Incidence of Mycotoxins (AFB₁ and AFM₁) in Feeds and Dairy Farms from Rio de Janeiro State, Brazil

Luiz Antonio Moura Keller, PhD¹; Marcos Aronovich, PhD²; Kelly Moura Keller, PhD³; Antonio Airton Castagna, DSc²; Lilia Renee Cavaglieri, PhD⁴; Carlos Alberto da Rocha Rosa, LD, PhD⁵*

¹Departmento de Zootecnia e Desenvolvimento Agrossocioambiental Sustentável, Universidade Federal Fluminense (UFF), Rua Vital Brasil Filho, 64, Vital Brasil, Niterói, RJ 24220-900, Brazil
²Empresa de Pesquisa Agropecuária do Estado do Rio de Janeiro (PESAGRO-RJ), Alameda São Boaventura, 770, Fonseca, Niterói, RJ 24120-191, Brazil
³Departamento de Medicina Veterinária Preventiva, Universidade Federal de Minas Gerais, Av. Presidente Carlos Luz, 4466, São Luiz, Belo Horizonte, MG 31250-810, Brazil
⁴Departamento de Microbiologia e Imunologia. Universidad Nacional de Río Cuarto. Ruta 36 km 601, Río Cuarto, Córdoba, 5800, Argentina
⁵Departmento de Microbiologia e Imunologia Veterinária, Universidade Federal Rural do Rio de Janeiro (UFRRJ); Conselho Nacional de Pesquisa Científica e Tecnológica (CNPQ), Rodovia BR 465 - Km 7 - Campus Universitário - Zona Rural, Seropédica, RJ 23890-000, Brazil

*Corresponding author
Carlos Alberto da Rocha Rosa , LD, PhD
Departamento de Microbiologia e Imunologia Veterinária
Universidade Federal Rural do Rio de Janeiro (UFRRJ)
Seropédica, RJ 23890-000, Brazil
Tel. +552126814610
Fax: +552126814610
E-mail: shalako1953@gmail.com

ABSTRACT

Brazil has regions located at the largest dairy production and milk derive industry concentration, supplying the major consumer markets, represented by São Paulo, Rio de Janeiro and Belo Horizonte Cities. The milk is the most important product of Brazilian agriculture, because it is always presents in daily diet, enhancing...
the quest for product quality shall be continuous.²

Mycotoxins are metabolites produced by certain species of filamentous fungus and can cause various toxic effects in animals.³ Human and animal exposure to aflatoxins (AFs) can occur primarily by ingesting contaminated food and feed, mainly cereals and grains, such as corn, wheat, and peanuts, among others. Eighteen (18) different types of AFs were identified, but only aflatoxin B₁(AFB₁) B₂(AFB₂) G₁(AFG₁) and G₂(AFG₂) were detected as natural contaminants and feed ingredients and AFB₁ toxin has a great toxicity.⁴⁶ A continuous intake of AFB₁ poses the final sample for each basin from selected region. These isolates. ³⁰ Quantitative enumeration was done using the surface-spread method. Ten grams of each sample were homogenized in 90 mL 0.1% peptone water solution for 30 min in an orbital shaker. Serial dilutions (10⁻² to 10⁻³) were made and 0.1 mL aliquots were inoculated in duplicates onto the culture media. Plates were incubated at 25 °C for 7-10 days in darkness. Nash-Snyder plates were incubated at 24 °C for 7 days under a 12 h cold white/12 h black fluorescent light photoperiod. Only plates containing 10-100 colony-forming units (CFU) were used for counting. The results were expressed as CFU per gram of sample (CFU.g⁻¹). Representative colonies of Aspergillus and Penicillium spp. were transferred for sub-culturing to tubes containing malt extract agar (MEA) and Fusarium spp. were transferred to carnation leaf agar (CLA). Fungal species were identified according to taxonomic specific protocols.²⁵⁻²⁷ The results were expressed as isolation frequency of the fungal genera (% of samples in which each genera was present) and relative density of each fungal species (% of isolation of each species among strains of the same genera).²³

