dairy

MDPI

Article

Multi-Mycotoxin Contamination of Concentrates Fed to Dairy Calves in Southeast Brazil: A Case Report

Rogério D'Antonio Pires [1], Aline Moreira Borowsky [1], Tobias Alves e Silva [1], Giovanna Canela Ruiz Castro Evangelista [1], Carla Maris Machado Bittar [1] and Carlos Humberto Corassin [2,*]

[1] Departament of Animal Science, Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo, Av. Pádua Dias, 11, Piracicaba 13418-900, SP, Brazil; dantonio@usp.br (R.D.P.); alineborowsky@usp.br (A.M.B.); tobiasalves@usp.br (T.A.e.S.); giovannacanelaruiz@usp.br (G.C.R.C.E.); carlabittar@usp.br (C.M.M.B.)

[2] Department of Food Engineering, Faculty of Animal Science and Food Engineering (FZEA), University of São Paulo (USP), Av. Duque de Caxias Norte, 225, Pirassununga 13635-900, SP, Brazil

* Correspondence: carloscorassin@usp.br; Tel.: +55-019-3565-4145

Abstract

The diet of dairy calves can be contaminated with mycotoxins, posing a potential risk to animal health. This case study report aimed to make the first assessment of the presence of multiple mycotoxins in concentrates fed to dairy calves in Brazil. A total of 19 concentrate samples intended for dairy calves were analyzed using liquid chromatography coupled with mass spectrometry. Aflatoxins, deoxynivalenol, and T-2 toxin were not detected in any samples, whereas fumonisins B_1 (FB_1) and B_2 (FB_2) were present in 100% of the samples, with mean concentrations of 2750.1 µg/kg and 834.9 µg/kg, respectively. Zearalenone (ZEN) was detected in 36.8% of samples, with a mean concentration of 929.9 µg/kg. Significant correlations were observed between FB_1 and FB_2 ($\rho = 0.978$; $p < 0.001$) and between FB_2 and ZEN ($\rho = 0.735$; $p = 0.05$). While the physical form of the concentrate did not influence ($p > 0.05$) mycotoxin concentrations, a trend was observed for FB_1 ($\rho = -0.417$; $p = 0.07$) and FB2 ($\rho = -0.395$; $p = 0.09$). These findings highlight the frequent occurrence of *Fusarium* mycotoxins, likely due to pre-harvest contamination, emphasizing the potential risk of additive or synergistic effects in dairy calves.

Keywords: *Fusarium*; bovine; fumonisins; zearalenone; animal health

Academic Editor: Gabriele Rocchetti

Received: 25 March 2025
Revised: 24 July 2025
Accepted: 24 July 2025
Published: 4 August 2025

Citation: Pires, R.D.; Moreira Borowsky, A.; Alves e Silva, T.; Evangelista, G.C.R.C.; Maris Machado Bittar, C.; Corassin, C.H. Multi-Mycotoxin Contamination of Concentrates Fed to Dairy Calves in Southeast Brazil: A Case Report. *Dairy* **2025**, *6*, 44. https://doi.org/10.3390/dairy6040044

1. Introduction

The diet of dairy animals, such as calves, is susceptible to various contaminants, such as mycotoxins. Mycotoxins are secondary toxic metabolites of different species of fungi, such as *Fusarium*, *Aspergillus*, and *Penicillium* [1]. Their production is influenced by environmental factors, including pH, temperature, humidity, and stressors such as plant damage caused by animals and insects [2].

The term "mycotoxin" is composed of the Greek word "*mykes*" and the Latin "*toxicum*", which mean, respectively, fungus and poison (or toxin) [3]. This term was only established in the second half of the 20th century. In 1959, the United Kingdom imported peanut feed from Brazil to be used as animal feed. After consumption, approximately 100,000 turkeys died. Subsequent studies identified contamination by aflatoxins, metabolites produced by the fungus *Aspergillus flavus*, as the cause of the deaths, giving rise to the study of mycotoxicology [4,5].

Ruminants are generally considered to have greater resistance to mycotoxins, that is, they require higher concentrations of mycotoxins in their diets when compared to mono-

gastric animals to show clinical signs of intoxication. This occurs due to the modification of some mycotoxins by ruminal microorganisms. However, this detoxification capacity is variable and will not necessarily result in the inactivation of toxins, and the extent of metabolization will depend on the diet, in addition to the characteristics of the animal, such as species, breed, sex, and age, as well as the microorganisms that inhabit the rumen and the exposure to mycotoxins itself, which can alter the microbial ecosystem of the rumen [2,6].

Among the most relevant mycotoxins in dairy production are aflatoxins, fumonisins (FBs), zearalenone (ZEN), and the trichothecenes T-2, HT-2, and deoxynivalenol (DON), which pose significant economic and health risks to both animals and consumers. While ZEN's molecular structure is similar to that of steroids and exerts agonist effects on estrogen receptors present in the uterus, mammary gland, liver, hypothalamus, and pituitary gland, and may cause a reduction in milk production, infertility, and hyperestrogenism in adult cattle, FBs are poorly degraded by the rumen and their acute and chronic ingestion are known to have hepatotoxic effects in cattle, with an increase in the hepatic biochemical enzymes alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT), as well as bilirubin and cholesterol levels [7].

