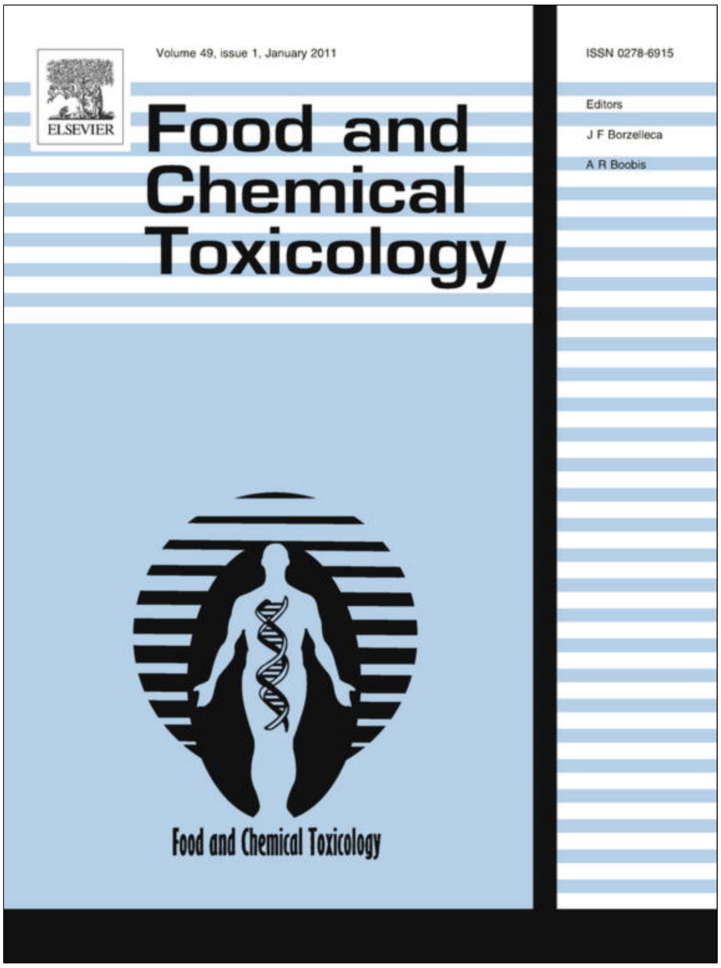

(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

Food and Chemical Toxicology 50 (2012) 250–257

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Exposure assessment of mycotoxins in cow's milk in Argentina

M.L. Signorini [a,b,*], M. Gaggiotti [a,1], A. Molineri [a,1], C.A. Chiericatti [c,2], M.L. Zapata de Basílico [c,2], J.C. Basílico [c,2], M. Pisani [a,1]

[a] Instituto Nacional de Tecnología Agropecuaria EEA Rafaela, Ruta 34 Km 227, Rafaela, C.P. 2300, Provincia de Santa Fe, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto Nacional de Tecnología Agropecuaria EEA Rafaela, Ruta 34 Km 227, Rafaela, C.P. 2300, Provincia de Santa Fe, Argentina
[c] Microbiology Laboratory of the School of Physics and Chemical Sciences in the Litoral National University, Santiago del Estero 2829, Santa Fe, C.P. 3000, Argentina

ARTICLE INFO

Article history:
Received 8 October 2010
Accepted 28 September 2011
Available online 10 October 2011

Keywords:
Mycotoxins
Milk
Exposure assessment

ABSTRACT

A stochastic simulation model was developed to carry out the first quantitative risk exposure assessment of the mycotoxin level in cow's milk produced in Argentina. The prevalence and concentration of aflatoxin M1 (AFM1), deoxynivalenol (DON) and zearalenone (ZEA) were modeled at various stages through milk processes complying with Argentinean practices. Concentration of AFM1 (0.059 ppb), DON (0.338 ppb) and ZEA (0.125 ppb) in dairy milk were estimated. The proportion of feed samples that exceeded the maximum level accepted by European regulations for AFB1, DON and ZEA were estimated at 25.07%, 0.0% and 8.9%, respectively. The percentage of milk samples that exceeded the maximum level accepted for AFB1 by the MERCOSUR (0.5 ppb) and the European Union regulations (0.05 ppb) were 0.81 and 32.65, respectively. The probability distribution of AFM1 concentration in milk was affected by the carry-over rate equations applied in the model. Mycotoxin levels in corn silage and concentrated feeds were the factors most correlated with mycotoxin concentrations in milk. Therefore, agricultural practices, crop management and feed production require prompt attention regarding mycotoxin issues.

1. Introduction

Mycotoxins are secondary metabolites produced by specific filamentous fungi that are common contaminants of agricultural commodities (Binder, 2007; Boudra and Morgavi, 2005; Pereyra et al., 2008). There are more than 300 different mycotoxins; however, the major classes of mycotoxins affecting feedstuffs include aflatoxins (AF), deoxynivalenol (DON) and zearalenone (ZEA), occurring the highest prevalence in corn and in ingredients for concentrate feeds (Driehuis et al., 2008; Binder, 2007).

Mycotoxins are capable of altering immune-mediated activities (Black et al., 1992), or producing acute toxic, carcinogenic, mutagenic, teratogenic and estrogenic effects on animals depending on the level of exposure (van Egmond, 1989). In cattle, mycotoxin consumption is associated with a decrease in feed intake, weight loss, reduced milk production, lack of response to diet change and therapies (Driehuis et al., 2008).

Mycotoxins, such as aflatoxins, can be also transferred to milk and its presence is considered to be undesirable (Yiannikouris and Jouany, 2002; Seglar, 2003). The occurrence of mycotoxins in dairy products and mainly in milk makes it a particular risk for humans because of their negative effects in foodstuff for adults and especially children (Prandini et al., 2009). The most prominent toxicological effects of the major classes of mycotoxins are recognized, but little is known about possible synergistic or antagonistic effects of mycotoxins and whether frequent exposure to low doses leads to chronic health problems (Driehuis et al., 2008).

Because mycotoxin contamination of foods and animal feeds highly depends on environmental conditions leading to mould growth and toxin production (van Egmond, 1989), as well as to multiple possibilities to produce animal diets, the inter-annual variability made the estimation of mycotoxin contamination a very complex task (FAO-WHO, 2001).

Quantitative exposure assessment is a methodology used to analyze scientific information to estimate the probability and severity of an adverse event. Risk assessment is now widely accepted as the preferred means to assess possible links between hazards in the food chain and actual risks to human health (FAO-WHO, 2006). The risk assessment results could be the scientific basis of risk management options.

The objective of this study was to develop a quantitative exposure assessment model for mycotoxins in cow's milk produced in Argentina, to improve the basis to decide on policy making and

* Corresponding author at: Instituto Nacional de Tecnología Agropecuaria EEA Rafaela, Ruta 34 Km 227, Rafaela, C.P. 2300, Provincia de Santa Fe, Argentina. Tel.: +54 3492 440121.
E-mail address: marcelo.signorini@gmail.com (M.L. Signorini).
[1] Tel.: +54 3492 440121.
[2] Tel.: +54 342 4571164.

0278-6915/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2011.09.036

research objectives, and to reduce human and animal health hazards due to mycotoxins.

