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# Histamine Level in Fishmeal and Shrimp Feed Marketed in India

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## Abstract

Twenty-five samples each of fishmeal and shrimp feed collected from the East and West coasts of India were screened for the level of histamine, total plate count and load of histamine forming bacteria. The average level of histamine in fishmeal samples was 138.2 $\mu g \cdot g^{-1}$ and in the shrimp feed samples 191 $\mu g \cdot g^{-1}$. Levels exceeding 200 $\mu g \cdot g^{-1}$ were observed in 4/25 samples of fishmeal and 11/25 samples of shrimp feed. The average bacterial load in fishmeal and shrimp feed samples was 1.08 x $10^6$ and 1.13 x $10^6$ cfu$\cdot g^{-1}$ respectively. *Bacillus, Staphylococcus* and *Micrococcus* were the major genera of bacteria isolated. The effect of feeding *Penaeus monodon* larvae with a diet containing histamine on the susceptibility to infection by *V.alginolyticus* was studied. There was no significant difference in the $LD_{50}$ between the treated and untreated larvae.

## Introduction

Feed forms an important input in aquaculture and its cost ranges from 30 to 60% of the production cost (Goddard 1996). Availability of good quality feed would be an important factor in deciding the success of the culture. In India, the annual feed requirement for aquaculture is estimated to be $3x10^5$ tonnes, valued approximately at US$ 210 million (Paulraj 1999). The occurrence of microbial toxins of both bacterial and fungal origin limits the shelf life and quality of the feed. The ingredients of most Indian feeds include groundnut oil cake, cottonseed cake, soyabean meal and fishmeal. All of these are prone to fungal or bacterial contamination and subsequent toxin production. Histamine is produced by the bacterial decarboxylation of histidine. Numerous bacteria have been reported to possess histidine decarboxylating activity. Members of the family Enterobacteriaceae like *Proteus, Escherichia, Klebsiella, Salmonella* and *Shigella* (Taylor and Speckhard 1983, Subburaj et al. 1984, Basavakumar

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1988, Georgnaras et al. 1995), *Vibrio* (Yoshinaga and Frank 1982, Middlebrooks et al. 1988, Okuzumi et al. 1990), *Pseudomonas* (Middlebrooks et al. 1988, Lopez et al. 1994), *Lactobacillus* (Maijala 1993, 1994), *Aeromonas* and *Staphyloccocus aureus* (Middlebrooks et al. 1988) and *Photobacterium* (Morii et al. 1986, 1988, Okuzumi et al. 1990) were all reported to produce histamine. Fishmeal prepared from fish like mackerel, sardine and anchovy, is the main sources of histamine in feed.

Histamine toxicity in humans, called 'scombroid poisoning' is well documented (Taylor et al. 1984, Middlebrooks et al. 1988, Veciana-Nogues et al. 1989, Hughes and Potter 1991, Rodriguez et al. 1994, Deng-Fwu et al. 1995). Jeya Shakila and Vasundhara (2002) reported the formation of histamine to significant levels in freshwater fishes like Catla (*Catla catla*) and Rohu (*Labeo rohita*). Avian black vomit disease, gizzard erosion, ulceration, proventricular distension and cellular necrosis was observed in chicks fed with diet containing fishmeal with high histamine content (Umemura et al. 1981, Okuzumi et al. 1984, Stuart et al. 1986, Wessels and Post 1989). In aquatic animals, though the effect of histamine toxicity is little studied, some observations of its effects have been recorded. Otake et al. (1978) observed pathological changes in the mackerel stomach caused by injection of histamine. High dietary histamine has been reported to cause growth reduction in fish (Lovell 1989). Fairgrieve et al. (1994) reported distended stomachs and stomach erosion in juvenile rainbow trout and Watanabe et al. (1987) documented the effect of histamine on the formation of gastric lesions in rainbow trout. However, no studies have been done on the effect of histamine on shrimp health. In this context, the present study on the level of histamine in fishmeal and shrimp feed marketed in India was undertaken.

