



International Journal of
Dairy Science

ISSN 1811-9743



Academic
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Aflatoxin Transfer from Naturally Contaminated Feed to Milk of Dairy Cows and the Efficacy of a Mycotoxin Deactivating Product

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Abstract: This study reports the results of an experiment, testing the aflatoxin B₁ (AfB₁) carry-over from naturally contaminated feed to the dairy cows' milk in the absence and in the presence of a mycotoxin deactivating product-Mycofix® Plus (MPL). The study was carried out using 18 healthy animals divided into 3 homogeneous groups of 6 animals each. The experimental design was a 3×3 Latin square with three periods of 7 days each without washout periods. The treatments were: (1) control diet without MPL (CTR); (2) control diet with 20 g/cow/day of MPL (T₁) and (3) control diet with 50 g/cow/day of MPL (T₂). The diet was a Total Mixed Ration (TMR) and 1 kg of a naturally contaminated maize meal (AfB₁ = 91.7±4.4 µg kg⁻¹) was included in the diet of each cow. Each animal ingested daily 97.3 µg kg⁻¹ AfB₁ since the analysis of the TMR before inclusion of the contaminated maize revealed however the presence of 0.24 µg kg⁻¹ DM of AfB₁, corresponding to 5.6 µg per cow per day. In T₁ and T₂ diets, MPL was mixed with the contaminated maize meal. Feed intake and individual daily milk production were recorded during the study. Morning and evening milk samples from each cow were collected on day seven of each week. Samples derived from individual cows were mixed in proportion to the morning and evening milk production and then again combined in proportion to the daily milk production of each cow to constitute a representative bulk milk sample of each group; these samples were analyzed to determine the aflatoxin M₁ (AfM₁) content. The addition of MPL did not influence feed intake and milk production. The addition of MPL to the diet reduced significantly (p<0.01) the milk AfM₁ content from 0.120 µg kg⁻¹ (CTR group) to 0.083 µg kg⁻¹ (-31%, T₁ group) and 0.072 µg kg⁻¹ (-41%, T₂ group).

Key words: Dairy cows, aflatoxin M₁, carry-over, deactivation

INTRODUCTION

Aflatoxins (B₁, B₂, G₁, G₂) are mycotoxins produced principally by *Aspergillus flavus* and *A. parasiticus* fungi (D'Mello and Macdonald, 1997), mainly in tropical and subtropical regions where the warm and humid weather provides optimum conditions for the growth of the molds (Rustom, 1997). They are considered as the mycotoxins that represent the greatest risk for human health. They occur in several products for human consumption, such as maize, almonds, peanuts and pistachio nuts. Nevertheless, their introduction in the food chain is greatly determined by the ingestion of contaminated feed such as maize and cotton seeds by livestock animals and their subsequent carry over into animal products for human consumption, particularly into milk and dairy products.

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Aflatoxins (Afs) have been shown to negatively affect production, immune system function and rumen metabolism in cattle (Hussein and Brasel, 2001). However, their carry over into milk in the form of the hydroxylated metabolites aflatoxin M₁ (AfM₁) and aflatoxin M₂ (AfM₂), and consequent carcinogenic risks in humans, are one of the most worrying aspects of this group of mycotoxins. Aflatoxins and AfM₁ have been included by the International Agency for Research on Cancer (IARC) in group 1 and 2B, as human and possible human carcinogens, respectively (IARC, 2002); their maximum levels both in animal feed and milk are regulated by European Commission's Directive (European Commission, 2003) and regulation (European Commission, 2006).

Although ruminants are globally more resistant to mycotoxins than most monogastric animals due to the partial detoxification capacity of ruminal microorganisms, aflatoxins' ruminal degradation is less than 10% for contaminations between 1 and 10 µg mL⁻¹ (Westlake *et al.*, 1989). AfM₁ is the main AfB₁ metabolite, which secretion depends on the animal's feed intake and lactation stage (Veldman *et al.*, 1992). The liver is the main site of AfB₁ biotransformation with the mitochondrial cytochrome P450 oxidative system (CYP) converting the AfB₁ into AfM₁ and other metabolites (Sudakin, 2003). A recent research demonstrated that Afs are quickly absorbed through the gastrointestinal tract of cows and hypothesized that an early absorption might also take place in mouth or esophageal mucous membranes, before the rumen compartment (Gallo *et al.*, 2008). Numerous strategies are described in the literature to avoid fungal growth and toxin production both in the field and during storage (Council for Agricultural Science and Technology, 2003). However, due to the existence of many factors that influence these processes (Ominsi *et al.*, 1994) and in order to counteract the negative impacts of Afs and their metabolites in animal and human health, the development of practical, cost-effective and safe detoxifying processes is of great importance.

