Research Notes

Sodium bentonite and monensin under chronic aflatoxicosis in broiler chickens

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ABSTRACT Clay feed additives have been increasingly incorporated into animal diets to prevent aflatoxicosis. Due to the nonselective nature of the binding interaction, many important components of the diets could also be made unavailable because of these feed additives. The anticoccidial monensin (MON) could also be sequestered by these clays. The use of sodium bentonite (Na-B) from a mine in the province of Mendoza, Argentina, was investigated as a sequestering agent to prevent the effects of 100 μg/kg of dietary aflatoxin B_1 (AFB_1). In vitro studies demonstrated that the above Na-B was a good candidate to prevent aflatoxicosis. They also showed that MON competes with AFB_1 for the adsorption sites on the clay surface and effectively displaces the toxin when it is in low concentration. Even though the levels of MON in diets, approximately 55 mg/kg, are high enough to not be significantly changed as a consequence of the adsorption, they can further affect the ability of the clays to bind low levels of AFB_1. An in vivo experiment carried out with poultry showed that 100 μg/kg of AFB_1 does not significantly change productive or biochemical parameters. However, liver histopathology not only confirmed the ability of this particular Na-B to prevent aflatoxicosis but also the decrease of this capacity in the presence of 55 mg/kg of MON. This is the first report stressing this fact and further research should be performed to check if this behavior is a characteristic of the assayed Na-B or of this type of clay. On the other hand, the presence of MON should also be taken into account when assaying the potential AFB_1 binding ability of a given bentonite.

Key words: aflatoxin B_1, monensin, competitive adsorption, detoxification, broiler chicken

2011 Poultry Science 90:352–357
doi:10.3382/ps.2010-00834

INTRODUCTION

Aflatoxins (**AF**), a group of potent mycotoxins produced by *Aspergillus* section *Flavi*, mainly *Aspergillus flavus* and *Aspergillus parasiticus* strains, are of major concern in poultry production. The natural incidence of potential aflatoxigenic strains and AF in Argentinean and Brazilian poultry feed was previously reported (Dalcero et al., 1997, 1998; Magnoli et al., 2002; Oliveira et al., 2006; Fraga et al., 2007). The most important member of the family is AFB_1, whose toxicity has been widely investigated by the determination of its carcinogenic, mutagenic, and teratogenic and immunosuppressive effects both in humans and animals (Council for Agricultural Science and Technology, 2003). Various symptoms have been associated with aflatoxicosis in poultry. The most common ones are lethargy, anorexia, lower growth rate, and poor feed utilization. Acute aflatoxicosis, performed with dietary AFB_1 doses ranging from 2.5 to 5 mg/kg, adversely affects relevant productive and biochemical-hematological parameters (Keçeci et al., 1998; Oğuz et al., 2000a; Oğuz and Kurtoglu, 2000; Rosa et al., 2001; Miazzo et al., 2005). Aflatoxin B_1 intake causes significant gross and microscopic changes in liver, such as hepatomegaly, paleness, hydropic degeneration, fatty change, bile duct hyperplasia, and periportal fibrosis (Fernandez et al., 1994;

Received April 9, 2010.
Accepted June 27, 2010.
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Kiran et al., 1998; Ledoux et al., 1999; Ortatatli and Oğuz, 2001; Ortatatli et al., 2005).

Different approaches, including physical, chemical, and biological treatments of contaminated feeds and feedstuffs, have been employed to detoxify AF (Jouany, 2007). Worldwide adsorbent-based methodologies have been used to reduce the effects of AF in contaminated feed and to minimize the toxicity of AF in poultry (Ibrahim et al., 2000; Parlat et al., 2001; Daković et al., 2005). The Argentine poultry feed industry has been using different national bentonites, either as binders or as AF-sequestering agents. These layered aluminosilicates have high swelling capacity and, therefore, are poorly adsorbate-selective (Pasha et al., 2005).