2. Materials and methods

2.1. Model development

The prevalence and concentration of mycotoxins were modeled at various stages along milk production processes. The conceptual model upon which the mathematical model was based is depicted in Fig. 1. The model was created in Microsoft Excel 2007 with the add-on package @Risk (version 4.0, Palisade Corporation, New York, USA).

The mycotoxins considered in this study were aflatoxin B1 (AFB1), deoxynivalenol (DON) and zearalenone (ZEA).

The model was developed using inputs from data collected at Argentinean dairy systems, and expert information and opinion, whenever possible. However, when Argentinean-specific data were not available, international data and scientific literature were consulted to improve the basis of the model.

The Monte Carlo Model Simulation Technique (applying 5000 iterations) was used to create the output distributions which reflect the inherent uncertainty and variability in each input variable. The number of iterations provided adequate convergence of the simulation statistics (<1%).

2.2. Model inputs

2.2.1. Animal diets

The amount of each ingredient in the diet (kg of dry matter per cow) for dairy cows in Argentina depends on two factors: (a) the season (S) (autumn and spring), and (b) the milk production level (low and high milk production) (MP).

The same probability was considered for each season, using a binomial distribution with probability = 0.5.

Cows in the first 3 months of lactation were considered as high milk production, and those from the fourth month to the end of the lactation were considered as low milk production. To develop the model, lactation cows from any of the 10 months of lactation (ML) were considered as having the same probability and using a discrete uniform distribution. The ingredients in each diet are depicted in Table 1. These diets (ingredients and quantities) were a reflection of the diets used in Argentina's central dairy region. Nevertheless, those feeding formulations can be changed regularly depending on ingredient availability and pricing.

2.2.2. Mycotoxin contamination in feed ingredients

Occurrence and concentration of each kind of mycotoxins found in the different ingredients were obtained from a data base generated by the Agricultural Experiment Station in Rafaela; Santa Fe, of the National Institute of Agricultural Technology and the Microbiology Laboratory of the School of Physics and Chemical Sciences,

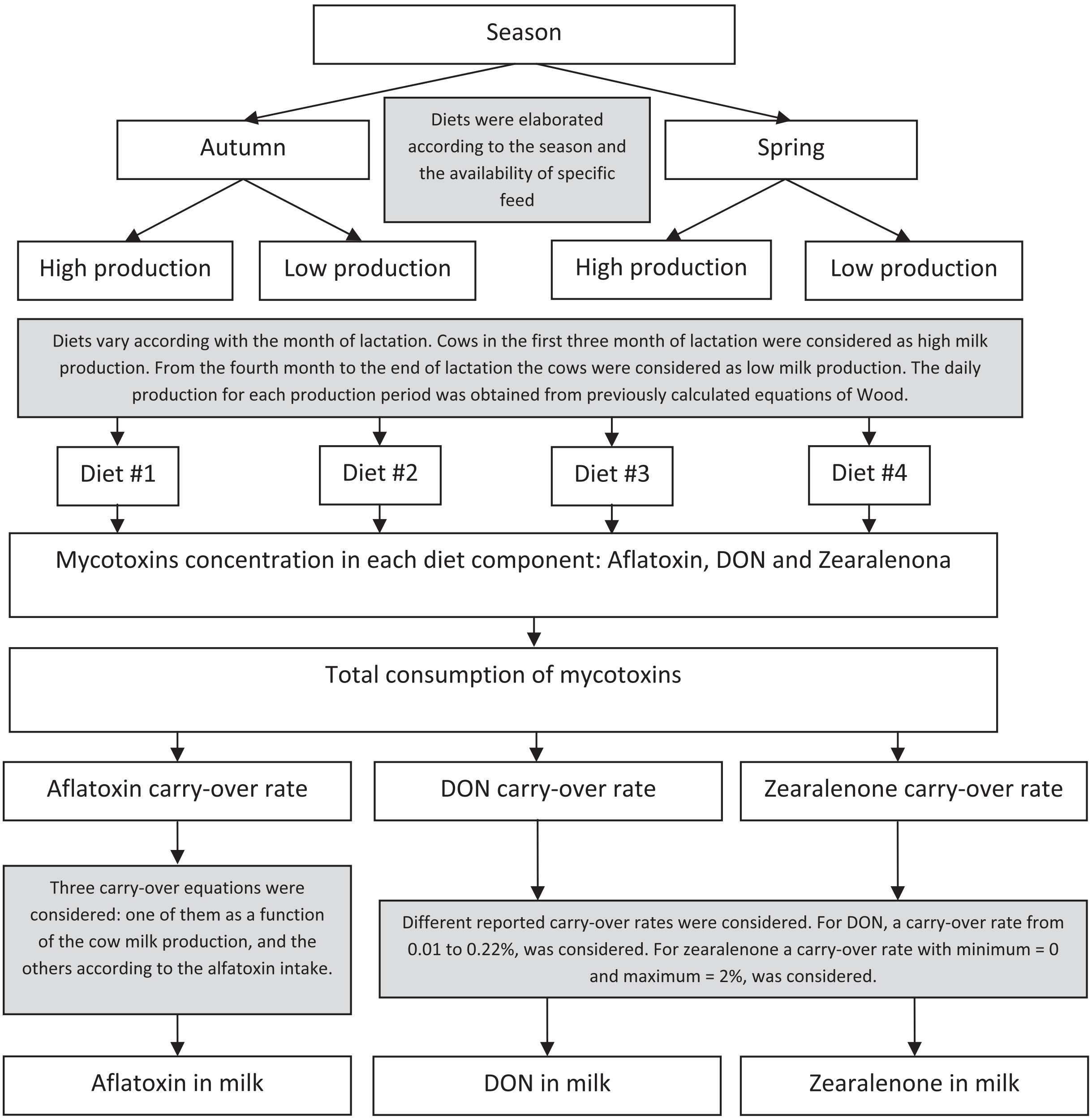

Fig. 1. Flow diagram of the mathematical model of exposure assessment of mycotoxins in bovine milk in Argentina.

Table 1
Diet composition of low and high milk production cows in autumn and spring seasons. Source: Romero et al., 2004.

Ingredient	Quantity in the diet (kg/DM) (CD)			
	Autumn		Spring	
	LP cows	HP cows	LP cows	HP cows
Alfalfa	4.0	6.5–7.0	14.0	10.0
Corn silage	7.0	7.3		3.0
Concentrate	4.5	6.5	4.0	6.0
Cotton seed	2.0			
Alfalfa hay		0.8		1.0
By-products		2.9		2.0

References: LP = low production; HP = high production.

Universidad Nacional del Litoral (Argentina). Eight hundred and forty-three feed samples (fresh grass, silage, hay, concentrated feed, cotton seed and industrial by-products), collected from 2000 to 2009 (Table 2) were tested. Feed samples were obtained from milk farms located in the central dairy region of Argentina (Gaggiotti et al., 2001, 2003). ELISA assays were used to obtain the mycotoxin concentration (μg/kg of feed, ppb). The limit of quantification for total AF, DON, and ZEA were 1.7, 200 and 50 μg/kg (Ridascreen® Fast, R-Biopharm, AG Darmstadt, Germany), respectively. The technique to quantify AF was not specific for AFB1, therefore, a correction factor of 0.8 was introduced to estimate the AFB1 concentration, considering the results presented by Penno (1995).

