

Presence of Mycotoxins in Feed and Dairy Products of Cattle in Paraná, Brazil

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Abstract

Milk contamination by mycotoxins is considered a public health problem. Therefore, the objective of this study was to identify these contaminants in concentrates and in the milk from 31 high-producing herds. Only two of the 55 concentrate samples analyzed showed the presence of aflatoxin G₁ (AFG₁, 3.2 and 3.6 µg·kg⁻¹). AFM₁ was detected in 93.5% of the 62 milk samples analyzed with a range from 0.045 to 0.442 µg·L⁻¹. All of the AFM₁ concentrations were below the maximum limit tolerated (0.5 µg·kg⁻¹) by the *Brazilian Agência Nacional de Vigilância Sanitária* (ANVISA). There was no difference in AFM₁ contamination levels for both sampling periods (summer and winter). In conclusion, the AFM₁ contamination in animal feed and, consequently, in milk is within the limits tolerated by ANVISA for Brazil ($\leq 0.5 \mu\text{g}\cdot\text{kg}^{-1}$). Furthermore, no season effect on AFM₁ levels was found.

Keywords: aflatoxins, contaminants, dairy farming, food security

1. Introduction

Dairy demand has grown more than the population growth in Brazil, and it is estimated that by 2025 the production will be at least 47.5 million tons of milk to supply a population of 219 million people (Vilela et al., 2017). In addition, there are concerns about the milk quality and its safety. Some risks associated with this product may be prevented or controlled by appropriate procedures related to food storage to avoid the appearance of fungi and the use of inorganic mycotoxin adsorbents in feed management.

The Normative Instructions of MAPA, No. 76 (IN-76) (BRASIL, 2018a) and the Normative Instruction of MAPA, No. 77 (IN-77) (BRASIL, 2018b) establish the identity and quality standards of pasteurized and raw refrigerated milk, and controls residues that are concern to public health. The contamination of milk with aflatoxin M₁ (AFM₁) is a consequence of poor

handling practices (ANVISA, 2011).

The season of the year affects the prevalence of mycotoxins in food, and hot and humid climates are known to favor the growth of aflatoxin-producing fungi. However, contradictory data have verified the seasonal effect of the presence of mycotoxins in feeds (El Marnissi et al., 2012; Diaz and Espitia, 2006).

The highest food AFB₁ contamination in the winter may be due to its storage under unsatisfactory conditions (Flores-Flores et al., 2015). It is also worth mentioning that the co-occurrence of more than one mycotoxin in the same food because fungi can produce several mycotoxins, which may affect their toxicity, shows an additive and even a synergistic effect (Gelderblom et al., 2002).

The objective of this study was to identify the levels of mycotoxin contaminants in concentrate animal feeds and in the milk. The effect of the milk collection season (summer and winter) on the levels of contamination by AFM₁ was also evaluated.

2. Materials and Methods

Milk and feed samples were collected from 31 dairy farms adopting an intensive production system in Paraná State, Southern Brazil. The evaluated animal feeds for lactating cows were corn silage, oats and rye, haylages, commercial concentrates, soybean meal, whole cottonseed, corn meal, and dried citrus pulp.

This study was conducted in two distinct periods: from June to July (winter season) and January to February (summer season). Four milk samples were collected from each farm, packaged in an isothermal environment with recyclable ice, and then frozen at -20 °C in a freezer until all the analysis were completed.

Concentrate samples were collected during the same period and similarly stored for mycotoxin analysis (AFB₁, AFB₂, AFG₁, AFG₂, and ochratoxin A) at the Veterinary Toxicology Laboratory from the Londrina State University as well as milk samples from each farm. Milk samples were also subjected to serum extraction to determine AFM₁ using Spectrometry and Chromatography Laboratory from Maringá State University.

For the detection of mycotoxins (AFB₁, AFB₂, AFG₁, AFG₂, and ochratoxin A) was used the thin layer chromatography described by Soares and Rodriguez-Amaya (1989). The following concentrations (mg/mL) were used AFB₁ (2.55), AFB₂ (2.62), AFG₁ (2.45), AFG₂ (4.55), and ochratoxin A (143.05) (Sigma-Aldrich Inc., USA), according to the AOAC methodology (AOAC, 2003). The detection limits of the method were 2 and 5 µg·kg⁻¹ and the limits of determination were 4 and 10 µg·kg⁻¹ for aflatoxin and ochratoxin, respectively.