Monensin (**MON**) is the sodium salt of a carboxylic acid ionophore. Its main therapeutic application is as a poultry feed additive to prevent coccidiosis (Schwarz et al., 2001). Because much of the economic losses associated with coccidiosis take place before diagnosis, prevention becomes more important than treatment (Elliott et al., 1998). There are limited data regarding clay feed additives and their effect on the efficacy of anticoccidials. It has been suggested that sodium bentonite (**Na-B**) may interfere with the efficacy of some coccidiostats when these compounds are added at half the minimum level approved for dietary inclusion (Gray et al., 1998). Thus, at the coccidiostat approved dietary inclusion level, the drug bioavailability should be high enough to prevent any practical problem. However, what is not known is how the binding of these coccidiostats to Na-B may affect the ability of the clays to bind AF.

In previous works, different bentonites showed high AFB_1 binding capacities both in vitro (Magnoli et al., 2008a) and in vivo (Rosa et al., 2001; Miazzo et al., 2005; Magnoli et al., 2008b). The adsorption ability of these clays varies from one geological deposit to another. All of those experiments were conducted with AF levels higher than 1 mg/kg. There is little information with respect to the protective effect of Na-B against experimentally induced chronic aflatoxicosis in the presence of MON in broiler chickens. Therefore, the objective of this study was to determine if MON affects the adsorption efficacy of a natural Na-B used to prevent aflatoxicosis in broiler chickens.

MATERIALS AND METHODS

Aflatoxins were produced via fermentation of rice by *A. parasiticus* NRRL 3000 (USDA, Agricultural Research Service, Peoria, IL) following previously described methodology (Miazzo et al., 2000). The AFB_1 levels in the ground culture material were measured by HPLC according to Trucksess et al. (1994) and AOAC (1995), respectively. The ratio of AFB_1 to AFG_1 in the culture was 2:1. The ground culture material was added to the basal diet to provide 100 μg of AFB_1/kg of feed. Commercial Na-B was obtained from a mine situated in the province of Mendoza, Argentina. The chemical, structural, and morphological characterization of the clay was previously reported (Magnoli et al., 2008a). A MON sodium salt (Sigma-Aldrich, St. Louis, MO) was used for in vitro study. Monensin standards were prepared in methanol and stored at 4°C until use.

In Vitro Study

For in vitro studies, the AFB_1 extract was purified according to AOAC (1995). The adsorption isotherms were carried down following the previously described methodology (Rosa et al., 2001). The AFB_1 concentration in each supernatant was determined by HPLC (Trucksess et al., 1994). Aflatoxin B_1 adsorption was estimated by standard solution-depletion method.

In Vivo Study

In the in vivo experiment, 1-d-old male chicks (Cobb) vaccinated against Marek's disease were obtained from a commercial hatchery. The birds were kept under continuous incandescent electric lamps with basal diet and water available for ad libitum consumption until they were 5 d old. A standard starter corn-soybean meal diet, which met NRC requirements, formulated without the coccidiostat MON, was fed from d 1 until the end of the experiment (NRC, 1994).

On d 6, a total of 240 birds were selected by weight and randomly distributed to 30 pens in five 3-tier batteries with raised floors. Each pen represents 1 replicate and each tier represents 1 block. Eight chickens were housed per pen (90 × 100 × 39 cm). All pens had a 1-cm^2 wire mesh bottom with a removal tray placed underneath to allow cleaning. The pens were also equipped with a feeding trough placed outside and a water cup placed inside them. Six replicas per treatment were placed in 2 vertical arrangements of the 3-tier batteries to minimize cross-contamination. These arrangements were randomly distributed.

From d 6 until 33, chickens received the corresponding experimental diets. The level of AFB_1 in the basal diet (4.2 μg/kg) was determined as described previously (Magnoli et al., 2002). The experimental diets for each treatment were as follows: treatment 1 = basal diet; treatment 2 = basal diet plus AFB_1 (100 μg/kg); treatment 3 = basal diet plus AFB_1 plus Na-B (0.3%); treatment 4 = basal diet plus AFB_1 plus Na-B plus MON (55 mg/kg); and treatment 5 = basal diet plus MON.

Chickens were monitored daily for signs of morbidity and mortality. The inhibitory effect of MON on the ability of Na-B to prevent aflatoxicosis was evaluated by measuring BW gain (BW), feed consumption, and feed:gain ratio during 3 periods evenly distributed over the entire experiment.

