Isolation of fungal agents from formulated and commercial feeds in three fish farms in humid tropical environments of Kenya.

NJAGI G ISAAC, TECHNICAL UNIVERSITY OF KENYA
5TH ANNUAL NATIONAL BIOSAFETY CONFERENCE
AUGUST 15-18, 2016 AT KENYA SCHOOL OF MONETARY STUDIES (KSMS), NAIROBI, KENYA
INTRODUCTION

1. Aquaculture is a fast growing sector contributed to economic development and food security worldwide (F.A.O, 2008).

2. E. U boost in Nov 2015 to Kenya at 1.4 b towards infrastructure and fish farmers timely-This has implications.

3. Disease Outbreaks are often related to management factors, such as quality and quantity of feeds.

4. Fungal contamination in foodstuffs has been a recurrent problem in Africa (Shepherd, 2003). Chronic exposure to aphyllatoxins has far reaching effects, has also not been well documented (Williams et al., 2004).
Factors involved

1. Kenyan climate is favorable for growth of moulds, the threat of mycotoxin related animals and human poisoning is real and of major concern.
2. The major constraints in raising fish include shortage and cost of feeds and the substantial economic losses.
3. This contamination has been a challenge posing a threat to food security and consequently, the livelihoods of the Kenyan population.
4. Conducive environmental parameters and poor feeds storage practices heighten the level of aflatoxin contamination.
5. In many parts of Africa, proper storage of foodstuff is a challenge hence predisposing these foods to fungal contamination.
6. Availability of raw materials for feed formulation.
7. Most are plant pathogens and can be found in soil.
1. Moulds will assimilate and utilize the most readily available nutrients in the materials they grow upon and spoilage may result in the loss of 5 to 100% of the nutrient in the feed.

2. Any feed material also contains various non-nutritional contaminants that may reduce its nutritional value or even exert adverse health effects in animals.

3. Increased moisture means increased mould production.

4. Growth of fungi can occur in feed having as low as 12% moisture.

5. Clear understanding of fungal organisms involved and the type of toxins they produce.
## Study Farms characteristics

<table>
<thead>
<tr>
<th>Fish Farm</th>
<th>Sagana Fish Farm (Government owned) Extensive</th>
<th>Jambo Fish Farm (Private owned by Dutch Nationals) Intensive</th>
<th>Mwea Fish Farm (private owned by a Kenyan) Extensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory in the farm</td>
<td>A laboratory in farm but does water quality only</td>
<td>No laboratory but the farm has fish specialist ichthyologist. Feed analysis externally Biosecure safe conditions in place.</td>
<td>laboratory in the farm. Owner is Fish expert.</td>
</tr>
<tr>
<td>Type of Fish Farm</td>
<td>Intensive Aquaculture: Highly controlled, high density, RAS, raceways, confined (industrialized)</td>
<td>Extensive Aquaculture: Minimal control, lower density, ponds, third world</td>
<td>Intensive Aquaculture: Highly controlled, high density, RAS, raceways, confined (industrialized)</td>
</tr>
<tr>
<td>Types Of Feeds</td>
<td>1. Formulated feeds 2. Commercial feeds (Kenyan) 3. Imported feeds</td>
<td>Imported Feeds of high quality.</td>
<td>1. Formulated feeds 2. Imported Feeds</td>
</tr>
</tbody>
</table>
Determination of moisture content
The moisture content (MC) was determined by drying 5 g of each sample for 2 hours at 105 oC to constant weight in an oven (ADP21/31 Yamato Scientific, America). The samples were then allowed to cool for 30 minutes in a desiccators after which dry weight was then recorded. The difference in weight was used to calculate moisture content, expressed (dry weight).

Mycoflora isolation
Quantitative enumeration of fungi was determined by use of direct plating technique as described by Pitt and Hocking (1997). Potato dextrose Agar was the media of choice. The prepared plates were incubated at a temperature of 28 oC up to 7 days with a photoperiodicity of 12 hours. During the incubation period, the cultural characteristics of the fungi were recorded and followed up to seven days of incubation (Samson et al., 2010). This isolation procedure was carried out in three replicates for each sample.

3.4.3.1 Morphological identification
Identification of all the recovered fungal isolates was achieved by use of Lactophenol blue stain. Macroscopic features that were observed include the colour of the colony, size of the spores, their texture and pattern. The microscopic features such as elevation of the philiades, the size of the conidiohores and protrusion of the hyphae were observed through a microscope mount. And confirmed via mycology charts.
Feed preparation practices in the three Farms

- FISH FARM 2

FISH FARM 1

FISH FARM 3
Good Pellets.
Note the uniform color and with no powdery substance

Feed storage practice in fish farm 1
Moldy Pellets.

