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Mycological and Bacteriological Survey on Fish Feed Ingredients and Mixed Fish Feeds in Elazig Province

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Abstract: This study was undertaken to determine the microbiological flora of 66 raw material samples and 24 of mixed feed samples. Thirty-three raw material samples were taken from different farms just after harvesting and 33 from different factories before processing. Of mixed feed samples 12 were collected from factories and 12 from stores. The raw materials taken from storage contained higher moisture levels than raw materials taken from factories. Moisture levels were higher in corn than other raw material. Parallel to moisture levels, the total bacteria and mold counts were higher in raw material samples taken from storage, ranging from 5.43 to 6.40 and 3.51 to 4.60 (log_{10}/g) per gram of diet with mean value of 5.913 (log_{10}/g) and 4.055 (log_{10}/g) respectively Total aerobic bacteria and mold counts were higher in corn than in other raw material. The bacterial and fungal counts and moisture levels in mixed feeds were significantly higher ($P \leq 0.05$) in samples taken from stores. Results obtained in this study indicated that temperature, moisture levels and duration of storage are critical factors which affect the microbiological quality of fish feeds.

Key words: Fish feed, mycology, bacteriology, aquaculture

Introduction

Aquaculture is an expanding sector in Turkey and parallel to this expansion demand for fish feed is increasing. Wide range of plant and animal originated feedstuffs has been used in compound fish feeds. Fish feeds are very valuable commodities and represents the largest cost item (40-50%) of the total operating cost of a fish farm. All types of ingredient, as well as completed compound feeds require special care during storage to prevent deterioration in quality.

It is well known that micro-organisms are biological contaminants of the natural environment and are present in all feeds. They persist after crops have been harvested from the fields and in animal carcasses prior to rendering. Processing of commodities after the harvest and animal renderings involving heat, chemical and mechanical extraction and dehydration eliminate most of the original contaminating micro flora. Fungi spores, which are resistant to harsh processing treatment, may remain dormant in the processed feeds until more favourable conditions once again permit their proliferation (Chow, 1980). Feeds are generally considered unsatisfactory when they contain a large population of bacteria and fungi. Although limited research has shown that a number species of bacteria are commonly found.

Contamination of feeds with mold or bacteria may decrease their nutritional value and affects animal health and contaminated feeds may serve to distribute pathogens to human especially in tropical countries where temperature and relative humidity are high (Bauduret, 1990). The earliest published work in this area concerned the transmission of bacterial kidney disease to juvenile salmon fed ground adult salmon carcasses and some authors reported the transmission of mycobacterial infections in salmon hatcheries where unprocessed adult salmon carcasses were fed to juveniles (Hardy, 1989). The presences of either bacteria or fungi in human and animal foods are well reported, there has been little information on micro flora of fish diets being produced in Turkey (Sarieyyupoglu, 1987; Sahin and Sari, 1996). In this survey we report the presence of viable microbes in fresh raw materials taken from harvesting farms and factories, mixed feeds taken from fish feed manufacturers and feed stores to compare the contamination rate in the stages of harvesting-storage-feeding chain.

Materials and Methods

A total of 66 raw material samples and 24 of mixed feed samples were examined. Thirty-three raw material samples were taken from different farms just after harvesting and 33 from different factories before processing. Fresh samples were collected on the production sites in Elazig province and several other regions of Turkey. Stored samples were collected from storage at Elazig province and this study continued for 24 months. Twelve mixed feed samples were collected from factories and 12 from stores. All materials sampled according to standard methods (Meyer *et al.*, 1983). Moisture levels of all samples were detected as soon as the samples arrived at laboratory and samples were kept at −20°C until further analysis. Ten grams of each sample were transferred to a dilution bottle containing 90 ml of a phosphate buffer saline, dissolved completely by rigorous shaking and allowed to stand for at least 30 min at 10°C. The resultant supernatant was used as an original solution and ten-fold serial dilutions were prepared in phosphate buffer saline. One ml of each diluted specimen was transferred into each of two sterilized petri-dishes. Plate count agar was used to estimate for total bacteria and Malt Extract Agar for fungi. Aerobic bacteria were incubated at 37°C for 48 h and fungi at 27°C for 48 h. The viable microorganisms present were enumerated by standard methods (Arda, 1983; 1985; Gürgün and Halkman, 1988). Student's t test was used for comparing means between groups (Snedecor and Cochran, 1989).