From this set of information for each mycotoxin in the different types of feed the results were adjusted using the appropriate probability distribution (Table 3). In those samples where the mycotoxin level was below the limit of detection, a uniform distribution was used, with a minimum value = 0 and a maximum value = technique limit of detection.

The amount of mycotoxin ingested by the selected cows was calculated as the sum of the mycotoxin level of each ingredient in the diet.

2.2.3. Carry-over rates of mycotoxins

Aflatoxin M1 (AFM1) is the principal oxidized metabolite of AFB1, and it can be easily found in milk and urine of most mammalians after the consumption of AFB1 (Creppy, 2002). In dairy cows the amount of AFM1 excreted into milk is affected by milk yield (Petterson et al., 1989; Veldman et al., 1992), and stage of lactation (Munksgaard et al., 1987; Petterson et al., 1989; Veldman et al., 1992). Three linear regression equations were used to model the carry-over rate, one of them as function of the milk yield (Masoero et al., 2007), and two equations related to AFB1 consumption (Petterson et al., 1989; Veldman et al., 1992).

Milk production per cow was estimated from a study reported by Romero et al. (2004), who adjusted the milk production curves from 79 cows in different seasons (autumn and spring), under the Argentinean production system, fitting for each season a Woods' equation (1969). Afterwards, the curves were incorporated into the model.

Deoxynivalenol level carry-over to bovine milk is estimated to be small but there is very little research done in the area (EFSA, 2004a). Transmission of DON to milk was confirmed in lactating dairy cows, but only extremely low amounts naturally occurring were detected (Côte et al., 1986; Yoshizawa et al., 1986). The total carry-over rates of ingested DON as DON and de-epoxy DON in milk ranged from 0.0001 to 0.0002 and from 0.0004 to 0.0024, respectively (Seeling et al., 2006; Fink-Gremmels, 2008). Two uniform distributions were developed for DON and de-epoxy DON carry-over rates according to the limits reported by Seeling et al. (2006), and the total DON carry-over was the addition of these distributions.

Several studies have been conducted to assess the ZEA carry-over into milk (Mirocha et al., 1981; Prelusky et al., 1990; Usleber et al., 1992; Galtier, 1998; Yiannikouris and Jouany, 2002; Seeling et al., 2005). These studies show that there is a low transmission rate into milk. The carry-over rate was modeled using a uniform distribution according to the values reported by Fink-Gremmels (2008).

2.2.4. Consumption data

The ingested doses (D) of each mycotoxin is a function of their concentration in raw milk and the quantities of milk consumed by Argentinean consumers. The mean consumption of pasteurized fluid milk (WM_c), ultra high temperature milk (UHT_c), powder milk (Pw_c) and other processed milk (Pr_c) (evaporated and concentrated milks), for adults was 81.09 g/day (±8.39 g/day), 26.97 g/day (±12.45 g/day), 7.89 g/day (±1.81 g/day), and 0.78 g/day (±0.27 g/day), respectively. A normal distribution was used with the mean and standard deviation for each product. Data on the quantities of milk consumed in Argentina were obtained from the Argentinean Ministry of Agriculture (MinAgri, 2010).

The human exposure assessment for each mycotoxin (g/kg body weight/day) was calculated based on the total exposure from each mycotoxin, assuming a body weight (BW) of 60 kg (Prandini et al., 2009).

2.2.5. Sensibility analysis

To determine the impact of each input variable on the outputs variable (for example, AFM1 in milk), a sensitivity analysis was conducted, using Pearson's correlation coefficient to determine the degree of association. Sensibility analysis was performed using the @Risk® version 4.5 software (Palisade, New York).

2.2.6. Model assumptions

The following assumptions were applied to the model. Because assumptions can impact on the obtained results, they should be taken into account when considering the outputs of the risk assessments:

(i) The model considered that the proportion of milking cows was the same for each season.
(ii) To develop the model, it was considered that the length of lactation was the same for all the cows, and each cow could be in any of the 10 months of lactation with the same probability.
(iii) Even considering that the diets (ingredients and quantities) were a reflection of the diets used in Argentina's central dairy region, they could change regularly depending on ingredient availability and pricing.
(iv) In those ingredients where the mycotoxin level was below the limit of detection, a uniform distribution was used, with a minimum value = 0 and, a maximum value = technique limit of detection.
(v) The dilution effect between whole milk with different levels of mycotoxins was not considered.
(vi) Considering that the mycotoxins are, in general, heat stable, it was assumed that the milk production processes (e.g. pasteurization, sterilization, evaporation, concentration), do not cause an appreciable change in the amount of mycotoxins in milk.

3. Results

3.1. Mycotoxins in different feedstuffs

In the database used to develop this model, AFB1 and ZEA were more frequently detected than DON. Incidence of AFB1 was particularly high in concentrated feed and cotton seed, being the AFB1 general prevalence of 78.9%. ZEA was found in 78.8% of the analyzed feed samples, especially in corn silage, industrial by-products and pasture. On the other hand, DON was particularly important in concentrated feeds and industrial by-products.

Co-occurrence of AFB1, DON and ZEA was frequently observed. Presence of AFB1 and ZEA was the most prevalent co-occurrence and the simultaneous presence of AFB1, DON and ZEA in the same feed was observed in 39.2% of the samples.

Table 2
Prevalence of mycotoxins in different diet ingredients expressed as number of samples with mycotoxin concentration greater than the limit of quantification/total number of samples. Source: Gaggiotti et al., 2001, 2003

Ingredient	AFB1	DON	ZEA	AFB1-DON	AFB1-ZEA	DON-ZEA	AFB1-DON-ZEA
Concentrate	110/131	37/62	45/69	31/59	38/63	25/54	23/53
Cotton seed	57/69	2/9	6/21	2/9	13/18	1/9	1/9
Silage	202/277	67/191	99/121	49/165	79/108	27/58	26/57
Alfalfa hay	7/11	3/8	8/11	0/4	5/8	0/3	0/3
By-products	31/39	14/27	34/37	10/27	25/36	13/27	9/27
Alfalfa	64/70	19/55	25/31	15/45	23/27	3/9	3/9
Total	471/597	142/352	217/290	107/309	183/260	69/160	62/158

References: AFB1 = aflatoxin B1, DON = deoxynivaleno, ZEA = zearalenone.

Table 3
Description of the input variables used in the model.