Many physical, chemical and biological techniques to neutralize mycotoxins have been developed and were reported in the literature throughout the years (Doyle *et al.*, 1982; Samarajeewa *et al.*, 1990). The adsorption of the aflatoxin molecule is possible due to its polarity. Deactivating agents used as feed additives are supposed to adsorb mycotoxins under the conditions within the digestive tract, whereby an absorption by the body is avoided (Dänicke, 2002). Chemisorbents have shown their capacity to tightly bind and immobilize Afs in the gastrointestinal tract of animals, resulting in a major reduction of the toxin bio-availability. Activated carbons (ACs) and Hydrated Sodium Calcium Alumino-Silicates (HSCAS) have shown a reduction of AfB₁ carry-over into milk of respectively 50 and 36% (Galvano *et al.*, 1996) in dairy cows with an inclusion rate of 2% in the pelleted contaminated feed. Although, *in vitro* experiments have shown promising results as regards to the use of HSCAS as effective AfB₁ adsorbing compounds (Ramos and Hernández, 1997) which have been corroborated by *in vivo* experiments (Harvey *et al.*, 1991; Smith *et al.*, 1994), special attention is recommended by several researchers regarding the use of ACs and HSCAS as feed additives (Galvano *et al.*, 1996; Ramos and Hernández, 1997) as the long-term effects of these materials on the utilization of essential nutrients are not well known yet and their effectiveness against other mycotoxins of importance in the animal production have not been thoroughly studied. If for other mycotoxins such as zearalenone, ochratoxin and trichothecenes the efficacy of adsorbing materials is very limited or even null, for AfB₁ adsorption these substances seem to be very efficient (Huwig *et al.*, 2001). Based on this knowledge, Vekiru *et al.* (2007) performed adsorption experiments in buffer solutions in order to evaluate the ability to bind AfB₁ at various pH-values. The chemisorption index was calculated, in order to understand if the adsorption was mainly physical adsorption/physisorption (relatively weak bonding involving Van der Waals interactions and hydrogen bonding) or chemical adsorption/chemisorption (stronger interaction which involves ionic or covalent bonding). Isothermal analysis was used to determine the values of maximum binding capacity (q_{max}, [mol kg⁻¹]) and the distribution constant (K_d), which describes the affinity of the binder to AfB₁ (Grant *et al.*, 1998). On the course of the same study, adsorption experiments in simulated gastrointestinal fluid and real gastric juice were also carried out. Furthermore,

binding capability of the materials regarding selected vitamins was examined. Based on the obtained *in vitro* results, highly promising sorbent materials were ranked for further *in vivo* studies.

However, due to the multi-factorial nature of AFB₁ carry-over into the milk, *in vivo* studies regarding the efficacy of the detoxifying product to avoid this phenomenon are always fundamental. Therefore, the main objective of this study was to determine whether the inclusion of Mycofix® Plus at different rates in the contaminated feed would reduce the carry-over of aflatoxin from feed into the milk of dairy cows.

MATERIALS AND METHODS

Tested Product

Mycofix® Plus (MPL) is a mycotoxin deactivator product, manufactured by BIOMIN GmbH, Herzogenburg, Austria, that presents as a beige greyish fine powder destined to be incorporated into the animal feed from 0.5 to 2.5% TMR, depending on the mycotoxin contamination level of feedstuffs. It has proven efficiency in the counteraction of the negative impacts of mycotoxins in animal health and performance (Cheng *et al.*, 2006; Diaz *et al.*, 2005; Politis *et al.*, 2005). Polar mycotoxins (e.g., aflatoxins) are adsorbed by the inorganic components: a blend of bentonites selected for inclusion in the product after thorough scientific tests ascertaining AFB₁-adsorption-properties (Vekiru *et al.*, 2007) (solo constituent of Mycofix® Secure). Non-adsorbable mycotoxins (e.g., trichothecenes, zearalenone) are degraded by biological constituents, namely a *Eubacterium* strain (BBSH 797) and a yeast strain affiliated to the *Trichosporon* genus (MTV) (Schatzmayr *et al.*, 2006). Finally, phycophytic substances and plant extracts act to compensate the adverse conditions caused by mycotoxins.

