Original Article

Toxigenic Fungal Contamination for Assessment of Poultry Feeds: Mashed vs. Pellet

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ABSTRACT

Background: Much attention has been paid to poultry feed processing and the contamination In Iran in order to improve the production and reduce the waste. No information is available on the fungal contamination and the strains found in processed (pellet) and non-processed (mash) poultry feeds. This study was designed to determine the hygienic condition and the risk of fungal contamination affecting the quality of poultry feeds (mashed vs. pellet).

Methods: A total of 90 samples of poultry feeds were collected from warehouses in Tehran and Alborz provinces. Samples were cultured on SDA, the CFUs were calculated, and the taxonomic identification of various fungal genera was made, both macroscopically and microscopically.

Results: Total mould counts for mashed feeds (15×10^3) was significantly ($P<0.05$) higher than pelleted feeds (11×10^2). The most frequent fungal genus recovered were *Fusarium* spp (90%) and *Aspergillus* spp (70%) in mashed and pellet feeds, respectively.

Conclusion: Heat processing reduced fungal contamination in poultry feeds. However, some fungal species are able to survive heat exposure and continue to form spores. We concluded that the assessment of fungal contamination in poultry feeds deserves a high attention to improve the quality, hygiene and safety of the foods originated from poultry .

Keywords: Fungal Contamination, Mashed and Pellet, Processed Poultry Feed, Starter and Finisher Feeds.

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INTRODUCTION

The poultry feed costs are highly variable and may constitute up to 60% of the poultry production expenses. Mould and mycotoxin contamination of feeds occur worldwide, and they cannot be totally eliminated from the feed ingredients. Almost all agricultural crops may become infested by moulds, during the growing season, transport and storage [1]. Agricultural practices aim to control or reduce the risk of fungal growth; however, these measures cannot entirely eliminate fungal growth [2]. Fungal invasion can reduce crop yield as well as altering the nutritional value of the crop; however, it is the formation of mycotoxins, which remains the major hazard for human and animal health [3]. Mycoflora (moulds) may lower seed germination, musty or sour odors, dry matter and nutrient loss, caking, mycotoxin formation and, ultimately, a reduction in the monetary value of the feeds [4]. The growth of mycoflora on crops is highly dependent upon climatic conditions, e.g., rainfall and temperature [5]. These moulds produce various toxic compounds but not all isolates of these species produce toxins. The genera of most concern globally are Aspergillus, Fusarium and Penicillium [6]. Some fungal species, such as Aspergillus, can also invade animal tissues and produce mycotoxins [7]. Mould can lower the value of feed ingredients through biochemical changes, physical damage or by the production of toxins, all of which are deleterious to the animal health. The widely used processing steps in feed manufacturing plants are: ***a)*** receiving the raw materials, ***b)*** grinding or particle size reduction, ***c)*** proportioning or batching, mixing, heating or thermal treatment (or pellet shaping), ***d)*** packaging, ***e)*** warehousing, and ***f)*** loading. Each of these steps can influence the feed quality and adversely affect the birds' health [8].

Pellet feed is a kind of feedstuffs made of raw material, vitamins, mineral and flavoring agents. It is estimated that over 80% of the feeds for poultry and pigs in the U.S. are pelleted [9]. Pellet is a form of mash feed that is compacted by heating, pressing and moisturizing processes [10]. Pelleting reduces microbial contamination and improves the digestibility, palatability and organoleptic characteristics of feeds [11]. The most critical point for microbial contamination at the feed mills is the post-processing heat treatment. The heating process is required to pellet the feed and usually kills most of the pathogens but inadequate operating temperatures for the pelleting equipment and feed conditioner are the major risk factors [12]. Despite some fungi which are sensitive to heat, those which are able to sporulate can survive and propagate even after the heat stress. Contamination of feeds before and after the heating process is common and can be attributed to

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many factors related to the feed mill factory. Un-hygienic feed production provides proper ground for fungal growth in the final product due to insufficient heat temperature in the initial mixture and not following the hygienic standards and recommended guidelines for feed production processes.

The aim of this study was to determine the hygienic condition and the risk of fungal contamination for poultry feeds (mashed vs. pelleted) in two important production regions of Iran.

MATERIAL AND METHODS

A total of 90 poultry feed samples (23 broiler chickens, 22 parents, 24 laying hens and 21 turkeys) comprising of mash (n=50) and pellet (n=40) feeds were collected randomly from feed mill factory in Alborz and Tehran provinces once every month throughout 2016. Sampling was also done randomly at feed mill factory. The mashed and pellet samples were collected at the warehouses of the production plants. For each sample, 500 g was collected from the poultry commercial feeds in a nylon bag, then transported to the laboratory for processing. Feed samples were stored at room temperature (22-25 C°) for a maximum of 24 hours prior to inoculation onto culture media.