Toxicogenic Profile of Fungal Isolates

The ability to produce ochratoxin A (OTA) by potentially producer strains isolated from samples (A. carbonarius, A. niger aggregate and A. ochraceus) was tested.²⁸ Aflatoxins production was evaluated in all Aspergillus section Flavi isolates.²⁹ Fusarium-toxins: primarily fumonisín B₁(FB₁) and zearalenone (ZEA), produced by the isolates.³⁰

Mycotoxins Analysis in Feed

For mycotoxins determination in the feed, the samples were evaluated for screening method to qualify toxigenic conditions and make a quantitative previous evaluation. The Vicam® fluorometer (4ex series) engaged with specific immunoaffinity columns (Vicam® Watertown, MA, USA). All positive samples were confirmed by a High Performance Liquid Chromatography (HPLC) evaluation.

The AFB₁ determination in the feed samples was done
in parallel of screening method, following the procedures recommended by the manufacturer of solid phase and cleanup phase columns Mycosep (Romer® Labs, Inc., Union, MO, USA) and evaluated by HPLC.

All feed samples were previously crushed and homogenized. Then, the analytical sample was placed in a blender along with specific solvent. The extract was filtered and collected for passage through the columns. The separation and quantification of mycotoxins was conducted on a HPLC system (JASCO LC 2000, Tokyo, Japan) equipped with a fluorescence detector (excitation: 360 nm and emission: 440 nm). The quantification of mycotoxins in the samples was performed by interpolation of the areas of the chromatographic peaks obtained in the samples in the calibration curve regression equation with specific external standards (Sigma-Aldrich, St. Louis, MO, USA).

**Analysis of aflatoxin M**: The extraction and purification of samples for the determination of AFM₁ were performed in duplicate according to the recommendations, with adjustments proposed by the manufacturer of immunoaffinity columns (Aflatest® Vicam, Watertown, MA, USA) as described by Oliveira et al. In summary, the analytical sample (25 mL) was preheated to 37 °C, added with 1 g of NaCl, and subject to centrifugation (2,500 g, 15 min), after which it was directly passed through immunoaffinity column connected to a vacuum system (flow 2-3 mL.min⁻¹). After the sample elution, the column was washed by passing 20 mL of ultrapure water (Milli Q, Millipore) and methanol (9:1, v:v⁻¹). The final purified elute was diluted with ultrapure water to form a solution of methanol-water (7:3, v:v⁻¹), similar to the HPLC mobile phase. The identification and quantification of AFM₁, residues were conducted with the injection of 20 µL of the extracts of the samples in the HPLC system (JASCO LC 2000) at a flow rate of 0.8 mL.min⁻¹. Under these conditions, the retention time for approximately 3.7 min. The calibration curve was prepared using AFM₁ standard (Sigma, St. Louis, MO, USA) previously solutions evaluated according to Scott (1990), at doses of 0.5, 1.0, 2.5, 5.0 and 10.0 ng.mL⁻¹.

**Statistical Analysis**

Data analysis were performed by analysis of variance (ANOVA). The test of least-significant differences (LSD) was used to determine the significant differences between means. Analysis was conducted using PROC GLM in SAS (SAS Institute, Cary, NC, USA). Statistical significance was indicated by p≤0.05.

**RESULTS**

Physical evaluation of the samples did not show significant differences in DM%, pH and a<sub>w</sub> values for seasonal samples. The mean (DM%) of 47.17±5.76%, pH values varied from 3.88 to 4.89 and a<sub>w</sub> varying between 0.729 and 0.985.