The milk samples were analyzed in duplicate using the Ridascreen® Fast AFM₁ immunoenzymatic kit (R-Biopharm®). This system comprises a "well" support coated with anti-IgG, five standard AFM₁ solutions (0, 0.25, 0.5, 1, and 2 µg·kg⁻¹) containing anti-AFM₁ polyclonal IgG, conjugated, chromogen, and blocking solution according to the protocol described in the manual.

The reading was performed using a spectrophotometer at a wavelength (λ) of 450 nm and the results were expressed as the average of the observed values for each duplicate. Absorbances were calculated for each observation according to:

A = absorbance at λ of 450 nm

A_{0ppt} = absorbance of standard 0 ($0 \mu\text{g}\cdot\text{kg}^{-1}$ of AFM_1)

A_i = observed absorbance of each sample (from i to n).

The absorbance values (%) of each observation were converted to concentration ($\mu\text{g}\cdot\text{kg}^{-1}$) based on the standard curve parameterized for each test provided by the Softmax-Pro® software, version 5.4. The analysis protocol was for competitive immunoaffinity assays [enzyme-linked immunosorbent assay (ELISA)] read at the endpoint of each reaction based on the protocol for melamine (Softmax-Pro v.5.4).

The data were statistically analyzed using the MIXED procedure of the Statistical Analysis System (SAS version 9.3) according to the following model:

$$Y_{ik} = \mu + t_i + e_{ik}$$

Y_{ik} = observed value

μ = overall mean

t_i = treatment effect ($i = \text{C1}$ and C2)

e_{ik} = residual error

3. Results

AFB_1 , AFB_2 , AFG_2 , and OCRA were not detected in all 22 samples of commercial concentrates analyzed. Only two samples showed AFG_1 both with low levels of contamination (3.2 and $3.6 \mu\text{g}\cdot\text{kg}^{-1}$, Table 1).

The results of the analysis of mycotoxins in samples of soybean meal, cottonseed, citrus pulp, corn meal, wheat bran, and brewers grains showed no aflatoxins (AFB_1 , AFB_2 , AFG_1 , and AFG_2) and ochratoxins (Table 1).

Table 1. Analysis of aflatoxins B₁ (AFB_1), AFB_2 , AFG_1 , AFG_2 and OCRA in dairy farm ingredients from Paraná – Brazil

Feed	No. of samples	Aflatoxins ($\mu\text{g}\cdot\text{kg}^{-1}$)				OCRA
		AFB_1	AFB_2	AFG_1	AFG_2	
Commercial concentrates	22	nd	nd	3.4*	nd	nd
Soybean meal	14	nd	nd	nd	nd	nd
Whole cottonseed	6	nd	nd	nd	nd	nd
Corn meal	5	nd	nd	nd	nd	nd
Citrus pulp	5	nd	nd	nd	nd	nd
Wheat bran	1	nd	nd	nd	nd	nd
Brewers grains	2	nd	nd	nd	nd	nd

* Mean of two positive samples: 3.2 and 3.6 $\mu\text{g}\cdot\text{kg}^{-1}$

Table 2 shows that the productivity of the farms varied from 17.5 to 34.6 $\text{kg}\cdot\text{cow}^{-1}\cdot\text{day}^{-1}$ and the occurrence of AFM₁ in two distinct collection periods (PC₁ – summer and PC₂ – winter).

Table 2. Productivity data and aflatoxin M₁(AFM₁) in cow's milk, values of two collection periods (Summer and Winter)

