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Silage Contribution to Aflatoxin B_1 Contamination of Dairy Cattle Feed

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1. Introduction

Dairy production systems have traditionally relied on direct utilization of pastures and annual soiling crop. This feeding strategy is complemented by the use of other feeds such as grains, balanced feed, silage, hay and industrial products, the level of use was variable and it defined in any way the degree of intensification of each dairy production systems.

Over recent decades, this intensification has been increasing at an accelerated rate, partly because the farms that remain, integrated into general agricultural-livestock mixed models, increasing land for agriculture, as a result of best price-cost and simplicity of production. This change in management practices in dairy cattle breeding, from the extended to semi-intensive or intensive form, has meant a change in the way animals are fed.

The change from grazing over large areas of land to cowshed feeding with grain-based concentrates and silage has greatly improved productivity increase on the number of animals per hectare and, in turn, improved performance and milk production per cow due to the nutritional advantages afforded by the new way of eating. The dairy industry has been driven to higher levels of efficiency and competitiveness. This management system makes storing feed necessary as it is used throughout the year whether it is produced in the same establishment or not. This raises the concern to protect these products from damage by insects, pests and fungal contamination in order to maintain an appropriate level of feed security. Storage systems for feed, both silage and whole grains are a man-made ecosystem in which quality and nutritive changes occur because of interactions between physical, chemical and biological factors.

The deterioration by fungi and mycotoxin contamination is one of the greatest risks of stored feed. Apart from reducing palatability and feed consumption, fungal growth leads to loss of nutrients and dry matter causing in animal performance (O´Brien et al., 2005). Fodder, cereals

and seeds used in feed for dairy cattle are naturally in contact with yeasts and filamentous fungi, the contamination of raw materials occurs frequently in the field, because of the infection of plant symbiotic fungi as phytopathogens. This contamination can also occur during harvesting, transport and storage of these products and post harvest mishandling can lead to rapid spoilage. In well-preserved forages fungal growth depends on moisture conditions of the plant during harvest. Stored feed, moisture, temperature and oxygen availability are key conditions that determine risk degree of fungal contamination. The critical water activity for safe storage is 0.7 to 0.8 (Magan & Aldred 2007; Scott, 1957). When this level is exceeded, large degrading ability fungi as *Eurotium sp.*, and species of *Aspergillus* and *Penicillium* can grow. Increase in respiratory activity, due to the development of these fungi, leads to an increase in the temperature of feed that can lead to the contamination by other fungi especially thermophilic fungi and, therefore, to further deterioration.
Silage is one of the main constituents in the diets of dairy cattle and its deterioration and aflatoxin contamination can lead to considerable production losses and a major impact on human health.

2. Breeding and feeding systems on dairy farms

In many systems of milk production mainly in the northern hemisphere, the dairy cows are housed in stockyard due to extreme weather conditions, either high or low temperatures. These intensive production systems use a minimal proportion of grass per cow. In other systems, where climates are more benign and temperate, the production system is typically extensive grazing.
In general, worldwide, the diversity of soils, climates and production scales do not allowa single production system; it is clear that there has been a gradual shift from purely pastoral models to semi-intensive systems (López 2008). In the first instance, the producers began to incorporate ration, preferably, corn grain or commercial feed and for this, they took the shackles of milking, where feeders are installed. Simultaneously, the corn silage began to spread, both as a reserve fodder as well as balanced diet. At this point, producers required new ways of providing meals.
This intensification is necessarily accompanied by a significant increase of the scale, this fact causes many people to use new technologies to keep the cows in confinement.
The development in milk production in recent years has followed an intensification which has resulted in a change in the use of feed, evolving from simple grazing feeding systems based on mixed feed formulation combining grains and forages.
Although the current systems of feeding in major milk producing areas in Argentina have particular differences in the degree of intensification, they can be considered supplement grazing systems (or semi-intensive). Through this enhancement, production level was able to grow extensively. The levels of milk production increased from 12 L to 20-30 L. However, animal numbers by hectare did not increase. That supplementation can not only avoid the seasonality of production due to the availability of pastures in different seasons, but also allow to balance the dietary components optimizing milk production per cow (West, 2003). However, many authors argue not to forget grass, which remains the staple feed "of ruminant herbivores" as well as the cheapest cost of production.
The composition of feed rations for dairy cows consists of:

- Pastures (including small grain winter and summer)
- Conserved forage (silage, hay)
- Concentrate

Figure 1 shows estimated components proportion, which may vary slightly according to season and geographical area. Perennial pastures are usually based on alfalfa pasture. Forages are used both for direct consumption of pasture (winter and summer soiling) and as conserved forage in the form of rolls or bales of hay. Typically, 10% of forage is intended for these purposes and often rye, oats, moha, wheat and sorghum are selected in dairy farms according to acreage and selected pasture. As concentrates, grain corn, grain sorghum, cotton seed, wheat bran, dregs of malt, peanut shells, and sunflower expeller, are used among others. It is also common to use commercial pelleted feed.

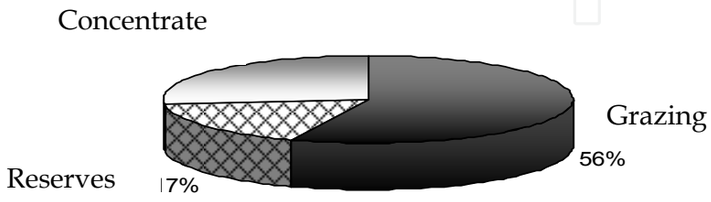


Fig. 1. Typical diet for milking cows (Chimicz & Gambuzzi 2007).

3. Corn silage

Corn (*Zea mays* L.) is the most widely grown crop in the Americas, extensively used for animal feeding and human consumption due to its nutritional value. A large percentage of the world corn production is destined to animal feeding. Silage is a widespread practice to preserve forages during extended time periods. The production of corn silage entails incorporation of the whole plant and its storage is based on the principle of preservation under anaerobic conditions with growth of lactic acid bacteria which promote a natural fermentation that lowers the pH to a level at which clostridia and most fungal growth are inhibited. In dairy cows, silage is a preferred food by the vast majority of producers.

As corn silage consists of grinding and storing the whole corn plant, it includes not just grain but a high percentage of stalks and stover and represents a new important bulky feed source for dairy and beef cattle. Nutritionally, corn silage, for example, has a balance between the energy density of the grain and fibber and digestibility of the green plant that makes it suitable for feeding ruminants in the phases of maximum nutritional needs (Molina et al., 2004).

4. Ensiling and storage conditions

Silage is a method of forage preservation based on lactic acid fermentation, usually spontaneous under anaerobic conditions, where the pH reaches values of 2-3 being an important indicator of forage conservation (Johnson et al., 2002). Air must be removed as much as possible from the silo in order to obtain good silage quality. To achieve this goal, certain management aspects must be emphasized. Forage should be harvested, chopped,

packed well and covered in the silo as fast as possible. Air and rain infiltration can cause poor fermentation and spoilage in the silo. Rain will increase moisture/seepage, favour growth of undesirable bacteria (for example *Clostridium sp.*), and wash nutrients away. The resulting silage will have low nutritional value and will likely be avoided by cows (low dry matter intake). Intake is directly related to milk production in lactating dairy cows, therefore low intake equals low milk yield.

