

Mycotoxin poisoning in an intensive beef-fattening system

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Abstract: In a beef cattle feedlot for human consumption, located in San Agustín village, Calamuchita department, Córdoba, Argentina, a marked mortality rate took place during the months of February 2013 to June 2014. Weather conditions were atypical for the area and season; there were high temperatures, droughts followed by periods of excess moisture and rain. The ration animals were fed with was composed of corn, alfalfa hay, corn burlanda, gluten feed, peanut shells and sunflower pellet. Affected animals presented various symptoms such as dyspnea, hemoglobinuria and hematuria, lack of coordination, death and, in many cases, sudden death. Bovines underwent necropsy by which jaundice, hepatitis with focal necrosis, gallbladder edema, hemoglobinuria, hematuria and kidney necrosis were found. The content of aflatoxins and ochratoxins (OTA) in the ration was determined, detecting a high amount of OTA which may have been the cause of cattle mortality.

Keywords: Beef Cattle, Feedlot, Ochratoxin, Aflatoxin

1. Introduction

Contamination of food and agricultural products of various types due to toxigenic mold is a serious problem. Fungi are present naturally and can contaminate food crops under favorable conditions of temperature, relative humidity, pH, nutrients availability and oxygen. Additionally, the loss in crops and food contamination by mycotoxins is a major problem since it has been associated with a wide range of negative effects on human health such as carcinogenic, immunotoxic, teratogenic, hepatotoxic and nephrotoxic effects.

In cattle, the new conditions of intensive management and use of subproducts in the industry enable fungal development and the use of increasingly concentrated diets modify cattle rumen microflora altering the metabolism of mycotoxins and making cattle more susceptible to them.

Mycotoxins can slow performance and cause poor diet, body weight loss and reproductive disorders. In particular, the species belonging to the genera *Aspergillus*, *Fusarium* and *Penicillium* are associated with the production of aflatoxin, fumonisin, ochratoxin, patulin, trichothecenes and zearalenone. Aflatoxins are toxic and carcinogenic compounds. The most toxic is B1 and the most affected

products are corn, peanuts, cotton seed, sorghum and most nuts. Ochratoxin (OTA) can be found in corn, barley, green coffee, wine, beer, cocoa, grapes, spices and dried fruits. OTA is a suspected carcinogen [1, 2] that affects the kidney in animals exposed to natural levels of contaminated food. It can be found along with aflatoxin. Human exposure to mycotoxins is mostly through plant foods. However, additional potential exposure may be through animal foods such as milk, cheese and meat from animals fed with mycotoxin contaminated foods. These can produce toxic waste and biotransformation products. In our country there are not many precedents for this type of research, for this reason we consider it useful for feedlots to take the necessary measures to control the portions they use as food.

2. Material and Methods

2.1. Weather Background

Table 1 shows temperature, humidity and rainfall records of the area studied, in which we can observe the weather conditions that took place, periods of drought with high

maximum temperatures, periods of heavy rainfall (higher than the monthly average) and relative humidity of 100% for many days. These conditions are not typical of this region

and contributed directly or indirectly to the proliferation / contamination of the different components of the ration with mycotoxin.

Table 1. Record of Temperature, Humidity and Precipitation.