Farms	Productivity ($\text{kg}\cdot\text{cow}^{-1}\cdot\text{day}^{-1}$)	Aflatoxin M ₁ ($\mu\text{g}\cdot\text{L}^{-1}$)		Mean \pm SD*
		Summer (C ₁)	Winter (C ₂)	
1	30.0	0.153	0.092	0.123 \pm 0.043
2	22.8	0.217	0.092	0.155 \pm 0.088
3	32.3	0.070	0.099	0.085 \pm 0.020
4	33.0	0.001	0.419	0.210 \pm 0.001
5	28.1	0.195	0.222	0.209 \pm 0.019
6	32.9	0.405	0.094	0.249 \pm 0.220
7	28.6	0.169	0.091	0.130 \pm 0.055
8	29.0	0.372	0.082	0.227 \pm 0.205
9	32.1	0.093	0.093	0.093 \pm 0.000
10	17.5	0.001	0.294	0.148 \pm 0.001
11	22.8	0.357	0.045	0.201 \pm 0.221
12	29.1	0.246	0.091	0.169 \pm 0.110
13	26.3	0.277	0.383	0.330 \pm 0.075
14	32.2	0.377	0.045	0.211 \pm 0.235
15	29.0	0.309	0.295	0.302 \pm 0.010
16	29.1	0.131	0.283	0.207 \pm 0.107
17	30.5	0.000	0.308	0.155 \pm 0.001
18	31.0	0.092	0.093	0.093 \pm 0.001
19	26.7	0.169	0.093	0.131 \pm 0.054
20	27.1	0.204	0.280	0.242 \pm 0.054
21	19.2	0.189	0.166	0.177 \pm 0.016
22	28.8	0.257	0.442	0.349 \pm 0.131
23	22.3	0.253	0.068	0.160 \pm 0.131
24	24.9	0.232	0.308	0.270 \pm 0.053
25	34.6	0.143	0.092	0.118 \pm 0.036
26	30.1	0.217	0.238	0.227 \pm 0.014
27	25.1	0.001	0.175	0.088 \pm 0.001
28	27.0	0.189	0.093	0.141 \pm 0.067
29	30.0	0.255	0.093	0.174 \pm 0.115
30	32.6	0.288	0.092	0.190 \pm 0.139
31	28.1	0.180	0.181	0.180 \pm 0.001
Mean	DP	AFM ₁ C1	AFM ₁ C2	P-value
	28.15	0.195	0.176	0.7927

SD = standard deviation of milk production in kg

Collection Periods (C)

C1 = Summer - Collection in January and February

C2 = Winter -Collection in June and July

* Mean \pm standard deviation of two collection periods (C1 and C2).

Figure 1 shows the AFM1 values of the two sampling periods (C1 and C2), and the maximum limit tolerated for aflatoxin in Brazil.

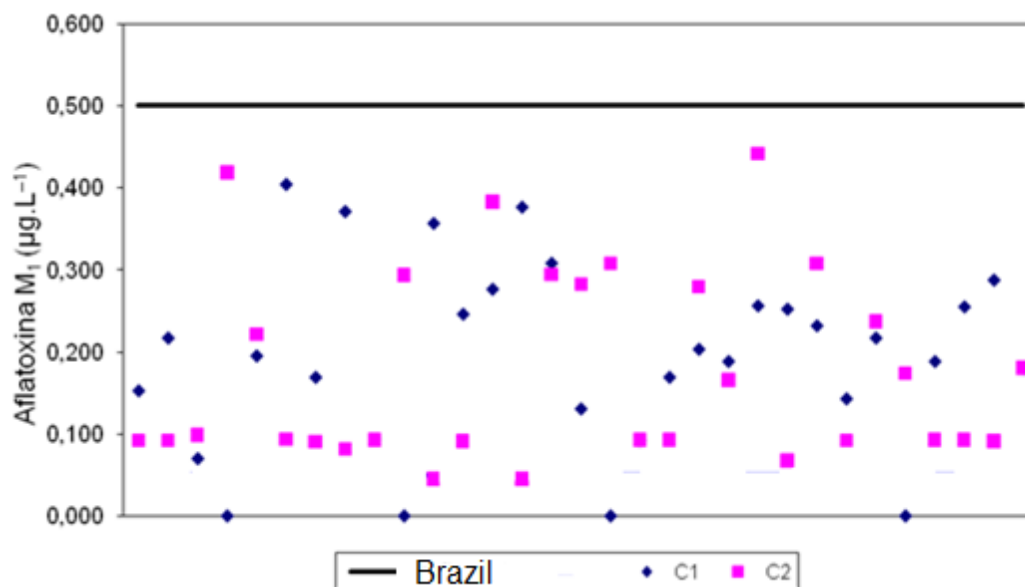


Figure 1. Distribution of aflatoxin M₁ values and the maximum limit tolerated for Brazil - C₁ - summer and C₂ - winter