Results

The raw materials and mixed feeds taken from storage contained higher moisture levels than raw materials and mixed feeds taken from factories (Table 1). Moisture level were higher in corn than other raw material. Parallel to moisture levels, the total bacteria and mold counts were higher in raw material samples taken from storage as log_{10}/g, ranging from 5.43 to 6.40 and 3.51 to 4.60 g^{-1} of diet with mean value of 5.913 and 4.055 respectively (Tables 2, 3). Total aerobic bacteria and mold counts were higher in corn than in other raw material.

The bacterial and fungal counts and moisture levels in stored mixed feeds were significantly higher in samples taken from stores. Total bacteria and mold counts were higher in stored mixed feed samples taken from storage as log_{10}/g, ranging from 4.10 to 4.26 and 3.93 to 4.05 g^{-1} of diet with mean value of 4.18 and 3.99 respectively (Tables 2, 3).

Discussion

In this study, predominant microbiological flora of feed ingredients and mixed feed samples sold at Elazig province was investigated. Moisture level was found higher in samples kept in stores after production than in samples taken from production facilities in feed ingredients and mixed feed samples. This results could be due to contamination of fresh and old samples, insufficient air-circulation and gridiron system. The counts of mold and aerobic bacteria

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Table 1: Moisture levels of feed ingredients and mixed fish feeds, % (±SEM)

Feed names	Fresh	Stored	P <
Wheat	9.74±0.8	12.18±0.2	0.01
Corn	10.33±0.4	13.22±0.3	0.01
Soybean meal	9.56±0.6	11.73±0.2	0.05
Cottonseed meal	9.63±0.7	12.14±0.1	0.01
Sunflower meal	10.14±0.9	12.27±0.5	0.05
Fish meal	10.23±0.5	12.44±0.4	0.01
Meat and bone meal	9.18±0.7	11.25±0.6	0.05
Corn gluten meal	8.93±0.2	10.86±0.7	0.05
Poultry by-product meal	9.24±0.4	11.26±0.7	0.05
Vitamin premix	8.38±0.4	10.08±0.8	0.05
Mineral premix	8.61±0.2	10.52±0.4	0.05
Trout pellets No.2	10.04±0.5	11.83±0.8	0.05
Trout pellets No.3	9.78±0.3	11.48±0.2	0.05
Trout pellets No.5	9.83±0.2	11.54±0.3	0.05
Carp pellets No.6	10.26±0.4	12.14±0.7	0.01

Table 2: Total bacterial counts of feed ingredients and mixed feeds, (log_{10}/g) (±SEM)

Feed names	Fresh	Stored	P <
Wheat	5.92±0.5	6.32±0.8	0.05
Corn	6.13±0.6	6.40±0.8	0.05
Soybean meal	5.96±0.6	6.26±0.9	0.01
Cottonseed meal	5.90±0.6	6.28±0.6	0.05
Sunflower meal	5.68±0.7	6.32±0.8	0.01
Fish meal	6.04±0.2	6.34±0.5	0.01
Meat and bone meal	5.87±0.3	6.27±0.7	0.01
Corn gluten meal	5.82±0.2	6.07±0.1	0.05
Poultry by-product meal	5.89±0.2	6.08±0.4	0.05
Mineral premix	5.28±0.1	5.43±0.3	0.05
Vitamin premix	5.30±0.3	5.55±0.2	0.05
Trout pellets No.2	3.66±0.8	4.22±0.6	0.05
Trout pellets No.3	3.58±0.2	4.18±0.1	0.05
Trout pellets No.5	3.60±0.4	4.10±0.4	0.05
Carp pellets No.6	3.69±0.4	4.26±0.6	0.01

