Microbial contamination in imported fish feed to Iraq

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Abstract. This study provides insight into the microbial contamination in imported fish feed to Iraq because this contaminated feed may cause a decrease in production of fish and increase mortality resulting economic losses. 125 samples were collected from Iraq border points; microbiological analysis were down. Bacteriological analysis results identified Gram-negative bacteria (45) isolates at (56.3%) of samples include: E.coli (17) isolates, Acinetobacter spp. (10) isolates, Citrobacter spp. (8) isolates, providencia spp. (6) isolates, Shigella spp. (5) isolates, and proteus spp. (2) isolates but we don’t detected (Salmonella spp., Klebsiella spp., Pseudomonas spp., Yersinia spp. and Enterobacter spp.). Mycological analysis results shown presence of mycotoxogenic fungi in (51) isolates at (63.8%) of samples include: Aspergillus flavus (16) isolates, Fusarium spp. (12) isolates, Penicillium spp. (10) isolates, Aspergillus niger (7) isolates, Aspergillus fumigates (3) isolates, Alternaria spp. (2) isolates and Rhizopus spp. (1) isolates. Mycotoxin results show total Aflatoxin in (45) samples, T2/HT2 in (56) samples and Ochratoxin A in (37) samples. This study concluded presence of microbial contamination in some tested samples, therefore, it must be assessed the microbiological tests of imported fish feed by responsible authorities to ensure its safety.

Keyword: fish feed, pathogens, mycotoxogenic fungi, mycotoxin
1. Introduction

Fish feed is rich source of protein as well as anther nutrients which are necessary for growth and health of fish and it composed often from plant ingredients which can contaminate with fungi and mycotoxin during crop production or storage which led to feed losses and opposite effects on health and production of fish. [1,2], therefore this concept is important for developing countries, especially those don’t apply suitable conditions for storage.

Bacteria such as Salmonella, E. coli and fungi can be contaminating fish feeds [3] especially when cereals use as a part of the fish meal [4] and when storage conditions were improper temperature and humidity, this would cause exposure the fish feed to microbial contamination. Mycotoxins are secondary metabolites produced by fungi which have different effect on feedstuffs safety. [5]

2. Materials and Methods

This study had carried out in Animal Resources Directorate / Quality Control on Feed Department in Baghdad, Iraq and (125) samples of imported fish feed were collected from border points about (3) Kg as representative sample, then transported to the laboratory to do bacteriological and mycological analysis, the media were prepared according to the manufacturer’s instructions on the labels and autoclaved at 121°C for 15 min, then.

2.1 Isolation of bacteria

For isolation of Salmonella spp., (25 g) of sample added to (225) ml peptone water, mix and incubated then transferred (1 ml) to (9 ml) of Selenite Cystine Broth and incubated at 37°C for 24 hours, then Xylose Desoxycholate agar and Hiktone Enteric agar were streaked and incubated at 37°C for 24 hours. Suspect colonies were select to do biochemical tests (triple sugar iron agar (TSI) and urease) [6], agglutination test (O&H antiserum) and isolates stained with gram stain to know microscopical characteristics [6]. For isolation of Escherichia coli and other bacteria used Eosine Methylen Blue agar (EMB agar), Nutrient agar and macconkey agar.

Identification of isolates by API kit (remel one rapid system)

2.2 Mycological analysis

2.2.1 Fungi identification:

sub-cultured were made on potato dextrose agar and incubated at 28°C for 7 days then identification of fungal species was depending on the macroscopic and microscopic characteristics of pure cultures of obtained isolates by slide cultures preparing for microscopic test [7]

22.2 Quantification of mycotoxins: it is conducted by using competitive direct enzyme-linked immunosorbent Assay (ELISA) by verotex kit. The procedure was done according to manufacturer’s instructions [8]
3 Results and discussion:

125 samples were collected and analyzed. Results showed that no bacteria growth in (68.8%) of tested samples, this could be attributed to use antibiotics concentration in these feed samples, or it collected in proper storage of feed but bacteria growth regard to Enterobacteriaceae family (Gram-negative bacteria) (45) isolates at (65.3%) of tested samples as it shown in table (1).

Results in Table (2) shows the bacteria growth percentage was as following: E. coli (17) isolates, Acinetobacter spp. (10) isolates, Citrobacter spp. (8) isolates, providencia spp. (6) isolates, Shigella spp. (5) isolates and Proteus spp. (2) isolates, but we don't found Salmonella spp., Klebsiella spp., Pseudomonas spp., Yersinia spp. and Enterobacter spp.