Maize, sorghum and barley malt are the main forages used for silage (Driehius & Oude Elferink, 2001). Ideal fermentation is dependent upon decisions and management practices implemented before and during the ensiling process. The primary management factors that are under the control of the producer are:

1. Stage of maturity of the forage at harvest.
2. The type of fermentation that occurs in the silo or bunker.
3. Type of storage structure used and methods of harvesting and feeding.

During the ensiling process, some bacteria are able to break down cellulose and hemicellulose to various simple sugars. Other bacteria break down simple sugars to smaller end products (acetic, lactic and butyric acids). The most desirable end products are acetic and lactic acid. As the bacteria degrade starches and sugars to acidic and lactic acids, dry matter is lost.

Quality silage is achieved when lactic acid is the predominant acid produced, as it is the most efficient acid fermentation and will drop the silage pH quickly. The faster the fermentation is completed, the more nutrients will be retained in the silage.

At least six phases can be described during the ensiling process (Table 1), in a first phase the aerobic bacteria predominant on the forage surface continue respiring within the silo structure. This phase is undesirable since the aerobic bacteria consume soluble carbohydrates that might otherwise be available for the beneficial lactic acid bacteria or for the animal consuming the forage. Phase I ends once the oxygen has been eliminated from the silage mass. Under ideal crop and storage conditions, this phase will last only a few hours.

After the oxygen in the ensiled forage has been used by the aerobic bacteria, Phase II begins. This is an anaerobic fermentation where the growth and development of acetic acid-producing bacteria occur. These bacteria ferment soluble carbohydrates and produce acetic acid as an end product. Acetic acid production is desirable as it can be utilized by ruminants in addition it initiates the pH drop necessary to set up fermentation phases. As the pH of the ensiled mass falls below 5.0, the acetic bacteria decline in numbers as this pH level inhibits their growth. This signals the end of Phase II. In forage fermentation, Phase II lasts no longer than 24 to 72 h. Phase III begins when the increasing acid inhibits acetic bacteria. The lower pH enhances the growth and development of another anaerobic group of bacteria, those producing lactic acid.

Phase IV is a continuation of Phase III as lactic-acid bacteria start to increase in number, ferment soluble carbohydrates and produce lactic acid. Lactic acid is the most desirable of the fermentation acids and for efficient preservation, should comprise greater than 60 percent of the total silage organic acids produced. When silage is consumed, lactic acid will also be utilized by cattle as an energy source. Phase IV is the longest phase in the ensiling process as it continues until the pH of the forage is low enough to inhibit the growth of all bacteria. When this pH is reached, the forage is in a preserved state. No further destructive processes will occur as long as oxygen is kept from the silage.

Phase V is the storage time when the final pH is reached, and the good conditions of anaerobiosis are supported.
Phase VI refers to the silage when it is cut to be used as feed. The Phase VI occurs on any surface of the silage that is exposed to oxygen during storage and in the feed bunk.

	Phase I	Phase II	Phase III	Phase IV	Phase V	Phase VI
Age of silage	0-2 days	2-3 days	3-4 days	4-21 days	21 days	
Activity	Cell respiration; production of CO_2, heat and water	Production of acetic acid and lactic acid ethanol	Lactic acid formation	Lactic acid formation	Material storage	Aerobic decomposition on re-exposure to oxygen
Temperature change	20-32 ºC	32-29 ºC	29 ºC	29 ºC	29 ºC	29 ºC
pH change	6.5-6.0	6.0-5.0	5.0-4.0	4.0	4.0	4.0-7.0
Produced by		Acetic acid and lactic acid bacteria	Lactic acid bacteria	Lactic acid bacteria		Mold and yeast activity

Table 1. Silage fermentation phases and storage

5. Influence of pH and water activity on the silage contamination

The current system of dairy animal production requires a thorough knowledge of production, processing and quality of all feed used. Contamination of feed intended for animal consumption usually reflects the incidence of fungal infection in the original crop.
Temperature, humidity, oxygen availability and pH conditions vary during the silage process and microbiota may also change from one stage to another. However, poor storage conditions - including excessive moisture or dryness, condensation, heating, leakage of rainwater and insect infestation - can lead to undesirable fungal contamination, mycotoxin production and the reduction of nutritional value.
The forage quality is evaluated through physicochemical and fermentative conditions such as pH, water activity (a_W), percentages of ammonium / total nitrogen (Teimouri Yansari et al. 2004). The water content of a substrate does not give a direct index of a_W for microbial growth. The availability of water in hygroscopic materials such as grains is measured as equilibrium relative humidity (ERH), a_W or water potential (ψ). The last two measures are most appropriate for situations where the availability of water in the substrate is the factor that controls growth.
The pH in the silage provides an indication of the type and range of the fermentation process. The acid pH resulting from fermentation prevents proper development of viable cells. Only a few yeasts, other microorganisms tolerant to this pH and spores as Clostridia and *Bacillus* can survive in dormant state (Driehius & Oude Elferink, 2001).
The silage can be contaminated and damaged by fungi from the soil and essentially can contaminate forages in various stages and plant management. The process of preservation by acidification, dehydration and exclusion of O_2 in the early stages of storage does effectively restrict the development of these microorganisms. Moreover, the improper extraction of silage (straight cut, little waste, little oxygenation) and the mixing of different

sections of the silo before being incorporated into the mixer, could enhance the final feed contamination with aflatoxigenic fungi and aflatoxins (Borreani & Tabacco, 2010).
Comparative multivariate statistical studies on the influence of pH and a_W on the fungal count and on the incidence of AFB_1 in dairy cattle feedstuff, were performed using principal component analysis. In Figure 2, the "biplot" graphic in which the variables: total fungal count, *Aspergillus* count, *A. fl*avus count and incidence of aflatoxin B_1 (AFB_1), depending on the type of food and a_W, are shown.
Corn silage at a_W 0.97 is closely related to total fungal count and *Aspergillus* spp. So is for that same feed at aw 0.98, 0.99, 0.96, and 0.93. This positive relationship shows that at a_W 0.93 or higher; the corn silage contributes to finished feed contamination by fungi such as aflatoxicogenic fungi.