As mentioned above, when ingested through contaminated feed, some mycotoxins are degraded in the rumen, while others can be excreted in biological fluids, such as milk. Aflatoxins, for instance, are commonly found in feed as aflatoxin B_1 (AFB_1) but undergo hepatic biotransformation into aflatoxin M_1 (AFM_1), a contaminant of cow's milk, posing a risk to both humans and calves [8].

Dairy calves play a crucial role in the industry, as they replace cows removed from the herd due to mastitis, reproductive issues, mobility problems, or low milk production. Additionally, they can be sold to other producers, representing an important source of income [9]. Calves are born with an underdeveloped rumen and initially rely entirely on a liquid diet (milk or milk replacer) to meet their nutritional needs for maintenance and growth [10]. However, to support proper rumen development and sustain performance post-weaning, early introduction of concentrates is essential, alongside economic considerations [11].

Cattle feed can be divided into two categories, roughage or concentrates. Roughage consists mainly of grasses, while concentrates consist of cereal and legume grains, such as corn, soybean and wheat, that are generally below the quality for human consumption [12]. In calves' diets, roughages are usually a minor proportion of the diet because of the low rates of fermentation and dry matter (DM) intake, while cereals in concentrate feeds are widely used [13], composing up to 60% of their diet [14].

Commercial concentrates, whether pelleted or multiparticulated, are widely used, with some farms preparing their own mixtures. While the physical form itself does not directly affect performance [15], ingredient quality control and storage conditions can impact nutrient composition and sanitary quality. Consequently, concentrates can serve as a potential source of mycotoxin exposure [16].

Mycotoxin ingestion can negatively affect calf growth by reducing dry matter intake, altering nutrient absorption and metabolism, disrupting endocrine functions, and inducing immunosuppression, ultimately leading to economic losses [17]. Despite the Brazilian dairy sector amounting to a total of BRL 80.4 billion in 2023 [18], there is a notable lack of studies addressing the occurrence and impact of mycotoxins in dairy calf nutrition. Therefore, this case study report aimed to make the first assessment of the presence of multiple mycotoxins (aflatoxins, fumonisins, deoxynivalenol, zearalenone, and T-2 toxin) in nineteen samples of concentrates fed to dairy calves in Brazil, providing insights into current challenges and guiding future research directions.

2. Materials and Methods

2.1. Sampling and Physical Form Characterization

Nineteen samples of commercial concentrate (a store-bought mixture of cereal and legume grains, e.g., corn, soybean, and cottonseed) destined for dairy calves were collected from nineteen farms located in the southeast region of Brazil (São Paulo and Minas Gerais States) that were willing to collaborate with the study.

The sampling period occurred between January and March of 2024, which corresponds to the summer season in Brazil. Despite the summer period, all samples were collected under dry and cool environmental conditions, with no rainfall during collection. One sample was collected from each farm.

The producers were instructed to randomly collect four 250g primary subsamples from the entire lot, in order to ensure that they were representative, forming a final sample weighing 1 kg. These samples were properly identified, packaged in polyethylene bags, and kept refrigerated until sent to the Mycotoxin Control and Decontamination Laboratory of the School of Animal Science and Food Engineering of the University of São Paulo (LCDM/ZEA/FZEA-USP) located in Pirassununga, SP, for chromatographic analysis. Upon arrival at the laboratory, the received samples were stored in a freezer at −20 °C until the time of analysis.

Samples were classified as being bran, pelleted, or multiparticle by visual evaluation, with posterior physical evaluation being performed. Density was measured by filling a volume test tube of 500 mL and weighing the amount of sample to calculate the value in g/L. Particle size evaluation was performed according to Zanotto and Bellaver [19], though using screens with sizes of 2, 1, 0.5, 0.3, and 0.18 mm. Samples (100 g) were placed on the top screen (2 mm mesh size) of a sieve shaker and vigorously agitated at approximately 60 shakes/min for 10 min. The amount of grain held on each screen was collected, weighed on a precision laboratory scale, and used for calculations performed according to Zanotto and Bellaver [19] for the average particle size, fineness modulus (FM), and the mean geometry diameter (MGD). The FM is an index from 0 to 6, with the index increasing as particles increase in size. The uniformity index (UI) refers to the relative relationship between large, medium, and small particles, categorized by their diameters: above 2 mm, between 2 mm and 0.60 mm, and below 0.60 mm, respectively; in the present study, the sieve of 0.60 mm was replaced by one of 0.51 mm.

Feed samples were then processed using a MA035 drying oven (Marconi, Piracicaba, Sao Paulo, Brazil) and a Saoy 1 mm screen Wiley Mill (Marconi, Piracicaba, Sao Paulo, Brazil).

2.2. Mycotoxin Extraction Procedure and Instrumentation

Once every sample was homogenized and had the same particle size after grinding, the extraction and determination of multiple mycotoxins (aflatoxins B_1—AFB_1, B_2—AFB_2, G_1—AFG_1, and G_2—AFG_2; deoxynivalenol—DON; fumonisins B_1—FB_1 and B_2—FB_2; toxin T-2; and zearalenone—ZEN) were conducted according to a previously validated method by our laboratory, as described by Sulyok et al. [20], with minor modifications.