These results indicated that fish feed samples are "critical feed", because it is rich nutrient source for bacterial growth when the growth conditions are favorable and we can conclude fecal contamination because presence of E. coli (17) isolates at (21.3%) of tested samples, which it may suggest animal waste using in feed compounding and presence of rodent feces during bad storage conditions which causing diseases by E. coli or other pathogens because of E. coli presence meaning the presence of other pathogens even if they were not detected.

Whereas prevalence of Shigella spp. (5) isolates at (6.3%) of tested samples, which it can cause infection for fish as well as reported by [9].

Also Proteus spp. was (2) isolates at (2.5%) of tested samples, which is an opportunistic pathogen and it can survive at refrigerator temperatures and in a wide range of pH [10] to cause infection mentioned by [11].

The finding shown Citrobacter spp. (8) isolates at (10%) of tested samples, this indicated to water which used in feed processing was contaminated with Citrobacter spp. because it is intestinal inhabitants and it existed in sewage and water [12] causing main disease for fish such as carp as it reported by [13].

Prevalence of Acinetobacter spp. was (10) isolates at (12.5%) of tested samples, which it is opportunistic bacteria for aquaculture and it causes infection and mortality for fish as it reported by [14,15].

Finelly providencia spp. was (6) isolates at (7.5%) of tested samples, which it can cause disease in cultured fish as it reported first once by [16] via fecal contamination because it presents in animal feces as well as it mentioned by [17].

| Tested samples | Percentage of samples with no bacteria growth% | Percentage of samples with bacteria growth% |
|----------------|----------------------------------------------|-------------------------------------------|
| 100            | 68.8%                                        | 65.3%                                     |
Table 2. Bacteria isolates in fish feed

| Bacteria species    | No. of isolates | %    |
|---------------------|-----------------|------|
| *Salmonella* spp.   | -               | -    |
| E. coli             | 17              | 21.3%|
| *Klebsiella* spp.   | -               | -    |
| *Shigella* spp.     | 5               | 6.3% |
| *Pseudomonas* spp.  | -               | -    |
| *Yersinia* spp.     | -               | -    |
| *Proteus* spp.      | 2               | 2.5% |
| *Citrobacter* spp.  | 8               | 10%  |
| *Acinetobacter* spp.| 10              | 12.5%|
| *Providencia* spp.  | 6               | 7.5% |
| *Enterobacter* spp. | -               | -    |
| **Total isolates**  | **45**          | **56.3%** |

Results in table (2) shown (51) fungal isolates at (63.8%) of tested samples, and these belonged to (5) fungi genera: *Aspergillus*, *Fusarium* spp., *Penicillium* spp., *Alternaria* spp. and *Rhizopus* spp., this fungi occurrence attributed to fungi growth conditions; moisture levels in the feed >14% and temperature >27 C were available during feed storage practices and processing methods and this may be resulting mycotoxin production [18] and the dominate genera belong to *Aspergillus* genus (26) isolates at (32.5%) of tested samples because this genus can tolerate a high temperature conditions and dry environment and little amount of oxygen that it is necessary to the growth, this result is less than results conducted by [1] who found *Aspergillus* genus at (48%) of fish feed samples, from this genus we could isolate *Aspergillus flavus* (16) isolates at (20%) which it less than study by [19] who isolate it at (54.5%) of samples whereas we isolate *Aspergillus niger* (7) isolates at (8.8%) of samples which it higher than study by [19] who isolate it at (6%) of samples and *Aspergillus fumigates* (3) isolates at (3.8%) of samples, which it needs warmer conditions during growth and mycotoxin production such as gliotoxin and fumagillin

secondly *Fusarium* spp. was (12) isolates at (15%) of samples, the results of [20] shown that *Fusarium* spp. were the most frequent fungi in the stored feedstuff of fish because it favors higher water activity and its growing at different temperature degrees. While *Penicillium* spp. (10) isolates at (12.5%) of samples which is less than study reported by [21] who reported prevalence of *Penicillium citrinum* at 71%. Also *Alternaria* spp. (2) isolates at (2.5%) of samples because its optimum temperature of growth range from 22 to 30°C but it can grow at low temperatures from
0 to −5°C and low water activity to produce mycotoxins which led to feedstuffs spoilage during transport and storage. Finally, *Rhizopus* spp. (1) isolates at (1.3%) of samples which is less than study reported by [22] who found *Rhizopus* spp. in (2) samples at (4%) of samples. This result indicate that this fungal contamination may be resulting from the grinding process of grains which contaminated with mycotoxigenic fungi, and this depending on processes of fish feed production and ingredients transfer from farm to the factories.