Experiment on Dairy Cows

The experiment was performed at the CERZOO research and experimental center (San Bonico, Piacenza, Italy) from April to May 2006.

A total of 18 healthy Italian Friesian dairy cows were used in this experiment. The average age of the animals given by the number of lactations was 1.9±0.9. Their average days in milk was 130±44 and the average daily milk production was 33.6±6.3 kg.

The treatment groups were identified by different codes: CTR, T₁ and T₂. The animals were housed in a free-stall barn randomly divided in three pens of 6 cows each.

Animals were fed once daily with free access to water. The composition and chemical analysis of the experimental diet are presented in Table 1 and 2.

The experimental design was a 3×3 Latin square with periods of seven days each without washout periods.

Table 1: Composition (% dry matter) of the TMR used in the trial

Dry matter composition	Values (%)
Maize silage	34.4
Whole cottonseed	6.4
Soybean meal	13.3
Mineral vitamin premix ¹	1.2
Maize meal (70%)+barley meal (30%)	17.5
Alfalfa hay	9.7
Alfalfa-rye grass hay mixture	5.8
Rye grass hay	3.9
Dehydrated beet pulp	3.9
Contaminated maize meal	3.9

¹The premix did not contain any clays

Table 2: Chemical analysis of DM for TMR used in the trial

Characteristics	Values (%)
Dry matter	53.24
Crude protein	15.62
Crude fibre	19.45
Ether extract	4.20
Ash	6.48
Nitrogen free extract	54.25
NDF	37.54
ADF	22.01
Starch	27.88
Zearalenone ($\mu\text{g kg}^{-1}$)	<5.00*
DON ($\mu\text{g kg}^{-1}$)	<10.00*
Aflatoxin B ₁ ($\mu\text{g kg}^{-1}$)	4.10

*Limit of detection (LOD)

The treatments were:

- Control diet containing 1 kg of contaminated maize meal, without MPL (CTR)
- Control diet containing 1 kg of contaminated maize meal, with 20 g/cow/day of MPL (T₁)
- Control diet containing 1 kg of contaminated maize meal, with 50 g/cow/day of MPL (T₂)

MPL was always mixed with the aflatoxin contaminated maize meal, before inclusion in the Total Mixed Ration (TMR).

Evaluation of Parameters

During the experiment the collection and recording of data were made as follows:

- **Feed intake:** The amount of TMR fed to the animals was measured with an electronic scale on a mixer-feeder wagon
- **Milk production:** The individual amount of milk produced (in kg) in each milking was recorded daily
- **Milk samples:** Morning and evening milk samples from each cow were collected on day 7 of each week. The samples of each cow were mixed proportionately to the morning and evening milk production and then again combined in proportion in the daily milk production of each cow to constitute a representative bulk milk sample of each group
- **Feed samples:** Samples of contaminated maize meal and of TMR were collected to determine composition and mycotoxin content
- **Health status:** Daily inspections were carried out by qualified personnel and by the veterinary responsible for the animal welfare throughout the whole period of the study. The consistency of the feces was also examined

Statistical Analysis

Data were analyzed by ANOVA using the GLM procedure of SAS (SAS Institute, 1999-2001) in a Latin square design (Steel and Torrie, 1980). Linear and quadratic contrasts were used to determine the nature of the response to the feeding of Mycofix® Plus. Differences were considered statistically significant at $p < 0.05$.

Analytical Procedures

A TMR sample (approximately 1.0 kg) was collected and analyzed for proximate composition, Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), starch and mycotoxins' content.

The proximate analysis of TMR was determined in compliance with the analytical methods of the Italian Ministero dell'Agricoltura e Foreste (supplement n. 2 of 1975) for moisture, ash and starch in animal feed. The crude protein content was determined according to the Gazzetta Ufficiale Serie Generale n. 92 of April 21, 1996; the crude fat according to the Directive EEC n. 84/4/EEC of December 12, 1983 (Gazzetta Ufficiale EC n. L 15 of January 1, 1984) and crude fibre according to the EEC Directive n. 92/89 of November 3, 1992.

