Determine if raw material freshness or processing conditions damage the nutritional quality of fish meal for shrimp feeds.
2. Evaluate fish meals with a gizzard erosion score (determined in chicks) - and also gizzerosine effect on shrimp physiology.
3. To develop a chemical method to determine digestibility.
4. Decrease environmental pollution using more digestible and assimilable feeds.
5. Investigate the effects of rancidity in fish oil and fish meal.
6. Establish quality control norms for fish meals used in shrimp nutrition.

PRACTICAL SHRIMP NUTRITION - NOT STRAIGHTFORWARD

Generally speaking, the smaller the animal the easier it is to work with in feeding trials. This is not the case with shrimp.

In practice, shrimp are reared in ponds under tropical conditions, where natural biomass, e.g. algae, etc., provide a certain amount of feed. This can be likened to turning cows into a field of grass and offering mixed feeds - the mixed feed provides only a part of the nourishment. In the case of shrimp, the natural biomass provides the greatest proportion of nourishment when the shrimp are small - under 0.5g, and at a low stocking density. As they get larger, and/or at higher stocking densities, the mixed feed plays a more important role. Contrary to land animals and indeed fin-fish, the quality of the mixed feed does not affect growth to the same extent in the young animal. However, when shrimp density exceeds 100g per metre³, that is typically when they exceed 1g to 2g liveweight in a semi-intensive pond system, quality of feed becomes more important

(Figure 1).

Another problem in shrimp feeding trials is that genetically they are not improved - farmed shrimp are essentially wild-shrimp derived, reared in captivity. This gives enormous variation between animals in growth rate. To add to these problems, they can exhibit very different behavioural patterns. In isolation they tend not to thrive; in groups, as they become bigger, cannibalism may become a problem.

During the first two weeks from hatching, shrimp go through several larval stages (Figure 2). They then metamorphose into postlarvae, looking like adult shrimp. It is the post larvae (PL) that consume significant amounts of mixed feeds. They are transferred to ponds (from tanks) about four weeks after the onset of the larval state, i.e. postlarval. Prior to this stage they consume very little mixed feed. They produce a shell or carapace which they regularly shed (moult), adding to the complexity of accurately measuring growth!

Growth rates achieved in laboratory tanks rarely exceed half that achieved by similar animals in ponds - probably because of the reasons given above.

With all these difficulties in undertaking feeding trials, it may be questioned if any progress at all has been made in establishing feed requirements; the answer is that progress made is very limited.

Nutrient requirements differ markedly between the major species, probably reflecting that shrimp range from carnivores, e.g. *Penaeus* (P.) *japonicus*, to herbivores, e.g. *Macrobrachium rosenbergii*. The EU projects will concentrate on *P.monodon* and *P.vannamei*. These species represented 56% and 19% respectively of world farmed shrimp last year.

Part of the Association's early involvement in shrimp feeding trials at Bangor centred around developing methodology. Subsequent work in Mexico and Tahiti has used tank rearing of shrimp in groups, selected initially on the basis of growth rate. This appears to give more accurate results than the early work at Bangor with individual animals where each acted as its own control.

1. FRESHNESS OF RAW MATERIAL

Both centres (Mexico and Tahiti) were supplied with fish meals that were prepared in a low temperature dryer in Chile from the same raw material (anchovy), fresh, moderately fresh or stale. Analysis of the raw material TVN and the meals are given in Table 1. Two trials were carried out in Mexico using in one trial four replicate tanks (60 litres capacity) each with 15 shrimp and in the other trial four replicate tanks (60 litres capacity) each with 8 shrimp. In both trials *P. vannamei* were used. Details of the diet formulation are given in Appendix 1. With the higher stocking density final weight and growth rate of the shrimp fed fish meal from fresh fish were significantly greater than the other two treatments (Table 2). Final liveweight and growth rate of the stale fish

treatment shrimp were lower than the moderately fresh fish treatment, though differences were not significant. Feed consumption was significantly higher for the fresh fish treatment ($P < 0.05$). With the low stocking density differences in final liveweight, growth and feed consumption were not significant (Table 3).