## Materials and Methods

### *Sample*

Fishmeal samples were collected from manufacturers along the coast of Mangalore, Karnataka. Shrimp feed samples were collected from shrimp farms along the East and West coasts of India. The samples were collected in sterile polythene bags. Plate count agar (Himedia, Mumbai) was used for the determination of viable bacterial numbers. Tryptone soy agar (TSA, Himedia, Mumbai) was used for purification of the isolated colonies. Histidine decarboxylating bacteria were enumerated on differential plating medium of Niven et al.(1981) and further purified on TSA.

### *Preparation of sample*

A sample of 10 g fishmeal/shrimp feed was homogenised in 90 ml-sterile physiological saline. The homogenate was used for the estimation of total plate count (TPC) by spread plate method and histamine formers count (HFC) by the method described by Niven et al. (1981). The plates were incu-

bated at room temperature for 24 to 48 h. Plates having a bacterial count between 30 to 300 were selected for enumeration. Colonies with varying morphology were picked for further identification.

Bacterial identification was done following the identification scheme for gram positive bacteria (Le Chevallier et al. 1980) and gram negative bacteria (Bain and Shewan 1968, Le Chevallier et al. 1980).

### *Estimation of histamine*

The levels of histamine in fishmeal and shrimp feed were estimated using the method of Hardy and Smith (1976). Briefly, the sample extract was prepared by blending 25 g of the sample with 225 ml of trichloroacetic acid (2.5%) for 2 minutes in a homogeniser followed by filtration through Whatman filter paper no.41 and adjusting the pH to 7 using 1N KOH.

Seventy five milliliters of the neutralized extract was added to an Amberlite CG50 (100 to 200 mesh) column and the bound histamine washed with 0.2N acetate buffer (pH 4.63) followed by elution with 25 ml 0.2N HCl. The histamine was diazotized with parabromoaniline and quantified colorimetrically at 495 nm using a spectrophotometer (Shimazdu–UV- 1601, Japan)

### *Effect of dietary histamine on the susceptibility of postlarvae to bacterial infection*

Two sets of 150 larvae (PL 17 to 20) each were taken in two plastic tubs (stock batch) of 5 l capacity filled with seawater. Larvae in one tub was fed with a test diet containing histamine at a level of 389.4 $\mu g \cdot g^{-1}$ and the other fed with the control diet containing negligible histamine (3.4 $\mu g \cdot g^{-1}$). The larvae were fed for 25 days. Larvae were drawn from the stock tubs to study the effect of short term (3 days) and long term (25 days) feeding of histamine on susceptibility of the larvae to *V. alginolyticus* infection. Five sets of ten larvae each from the test and control group were taken in a 1.5 l beaker containing 1 l of UV sterilized seawater and acclimatized for 24 h. A young culture (12 to 18h) of *V. alginolyticus* was inoculated into each beaker at a concentration ranging from $10^4$ to $10^7$ $cfu \cdot ml^{-1}$. A control consisting of 10 larvae (not challenged with *V. alginolyticus*) was included in both experiments. The larvae were observed for mortality (after 48 h) and $LD_{50}$ value was calculated following the method of Reed and Muench (1938).

## Results

### *Histamine level in fishmeal and shrimp feed*

Fishmeal: 25 samples were analyzed for histamine and the level ranged from a low of 24.1 $\mu g \cdot g^{-1}$ to a high of 383.4 $\mu g \cdot g^{-1}$ (Table 1). The average histamine level was 138.2 $\mu g \cdot g^{-1}$. Out of the 25 samples analyzed, four samples showed appreciably high levels of more than 200.0 $\mu g \cdot g^{-1}$.

Shrimp feed: 25 samples of shrimp feed were analyzed for histamine level which ranged from 34.0 $\mu g \cdot g^{-1}$ to 389.4 $\mu g \cdot g^{-1}$ with an average level of 191.0 $\mu g \cdot g^{-1}$. Levels exceeding 200 $\mu g \cdot g^{-1}$ were observed in 11/25 samples with 7/25 having more than 300 $\mu g \cdot g^{-1}$.

### *Total plate count in fishmeal and shrimp feeds*

The total plate count (TPC) of the fishmeal samples ranged from 4.5 x $10^3$ to 1.33 x $10^7$ $cfu \cdot g^{-1}$ with an average count of 1.08 x $10^6$ $cfu \cdot g^{-1}$ (Table 1). Sample 13 showed an abnormally high count of > 1.33 x $10^7$. The rest had counts of four to six log units.