At the end of the trial, 3 chickens from each treatment were randomly selected, weighed, and killed by cervical dislocation. Blood samples, taken by cardiac puncture, with anticoagulant addition were collected before eutha-

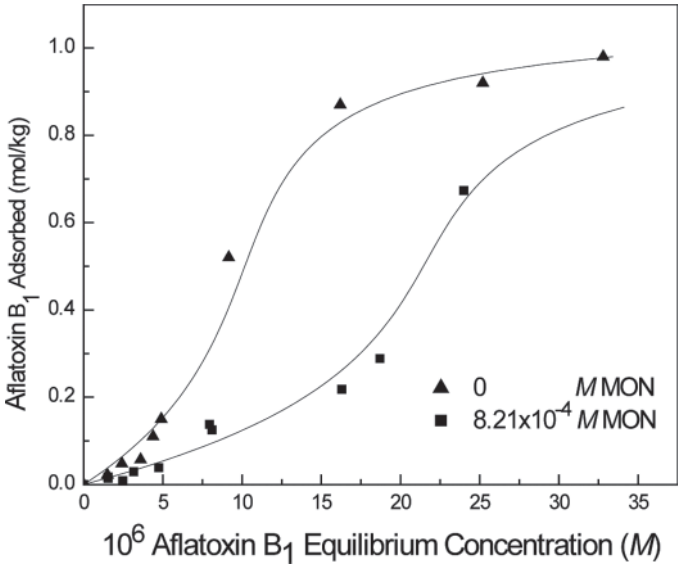

Figure 1. Effect of monensin (MON) on the adsorption isotherm of aflatoxin B_1 on sodium bentonite at pH 2 and 39.5 ± 0.5°C.

nasia, centrifuged, and the plasma was stored at −20°C until use. Total protein, albumin (**ALB**), and globulin (**GLOB**) concentrations and ALB:GLOB ratio for each group were determined with a clinical chemistry analyzer (commercial kit, Wiener Laboratory, Rosario, Argentina) according to the manufacturer's recommended procedure (Rojkin and Alguin, 1974).

After necropsy, 5 livers per treatment (1 liver for each replicate) were excised, weighed, and fixed in 10% neutral-buffered formalin. Fixed tissues were trimmed, embedded in paraffin, and stained with hematoxylin and eosin for histopathological examination. As suggested by Oğuz et al. (2003), the hepatocellular degeneration in livers was graded in a blinded test according to the following score: slight (degree 1) = mild hepatocellular swelling due to hydropic degeneration and fatty changes only in centrilobular areas; moderate (degree 2) = clear hepatocellular swelling in both centrilobular and midzonal areas; and severe (degree 3) = diffuse and severe hepatocellular swelling, cytoplasmic paleness, and rupture.

The experiment was conducted as a randomized complete block design with the single pen representing the experimental unit (replicate). Data were subjected to ANOVA for randomized complete block design statistical analysis to determine whether significant differences exist in the group means of the observed variables (SAS Institute, 1997). All statements of significance were based on the 0.05 level of probability (Quinn and Keough, 2002).

RESULTS

In this study, the interference of MON in the adsorption efficacy of a natural bentonite to prevent the effects of low levels of AFB_1 in broiler chicken diets was undertaken. To in vitro test the AFB_1 adsorption ability of Na-B, isotherms were determined at pH 2 and 39.5°C (Figure 1). The AFB_1 coverage for a saturated surface was 1.07 mol of AFB_1/kg of Na-B.

As can be observed from the isotherm data, 8.21×10^{-4} M of MON remarkably reduces the adsorption of AFB1 by this particular Na-B. In fact, for a toxin equilibrium concentration of approximately 20×10^{-6} M, which is 20 times lower than that of MON, a reduction of approximately 25% in the adsorption was observed. This competition between adsorbates for the adsorbent is likely to be more important when the relationship between MON and AFB1 increases. This fact is particularly important because, in poultry diets, this relationship could be much greater than the one used in the isotherm.

In vivo assay did not show significant differences on productive parameters (BW gain, feed intake, and feed:gain ratio) compared with controls either among blocks or treatments ($P < 0.05$; data not shown). Mortality did not occur, nor were significant changes observed in biochemical parameters (total protein, ALB, and GLOB concentrations and ALB:GLOB ratio in blood plasma) nor in the weight of livers.

