

Original Research Article

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## Evaluation of the Quality of Commercial Fish Feeds in India with Respect to Microbiological Parameters

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### ABSTRACT

This paper describes the first comprehensive study of the quality of commercial fish feeds in India with regard to microbiological indices. Quality of feed is an important parameter that has a direct impact on the outcome of any aquaculture system. Microbiological parameters such as total plate count (TPC), *Escherichia coli* (CFUg<sup>-1</sup>), coliforms (CFUg<sup>-1</sup>), *Enterobacteriaceae* (CFUg<sup>-1</sup>) and yeast and mould (CFUg<sup>-1</sup>) counts were analysed using 3M™ Petrifilm™ as per guidelines. The TPC ranged from  $2.0 \times 10^2$  to  $3.13 \times 10^4$  CFUg<sup>-1</sup> in different feeds. Presence of *E. coli* was detected in one of the feeds with  $1.15 \times 10^2$  CFUg<sup>-1</sup>. Coliform bacteria were not detected in any of the feeds. *Enterobacteriaceae* was present in three feeds in the range of  $5.45 \times 10^2$  to  $1.58 \times 10^3$  CFUg<sup>-1</sup>. Yeast and mould count ranged from <10 to  $1.68 \times 10^4$  CFUg<sup>-1</sup> in the feeds analyzed. The results obtained from the present study indicate that the feeds were contaminated with micro-organisms. As far as Indian scenario is concerned, there exist several feed companies which do not comply with the quality regulations and specifications as laid down by the Bureau of Indian Standards (BIS). In addition, specifications are not available for aqua feeds regarding the acceptable levels of microbiological parameters. Hence the present study calls for a standardized code of quality to be observed by feed manufacturing companies for quality products.

#### Keywords

Microbiological quality, Fish feeds, pathogens, Feed quality and safety

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### Introduction

Formulated feed plays a significant role in semi intensive fish farming where a high stocking density of fish is maintained than the natural fertility of the water can support (Jhingran, 1991). The use of commercial feeds with well-balanced nutritional profile has become crucial for the success of aquaculture

under intensive conditions (Abid and Ahmed, 2009). Formulated feed is found to be useful in providing adequate nutrient components and energy required for better fish growth and production and is known to increase the carrying capacity of culture system thereby enhancing fish production by several folds (Nazish and Mateen, 2011). The net fish production obtained when fed with

supplementary feed was 7.7 times greater as compared to the treatment without feed in polyculture system (Kabir *et al.*, 2009). There is a growing interest among the farmers to opt for pelleted feed over traditional feeds such as mash for fish culture which are comparatively superior to mash feed since, loss of nutrients out of leaching and wastage of feed is less. Moreover, as the feed wastage is less, physico-chemical and biological conditions of the aquaculture system under application of pellet feed might be better because of less decomposition and organic loading. Feed comprises about 60% of the total cost of any aquaculture venture. The global demand for formulated feeds for fish was estimated to be 29.3 million tonnes in 2008 and is expected to grow with increasing aquaculture (FAO, 2011).

Microbiological parameters are important to determine the safety and quality of feeds. Feed may be contaminated during processing, storage, transport or handling. Contaminated feed frequently causes disease and for that reason, it is necessary to establish surveillance programs for microbiological feed hazards (Radanov *et al.*, 1999). Feed ingredients must be safe and suitable for storage and consumption. Even a single unsafe component may jeopardize the quality of compound feed. Some microorganisms introduced during storage, primarily moulds, can negatively affect feed quality including reducing dry matter and nutrients, causing musty or sour odours, and producing toxins (Meeusen, 1997).

The microbiological indices of feed quality include Total plate count (TPC), *Escherichia coli* count, coliforms, Enterobacteriaceae count, yeast and mould count etc. Therefore, in the present study the microbiological quality of commercial fish feeds was analysed to understand the quality of fish feed available in the country.

## Materials and Methods

### Feed collection and sample preparation

Eight different brands of commercial fish feeds were selected for the experiment. The feeds were procured from the retail markets of Kochi, India. Triplicate samples of the eight feeds were aseptically transferred to the microbiology laboratory where the analyses were carried out.