The TPC in the case of shrimp feeds ranged from 4.0 x $10^3$ to 6.28 x $10^6$ $cfu \cdot g^{-1}$. The average load was estimated to be 1.13 x $10^6$ $cfu \cdot g^{-1}$. Six out of 25 samples showed counts above 6 log units, while the rest had counts ranging between four and five log units.

### *Histamine formers count in fishmeal and shrimp feed*

The histamine formers count (HFC) in the fishmeal samples, ranged from < 1x$10^2$ $cfu \cdot g^{-1}$ to 4.2 x $10^4$ $cfu \cdot g^{-1}$, with an average count of 6.45 x $10^3$ $cfu \cdot g^{-1}$ (Table 1). The HFC as percentage of total flora varied between 0.1% to 5.86% with an average contribution of 1.45% (Table 1).

Table 1. Results of 25 samples of fishmeal and shrimp feed samples analyzed for histamine content, total plate count (TPC), histamine formers count (HFC) and histamine formers as percentage of total plate count.

| Sample No | Fishmeal | | | | Shrimp feed | | | |
|---|---|---|---|---|---|---|---|---|
| | Histamine Content ($\mu g \cdot g^{-1}$) | TPC ($cfu \cdot g^{-1}$) | HFC ($cfu \cdot g^{-1}$) | HFC as percentage of TPC (%) | Histamine Content ($\mu g \cdot g^{-1}$) | TPC ($cfu \cdot g^{-1}$) | HFC ($cfu \cdot g^{-1}$) | HFC as percentage of TPC (%) |
| 1 | 111.3 | $7.53x10^4$ | $2.35x10^2$ | 0.31 | 72.3 | $2.76x10^5$ | $3.55x10^3$ | 1.28 |
| 2 | 86.4 | $7.40x10^4$ | $8.60x10^2$ | 1.16 | 60.7 | $3.05x10^4$ | $1.95x10^3$ | 6.39 |
| 3 | 128.2 | $8.50x10^4$ | $6.70x10^2$ | 0.78 | 208.9 | $5.45x10^4$ | $4.50x10^2$ | 0.82 |
| 4 | 130.5 | $5.00x10^4$ | $4.85x10^2$ | 0.97 | 389.4 | $1.28x10^5$ | $2.50x10^2$ | 0.19 |
| 5 | 42.4 | $7.00x10^3$ | $1.00x10^2$ | 1.42 | 374.9 | $1.45x10^5$ | $2.00x10^2$ | 0.13 |
| 6 | 164.9 | $3.00x10^5$ | $4.45x10^3$ | 1.48 | 386.8 | $4.00x10^3$ | $1.00x10^2$ | 2.50 |
| 7 | 158.5 | $1.20x10^4$ | $1.00x10^2$ | 0.83 | 36.9 | $5.70x10^4$ | $5.80x10^3$ | 10.17 |
| 8 | 72.3 | $1.14x10^6$ | $5.55x10^3$ | 0.48 | 107.7 | $3.64x10^6$ | $2.97x10^4$ | 0.81 |
| 9 | 79.4 | $1.00x10^6$ | $3.25x10^3$ | 0.32 | 88.0 | $2.96x10^6$ | $2.83x10^4$ | 0.95 |
| 10 | 133.4 | $1.71x10^6$ | $6.60x10^3$ | 0.38 | 374.0 | $1.80x10^4$ | $1.00x10^2$ | 0.55 |
| 11 | 80.3 | $5.40x10^5$ | $6.00x10^2$ | 0.11 | 121.8 | $3.30x10^5$ | $3.05x10^3$ | 0.92 |
| 12 | 83.5 | $1.23x10^6$ | $1.25x10^3$ | 0.10 | 122.8 | $2.80x10^6$ | $1.67x10^4$ | 0.59 |
| 13 | 24.1 | $1.33x10^7$ | $3.23x10^4$ | 0.24 | 127.8 | $5.01x10^5$ | $3.90x10^4$ | 7.77 |
| 14 | 300.6 | $4.90x10^5$ | $5.00x10^3$ | 1.02 | 117.5 | $1.70x10^4$ | $1.35x10^3$ | 7.94 |
| 15 | 183.9 | $1.49x10^6$ | $4.20x10^3$ | 2.80 | 34.0 | $5.45x10^4$ | $1.70x10^4$ | 3.11 |
| 16 | 114.7 | $1.68x10^6$ | $1.15x10^3$ | 0.70 | 323.8 | $5.00x10^5$ | $3.75x10^4$ | 7.50 |
| 17 | 128.4 | $3.06x10^5$ | $4.60x10^3$ | 1.50 | 129.5 | $8.35x10^5$ | $5.35x10^3$ | 0.64 |
| 18 | 30.8 | $9.45x10^5$ | $3.74x10^4$ | 3.91 | 102.4 | $3.20x10^4$ | $2.45x10^3$ | 7.65 |
| 19 | 40.2 | $2.30x10^6$ | $5.15x10^3$ | 0.22 | 77.4 | $3.30x10^4$ | $2.55x10^3$ | 7.72 |
| 20 | 38.8 | $2.90x10^4$ | $1.70x10^3$ | 5.86 | 234.1 | $8.05x10^5$ | $6.00x10^2$ | 0.07 |
| 21 | 132.1 | $1.71x10^5$ | $4.25x10^3$ | 2.48 | 238.9 | $3.05x10^5$ | $1.00x10^1$ | 0.003 |
| 22 | 127.6 | $5.40x10^4$ | $2.75x10^3$ | 5.09 | 378.6 | $5.10x10^6$ | $1.05x10^5$ | 2.05 |
| 23 | 304.8 | $2.40x10^4$ | $3.40x10^2$ | 1.41 | 266.6 | $4.80x10^5$ | $6.15x10^3$ | 1.28 |
| 24 | 383.4 | $4.50x10^3$ | $1.00x10^2$ | 2.22 | 310.9 | $6.28x10^6$ | $3.20x10^4$ | 0.50 |
| 25 | 374.6 | $6.10x10^4$ | $4.00x10^2$ | 0.65 | 92.2 | $2.88x10^6$ | $6.75x10^3$ | 0.23 |
| Average | 138.2 | $1.08x10^6$ | $6.45x10^3$ | 1.45 | 191.0 | $1.13x10^6$ | $1.32x10^4$ | 2.86 |