In Tahiti two trials were carried out, one with *P. monodon* and one with *P. stylirostris*. Full details are not yet available, but growth rates are given in Table 4 which summarises the trials at both centres. With both species used in Tahiti, growth was significantly better with the fresher raw material.

2. EFFECT OF FISH MEALS WITH A GIZZARD EROSION SCORE

2.1 Gizzerosine

At the University of Nuevo Leon a shrimp growth trial was carried out with fish meals which had different gizzard erosion scores determined in poultry and also diets spiked with gizzerosine. The first four treatments used fish meals which were either normal (NFM) (a and b - from fresh fish giving no gizzard erosion), treatments D₁ and D₂ respectively, or fish meal from stale fish with gizzard erosion score (GE1) with high amines, treatment D₃, or fish meal from fresh fish with gizzard erosion score (GE2) with low amines, treatment D₄ (see Table 5). The other four treatments used the normal fish meal (NFM-a) with 1, 3, 6 or 9 ppm gizzerosine added - treatments D₅, D₆, D₇ and D₈ respectively. For each treatment three tanks each with 15 shrimp (*P. vannamei*) were used. The shrimp weighed around 0.7g initially, and were on trial 28 days.

Weight gain and feed conversion did not differ between treatments (Table 6). Mortality was higher with fish meals with higher gizzard erosion scores. It also increased with increasing gizzerosine, except for the lowest level added - 1 ppm which gave very high mortality - equivalent to the fish meal with highest gizzard erosion score. It was difficult to explain this treatment (D₅) response. However, there was an indication that adding gizzerosine increased mortality.

The results with gizzerosine added to the feeds are equivocal. It was not possible to check that the addition to the diets was correct as there is currently no satisfactory and straight forward method to determine gizzerosine. The possibility that leeching occurred - feed pellets can remain in tanks for some hours before being eaten, cannot be ruled out. Leeching could create two problems - less gizzerosine in the feed than planned and the possibility that the chemical could have got into water recycled through other tanks, confounding treatments.

It was recommended that future work should concentrate on fish meals with known gizzard erosion scores in chicks, and that these should be fed to shrimp in tanks with separate (not recycled) water supplies.

2.2 Effect of Fish Meals With A Gizzard Erosion Score

A further trial has been conducted with shrimp (*P. vannamei*) at Nuevo Leon to

investigate fish meals with different gizzard erosion scores determined in chickens, Details of the fish meals used and the diet composition are given in Appendix Tables 1 and 2. The mortality figures represent the chickens dying which showed symptoms of black vomit - an extreme condition arising from haemorrhaging gizzards. Because it was not possible to find a commercial fish meal with a gizzard erosion score of more than 1.3, a higher score was achieved by reheating the fish meal to give the highest value used (treatment DFM). The fish meals were included at 40% of the diet.

The results of this trial are shown in Table 7 and Figures 3a and 3b. There were no differences in mortality which was generally low. There were growth rate differences with meals with higher gizzard erosion scores depressing growth significantly, especially with the treatment with the fish meal with the highest gizzard erosion score (treatment DD). The fish meal with the low gizzard erosion score (0.9) showed no effect in terms of the growth rate and feed conversion of shrimp; however, with a slightly higher score (1.3) growth rate was significantly poorer, and feed conversion ratio was numerically poorer though this difference was not significant.

The two trials did differ in a number of respects. Comparing these trials, the differences between them were as shown in Table 8

The most likely explanation for the difference in the two trials was believed to be the size and age of the shrimp. Because in the first trial the shrimp were smaller and younger, it is likely that they were more susceptible to the gizzard erosion producing factors.

