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EVALUATION OF FUNGI AND FUMONISINS IN SWINE FEED AND ITS INGREDIENTS ON A FARM IN SANTA CATARINA, BRAZIL

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ABSTRACT: Fungi are filamentous multicellular organisms that can grow in/on grains and foods and produce toxic substances called mycotoxins. The aim of our research was to assess the safety of ingredients (soybean meal, corn, rice meal) and *final products* (feed) for off-spring (piglets) and pregnant adult swine (gilts/sows) from a small farm in Southern Brazil. For fungi, the residue of pre cleaned corn showed the highest total number of counts with 3.5 (3-3.8), followed by the corn sample with 2.8 (1.7- 4.0) log CFU/g. The rice and soybean meal samples had lower counts of 2.9 (1.7-3.9) and 1.3 (0-2.9) log CFU/g, respectively. The pregnancy and offspring feed samples showed counts of 2.6 (0-3.7) and 2.1 (0-3.7) log CFU/g, respectively. The main fungal genera found were *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp for all samples. FBs were found in samples of corn, rice and soybean meal with an average of 795.9, 138.8 and 198.3 μg/Kg, respectively. The residue of pre cleaned corn had the highest rate of contamination of 83% and 100% for FB_1 and FB_2, respectively. The total for both FBs was 3915 μg/Kg. More than 50% of the samples of feed for swine analysed were contaminated by FB_1.The rate of contamination was higher for off-spring feed than for pregnancy feed, with an average of 411.3 (171.1-728.1) μg/Kg and 296.9 (58.5-531.2) μg/Kg, respectively. The rate of contamination by FB_2 in samples was higher for off-spring feed (33.33%) than for pregnancy feed (25%), and the average levels of contamination were 363.8 (51.1-792) and 457.4 (81.2-997.6) μg/Kg, respectively. It should be noted, therefore, that continuous exposure to seemingly low levels of contamination can lead to serious problems in the product chain. Thus, our research suggests the need for constant monitoring, both during processing and storage of animal feed and its ingredients. **Keywords:** feed; fumonisin; mycology; swine

RESUMO: Fungos são organismos multicelulares filamentosos que podem crescer em grãos e alimentos e produzem substâncias tóxicas chamadas de micotoxinas. O objetivo de nossa pesquisa foi avaliar a segurança de ingredientes (farelo de soja, milho, farelo de arroz) e produtos finais (rações) de leitões e suínos gestantes de uma pequena fazenda do Sul do Brasil. Para fungos, o resíduo da pré-limpeza do milho apresentou o maior número na contagem total 3.5 (3-3.8), seguido da amostra de milho 2.8 (1.7 a 4.0) log UFC/g. As amostras de farelo de arroz e de soja tiveram as contagens mais baixas 2.9 (1.7-3.9) e 1.3 (0-2.9) log UFC/g, respectivamente. As amostras de ração gestação e inicial apresentaram contagem de 2.6 (0-3.7) e 2.1 (0-3.7) log UFC/g, respectivamente. Os principais gêneros fúngicos encontrados foram *Aspergillus* spp, *Penicillium* spp e *Fusarium* spp em todas as amostras. FBs foram encontradas em amostras de milho, farelo de arroz e soja com uma media de 795.9, 138.8 e 198.3 μg/Kg, respectivamente. O resíduo da pré-limpeza do milho teve a mais alta taxa de contaminação 83% e 100% para FB_1 e FB_2, respectivamente. O total para ambas FBs foi 3915 μg/Kg. Mais de 50% das amostras de rações de suínos analisadas estavam contaminadas por FB_1. A taxa de contaminação foi maior para ração inicial do que para ração gestação com a média de 411.3 (171.1-728.1) μg/Kg and 296.9 (58.5-531.2) μg/Kg, respectivamente.