In the 25 shrimp feed samples analyzed, the HFC ranged from $1.0x10^2$ $cfu \cdot g^{-1}$ to $1.05 \times 10^5$ $cfu \cdot g^{-1}$ with an average load of $1.32 \times 10^4$ $cfu \cdot g^{-1}$. Histamine formers accounted for 0.003% to 10.17% (Table 1) with an average of 2.86%.

### *Generic distribution of bacteria in fishmeal and shrimp feed samples*

Tables 2 and 3 show the bacterial genera identified in the fishmeal and feed samples analyzed. The fishmeal samples had predominantly gram-positive bacteria. *Bacillus* could be detected in 14/15 samples analyzed. The other species detected were *Staphylococcus* (10/15 samples) and *Micrococcus* (6/15 samples). Gram-negative bacteria were detected in 6/15 samples. The flora observed in the feed sample also did not show any great variation in the generic composition. Gram-positive bacteria were the dominant species observed. *Bacillus* was found in 17/19 samples, *Staphylococcus* in 11/19 samples and *Micrococcus* in 9/19 samples. Gram-negative bacteria were found in only 2/19 samples which comprised *Pseudomonas* sp. in one sample and *Moraxella* sp. in the other.

### *Larval experiments*

The results of the experiments conducted to study the effect of dietary histamine on the susceptibility of the PL of *P.monodon* to *V. alginolyticus* infection are presented in table 4. Percentage mortality in larvae fed with diet containing histamine was similar to that in the control group. The results were similar in both long term and short term feeding.

### *Statistical analysis*

No significant relationship was observed between the HFC and histamine levels or between the TPC and histamine levels (Table 5).