**Table 3.** No. of fungi isolates in fish feed

| Fungi species       | No. of isolates | Percentage of samples with fungi isolates % |
|---------------------|-----------------|--------------------------------------------|
| *Aspergillus* (Total) | 26              | 32.5%                                       |
| *Aspergillus niger*  | 7               | 8.8%                                        |
| *Aspergillus flavus* | 16              | 20%                                         |
| *Aspergillus fumigates* | 3              | 3.8%                                        |
| *Fusarium spp.*      | 12              | 15%                                         |
| *Penicillium spp.*   | 10              | 12.5%                                       |
| *Alternaria spp.*    | 2               | 2.5%                                        |
| *Rhizopus spp.*      | 1               | 1.3%                                        |
| Total isolates       | 51              | 63.8%                                       |

because of fish exposure to mycotoxin would reduce its growth, causing liver damage, reduce immunity system and increase mortality, we do mycotoxin test to these samples and results in table (4) shown the highest positive samples was that of ochratoxin A (63 samples) and the maximum level was 2.1 ppb but minimum level was 0.1 ppb. The lower positive sample was that of T2/HT2toxin (56 samples) and the maximum level was 18.5 ppb but minimum level was 1.7 ppb. The lowest positive sample was that of total aflatoxin (45 samples) and the maximum level was 5.5 ppb but minimum level was 0.2 ppb.

**Table 4.** Mycotoxin type in fish feed

| Mycotoxin type   | No. of negative samples (%) | No. of positive samples (%) | Maximum level (ppb) | Minimum level (ppb) |
|------------------|-----------------------------|-----------------------------|---------------------|---------------------|
| Total Aflatoxin  | 55 (68.8%)                  | 45 (65.3%)                  | 5.5                 | 0.2                 |
| T2/HT2toxin      | 44 (55%)                    | 56 (70%)                    | 18.5                | 1.7                 |
| Ochratoxin A     | 37 (46.3%)                  | 63 (78.8%)                  | 2.1                 | 0.1                 |
Table (5) shows the distribution of total aflatoxin concentrations in the tested samples as following: 20% of the tested samples had total aflatoxin concentrations between 0.2-2.9 ppb, which are the highest number of contaminated samples followed by 14% of the tested samples between 3.1-4.0 ppb and 11% of the tested samples between 4.6-5.5 ppb, but 55% of the tested samples were negative samples. All these total aflatoxin concentrations in positive samples were within the allowed levels according to Iraqi agriculture ministry regulations and European commission regulations.

Table 5. Total Aflatoxin concentration (ppb) in fish feed

| Percentage of samples | Total Aflatoxin concentration (ppb) |
|-----------------------|------------------------------------|
| 55%                   | 0.0                                |
| 20%                   | 0.2-2.9                            |
| 14%                   | 3.1-4.0                            |
| 11%                   | 4.6-5.5                            |

While table (6) shows T2/HT2 toxin concentrations as following; 18% of the tested samples had T2/HT2 toxin concentrations between 1.7-5.0 ppb, followed by 33% of the tested samples between 8.0-12.5 ppb which are the highest number of contaminated samples and 5% of the tested samples between 13.7-18.5 ppb, but 44% of the tested samples were negative samples. All these T2/HT2 toxin concentrations in positive samples were within the allowed levels according to Iraqi agriculture ministry regulations and European commission regulations.

Table 6. T2/HT2 toxin concentration (ppb) in fish feed

| Percentage of samples | T2/HT2 toxin concentration (ppb) |
|-----------------------|----------------------------------|
| 44%                   | 0.0                              |
| 18%                   | 1.7-5.0                          |
| 33%                   | 8.0-12.5                         |
| 5%                    | 13.7-18.5                        |

Finally, Table (7) shows ochratoxin A concentrations as following; 32% of the tested samples had ochratoxin A concentration between 0.1-0.5 ppb, which are the highest number of contaminated samples followed by 10% of the tested samples between 1.3-1.6 ppb and 21% of the tested samples between 1.8-2.1 ppb but 63% of the tested samples were negative samples. All these concentrations were within the allowed levels according to Iraqi agriculture ministry regulations and European commission regulations.

Table 7. Ochratoxin A concentration (ppb) in fish feed

| Percentage of samples | Ochratoxin A concentration (ppb) |
|-----------------------|----------------------------------|
|                       |                                   |
From all results of mycotoxins, we found presence of total aflatoxin, T2/HT2 toxin and ochratoxin A together in the same feed sample which cause multiply risk of mycotoxin on fish health when it consumed the contaminated feed as well as human health by fish consumption, because mycotoxin residues can have concentrated in fish muscles as it reported by [1].

4 Conclusion

Some feed samples in the present study fell below international microbiological standards because of presence of pathogenic bacteria, fungi and mycotoxins. Therefore, it is necessary to do microbiological tests to decrease the contamination and prevent animal diseases and death addition human diseases.

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