Note (i) the colour of pellets is not uniform, (ii) the powdery substance that remains on the hand and (iii) the holes in the pellets. The whitish tinge and powdery substance that remains on the hand are due to mold. The holes are due to insects.
The presence of the aforementioned fungi in all the feed samples calls for attention in the storage methods employed by the poultry farmers, livestock feed manufacturers, distributors and the retailers.
Abnormalities identified on fingerlings in the Fish farms

experimental infections Nile tilapia behavioral signs “C” shape/ erratic

a dead catfish with eroded abdomen

Body and fin hemorrhagic lesions and poe eye syndrome

bubbles formation in hatchery tank due to low dissolved oxygen

Fungal infection due to saprolegniasis notice the erosion of scales
Percentage culture positive feed samples.

<table>
<thead>
<tr>
<th>Sample Types</th>
<th>feed Types</th>
<th>Number Tested</th>
<th>Number positive of fungi</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial feeds</td>
<td>104</td>
<td>49</td>
<td></td>
<td>79.9</td>
</tr>
<tr>
<td>Formulated feeds</td>
<td>17</td>
<td>17</td>
<td></td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>66</td>
<td></td>
<td>82.8</td>
</tr>
<tr>
<td>Season</td>
<td>Number of feed sample tested</td>
<td>Number positive for growth (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------------</td>
<td>-------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rainy season</td>
<td>42</td>
<td>95.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold season</td>
<td>32</td>
<td>64.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot &amp; Dry season</td>
<td>45</td>
<td>84.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>91.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungal agent isolated</td>
<td>Formulated feeds</td>
<td>Commercial feeds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------------</td>
<td>------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus</em> spp</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39.0±2.8^b</td>
<td>11.5±4.9^ab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mucor</em> spp</td>
<td>37.0±7.1^c</td>
<td>19.0±7.1^b</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rhizopus</em> spp</td>
<td>36.0±1.4^a</td>
<td>13.5±2.1^a</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Saprolegnia</em> spp</td>
<td>32.0±7.1^abc</td>
<td>10.5±3.5^b</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Penicillium</em> spp</td>
<td>26.5±7.8^bc</td>
<td>4.5±3.5^b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p-value: 0.016

p-value: 0.007
These findings were similar to those previous obtained in Kenya (Alakonya et al., 2009), Nigeria (Bankole, 2003) and India (Janardhana et al., 2011). Even though previous studies have shown differences in geographical locations and environmental conditions could be responsible for the differences in fungal distribution (Fandohan et al., 2003).
To compare fungi infections in two sites of fish body after feeding with formulated feeds.
1. Fungal contamination levels was shown to differ significantly within the three study farms. The isolation of four prominent fungal agents in the study areas demonstrates a clear need for tools to manage contamination of locally produced feeds.

2. Formulated Feeds were a source of fungal infections because of quality and storage methods.

3. This study showed that fungi isolated from feed are implicated in production of Toxins in Animals and Human.

4. Moisture & Temp levels in various food commodities are directly related to the resultant feed contamination.

5. The study showed fungal infection is favoured by aerobic environments. Environmental conditions can be controlled in storage facilities but this comes with a high additional cost.

6. Lack of biosecurity, poor or no Training of staff in fish farms also contributed to the human pathogens that were isolated on the farms therefore potential Fungal zoonosis.

7. Study confirmed that Fish just like any animal is susceptible to fungal infections especially if fed on poor quality feeds.
Achieving Excellence in Biosafety in the Farms

- proper preparation, packaging and storage of Formulated feeds and commercial feeds.
- Use of personal protective equipment after or between use and personnel training.
- Foot baths and hand disinfection, PPE’S required especially by workers before entering and working in the fish farms.
- Setting up Animal Laboratory in each county to strengthen capacities.
- Subsidy of commercial feeds to affordable cost
- The use of grains that are tolerant to fungal disease, properly dried grains and toxin binders for poultry feed production should be encouraged
Best practices

Feed Cage.
A simple cage made of timber and coffee wire mesh all around to keep out insects etc

Bags of Feed in Store.
On top of pallets and off the walls of the building to prevent moisture coming in contact with the bags. This provides protection against rodents.

THANKYOU