Table 3: Total mould counts of feedstuffs and mixed fish feeds (log_{10}/g) (±SEM)

Feed names	Fresh	Stored	P <
Wheat	3.94±0.07	4.40±0.08	0.05
Corn	4.08±0.03	4.60±0.06	0.01
Soybean meal	3.80±0.06	4.30±0.09	0.05
Cottonseed meal	3.86±0.04	4.33±0.03	0.05
Sunflower meal	3.72±0.03	4.35±0.08	0.05
Fish meal	3.93±0.04	4.25±0.05	0.05
Meat and bone meal	3.85±0.05	4.33±0.06	0.01
Corn gluten meal	3.78±0.01	4.22±0.02	0.05
Poultry by-product meal	3.91±0.02	4.28±0.04	0.05
Mineral premix	3.22±0.04	3.81±0.06	0.05
Vitamin premix	3.12±0.06	3.51±0.08	0.01
Trout pellets No.2	3.14±0.03	3.93±0.06	0.05
Trout pellets No.3	3.25±0.11	3.96±0.14	0.05
Trout pellets No.5	3.07±0.05	4.03±0.22	0.01
Carp pellets No.6	3.23±0.06	4.05±0.06	0.01

were found parallel to the level of moisture in all samples. This is the results of positive correlation between moisture level and the number of microorganisms. In addition, due to the negative factors mentioned above, stores provide a medium for bacteria and mold to grow. Similar to our results, Sahin and Sari (1996) reported significant correlation between the level of moisture and aerobic bacteria (r=0.73) and also duration of storage and bacteria and mould counts (r= 0.95) in feed ingredients of poultry.

In all samples taken from either production facilities or stores the highest counts of aerobic bacteria and mould was detected in corn, the lowest level was determined in vitamin and mineral premixes. The higher counts of bacteria and mould in corn could be due to high moisture level, fractured content and the hygroscopic characteristics of corn. Similar to our results, Russel *et al.* (1991) reported that moisture level of 10.5-13.3% and 26.3 x 10^3 mould/g for corn stored for a duration of 12 months. Flatscher and Willinger (1981) reported that corn was responsible for 19% of mycological contamination of mixed feed. Mold and bacterial flora of corn detected in this study approached those reported in France (Bauduret, 1990) and in USA (Bothast *et al.*, 1974).

Temperature is a predominant factor which affects microbiological quality of feeds during their storage. In this study it is observed that the bacterial and fungal contamination gradually increased from factories to consumption (Tables 2, 3). This is consistent with previous studies (Jones *et al.*, 1982; Sahin and Sari, 1996; Zmysłowska, 2000). Moreover, Sahin and Sari (1996) were found significant correlation's between duration of storage and bacteria (0.75) and mould counts (r = 0.92) in mixed feeds of poultry and cattle. Trust (1971) found that total count of aerobic bacteria in commercial fish feeds ranged from 10^3 to 10^7 bacteria/g. Trust and Money (1972) reported that the number of aerobic bacteria varied from 9 x 10^2 to 1.3 x 10^6/g of 24 fish feeds with mean value of 1.5 x 10^5/g. Kitao and Aoki (1976) also reported that the total counts of aerobic bacteria ranged from 1.8 x 10^3 to 8.0 x 10^5 /g of diet with a mean value of 1.4 x 10^5 and total fungal counts ranged from 4.0 x 10 to 9.6 x 10^4/g of diet with a mean value of 1.1 x 10^4 in 20 different fish diets.

Results obtained in this study indicated that temperature and duration of storage are a critical factor which affects the microbiological quality of fish feeds and suggest that a moisture level above 13% in stores provides a medium for bacteria to grow, thus the feeds should be stored at conditions at which the moisture level is below 13%.

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