NDF and ADF contents were determined according to the methods reported in the literature (Martillotti *et al.*, 1987; Mertens, 1992; Van Soest *et al.*, 1991).

Aflatoxin B₁, after extraction and purification by an immunoaffinity column, was determined by reversed-phase HPLC with fluorescence detection after post column derivatization involving bromination with pyridinium hydrobromide perbromide (Stroka *et al.*, 2003).

Deoxynivalenol, after extraction and purification by a commercial (Mycosep®) clean-up column (Radova *et al.*, 1998), was determined by GLC-MS after derivatization to trimethylsilylether (Eskola and Rizzo, 2001). Zearalenone, after extraction purification by an immunoaffinity column, was determined by reversed-phase HPLC with fluorescence detection (Kruger *et al.*, 1999). These analysis were performed to certify that no mycotoxins other than aflatoxins were present in the rations fed to the animals.

AfM₁ in milk, after extraction/purification by an immunoaffinity column, was determined by reversed-phase HPLC with fluorescence detection (Dragacci and Grosso, 2001).

RESULTS AND DISCUSSION

Effect of the Treatments on the Health Condition of the Animals

Daily inspections carried out by qualified personnel and by the veterinary responsible for animal welfare and examination of feces consistency reflected a good animal health condition throughout the experimental period. The fact that the animals were in contact with a low level of the mycotoxin for a short period might be an explanation for this fact, since a field experiment with natural contamination of feedstuffs and a longer period of aflatoxins intake, reported problems with the health condition of the herd, as animals had a higher incidence of lameness (subclinical laminitis) and impaired fertility (cystic ovaries) (Özsoy and Altunatmaz, 2005). On the other hand, in controlled experiments, animals generally show a high tolerance to contaminated feeds (Fink-Gremmels, 2008).

Effect of the Treatments on Feed Intake, Milk Yield and Milk AfM₁ Content

Addition of MPL did not influence dry matter feed intake (Table 3) or milk production, which evidences the non-interference of the feed additive with the animals' performance in a negative way, demonstrating that it may be added to feed in practical conditions.

Aflatoxin B₁ in Feed

The mean content of AfB₁ in contaminated maize meal fed to the animals was 91.7±4.4 µg kg⁻¹. The analysis of the TMR before inclusion of the contaminated maize revealed however the presence of 0.24 µg kg⁻¹ DM of AfB₁, corresponding to 5.6 µg per cow per day. As each animal ingested

Table 3: Feed intake (kg DM/cow/day)

Weeks	CTR	T ₁ group	T ₂ group
1	23.8±2.1	23.0±3.4	22.9±3.3
2	22.1±4.0	24.4±2.0	23.3±1.7
3	24.1±0.9	23.2±1.2	24.8±0.3
Average (Weeks 1-3)	23.3±2.7	23.5±2.3	23.7±2.2

one kg per day of contaminated maize meal, the sum of these two values represents the mean individual daily ingestion (μg) of AfB_1 , meaning that each animal ingested daily $97.3 \mu\text{g kg}^{-1} \text{AfB}_1$.

AfB₁ Intake and Effect of Treatments on Milk Yield and Aflatoxin M₁ in Milk

Aflatoxin B₁ carry-over into the milk as AfM_1 is known to occur quickly, as this metabolite is detected in milk from the first milking after the animal has ingested AfB_1 -contaminated feed (Diaz *et al.*, 2004; Masoeroa *et al.*, 2007). Based on this information and since the main objective of this study was to determine whether the inclusion of MPL would reduce the carry-over of aflatoxin from feed into the milk of dairy cows, the occurrence of AfM_1 in milk was recorded every 7th day of the experiment.

Raw data are shown in Table 4 and calculations are presented in Table 5. The continuous administration of aflatoxin contaminated maize meal to dairy cows has shown to negatively affect the milk quality regarding the content of the AfM_1 , resulting in higher contents of this toxic metabolite into the milk. The addition of MPL to the diet reduced significantly ($p < 0.01$) the milk AfM_1 content from $0.120 \mu\text{g kg}^{-1}$ (CTR group) to $0.083 \mu\text{g kg}^{-1}$ (-31%; T₁ group = 20 g MPL day⁻¹) and $0.072 \mu\text{g kg}^{-1}$ (-41%; T₂ group = 50 g MPL day⁻¹).