A taxa de contaminação por FB_2 nas amostras foi maior para ração inicial (33.33%) do que para gestação (25%), e os níveis médios de contaminação foram 363.8 (51.1-792) e 457.4 (81.2-997.6) μg/Kg, respectivamente. Deve-se notar, portanto, que a exposição continua a níveis aparentemente baixos de contaminação podem causar graves problemas na cadeia de produção. Assim, nossa pesquisa sugere a necessidade de monitoramento constante durante o processamento e armazenamento da ração animal e seus ingredientes. **Palavras-chave:** fumonisinas; micologia; ração; suínos

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INTRODUCTION

Moulds infect most agricultural commodities, and the mycotoxins they produce represent a major challenge in the control and inspection of foodstuffs (Maul et al., 2012). The growth of moulds and resulting production of mycotoxins depends on the interaction of multiple variables such as pH, water activity (aw), solute concentration, temperature, atmosphere andtime (**Garcia et al., 2011).** *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. are the most common filamentous moulds found in stored cereal grains and feeds (Rosa et al., 2009; Lee et al., 2010). Although the detection of fungi does not necessarily imply the presence of mycotoxins, several studies around the world have reported a high incidence of mycotoxins in feed for animal consumption (Placinta et al., 1999). Fumonisin (FBs) is one of the mycotoxins produced by fungi of the genus *Fusarium* in favorable conditions of high moisture content and temperature (Dilkin et al., 2002).

Symptoms of contamination by FBs depend on the type of mycotoxin, the amount and duration of the exposure, the age, health and sex of the exposed individual, as well as dietary status and interactions with other toxins (Bennett and Klich, 2003). The negative effect of FBs on the growth and health of livestock makes them a major problem for many production systems (Roigé et al., 2009). Fumonisins cause various swine diseases: liver and kidney toxicity and carcinogenicity, pulmonary edema, immunosuppression and neurotoxicity (Gazzotti et al., 2011, Grenier et al., 2012). Swine feed contaminated by FBs can cause loss of appetite, depression, cardiovascular toxicity and induced pulmonary edema (Bryden, 2012).

Considering that the region where the collections were made has a humid subtropical climate, and that factors of humidity and temperature are essential for toxigenic fungi growth the aim of this work was to assess the safety of *ingredients* (soybeans, corn, rice bran), *final products* (feed) for off-spring (piglets) and pregnant adult swine (gilts/sows) and *residue* (pre cleaned corn) stored in a small swine farm in the South of Brazil. This was achieved by testing for the presence of toxigenic fungi and their correlation with the presence of FBs.

MATERIAL AND METHODS

Samples: Two types of swine feed samples were collected for analysis *feed ingredients* (*grain*: corn; *meal*: soybeans and rice) used feed *final products* for pregnant swine (gilts/sows) and piglets from a small farm, located in Southern Brazil.

Reagents, Solvents, Standards and Culture Media *Reagents* - methanol, phosphoric acid, 2-mercaptoethanol, acetic acid, sodium dihydrogen phosphate solution, ophthaldialdehyde (OPA). All HPLC grade, from (Vetec). *Solvents* - methanol Vetec. All HPLC grade. Ultrapure water (MilliQ system - Millipore), *Standards:* FB_1 and FB_2 (Sigma). *Culture media:* malt extract agar-MEA; peptone agar, dichloran rose bengal chloranphenicol agar (DRBC) (Himedia).

Methods

***Samples collection and preparation*:** *Collection* - samples were collected from August 2010 to February 2011 in a small farm located in Santa Catarina state, Southern Brazil, at Doutor Pedrinho town. They were collected from silos (stored in bulk/loose) and storehouses (stored in bags). Corn and soybean meal were collected from silos of 50 and 20 ton capacity, respectively. Rice meal from local suppliers and feed for *pregnant* swine and piglets prepared on the premises were collected from 25 Kg bags. 200 g of each sample type were gathered from different collection points to get a total sample of 1 Kg (composite sample) of each ingredient and feed. *Preparation* - each sample was homogenized and divided into smaller portions (analytical samples) for mycological and mycotoxin (FBs) analysis.

Mycology tests and FBs analysis: *Mycology:* For total fungi count the method used was Pitt and Hocking (1997), applying serial dilutions (10^{-1} to 10^{-3}) and staining by dichloran rose bengal chloramphenicol agar (DRBC). The identification of fungi gender was carried out according to Samson et al. (2002). *FBs:* analysed by liquid chromatography and fluorescence detector (LC/FD) at 335 and 440 nm wavelength (excitation and emission, respectively), as described by AOAC (2005). **LOD was 0.5** and LOQ was 1 µg/Kg for both, FB_1 and FB_2.