25g of each powdered feed was added to 250mL of phosphate buffer in a plastic pouch and blended in a stomacher to form a homogenous mix. Aliquots of different dilutions ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$ ) were prepared using phosphate buffer.

### The 3M™ Petrifilm aerobic count plates

The 3M™ Petrifilm plate is an all-in-one plating system manufactured and patented by the Food Safety Division of the 3M Corporation. They are widely used in many microbiology related fields and are an efficient method for detection and enumeration of microorganisms compared to conventional plating techniques. The 3M petrifilms are specifically available for estimation of various parameters including Total plate count, *E. coli*, coliforms, Enterobacteriaceae, yeast and mould etc. (Fig. 1).

In order to inoculate, the top layer of petrifilm was lifted to expose the plating surface, and with a pipette, one mL of each dilution was transferred to the plating surface.

The top film was then slowly rolled down and a 'spreader' was used for even distribution. After inoculation, the petrifilms were kept in incubator for a specified period of time for each test. After incubation, the colonies formed in films were counted using a colony counter.

## Microbiological parameters

The commonly acceptable microbiological techniques such as Total Plate count (TPC), *Escherichia coli* count, Coliforms count, Enterobacteriaceae count and yeast and mould count were performed in the present study to describe the microbiological quality of commercial aqua feeds.

## Results and Discussion

The results of microbiological evaluation of commercial fish feeds are summarized in Table 1. Microbiological evaluation of fish feeds gives qualitative as well as quantitative indication of the microbial load associated with them. The microorganisms detected in the feeds may be due to defective processing conditions of the feed and/or due to improper storage conditions. *E. coli* is implicated in disease conditions such as colibacillosis which occurs in forms such as enteric and septicaemic colibacillosis whereas *Salmonella* sp., *Listeria* sp. and *Staphylococcus aureus* are capable of producing acute and chronic infections in all or most types of birds and animals (Mallinson, 1984).

Ogbulie (1998) reported that bacterial genera may have originated from nitrogenous waste products in compounding animal feeds such as dung, chicken excreta etc., and fungal species may have resulted from carry-over of over seasoned fungal species from the field.

Handling and other post-harvest process may also contribute as the primary sources of contamination. Most of the fungal species have been isolated from cereals (Pitt *et al.*, 1994).

### Total Plate Count

The TPC is one of the most common tests applied to indicate the sanitary quality,

organoleptic acceptability, adherence to good manufacturing practices, and to a lesser extent, as an indicator of safety of food/ feed. It is also known as aerobic colony count, standard plate count, mesophilic count or total viable count. It gives an indication of the bacterial populations in a sample. The TPC defines how many aerobic, mesophilic microorganism colonies, such as bacteria, yeast, and mould fungi will grow for 72 h on an agar plate that was normalized for microbiological testing at 30°C. In this study, the TPC ranged from  $2.0 \times 10^2$  to  $3.13 \times 10^4$  CFU g<sup>-1</sup> in different feeds.

### *Escherichia coli* count

The presence of *E. coli* in feeds is undesirable because it indicates poor hygienic conditions which have led to contamination or inadequate heat treatment. Ideally *E. coli* should not be detected and as such a level of <3 per gram (the limit of the Most Probable Number test) has been given as the satisfactory criteria for this organism. Levels exceeding 100 per gram are unacceptable and indicate a level of contamination which may have introduced pathogens or that pathogens, if present in the food prior to processing, may have survived (ICMSF, 1996). In the present study, the level of *E. coli* was within the acceptable limits except in feed D with  $1.15 \times 10^2$  CFU g<sup>-1</sup>.