In the "dilute-and-shoot" method, 1 g of the sample was weighed in a 15 mL Falcon tube, followed by the addition of 4 mL of an extraction solution composed of 79% acetronitrile, 20% water, and 1% acetic acid. The Falcon tube was agitated on a shaker table for 60 min at 250 rpm, followed by centrifugation at 23 G for 5 min. The resulting supernatant was filtered using polytetrafluoroethylene (PTFE) membranes (13 mm; 0.22 µm) in 4 mL vials. Subsequently, 40 µL of the sample along with 10 µL of a solution containing reference material were transferred to glass inserts inserted into 8 mL vials, which were stirred in a vortex prior to analysis.

The analytical method performance was based on calibration curves constructed from the data obtained from spiking samples before extraction, spiked extracts, and standards diluted in solvents. The reference material solution containing a mixture of mycotoxins was prepared in a water–acetonitrile ratio of 50:50, with concentrations of AFB_1, AFB_2, AFG_1, AFG_2, FB_1, FB_2, and ZEN at 5 ng/mL, while the DON mycotoxin was prepared at a concentration of 100 ng/mL. This solution was used to prepare five reference calibration tests corresponding to the matrix at different concentration levels. Additionally, possible matrix effects of the sample extract were compensated by corresponding the signal variation of the isotopically labeled standards [21].

The determination and quantification of multi-mycotoxins was performed using a Waters Acquity I-Class (Waters®, Milford, MA, USA) ultra-performance liquid chromatography (UPLC) system equipped with a BEH C18 column (2.1 × 50 mm, 1.7 µm) and coupled to a Xevo TQ-S Mass Spectrometer (Waters®, Milford, MA, USA). The experimental parameters used in the analyses were as follows: capillary voltage for the positive mode of 3.00 V, and for the negative mode one of 2.00 V; source temperature of 150 °C; desolvation temperature of 500 °C; cone gas flow of 150 L/h; desolvation gas flow of 700 L/h; collision gas flow of 0.15 mL-1; nebulizer gas pressure of 7.00 bar. The injection volume of standards and samples was 5 mL.

Mass spectrometry (MS) analyses were performed in multiple reaction monitoring (MRM) mode, using electrospray ionization in positive ion mode. The chromatographic procedure, the MS parameters, and the MRM transitions (Table 1) were the same as those adopted by Franco et al. [21]. All data was processed using Masslynx 4.1® software and the TargetLynx® platform (Waters®, Milford, MA, USA).

Table 1. Parameters used for mass spectrometry (MS) and tandem mass spectrometry (MS/MS) in conjunction with the methodology applied for the determination of mycotoxins in concentrate samples (n = 19).

Mycotoxins	RT (min.)	Mass (g/mol)	Molecular Ion	Transition (*m*/*z*)	LOD (µg/kg)	LOQ (µg/kg)
AFB_1	4.80	312.3	$[M+H]^+$	312.7 > 284.9 [a] 312.7 > 241.1 [b]	0.4	0.8
AFB_2	4.50	314.3	$[M+H]^+$	314.7 > 259.0 [a] 314.7 > 287.0 [b]	0.4	0.8
AFG_1	4.46	328.3	$[M+H]^+$	328.9 > 243.0 [a] 328.9 > 199.5 [b]	0.4	0.8
AFG_2	4.18	330.3	$[M+H]^+$	330.9 > 245.0 [a] 330.9 > 188.9 [b]	0.5	1.0
DON	1.98	296.3	$[M+H]^+$	297.3 > 249.1 [a] 297.3 > 231.1 [b]	6.1	18.0
FB_1	5.40	721.8	$[M+H]^+$	722.5 > 334.0 [a] 722.5 > 352.1 [b]	0.9	2.5
FB_2	3.74	705.8	$[M+H]^+$	706.5 > 336.2 [a] 706.5 > 318.3 [b]	0.7	2.0
T2	4.49	489.2	$[M+NH4]^+$	484.2 > 541.1 [a] 484.2 > 542.0 [b]	5.1	15.0
ZEN	5.98	318.1	$[M-H]^-$	317.1 > 175.1 [a] 317.1 > 130.9 [b]	6.1	18.0

RT: retention time; LOD: limit of detection; LOQ: limit of quantification; AF: aflatoxin; DON: deoxynivalenol; FB: fumonisin; T2: T-2 toxin; ZEN: zearalenone. [a] Transitions used in quantification. [b] Transitions used in confirmation.

2.3. Statistical Analysis

After the identification and quantification of the mycotoxins present in the samples, the means, standard errors, and corresponding maximum and minimum limits were calculated

using the PROC MEANS procedure of the SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). The data was subjected to variance analysis using the PROC GLM procedure, considering the effect of different types of physical evaluation. In addition, a correlation analysis between mycotoxins was performed using the PROC CORR procedure. For all tests, a significance level of 5% was used.

2.4. Reagents

Biopure® mycotoxin analytical standards (Biopure®, Getzersdorf, Austria) were employed in this study. Specifically, aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), zearalenone (ZEN), deoxynivalenol (DON), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), T-2 toxin, Mix 11 (13C Aflatoxins), U-[13C18] Zearalenone, and Mix10 (13C Fusarium Toxins) were used.

