Biotechnology in Animal Husbandry 27 (1), p 93-100 , 2011
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 639.3.043
DOI: 10.2298/BAH1101093A

MYCOBIOTA IN FEED FOR FARMED SEA BASS (*Dicentrarchus labrax*)

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Original scientific paper

Abstract: Aquaculture producers, feed manufactures, farmers and distributors and their feed quality-control are, nowadays, placed in the centre of feed safety issues due to possible repercussions of residues in food. The aim of this preliminary study was to evaluate fungi contamination in 87 samples finished fish feed samples for sea bass (52 extruded feed and 35 pellet), were randomly collected from different factories in Portugal. All extruded samples revealed to be negative for fungi contamination. Concerning to 35 pelleted samples, mould counts were around 1.0×10 to 6.5×10^2 cfu.g^{-1}. Six moulds genera were recovered. *Aspergillus flavus* had the highest incidence appearing in 35 samples (100 %), with a range of $1.0 \times 10^2 - 1.5 \times 10^3$ and a mean value of 5.3×10^2. In the second order were the moulds from the specie *Aspergillus niger* in 34 samples (97 %) with a range of $1.0 \times 10 - 4.5 \times 10^2$ and a mean value of 1.6×10^2. *Aspergillus glaucus* had a percentage of 74 % with a levels ranging between 1.0×10 to 2.5×10^2. *Penicillium* and *Cladosporium* both recovered from 25 samples (71.4 %) with a range of $1.0 \times 10^2 - 6.5 \times 10^2$ and $1.2 \times 10^2 - 2.0 \times 10^3$ and a mean of 1.2×10^2 and 9.8×10^1, respectively. To the fifth order was mould from the genera *Fusarium* contamination from 22 samples (62.8 %) with a range of $1.0 \times 10 - 1.9 \times 10^2$ and a mean of 7.1×10^1.

Key words: aquaculture, fungi, fish feed, quality control

Introduction

Information about fungi associated with food and feeds is important in assessing risk of mycotoxin contamination. They are usually in form of dry complex feeds composed of plant (cereal seeds, bran, rapeseed or soybean meal or

cake, legume seeds) and animal components (meat-bone and fish meal, poultry off-fall, meat, powdered milk, animal fats) supplemented with vitamins and minerals *(Zmyslowska and Lewandowska., 2000)*. This type of raw materials have been associated with contaminants produced by moulds during the initial stages of the crop production.

Feed manufacturing is concerned with the physical transformation of a written formulation into a compounded "edible" diet. A wide variety of techniques exist for the manufacture of complete aquaculture feeds (straight mixing/blending; flaking; dry or steam compaction pelleting; extrusion/expansion pelleting; and so on). In our study the samples analysed were processed using extrusion/expansion pelleting (dry, moist or rehydratable expanded pellets). According to *FAO/WHO (2004)*, the efficiency of manufacturing process employed will depend on the feeding habit of the fish to be fed (ie. benthic, pelagic or surface feeder; visual or olfactory feeder; moist or dry diet feeder; rapid or slow feeder) and its physical feed requirements (ie. feed size, buoyancy, texture, palatability, and desired water stability) for all stages of the culture cycle. These technical factors will have then to be balanced with the market value of the cultured species and the availability of cash funds, feed ingredients and services.

Aquatic animals cannot digest starch effectively resulting in excessive excrement which can causes physiological problems such as excessive gas, bloating diarrhoea and these apart, from affecting the growth of the fish, will also lead to water pollution. The extrusion, high temperature and short duration process, cooks the raw materials, eliminating microorganisms including pathogens, and feed became more digestible.The use of extruded floating fish feed comes with a host of advantages in terms of digestion, growth, water protection, zero water pollution , optimized labour usage and zero wastage of raw materials, encouraging the use of this type of feed by fish farmers *(Hashimoto et al., 2003)*.

During manufacturing, feeds can be contaminated with mould spores, especially when the cereal grains are ground and the feeds are pelleted *(Suárez, 1999)*. Mould contamination not only causes deterioration of food and feeds, but also can adversely affect the health of humans and animals, once they may produce toxic metabolites *(Joseph, 1971; Cole and Cox, 1981)*. The filamentous moulds most commonly found in stored cereal grains are *Aspergillus, Penicillium* and *Fusarium* species *(Abarca et al, 1994; Joosten et al, 2001; Martins et al, 2001)*. *Aspergillus* is common contaminants of oilseed, crops, such as cottonseed, peanut meal and corn. Wheat, sunflower, soybean, fish meal, and nutritionally complete feeds can also be contaminated with aflatoxins and other mycotoxins.

The contamination of agriculture commodities with toxigenic fungi under favourable conditions may lead to mycotoxin build-up reaching to injurious levels for farm animals and human health. It can cause impairment of immune and acquired resistence to infections causing health problems which lead to economic and productivity losses Effective post-harvest management of stored commodities

requires clear monitoring criteria and effective implementation in relation to abiotic and biotic factors, hygiene and monitoring to ensure that mycotoxin contamination is minimised and that stored grain can proceed through the food chain for processing *(Magan and Aldred, 2007)*.