Tehran and Alborz provinces lie within latitudes 34'36" and 36'18" N and longitudes 50'14" and 53'12" E, with the average annual rainfall is 187 mm. Herd population of broiler chickens, parents, laying hens and turkeys are about 130, 13, 120, and 5 in Tehran province, and 265, 14, 125, 5 in Alborz province, respectively. (GIS IVO, 2016) These provinces have the highest production potential in the Iranian poultry industry.

All samples were collected based on the Iranian Veterinary Organization recommended method (Method, 2015) for the evaluation of mycobiota contamination. First, the samples were homogenized, and laboratory samples (20 mg each) were prepared and blended in 180 ml of saline solution (0.85% sodium chloride) and 0.05% of twin culture media. The mixture was subsequently shaken for 30 minutes. Second, 1 ml of the above mentioned solution was transferred to 10 ml Sabouraud dextrose agar (SDA, E. Merck, Germany) plates for enrichment and selective culture, and was incubated at 27˚C for 96 hours. The subcultures from the samples were transferred separately to a selective isolation media, using spread plating method [13]. Identification of different genera was verified based on microscopic criteria and appropriate guidelines [14, 15]. The frequency (Fr) and relative density (RD) of the isolated species were determined based on an established method [16] as follows:

Fr (%) = Number of samples with at least one genus or species divided by the total number of samples ×100.

RD (%) = Number of isolates of a genus or species divided by the total number of fungi isolated ×100. The data were statistically analyzed, using SPSS version 16 and Microsoft Excel, 2007 packages. The means for the various groups were compared using one-way ANOVA and standard *t*- test. In all tests, a $p<0.05$ was considered as significant.

RESULTS

The fungal counts are shown in Tables 1 and 2. The total mould counts per gram in mash form was 1.2×10^2 to 63×10^3, in pellet form was 1×10^1 to 75×10^2, and in mashed poultry feed was significantly different from pelleted feed (P=0.03). Among the feed samples, 26 (56%) mash and 10 (25%) pellet samples exceeded the accepted European standard for finished poultry feed (1×10^3 CFU g^{-1}), which means that among all 90 finished feed samples 36 (40%) samples exceeded the standard limits.

As shown in Figure 1, 20% of poultry mashed starter feeds (Broiler Chicken, Parent stock, Laying Hen and turkey feed) and 32% of mashed finisher feeds, 2.5% of pellet starter feed and 22.5% of finisher pellets were contaminated with fungi. There was a significant difference between the fungal contamination in poultry feed starter and the finisher feed samples (P=0.02).

The fungal genera counts in 90 poultry feed samples are shown in Table 3. Seven mould genera were collected, three of which, *Aspergillus*, *Fusarium* and *Penecillium*, are known to be potentially mycotoxigenic fungi.

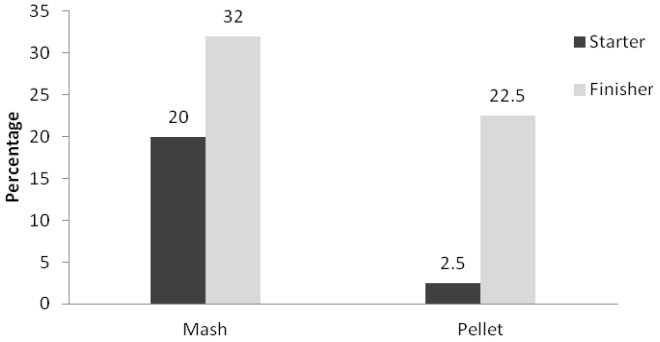

Figure 1. Fungal Comparison of mash and pellet forms in total starter and finisher of commercial poultry feeds.

The frequency of toxicogenic fungi was consistently higher (p <0.05) in the mashed compared to the pellet feed (Fig. 2 & Table 3). In the potentially toxigenic fungi, the frequencies were 72.4% and 54.2%, respectively, in the mashed and pellet feeds. The frequencies for the non-toxigenic group were 27.6%; and 45.8% (mashed vs. pellet feed). Figures 3a and 3b represent the frequencies and relative density of various fungal genera in mashed and pellet feeds. A significant difference was observed between the frequency of *Fusarium* in mashed and pellet feeds, which was lower in pellet feed. The most frequent recovered genus in pellet feed was *Aspergillus* and yeast while the most frequent genus in mashed feed was *Fusarium* and *Aspergillus*. Details of the fungal species and the frequencies are shown in Table 3. The fungal contamination levels found in poultry feeds produced by different countries are presented in Table 4.