It was agreed that a further trial will be required before this work could be concluded, and that the design of a further trial should take into account the following:-

1. Shrimp of different sizes should be used.
2. The shrimp should be subject to an acclimatisation period.
3. An open system should be used to avoid cross contamination from feeds.
4. A longer bio-assay period should be used for the larger shrimp.
5. Binders should be used to improve diet stability.
6. Fundación Chile will supply fish meals with gizzard erosion scores as close as possible to 0.1, 0.7, 0.9, 1.3-1.5 and 2 or over, where the two with the highest scores also give black vomit. In addition, fish meals should be selected which have a maximum content of the four major biogenic amines of less than 2,500 ppm. If possible, fish meals from the same species of fish should be used.
7. If possible, trout digestibility should be done on the fish meals.
8. The peritrophic membrane of the shrimps should be examined and the content

of the glycine determined as this is likely to show abnormalities if changes to the intestine occur as a result of the gizzard eroding factors in the fish meals.

In summary, shrimp require fish meal made from fresh fish. It should be processed to avoid producing meals which can give gizzard erosion in chickens as shrimp appear to be sensitive to the same factors causing gizzard erosion. Gizzard erosion score is an important indicator of the suitability of fish meal for shrimp.

3. DETERMINATION OF DIGESTIBILITY

Progress has been made in developing a moving screen procedure for the rapid collection of faeces to determine feed digestibility in shrimp. Both centres (Tahiti and Mexico) will confirm the methodology gives comparable results. They will then proceed with the development of a chemical method using the proteolytic enzyme trypsin which is the main proteolytic enzyme in shrimp. Early results suggest that fish meals with different digestibilities in salmon show differences in shrimp similar to those in salmon. More data to confirm these results is being sought.

4. OTHER SHRIMP WORK

Fish oils which have been subjected to different degrees of oxidation are to be incorporated in shrimp diets and growth and mortality determined. This work is now in progress but no results are available. In a later trial fish meals where the lipid component has undergone different degrees of oxidation will be tested.