Symbol	Name	Units	Assumption/distribution
S	Season		Binomial(1,0.5)
ML	Month of lactation		Discrete uniform({1;2;3;4;5;6;7;8;9;10})
$AFB1_{aa}$	Level of AFB1 in alfalfa	µg/kg	Triangular(0, 1.7, Cumulative(0, 22, {1.7;2.5;3.5;5.5;7;9;13.2}, {0.114;0.286;0.457;0.614;0.743;0.9;0.986}))
$AFB1_{MS}$	Level of AFB1 in corn silage	µg/kg	Cumulative(0,69.6,{1.7;3.4;5.5;8;11;18.7;66.4}, {0.27;0.50;0.69;0.83;0.92;0.97;0.99})
$AFB1_{AH}$	Level of AFB1 in alfalfa hay	µg/kg	Cumulative(0, 26, {1.7;2;2.6;3.2;5}, {0.36;0.54;0.72;0.81;0.91})
$AFB1_{BC}$	Level of AFB1 in concentrate	µg/kg	Cumulative(0, 139, {1.7;3;4.5;7;22}, {0.16;0.35;0.55;0.76;0.91})
$AFB1_{CS}$	Level of AFB1 in cotton seed	µg/kg	Cumulative(0116,{1.7;3.5;9},{0.17;0.42;0.71})
$AFB1_{BP}$	Level of AFB1 in by-products	µg/kg	Cumulative(0, 76.8, {1.7;3;4.5;13.8}, {0.23;0.46;0.66;0.97})
DON_{aa}	Level of DON in alfalfa	µg/kg	Triangular(0, 200, IF(E98 < 200,200, Cumulative(0, 400, {200;300}, {0.85;0.94})))
DON_{MS}	Level of DON in corn silage	µg/kg	Cumulative(0, 2500, {200;300;400;600;1200}, {0.78;0.86;0.91;0.95;0.99})
DON_{AH}	Level of DON in alfalfa hay	µg/kg	Uniform(0, 200)
DON_{BC}	Level of DON in concentrate	µg/kg	Cumulative(0,3800,{200;400;900},{0.48;0.75;0.88})
DON_{CS}	Level of DON in cotton seed	µg/kg	Cumulative(0, 800, {200;800}, {0.88;1})
DON_{SI}	Level of DON in by-products	µg/kg	Cumulative(0,1900,{200;500;800;1300;1500;1600}, {0.59;0.70;0.81;0.88;0.92;0.96})
ZEA_{aa}	Level of ZEA in alfalfa	µg/kg	Triangular(0, 50, IF(ZEA_{aa} < 50,50, Cumulative(0, 623, {53;131.2;236;386;588.5}, {0.22;0.45;0.67;0.90;0.96}))
ZEA_{MS}	Level of ZEA in corn silage	µg/kg	Cumulative(0,4005,{50;100;180;293;400;801;1500;2500}, {0.18;0.33;0.48;0.67;0.82;0.94;0.96;0.99})
ZEA_{AH}	Level of ZEA in alfalfa hay	µg/kg	Cumulative(0350,{50;73.6;95.5;100;228;300}, {0.27;0.36;0.54;0.72;0.81;0.90})
ZEA_{BC}	Level of ZEA in concentrate	µg/kg	Cumulative(0, 1500, {50;116;175}, {0.36;0.66;0.85})
ZEA_{CS}	Level of ZEA in cotton seed	µg/kg	Cumulative(0,6000,{50;120;572.3;6000},{0.23;0.5;0.69;1})
ZEA_{SI}	Level of ZEA in by-products	µg/kg	Cumulative(0, 5782.3, {50;132.7;200;344;502;723.2}, {0.08;0.29;0.56;0.86;0.91;0.97})
Con_{AFB1}	Total consumption of AFB1	µg	$(\Sigma(CD \times AFB1_{(in\ each\ ingredient)})) \times 0.8$
Con_{DON}	Total consumption of DON	µg	$\Sigma(CD \times DON_{(in\ each\ ingredient)})$
Con_{ZEA}	Total consumption of ZEA	µg	$\Sigma(CD \times DON_{(in\ each\ ingredient)})$
MP_{autumn}	Milk production (autumn)	Lts	$22.82 * LM^{0.141} * 2.718^{(-0.051*LM)}$
MP_{spring}	Milk production (spring)	Lts	$29.12 * LM^{0.483} * 2.718^{(-0.204*LM)}$
$AFM1_{CO1}$	AFM1 carry-over rate (yield production)	%	$-0.3255 + 0.0769*(MP_{autumn}$ or $MP_{spring})$
$AFM1_{milk1}$	AFM1 in milk	µg/lt	$Con_{AFB1}*(AFM1_{CO1}/100))/(MP_{autumn}$ or $MP_{spring})$
$AFM1_{milk2}$	AFM1 in milk (AFB1 consumption)	µg/lt	$10.95+(0.787*Con_{AFB1}))/1000$
$AFM1_{CO2}$	AFM1 carry-over rate (AFB1 consumption)	%	$AFM1_{milk2} * (MP_{autumn}$ or $MP_{spring})*100)/Con_{AFB1}$
$AFM1_{milk3}$	AFM1 in milk (AFB1 consumption)	µg/lt	$1.9+(1.2*Con_{AFB1})/1000$
$AFM1_{CO3}$	AFM1 carry-over rate (AFB1 consumption)	%	$AFM1_{milk3} * (MP_{autumn}$ or $MP_{spring})*100)/Con_{AFB1}$
DON_{CO}	DON carry-over rate		Uniform(0.0001, 0.0002) + Uniform(0.0004, 0.0024)
DON_{milk}	DON in milk	µg/lt	$Con_{DON} * DON_{CO}$ / $(MP_{autumn}$ or $MP_{spring})$
ZEA_{CO}	ZEA carry-over rate	%	Uniform(0.06.0.08)
ZEA_{milk}	ZEA in milk	µg/lt	$Con_{ZEA} * (ZEA_{CO}$ / 100) / $(MP_{autumn}$ or $MP_{spring})$
WM_c	Pasteurized milk consumption	g/day	Normal(81.09,8.39)
UHT_c	UHT milk consumption	g/day	Normal(26.97,12.45)
Pw_c	Powder milk consumption	g/day	Normal(7.89,1.81)
Pr_c	Processed milk consumption	g/day	Normal(0.78,0.27)
TE	Total milk exposure	g/day	$WM_c + UHT_c + Pw_c + Pr_c$
BW	Body weight	kg	60 (fixed value)
D	Total exposure	g/kg bw/day	((Concentration of each mycotoxin/1000) x TE) / IW

3.2. Prediction model

Concentration of AFB1, DON and ZEA in the dairy cattle diets was estimated in 4.7, 230.5 and 194.9 ppb, respectively (Fig. 2). The uncertainty about the true mean value (95% confidence interval) was calculated for AFB1 (0.832–23.14 ppb), DON (48.34–880.35 ppb), and ZEA (26.74–770.15 ppb).

The average concentration of AFM1 in bovine milk varied considering the carry-over rate equation applied. When the carry-over rate equation that considered milk production was used, AFM1 concentration in bovine milk was estimated in 0.059 ppb (95% CI 0.032–0.323 ppb). However, when the other two equations that considered the AFB1 consumption were used, the AFM1 concentration in bovine milk was increased to 0.082 ppb (95% CI 0.023–0.354 ppb), and 0.111 (95% CI 0.02–0.526) (Fig. 3).

The average concentration of DON and ZEA in bovine milk was estimated in 0.338 ppb (95% CI 0.049–1.396 ppb), and 0.125 ppb (95% CI 0.016–0.469 ppb), respectively (Fig. 4).

The AFM1 concentration in bovine milk was sensitive to the AFB1 level on concentrate ($r = 0.735$), the carry-over rate ($r = 0.351$), the AFB1 level on corn silage ($r = 0.186$), the season ($r = -0.143$), and the AFB1 level on cotton seed ($r = 0.119$) (Fig. 4). The DON concentration in bovine milk was sensitive to the DON levels on concentrate ($r = 0.65$), the carry-over rate ($r = 0.373$), corn silage ($r = 0.155$), pasture ($r = 0.096$), and cotton seed ($r = 0.078$). Finally, the ZEA concentration in bovine milk was sensitive to the ZEA level on corn silage ($r = 0.439$), the ZEA level on concentrate ($r = 0.398$), the season ($r = -0.35$), the ZEA level on pasture ($r = 0.182$), and the carry-over rate ($r = 0.072$), (Fig. 5).