## Discussion

Presence of high levels of histamine in feed is undesirable. Histamine levels exceeding 100 mg% induces gizzard erosion and proventricular abnormalities in chicken (Harry et al. 1975, Toyama et al. 1981, Miyazaki and Umemura 1987) and abnormalities of stomach in case of trout (Okuzumi et al. 1984, Watanabe et al. 1987, Fairgrieve et al. 1994). Of the 25 samples of fishmeal analyzed for histamine, 4/25 samples had levels exceeding 30 mg%, which was significant. Presence of high levels of histamine observed in animal and fish feeds could be due to the use of red meat fish such as mackerel and sardine which contain high level of free histidine for preparation of fishmeal. Interestingly, fishmeal samples (Number 1-22, Table 1) mainly manufactured from shrimp waste and Squilla were found to have low levels of histamine as shown by the study. The free histidine content of shrimp

Table 2. Percentage distribution of bacterial genera in fishmeal samples (number of colonies picked up for identification, n=30)

| Genus | Sample code | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FM1 | FM2 | FM3 | EM1 | EM2 | RF | HCa | Ha | G | CS | CNS | MU | BB | B41 | B47 |
| *Bacillus* sp. | 20 | 80 | 26.8 | 20 | - | 20 | 85.7 | 40 | 20 | 28.6 | 38.8 | 25 | 60 | 100 | 60 |
| *Staphylococcus* sp. | 80 | 10 | 6.6 | - | - | 66.6 | - | 50 | 53.4 | 71.4 | 47.8 | 55 | 13.4 | - | - |
| *Micrococcus* sp. | - | - | 66.6 | 80 | 100 | - | - | - | - | - | 13.4 | 20 | - | - | 40 |
| *Lactobacillus* sp. | - | - | - | - | - | - | - | 5 | - | - | - | - | 20 | - | - |
| *Pseudomonas* sp. | - | - | - | - | - | 13.4 | - | - | - | - | - | - | - | - | - |
| *Hafnia* sp. | - | 10 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *Flavobacterium* sp. | - | - | - | - | - | - | 14.3 | - | - | - | - | - | - | - | - |
| *Moraxella* sp. | - | - | - | - | - | - | - | - | 26.6 | - | - | - | 6.6 | - | - |
| *Plesiomonas* sp. | - | - | - | - | - | - | - | 5 | - | - | - | - | - | - | - |

Table 3. Percentage distribution of bacterial genera in shrimp feed samples (number of colonies picked up for identification, n=30)

| Genus | Sample code | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Su | Vis | ST-7 | GB3 | AVN | LUX | HI | ARN | GB | HG | HI | CP | A1 | A5 | A6 | 584 | 582 | SD | HI |
| *Bacillus* sp. | 100 | 80 | 6.6 | 66.6 | 80 | 93.3 | 70 | 70 | 100 | 80 | 71.6 | 13.3 | 54.5 | 50.2 | - | - | 50 | 73.4 | 20.0 |
| *Staphylococcus* sp. | - | - | 40 | 33.4 | - | 6.7 | 30 | - | - | - | 28.4 | 56.7 | 27.7 | 16.6 | - | 100 | - | 26.6 | 60.0 |
| *Micrococcus* sp. | - | 20 | 53.4 | - | 6.6 | - | - | 30 | - | 13.3 | - | 30 | 17.8 | 16.6 | 70 | - | 30 | - | 13.3 |
| *Lactobacillus* sp. | - | - | - | - | 13.4 | - | - | - | - | 6.7 | - | - | | 16.6 | 30 | - | - | - | 6.7 |
| *Pseudomonas* sp. | - | - | - | - | - | - | - | - | - | - | - | - | | | - | - | - | - | - |
| *Hafnia* sp. | - | - | - | - | - | - | - | - | - | - | - | - | | | - | - | - | - | - |
| *Flavobacterium* sp. | - | - | - | - | - | - | - | - | - | - | - | - | | | - | - | - | - | - |
| *Moraxella* sp. | - | - | - | - | - | - | - | - | - | - | - | - | | | - | - | 20 | - | - |
| *Plesiomonas* sp. | - | - | - | - | - | - | - | - | - | - | - | - | | | - | - | - | - | - |

and Squilla is very low (Lukton and Olcott 1958, Hibiki and Simidu 1959, Shewan 1974) which probably was the reason for the low levels observed. The commercial samples (Nos. 22 to 25), probably manufactured from red meat fish had a high content of histamine as these fish have a high level of histidine in the muscle (Suyama and Yoshizawa 1973, Arnold and Brown 1978, Frank et al. 1981, Pan and James 1985).