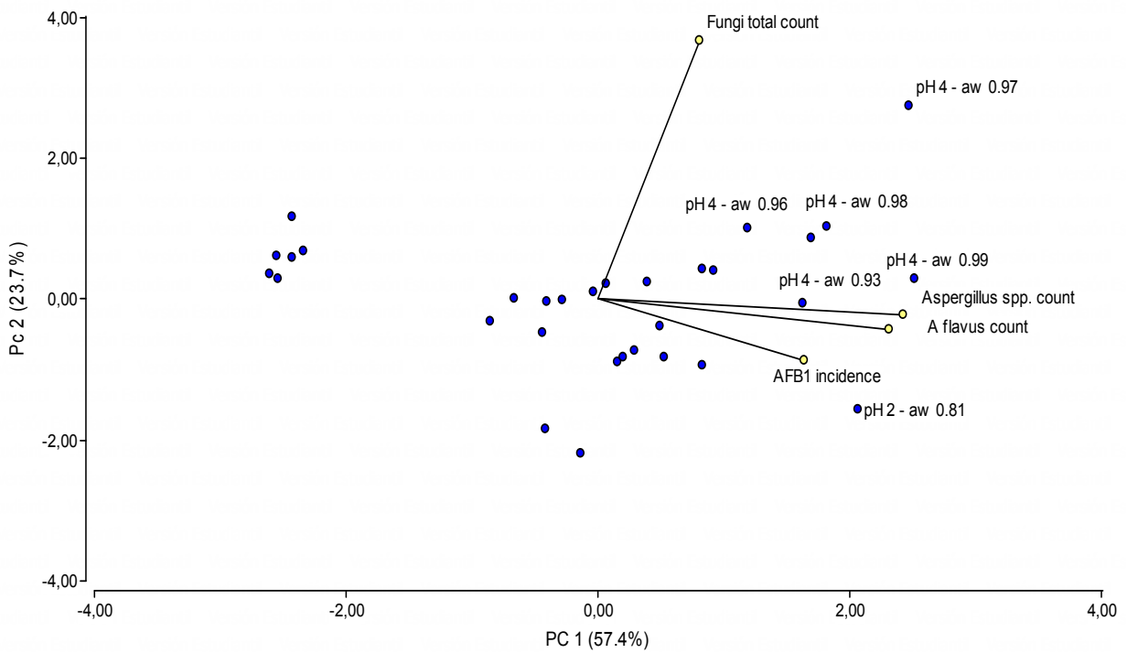


Fig. 2. Graph "biplot" principal component analysis to study variables (total fungal count, *Aspergillus* spp count of, *A. flavus* count, incidence of AFB_1) depending on the type of food and a_W.

A multivariate statistical comparative study in terms of the type of feedstuff and pH among the variables total fungal count, *Aspergillus spp* count, *A. flavus* count, and AFB_1 incidence are shown in Figure 3.
According to the principal component analysis, the contribution of total fungi to finished feed is mainly given by the silage at pH 4 and 5.
The contribution of *Aspergillus* spp. and *A. flavus* corresponds mainly to the silage at pH 4.5. These studies allow to highlight that silage, when reaches these pH values, will be affected by contamination with *Aspergillus* spp.s and *A. flavus.* This fact will determine the contribution of fungal contamination from silage to finished feed that will be consumed by dairy cattle.

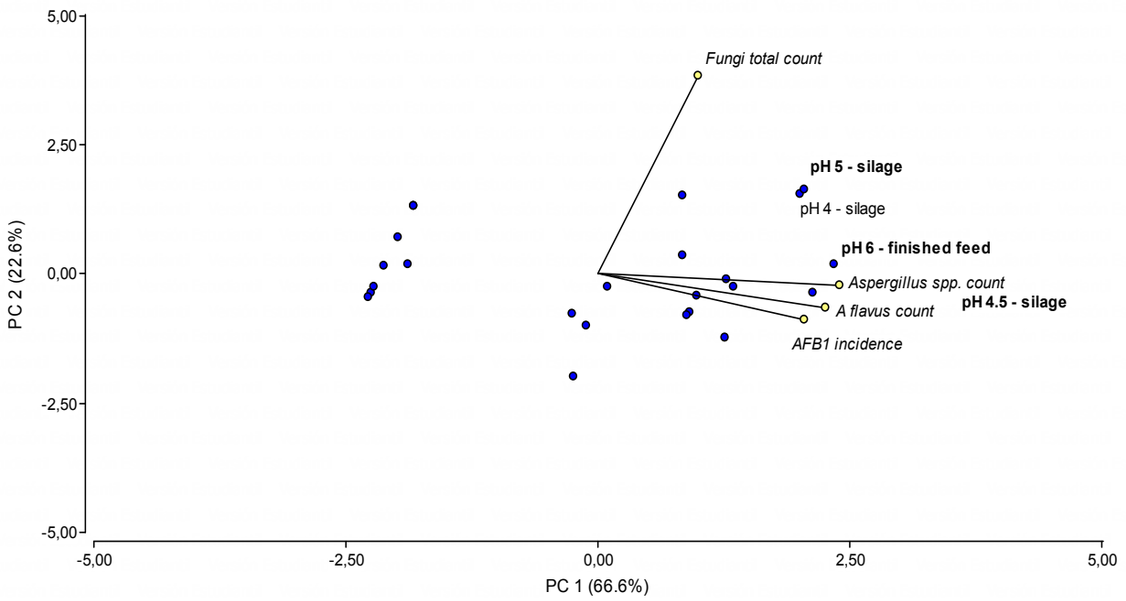


Fig. 3. Graph biplot of principal component analysis to study variables (total fungal count, *Aspergillus* spp count, *A. flavus* count, AFB_1 incidence) depending on the type of food and pH.

6. Silage mould pathogens

Fungi growing in silage expose animals to respiratory problems, abnormal rumen fermentation, decreased reproductive function, kidney damage, skin and eye irritation (Akande, 2006; Scudamore & Livesey, 1998). Fungal concentrations in forage above 1×10^4 CFU g^{-1} may be the reason for these problems. Thus, the fungal colony count is an indicator of forage quality (Di Costanzo et al., 1995). Currently, the Good Manufacturing Practises International (GMP 2008) recommends a limit set as 1×10^4 CFU g^{-1} in feedstuff.

The major fungal species isolated from feed for dairy cattle, belong to *Aspergillus*, *Penicillium* and *Fusarium* genera (El-Shanawany et al., 2005, Garon et al., 2006; Gonzalez Pereyra et al., 2008; Rosa et al., 2008; Simas et al., 2007).

Several species within these genera are capable of producing mycotoxins, in exposed animals or humans.

Strains of *A. flavus* and aflatoxins are the main grains and corn plant contaminants (Chulze 2010). *A. flavus* can infect pre-and post-harvest corn and a significant increase in the content of aflatoxins may occur if the drying and storage phases do not perform correctly.

In Argentina, in studies on the fungal contamination in dairy cattle feed it can be seen how corn silage influences the degree of contamination of the ration supplied to livestock (Gonzalez Pereyra et al., 2008).

The multivariate analysis through principal component analysis (PCA) allows biplot graph (Figure 4) expressing the associations between finished feed contamination and raw materials that mainly contribute with fungal contamination. It can be seen that the obtained silage fungal counts are strongly correlated with the finished feed contamination. A similar correlation was observed between the finished feed and cotton seed. Raw materials such as corn and brewer's grains, do not have correlation with finished feed, in other words, do not contribute to the increase of fungal contamination.

In relation to sampling periods, it can be seen in the same figure that December is associated with a higher contamination in silage and in finished feed. The prediction ellipse confirms that during December all feed adds high fungal contamination to finished feed (98% confidence ellipse).