The total daily intake (TDI) estimated for AFM1, DON, and ZEA in milk were 1.22×10^{-4} µg/kg bw (95% IC 7×10^{-6}–6.33×10^{-4} µg/kg bw), 4.44×10^{-4} µg/kg bw (95% IC 3×10^{-5}–1.96×10^{-3} µg/kg bw), and 1.68×10^{-3} µg/kg bw (95% IC 9×10^{-9}–1.27×10^{-2} µg/kg bw), respectively.

4. Discussion

This is the first quantitative risk assessment for mycotoxins in dairy milk done in Argentinean practice farming conditions.

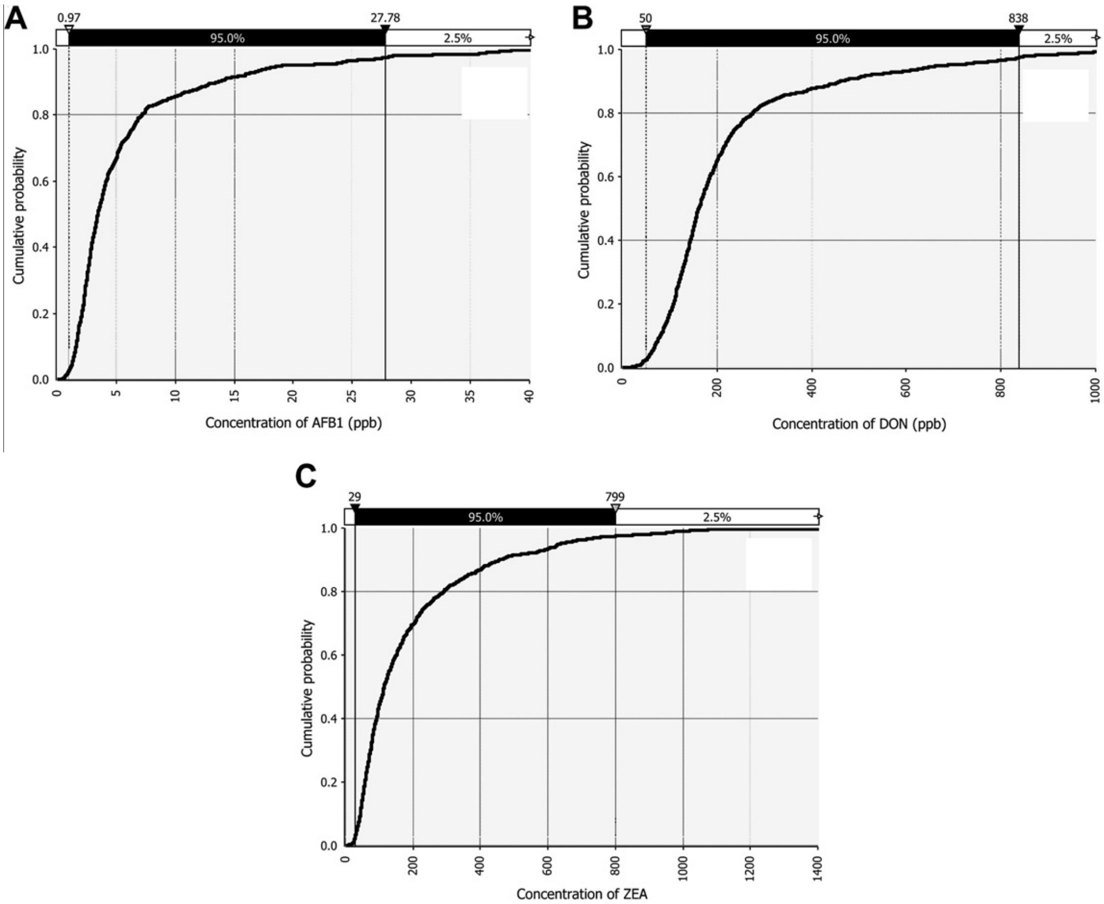

Fig. 2. Cumulative probability distribution for aflatoxin B1 (A), deoxynivalenol (B) and zearalenone (C) concentration in feedstuff.

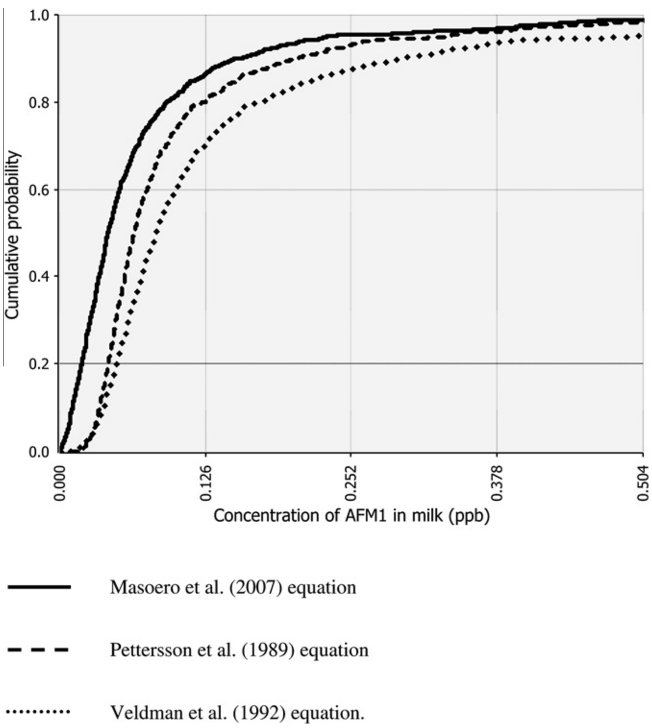

Fig. 3. Concentration of aflatoxin M1 in dairy milk according to the equation to estimate the carry-over rate.

The European Union (EU) (Directive 2002/32/EC), and MERCOSUR (MERCOSUR GMC/RES. N° 25/02), regulations determine a maximum level of 5 and 20 ppb AFB1 in feed for dairy cattle, respectively. According to the estimates generated by this risk model, approximately 25.07% and 3.76% of the diets offered to dairy cattle in Argentina's central dairy region would present higher levels than those established by the international regulations.

The maximum level of 5000 and 500 ppb for DON and ZEA in feed, respectively, were established by the European Union (EC N° 876/2006a). It was estimated that none of the diets offered to dairy cattle exceeded the maximum level for DON, but 8.9% of the diets showed higher levels of ZEA than those established by the international regulation. There is overwhelming evidence of global contamination of cereals and animal feeds with *Fusarium* mycotoxins particularly trichothecenes, zearalenone and fumonisins. Developing countries, such as India, South Africa, Philippines, and Thailand present high incidence of DON and ZEA, but also there have been documented high concentrations in developed countries (e.g. New Zealand, USA, Canada, Germany, Norway and the Netherlands) (Placinta et al., 1999).