Statistical Analyses: Total fungal counts data were transformed using a logarithmical function $\log_{10}(x+1)$. Performed by variance analysis (ANOVA) and Turkey´s test, to evaluate significant differences among the means ($P<0.05$) using GraphPad Prism 4.0 software. The results were expressed as the mean values and standard errors.

RESULTS AND DISCUSSION

Molds and yeasts were found in more than 95% of the samples. The main fungal genera were *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. Conditions of moisture and water activity in the samples appeared altered, which can cause fungal growth and production of FBs.

Total count of molds and yeasts

The residue from pre-cleaning corn showed the highest total number of mold counts 3.5 (3 to 3.8) log CFU/g, followed by corn with 2.8 (1.7 to 4.0) log CFU/g (Table 1).

This result proves that pre-cleaning can reduce mold counts in corn. Values higher than those in our corn samples had been found by Rosa et al (2009), (4.44 log CFU/g). Rice and soybean meal samples had counts of 2.9 (1.7 to 3.9) log CFU/g and 1.3 (0 to 2.9) log CFU/g, respectively. Pregnancy and off-spring feed samples showed counts of 2.6 (0 to 3.7) and 2.1 (0 to 3.7) log CFU/g, respectively. Pereyra et al. (2008) found total mold counts over 5.0 log CFU/g in compound feed intended for fattening swine. None of our samples counts exceeded 5.0 log CFU/g, which may indicate the hygienic quality of feed is in accordance with GMP (2005).

Table 1 – Total count for molds and yeasts in ingredients and feed samples.

	Ingredients (log CFU/g)			*Residue****	*Feed(logcfu/g)*		*Collection data*	
	Soybean*	***Rice****	***Corn****		***Pregnancy ****	***Off-spring*****	***Month***	***Year***
	0	3.1	4.0	3.8	0	0	Aug	2010
	0	3.3	1.7	3.8	0	0	Aug	
	1.7	2.8	1.7	3.4	3.6	3.0	Sept	
	2.9	1.7	2.2	3.6	2.5	3.3	Sept	
	0	3.8	3.2	3.3	3.4	2.0	Oct	
	2.3	2.9	2.8	3.6	2.8	2.2	Nov	
	2.0	1.7	2.6	3.0	3.0	2.5	Nov	
	1.7	3.9	3.9	3.4	3.7	3.2	Dec	
	0	2.8	3.2	3.7	3.4	3.7	Dec	
	2.5	2.9	2.1	3.5	2.9	0	Jan	2011
	2.8	2.7	2.5	3.0	3.0	3.0	Jan	
	0	3.0	3.9	3.8	3.2	2.5	Feb	
Average:	1.3	2.9	2.8	3.5	2.6	2.1	na	
Max:	2.9	3.9	4.0	3.8	3.7	3.7	na	
Min:	0	1.7	1.7	3.0	0	0	na	

na: not applied * significant differences soybean with rice, corn, residue and pregnancy (P<0.05) ** significant differences residue with off-spring (P<0.05)

Fungal genera identified

In this work, the following were identified: (a) five storage fungi (*Cephalosporum, Geotrichum*, *Mucor, Peziza*and, *Rhizopus*), (b) three harvest fungi *(Alternaria, Fusarium, Trichoderma)* and (c) three harvest and storage fungi (*Aspergillus*, *Botrytis*, *Penicillium*). The largest amount of fungal genera identified in the samples corresponded to *Aspergillus*, *Fusarium* and *Penicillium* sp. Many studies have shown that these genera are found in most feeds (Bragulat et al., 1995; Rosa et al., 2006). *Fusarium* fungi were found in lower quantities in samples of soybean meal and corn, which may be due to the process of harvesting the grain and to weather conditions. *Fusarium* toxins are produced in cereal grains due to high moisture conditions around harvest (Bryden, 2012).

Table 2 – Identification of fungal genera in ingredient and feed samples.