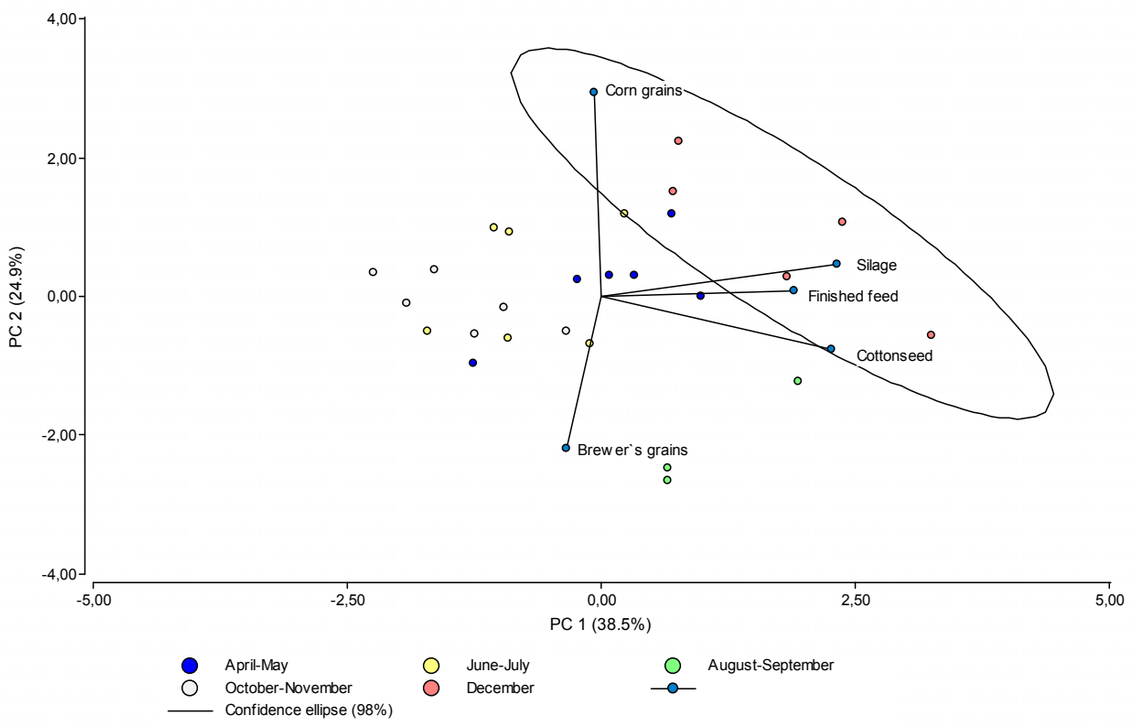


Fig. 4. Graph "biplot" principal component analysis of the feed materials and sampling periods in terms of total fungal counts.

7. Corn silage sections

Corn silage can be divided into three main sections corresponding to the upper (generally more exposed to fungal contamination) middle (best-preserved portion) and lower portions. Statistical analysis based on fungal counts were performed on the three sections of the silo showed that levels of found contamination were significantly different in each studied section ($p < 0.05$). Table 2 shows the associations between different levels of silage for fungal contamination. The upper and lower sections had the same levels of fungal contamination whereas pollution in the middle section did not show association with low levels. This was an expected result since the anaerobic environment and low pH silage allow good conservation in half portions of the silage that do not have contact with air or ground as in upper and lower section.

Proper storage is related the state of compaction. The most compact the silo, the teast possibility of losing reduced pH and anaerobic conditions. The extraction method is also highly important. Even if the upper section is in contact with air, it is less affected when the silo is firmly packed. In silos visibly unarmed, the upper and lower sections (more than 10 cm) are visibly contaminated and altered in colour and smell.

Silage	Total fungal count (log $_{10}$CFU g^{-1})	
	Mean ± Standard Error	LSD (p < 0.05)
Upper	7.13 ± 0.58	b
Middle	4.83 ± 0.57	a
Lower	6.99 ± 0.58	b

Different letters indicate significant differences (p <0.05) according to the test of Fisher's least significant difference (LSD). The count data were transformed to $\log_{10}$ (x +1) to achieve homogeneity of variance

Table 2. Total fungal counts (CFU g^{-1}) present in samples of silages at different sampling sections of the silo.

Table 3 details the total fungal counts, expressed in CFU g^{-1}, obtained for each raw material and finished feed at different sampling periods. The silage was considered by averaging the counts obtained in the three sections for each sampling period.
In corn, the average values in total fungal counts during all sampling periods ranged from a minimum of 2.36 x 10^3 to a maximum of 7.00 x 10^5 CFU g^{-1}, corresponding to the periods August-September and October-November, respectively. In general, the fungal counts showed low variability throughout the year, finding associations in contamination levels during the first three bimonthly sampling. Table 4 shows the percentage of samples whose total fungal counts exceeded hygienic limit of 1 x 10^4 CFU g^{-1} established by Good Manufacturing Practices (GMP, 2008) for feedstuff. The levels of fungal contamination in silage were among the highest. All tested samples were positive for low levels of fungi, with a maximum of 2.10 x 10^5 CFU g^{-1} and 80% had values of total fungal counts greater than 1 x 10^4 CFU g^{-1} (Table 4).

Sampling period	Total fungal count (CFU g^{-1})				
	Dairy cows feedstuff				
	Ingredients				Finished feed
	Corn grains	Cotton seed	Brewer's grains	Corn Silage	
April-May	5,50 x 10^5 **c**	2,10 x 10^5 **ab**	1,70 x 10^6 **b**	5,13 x 10^7 **a**	9,17 x 10^6 **b**
June-July	3,65 x 10^5 **c**	5,00 x 10^4 **bc**	2,12 x 10^4 **a**	1,33 x 10^7 **a**	2,49 x 10^5 **b**
August-September	2,36 x 10^3 **c**	2,40 x 10^5 **c**	8,90 x 10^6 **a**	4,22 x 10^7 **a**	2,27 x 10^7 **b**
October-November	7,00 x 10^5 **a**	3,00 x 10^3 **a**	4,12 x 10^6 **a**	2,64 x 10^6 **a**	4,80 x 10^5 **a**
December	2,30 x 10^5 **b**	5,75 x 10^5 **b**	3,80 x 10^5 **a**	2,18 x 10^8 **b**	3,08 x 10^6 **b**

Different letters indicate significantly different values according to Fisher's least significant difference test (LSD) p=0.05. The count data were transformed to $\log_{10}$ (x +1) to achieve homogeneity of variance. Statistical results should be read vertically to each food type separately.

Table 3. Total fungal colony count (CFU g^{-1}) from food samples for dairy cows during different sampling periods.

The mean values of total fungal counts in the finished feed, varied between 10^5 and 10^7 CFU g^{-1}. During October and November the count levels found were low, while during other periods of the year the pollution levels were consistent with each other. As it can be seen, 100% of the silage samples exceeded the limit of hygienic quality. It is important to observe that silage samples exceeded 100% the GMP recommendation in lower and upper sample sections.

Dairy cattle feedstuff		Total fungal count (CFU g^{-1})	
		Contaminated samples (%)	Simples exceeding HLQ (%)[b]
Ingredients	Corn grains	80	90
	Cotton seed	100	80
	Brewer's grains	100	100
	Corn silage Upper	92	100
	Corn silage Middle	85	81
	Corn silage Lower	100	100
Finished feed		90	100

[b] LCH: Hygienic limit quality by GMP (2008) $1x10^4$ CFU g^{-1}.

Table 4. Samples percentage that exceeded hygienic limit according to good manufacturing practices (GMP, 2008).