The gross and microscopic changes in the livers during chronic aflatoxicosis were also evaluated. The macroscopic appearances of livers from chickens fed different diets (not shown) were similar, except for treatment 3 (AFB_1 plus Na-B), which was slightly darker in color.

Figure 2 shows the photomicrographs of hematoxylin-and-eosin-stained liver sections of chickens fed dietary treatments. The livers of chickens fed the basal diet showed normal hepatocytes (Figure 2a). Livers of chickens fed the diet containing 100 μg/kg of AFB_1 showed slight lesions, such as multifocal and varied cytoplasmatic vacuolation with perilobular location (Figure 2b), whereas the livers of broilers consuming the AFB_1 plus Na-B diet showed only a moderate hydropic degeneration (Figure 2c). Birds fed with AFB_1 plus Na-B and MON showed moderate lesions showing hepatocytes with small fatty vacuoles and diffuse and severe necrosis were observed (Figure 2d). Normal hepatocytes were observed in the basal plus MON treatment (Figure 2e). None of the treatments showed severe lesions.

DISCUSSION

Results of the in vitro study showed that the Na-B was a good candidate to adsorb low levels of AFB_1 in vitro (Magnoli et al., 2008a). However, the adsorption isotherm of AFB_1 was significantly changed by the presence of MON in the system. As can be observed in Figure 1, the competition of MON for adsorption sites on the surface diminishes both the adsorption ability and the maximum capacity of the Na-B for the AF. The decrease is important at low toxin concentrations. It should be noted that the in vitro assay was conducted using a MON concentration around the recommended levels of the coccidiostat in prophylactic management