The total Fungal Counts are specified at Table 1 (7.3*10⁻¹-1.4*10⁵ CFU.g⁻¹). The profiles of isolated strains, Table 2, approximately sixty percent of *A. flavus* and *A. parasiticus* (79 out of 136 isolates), were able to produce AFB 1 and AFB 2 at ranges from 0.2 to 8.0 µg.g⁻¹. Twenty five strains of *A. niger* aggregate isolates (23%) showed ability to produce at ranges from 0.05 to 10.0 µg.g⁻¹ of OTA. All strains of *F. verticillioides* able to produce FB, at ranges from 0.2 to 8.0 µg.g⁻¹.

When the samples of feed contaminated with AFB, were compared, the range levels (0.2-50.0 µg.kg⁻¹) and the frequency of the contaminated milk samples showed in 75% with AFM₁, at range levels (0.05-1.50 µg.L⁻¹). The quantification limits for AFB, and AFM₁ were 0.02 and 0.05 µg.L⁻¹, respectively, considering the minimum amount of toxin that could produce a chromatographic peak three times the baseline standard deviation. The Table 2 shows data on milk yield level, the number of posi-

| Samples       | Season | Total fungal counts (CFU g⁻¹) Mean ± SD       | Culture media |
|---------------|--------|-----------------------------------------------|---------------|
|               |        | DRBC                                              | DG18                                                     |
| Corn and Corn meal | Su  | 5.8x10⁵±1.0x10⁴ <i>b</i> 5.8x10⁵±1.0x10⁴ <i>b</i> |               |
|                | Au    | 4.3x10⁴±1.6x10<i>b</i> 4.3x10⁴±1.6x10<i>b</i> |               |
|                | Wi    | 3.4x10⁴±1.5x10⁴ <i>b</i> 4.4x10⁴±1.5x10⁴ <i>b</i> |               |
|                | Sp    | 5.7x10⁴±1.5x10⁴ 5.7x10⁴±1.5x10⁴ |               |
| Corn Silage and Wheat Brew Silage | Su  | 7.3x10⁵±2.8x10⁵ 3.4x10⁴±1.4x10⁴ |               |
|                | Au    | 9.2x10⁴±9.1x10⁴ 3.6x10⁴±1.0x10⁴ |               |
|                | Wi    | 7.3x10⁴±1.0 x 10⁴ 3.6x10⁴±1.3x10⁴ |               |
|                | Sp    | 1.3x10⁵±6.5x10⁴ 3.8x10⁴±1.1x10⁴ |               |

<i>b</i> Values indicated with different letters are significantly different according to LSD test (<i>p</i>&lt;0.05 for seasonal period samples). Su: Summer, Au: autumn, Wi: winter, Sp: spring.

Table 1: Total fungal counts (CFU g⁻¹) found in feed samples collected from different farms in four (4) seasonal periods of the year and evaluated on DRBC and DG18 media.
The incidence of AFM\textsubscript{1} in all milk samples was 25.45% at concentrations ranging from 0.05 to 1.500 μg.L\textsuperscript{-1}. This frequency was consistent with the detection of AFM\textsubscript{1} at all times of milk samples selected. Farm samples with milk production between 150 and 300 L/day showed also positive samples for AFM\textsubscript{1} (38.90%).

**DISCUSSION**

The results of differences in DM%, pH and a\textsubscript{w} values for seasonal samples are comparable to those obtained by Keller et al\textsuperscript{33} in Brazil and González Pereyra et al\textsuperscript{34} in Argentina.

Total fungal counts present in 67% of feed samples, Table 1, exceeded the limit recommended as quality standard (1×10\textsuperscript{4} UFC.g\textsuperscript{-1}) proposed by GMP\textsuperscript{16} and Brazilian regulation.\textsuperscript{35} The total fungi isolated were increased during rainy season and temperature rise similar as those reported for other regions.\textsuperscript{36,37} Mycobiota isolated from several feed samples were comparable to species found by other researchers from the same substrate in Brazil, Argentina, France and Egypt being potentially toxigenic species A. flavus, A. parasiticus, A. fumigatus, A. niger aggregate, P. citrinum, F. verticillioides and F. graminearum prevailed on this substrate. Aspergillus flavus and A. fumigatus relative density in post-fermentation silage samples was higher than in pre-fermentation samples.\textsuperscript{36,40}