4. Discussion

In contrast to the results observed in this work, some studies showed concerning levels of aflatoxin contamination (Sassahara et al., 2003; 2005). Sassahara et al. (2003) reported AFB₁ contamination in 13.6% of 272 samples initially produced to feed dairy cattle in Paraná. In another study, the presence of AFB₁ was detected in 7 (25%) of the 27 commercial feed samples analyzed in Northern Paraná (Sassahara et al., 2005). Oliveira et al. (2010) verified AFB₁ contamination in 40% of animal feed concentrates, obtaining levels between 1.0 and 19.5 $\mu\text{g}\cdot\text{kg}^{-1}$. Pontes Neto et al. (2002) evaluated feed samples supplied to dairy cows in the Northern region of Paraná and found that 31.08% were contaminated with aflatoxins. Furthermore, Motta et al. (2015) analyzed 288 total mixed samples (forage plus concentrate) and verified the occurrence of AFB₁ in 31.44% with contents between 1.68 and 194.51 $\mu\text{g}\cdot\text{kg}^{-1}$.

The results of the analysis of five mycotoxins in samples of soybean meal, cottonseed, citrus pulp, corn meal, wheat bran, and brewer's grains showed no aflatoxins (AFB₁, AFB₂, AFG₁ and AFG₂) and ochratoxins (Table 1).

Globally, mycotoxins in animal feeds are serious threats to the health of humans and animals. A higher incidence of contaminated dairy cow feeds in other countries was also observed by Xiong et al. (2018) in China, who evaluated 174 samples (corn, wheat bran, soybean meal,

peanut meal, and cottonseed) and found that 35.1% were positive for AFB₁.

Granados-Chinchilla et al. (2017) evaluated 970 feed samples for animals in Costa Rica and detected a 24% rate of aflatoxin contamination. Diehrius et al. (2008) analyzed 169 feed samples from 24 dairy farms for contamination by 20 mycotoxins found a high prevalence of deoxynivalenol, zearalenone, roquefortina C, and mycophenolic acid. In the present study, milk samples were collected on farms that produced between 800 and 10,000 L of milk day⁻¹, and we observed detectable levels of AFM₁, which were below the maximum limit tolerated by ANVISA (ANVISA, 2011).

We observed that from 62 samples analyzed, 93.5% showed detectable levels of AFM₁ ranging from 0.001 to 0.442 µg·L⁻¹ but below the maximum limit allowed by ANVISA (2011). Sabino et al. (1989) found 18% of positive samples at levels of 0.10 to 1.68 µg·L⁻¹. For some samples, the limit established by ANVISA is ≤ 0.5 µg·L⁻¹. Sassahara et al. (2005) observed AFM₁ levels of 0.29 to 1.97 µg·L⁻¹ in 24% of raw milk samples collected from farms in the State of Paraná, and only three (7%) were above the limit of 0.5 µg·L⁻¹. Shundo and Sabino (2006) analyzed 107 milk samples, and 79 (73.8%) showed AFM₁ levels varying between 0.02 and 0.26 µg·L⁻¹. Of these 107 samples, 22, 42, and 43 were raw, ultra-high temperature (UHT) and pasteurized milk samples, of which 13, 34, and 32, respectively, showed some AFM₁ contamination.

Several studies have demonstrated a seasonal trend in milk contamination by AFM₁, and usually higher incidence occurs during winter when the animals are fed with more concentrates than during other seasons (Patterson et al., 1980; Galvano et al., 1986; Kamkar, 2005; Diaz and Espitia, 2006). The effect of the climactic season on contamination by mycotoxins was also studied by Fallah et al. (2016) who reported contradictory results. Seasonal variations were not observed in this study probably because these herds do not change their feed management around the year, because the supply of concentrates to the animals remains constant.

Many countries have legislation to control the maximum contamination limit of exported dairy products, and that of AFM₁ is 0.5 µg·L⁻¹. This demonstrates that 100% of the analyzed milk samples would be approved based on this parameter relating to AFM₁ for dairy export to these countries. Milk processing, whether by pasteurization or UHT, does not destroy AFM₁ and has a cumulative effect on the body and, therefore, care must be taken to maximally reduce the level of AFM₁ in milk. Similarly with our study and sampling milk samples in the spring and summer months, Oliveira et al. (2010) obtained only one sample with a level > 0.5 µg·L⁻¹. However, 20% of the samples showed AFM₁ levels higher than the TUL of the European Union legislation (0.05 µg·L⁻¹). Gonzalez et al. (2005) found 17 contaminated samples from the 43 analyzed, and 11 (64.7%) of them showed concentrations above the maximum limit allowed by Brazilian legislation.