FIG. 1 CONTRIBUTION OF NATURAL BIOMASS TO THE GROWTH OF SHRIMP

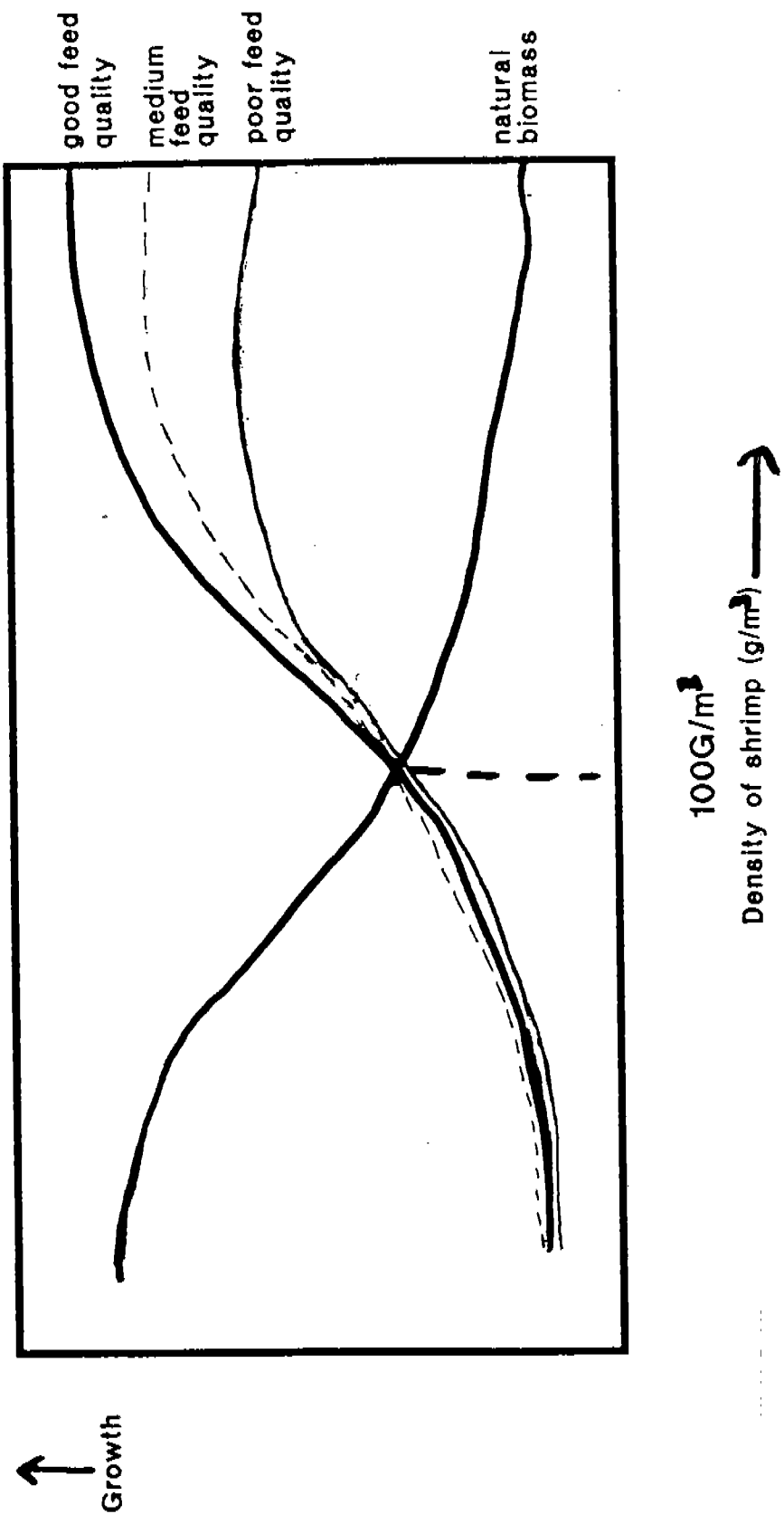


FIGURE 2

Life Cycle of Penaeid Shrimp

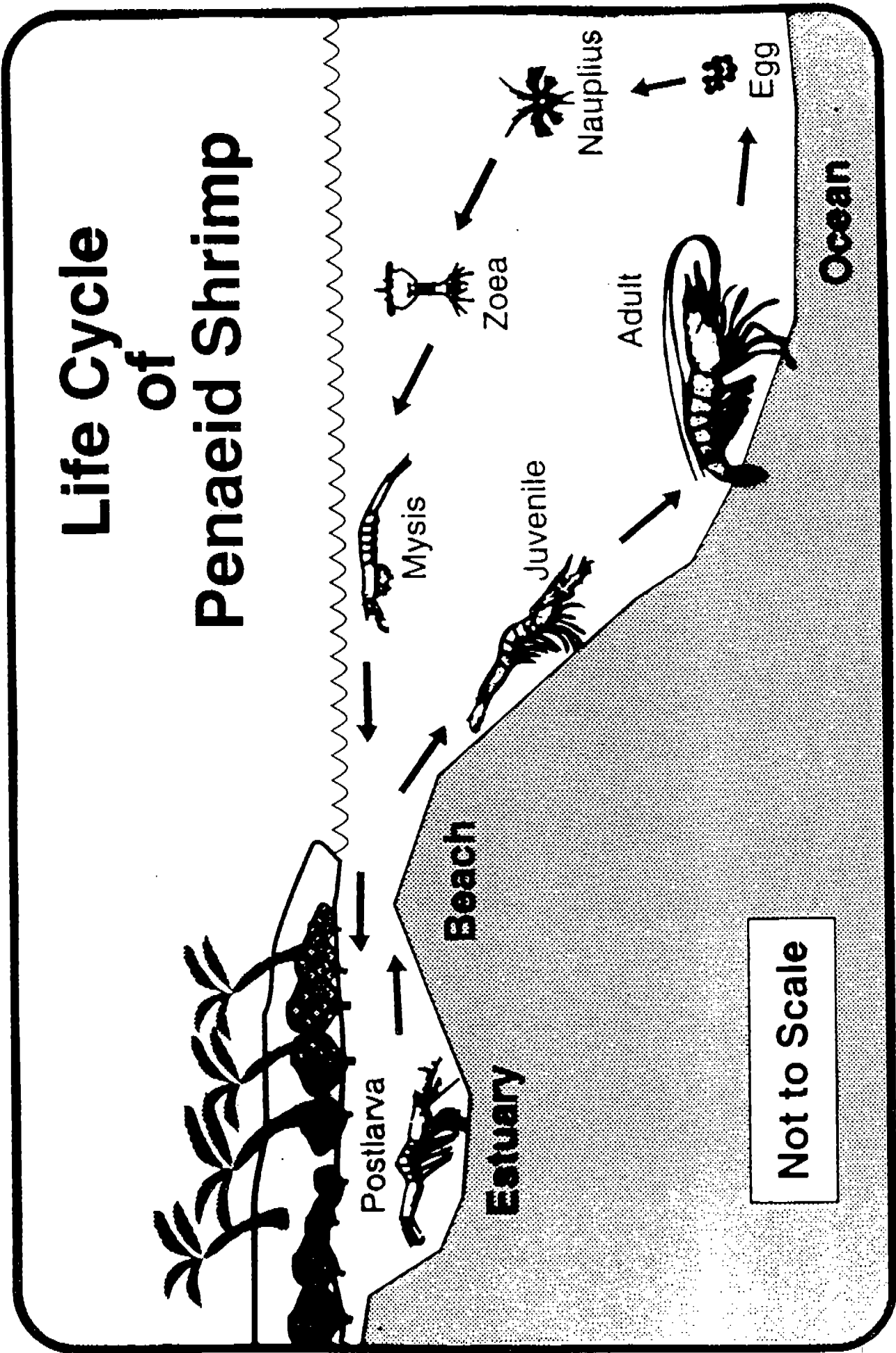


FIG. 3a SECOND GIZZARD EROSION SCORE TEST
GROWTH RATE - 28 DAYS

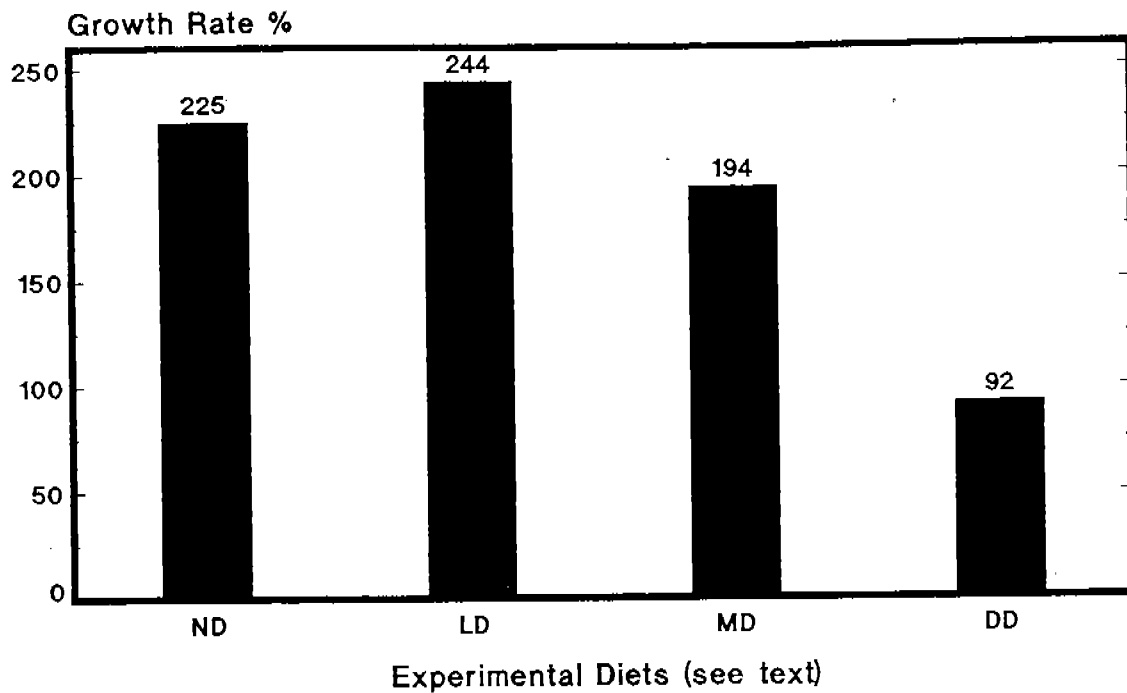