Acetonitrile, methanol, HPLC-grade isopropanol, and Milli-Q® ultrapure water (Merck Millipore® Darmstadt, Germany) were used as solvents. In the mobile phase, acetic acid, formic acid, and ammonium acetate were used as additives for liquid chromatography coupled to mass spectrometry (LC-MS/MS) analyses.

3. Results

3.1. Characterization of Physical Form of Concentrates

The majority of the analyzed samples were in bran form (eleven samples; 58%), followed by five pelleted samples (26%) and three multiparticulate samples (16%). The evaluation of the physical form of the concentrates revealed an average FM of 3.7, an MGD of 1.7 mm, and a density of 591 g/L. However, variations were observed when these parameters were analyzed according to the physical form of the concentrate (Table 2).

Table 2. Physical form evaluation of concentrates fed to dairy calves in Brazil (n = 19).

	Overall		Bran		Multiparticle		Pelleted	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
FM	3.7	0.2	3.0	0.1	4.4	0.6	4.9	0.1
MGD, mm	1.7	2.7	0.9	0.9	2.5	7.4	3.2	0.1
Density, g/L	591	15	581	18	602	66	606	28

FM: Fineness modulus; MGD: Mean geometric diameter; SE: Standard error.

3.2. Mycotoxins

Aflatoxins (AFs), as well as DON and T-2 toxin, were not identified in any of the analyzed samples. In contrast, FB_1 and FB_2 were detected in 100% of the samples, with mean values of 2750.1 µg/kg and 834.9 µg/kg, respectively (Tables 3 and 4). On the other hand, ZEN was found in 36.8% of the samples, with an average of 929.9 µg/kg. A correlation was observed between the presence of FB_1 and FB_2 ($\rho = 0.978$; $p < 0.001$) and FB_2 and ZEN ($\rho = 0.735$; $p = 0.05$).

Table 3. Means, standard errors (SEs), and minimum and maximum limits of mycotoxins in dairy calf concentrates in Brazil (n = 19).

Mycotoxin (µg/kg)	Mean ± SE	Minimum	Maximum
FB_1	2750.1 ± 600.9	467.8	9443.6
FB_2	834.9 ± 197.6	134.7	3428.0
ZEN	929.9 ± 796.6	78.3	5708.5

Table 4. Levels of mycotoxins found in dairy calf concentrates in Brazil above the limit of quantification (n = 19).

Sample	FB_1 (µg/kg)	FB_2 (µg/kg)	ZEN (µg/kg)
1	467.8	134.7	<LOQ
2	895.7	232.3	<LOQ
3	3332.8	1242.7	<LOQ
4	4537.9	1436.9	5708.5
5	9443.6	3428.0	<LOQ
6	2480.0	699.6	<LOQ
7	4202.6	1160.8	100.9
8	6814.4	1823.3	<LOQ
9	1776.4	701.5	<LOQ
10	2159.8	410.4	<LOQ
11	1089.3	352.2	<LOQ
12	780.9	234.5	111.3
13	520.7	143.4	78.3
14	1233.5	410.7	<LOQ
15	761.1	207.9	<LOQ
16	676.8	209.1	174.5
17	1471.0	497.7	135.8
18	7475.4	2109.9	<LOQ
19	2132.4	427.6	200.4

LOQ: Limit of quantification; FB: Fumonisin; ZEN: Zearalenone.

When considering the physical presentation of the concentrates, a higher concentration of mycotoxins was observed in the bran feed (Table 5 and Figure 1), although the physical form did not influence ($p > 0.05$) the concentration of mycotoxins. Even so, a trend of correlation was found between the physical form and concentration of FB_1 ($\rho = -0.417$; $p = 0.07$) and FB_2 ($\rho = -0.395$; $p = 0.09$).

Table 5. Means ± standard errors (SEs) of mycotoxins considering the physical evaluation of dairy calf concentrates in Brazil (n = 19).

Mycotoxin (µg/kg)	Feed Presentation			*p*-Value
	Bran	Multiparticle	Pellet	
FB_1	3774.57 ± 920.01	1537.63 ± 12.41	1223.73 ± 282.41	0.1321
FB_2	1168.61 ± 304.53	440.32 ± 444.41	337.44 ± 68.42	0.1378
ZEN	1973.53 ± 1867.49	174.48 ± 142.95	138.16 ± 35.26	0.6169

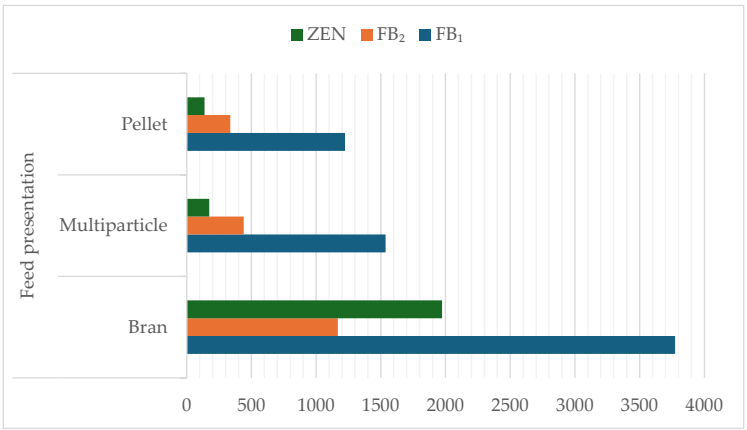

Figure 1. Means of mycotoxins considering the physical evaluation of dairy calf concentrates in Brazil.