Figure 5 shows the log_{10} CFU g^{-1} for each type of food. Silage was the substrate with higher levels of pollution, followed by cotton seed. These substrates have a difference of almost two log_{10} units in relation to the other contaminated foods. The principal component analysis indicates that both components made the greatest contribution of fungal contamination to finished feed (Figure 4).

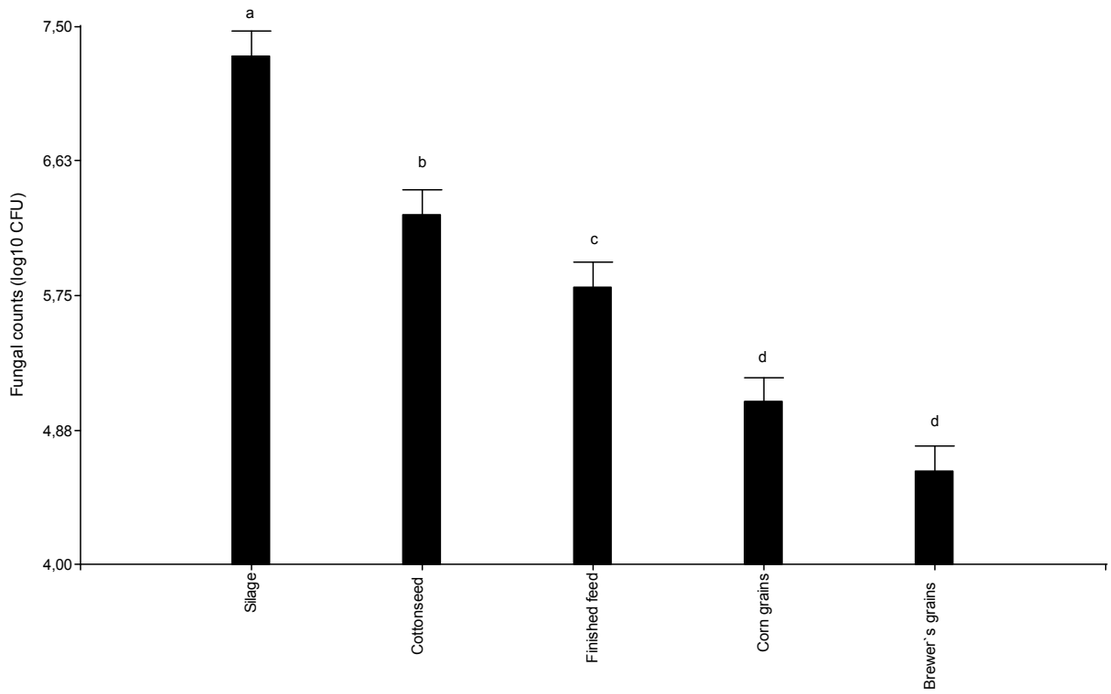


Fig. 5. total fungal count (Log_{10}CFU g^{-1}) of ingredients and finished feed for dairy cattle consumption.

8. Fungal genera distribution

Mycological survey of the strains isolated from different feeds, showed that the main toxigenic genera were present at high levels and in all types of feed samples.

Finished feed samples showed a high variability in their isolation, finding a high frequency of *Aspergillus spp*, in all sampling periods. They were isolated in 100% of the samples during the periods April-May, June-July and December (Figure 6).
Fusarium spp. were also one of the most frequent followed by yeasts. Penicillium spp were isolated throughout the sampling, although less frequent. They were isolated from 50% samples during June to July and August-September. December had further fungal diversity.

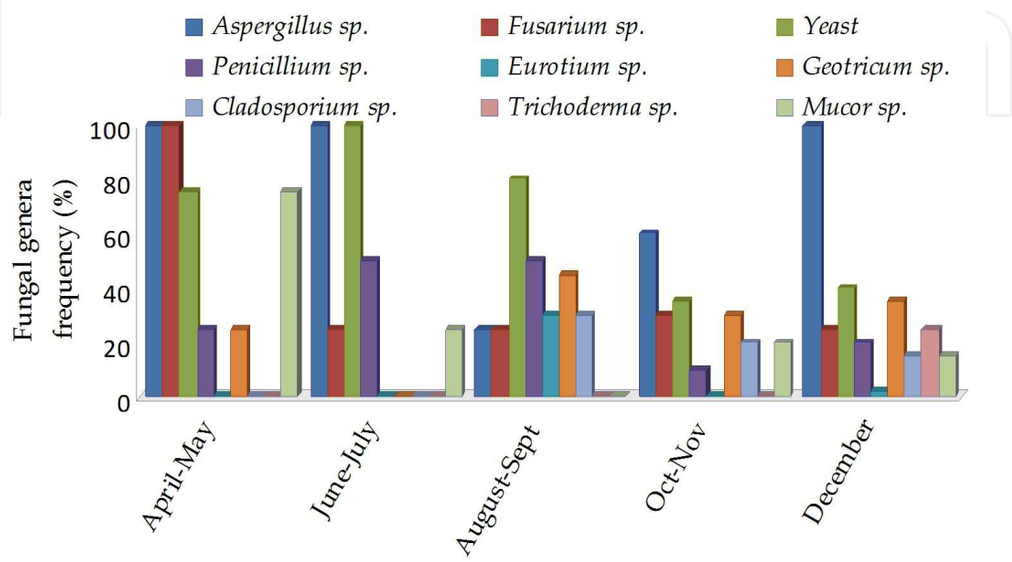


Fig. 6. Frequency of isolation of fungal genera (%) in finished feed during different sampling periods.

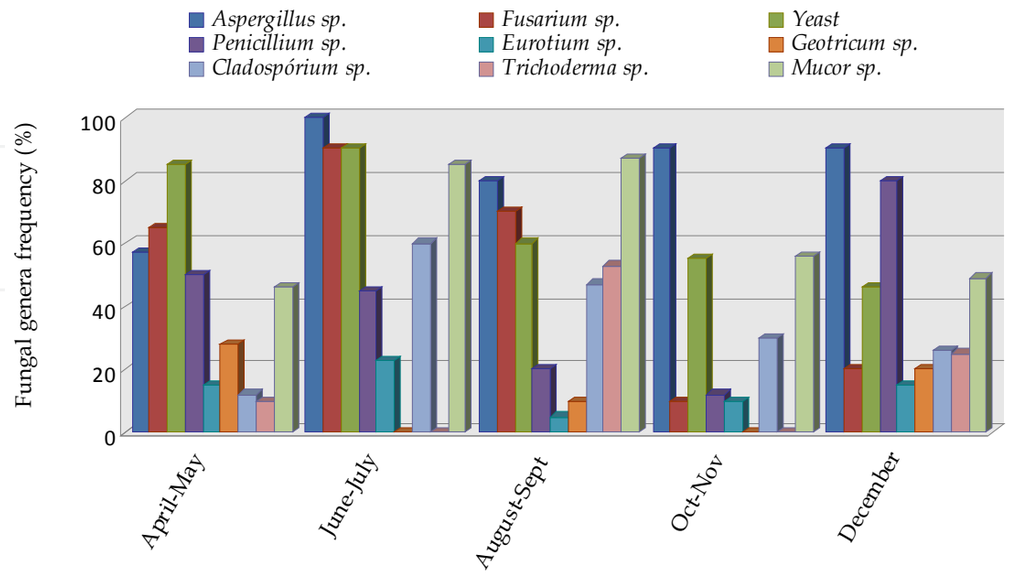


Fig. 7. Frequency of isolation of fungal genera (%) present in silage maize at different sampling periods.