The average level of histamine in shrimp feed was found to be 191.0 $\mu g \cdot g^{-1}$. Levels exceeding 200 $\mu g \cdot g^{-1}$ were observed in 11/25 samples. Histamine content of 200 $\mu g \cdot g^{-1}$ and more is indicative of poor fish handling. In this study, the histamine level was more in feeds than in fishmeal. Since the moisture content of shrimp feed is low (<10%), it is unlikely that histamine is formed after manufacture, and thus suggests that the histamine levels detected might represent the level found in the raw material.

Histamine has been found to be thermally stable (Huss 1993) and can withstand the heating temperature during feed processing. Pike (1991) reported that fishmeal made from stale herring contained 830 $\mu g \cdot g^{-1}$ histamine, compared to < 3.0 $\mu g \cdot g^{-1}$ histamine in fishmeal made from fresh herring. Fishmeal, when produced from decomposed fish had histamine levels as high as 4800 $\mu g \cdot g^{-1}$, which would result in approximately 2000 $\mu g \cdot g^{-1}$ histamine in typical salmonid feed (Pike et al. 1990). Thus, it is important to use good quality raw material in the manufacture of formulated feed.

Fishmeal and shrimp feed are products with low moisture content. Therefore, though bacteria are detected in these products, it is unlikely that these bacteria are active in these products. However, the microflora would assume significance whenever there is bad storage. The total viable count in

Table 4. $LD_{50}$ value *P. monodon* larvae (10 in each set) challenged with *V.alginolyticus* by immersion.

| Histamine concentration ($\mu g \cdot g^{-1}$) | Mortality at 48 h with different concentration of bacteria $ml^{-1}$ | | | | | $LD_{50}$ |
|---|---|---|---|---|---|---|
| | Control | $10^4$ | $10^5$ | $10^6$ | $10^7$ | |
| 3.4[a] | 0 | 1 | 3 | 5 | 8 | $2.14x10^{5.82}$ |
| 3.4[b] | 1 | 1 | 3 | 4 | 9 | $2.76x10^{5.89}$ |
| 389.4[a] | 1 | 0 | 2 | 4 | 9 | $2.14x10^{6.09}$ |
| 389.4[b] | 1 | 1 | 2 | 5 | 7 | $2.76x10^{6.0}$ |

[a]larvae fed at this level for 3 days
[b]larvae fed at this level for 25 days.

Table 5. Correlation between TPC and histamine level and HFC and histamine level in fishmeal and shrimp feed.

| Sample | Parameter | r* (not significant at 5% level) |
|---|---|---|
| Fishmeal | TPC Vs Histamine level | 0.34 |
| | HFC Vs Histamine level | 0.27 |
| Shrimp feed | TPC Vs Histamine level | 0.09 |
| | HFC Vs Histamine level | 0.25 |

*r – correlation coefficient

the fishmeal samples analyzed averaged $1.08x10^6$ cfu·g$^{-1}$, 13/25 samples had a load exceeding five log units (Table 1). The TPC in the feed samples analyzed had an average level of $1.13x10^6$ cfu·g$^{-1}$, 16/25 samples had a load exceeding 5 log units. In the fishmeal, the HFC contributed to 0.1% to 5.8% of the total flora. The level did not exceed 4 log units and had an average count of $6.45x10^3$ cfu·g$^{-1}$, while the histamine level was found to be between 24.1 µg·g$^{-1}$ to 383.4 µg·g$^{-1}$. In the shrimp feeds analyzed, the average HFC was $1.32x10^4$ cfu·g$^{-1}$, contributing a maximum of 10.17% to the total microflora with the histamine level varying from 34.0 µg·g$^{-1}$ to 389.4 µg·g$^{-1}$ (Table 1). No correlation was observed between the TPC and the histamine level and between histamine level and HFC in both fishmeal and shrimp feed. Many attempts have been made to correlate histamine levels with bacterial load. Shifrine et al. (1959) showed that histamine content in tuna fish varied from 0 to 20 µg·g$^{-1}$, when the bacterial load was as high as $4.6x10^8$ cfu·g$^{-1}$ to $6.3x10^9$ cfu·g$^{-1}$. Subburaj et al. (1984) also did not find a direct relationship between bacterial numbers and histamine level and indicated that the count alone may not be directly related to the level of histamine but may also depend on the generic composition. Nagendra (1987) reported that there was no relation between the two in commercial fish like mackerel and seerfish. Niven et al. (1981) reported that the disadvantage of their medium was the high number of false positives. For example, *Staphylococcus* sp. which is not a histamine producer, was positive in Niven's and modified Niven's media (Rodriguez-Jerez et al. 1994). These could also be contributing to the non-existence of a relationship between the level of histamine forming bacteria and level of histamine.