4. Discussion

Toxicogenic fungi are usually classified in two different groups: those produced in the field, which can contaminate pre-harvested crops, and those produced during storage, which produce toxins primarily after harvesting. *Aspergillus* species, which produce aflatoxins, are known to contaminate feed during the storage phase, while *Fusarium*, responsible for the production of fumonisins, DON, ZEN, and T-2 toxin, usually produces toxins while in the field [22].

The analyzed concentrate samples showed contamination by fumonisins and ZEN, both considered field mycotoxins. This indicates that the contamination of the samples analyzed probably occurred before the harvest and not during storage. Since we do not have data on the origin and field characteristics of the inputs that composed the concentrates, we can only make assumptions regarding the causes of contamination. Some of the factors that may explain this presence include biological and environmental factors, such as crop susceptibility, temperature, moisture availability, and physical damage, and harvest practices, including crop maturity, handling, temperature, and moisture at the time of harvest [23]. Other than the growth of new toxigenic fungi, sources of mycotoxins in field soils include plant residues remaining in the field after harvest, runoff from infected crops after heavy rain events, and even mycotoxins that have already been fed to animals through the use of manure [24].

Although there is a lack of studies regarding mycotoxins specifically in calves' diets, we can draw parallels between studies regarding adult cattle diets. In Brazil, a five-year study found that 97% of cattle feed samples were contaminated with at least one mycotoxin, but, in disagreement with our findings, the most frequently found mycotoxin was DON (67.8%), followed by ZEN (62.5%) and AFs (58.6%) [25]. In Total Mixed Ration (TMR) samples fed to beef cattle, a study highlighted a 100% contamination rate by mycotoxins, with fumonisins being the most prevalent and found at the highest concentrations [26]. Similarly, in TMR for dairy cows, fumonisins were found in 100% of the samples [26], corroborating our findings. This consistency across different studies emphasizes the widespread nature of fumonisin contamination in cattle feed and highlights the urgent need for effective strategies to monitor and mitigate the impact of mycotoxins on livestock health and productivity.

A study conducted in Thailand also encountered a co-occurrence of ZEN and FB_1 in cattle feed, found in 65.9% of the samples [27]. Similarly, our research identified a correlation between FB_2 and ZEN, providing further evidence that a single fungal species is capable of producing multiple mycotoxins simultaneously. Additionally, different fungal species may colonize the same substrate, leading to the co-occurrence of several mycotoxins [28]. These findings underscore the complexity of feed contamination and highlight the critical need for robust monitoring and management practices to address the risks associated with multi-mycotoxin exposure in livestock production systems.

Fumonisins (FBs) are mainly produced by *Fusarium verticillioides* and other related fungi species [7]. In calves, a study conducted by Osweiler et al. [29] also found an increase in hepatic enzymes after feeding fumonisin-contaminated corn in concentrations of 148 µg/g, which also led to alterations in immune function, with an impairment of lymphocyte blastogenesis, leading to a higher susceptibility to pathogens. Immunosuppression is of particular importance in calves due to their innate lack of full immune competence [30]. Furthermore, changes in the heart rate and respiratory rate of cattle have also been observed, both indicating a compromise in the general health condition when exposed to fumonisins [6].

Zearalenone is produced by the *Fusarium* species *graminearum*, *culmorum*, *crookwellense*, *equiseti*, and *semitectum*, being frequently present in corn, wheat, oats, and barley. While it is known for its effects on estrogen receptors [7], it has also been shown to cause a diffuse

immune response, characterized by an increase in body temperature [6], which could disrupt calf growth. But it is important to take into consideration that, even though it is thought that the ruminal microbiota has the ability to convert ZEN into α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL), with α-ZEL being a metabolite with greater toxicity, presenting a greater affinity for estrogen receptors, while β-ZEL represents a metabolite with less toxicity [31], at birth, the rumen is not completely developed metabolically or physically, and thus does not present full capabilities for ZEN conversion [32]. The physiological difference between calves and adult cattle makes it necessary for more in vivo studies regarding the effects of mycotoxins in calves to more correctly estimate their effects.

Despite bran feed presenting a higher concentration of mycotoxins, we found that the physical presentation did not influence ($p > 0.05$) the concentration of mycotoxins. This is corroborated by the fact that the found mycotoxins are not usually produced during storage; hence, the physical presentation of feed would not have an effect on ZEN and FB concentrations. However, it is important to note that mycotoxins can be present in all types of feed, posing various risks to animal health and productivity.

Heterogeneity of mold and mycotoxin distribution in raw materials and final products represents a difficulty in mycotoxin identification and quantification. The smaller particle sizes in bran feed make for a more homogeneous sample, thus facilitating mycotoxin quantification [33]. This could help explain the negative correlation observed between FBs and physical evaluation, as finer particles might allow for a higher bioavailability and absorption of mycotoxins.