The screening of feed samples allowed evaluating a concurrence of several mycotoxins. The AFB\textsubscript{1} contamination was detected in all samples, areas and seasonal periods, many of them were exceeded the recommended limit for AFB\textsubscript{1} in cattle feed (20 μg.kg\textsuperscript{-1}) proposed by GMP. Also, OTA, FB\textsubscript{1}, and ZEA were detected. However, when the increase of the mycotoxin level on severe seasonal conditions was evaluated, the statistical differences were not found.

This evaluation suggests that mycotoxins contamination was enhanced during storage.\textsuperscript{41} The ensiling process supposes control fungal contamination since pH is reduced to an extremely acid condition and oxygen is consumed to anaerobiosis. However, bad storing condition and practices during the ensiling process or even after the silo is opened for feeding-out, can lead to this kind of contamination.\textsuperscript{42}

The AFB\textsubscript{1} levels observed in the feed samples remained below the tolerance limit recommended (50.0 μg.kg\textsuperscript{-1}) for feed ingredients in Brazil.\textsuperscript{53} The research reveals that toxigenic fungi and their mycotoxins are present in feed intended for bovine fed in Rio de Janeiro farms, as occurs in other Brazilian States. Subsequent evaluations of mycotoxin levels are important to provide information, so that the assessments of risk for animal feed and livestock environment can be made.

Sabino et al\textsuperscript{44} found 18% positive samples at levels from 0.10 to 1.68 μg.L\textsuperscript{-1} in the San Pablo State. Recently, Sassahara et al\textsuperscript{45} and Oliveira et al\textsuperscript{46} found AFM\textsubscript{1} at levels from 0.29 to 1.15 μg.L\textsuperscript{-1} in the San Pablo State.
to 1.97 μg.L⁻¹ in 24% of raw milk samples collected from farms in the Paraná and São Paulo States, respectively. The process of modernization of the milk chain production in order to conform to current standards regulations in Brazil, has promoted a significant increase in milk production, enabling the export of dairy products. This increase is due in part to the extensive supply of rations to animals, especially in the off season months (autumn and winter), which may have contributed to obtain detectable levels of AFM₁ in milk of the studied regions.

Sampling performed during the months of spring and summer obtained all samples with AFM, above 0.5 μg.L⁻¹. However, 10% of the milk samples collected on other mouths had higher levels of AFM₁ tolerance limit adopted by the European Union (0.050 μg.L⁻¹).

This study shows the need to revise the legislation for AFs in rations and AFM₁ in raw milk in Brazil to prevent the occurrence of AFB₁ and other mycotoxins in feed ingredients for dairy cattle and consequently human toxicity. On addition reinforces the importance of the revision of standard, in order to establish consistent limits for mycotoxins in feed ingredients intended for dairy cattle.

ACKNOWLEDGEMENTS

This work was conducted with support from CNPq (EDITAL UNIVERSAL - 480548/2011-0), FAPUR/UFRRJ, PESAGRO-RJ.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. EMBRAPA, Empresa Brasileira de Pesquisa Agropecuária. O Agronegócio do Leite no Brasil. Juiz de Fora, MG, Brazil: Gado de Leite; 2006: 262.

2. IBGE. Pesquisa Pecuária Municipal. Rio de Janeiro, Brazil 2014.

3. CAST, Council for Agriculture Science and Technology. Mycotoxins: Risks in Plant, Animal, and Human Systems. Ames, Iowa, USA: CAST; 2003.

4. Hussein HS, Brasel JM. Toxicity metabolism and impact of mycotoxins on humans and animals. Toxicology; 2001; 167: 101-134. Web site: http://www.ncbi.nlm.nih.gov/pubmed/11567776. Accessed April 16, 2016

5. Jonker MA, Van Egmond HP, Stephany RW. Mycotoxins in Food of Animal Origin: A Review in CRL, Document 389002/095 from European Commission. Bithoven, Netherlands, England: European Union Community reference laboratory and National Institute of Public Health and the Environment; 1999: 1: 1-39.