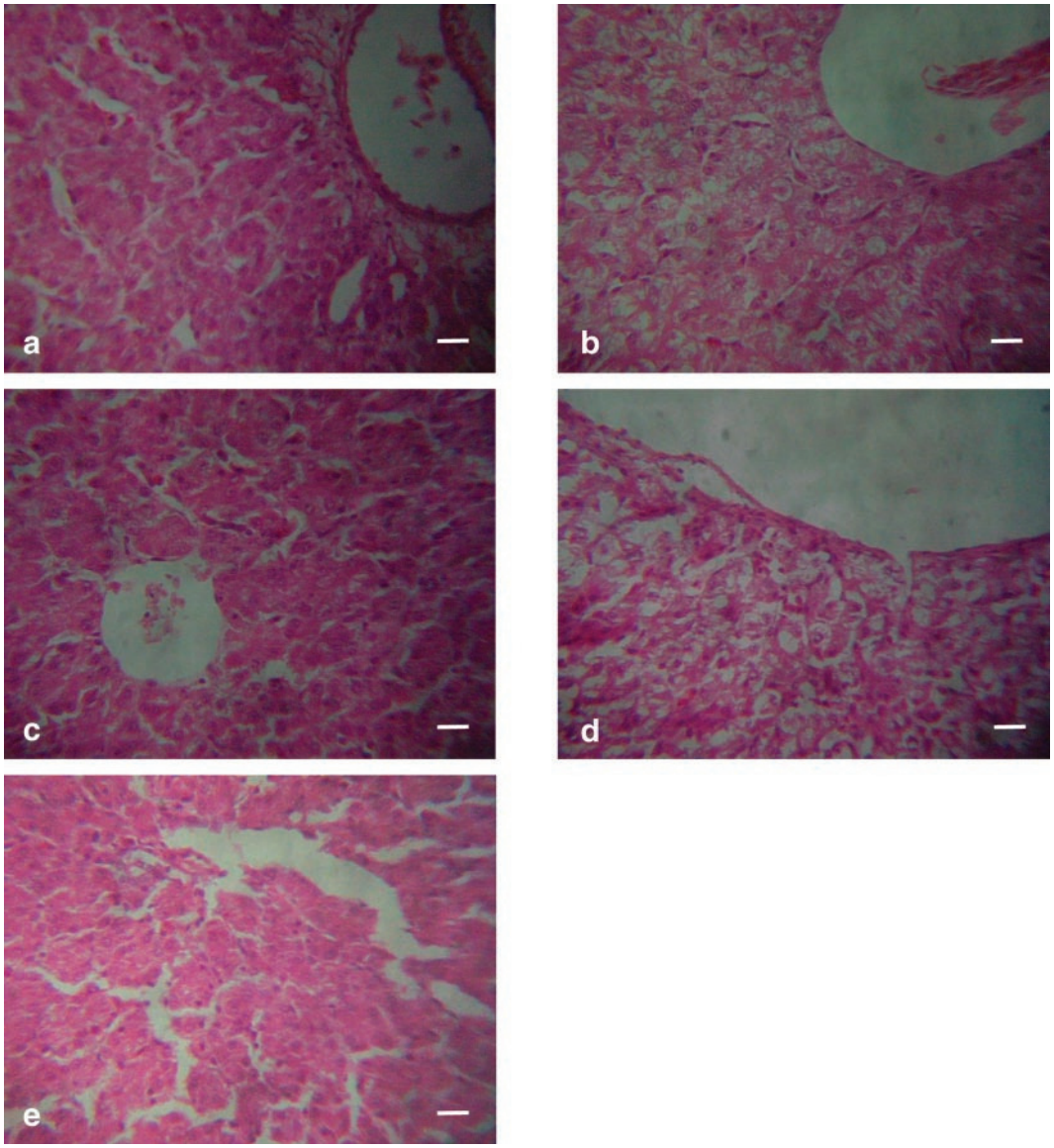

Figure 2. Photomicrographs of hematoxylin-and-eosin-stained chicken liver sections: (a) basal diet, (b) basal diet plus aflatoxin B_1 (AFB_1), (c) basal diet plus AFB_1 plus sodium bentonite (Na-B), (d) basal diet plus AFB_1 plus Na-B plus monensin (MON), (e) basal diet plus MON, normal hepatocytes. Bar equals 10 μm. Color version available in the online PDF.

of broiler chickens (Dusi and Gamba, 1999). Gray et al. (1998) reported the interactive effect of Na-B and MON in coccidiosis-infected chickens. In fact, they found that dietary Na-B may reduce the efficacy of MON when it is added below recommended levels. The present results agree with our in vitro findings suggesting a decrease in the bioavailability of MON due to Na-B adsorption.

Most previously reported in vivo studies have been performed under acute aflatoxicosis (i.e., 1 to 5 mg/kg of dietary AFB_1; Keçeci et al., 1998; Oğuz et al., 2000a; Oğuz and Kurtoglu, 2000; Rosa et al., 2001; Karaman et al., 2005, Miazzo et al., 2005). Less information is available dealing with lower AFB_1 dose exposure (i.e., 30 to 185 μg/kg; Oğuz et al., 2000b, 2003; Ortatatli et al., 2005; Magnoli et al., 2008b). It would be very important to determine the chronic AFB_1 levels that affect broilers in terms of performance, hematology, enzyme biochemistry, immunology, and histology. It has been suggested that clinical, hematological-biochemical, and histopathological changes in broilers began at AFB_1 concentrations ranging from 90 to 100 μg/kg (Giambrone et al., 1985; Marquez and Hernandez, 1995; Oğuz et al., 2000b; Shi et al., 2009). Our results differ from others reported in the literature, in which productive parameters were affected by 100 μg/kg of AFB_1 dietary intake (Marquez and Hernandez, 1995; Oğuz et al., 2000b; Shi et al., 2009). The biochemical parameters, serum total protein, ALB, and GLOB were also not significantly different among treatments. Johri et al. (1990) and Abdelhamid et al. (1994) also reported no significant changes in serum biochemistry for induced aflatoxicosis ranging from 50 to 200 μg/kg.

From macroscopic examination of livers, a beneficial effect of Na-B might be occurring because a slightly