January			February			March			
Days	Maximum temperature °C	Maximun relative humidity %	Rainfall mm	Maximum temperature °C	Maximun relative humidity %	Rainfall mm	Maximum temperature °C	Maximun relative humidity %	Rainfall mm
1	32.7	100		29,8	100		24,7	100	20
2	26.9	100	36	26,1	100	28	18,6	100	
3	27.3	100		25,2	100	11	21,9	100	
4	32.6	90		25,5	100		24,5	100	
5	33.5	77		31,8	100	63	29,2		
6	34.5	94		22,1	100				17
7	35.9	96		30,0	100				
8	34.5	93		33,2	100	13			
9	36.3	97	4	23,6	100	11			9
10	32.4	92		26,9	100				
11	30.5	92		27,9	100	11			
12	27.8	96		29,8	100				
13	30.8	100		27,0	100	5			55
14	33.4	97		23,6	100	7			18
15	37.7	88		24,3	100				
16	36.3	92		24,0	100	17			
17	36.4	92		21,8	100				
18	37.7	92	2	25,5	100				
19	31.9	98		28,8	100				
20	35.5	97		29,1	100				
21	33.9	84		23,0	100				
22	37.5	100		24,7	100	18			
23	38.4	100	44	27,0	100				
24	24.1	100		25,4	100	50			
25	23.6	100		17,4	100				
26	27.6	100		19,5	100				
27	26.3	99	8	22,9	100				
28	30.8	100		23,6	100				
29	30.5	100			100				
30	31.5	100							
31	34.3	100							

Source: Department of Agro-meteorology of Experimental Station Manfredi of INTA (National Agricultural Technology Institute).

In a direct way because the ideal conditions for the growth of different fungal species were generated: *humidity*, the more moisture, the more proliferation. *Temperature*, the temperature range for optimum fungi growth is from 25°C to 35°C, with species that are tolerant up to 55 ° C and 4 ° C minimum. [3-5]. In an indirect way because the raw materials (burlanda) in the ration could not be transferred to the facilities and could not be consumed in time and quality. We should also keep in mind that fungal stress processes favors their mycotoxin production.

Most animals (steers and heifers in final fattening phase) affected died after more than 30 days on site, and the ration they were fed on consisted of alfalfa hay, cracked corn, sunflower pellet, corn burlanda and / or gluten feed. These last in 40% of the gross weight of the ration. The different components of the ration have already been mentioned: corn, alfalfa hay, sunflower pellets, corn burlanda, gluten feed. It is important to remember that corn burlanda is a byproduct of the production of ethanol and that it concentrates loads of mycotoxins or fungi that could be brought from the corn from which it originated. Gluten feed is the byproduct of the

extraction of glucose from the corn grain. Burlanda and gluten feed have 67% humidity and a high Aw (water activity) favoring the development of different species of fungi [5, 6].

2.2. Mycotoxin Content of the Ration

The mycotoxin aflatoxin were analzed with the Veratox Quantitative Aflatoxin test, ELISA kit, Neogen (USA) and by the Tam et al method (7) and ochratoxin were determined with the Veratox Quantitative Ochratoxin test, ELISA kit and by the Ng et al. method [8].

2.3. Cattle Study

The clinical signs presented were: sudden death, dyspnea, anorexia, polydipsia, jaundice, lethargy, ambulation, anuria, red urine (hematuria and hemoglobinuria), and there was no increase in body temperature.

3. Results

Table 2 shows that during 2013 and 2014 cattle deaths

occurred on site. It can be seen how they increased during the months of January, February and March 2014.

Table 2. Cattle death.

	January	February	March	April	May	June	July	August	September	October	November	December
2013		3		4		2	2	1			1	5
2014	11	50	15	2	1	0						

The values found of mycotoxins in different samples can be in Table 3.

Table 3. Content of mycotoxins in different samples.

Substratum	Aflatoxin ppb	Ochratoxin ppb
Ration	5.2	29.5
Corn Burlanda	4.3	29.9
Gluten feed	4.1	32.5
Corn	3.3	5.9

The content of mycotoxins with mortality in the months of January to April 2014 are shown in Table 4.

Table 4. Relationship of mycotoxins and monthly mortality.