FIG. 3b SECOND GIZZARD EROSION SCORE TEST
FEED CONVERSION RATIO - 28 DAYS

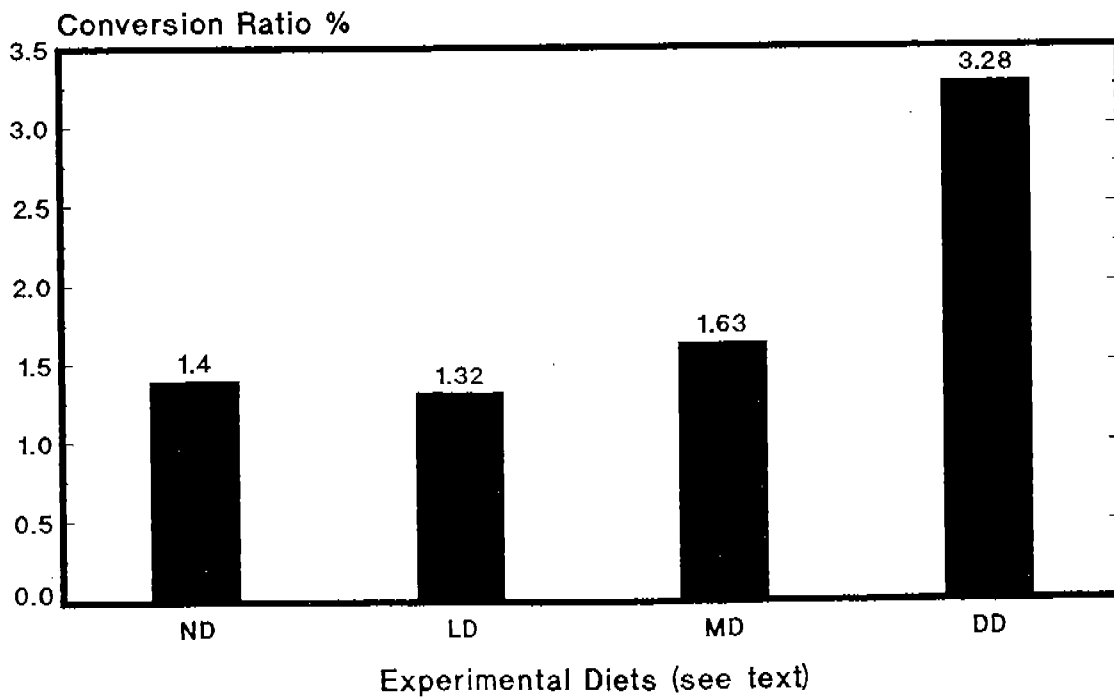


TABLE 1

EXPERIMENTAL FISH MEAL COMPOSITION (AS FED)			
	Fresh	Moderately Fresh	Stale
Moisture (%)	7.8	9.3	9.7
Ash (%)	11.3	11.4	10.7
Crude fat (%)	8.3	7.8	9.8
Crude protein (%)	66.9	64.8	63.0
TVN (mg N/100g of raw material)	14	30	50
Histamine (ppm)	28	1850	4701
Cadaverine (ppm)	51	803	1599
Putrescine (ppm)	35	446	916
Tyramine (ppm)	-	285	657
Digestibility in mink (%)	91.4	89.7	89.8

TABLE 2

**RESULTS OF GROWTH, CONSUMPTION, FOOD
CONVERSION RATIO AND SURVIVAL.**

Bioassay I (15 shrimps/tank), 14 days.