### *Generic distribution of bacteria*

The most abundant genera in fishmeal samples were *Bacillus* and *Staphylococcus*. These were isolated from nearly all samples and contributed about 20 to 100% and 6.6 to 80% respectively of the total species identified. The other genera identified were *Micrococcus* (11.11 to 100%), *Lactobacillus* (5 to 20%), *Pseudomonas* (13.3%), *Hafnia* (10%), *Flavobacterium* (14.25), *Moraxella* (6.6 to 26.66%) and *Plesiomonas* (5%) (Table 2). In shrimp feed, the histamine formers were dominated by *Bacillus* (6.6 to 100%), *Staphylococcus* (6.6 to 100%), *Micrococcus* sp. (6.6 to 100%), *Lactobacillus* sp. (6.6 to 13.3%), *Pseudomonas* sp. (6.6%) and *Moraxella* sp. (20%) (Table 3). The types of bacteria isolated from both fishmeal and shrimp feed were similar though three genera viz., *Hafnia*, *Flavobacterium* and *Plesiomonas* were not detected in shrimp feeds. The low moisture content and the thermal processing of both samples may have resulted in a low microbial diversity. Rodriguez-Jerez et al. (1994) reported that *Bacillus* sp. and *Staphylococcus* sp. isolated by them did not produce histamine, though they were positive on Niven's medium. Basavakumar (1988) however, demonstrated that both *Bacillus* and *Staphylococcus* could produce histamine in fresh mackerels. Ameeta and Shetty (1992) and Maijala (1993, 1994) demonstrated the histamine forming ability of *Lactobacillus*. The histidine decarboxylating ability of *Micrococcus*

sp., *Hafnia* sp., *Pseudomonas* sp., *Moraxella* sp. and *Plesiomonas* sp. is well documented (Basavakumar 1988, Middlebrooks et al. 1988, Okuzumi et al. 1990, Rodriguez-Jerez et al. 1994)

There is limited data on the effect of histamine containing diet on the health of *P. monodon* larvae. In this study, feeding *P. monodon* larvae with a diet containing 389.4 $\mu g \cdot g^{-1}$ histamine for 25 days did not increase the susceptibility of larvae to infection with *V. alginolyticus*. Watanabe et al. (1987) in their experiments on rainbow trout reported that histamine at levels of 700 $\mu g \cdot g^{-1}$ in diets, increased the rate of dietary protein digestion by stimulating the secretion of pepsin, thereby increasing the rate of feed consumption without having any deleterious effect as seen with higher histamine levels. It is also possible that the quantity of pelleted feed taken by the larvae is very less which would mean the intake of very less quantity of histamine. We also surmise that some of the histamine in the feed may be lost by leaching in water. Further studies are required to understand the effect of histamine in the diet of *P. monodon* larvae and adult animals and to arrive at a hazard level.

## Conclusion

The histamine levels in shrimp feed and fish meal tested in this study were not very high to cause serious health problems to shrimp. However, the conditions under which the feed would be stored will decide if further decomposition of the feed can occur due to the bacteria present in it. Also, the level of histamine did not make the shrimp susceptible to challenge by *V. alginolyticus*. Further research is needed to ascertain the toxic levels of histamine that would affect the growth and health of shrimp.

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