6. Moss MO. Recent studies of mycotoxins. Journal of Applied Microbiology Symposium. 1998; 84: 62S-76S. doi: 10.1046/j.1365-2672.1998.0840s162S.x

7. Park DL, Pohland AE. The rationale for the control of aflatoxin in the animal feeds. In: Steyn PS, Vleggaar R, eds. Mycotoxins and Phycotoxins. Amsterdam, The Netherlands: Elsevier Applied Science; 1986: 473-482.

8. Riley RT, Pestka J. Mycotoxins: Metabolism, mechanisms and biochemical markers. In: Diaz DE, ed. The Mycotoxin Blue Book. Nottingham, England: Nottingham University Press; 2005: 279-294.

9. IARC, International Agency for Research on Cancer. Some Naturally Occurring Substances: Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins Food. Lyon, France: IARS; 1993: 56.

10. Alonso VA, Monge MP, Larriestra A, Dalcero AM, Cava- glieri LR, Chiacchiera SM. Naturally occurring aflatoxin M₁ in raw bulk milk from farm cooling tanks in Argentina. Food Addit Contam. 2010; 27: 373-379. doi: 10.1080/1944040903403362

11. Garrido NS, Iha MH, Orlotani MRS, Fávaro RMD. Occurrence of aflatoxins M₁ and M₂ in milk commercialized in Ri-beirão Preto-SP, Brazil. Food Addit Contam. 2003; 20: 70-73. doi: 10.1080/0265203021000035371

12. Lópeze CE, Ramos LL, Ramadá SS, Bulacio LC. Presence of aflatoxin M₁ in milk for human consumption in Argentina. Food Control. 2003; 14: 31-34. Web site: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3908700/. Accessed April 16, 2016

13. Oliveira C, Rosemary J, Rosim R. Aflatoxin M₁ and cyclopi- azonic acid in fluid milk traded in São Paulo, Brazil. Food Addit Contam. 2006; 23: 196-201. doi: 10.1080/02652030500398379

14. Prado G, Oliveira MS, Abrantes FM, Santos LG, Soares CR, Veloso T. Ocorrência de aflatoxina M₁ em leite consumido na cidade de Belo Horizonte, Minas Gerais, Brasil, agosto/98 to abril/99. Ciencia Tecnol Alim. 2009; 19(3): 420-423. doi: 10.1590/S0101-20611999000300022

15. Sabino M, Purchio A, Zorzetto AP. Variations in the level of aflatoxin in milk cows Consumed in the city of São Paulo, Brazil. Food Addit Contam. 1989; 6: 321-326. doi: 10.1080/02652038909373786

16. Brazil. Ministry of Agriculture, Livestock and Supply - MAPA. Normative Instruction n. 51 of 18 September 2002b. Approves technical regulations of production, identity and quality of the milk type, milk type B, type C milk, pasteurized and
refrigerated raw milk and the technical regulations of cooled raw milk collection and bulk transport. Official Gazette, Executive Branch, Brasilia, DF, September 20, 2002. Web site: http://www.scielo.br/pdf/v70n4/a03v70n4.pdf. Accessed April 16, 2016

17. Keller LAM, Keller KM, Monge MP, et al. Glutathione in and pre- and post-fermented corn, sorghum and wet betters’ grains silage in Sao Paulo and Rio de Janeiro State, Brazil. J Appl Microbiol. 2012; 112: 865-873. doi: 10.1111/j.1365-2672.2012.05273.x

18. Keller LAM, Keller KM, Monteiro BS, et al. Micobiota and micotoxins em resíduo úmido de cervejaria [In Portuguese]. Rev Bras Med Vet. 2010; 31: 247-252.