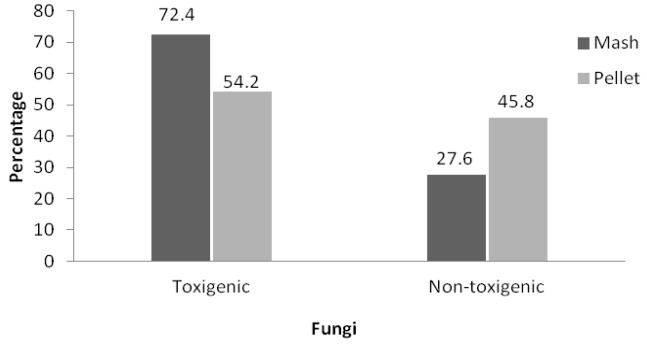

Figure 2. The ratio of fungi (toxigenic vs. non-toxigenic) to total isolated numbers in poultry feeds.

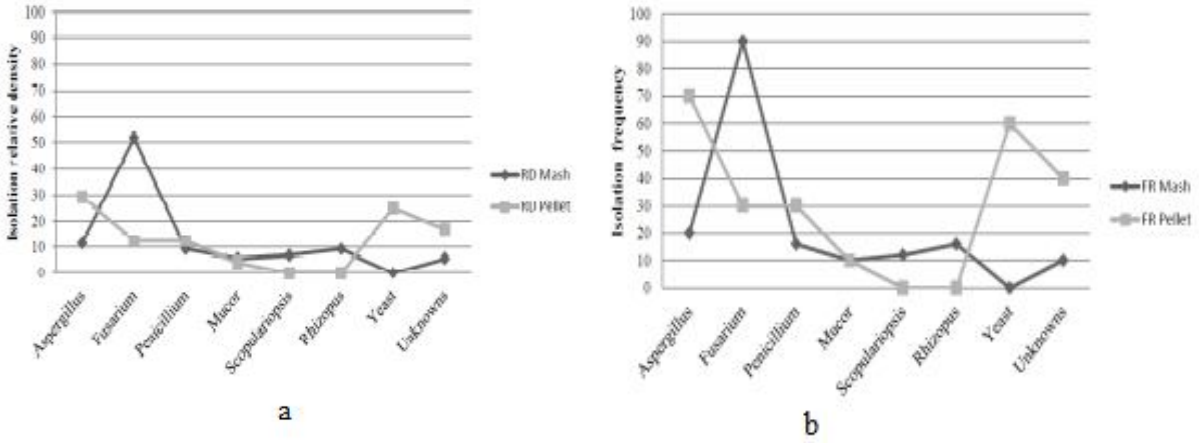

Figure 3. a: Fungal genera relative densities from 50 mashed and 40 pellet feed samples, **b:** Fungal genera frequencies from 50 mashed and 40 pellet feed samples.

Table 1. Counts of fungal test in the mashed form of poultry feed samples.

Poultry feed	No	Fungal Mycroflora (CFU g^{-1})		$>10^3$	$<10^3$
		Range	Mean	(٪)No.	(٪)No.
Broiler Chicken Starter	7	1.8×10^2-2.5×10^3	1.1×10^3	3(6)	4(8)
Broiler Chicken Finisher	6	2.1×10^2-35×10^3	21×10^3	4(8)	2(4)
Parent stock Starter	6	1.2×10^2-1.5×10^3	3.1×10^3	1(2)	5(10)
Pre-Breeder(5% production)	5	1.4×10^2-18×10^3	7.7×10^3	2(4)	3(6)
Laying Hen Starter	8	2.5×10^2-3.5×10^3	2.6×10^3	4(8)	4(8)
Laying Hen Finisher	9	2.8×10^2-55×10^3	34×10^3	6(12)	3(6)
Turkey Starter	4	3.1×10^2-8.5×10^3	5.5×10^3	2(4)	2(4)
Turkey Finisher	5	4.3×10^2-63×10^3	45×10^3	4(8)	1(2)
Total mash	50	1.2×10^2-63×10^3	15×10^3	26(52)	24(48)

*Acceptable level is $<10^3$ (CFU/g) EU Standard.

Table 2. Counts of fungal test in the pellet form of poultry feed samples.