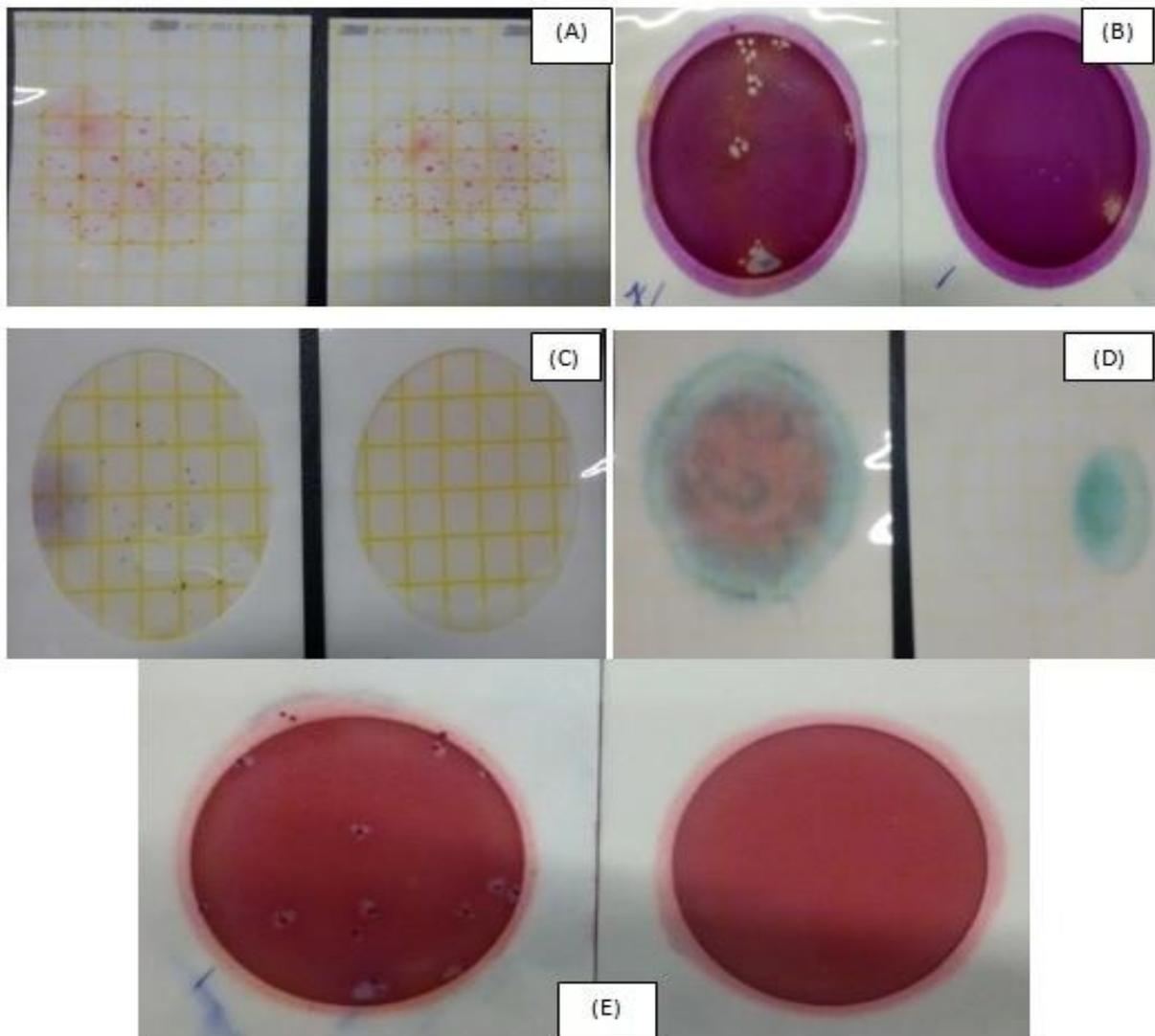
### Coliforms count

Coliform bacteria are a commonly used bacterial indicator of sanitary quality of foods and water. Coliforms can be found in the aquatic environment, in soil and on vegetation; they are universally present in large numbers in the feces of warm-blooded animals. They are easy to culture, and their presence is used to indicate that other pathogenic organisms of fecal origin may be present (ICMSF, 1996). Coliform bacteria were within satisfactory limits in the commercial fish feeds analyzed in this study.