	Aflatoxin / ppb	Ochratoxin / ppb	Number of dead animals
January/14	8.1	7.3	11
February/14	5.0	29.5	50
March/14	3.3	8.2	15
April/14	3	1.3	2

Urinalysis:

Appearance: Cloudy

Color: Reddish

Density: 1018

Ph: 7

Hemoglobin: ++++

Proteins: ++

Bilirubin: none

Urobilinogen: none

Glucose: none

Ketones: none

Nitrite: none

Sediment

Cylinders: none

Thin epithelial cells: none

Round transition Cells: none

Renal and tubular cells: Frequently isolated and grouped 7

/ C

Leukocytes: Some isolated and grouped 2 / C

Erythrocytes: Some isolated and grouped 2 / C

Bacteria: none

Crystals: none

Findings at necropsy:

Highly marked Jaundice in mucosa and subcutaneous adipose tissue.

Dark intestines, congestive and hemorrhagic mucosa with arborization. Bloody feces. Hemorrhagicenteritis. Gas production is observed.

Dark Kidneys from gray to deep black, friable and in some cases amorphous even autolytic (Fig.1) hematuria and hemoglobinuria (not always), full-fledged bladder (anuria)

very strong generic *smell*.

Liver with an increased size (Hepatomegaly), a deep ocher color, areas of necrosis, friable to touch, full-fledged gallbladder with emphysema and edema, very dense umber bile with the lumpy appearance of clay (Fig.2)

In some cases only the liver injury was found.

Muscles have a diminished color (pale pink).

Macroscopically livers with a firm consistency, regular edges and smooth surfaces were observed, some exhibited a pale color, others had a marked lobular pattern and most of them had jaundice of varying intensity. The pieces sent were of various sizes.



Fig. 1. Kidney black, friable, autolytic.



Fig. 2. Hepatitis, liver ocher gallbladder eventful, emphysema perivesicular.

Microscopically the liver parenchyma showed necrosis with centrilobular predominance (Fig.3), varying amounts of fat change, lipidosis, biliary hyperplasia, condensation of fibrous stroma (Fig.4) and eventually regenerating nodules. Hepatocytes showed atypia, vacuolization, degeneration and some had extremely large cores and larger volume (megakocytes) (Fig.5).

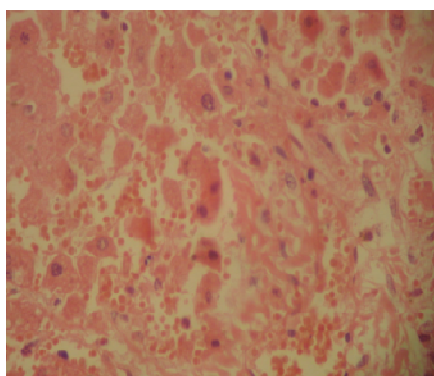


Fig. 3. Centrilobular necrosis HE 40 X.



Fig. 4. Stromal diffuse fibrosis HE 40 X.

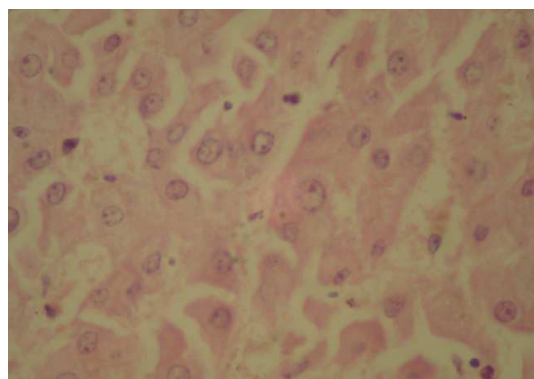


Fig. 5. Megalocytes. HE. 40 X.

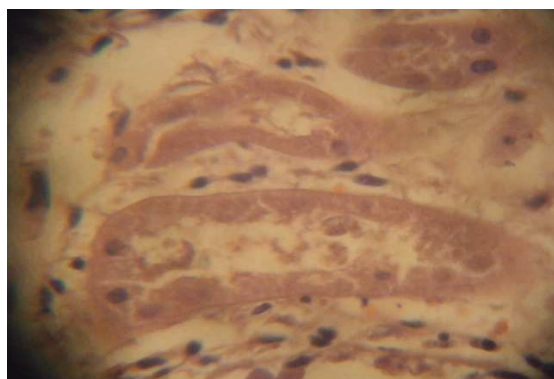


Fig. 6. Necrosis diffuse HE 40 X.