The inclusion of different quantities of MPL to the contaminated feed had positive effects, significantly reducing ($p < 0.01$) the concentration of AfM_1 in the treated groups (T₁ and T₂ groups). This reflects the efficacy of the product in the reduction of aflatoxin carry-over from feed to milk. A 31 and 41% reduction on the carry-over of AfB_1 from feed to milk was achieved with the inclusion of respectively, 20 and 50 g MPL/cow/day. In percentage of TMR, these numbers represent a 0.047 and a 0.12% inclusion rate, respectively.

In comparison with earlier studies where other binding agents were tested, the results of this trial are very positive in a milk producer's point of view as the inclusion rate of the product is much lower. A 24% decrease on the secretion of AfM_1 into the milk was obtained by the addition of 1% HSCAS (Harvey *et al.*, 1991) to the feed. Veldman (1992) reported no effects on the AfM_1 content of milk when animals were fed 1% HSCAS. Galvano *et al.* (1996) achieved a 36% of carry-over reduction with the addition of 2% HSCAS. The results of this trial were positive as regards to the product's ability to bind aflatoxins; however, the high initial contamination of the TMR impeded the fulfilment of the European Commission requirement of $0.050 \mu\text{g kg}^{-1}$ of AfM_1 in the milk. Nevertheless, it should be considered that the AfB_1 daily intake of each dairy cow was $97.3 \mu\text{g}$; this amount represents a level of $8.3 \mu\text{g kg}^{-1}$, if calculated on the concentrate part of the TMR and therefore exceeds the $5 \mu\text{g kg}^{-1}$ level permitted by the EU Directive for dairy complete feeding stuffs. It should be also considered that there is a linear correlation between the AfM_1 content of milk and the

Table 4: Aflatoxin M₁ in milk (raw data in $\mu\text{g kg}^{-1}$)

Weeks	CTR	T ₁ group	T ₂ group
1	0.127	0.083	0.078
2	0.117	0.084	0.066
3	0.117	0.083	0.071
Average (Weeks 1-3)	0.120	0.083	0.072

Table 5: Reduction of AfB_1 carry-over as AfM_1 into milk over a 3 week period in three groups of cows with different treatments

Treatments	MPL inclusion (g/cow/day)	AfB_1 intake (μg)	Average AfM_1 ($\mu\text{g kg}^{-1}$)	Pooled SEM	P of the model	Milk yield (kg/cow/day)	AfM_1 excreted ($\mu\text{g day}^{-1}$)	Carry-over (%)	Reduction carry-over (%)
CTR	0	97.3	0.120 ^a	1.347	0.0081	31.2	3.744	3.85	na*
T ₁	20	97.3	0.083 ^b			31.2	2.590	2.66	31
T ₂	50	97.3	0.072 ^b			30.7	2.210	2.27	41

^{a,b}Values within the same column with different superscripts differ significantly ($p < 0.01$), *na: Not applicable

AfB₁ intake, expressed by the regression equation (Veldman *et al.*, 1992):

$$\text{AfM}_1 \text{ (ng kg}^{-1} \text{ of milk)} = 1.19 \text{ AfB}_1 \text{ intake (}\mu\text{g/cow/day)} + 1.9$$

From this equation it can be calculated that the average daily AfB₁ intake in a herd has to be below 40 μg per cow in order to produce milk with less than 0.050 $\mu\text{g kg}^{-1}$ of AfM₁. In this trial, the AfB₁ amount ingested by each cow was 2.43 times higher (97.3 μg), but the T₂ group (50 g MPL/cow/day) produced milk exceeding only 1.44 times the AfM₁ limit fixed by the EC.