The distribution of fungal genera in corn silage is presented in Figure 7. The incidence of important toxigenic genera was very high throughout the period. The rates of isolation of *Aspergillus spp* ranged between 90 and 100% in all sampling months, except for April-May, when the incidence, although lower, was also important (60%). For *Penicillium* spp. the isolation frequency was from 12 to 80% in December.
The incidence of *Fusarium spp* was high during the first three bimonthly sampling. They were isolated at 90% during the period June-July.

9. Incidence and toxigenic potential of *Aspergillus* section *Flavi*

It is of particular interest to describe the behaviour of the population of *Aspergillus* Section *Flavi*, its ability to produce AFs in silage for dairy cows, as it gives the possibility of contamination with aflatoxin B_1 in feedstuff. A widespread population of aflaoxicogenic *Aspergillus* has been described in raw materials and especially in silage samples intended for dairy cattle.

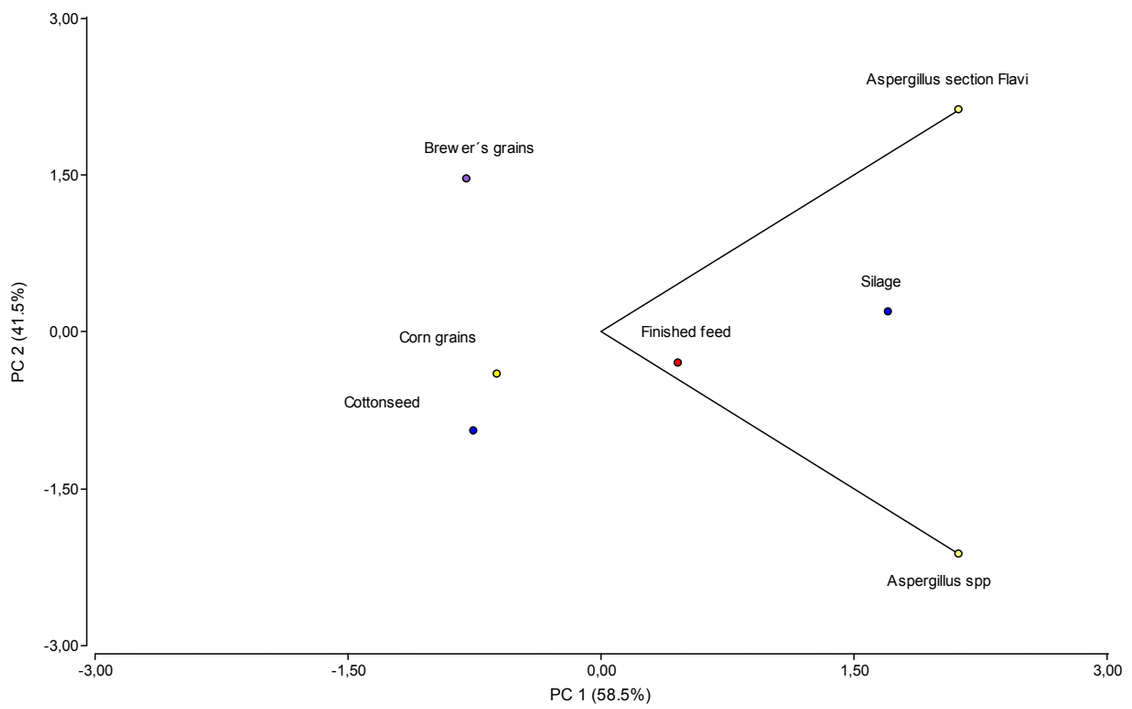


Fig. 8. Relative density of *Aspergillus* spp isolated from feed

Aspergillus flavus was also predominant in the other studied raw materials: 74% in cottonseed, 60.5% in corn and 39.7 in finished feed. Aspergillus parasiticus, although less frequent, it was present in all the substrates with rates of 5.6% in silage and 10% in cotton seed.
The finished feed showed a wide diversity of species, and *A. fumigatus* (19.7%) followed *A. flavus*. Analyzing the obtained results, it is estimated that the major contribution of this fungus to the finished feed comes from corn silage.

	Dairy cattle feedstuff	Total fungal count (CFU g^{-1})	
		Aspergillus genera	*Aspergillus* section *Flavi*
Ingredients	Corn grains	3,20 x 10^4 **b**	7,07 x 10^3 **b**
	Cotton seed	1,28 x 10^5 **bc**	3,03 x 10^4 **bc**
	Brewer's grains	0 **a**	0 **a**
	Corn silage	1,95 x 10^7 **d**	1,20 x 10^6 **d**
	Finished feed	6,06 x 10^5 **c**	3,72 x 10^4 **c**

Table 5. Total fungal counts of *Aspergillus* section *Flavi* and *Aspergillus sp* (CFU g^{-1}) from dairy cattle feedstuff during different sampling periods

Figure 9 shows the principal component analysis for *Aspergillus* spp variables depending on the kind of the studied feedstuff. This analysis shows that pattern of behaviour in relation to the kind of feed between the species of *Aspergillus* and *Aspergillus* section *Flavi*. There was a positive correlation between the presence of *Aspergillus* section *Flavi* and *Aspergillus* genera, according to the kind of feed (Figure 9). It is important to emphasize that silage was the ingredient with a greater presence of these fungi. Thus, this is ingredient that contributes with the greatest contamination to finished feed.

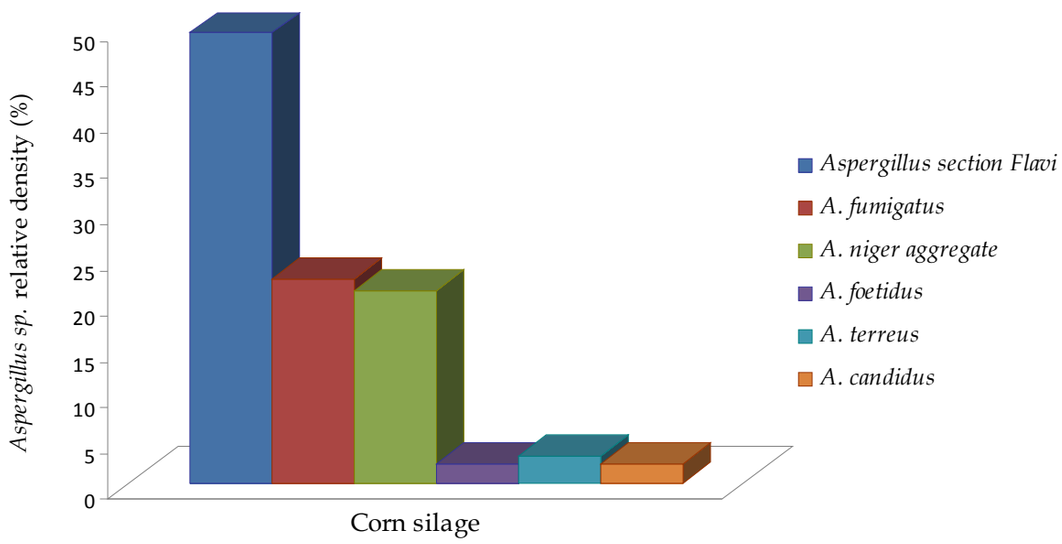


Fig. 9. Graph "biplot" principal analysis component of variable *Aspergilllus spp*. and *Aspergillus* section *Flavi* in relation to the kinds of feed.