It is necessary to highlight that, despite the collection of samples being conducted within the southeast region of Brazil, this does not mean that the inputs that made the concentrates came from that region. Brazil is a country of continental proportions that makes trade possible between regions with different climates, which makes the task of pinpointing a region with field mycotoxin problems difficult [34]. Further studies are necessary.

Brazil currently does not have regulations regarding mycotoxins in animal feed, but taking into consideration the European Union's established thresholds for fumonisins (20,000 μg/kg) and zearalenone (500 μg/kg) [35], none of the analyzed samples exceeded the recommendations. Nonetheless, it is important to consider the effects of exposure to multi-mycotoxins. When simultaneously present, they may present additive or synergistic effects, thus increasing the risk to animals [36].

Mycotoxins can also contaminate milk, which can become another source of contamination to calves. In our study, 43% of the properties reported using discarded cow milk, the quality of which can be highly variable, being a source of other pathogens, such as bacteria [37]. Studies regarding the presence of mycotoxins in cow's milk in Brazil found the presence of multiple mycotoxins. A study focused on the southeast region identified FB_1, FB_2, DON, AFM_1, α-ZEL, and β-ZEL, with 43% of the samples containing at least two mycotoxins [38]. Another study that evaluated the presence of mycotoxins in commercial milk found that 91.2% of the samples had quantifiable concentrations of one or more types of mycotoxins [39]. This data indicates that even though only FBs and ZEN were detected in the present study, below recommended threshold levels, calves' exposure is probably wider and higher.

Although there are studies on milk contamination, the presence of mycotoxins specifically in discarded cow milk is still largely unknown, and further studies would help to provide a clearer picture of the total mycotoxin intake and its effects on calves' health and growth.

5. Conclusions

This case study report is the first to provide information about the occurrence and concentration of multi-mycotoxins in calf concentrates in the southeast region of Brazil.

The results indicate that major *Fusarium* mycotoxins were frequently found, but with no effect on the physical form of the concentrates. Due to *Fusarium*'s growth characteristics, it can be safely assumed that it represents pre-harvest contamination. The high incidence and co-occurrence of these metabolites should be taken into consideration due to the potential for additive or synergistic effects, considering that calves' liquid diets can also be a source of exposure to other mycotoxins.

Further research with larger sample sizes of calves' solid and liquid diets is needed to establish systematic tracking of mycotoxins, as well as studies regarding the effects of the interactions of different types and concentrations of mycotoxins on calves' health.

Author Contributions: Conceptualization, C.M.M.B. and C.H.C.; methodology, G.C.R.C.E., R.D.P., and T.A.e.S.; software, R.D.P. and A.M.B.; validation, R.D.P. and G.C.R.C.E.; formal analysis, R.D.P. and T.A.e.S.; investigation, R.D.P., A.M.B., T.A.e.S. and G.C.R.C.E.; resources, C.M.M.B. and C.H.C.; data curation, C.M.M.B. and C.H.C.; writing—original draft preparation, R.D.P., A.M.B., T.A.e.S., and G.C.R.C.E.; writing—review and editing, R.D.P., C.M.M.B. and C.H.C.; visualization, C.M.M.B. and C.H.C.; supervision, C.M.M.B. and C.H.C.; project administration, C.M.M.B. and C.H.C.; funding acquisition, C.M.M.B. and C.H.C. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financed, in part, by the São Paulo Research Foundation (FAPESP), Brasil—Process Numbers 2025/04043-3, 2023/16007-6 and 2022/07439-7; the National Council for Scientific and Technological Development (CNPq)—Grant #304262/2021-8; and by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasil (CAPES)—Finance Code 001.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors thank the São Paulo Research Foundation (FAPESP), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and the National Council for Scientific and Technological Development (CNPq) for partly financing this study.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Corassin, C.H.; de Oliveira, C.A.F. Mycotoxins in the Dairy Industry. *Dairy* **2023**, *4*, 392–394. [CrossRef]
2. Vedovatto, M.G.; Bento, A.L.; Kiefer, C.; Souza, K.M.R.; Franco, G.L. Micotoxinas Na Dieta de Bovinos de Corte: Revisão. *Arch. De Zootec.* **2020**, *69*, 234–244. [CrossRef]
3. Goldblatt, L.A. Implications of Mycotoxins. *Clin. Toxicol.* **1972**, *5*, 453–464. [CrossRef] [PubMed]
4. Cole, R.J. Etiology of Turkey "X" Disease in Retrospect: A Case for the Involvement of Cyclopiazonic Acid. *Mycotoxin Res.* **1986**, *2*, 3–7. [CrossRef] [PubMed]
5. El-Sayed, R.A.; Jebur, A.B.; Kang, W.; El-Demerdash, F.M. An Overview on the Major Mycotoxins in Food Products: Characteristics, Toxicity, and Analysis. *J. Future Foods* **2022**, *2*, 91–102. [CrossRef]
6. Hartinger, T.; Grabher, L.; Pacífico, C.; Angelmayr, B.; Faas, J.; Zebeli, Q. Short-Term Exposure to the Mycotoxins Zearalenone or Fumonisins Affects Rumen Fermentation and Microbiota, and Health Variables in Cattle. *Food Chem. Toxicol.* **2022**, *162*, 112900. [CrossRef]
7. Fink-Gremmels, J. The Role of Mycotoxins in the Health and Performance of Dairy Cows. *Vet. J.* **2008**, *176*, 84–92. [CrossRef]
8. Campagnollo, F.B.; Ganev, K.C.; Khaneghah, A.M.; Portela, J.B.; Cruz, A.G.; Granato, D.; Corassin, C.H.; Oliveira, C.A.F.; Sant'Ana, A.S. The Occurrence and Effect of Unit Operations for Dairy Products Processing on the Fate of Aflatoxin M1: A Review. *Food Control* **2016**, *68*, 310–329. [CrossRef]
9. Erickson, P.S.; Kalscheur, K.F. Nutrition and Feeding of Dairy Cattle. In *Animal Agriculture: Sustainability, Challenges and Innovations*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 157–180, ISBN 9780128170526.