darker brownish color can be appreciated in livers of birds fed the basal diet plus Na-B plus AFB_1. However, because no macroscopic differences in liver appearance of birds fed the basal diet and basal diet plus AFB_1 were observed, the color may have had nothing to do with AFB_1 exposure. The liver histopathology of chicks receiving 100 μg/kg of AFB_1 alone showed the typical pattern of subclinical aflatoxicosis. The present results agree with those reports by Giambrone et al. (1985), who reported only microscopic lesions in birds given 100 μg/kg of dietary AFB_1. On the other hand, a rather mild aflatoxicosis was observed in liver of chicks fed the basal diet plus Na-B. These results suggest the ability of Na-B to counteract low levels of AFB_1 at least when no MON is added to the diet. Moreover, the major histopathologic changes were observed in livers of chicks fed diets with Na-B (0.3%) plus AFB_1 (100 μg/kg) plus MON (55 mg/kg). The present results indicate that Na-B in the presence of MON does not ameliorate the effects of the 100 μg/kg of AFB_1. Therefore, the histopathologic findings agree with those resulting from in vitro studies, that is, MON competes and displaces AFB_1 from adsorption sites at the adsorbent surface at least when AFB_1 levels are low. These results are the first evidence of competition at least for this particular Na-B from a mine in the province of Mendoza in Argentina. This type of inhibition in the binding ability of Na-B due to MON could be also present in other animals, like pigs and cows. These species, which are also sensitive to AFB_1 usually, receive MON as a growth promotant or as control for rumen acidosis. Moreover, the longer life span of these species compared with poultry could make the aflatoxicosis signs worse. Because the adsorbent supplementation levels used worldwide range from 0.05 to as high as 1%, the present results could be of a great concern for the doses lower than 0.3% (Dixon et al., 2008). Results of this study would be less of a concern at higher adsorbent concentrations. It should be stressed that there is no reference in the literature about the existence of such competition, although care should be taken because it could be specific to the particular assayed Na-B. Therefore, the presence of MON should be considered when testing related adsorbents for prevention of aflatoxicosis. These results might reopen discussions about the efficacy of nonselective adsorbents, like this specific Na-B, to prevent subclinical aflatoxicosis and the need to find selective AFB_1 binders. However, taking into account that the clinical signs for aflatoxicosis depend on bird age and both nutritional or health status at the time of exposure to contaminated feed, we suggest that further in vivo experiments should be conducted to confirm the present results.

ACKNOWLEDGMENTS

The authors are grateful to the Secretaría de Ciencia y Técnica (SECyT), Universidad Nacional de Río Cuarto, and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina), who supported this study through grants. Alejandra Magnoli thanks CONICET for fellowship support. AMD, CEM, LRC, and SMC held positions at CONICET. We thank CEMA SAICF from Ciudad Autónoma de Buenos Aires, Argentina, for providing us the Na-B.

REFERENCES

Abdelhamid, A. M., T. M. Dorra, S. E. Mansy, and A. E. Sallam. 1994. Effect of raising dietary protein, amino acids, and/or energy levels as an attempt to alleviate severity of chronic aflatoxicosis by broiler chicks. 2. Biochemical characteristics. Arch. Tierernahr. 46:347–355.

AOAC. 1995. Sections 975.35 and 976.22 in Official Methods of Analysis. AOAC International, Gaithersburg, MD.

Council for Agricultural Science and Technology. 2003. Mycotoxins: Risk in Plant, Animal, and Human Systems. Task Force Report No. 139. Council for Agricultural Science and Technology, Ames, IA.

Daković, A., M. Tomasevic-Canovic, V. Dondur, G. E. Rottinghaus, V. Medakovic, and S. Zaric. 2005. Adsorption of mycotoxins by organozeolites. Colloids Surf. B Biointerfaces 46:20–25.

Dalcero, A., C. Magnoli, S. Chiacchiera, G. Palacios, and M. Reynoso. 1997. Mycoflora and incidence of aflatoxin B_1, zearalenone and deoxynivalenol in poultry feeds in Argentina. Mycopathologia 137:179–184.

Dalcero, A., C. Magnoli, M. Luna, G. Ancasi, M. M. Reynoso, S. Chiacchiera, R. Miazzo, and G. Palacio. 1998. Mycoflora and naturally ocurring mycotoxins in poultry feeds in Argentina. Mycopathologia 141:37–43.

Dixon, J. B., I. Kannewischer, M. G. Tenorio Arvide, and A. L. Barrientos Velazquez. 2008. Aflatoxin sequestration in animal feeds by quality-labeled smectite clays: An introductory plan. Appl. Clay Sci. 40:201–208.

Dusi, G., and V. Gamba. 1999. Liquid chromatography with ultraviolet detection of lasalocid, monensin, salinomycin and narasin in poultry feeds using pre-column derivation. J. Chromatogr. A 835:243–246.