19. Pereira MMG, Carvalho EP, Prado G, et al. Aflatoxins contamination in and pre- and post-fermented corn, sorghum and wet brewer’s grains silage in Sao Paulo and Rio de Janeiro State, Brazil. J Appl Microbiol. 2012; 112: 865-873. doi: 10.1111/j.1365-2672.2012.05273.x

20. Sassahara M, Yanaka EK, Neto DP. Ocorrência de aflatoxina e zearealenona em alimentos destinados a bovinos e em amostras de leite da região de Lavras, Minas Gerais, Brasil [In Portuguese]. Ciencia Agrotec, Lavras. 2005; 29: 106-112. doi: 10.1590/S1413-70542005000100013

21. Ohyama Y, Masaki S, Hara S. Factors influencing aerobic deterioration of silages and changes in chemical composition after opening silos. J Sci Food Agric. 1975; 26: 1137-1147. doi: 10.1002/jsfa.2740260811

22. Abarca ML, Bragulat MR, Castellá G, Cabañes FJ. Mycobiota and aflatoxin-producing strains in animal mixed feeds. J Food Prot. 1994; 57: 256-258.

23. Pitt JI, Hocking AD. Fungi and Food Spoilage. 2nd ed. London, UK: Blackie Academic Press; 1997.

24. Nelson PE, Toussoun TA, Marasas WFO. Fusarium Species: An Illustrated Manual for Identification. University Park, PA, USA: The Pennsylvania State University Press; 1983.

25. Pitt JL, Hocking AD. Fungi and Food. London, UK: Black Academia and Professional Chapman and Hall; 1997: 593.

26. Nelson PT, Toussoun TAA, Marasas WEO. Fusarium Species: An Illustrated Manual for Identification. University Park, PA, London: The Pennsylvania State University Press; 1983: 479.

27. Samson RA. Introduction to Food and Airborne Fungi. 6th ed. Utrecht, The Netherlands: Central bureau Voor Schimmelcultures, Institute of the Royal Netherlands Academy of Arts and Sciences. 2000: 388.

28. Téren J, Varga J, Hamari Z, Rinyu E, Kevei F. Immunochemical detection of ochratoxin A in black Aspergillus strains. Mycopathologia. 1996; 134: 171-176. Web site. http://www.ncbi.nlm.nih.gov/pubmed/8981783. Accessed April 16, 2016.

29. Abarca ML, Bragulat MR, Castellá G, Cabañes FJ. Mycoflora and aflatoxin-producing strains in animal mixed feeds. J Food Prot. 1994; 57: 256-258. Web site. http://www.ncbi.nlm.nih.gov/pubmed/8981783. Accessed April 16, 2016.

30. González Pereyra ML, Alonso VA, Sager R, et al. Fungi and selected mycotoxins from pre- and post-fermented corn silage. J Appl Microbiol. 2008; 104: 1034-1041. doi: 10.1111/j.1365-2672.2007.03634.x

31. Tuijnstra LG, Roos AH, Van Trijp JM. Liquid chromatographic determination of aflatoxin M1 in milk powder using immunoaffinity columns for clean-up: Interlaboratory study. J AOAC. 1993; 76: 1248-1254. Web site. http://www.ncbi.nlm.nih.gov/pubmed/8286964. Accessed April 16, 2016.

32. Oliveira CAFS, Fagundes LS, Rosim H, Eliana R, Fernandes AM. Determinação de aflatoxina B1 em rações e aflatoxina M1 no leite de propriedades do Estado de São Paulo [In Portuguese]. C Tecnol Alim Campinas. 2010; 30(Suppl 1). doi: 10.1590/S0101-206120100005000034

33. Keller LAM, Pereyra M, Keller KM. Fungal and myco-toxins contamination in corn silage: Monitoring risk before and after fermentation. Journal of Stor Prod Res. 2013; 52: 42-47.