	Fresh	Moderately Fresh	Stale
Replicates	4 x 15	4 x 15	4 x 15
Initial Mean Weight (G)	0.937	0.935	0.937
Weight Gain (G)	0.59	0.50	0.44
Growth Rate (%)	63.04^b	54.01^a	46.93^a
Consumption (G)	1.75^b	1.50^a	1.55^a
Food Conversion Ratio	2.99^a	3.02^a	3.56^a

a, b: Different letters indicate different subsets as defined by the Scheffe test at a 5% risk.

TABLE 3

**RESULTS OF GROWTH, CONSUMPTION, FOOD
CONVERSION RATIO AND SURVIVAL.
Bioassay IIa (8 shrimps/tank) 14 days.**

	Fresh	Moderately Fresh	Stale
Replicates	4 x 8	4 x 8	4 x 8
Initial Mean Weight (G)	1.52	1.56	1.52
Weight Gain (G)	0.817	0.735	0.827
Growth Rate (%)	53.64 ^a	47.45 ^a	54.24 ^a
Consumption (G)	2.65 ^a	2.31 ^a	2.54 ^a
Food Conversion Ratio	3.24 ^a	3.16 ^a	3.09 ^a
Survival (%)	100.00 ^a	90.62 ^a	93.75 ^a

a: The same letter indicates values belong to the same subset as defined by the Scheffe test at a 5% risk.

TABLE 4

EFFECT OF RAW MATERIAL FRESHNESS - ANCHOVY MEAL
GROWTH OF SHRIMP

	FRESH (F)	MOD FRESH (MF)	STALE (S)	Significance
		Nuevo Leon	P.vannemi	
Trial 1 15 days	0.59	0.50	0.47	F>MF&S*
Trial 2 28 days	1.92	1.64	1.63	G>MF&S*
		Tahiti	P. monodon	
Trial 1 30 days	3.3	3.1	2.8	F>S*
		Tahiti	P.stylirostris	
Trial 1 31 days	6.7	6.0	5.6	F>MF&S*

*Sign P = 0.05

TABLE 5

COMPOSITION OF FISH MEALS INCLUDING CHICK ASSESSMENT					
PARAMETER	NFM(a)	NFM(b)	GE1FM(a)	GE2FM(b)	
Biotoxicological score	0.1	0.1	1.1	1.4	
Chickens mortality %	0.0	0.0	7.0	23.0	
Raw material	Jurel	Jurel	Anchovy	Jurel	
Drying Process	Indirect	Indirect	Direct		
Direct Solubles []	Yes	Yes	Yes	-	
Cook Blood liquor	No	No	Yes	No	
Raw material TVN mg N/100g sample	19.2	14.2	50.0	-	
Histamine (ppm)	320.0	230.0	4840.0	120.0	
Cadaverine (ppm)	720.0	850.0	4040.0	580.0	
Putrescine (ppm)	180.0	150.0	1760.0	160.0	
Tyramine (ppm)	n.d.	80.0	240.0	n.d.	
NFM(a) = Normal fish meal (a) CONTROL			GEFM(b) = Gizzard erosion fish meal (b), low histamine content.		
NFM(b) = Normal fish meal (b)			TVN = Total volatile nitrogen.		
GEFM(a) = Gizzard erosion fish meal (a), high histamine content					

TABLE 6

FEEDING TRIAL RESULTS - FIRST GIZZARD EROSION SCORE TEST								
TREATMENT	D ₁	D ₂	D ₃	D ₄	D ₅	D ₆	D ₇	D ₈
INITIAL MEAN WEIGHT (g)	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
WEIGHT GAIN (g)	0.250	0.237	0.237	0.254	0.260	0.230	0.234	0.260
CONSUMPTION (g)	0.373	0.377	0.392	0.394	0.381	0.388	0.378	0.381
FCR	1.50	1.70	1.62	1.56	1.46	1.69	1.61	1.47
GROWTH RATE (%)	379.79	359.39	359.59	384.84	394.94	349.49	354.54	394.94
SURVIVAL (%)	97.77	91.10	84.44	80.00	80.80	93.33	88.88	86.66
MORTALITY (%)	2.22	8.89	15.56	20.00	20.00	6.67	10.17	13.33