Establishing the importance of the Brazilian dairy sector in the international market will require producers, industries, research institutions, and government leaders to be aware of the need for sustainability.

5. Conclusion

In conclusion, no AFB₁, AFB₂, and AFG₂ contamination was detected in the feed produced for animal consumption and only two concentrate samples presented low AFG₁ values. The milk samples analyzed for AFM₁ were all within the limit tolerated by Brazilian legislation, which is $\leq 0.5 \mu\text{g}\cdot\text{kg}^{-1}$. There were no differences in the AFM₁ contamination levels between the two sampling periods (summer and winter).

Conflict of interest statement

The authors declare that they have no conflict of interest.

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References

- ANVISA (2011). AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. Resolução – RDC no 07. Anvisa estabelece limites para presença de micotoxinas em alimentos. In: http://portal.anvisa.gov.br/documents/10181/2968262/RDC_07_2011_COMP.pdf/afe3f054-bc99-4e27-85c4-780b92e2b966. Acessado: em 23/05/2018.
- AOAC (2003). Official Methods of Analysis of AOAC International. 17^{ed}. Gaithersburg: AOAC International. 141 p.
- BRASIL (2018a). *Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 76, de 30 de novembro de 2018*. Dispõe sobre regulamentos técnicos de produção, identidade, qualidade, coleta e transporte de leite. Edição 230, Seção 1, página 9 do Diário Oficial da União, Brasília (DF), 26 Nov. (2018), 6p. Google Scholar.
- BRASIL (2018b). *Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº 77 de novembro de 2018*. Brasília (DF): Diário Oficial da União (in Portuguese). Google Scholar.
- Diaz, G. J., & Espitia, E. (2006). Occurrence of aflatoxin M₁ in retail milk samples from Bogota, Colombia. *Food Additives & Contaminants*, 23, 811-815. <https://doi.org/10.1080/02652030600681617>
- Driehuis, F., Spanjer, M. C., Scholten, J. M., & Giffel, M. C. (2008). Occurrence of Mycotoxins in Feedstuffs of Dairy Cows and Estimation of Total Dietary Intakes. *Journal of Dairy Science*, 91, 4261-4271. <https://doi.org/10.3168/jds.2008-1093>
- El Marnissi, B., Belkhou, R., Morgavi, D. P., Bennani, L., & Boudra, H. (2012). Occurrence of aflatoxin M₁ in raw milk collected from traditional dairies in Morocco. *Food and Chemical Toxicology: An International Journal Published for the British Industrial*

Biological Research Association, 50, 2819e28. <https://doi.org/10.1016/j.fct.2012.05.031>

Fallah, A., Fazlollahi, R., & Emami, A. (2016). Seasonal study of aflatoxin M₁ contamination in milk of four dairy species in Yazd, Iran. *Food Control*, 68, 77-82. <https://doi.org/10.1016/j.foodcont.2016.03.018>

Flores-Flores, M. E., Lizarraga, E., Cerain, A. L., & Gonzalez-Penas, E. (2015). Presence of mycotoxins in animal milk: A review. *Food Control*, 53, 163-176. <https://doi.org/10.1016/j.foodcont.2015.01.020>

Galvano, F., Galofaro, V., & Galvano, G. (1996). Occurrence and stability of aflatoxin M₁ in Milk and milk products: a worldwide review. *Journal of Food Protection*, 59, 1079-1090. <https://doi.org/10.4315/0362-028X-59.10.1079>

Gelderblom, W. C., Marasas, W. F., Lebepe-Mazur, S., Swanevelder, S., Vessey, C. J., & Hall P. de, L. (2002). Interaction of fumonisin B (1) and aflatoxin B(1) in a shortterm carcinogenesis model in rat liver. *Toxicology*, 171, 161-173. [https://doi.org/10.1016/S0300-483X\(01\)00573-X](https://doi.org/10.1016/S0300-483X(01)00573-X)

Gonçalves, E., Felício, J. D., Pinto, M. M., Rossi, M. H., Nogueira, J. H. C., & Manginelli, S. (2005). Ocorrência de aflatoxina M₁ em leite comercializado em alguns municípios do Estado de São Paulo. *Arquivos do Instituto Biológico*, 72, 435-438.