Of particular concern is the co-occurrence of several mycotoxins in the same sample of grain or animal feed. The toxicity of mycotoxin mixtures cannot be accurately predicted only on the basis of the effect of the individual toxins. Some authors (Heussner et al., 2006), hypothesized the existence of a super additive or synergistic mode of action of different mycotoxins (ochratoxin A, ochratoxin B, citrinin and patulin) in renal cells. These types of synergistic or additive effects were observed in other studies, thus these aspects should be considered in future risk assessment studies on animal health (Speijers and Speijers, 2006).

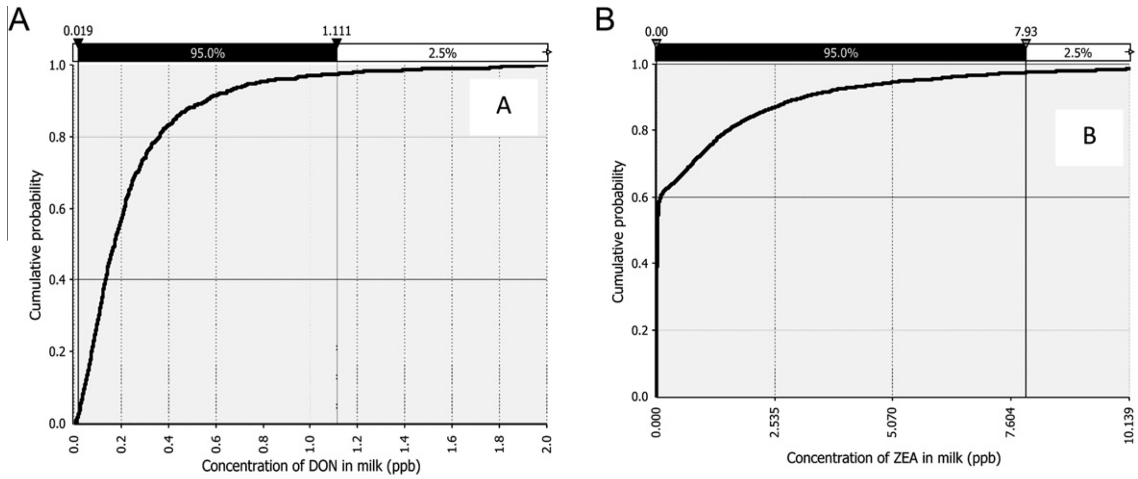

Fig. 4. Concentration of deoxynivalenol (A) and zearalenone (B) in dairy milk.

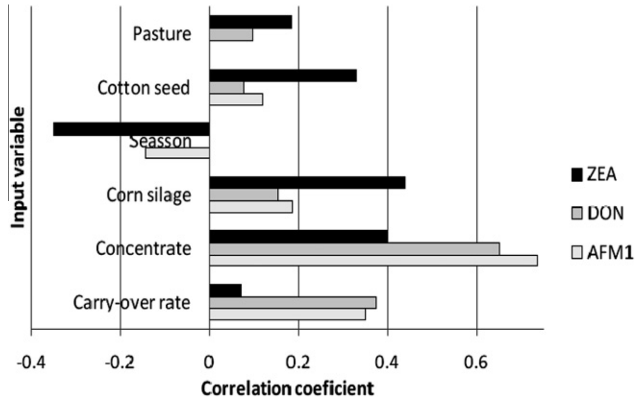

Fig. 5. Sensitivity analysis for mycotoxins concentration in bovine milk.

The estimated average level of AFM1 in bovine milk was within the maximum level accepted by the MERCOSUR regulation (0.5 ppb), (MERCOSUR GMC/RES. N° 25/02), but it was higher than the one established by the European regulations (0.05 ppb), (EC N° 1881/2006). Taking into account the different carry-over rates (considering the AFB1 consumption or the milk production), between 0.66% and 5.06% of the milk produced in Argentina's central dairy region exceeds the maximum level accepted by MERCOSUR. Considering the European regulations, the percentages of samples that exceed the maximum level were estimated between 32.65% and 66.84%. About this discrepancy in the international legislation, the Expert Committee on Food Additives's intervention was requested by the Codex Committee on Food Additives and Contaminants (FAO-WHO, 2001), and it was estimated that the projected risks for liver cancer at the maximum levels of AFM1 of 0.05 and 0.5 μg/kg are very small. For example, in a population with a prevalence of hepatitis B virus infection of 1%, the additional numbers of liver cancer cases associated with contamination of all milk with AFM1 at 0.5 versus 0.05 μg/kg would be 29 cancers/1000 million persons per year. Agreement and setting of international regulatory standards are very difficult, as not only does potential health benefit but also political and economical issues have to be considered (Binder, 2007).

Studies in Argentina are controversial. Some authors reported that 33% of the samples had AFM1 levels from 0.05 to 0.5 ppb in raw milk (FAO-WHO, 2001), while other authors reported an average level of 0.016 ppb (SD = 0.007 ppb) (López et al., 2003). Both studies were conducted in different dairy regions in Argentina, so those differences could be considered a consequence of different ingredients (especially concentrated feeds and pasture), and/or quantities in the diets. A study conducted in the Argentina's central dairy region (Basílico and Zapata de Basílico, 2005), identified that out of 33 samples of raw milk, two of them had AFM1 levels above 1 ppb (6.06%), a percentage that is similar to the one estimated by this simulation model. In Argentina, the National Plan for Residue Management and Food Safety (CREHA) monitors various raw materials of animal origin for residues of veterinary drugs, pesticides and mycotoxins. From 2003 through 2009, 1777 samples of raw milk in dairy industry were analyzed, of which 550 (30.95%), had AFM1 values higher than 0.025 ppb, but lower than 0.5 ppb (CREHA, 2010). These values, although lower, are not very different from those predicted by this model. CREHA Plan's samples were taken directly from the dairy industry, situation which could generate a dilution effect of mycotoxins in milk and, as a consequence present lower concentrations. By contrast, this model estimated the mycotoxins concentration in dairy farm level, so it is more likely to find higher concentrations individually.

The AFB1 carry-over rate was an important source of uncertainty in the model identified by the sensibility analysis. The equation developed by Veldman et al. (1992) predicted AFM1 levels in milk (approximately two times) higher than the levels estimated by the equation that considered the dairy milk production (Masoero et al., 2007). The probability distribution of AFM1 concentration in milk derived from the different carry-over rate equations could be considered as extreme values and include the uncertainty value in the result.

Concentrated feeds and corn silage were two ingredients highly correlated with the level of mycotoxins in dairy milk. The AFB1 proportion from these two ingredients was, on average, 57.13% of the total diet. In the case of DON and ZEA, the proportion provided by corn silage and concentrated feeds was estimated at 55.5% and 50.64% of the total diet, respectively. For those reasons, the conditions of harvest, storage and feed production derived from corn, should be carefully controlled in order to reduce the exposure of dairy cattle to mycotoxins and subsequently reduce their concentration in milk. During harvest time, it is important to prevent excess damage to kernels, which may predispose them to be infected during storage. Post-harvest mycotoxin control prevention of conditions favoring fungal growth and subsequent toxin production such as water activity of stored products, temperature, rodent and insect damage, and microbial interactions need to be considered (Binder, 2007). Dilution of contaminated grain with other feed components is another option, providing that monitoring is performed before grains are incorporated into compound feeds (Placinta et al., 1999).