GENERA	*SAMPLES (%)*					
	Ingredients			*Residue*	*Feed*	
	Soybean	*Rice*	*Corn*		*Pregnancy*	*Off-spring*
Storage						
Cephalosporum	+	ni	ni	+	ni	+
Geotrichum	+	ni	ni	+	ni	ni
Mucor	+	+++	+	+	+	+
Peziza	ni	+	ni	ni	ni	ni
Rhizopus	ni	+	+	+	+	+
Harvest						
Alternaria	ni	+	ni	Ni	ni	ni
Fusarium	+	++	+	++	++	++
Trichoderma	+	+	++	++	++	++
Storage and Harvest						
Aspergillus	++	+++	+++	+++	+++	+++
Botrytis	+	ni	+	+	ni	ni
Penicillium	++	+++	+++	+++	+++	+++

ni not identified + found in 1 to 4 samples ++ found in 5 to 8 samples +++ found in 9 to 12 samples

Levels of fumonisins

Regarding the presence of FBs, corn was the ingredient that had the highest rate of contamination. 83.33% of our samples showed contamination by FB_1 and 58.33% by FB_2, showing rates of 121.3-1405 µg/Kg and 49.8-680.9 µg/Kg, respectively. Similarly, a study published by Prandini et al. (2011) found contamination by FBs in corn samples ranging from 300 to 1200 µg/kg FB_1 and FB_2 were detected in all feed and corn samples (100%). In another study by Pereyra et al. (2008) the mean values ranged from 3210 to 4020 µg/Kg for FB_1 and from 1300 to 1950 µg/Kg for FB_2. It was interesting to observe that the cleaning of corn leads to a significant reduction in the presence of FBs. The average contamination of the residue of pre cleaning corn was 2093 and 1822 µg/kg for FB_1 and FB_2, respectively. After cleaning, the average levels found in corn fell to 526.2 and 269.7 µg/Kg, respectively. By the same token, it can be concluded that damaged grains separated by the process of sifting, are more susceptible to contamination by FBs (Scussel 2002).

More than 50% of the samples of feed for swine analysed were contaminated by FB_1. The average rate of contamination was higher for off-spring feed (411.3 µg/Kg) than for pregnancy feed (296.9 µg/Kg) (Table 3).

In a study by Martins et al. (2008) no contamination by FB_1 was found in any of the 285 samples of swine feed evaluated. However, and in accordance with our results, Martins et al., (2011) found contamination by FBs in swine feed. in 32.9% of 82 samples. The percentage of contamination by FB_2 was higher in off-spring feed (33.33%) than in pregnancy feed (25%), with an average level of contamination of 363.8 (51.1-792) and 457.4 (81.2-997.6) µg/Kg, respectively. Pereyra et al. (2008) found that more than 90% of swine feed samples were contaminated with FB_2.

CONCLUSION

The United States of America is the only country in the world to have established parameters for the presence of FBs (20000 µg/Kg) (FAO, 2004). The results for all ingredients and feed in this study are within these parameters. In spite of this, it is important to emphasize that constant exposure of the animals to FBs, even at low levels of concentration, can have a great impact, both sanitary and consequently financial, on the production chain. For this reason, periodic monitoring for the presence of FBs in ingredients and feed is required to reduce the risk of dangerous diseases and protect profitability.

Many factors contribute to the proliferation of molds and the consequent production of mycotoxins. These include cultural practices, harvesting practices, transport and storage conditions, relative humidity, aeration and the presence of insects. Alternatives for the elimination of mycotoxins in feed have been studied. However, it still seems most productive to constantly monitor the quality of both ingredients and feeds within a property.

Table 3 – Presence of FBs in *ingredients* and *feeds* herds.

Sample	*Number*	FB_1 *(µg/kg)* (%)	FB_1 *Average*	FB_1 *Range*	FB_2 *(µg/kg)* (%)	FB_2 *Average*	FB_2 *Range*	*FBs (µg/Kg)*
Ingredients								
Soybeans	12	33.33	198.3	40-537.8	nd	nd	nd	198.3
Rice	12	33.33	138.8	68.4-264.9	8.33	nd	nd-174.7	138.8
Corn	12	83.33	526.2	121.3-1405	58.33	269.7	49.8-680.9	795.9
Feed								
Pregnancy	12	58.33	296.9	58.5-531.2	25.00	457.4	81.2-997.6	754.3
Off-springs	12	58.33	411.3	171.1-728.1	33.33	363.8	51.1-792	775.1
Residue								
Pre-cleaning	12	83.33	2093	57-6051	100.00	1822	223.5-4844	3915
Total sample:	***72***	na	na	na	na	na	na	na

nd: not detected; na: not applied

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