Granados-Chinchilla, G., Molina, A., Chavarría, G., Alfaro-Cascante, M., & Murillo-Williams, A. (2017). Aflatoxins occurrence through the food chain in Costa Rica: Applying the one Health approach to mycotoxin surveillance. *Food Control*, 82, 217-226. <https://doi.org/10.1016/j.foodcont.2017.06.023>

Kamkar, A. (2005). A study on the occurrence of aflatoxin M₁ in raw milk produced in Sarab city of Iran. *Food Control*, 16, 593-599. <https://doi.org/10.1016/j.foodcont.2004.06.021>

Motta, T. P., Frizzarin, A., Martins, T., Miranda, M., Arcaro, J. M. P., Ambrosio, L. A., & Pozza, C. R. (2015). Estudo sobre a ocorrência de fungos e aflatoxina B₁ na dieta de bovinos leiteiros em São Paulo. *Pesquisa Veterinária Brasileira*, 35, 23-28. <https://doi.org/10.1590/S0100-736X2015000100006>

Oliveira, C. A. F., Sebastião, L. S., Fagundes, H., Rosim, R. E., & Fernandes, A. M. (2010). Determinação de aflatoxina B₁ em rações e aflatoxinas M₁ no leite de propriedades do estado de São Paulo. *Ciência e Tecnologia de Alimentos*, 30(Supl.1), 221-225. <https://doi.org/10.1590/S0101-20612010000500034>

Patterson, D. S. P., Glancy, E. M., & Roberts, B. A. (1980). The “carry over” of aflatoxin M₁ into the Milk of cows fed rations containing a low concentration of aflatoxin B₁. *Food Cosmetic Toxicology*, 18, 35-37. [https://doi.org/10.1016/0015-6264\(80\)90008-5](https://doi.org/10.1016/0015-6264(80)90008-5)

Pontes Netto, D., Zanluchi, A. T., Sassahara, M., & Yanaka, E. K. (2002) Micotoxinas em alimentação animal no período de maio/1997 a março/2001 no Laboratório de Toxicologia Veterinária da Universidade Estadual de Londrina – Londrina – PR. *SEMINA: Ciências Agrárias*, 23, 63-69. <https://doi.org/10.5433/1679-0359.2002v23n1p63>

Sabino, M., Purchio, A., & Zorzetto, A. P. (1989). Variations in the level of aflatoxin in cows milk consumed in the city of São Paulo, Brazil. *Food Additives and Contaminants*, *6*, 321-361. <https://doi.org/10.1080/02652038909373786>

Sassahara, M., Pontes Netto, D. P., & Yanaka, E. K. (2005). Aflatoxin occurrence in foodstuff supplied to dairy cattle and aflatoxin M₁ in raw milk in the north of Paraná state. *Food Chemical Toxicology*, *43*, 981-984. <https://doi.org/10.1016/j.fct.2005.02.003>

Sassahara, M., Yanaka, E. K., & Pontes Netto, D. P. (2003). Ocorrência de aflatoxina e zearalenona em alimentos destinados ao gado leiteiro na região Norte do Estado do Paraná. *Semina. Ciências Agrárias*, *24*, 63-72. <https://doi.org/10.5433/1679-0359.2003v24n1p63>

Shundo, L., & Sabino, M. (2006). Aflatoxin M₁ in milk by immunoaffinity column cleanup with TLC/HPLC determination. *Brazilian Journal of Microbiology*, *37*, 164-167. <https://doi.org/10.1590/S1517-83822006000200013>

Soares, L. M. V., & Rodriguez-Amaya, D. B. (1989). Survey of aflatoxins, ochratoxin A, zearalenone and sterigmatocystin in some Brazilian foods by using multi-toxin thinlayer chromatographic method. *Journal of Association of Official Analytical Chemists International*, *72*, 22-26. <https://doi.org/10.1093/jaoac/72.1.22>

Vilela, D., Resende, J. C., Leite, J. B., & Alves, B. (2017). A evolução do leite no Brasil em cinco décadas. *Revista de Política Agrícola*, *XXVI*, 5-24.

Xiong, J., Xing, L., Zhou, H., Liu, Y., & Wu, L. (2018). Occurrence of aflatoxin B₁ in dairy cow feedstuff and aflatoxin M₁ in UHT and pasteurized milk in central China. *Food Control*, *9*, 386–390. <https://doi.org/10.1016/j.foodcont.2018.05.022>

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