The season was another factor associated with the presence of mycotoxins in milk which showed that micotoxin's concentration during autumn was higher than in spring. This may be due to the diet composition in each season, with a greater involvement of concentrated feeds and corn silage (ingredients very susceptible to mycotoxigenic fungi), during the autumn. According to the milk production levels of the dairy cattle, in autumn, these ingredients mean 57.26% and 65.71% of the total diet, whereas during spring, both components accounted for 22.22–40.9% of the diet. Given this differential composition of the diet according to the season, it was estimated that the consumption of AFB1 from corn silage and concentrated feeds represented 66% of AFB1 in the diet during autumn, while during the spring this proportion was reduced to 48.18%. In the case of DON and ZEA, the contribution of these mycotoxins from corn silage and concentrated feeds were, for autumn and spring, 69.6% and 41.4%; and 62.9% and 39.13%, respectively.

Although the concentrated feeds and corn silage were identified as sources of ZEA, pastures seem to exert a significant impact in the total intake of this mycotoxin. In autumn, pastures represent 22–25% of the dry matter offered to cows and provide approximately 13% of ZEA consumed. During spring the pastures are abundant in the Argentina's central dairy region contribute with 45–77% of the diet and provide 56% of the daily intake of ZEA. Zearalenone is common in pastures in New Zealand and it is causing fertility problems in sheep (EFSA, 2004b). Engels and Kramer (1996) reported the occurrence of ZEA in ryegrass at a level from 40 to 2780 ppb. Data on ZEA occurrence in pastures are less well documented, but there are indications that this exposure route needs to be considered (Binder et al., 2007; EFSA, 2004b).

Due to their very low carry-over rate from feed to milk, DON and ZEA are not of significant concern with respect to the safety of dairy products for consumers (Coffey et al., 2009); DON and ZEA are of concern to the dairy sector primarily because of their potential adverse effects on cattle health (Galtier, 1998).

Levels of DON estimated in this study were within ranges reported by studies conducted in the Netherlands (Driehuis et al., 2008). However, ZEA levels estimated for Argentina's central dairy region were higher than those found in studies conducted in the Netherlands (Driehuis et al., 2008).

For genotoxic carcinogen, a tolerable daily intake (TDI) is generally not determined. However, Kuiper-Goodman (1990) have estimated a TDI for AFM1 of 0.2 ng/kg bw, a value equivalent to a risk level of 1:100,000. The estimated ingested dose for AFM1 by this exposure assessment were, in average, 1.64 times lower than this tolerable estimated daily intake. Considering data from milk consumption in Latin American, JECFA (2001) estimated that the AFM1 dietary intake of AFM1 concentrations in milk was 0.058 μg/kg bw. The TDI estimated by JECFA (which used a similar milk product daily consumption compared with our model), was 475 times higher than the TDI observed in this risk assessment. Even considering the maximum TDI estimated in this risk assessment, it was 32 times lower than the TDI reported by JECFA.

Based on recent data in the most sensitive animal species (pig), and taking into account comparisons between pigs and humans, the Panel on Contaminants in the Food Chain of the European Food Safety Agency (EFSA, 2011), established a TDI for ZEA of 0.25 μg/kg bw The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2010) established a TDI for DON of 1 μg/kg bw. The estimated ingested doses for DON and ZEA by this exposure assessment were below the international regulatory limits. The ratio regulatory limit: TDI was 2252.25 and 148.81 for DON and ZEA, respectively.

Results from the exposure assessment model suggest that the presence of mycotoxins in bovine feed in Argentina should not give rise to significant mycotoxin levels in milk and, thus the risk to human beings could be considered as negligible.

5. Conclusions

This study showed that in the diet of Argentinean dairy cattle, corn silage and concentrated feeds are the major source of mycotoxins and, therefore, it is recommended to include these ingredients in monitoring and control programs. More certain knowledge regarding the mycotoxin contamination indicates the need to inhibit fungal growth (to improve the conditions of harvest and post harvest, storage and feed production), to reject contaminated food (or blending non-contaminated grain), or to add additives in the diets that block the action of mycotoxins. Although animal diets contain significant levels of mycotoxins, milk produced was not significantly outside the maximum allowable international limits. However, since the quality requirements are increasingly stringent, any reduction in established international regulatory limits would be a serious impact on domestic production. For that reason, Argentina should improve its monitoring program on mycotoxins in animal feed and milk.

Conflict of Interest

The authors dcelare that there are no conflict of interest.

References

Basílico, J.C., Zapata de Basílico, M.L., 2005. Aflatoxina M1. In: *Manual de referencias técnicas para el logro de leche de calidad*. Ed. Instituto Nacional de Tecnología Agropecuaria, Buenos Aires (Argentina), pp. 63-68. ISBN 987-521-165-6.

Binder, E.M., 2007. Managing the risk of mycotoxins in modern feed production. Anim. Feed Sci. Technol. 133, 149–166.

Binder, E.M., Tan, L.M., Chin, L.J., Handl, J., Richard, J., 2007. Worldwide occurrence of mycotoxins in commodities feeds and feed ingredients. Anim. Feed Sci. Technol. 137, 265–282.

Black, R.D., McVey, D.S., Oehme, F.W., 1992. Immunotoxicity in the bovine animal. Vet. Hum. Toxicol. 34, 438–442.

Boudra, H., Morgavi, D.P., 2005. Mycotoxin risk evaluation in feeds contaminated by *Aspergillus fumigatus*. Anim. Feed Sci. Technol. 120, 113–123.

Coffey, R., Cummins, E., Ward, S., 2009. Exposure assessment of mycotoxins in dairy milk. Food Control 20, 239–249.

Côte, L.M., Dahlem, A.M., Yoshizawa, T., Swanson, S.P., Buck, W.B., 1986. Excretion of deoxynivalenol and its metabolite in milk, urine, and feces of lactating dairy cows. J. Dairy Sci. 69, 2416–2423.

CREHA (Residue and Food Hygiene National Control Plan). 2010. Annual reports: years 2003–2009. http://www.senasa.gov.ar/contenido.php?to=n&in=1125&io=2949.

Creppy, E.E., 2002. Uptake of survey, regulation and toxic effects of mycotoxins in Europe. Toxicol. Lett. 127, 19–28.

Driehuis, F., Spanjer, M.C., Scholten, J.M., te Giffel, M.C., 2008. Occurrence of mycotoxins in feedstuffs of dairy cows and estimation of total dietary intakes. J. Dairy Sci. 91, 4261–4271.

EFSA (European Food Safety Authority), 2004a. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Deoxynivalenol (DON) as undesirable substance in animal feed. The EFSA Journal, 73, 1–42.

EFSA (European Food Safety Authority), 2011. Scientific Opinion on the risks for public health related to the presence of zearalenone in food. The EFSA Journal, 9(6), 2197.

EFSA (European Food Safety Authority), 2004b. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Zearalenone as undesirable substance in animal feed. The EFSA Journal, 89, 1–35.

Engels, R., Kramer, J., 1996. Incidence of Fusaria and occurrence of selected Fusarium mycotoxins on *Lolium* spp. in Germany. Mycotox. Res. 12, 31–40.

European Community, 2002. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.

European Community, 2006. 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.

European Community, 2006. Commission Regulation No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

FAO-WHO (Food and Agriculture Organization of the United Nations-World Health Organization), 2001. Safety evaluation of certain mycotoxins in food. FAO Food and Nutrition Paper N° 74.