10. Aflatoxin in silage

In cattle, chronic ingestion of mycotoxins causes various adverse effects such as increased susceptibility to disease, loss of reproductive performance, and in case of dairy cattle, a decrease in yield and quality of milk production. These effects are caused because the exposure of animals to mycotoxins causes a decrease in consumption or feed refusal,

a reduction of nutrient absorption, an impairment of metabolism, and changes in the endocrine and immune system suppression. Exposure of cattle to mycotoxins generally occurs through consumption of contaminated feed. Nelson et al., (1993) described as "mycotoxicosis" to diseases caused by exposure to food mycotoxin-contaminated rations.

Aflatoxins, particularly AFB_1 have been described both acute and chronic (Meggs, 2009). In June 2004, in Kenya there was an outbreak of acute aflatoxicosis, high levels of AFB_1 in stored corn at high humidity conditions were found (Lewis, 2005). Aflatoxin B_1 has been found in different countries as a contaminant in feed of dairy, cottonseed, barley, soy bran, pellet wheat, peanut shells, corn silage and sorghum silage (Decastelli et al., 2007; Sassahara et al., 2005).

For dairy cattle the problem does not end in animal disease or production losses since the mycotoxins in feed can lead to their presence or their metabolic products in dairy products which will be eventually affecting human health.

In the case of AFB_1, its presence in the food of dairy cattle leads to the emergence of AFM_1 in milk and dairy products (Boudra et al., 2007; Veldman et al., 1992).

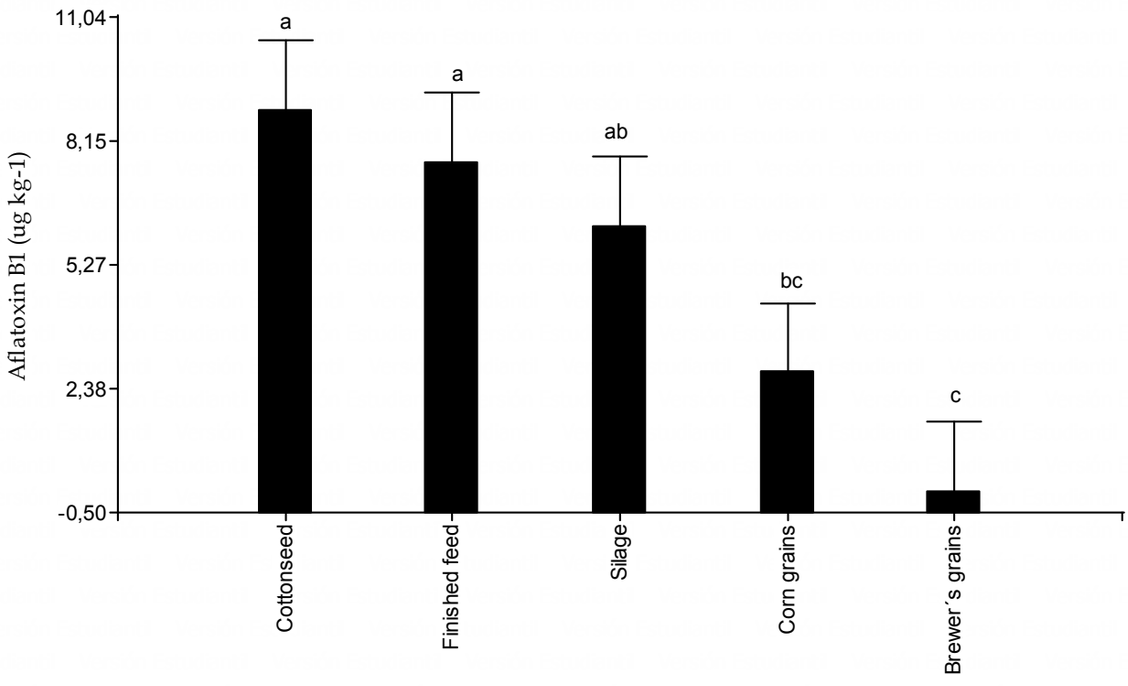


Fig. 10. AFB_1 levels in raw materials and finished feed intended for dairy cattle.

The natural occurrence of AFB_1 in feeds for dairy cows has shown that, in many cases aflatoxin levels exceeded regulation limits. Multivariate statistical studies show that silage makes the main contribution of AFB_1 to finished feed (Figure 11).

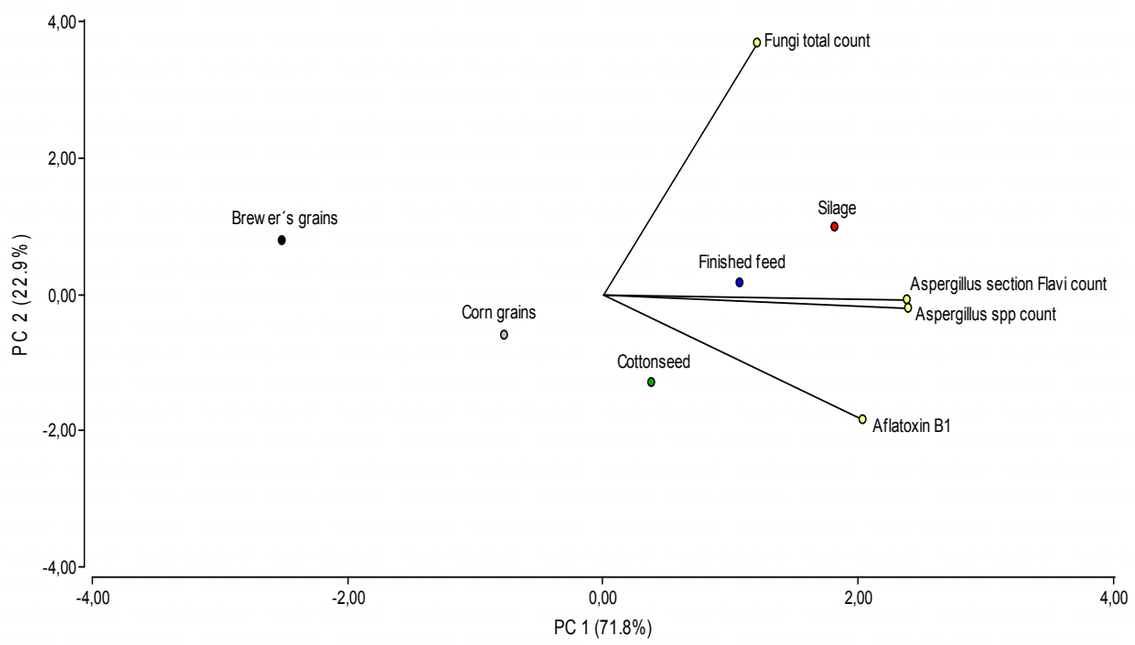


Fig. 11. Principal component analysis for variables: total fungal count, *Aspergillus spp* count, *Aspergillus* Section *Flavi* count and AFB_1 incidence.