34. González Pereyra ML, Alonso VA, Sager R, et al. Fungi and selected mycotoxins from pre- and post-fermented corn silage. J Appl Microbiol. 2008; 104: 1034-1041. doi: 10.1111/j.1365-2672.2007.03634.x

35. GMP, Good Manufacture Practice. Certification Scheme Animal Feed Sector 2006, Including Residue Standards. The Netherlands: Productschap Diervoeder; 2008.

36. Jouany JP, Díaz DE. Effects of mycotoxins in ruminants. In: Díaz DE, ed. The Mycotoxin Blue Book. Nottingham, England: Nottingham University Press; 2005: 295-321.

37. Moreno EC, García GT, Ono MA, et al. Co-occurrence of mycotoxins in corn samples from the Northern region of Paraná State, Brazil. Food Chem. 2009; 116: 220-226. Web site. http://agris.fao.org/agris-search/search.do?recordID=US201301622144. Accessed April 16, 2016.

38. El-Shanawany AA, Eman Mostafa M, Barakat A. Fungal populations and mycotoxins in silage in Assiut and Sohag governates in Egypt, with a special reference to characteristic Aspergilli toxins. Mycopathologia. 2005; 159: 281-289. doi: 10.1007/s11046-004-5494-1

39. Richard E, Heutte N, Bouchart V, Garon D. Evaluation of
fungal contamination and mycotoxin production in maize silage. *Animal Feed Sci Technol.* 2009; 148: 309-320.

40. Richard E, Heutte N, Sage L, et al. Toxigenic fungi and mycotoxins in mature corn silage. *Food Chem Toxicol.* 2007; 45: 2420-2425. Web site. [http://www.ncbi.nlm.nih.gov/pubmed/17655998](http://www.ncbi.nlm.nih.gov/pubmed/17655998). Accessed April 16, 2016

41. Dos Santos VM, Dorner JW, Carreira F. Isolation and toxigenicity of Aspergillus fumigatus from moldy silage. *Mycopathologia.* 2003; 156: 133-138. Web site. [http://www.ncbi.nlm.nih.gov/pubmed/12733634](http://www.ncbi.nlm.nih.gov/pubmed/12733634). Accessed April 16, 2016

42. Keller LAM, Keller KM, Monge MP, et al. Gliotoxin contamination in and pre- and post-fermented corn, sorghum and wet brewer’s grains silage in Sao Paulo and Rio de Janeiro State, Brazil. *Journal of Appl Microbiol.* 2012; 112: 865-873. doi: 10.1111/j.1365-2672.2012.05273.x

43. BRASIL - Ministério da Agricultura, Pecuária e Abastecimento (MAPA). Padrões mínimos de matéria prima empregada na alimentação animal. 1988.

44. Sabino M, Purchio A, Zorzetto AP. Variations in the level of aflatoxin in milk cows consumed in the city of São Paulo, Brazil. *Food Addit Contam.* 1989; 6: 321-326. doi: 10.1080/02652038909373786

45. Sassahara M, Yanaka EK, Netto DP. Ocorrência de aflatoxina e zearalenona em alimentos destinados ao gado leiteiro na Região Norte do Estado do Paraná [In Portuguese]. *Semina: Ciências Agrárias, Londrina.* 2003; 24: 63-72. doi: 10.5433/1679-0359.2003v24n1p63

46. Oliveira CAFS, Fagundes LS, Rosim H, Eliana R, Femandes AM. Determinação de aflatoxina B, em rações e aflatoxina M, no leite de propriedades do Estado de São Paulo [In Portuguese]. *C Tecnol Alim, Campinas.* 2010; 30(Suppl 1). Web site. [http://vufind.uniovi.es/Record/oai%3Adoaj.orgarticle%3Adc-7188969c84df5a38039ba98838c5a](http://vufind.uniovi.es/Record/oai%3Adoaj.orgarticle%3Adc-7188969c84df5a38039ba98838c5a). Accessed April 14, 2015