Elliott, C. T., D. G. Kenned, and W. J. McCaughey. 1998. Methods for the detection of polyether ionophore residues in poultry. Analyst (Lond.) 123:45R–56R.

Fernandez, A., M. T. Verde, M. Gascon, J. Ramos, J. Gomez, D. F. Lucco, and G. Chavez. 1994. Variations of clinical biochemical parameters of laying hens and broiler chickens, fed aflatoxin-containing feed. Avian Pathol. 23:37–47.

Fraga, M. E., F. Curvello, M. J. Gatti, L. R. Cavaglieri, A. M. Dalcero, and C. A. da Rocha Rosa. 2007. Potential aflatoxin and ochratoxin A production by *Aspergillus* species in poultry feed processing. Vet. Res. Commun. 31:343–353.

Giambrone, J. J., U. L. Diener, N. D. Davis, V. S. Panangala, and F. J. Hoerr. 1985. Effects of aflatoxin on young turkeys and broilers chickens. Poult. Sci. 64:1678–1684.

Gray, S. J., T. L. Ward, L. L. Southern, and D. R. Ingram. 1998. Interactive effects of sodium bentonite and coccidiosis with monensin or salinomycin in chicks. Poult. Sci. 77:600–604.

Ibrahim, I. K., A. M. Shareef, and K. M. T. Al-Joubory. 2000. Ameliorative effects of sodium bentonite on phagocytosis and Newcastle disease antibody formation in broiler chickens during aflatoxicosis. Res. Vet. Sci. 69:119–122.

Johri, T. S., V. R. Sadagopan, H. P. Shrivastava, and S. Jumdar. 1990. Effect of aflatoxin on the performance of purebred chicks. Indian J. Anim. Sci. 60:1246–1248.

Jouany, J. P. 2007. Methods for preventing, decontaminating and minimizing the toxicity of mycotoxins in feeds. Anim. Feed Sci. Technol. 137:342–362.

Karaman, M., H. Basmacioglu, M. Ortatatli, and H. Oğuz. 2005. Evaluation of the detoxifying effect of yeast glucomannan on aflatoxicosis in broilers as assessed by gross examination and histopathology. Br. Poult. Sci. 46:394–400.

Keçeci, T., H. Oğuz, V. Kurtoglu, and O. Demet. 1998. Effects of polyvinylpoly-pyrrolidone, synthetic zeolite and bentonite on se-