10. Khan, M.A.; Bach, A.; Weary, D.M.; von Keyserlingk, M.A.G. Invited Review: Transitioning from Milk to Solid Feed in Dairy Heifers. *J. Dairy. Sci.* **2016**, *99*, 885–902. [CrossRef]
11. Bittar, C.M.M.; Gallo, M.P.; Silva, J.T.; de Paula, M.R.; Poczynek, M.; Mourão, G.B. Gradual Weaning Does Not Improve Performance for Calves with Low Starter Intake at the Beginning of the Weaning Process. *J. Dairy. Sci.* **2020**, *103*, 4672–4680. [CrossRef]
12. Schöne, F.; Rajendram, R. Iodine in Farm Animals. In *Comprehensive Handbook of Iodine*; Elsevier: Amsterdam, The Netherlands, 2009; pp. 151–170.
13. Suárez, B.J.; Van Reenen, C.G.; Gerrits, W.J.J.; Stockhofe, N.; van Vuuren, A.M.; Dijkstra, J. Effects of Supplementing Concentrates Differing in Carbohydrate Composition in Veal Calf Diets: II. Rumen Development. *J. Dairy. Sci.* **2006**, *89*, 4376–4386. [CrossRef] [PubMed]
14. Ribeiro, M.D.; Pereira, J.C.; Bettero, V.P.; de Queiroz, A.C.; Costa, M.G.; de Paula Leonel, F. Níveis de Concentrado Na Dieta de Bezerros. *Rev. Bras. De Zootec.* **2009**, *38*, 1133–1141. [CrossRef]
15. Bittar, C.M.M.; Ferreira, L.S.; Santos, F.A.P.; Zopollatto, M. Desempenho e Desenvolvimento Do Trato Digestório Superior de Bezerros Leiteiros Alimentados Com Concentrado de Diferentes Formas Físicas. *Rev. Bras. De Zootec.* **2009**, *38*, 1561–1567. [CrossRef]
16. Van Gastelen, S.; Mens, A.J.W.; Binnendijk, G.P.; Ellis, J.L.; Powell, C.D.; Gerrits, W.J.J. Effect of Solid Feed Level and Types of Roughage on Passage Kinetics of Milk Replacer, Concentrate, and Roughage in Veal Calves. *J. Dairy. Sci.* **2021**, *104*, 7871–7887. [CrossRef]
17. Gallo, A.; Giuberti, G.; Frisvad, J.C.; Bertuzzi, T.; Nielsen, K.F. Review on Mycotoxin Issues in Ruminants: Occurrence in Forages, Effects of Mycotoxin Ingestion on Health Status and Animal Performance and Practical Strategies to Counteract Their Negative Effects. *Toxins* **2015**, *7*, 3057–3111. [CrossRef]
18. Brasil Produção da Pecuária Municipal; Brasil Ministério da Agricultura; IBGE. Diretoria de Agropecuária, Recursos Naturais e Geografia. *Prod. Pec. Munic. Rio de Janeiro* **2023**, *51*, 1–12.
19. Zanotto, D.L.; Bellaver, C. *Método de Determinação Da Granulometria de Ingredientes Para Uso Em Rações de Suínos e Aves*; EMBRAPA-CNPSA: Concórdia, Brazil, 1996.
20. Sulyok, M.; Krska, R.; Schuhmacher, R. A Liquid Chromatography/Tandem Mass Spectrometric Multi-Mycotoxin Method for the Quantification of 87 Analytes and Its Application to Semi-Quantitative Screening of Moldy Food Samples. *Anal. Bioanal. Chem.* **2007**, *389*, 1505–1523. [CrossRef]
21. Franco, L.T.; Petta, T.; Rottinghaus, G.E.; Bordin, K.; Gomes, G.A.; Oliveira, C.A.F. Co-Occurrence of Mycotoxins in Maize Food and Maize-Based Feed from Small-Scale Farms in Brazil: A Pilot Study. *Mycotoxin Res.* **2019**, *35*, 65–73. [CrossRef]
22. Kemboi, D.C.; Antonissen, G.; Ochieng, P.E.; Croubels, S.; Okoth, S.; Kangethe, E.K.; Faas, J.; Lindahl, J.F.; Gathumbi, J.K. A Review of the Impact of Mycotoxins on Dairy Cattle Health: Challenges for Food Safety and Dairy Production in Sub-Saharan Africa. *Toxins* **2020**, *12*, 222. [CrossRef]
23. Neme, K.; Mohammed, A. Mycotoxin Occurrence in Grains and the Role of Postharvest Management as a Mitigation Strategies. A Review. *Food Control* **2017**, *78*, 412–425. [CrossRef]
24. Juraschek, L.M.; Kappenberg, A.; Amelung, W. Mycotoxins in Soil and Environment. *Sci. Total Environ.* **2022**, *814*, 152425. [CrossRef]
25. Biscoto, G.L.; Salvato, L.A.; Alvarenga, É.R.; Dias, R.R.S.; Pinheiro, G.R.G.; Rodrigues, M.P.; Pinto, P.N.; Freitas, R.P.; Keller, K.M. Mycotoxins in Cattle Feed and Feed Ingredients in Brazil: A Five-Year Survey. *Toxins* **2022**, *14*, 552. [CrossRef]
26. Custódio, L.; Prados, L.F.; Yiannikouris, A.; Holder, V.; Pettigrew, J.; Kuritza, L.; de Resende, F.D.; Siqueira, G.R. Mycotoxin Contamination of Diets for Beef Cattle Finishing in Feedlot. *Rev. Bras. De Zootec.* **2019**, *48*, e20190079. [CrossRef]
27. Awapak, D.; Petchkongkaew, A.; Sulyok, M.; Krska, R. Co-Occurrence and Toxicological Relevance of Secondary Metabolites in Dairy Cow Feed from Thailand. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* **2021**, *38*, 1013–1027. [CrossRef]
28. Feijó Corrêa, J.A.; Orso, P.B.; Bordin, K.; Hara, R.V.; Luciano, F.B. Toxicological Effects of Fumonisin B1 in Combination with Other Fusarium Toxins. *Food Chem. Toxicol.* **2018**, *121*, 483–494. [CrossRef]
29. Osweiler, G.D.; Kehrli, M.E.; Stabel, J.R.; Thurston, J.R.; Ross, P.F.; Wilson, T.M. Effects of Fumonisin-Contaminated Corn Screenings on Growth and Health of Feeder Calves. *J. Anim. Sci.* **1993**, *71*, 459–466. [CrossRef]
30. Chase, C.C.L.; Hurley, D.J.; Reber, A.J. Neonatal Immune Development in the Calf and Its Impact on Vaccine Response. *Vet. Clin. N. Am. Food Anim. Pract.* **2008**, *24*, 87–104. [CrossRef]
31. Kennedy, D.G.; Hewitt, S.A.; McEvoy, J.D.G.; Currie, J.W.; Cannavan, A.; Blanchflower, W.J.; Elliot, C.T. Zeranol Is Formed from Fusarium Spp. Toxins in Cattle in Vivo. *Food Addit. Contam.* **1998**, *15*, 393–400. [CrossRef] [PubMed]
32. Baldwin, R.L.; McLeod, K.R.; Klotz, J.L.; Heitmann, R.N. Rumen Development, Intestinal Growth and Hepatic Metabolism in the Pre- and Postweaning Ruminant. *J. Dairy. Sci.* **2004**, *87*, E55–E65. [CrossRef]
33. Pleadin, J.; Frece, J.; Markov, K. Mycotoxins in Food and Feed. In *Advances in Food and Nutrition Research*; Academic Press Inc.: New York, NY, USA, 2019; Volume 89, pp. 297–345, ISBN 9780128171714.