REFERENCES

- Cheng, Y.H., C.F. Weng, B.J. Chen and M.H. Chang, 2006. Toxicity of different Fusarium mycotoxins on growth performance, immune responses and efficacy of a mycotoxin degrading enzyme in pigs. *Anim. Res.*, 55: 579-590.
- Council for Agricultural Science and Technology, 2003. *Mycotoxins: Risks in Plant, Animal and Human Systems*, No. 139. Council for Agricultural Science and Technology, Ames, Iowa, ISBN: 1-887383-22-0.
- Dänicke, S., 2002. Effects of fusarium toxin contaminated wheat grain and of a detoxifying agent on rumen physiological parameters and in sacco dry matter degradation of wheat straw and lucerne hay in wethers. *J. Anim. Feed Sci.*, 11: 437-451.
- Diaz, G.J., A. Cortés and L. Roldán, 2005. Evaluation of the efficacy of four feed additives against the adverse effects of t-2 toxin in growing broiler chickens. *J. Applied Poult. Res.*, 14: 226-231.
- D'Mello, J.P.F. and A.M.C. Macdonald, 1997. *Mycotoxins*. *Anim. Feed Sci. Technol.*, 69: 155-166.
- Doyle, M.P., R.S. Applebaum, R.E. Brackett and E.H. Marth, 1982. Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities. *J. Food Protect.*, 45: 964-971.
- Dragacci, S. and F. Grosso, 2001. Immunoaffinity column cleanup with liquid chromatography for determination of aflatoxin M1 in liquid milk: Collaborative study. *J. AOAC Int.*, 84: 437-443.
- Eskola, M. and A. Rizzo, 2001. Sources of variation in the analysis of trichothecenes in chromatography-mass spectrometry. *Mycotoxin Res.*, 17: 68-87.
- European Commission, 2003. Commission directive 2003/100/EC of 31 October 2003 amending annex 1 to directive 2002/32/EC of the European parliament and of the council on undesirable substances in animal feed. *Off. J. Eur. Union*, L285: 337-37.
- European Commission, 2006. Commission regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Union*, L364: 5-24.
- Fink-Gremmels, J., 2008. The role of mycotoxins in the health and performance of dairy cows. *Vet. J.*, 176: 84-92.
- Gallo, A., M. Moschini and F. Masoero, 2008. Aflatoxins absorption in the gastro-intestinal tract and in the vaginal mucosa in lactating dairy cows. *Ital. J. Anim. Sci.*, 7: 53-63.
- Galvano, F., A. Pietri, T. Bertuzzi, G. Fusconi, M. Galvano, A. Piva and G. Piva, 1996. Reduction of carry-over of aflatoxin from cow feed to milk by addition of activated carbons. *J. Food Protect.*, 59: 551-554.
- Grant, P.G., S.L. Lemke, M.R. Dwyer and T.D. Phillips, 1998. Isothermal adsorption of aflatoxin B1 on HSCAS clay. *J. Agric. Food Chem.*, 46: 599-605.
- Harvey, R.B., T.D. Phillips, J.A. Ellis, L.F. Kubena, W.E. Huff and H.D. Petersen, 1991. Effects on aflatoxin M1 residues in milk by addition of hydrated sodium calcium aluminosilicate to aflatoxin-contaminated diets of dairy cows. *Am. J. Vet. Res.*, 52: 1556-1559.