FAO-WHO (Food and Agriculture Organization of the United Nations-World Health Organization), 2006. Food safety risk analysis. A guide for national food safety authorities. FAO Food and Nutrition Paper N° 87.

Fink-Gremmels, J., 2008. Mycotoxins in cattle feeds and carry-over to dairy milk: a review. Food Addit. Contam. 25, 172–180.

Gaggiotti, M.C., Caffaratti, S., Romero, L., Basílico, J.C., Basílico, M.L.Z. de, Comerón, E., 2001. Ensilajes de maíz y sorgo forrajero: influencia de la compactación sobre la calidad micotoxicológica. In: *24°Congreso Argentino de Producción Animal*. Rafaela, 19 al 21 de setiembre de 2001.

Gaggiotti, M.C., Basílico, J.C., Romero, L.A., Basílico, M.L.Z. de., 2003. Eficacia del uso de vomitoxina como indicadora de la presencia de otras micotoxinas en silajes. In: *26°Congreso Argentino de Producción Animal*. Mendoza, 23 y 24 de Octubre de 2003.

Galtier, P., 1998. Biological fate of mycotoxins in animals. Rev. Méd. Vét. 149, 549–554.

Heussner, A.H., Dietrich, D.R., ÓBrien, E., 2006. In vitro investigation of individual and combined cytotoxic effects of ochratoxin A and other selected mycotoxins on renal cells. Toxicol. in Vitro 20, 332–341.

JECFA, 2001. Safety Evaluation of Certain Mycotoxins in Food – Prepared by the 56th meeting of the JECFA (Join FAO/WHO Expert Committee on Food Additives) – FAO Food and Nutrition Paper 74/WHO Foods Additives Series 47.

JECFA, 2010. Summary report of the seventy-second meeting of Joint FAO/WHO Expert Committee on Food Additives (JECFA). Document JECFA/72/SC.

Kuiper-Goodman, T., 1990. Uncertainties in the risk assessment of three mycotoxins: aflatoxin, ochratoxin and zearalenone. Can. J. Physiol. Pharmacol. 68, 1017–1024.

López, C.E., Ramos, L.L., Ramadan, S.S., Bulacio, L.C., 2003. Presence of aflatoxin M1 in milk for human consumption in Argentina. Food Control 14, 31–34.

Masoero, F., Gallo, A., Moschini, M., Piva, G., Díaz, D., 2007. Carryover of aflatoxin from feed to milk in dairy cows with low or high somatic cell counts. Animal 1, 1344–1350.

MERCOSUR. Reglamento técnico MERCOSUR sobre límites máximos de aflatoxinas admisibles en leche, maní y maíz. GMC/RES. N° 25/02.

MinAgri, 2010. Ministerio de Agricultura, Ganadería y Pesca de la Nación (Argentina). Consumo de lácteos por productos (2000–2009). http://www.alimentosargentinos.gov.ar/lacteos/docs/06_Consumo/Consumo_03.htm.

Mirocha, C.J., Pathre, S.V., Robison, T.S., 1981. Comparative metabolism of zearalenone and transmission into bovine milk. Food Cosmet. Toxicol. 19, 25–30.

Munksgaard, L., Larsen, J., Werner, H., Andersen, P.E., Viuf, B.T., 1987. Carry over of aflatoxin from cows' feed to milk and milk products. Milchwissenschaft 42, 165–167.

Penno, J., 1995. Presencia de aflatoxinas, fusaproliferina y beauvericina en maíz proveniente de la provincia de Santa Fe. Thesis for Master of Science, Chemical Engineering Faculty, Litoral National University, Argentina.

Pereyra, C.M., Alonso, V.A., Rosa, C.A.R., Chiacchiera, S.M., Dalcero, A.M., Cavaglieri, L.R., 2008. Glitoxin natural incidence and toxigenicity of Aspergillus fumigates isolated from corn silage and ready dairy cattle feed. World Mycotoxin J. 1, 463–467.

Petterson, H., Bertilsson, J., Wennberg, O., 1989. Carry-over of aflatoxin from dairy cattle feed to milk. Healthy animals, safe foods, healthy man. Proceedings of the World Association of Veterinary Food Hygienists Symposium, Stockholm, pp. 97–102.

Placinta, C.M., ĎMello, J.P.F., Macdonald, A.M.C., 1999. A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. Anim. Feed Sci. Technol. 78, 21–37.

Prandini, A., Tansini, G., Sigolo, S., Filippi, l., Laporta, M., Piva, G., 2009. On the occurrence of aflatoxin M_1 in milk and dairy products. Food Chem. Toxicol. 47, 984–991.

Prelusky, D.B., Scott, P.M., Trenholm, H.L., Lawrence, G.A., 1990. Minimal transmission of Zearalenone to milk of dairy cows. J. Environ. Sci. Health B 25, 87–103.

Romero, L.A., Cuatrin, A., Comeron, E.A., Maciel, M., 2004. Modelos de ajuste de curvas de primeras lactancias de vacas lecheras de un sistema de parición biestacionado. Rev. Argent. Prod. Anim. 24 (Supl. 1), 323–324.

Seeling, K., Danicke, S., Ueberschar, K.H., Lebzien, P., Flachowsky, G., 2005. On the effects of *Fusarium* toxin-contaminated wheat and the feed intake level on the metabolism and carry over of zearalenone in dairy cows. Food Anim. Contam. 22, 847–855.

Seeling, K., Danicke, S., Valenta, H., Van Egmond, H.P., Schothorst, R.C., Jekel, A., Lebzien, P., Schollenberger, M., Razzazi-Fazeli, E., Flachowsky, G., 2006. Effects of *Fusarium* toxin-contaminated wheat and feed intake level on the biotransformation and carry-over of deoxynivalenol in dairy cows. Food Addit. Contam. 23, 1008–1020.

Seglar, W.J., 2003. Fermentation analysis and silage quality testing. In Proceedings of the Minnesota Dairy Health. Conference, College of Veterinary Medicine, University of Minnesota, May 2003.

Speijers, G.J.A., Speijers, M.H.M., 2006. Combined toxic effects of mycotoxins. Toxicol. Lett. 153, 91–98.

Usleber, E., Renz, V., Märtlbauer, E., Terplan, G., 1992. Studies on the application of enzyme immunoassays for the *Fusarium* mycotoxins deoxynivalenol, 3-acetyldeoxynivalenol, and zearalenone. J.Vet. Med. 39, 617–627.

Van Egmond, H.P., 1989. Mycotoxins in dairy products. Elsevier Applied Science, London (UK), pp. 272.

Veldman, V.A., Meijs, J.A.C., Borggreve, G.J., Heeres van der Tol, J.J., 1992. Carry-over of aflatoxin from cows' food to milk. Anim. Prod. 55, 163–168.

Woods, P.D.P., 1969. Factors affecting the share of the lactation curve in cattle. Anim. Prod. 11, 307–316.

Yiannikouris, A., Jouany, J.P., 2002. Mycotoxins in feeds and their fate in animals: a review. Anim. Res. 51, 81–89.

Yoshizawa, T., Cote, L.M., Swanson, S.P., Buck, W.B., 1986. Confirmation of DOM-1, a de-epoxidation metabolite of deoxynivalenol, in biological fluids of lactating cows. Agric. Biol. Chem. 50, 227–229.