Poultry feed	No	Fungal Mycroflora (CFU g^{-1})		$>10^3$	$<10^3$
		Range	Average	(٪)No.	(٪)No.
Broiler Chicken Starter	4	1.5×10^1-1.7×10^2	1.3×10^2	0(0)	4(10)
Broiler Chicken Finisher	6	2.7×10^1-58×10^2	12×10^2	2(5)	4(10)
Parent stock Starter	5	1.1×10^1-1.2×10^2	8.8×10^1	0(0)	5(12.5)
Pre-Breeder(5% production)	6	3.1×10^1-37×10^2	14×10^2	1(2.5)	5(12.5)
Laying Hen Starter	3	1.8×10^1-3.2×10^2	1.7×10^2	0(0)	3(7.5)
Laying Hen Finisher	4	2.9×10^1-56×10^2	18×10^2	2(5)	2(5)
Turkey Starter	5	3.5×10^1-47×10^2	9.2×10^2	1(2.5)	4(10)
Turkey Finisher	7	4.3×10^1-75×10^2	31×10^2	4(10)	3(7.5)
Total pellet	40	1.1×10^1-75×10^2	11×10^2	10(25%)	30(75%)

*Acceptable level is $<10^3$ (CFU/g).

Table 3. Fungal genus present in poultry feed samples.

Genus	Mash (n=50)				Pellet (n=40)			
	Range	Average	(%)*	(%)**	Range	Average	(%)*	(%) **
*Aspergillus*Ω	0-18	10	20	11.5	1-54	28	70	29.2
*Fusarium*Ω	0-82	45	90	51.7	0-26	12	30	12.5
*Penicillium*Ω	1-14	8	16	9.2	0-28	12	30	12.5
Mucor	0-10	5	10	5.7	1-10	4	10	4.2
Scopulariopsis	0-8	6	12	6.9	0	0	0	0
Rhizopus	0-15	8	16	9.2	0	0	0	0
Yeast	0	0	0	0	1-45	24	60	25
Unknowns	0-7	5	10	5.7	1-35	16	40	16.7
Total		87				96		

* Isolation frequency, **Isolation relative density, potentially mycotoxigenicfungi

Table 4. Fungal contamination level (CFU g^{-1}) in poultry feed from different countries.

Researchers	Local	Sample	(CFU g^{-1})	Genus
Dalcero, et al. (1998)	Argentina	130	6.6×10^3-6.3×10^5	*Asp> Fus*
Magnoli et al. (2002)	Argentina	120	2×10^3 -3×10^5	*Fus, Pen> Asp*
Rosa et. al (2006)	Brazil	96	2.05×10^2 -4.06×10^5	*Asp> pen*
Labuda et al. (2006)	Slovakia	100	1×10^3 to 200×10^5	*Pen >Asp > Muc*
Okoli, et al. (2007)	Nigeria	54	------	*Asp >Pen > Muc*
Saleemi et al. (2010)	Pakestan	119	------	*Asp>Pen >Fus>Alt*
Shareef et al. (2010)	Iraq	45	0.1-10^1 -6.5×10^6	*Asp> Pen, Muc > Rhi*
Astoreca et. al. (2011)	Argentina	35	4×10^4-1.6×10^5	*Asp> Pen > Fus*
Cegielska-Radziejewska (2013)	Poland	45	5.5×10-7×10^3	Finisher feed> starter feed
Krnjaja et. al. (2014)	Serbia	30	------	layer feed> broiler feed
Present study	Iran	mash	15×10^3	*Fus> Asp> Pen*
Present study	Iran	pellet	11×10^2	*Asp> Fus, Pen*

DISCUSSION

The assessment of fungal contamination in poultry feeds is one of the important steps to control the feeds quality and hygiene. Many studies have been conducted on poultry feeds fungal contamination, species and frequencies. However, comprehensive and stratified studies comparing pellet form (processed feeds) and mashed form (non-processed feeds) for fungal contamination are scarce. We compared our results with those of other studies on the general characteristics and fungal contamination of poultry feeds (Table 4).