Table 7

RESULTS - SECOND GIZZARD EROSION SCORE TEST
(28 day trial)

	ND	LD	MD	DD
Replicates	5X7	5X7	5X7	5X7
Initial Mean Weight (g)	0.259	0.259	0.260	0.260
Final Mean Weight (g)	0.845	0.880	0.764	0.502
Growth Rate (%)	225.0 ^c	244.0 ^c	194.3 ^b	92.1 ^a
Consumption (g)	0.811 ^b	0.837 ^c	0.826 ^{bc}	0.766 ^a
Food Conversion Ratio	1.40 ^a	1.32 ^a	1.63 ^a	3.28 ^b
Survival (%)	94.2 ^a	97.1 ^a	97.1 ^a	97.1 ^a

Figures with different superscripts differ significantly

Table 8

**DIFFERENCES BETWEEN TWO TRIALS TO INVESTIGATE FISH MEALS
WITH A GIZZARD EROSION SCORE FED TO SHRIMP**

	FIRST TRIAL	SECOND TRIAL
Weight of Shrimps	66mg	260mg
Circulation System	Closed	Open
Stocking Density	15 shrimp/tank (83/m ²)	7 shrimp/tank (116.6m ²)
Origin	Escvinapa-natural	Laboratory SPF (Spec pathogen free)
Date	October 1992	October 1994
Water Temperature	26-28°C	26-28°C
Fish Meal GE Scores	0.1, 0.1, 1.1, 1.4	0.1, 0.9, 1.3, 2.0
Replication	4 → 3	6 → 5
Feed	Ad Lib	Ad Lib
Fish Meal Inclusion - Dietary Protein	30%	40%

APPENDIX TABLE 1

COMPOSITION OF FISH MEAL (AS FED)

	NFM	LFM	MFM	DFM
Biotoxicological score	0.1	0.9	1.3	2.0
Mortality	0.0	0.0	7%	20%
Moisture (%)	10.2	9.8	7.9	1.3
Ash (%)	13.6	14.2	16.6	17.8
Crude fat (%)	8.5	9.6	10.2	10.4
Crude protein (%)	67.5	65.9	65.3	67.3
Histamine (ppm)	141	2471	1347	
Cadaverine (ppm)				
Putrescine (ppm)				
Tyramine (ppm)				
TVN of fish meal	82.6	82.2	217	
Free fatty acids	4.6	3.3	10.5	
Digestibility				
Modified Torry (%)	97.5	97.8	96.6	

APPENDIX TABLE 2

COMPOSITION OF EXPERIMENTAL DIETS

Ingredient (%)	ND	LD	MD	DD
Normal fish meal	40	-	-	-
Light fish meal	-	40	-	-
Medium fish meal	-	-	40	-
Dangerous FM	-	-	-	40
Wheat meal	51.7	51.9	51.8	52.4
Wheat gluten	5.0	5.3	5.6	5.0
Soybean lecithin	2.9	2.4	2.2	2.2
Vitamin mixture ^a	0.2	0.2	0.2	0.2
Ascorbic acid (Stay C)	0.02	0.02	0.02	0.02

PROXIMAL ANALYSIS OF THE EXPERIMENTAL DIETS

Parameter (%)	ND	LD	MD	DD
Crude protein	37.9	38.2	37.6	37.8
Crude fat	6.8	7.3	7.1	7.0
Ash	6.5	6.4	7.4	8.1
Crude fibre	0.63	0.48	0.44	2.28
NFE				
Water stability LDM (%) (1 hour)	7.1	8.3	15.4	8.8