rum biochemical and haematological characters of broiler chickens during aflatoxicosis. Br. Poult. Sci. 39:452–458.
Kiran, M. M., O. Demet, M. Ortatatli, and H. Oğuz. 1998. The preventive effect of polyvinylpolypyrrolidone on aflatoxicosis in broilers. Avian Pathol. 27:250–255.
Ledoux, D. R., G. E. Rottinghaus, A. J. Bermudez, and M. Alonso-Debolt. 1999. Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poult. Sci. 78:204–210.
Magnoli, A., L. Tallone, R. C. A. Rosa, A. M. Dalcero, S. M. Chiacchiera, and R. M. Torres Sanchez. 2008a. Commercial bentonites as detoxifier of broiler feed contaminated with aflatoxin. Appl. Clay Sci. 40:63–71.
Magnoli, A. P., L. Cavagglieri, C. Magnoli, J. C. Monge, R. Miazzo, M. F. Peralta, M. Salvano, C. A. R. Rosa, A. Dalcero, and S. M. Chiacchiera. 2008b. Bentonite performance on broiler chickens fed with diets containing natural levels of aflatoxin B_1. Rev. Bras. Med. Vet. 30:55–60.
Magnoli, C., S. Chiacchiera, R. Miazzo, G. Palacio, A. Angeletti, C. Hallak, and A. Dalcero. 2002. The mycoflora and toxicity of feedstuffs from a production plant in Córdoba, Argentina. Mycotox. Res. 18:7–22.
Marquez, R. N. M., and I. T. Hernandez. 1995. Aflatoxin adsorbent capacity of two Mexican aluminosilicates in experimentally contaminated chick diet. Food Addit. Contam. A 12:431–433.
Miazzo, R., M. F. Peralta, C. Magnoli, M. Salvano, S. Ferrero, S. M. Chiacchiera, E. C. Q. Carvalho, C. A. Rosa, and A. Dalcero. 2005. Efficacy of sodium bentonite as a detoxifier of broiler feed contaminated with aflatoxin and fumonisin. Poult. Sci. 84:1–8.
Miazzo, R., C. A. R. Rosa, E. C. De Queiroz Carvalho, C. Magnoli, S. M. Chiacchiera, G. Palacio, M. Saenz, A. Kikot, E. Basaldela, and A. Dalcero. 2000. Efficacy of synthetic zeolite to reduce the toxicity of aflatoxin in broiler chicks. Poult. Sci. 79:1–6.
NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy of Science-National Research Council, Washington, DC.
Oğuz, H., H. H. Hadimli, V. Kurtoglu, and O. Erganis. 2003. Evaluation of humoral immunity of broilers during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure. Rev. Méd. Vet. 154:483–486.
Oğuz, H., T. Keçeci, Y. O. Birdane, F. Onder, and V. Kurtoglu. 2000a. Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Res. Vet. Sci. 69:89–93.
Oğuz, H., and V. Kurtoglu. 2000. Effect of clinoptilolite on fattening performance of broiler chickens during experimental aflatoxicosis. Br. Poult. Sci. 41:512–517.
Oğuz, H., V. Kurtoglu, and B. Coskun. 2000b. Preventive efficacy of clinoptilolite in broilers during chronic aflatoxin (50 and 100 ppb) exposure. Res. Vet. Sci. 69:197–201.
Oliveira, G. R., J. M. Ribeiro, M. E. Fraga, L. R. Cavaglieri, G. M. Direito, K. M. Keller, A. M. Dalcero, and C. A. Rosa. 2006. Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. Mycopathologia 162:355–362.
Ortatatli, M., and H. Oğuz. 2001. Ameliorative effects of dietary clinoptilolite on pathological changes in broiler chickens during aflatoxicosis. Res. Vet. Sci. 71:59–66.
Ortatatli, M., H. Oğuz, F. Hatipoglu, and M. Karaman. 2005. Evaluation of pathological changes in broiler during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure. Res. Vet. Sci. 78:61–68.
Parlat, S. S., M. Ozcan, and H. Oğuz. 2001. Biological suppression of aflatoxicosis in Japanese quail (*Coturnix coturnix japonica*) by dietary addition of yeast (*Saccharomyces cerevisiae*). Res. Vet. Sci. 71:207–211.
Pasha, T. N., M. U. Farooq, F. M. Khattak, M. A. Jabbar, and A. D. Khan. 2005. Effectiveness of sodium bentonite and two commercial products as aflatoxin absorbents in diets for broiler chickens. Anim. Feed Sci. Technol. 132:103–110.
Quinn, G. P., and M. J. Keough. 2002. Experimental Design Data Analysis for Biologists. Cambridge University Press, UK.
Rojkin, M. L., and D. E. M. Alguin. 1974. Proteínas totales del suero. Causas mas frecuentes del error en la reacción del Biuret. Nuevo reactivo cuproalcalíno estable. Vigo Atlántico VI/63:1931.
Rosa, C. A., R. Miazzo, C. Magnoli, M. Salvano, S. M. Chiacchiera, S. Ferrero, M. Saenz, E. C. Carvalho, and A. Dalcero. 2001. Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of aflatoxin in broilers. Poult. Sci. 80:139–144.
SAS Institute. 1997. SAS/STAT Software: Changes and Enhancement Through Release 6.12. SAS Institute Inc., Cary, NC.
Schwarz, S., C. Kehrenberg, and T. R. Walsh. 2001. Use of antimicrobial agents in veterinary medicine and food animal production. Int. J. Antimicrob. Agents 17:431–437.
Shi, Y., Z. Xu, Y. Sun, C. Wang, and J. Feng. 2009. Effects of two different types of montmorillonite on growth performance and serum profiles of broiler chicks during aflatoxicosis. Turk. J. Vet. Anim. Sci. 33:15–20.
Trucksess, M. W., M. E. Stack, S. Nesheim, R. Albert, and T. Romer. 1994. Multifunctional column coupled with liquid chromatography for determination of aflatoxins B_1, B_2, G_1, and G_2 in corn, almonds, Brazil nuts, peanuts, and pistachio nuts: Collaborative study. J. AOAC Int. 77:1512–1521.