Macroscopically pieces belonging to the kidney were observed, some had a solid consistency, when cut some

revealed a semi-soft consistency, with regular edges, smooth surfaces and different measures (3x2, 2x1, 6cm; 3x2, 7x2cm; 2,5x2x2cm; 4x2x2cm and 3x2x1, 7cm)

Microscopically renal parenchyma with interstitial nephritis was observed there was also presence of interstitial, intertubular and interglomerular fibrin. The cells of the renal tubules are degenerated, vacuolated. Diffuse necrosis (Fig. 6). Other sections showed congestion with preserved parenchyma and stroma.

These histopathological lesions corresponding to diffuse hepatic fibrosis and renal lesions are not pathognomonic of a particular disease as they occur in different toxins (aflatoxins, OTA, pyrrolizidine alkaloids, nitrosamines) [9, 10] but it is important to accompany visible histopathological lesions with the context, rodeo clinical characteristics and other confirmatory studies.

Diagnosis:

It was made from injuries and the confirmation of mycotoxins in the rations given to the animals.

Differential Diagnosis:

Leptosirosis:

Seorología: Martin and Petit Microagglutination was conducted to investigate *Leptospira interrogans*, serovars: pomona, wolffi, Icterohemorrhagic and canicola. The results were NEGATIVE

Bacillary hemoglobinuria: No consistent lesions were found.

4. Discussion

In general there is no data of OTA poisoning in cattle, due to the ability of the rumen protozoa microflora to metabolize and hydrolyse OTA to ochratoxin-alpha that is not toxic and does not degrade. This ability is affected by the variation of rumen microflora due to the consumption of the different food types that are assigned to cattle. There are studies in which it is proved that different types of diets have a large influence on the metabolism of some mycotoxins. Thus, a diet based on 100% hay leads the rumen fluid to a pH of 7.1, ochratoxin A is hydrolyzed to ochratoxin alpha (non-toxic) in 36 minutes. When the diet contains 70% hay and 30% concentrate the pH of rumen liquid is 6.5, OTA hydrolysis takes longer (1:18 hours). If diets with 100% concentrate are consumed, this hydrolysis takes 3:18 hours [4]. This could explain the absorption of unhydrolyzed mycotoxins and cause the pathologies described below.

Although prevention of mycotoxin contamination in the field is the main objective of the agriculture and food industries, methodologies must be developed during the processing of food for slowing the growth of fungi.

Post-harvest strategies aim at the reduction of fungal contamination and, consequently, the levels of mycotoxins in agricultural products during storage, handling, processing and transportation. Such strategies include the improvement of the conditions of drying and storage, the use of chemical and natural agents, and irradiation. Unfortunately, contamination cannot be completely avoided. Therefore,

there is an increased focus on efficient detoxification methods for mycotoxins present in foods, and in inhibiting mycotoxin absorption in the gastrointestinal tract. It is also possible to prevent the toxic effects of mycotoxins using feed additives, such as antioxidants, sulfur-containing amino acids, vitamins and trace elements. A new strategy to control mycotoxicosis is the application of microorganisms (lactic acid bacteria, yeast) capable of biotransforming some mycotoxins into less toxic metabolites.

5. Conclusions

After confirmation of the presence of toxins in the feed ration, the component of the ration that presented a higher load of mycotoxins was identified in order to limit its inclusion or to remove it. Mycotoxin adsorbent was used in the ration [5].

A control plan was implemented through periodic surveys directed at the mycotoxin content of the ration to establish its seasonal incidence and to anticipate the occurrence of poisoning.

In this investigation the levels of mycotoxins in bovine feed was reduced using absorbents, as a result of this cattle mortality in the establishment ceased.

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