11. References

Akande KE, Abubakar MM, Adegbola TA, Bogoro SE. (2006). "Nutricional and health implications of mycotoxins in animal feed: a review". Pakist J Nutrit. 5, 398-403.

Borreani G, Tabacco E. (2010). "The relationship of silage temperature with the microbiological status of the face of corn silage bunkers". J Dairy Sci. 93, 2620-2629.

Boudra H, Barnouin J, Dragacci S, Morgavi DP. (2007). "Aflatoxin M_1 and ochratoxin A in raw bulk milk from French dairy herds." J. Dairy Sci. 90, 3197-3201.

Chulze SN. (2010). "Strategies to reduce mycotoxin levels in maize during storage: a review". Food Additives and Contaminants" 27, 651–657

Decastelli L, Lai J, Gramaglia M, Monaco A, Nachtmann C, Oldano F, RuYer M, Sezian A, Bandirola C. (2007). "Aflatoxins occurrence in milk and feed in Northern Italy during 2004–2005". Food Control 18, 1263–1266.

Di Costanzo A, Johnston L, Felice L. Murphy M. (1995). "Effect of molds on nutrient content of feeds reviewed". Feed Stuffs. 16, 17-20.

Driehuis F, Oude Elferink SJ. (2001). "The impact of the quality of silage on animal health and food safety: a review". Vet Q. 22, 212-216.

El-Shanawany A, Eman A, Mostafa M. Barakat A. (2005). "Fungal populations and mycotoxins in silage in Assiut and Sohag governorates in Egypt, with a special reference to characteristic Aspergilli toxins". Mycopathologia. 159, 281–289.

Garon D, Richard E, Sage L, Bouchart V, Pottier D, Lebaill P. (2006). "Mycoflora and multimycotoxin detection in corn silage: experimental study." J Agricult. Food Chemist. 54, 3479-3484.

GMP (2008). Certification Scheme Animal Feed Sector, 2006. Version Marzo 2008. Appendix 1: Product standards (including residue standards). The Hague, the Netherlands: Productschap Diervoeder. pp 1 – 39.

Gonzalez Pereyra ML, Alonso VA, Sager R, Morlaco MB, Magnoli CE, Astoreca AL, Rosa CA, Chiacchiera SM, Dalcero AM, Cavaglieri LR. (2008a). "Fungi and selected mycotoxins from pre- and postfermented corn silage". J of App Microb. 104, 1034–1041.

Johnson LM, Harrison JH, Davidson D, Mahanna WC, Shinners K, Linder D. (2002). "Corn silage management: effects of maturity, inoculation, and mechanical processing on pack density and aerobic stability". J Dairy Sci. 85, 434-44

Lewis L, Onsongo M, Njapau H, Schurz-Rogers H, Luber G, Kieszak S, Nyamongo J, Backer L, Dahiye AM, Misore A, DeCock K, Rubin C. (2005). "Aflatoxin Contamination of Commercial Maize Products during an Outbreak of Acute Aflatoxicosis in Eastern and Central Kenya". Environ Health Perspect. 113, 1763-1767.

López A. (2008). Argentina Milk production systems. Red ICAARG. Cattle Milk Production Area. Veterinary Science Faculty of Buenos Aires, UBA.

Magan N, Aldred D. (2007). "Post-Harvest Control Strategies: Minimizing Mycotoxins In The Food Chain". Int J of Food Microbiol. 119, 131-139.

Meggs W. (2009). "Epidemics of mold poisoning past and present". Toxicol and Indust Health 25, 9-12.

Molina AM, Roa LB, Alzate SR, Serna de León JG, Arango AF. (2004). "Silage as a livestock feed source". Rev Lasall Investig. 1, 66-71.

O´Brien M, O`Kiely P, Forristal P, Fuller HT. (2005). "Fungi isolated from contaminated baled grass silage on farms in the Irish Midlands". Microbiology Letters. 247, 131–135.

Rosa CA, Cavaglieri LR, Ribeiro JMM, Keller KM, Alonso VA, Chiacchiera SM, Dalcero AM, Lopes CWG. (2008). "Mycobiota and naturally-occurring ochratoxin A in dairy cattle feed from the Río de Janeiro State, Brazil." World Mycot J. 1, 195-201.

Sassahara M, Pontes D, Yanaka K. (2005). "Aflatoxin occurrence in foodstuff supplied to dairy cattle and aflatoxin M1 in raw milk in the north of Paraná state". Food and Chem Toxicol. 43, 981-984.

Scott WJ. (1957) "Water relations of food spoilage micro-organisms". Advan Food Res 7, 83-127.

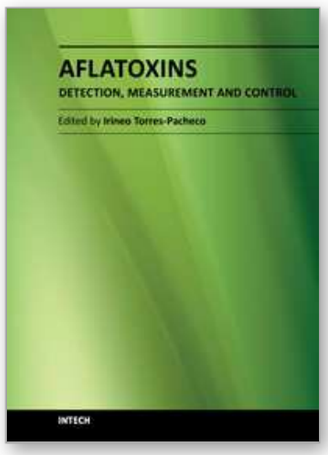
Scudamore K A, Livesey CT. (1998). "Occurrence and significance of mycotoxins in forage crops and silage: A review". J. Sci. Food Agric. 77, 1–17.

Simas MS, Botura MB, Correa B, Sabino M, Mallmann CA, Bitencourt T, Batatinha M. (2007). "Determination of fungal microbiota and mycotoxins in brewers grain used in dairy cattle feeding in the State of Bahia, Brazil". Food Control. 18, 404-408.

Teimouri Yansari A, Valizadeh R, Naserian A, Christensen DA, Yu P, Eftekhari Shahroodi F. (2004). "Effects of alfalfa particle size and specific gravity on chewing activity, digestibility, and performance of Holstein dairy cows". J Dairy Sci. 87, 3912-24.

Veldman AJ, Meijs AC, Borggreve GJ, Heeres van der Tol JJ. (1992). "Carry-over of aflatoxina from cows' food to milk." Anim Prod. 55, 163–168.

West JW. 2003. "Effects of Heat-Stress on Production in Dairy Cattle". Dairy Science, 86:2131-2144.



Aflatoxins - Detection, Measurement and Control

Edited by Dr Irineo Torres-Pacheco

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This book is divided into three sections. The section called Aflatoxin Contamination discusses the importance that this subject has for a country like the case of China and mentions examples that illustrate the ubiquity of aflatoxins in various commodities The section Measurement and Analysis, describes the concept of measurement and analysis of aflatoxins from a historical prespective, the legal, and the state of the art in methodologies and techniques. Finally the section entitled Approaches for Prevention and Control of Aflatoxins on Crops and on Different Foods, describes actions to prevent and mitigate the genotoxic effect of one of the most conspicuous aflatoxins, AFB1. In turn, it points out interventions to reduce identified aflatoxin-induced illness at agricultural, dietary and strategies that can control aflatoxin. Besides the preventive management, several approaches have been employed, including physical, chemical biological treatments and solvent extraction to detoxify AF in contaminated feeds and feedstuffs.

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