In a study [17], the authors reported the presence of 15 genera of filamentous fungi in feeds, *Fusarium* and *Penicillium* were isolated in 67.5% of the samples and *Aspergillus* in 57.5% of them. Another study [18] carried out on 96 finished feeds from four feed mills in Brazil, showed more than 1×10^5 CFU.g^{-1} fungal contamination of poultry feed, in which *Aspergillus* and *Penicillium* were the most frequent isolated genera. Another study conducted on 130 samples from two feed mills in Argentina [19], demonstrated a range of 6.6×10^3 to 6.3×10^5 feed fungal contamination, and the most frequent isolates were *Aspergillus* and *Fusarium.* A survey on 35 feed ingredients and finished feed samples in Buenos Aires [20], showed that the contamination loads were 4×10^4 to 1.6×10^5 and *Aspergillus, Penicillium* and *Fusarium* were the most common genera. Analysis of mycobiota in commercial poultry feeds in Nigeria has detected common moulds isolated, *Aspergillus* spp., *Penicillium* spp., *Mucor* species, yeast, and bacteria [21].

Labuda and Tancinova (2006) analyzed more than 100 samples of poultry feed mixtures in Slovakia for overall fungal counts, and found penicillium as the most frequent genus followed by Aspergillus and Mucor [22]. In Pakistan, a study found [16] *Aspergillus* 44.5%, species as the most predominant fungi, followed by *Penicillium* 22.7%, *Fusarium*, 6.7%, and *Alternaria*. In Iraq [23], fourteen different mould genera have been isolated from poultry feeds and the most frequent contaminant fungi were *Aspergillus* 88.8%, followed by *Penicillium* 62.2%, *Mucor* 62.2%, *Rhizopus*, *and Scopulariopsis*.

In our study, *Fusarium* frequencies and relative densities on mashed poultry feeds was more prevalent than *Aspergillus and Penicillium (*Fig. 3a-b). *Aspergillus* frequencies and relative densities on pellet form of poultry feed were more than those for *Fusarium and Penicillium.* (Fig. 3a-b). Another study has shown that the frequency of *Aspergillus* after pelleting (29.1%) exceeded that of pre-pelleting (5.5%). This can be due to the thermal resistance and thermophilic property of *Aspergillus* [12].

In the present study, the average colony forming units in mashed feeds was 15×10^3 CFUg^{-1} and 52% of samples more contaminated than the accepted levels based on the European standard. In pellet feeds, the contamination average was 11×10^2 CFU/g^{-1} and 25% of them were more contaminated than the accepted levels for

European standard [24]. Applying optimal heat on raw material, especially in corn, helps to control fungal contamination in the ingredients, and promote hygienic quality of the final products. Inappropriate waste disposal, lack of access to hygienic water and insufficient heat process on the initial mixture are the main causes of exceeding fungal loads and spreading the pathogenic microorganisms to the final products. Furthermore, using air for the cooling process can recontaminate the feeds by letting additional pathogens to reach the feeds [25]. Thus, feed mills need to have a functional biosecurity program to minimize or eliminate all contaminants, including fungi.

In poultry feeds, many studies suggest that the presence of *Aspergillus* fungi might potentially lead to mycotoxin production when the storage and transportation processes are not appropriate. Evidently, the fungi are able to produce toxins and contaminate chickens during growth. In a study on 14 broiler and 16 layer feed samples, most contaminated layer feed samples belonged to the range of 1.4 - 4.8×10^8 $CFUg^{-1}$ contamination while, the most frequent contaminated broiler feed samples were in the range of 1-3×10^2 $CFUg^{-1}$ [26].

The result of our study also confirmed that layer feeds were more contaminated than others. A study on broilers feed, 45 feed samples were analyzed, feed fungal contamination level was 5.5×10-7×10^3; finisher feed was the most contaminated (6.6×10^3) and starter feed (3×10^2) was the least contaminated feed [27]. In this study, we had similar results; finisher fungal load was higher than that in the starters. This can be due to reducing the amount of soybean meal while increasing other ingredients in finisher feeds. It appears that corn and other ingredients are the main sources of feed contamination because they are not processed and stored appropriately [28].

CONCLUSION

High-risk ingredients (corn and other non-processed ingredients) should be screened carefully. They are the reason for the current diversity in types and loads of fungal contamination in poultry feeds. The process of converting mashed to pellet feeds is hazardous where there is not enough time and high temperature to destroy fungi. Therefore, machinery for manufacturing and packaging poultry pellet feeds is a possible source of fungal contamination in poultry feeds.

However, fungal load in processed feeds (pellets) was lower, in comparison to the unprocessed feed (mashed). Some toxicogenic fungi, such as *Aspergillus,* can survive from during the thermal process. Water fog, flies and bugs, dirty machinery and environmental air play the initial roles, causing pellet contamination by spreading pathogenic organisms. Poor or lack of hygienic processes in facilities and inappropriate storage conditions not only reduce feeds nutritional value but also endanger the health of humans and animals due to the toxins produced and released by fungi in poultry feeds.

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