- Hussein, H.S. and J.M. Brasel, 2001. Toxicity, metabolism and impact of mycotoxins on humans and animals. *Toxicology*, 167: 101-134.
- Huwig, A., S. Freimund, O. Kappeli and H. Dutler, 2001. Mycotoxin detoxication of animal feed by different adsorbents. *Toxicol. Lett.*, 122: 179-188.
- IARC, 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Summary of Data Reported and Evaluation. IARC Monographs on the evaluation of the carcinogenic Risk to Humans. Vol. 82. International Agency for Research on Cancer, Lyon, France.
- Kruger, S.C., B. Kohn, C.S. Ramsey and R. Prioli, 1999. Rapid immunoaffinity-based method for determination of zearalenone in maize by fluorometry and liquid chromatography. *J. AOAC Int.*, 82: 1364-1368.
- Martillotti, F., M. Antongiovanni, L. Rizzi, E. Santi and G. Bittante, 1987. Methods for chemical analysis of feed-stuffs. International Public Relations Association No. 8, CNR-IPRA, Roma.
- Masoeroa, F., A. Galloa, M. Moschinia, G. Pivaa and D. Diaza, 2007. Carry over of aflatoxin from feed to milk in dairy cows with low or high somatic cell counts. *Animal*, 1: 1344-1350.
- Mertens, D.R., 1992. Critical conditions in determining detergent fibers. Proceedings of the NFTA Forage Analysis Workshop, Sept. 16-17, Denver, pp: C1-C8.
- Ominsi, K.H., R.R. Marquardt, R. Sinha and D. Abramson, 1994. Ecological Aspects of Growth and Mycotoxin Production by Storage Fungi. In: *Mycotoxins in Grain Compounds Other Than Aflatoxin*, Miller, J.D. and H. Trenholm (Eds.). Eagan Press, St. Paul, MN., ISBN: 0962440752, pp: 287-312.
- Özsoy, S. and K. Altunatmaz, 2005. The relationship between lameness, fertility and aflatoxin in a dairy cattle herd. *Turk. J. Vet. Anim. Sci.*, 29: 981-986.
- Politis, I., K. Fegeros, S. Nitsch, G. Schatzmayr and D. Kantas, 2005. Use of *Trichosporon mycotoxinivorans* to suppress the effects of ochratoxicosis on the immune system of broiler chicks. *Br. Poult. Sci.*, 46: 58-65.
- Radova, Z., K. Holadova and J. Hajslova, 1998. Comparison of two clean-up principles for determination of trichothecenes in grain extract. *J. Chromatogr. A*, 829: 259-267.
- Ramos, A.J. and E. Hernández, 1997. Prevention of aflatoxicosis in farm animals by means of hydrated sodium calcium aluminosilicate addition to feedstuffs: A review. *Anim. Feed Sci. Technol.*, 65: 197-206.
- Rustom, I.Y.S., 1997. Aflatoxin in food and feed: Occurrence, legislation and inactivation by physical methods. *Food Chem.*, 59: 57-67.
- Samarajeewa, V., A.C. Sen, M.D. Cohen and C.I. Wey, 1990. Detoxification of aflatoxins in foods and feeds by physical and chemical methods. *J. Food Protect.*, 53: 489-501.
- SAS Institute, 1999-2001. Users Guide Statistics. 8th Edn., SAS Institute Inc., Cary, NC.
- Schatzmayr, G., F. Zehner, M. Täubel, D. Schatzmayr, A. Klimitsch, A.P. Loibner and E.M. Binder, 2006. Microbiologicals for deactivating mycotoxins. *Mol. Nutr. Food Res.*, 50: 543-551.
- Smith, E.E., T.D. Phillips, J.A. Ellis, R.B. Harvey and L.F. Kubena, 1994. Dietary hydrated sodium calcium aluminosilicate reduction of aflatoxin M1 residue in dairy goat milk and effects on milk production and components. *J. Anim. Sci.*, 72: 677-682.
- Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn., McGraw Hill, New York, USA., ISBN-13: 978-0070609259.
- Stroka, J., C. Von-Holst and E. Anklam, 2003. Immunoaffinity column cleanup with liquid chromatography using post-column bromination for determination of aflatoxin B1 in cattle feed: Collaborative study. AOAC official method 2000.02. *J. AOAC Int.*, 86: 1179-1186.
- Sudakin, D.L., 2003. Dietary aflatoxin exposure and chemoprevention of cancer: A clinical review. *J. Toxicol. Clin. Toxicol.*, 41: 195-204.

- Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, 74: 3583-3597.
- Vekiru, E., S. Fruhauf, M. Sahin, F. Ottner, G. Schatzmayr and R. Krska, 2007. Investigation of various adsorbents for their ability to bind Aflatoxin B1. *Mycotoxin Res.*, 23: 27-33.
- Veldman, A., 1992. Effect of sorbentia on carry-over of aflatoxin from cow feed to milk. *Milchwissenschaft*, 47: 777-780.
- Veldman, A., J.A.C. Meijjs, G.J. Borggreve and J.J. Heeres-van-der-Tol, 1992. Carry-over of aflatoxin from cows' food to milk. *Anim. Prod.*, 55: 163-168.
- Westlake, K., R.I. Mackie and M.F. Dutton, 1989. *In vitro* metabolism of mycotoxins by bacterial, protozoal and ovine ruminal fluid preparations. *Anim. Feed Sci. Technol.*, 25: 169-178.