34. Taniwaki, M.H.; Pitt, J.I.; Copetti, M.V.; Teixeira, A.A.; Iamanaka, B.T. Understanding Mycotoxin Contamination Across the Food Chain in Brazil: Challenges and Opportunities. *Toxins* **2019**, *11*, 411. [CrossRef] [PubMed]
35. Kyprianou, M. European Commission Commission Recommendation of 17 August 2006 on the Presence of Deoxynivalenol, Zearalenone, ochratoxin A, T-2 and HT-2 and Fumonisins in Products Intended for Animal Feeding (2006/576/EC). *Off. J. Eur. Union* **2006**, *49*, L299.
36. Oyesigye, E.; Cervini, C.; Oluwakayode, A.; Mahuku, G.; Medina, A. First Evidence on the Occurrence of Multi-Mycotoxins and Dietary Risk Exposure to AFB1 along the Cassava Value Chain in Uganda. *Mycotoxin Res.* **2024**, *40*, 693–708. [CrossRef]
37. Moore, D.A.; Taylor, J.; Hartman, M.L.; Sischo, W.M. Quality Assessments of Waste Milk at a Calf Ranch. *J. Dairy. Sci.* **2009**, *92*, 3503–3509. [CrossRef]
38. Borowsky, A.M.; Rosim, R.E.; Tonin, F.G.; de Oliveira, C.A.F.; Corassin, C.H. Co-Occurrence of Mycotoxins in the Diet and in the Milk of Dairy Cows from the Southeast Region of Brazil. *Toxins* **2024**, *16*, 492. [CrossRef]
39. Frey, M.; Rosim, R.; Oliveira, C. Mycotoxin Co-Occurrence in Milks and Exposure Estimation: A Pilot Study in São Paulo, Brazil. *Toxins* **2021**, *13*, 507. [CrossRef]