**Table.1** Microbiological evaluation of commercial fish feeds

PARAMETERS	FEEDS							
	A	B	C	D	E	F	G	H
TPC (CFU g <sup>-1</sup> )	5.5×10 <sup>2</sup>	3.50×10 <sup>2</sup>	1.66×10 <sup>4</sup>	1.65×10 <sup>4</sup>	3.13×10 <sup>4</sup>	2.0×10 <sup>2</sup>	6.00×10 <sup>2</sup>	1.76×10 <sup>4</sup>
<i>E.coli</i> (CFU g <sup>-1</sup> )	<10	<10	<10	1.15×10 <sup>2</sup>	<10	<10	<10	<10
Coliformes (CFU g <sup>-1</sup> )	<10	<10	<10	<10	<10	<10	<10	<10
Enterobacteriaceae (CFU g <sup>-1</sup> )	<10	5.45 × 10 <sup>2</sup>	1.58×10 <sup>3</sup>	1.58×10 <sup>3</sup>	<10	<10	<10	<10
Yeast and mould (CFU g <sup>-1</sup> )	1.5×10 <sup>1</sup>	3.2×10 <sup>1</sup>	1.25×10 <sup>3</sup>	5.4×10 <sup>2</sup>	2.2×10 <sup>2</sup>	<10	2×10 <sup>2</sup>	1.68×10 <sup>4</sup>

**Fig.1** Petrifilms specific for (A) TPC (B) *Enterobacteriaceae* (C) Coliforms (D) Yeast and mould (E) *E. coli*



### **Enterobacteriaceae count**

The family Enterobacteriaceae includes gram negative bacteria including harmless symbionts and human pathogens such as *Salmonella* sp. and *Shigella* sp. They are useful indicators of hygiene and of post-processing contamination of heat processed foods/ feed. High numbers of Enterobacteriaceae (>10<sup>4</sup> per gram) in ready-to-eat foods indicates an unacceptable level of contamination or inadequate processing conditions (FSANZ, 2001). (e.g. inadequate cooking). In feeds B, C and D, a higher load of Enterobacteriaceae was detected.

### **Yeast and mould count**

Yeasts are eukaryotic microorganisms classified as members of the kingdom fungi. Some species of yeast are opportunistic pathogens that can cause infection. They are able to grow in feeds with low pH (5.0 or lower) and in the presence of sugars, organic acids, and other easily metabolized carbon sources. During their growth, yeasts metabolize some feed components and produce metabolic end products which causes spoilage due to alteration in physical, chemical, and sensible properties of feed. A mould is a fungus that grows in the form of multicellular filaments called hyphae. Some mould varieties present in feed produce mycotoxins that can pose health risks. Improper storage of feed without proper packaging and under humid condition accelerate the growth of moulds. Except in feed F, yeast and mould colonies were detected in all other feeds. Exceptionally high concentration of yeast and mould colonies (1.68x10<sup>4</sup> CFU g<sup>-1</sup>) were detected in feed H. Although it is not possible to produce microbe-free feed without adversely affecting its nutritional value, hazards due to microbial action can be minimized to a significant extent through appropriate quality control

measures in different stages of feed processing and storage.

Feed materials may serve as carriers for a wide variety of microorganisms. It is believed that lower number of microorganisms decreases the probability of pathogens occurrence (Bryan, 1982). The presence of microorganisms is unavoidable on account of the permanent contact of plant feed materials with soil and dust during the growing season and harvesting. However, the efforts to reduce the number of microorganisms in feed as much as possible show that the ultimate goal is not sterile feed but feed with "safe contamination level". In India, quality specification of feeds for most cultured fish (except for carps and cat fish) and ornamental fish is lacking. Specifications are important since they act as measuring sticks, which forms the foundation of a quality assurance programme because they provide accurate information to all the stakeholders involved in the chain of fish feed manufacture and utilization. In the present study, some of the fish feeds were not found to be satisfactory/ conforming to existing quality requirements as laid down by BIS. Even though the commercial feed manufacturers have followed most of the quality parameters; still further scope of improvement is possible. In BIS specifications for fish feeds, some more parameters of relevance such as the microbiological parameters, maximum allowable limit of pesticide residues and heavy metals should also be included for the production and supply of quality feeds.

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