The aim of this study was to make a preliminary screening of mycological analysis in fish feed using standard method of microbiology.

Material and Methods

A total of 87 samples finished fish feed samples for sea bass (52 extruded feed and 35 pellet), were randomly collected from different factories in Portugal. All packaged samples were aseptically transport to laboratory and tested within 24 hours after collection or, if necessary, they were stored for 2-3 days in paper bags at room temperature (22-25ºC).

Ten grams of each sample were homogenizing for 3 min in 90 ml (10^{-1} suspension) peptone water (Oxoid, code CM 9, Basingstoke, England). Ten–fold dilutions were prepared till 10^{-3}**.**

From each dilution in peptone water 1 ml was spread onto plates of two media: Dichloran Rose Bengal Chlortetracycline Agar (DRBC) and Sabourad's agar with choramphenicol (BD Diagnostic Systems) with 0.25 ml per plate; the plates were incubated at 25ºC in the upright position for 5 days according to the *King et al. (1984).*

Taxonomic identification of all colonies considered as different was achieved through macroscopic and microscopic studies. Aspergillus species were identified according to *Pitt and Hocking (1985)* and *Raper and Fennel (1965)*. Other moulds genera were identified according to *Domsch and Gams (1980).*

Results and Discussion

The results obtained in this first survey showed that of fifty two extruded samples analyzed, none were contaminated by fungi. Otherwise, the 35 pellet samples presented fungi contamination (Table 1).

Table 1. Number and percentage of positive fish fed samples analysed

Matrix	Nº of analysed samples	Positive samples	Percentage (%)	Fungal counts		
				$< 1\times10^{1}$	10^{2}- 10^{3}	10^{3} - 10^{4}
Extruded	52	0/52	0	52	0	0
Pelleted	35	35/35	100	0	21	14

Table 2. Frequency and average count of mould genera from 35 fish pellet feed samples

Moulds	Frequency of positive samples	Percentage of mould genera frequencies (%)	Range	Average counts cfu g^{-1} (*)
Aspergillus flavus	35	100	$1.0 \times 10^2 - 1.5 \times 10^3$	5.3×10^2
Aspergillus niger	34	97	$1.0 \times 10 - 4.5 \times 10^2$	1.6×10^2
Aspergillus glaucus	26	74	$1.0 \times 10 - 2.5 \times 10^2$	8.7×10^1
Penicillium spp.	25	71.4	$1.0 \times 10^2 - 6.5 \times 10^2$	1.2×10^2
Cladosporium spp.	25	71.4	$1.2 \times 10^2 - 2.0 \times 10^3$	9.8×10^1
Fusarium spp.	22	62.8	$1.0 \times 10 - 1.9 \times 10^2$	7.1×10^1

Legend: (*) cfu- colony forming units per g of sample

Total number of cultivable fungi varied from 4.5×10^1 to 4.1×10^3 CFUs g^{-1} (colonies forming units). The counts should not exceed the values of 10^5 CFU^{-1}. The most predominant genera identified were *Aspergillus, Penicillium, Cladosporium* and *Fusarium. Aspergillus flavus* had the highest incidence appearing in 35 samples (100 %), with a range of $1.0 \times 10^2 - 1.5 \times 10^3$ and a mean value of 5.3×10^2. In the second order were the moulds from the specie *Aspergillus niger* in 34 samples (97 %) with a range of $1.0 \times 10 - 4.5 \times 10^2$ and a mean value of 1.6×10^2. *Aspergillus glaucus* had a percentage of 74 % with a levels ranging between 1.0×10 to 2.5×10^2. Other genera were isolated at lower frequency, *Penicillium* and *Cladosporium* both recovered from 25 samples (71.4 %) with a range of $1.0 \times 10^2 - 6.5 \times 10^2$ and $1.2 \times 10^2 - 2.0 \times 10^3$ and a mean of 1.2×10^2 and 9.8×10^1 , respectively. To the fifth order was mould from the genera *Fusarium* contamination from 22 samples (62.8 %) with a range of $1.0 \times 10 - 1.9 \times 10^2$ and a mean of 7.1×10^1. The *Fusarium* percentage counts were the lowest one with 72 % (Table 2).

This is the first study in Portugal identifying and enumerating fungi infecting fish farmed feed. Hence, it is very difficult to compare the results from this study with those from other authors in Portugal or by international researchers. However, many reports about mycodiversity have been published earlier at different animal feed (bovine, swine, poultry). The results obtained in this study have provided, for the first time, information about the presence and distribution of mycotoxigenic fungi in fish feed.

Ones that the composition in fish feed as many ingredients in common (wheat, soy, barley, maize, sunflower seed and fat) with other animal feed, we opted to discuss the results of this screening with the results found for other authors in different feed.

In a previous study effectuated in feed by *Almeida et al. (2007)* they found contamination with levels ranging between 1.7 $\log_{10}$ cfu/g up to 4.6 $\log_{10}$ cfu/g in samples of corn and levels 1.1 $x10^2$ to $5.0x\ 10^4$ in feeds (bovine, swine and poultry). Regarding others previous studies effectuated by others authors, *Martins*

and Martins (2001) it was concluded that the levels and frequency of mycobiota contamination are decreasing judging the results obtained in the last ten years, in Portugal. According to *Almeida et al. (2010),* in 31 samples of rat feed mould counts ranged from 3 to 4.2 $\log_{10}$ cfu/g, highlighted once again the low levels among this kind of contamination.

Dalcero et al (1998) the presence of mycobiota in 180 samples of poultry had mean value from 1x 10^3 to 9.5x 10^4 CFU/g for the *Aspergillus* spp. and 1.2 x 10^3 to 2.5 x10^5 cfu/g for the *Penicilium* spp. These results agree with those obtained by screening effectuated by *Martins and Martins (2001).*

In 2010 a similar study done by *Saleemi et al*., also showed that the fungi contamination was low. Data related to the mould incidence in maize kernels from Swat Valley *(Shah et al 2010)* are in agreement with results found in corn-based food by *Almeida el at (2007).*

The total fungal loads in finished feed samples analyzed by *Almeida et al (2007)* were 10^4 cfug^{-1} which is higher than the ones found in Iraq of 10^5 *(Shareef, 2010).*

The knowledge of mycobiota and its evolution on feed chain is an essential tool to manage production of animal production systems.

These include cultural practices of cereals in fields as well as harvest, transport and storage conditions. Physical, chemical or biological treatments of contaminated feed have poor efficacies and are not economically viable. Organic and inorganic adsorbents could be used to decrease the deleterious effects of contaminated animal feeds, they bind to mycotoxins and decrease their bioavailability which shows a great deal of promise in strategies that attenuate mycotoxin-induced toxicosis. The high affinity and high adsorption capacity of yeast-derived glucomannan preparations make their use as adjuncts for controlling naturally occurring mycotoxins in feeds attractive. The problem is that they have not yet been allowed for such purpose in the European Union *(Jouany, 2007).*

Conclusion

Plant ingredients pose a high risk for mycotoxin contamination. Since the aquafeed industry is moving towards using more plant ingredients, both risk assessment of mycotoxins as well as the development of appropriate protection strategies will become an integral part of aquaculture nutrition. The presence of toxigenic fungi does not indicate the mycotoxin production, because the toxins may persist long after vegetative growth has occurred and the moulds have died. Therefore, the presence of determinates fungi implicates a potential risk for animal health *(Bueno et al., 2001).*

Good quality of the products used and proper hygiene of the technological processes decrease the risk of microbiological contamination of fish feeds.

According to *Zmyslowska and Lewandowska (*2000*)*, storage conditions, especially temperature and humidity represent another important factor affecting microbiological quality of feeds. Improper storage temperature may prolong survival of the micro-organisms present in the feed, or even enhance their multiplication and production of toxic substances

Mikobiota u hrani za brancina koji se gaji u ribnjacima (*Dicentrarchus labrax*)

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Rezime

Proizvođači u akvakulturi, proizvođači hrane, farmeri i distributeri i njihove kontrole kvaliteta hrane, danas, se nalaze u centru svih pitanja koja se odnose na bezbednost hrane zbog mogućih posledica rezidua u hrani. Cilj ovog preliminarnog istraživanja je bio da se uradi procena kontaminacije gljivama 87 uzoraka gotove hrane za brancina (52 ekstrudiranih uzoraka i 35 peletiranih), koji su slučajno odabrani iz različitih fabrika u Portugaliji. Svi ekstrudirani uzorci su se pokazali negativni u smislu kontaminacije gljivama. U vezi sa 35 peletiranih uzoraka, broj plesni je bio oko 1.0 ×10 to 6.5 $\times 10^2$ cfu.g^{-1}. Određeno je šest rodova plesni. Za vrstu *Aspergillus flavus* je utvrđena najveća učestalost i to u 35 uzoraka (100 %), u opsegu od 1.0 $\times 10^2$ – 1.5 $\times 10^3$ i srednjom vrednošću od 5.3 $\times 10^2$. Na drugom mestu je bila vrsta *Aspergillus niger* u 34 uzorka (97 %) opsega od 1.0 ×10 – 4.5 $\times 10^2$ i srednjom vrednošću od 1.6$\times 10^2$. *Aspergillus glaucus* je imao procenat of 74 % sa nivoima u opsegu od 1.0 ×10 to 2.5 $\times 10^2$. *Penicillium* i *Cladosporium* su registrovani u 25 uzoraka (71.4 %) u opsegu od 1.0 $\times 10^2$ – 6.5 $\times 10^2$ i 1.2 $\times 10^2$ – 2.0 $\times 10^3$ i srednjom vrednošću od 1.2 $\times 10^2$ i 9.8 $\times 10^1$, respektivno. Na petom mestu se nalazila vrsta iz roda *Fusarium* u 22 uzorka (62.8 %) u opsegu od 1.0 ×10 – 1.9 $\times 10^2$ i srednjom vrednošću od 7.1 $\times 10^1$.

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Received 8 November 2010; accepted for publication 16 October 2010