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Full Length Research Paper

Ameliorative effect of seed extract of *Pterocarpus santalinus* on coragen induced haematological alterations and serum biochemical changes in Charles Foster rats

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In the present study, the ameliorative effect of an aqueous extract of *Pterocarpus santalinus* against coragen induced haematological changes, biochemical alterations and oxidative damage in Charles foster rats was undertaken. Coragen administration (1000 mg kg^{-1} body weight orally for 6 weeks) was associated with significant rise in serum levels of alkaline phosphatase, urea, uric acid and creatinine and enhanced lipid peroxidation which is evident by significant increase in malondialdehyde (MDA) levels. Furthermore, significant changes in the haematological indices (red blood cell (RBC) count, haemoglobin percentage, haematocrit, mean corpuscular volume of RBCs, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and white blood cell (WBC) count) were observed. Treatment with aqueous seed extract of *P. santalinus* (300 mg kg^{-1} body weight orally for 30 days) attenuated the oxidative stress and improved haematological as well as biochemical alterations evoked by Coragen. Thus, *P. santalinus* possesses ameliorative effect against coragen induced toxicity.

Key words: *Pterocarpus santalinus,* coragen, lipid peroxidation, oxidative damage, MDA.

INTRODUCTION

Pesticides are widely used by farmers for agricultural purposes. It has conferred immense benefits to mankind by improving health and nutrition. Pesticides fall into numerous chemical classes, which have widely differing biological activities and thus differing potential to produce adverse effects in living organisms, including humans (Timothy and Ballantyne, 2004).

Ryanodine receptor-targeting insecticides (Ryanoids) are a novel class of insecticides acting selectively on ryanodine receptors of a broad spectrum of lepidopteran species. Ryanoids are synthetic analogues with the same mode of action as ryanodine, a naturally occurring insecticide extracted from *Ryania speciosa*. They bind to calcium channels in cardiac and skeletal muscle, blocking nervous transmission. Ryanodine receptors are intracellular channels in insects, responsible for the

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control of calcium ion release (Ebbinghaus et al., 2006). Ryanodine receptor plays an important role in controlling the release of calcium ions, universal intracellular transmitter, from intracellular stores. The flow of Ca^{2+} is regulated by ryanodine receptors, which mediate in several metabolic and physiological cellular processes such as neurotransmission, hormones secretion, muscles excitation-contraction coupling (Copping and Duke, 2007). A class of antranilicdiamides (antranilodiamides) were being developed; with representatives Chlorantraniliprole and Cyazypyr. Two insecticidal preparates containing Chlorantraniliprole were being promoted; Altacor and Coragen. Coragen 18.5 SC is a concentrated suspension of Chlorantraniliprole, being applied for use in apples against codling moth (*Cydiapomonella*), apple fruit moth (*Argyresthia conjugella*) and free leaf living larvae (Kar et al., 2013).

Pesticides are known to increase the production of reactive oxygen species (ROS), which in turn, generate oxidative stress in different tissues (Heikal et al., 2012; Heikal and Soliman, 2010; Heikal et al., 2011; Rai and Sharma, 2007). Many studies have implicated oxidative damage as the central mechanism of toxicity (Celik et al., 2009; Halliwell and Gutteridge, 2002; Kalender et al., 2010). Oxidative damage primarily occurs through production of reactive oxygen species (ROS), including hydroxyl radicals and hydrogen peroxide that are generated during the reaction and react with biological molecules, eventually damaging membranes and other tissues. High oxidative stress depletes the activity of antioxidant defense system and thus promotes free radicals generation. Oxygen free radicals could react with polyunsaturated fatty acids which lead to lipid peroxidation (LPO) (Gandhi et al., 2012; Messarah et al., 2010).

Many insecticides are hydrophobic molecules that bind extensively to biological membranes, especially phospholipid bilayers, and they may damage membranes by inducing lipid peroxidation (Heikal et al., 2011; Celik et al., 2009; Kalender et al., 2010). Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules in healthy human cells and cause protein and DNA damage along with lipid peroxidation (Mishra et al., 2011). Herbal medicines derived from plant extracts are being increasingly utilized as adjunct treatment options for a wide variety of clinical diseases. Many phytochemicals have been found to play an important role as potential antioxidants and antimicrobials (Mishra et al., 2011).

Pterocarpus santalinus (Linn Fabaceae), commonly known as “Red sanders” is a small to medium-sized deciduous tree, 7.5 m high, with an extremely hard dark purple heartwood with a bitter flavor. In the traditional system of medicine, the decoction prepared from the heartwood is attributed various medicinal properties, it has been used as a anti-pyretic, anti-inflammatory, anthelmintic, tonic, hemorrhage, dysentery, aphrodisiac, diaphoretic as well as to induce vomiting, to treat eye diseases, mental aberrations and ulcers (Kirtikar and Basu, 1987). The wood in combination with other drugs is also prescribed for snake-bites and scorpion-stings (Warrier et al., 1995). Decoction of the heartwood has been reported as a central nervous system (CNS) depressant and also shown to have anti-inflammatory activity for induced hand paw edema in rats when prepared in formalin (3%). Heartwood contains pterocarpol, santalin A, B, pterocarptriol, isopterocarpolone, pterocarpodiolones with β-eudeslol and crytomeridol (Yoganarasimhan, 2000). In addition, Auron glycosides viz., 6-OH-1-Methyl-3’,4’,5’-trimethoxy-aurone-4-O-rhamnoside and 6,4’-dihydroxyaurone-4-O-neohesperidoside, and isoflavone glycoside 4’,5-dihydroxy-7-O-methyl isoflavone 3’-O-beta-D-glucoside (Krishnaveni and Rao, 2000) are also present. However, the species has remained unexplored for many pharmacological activities claims. The phytochemical investigation of the seed extract has not been demonstrated so far. Hence, the present investigation was carried out to analyze the therapeutic effect of seed extract of *P. santalinus* on alterations in haematological and biochemical parameters caused upon sub-chronic exposure to Coragen.

MATERIALS AND METHODS

Animals

Charles Foster rats (n = 30), weighing 180 to 200 g of 8 weeks old, were obtained from animal house of Mahavir Cancer Institute and Research Centre, Patna, India (CPCSEA Regd-No. 1129/bc/07/CPCSEA). The research work was approved by the (IAEC, Institutional Animal Ethics Committee) with IAEC No. IAEC/2011/12/01. Food and water to rats were provided *ad libitum* (prepared mixed formulated food by the laboratory itself). The experimental animals were housed in conventional polypropylene cages in small groups (2 each). The rats were randomly assigned to control and treatment groups. The temperature in the experimental animal room was maintained at 22±2°C with 12 h light/dark cycle.

Chemicals

Coragen 18.5 (SC 18.5% w/w) was obtained from M/S Krishi Seeds Niketan, Patna, India. Other reagents used were of analytical grade and were prepared in all glass-distilled water.

Preparation of seed extract of *P. santalinus*

In the present study, dry seeds of *P. santalinus* were procured from a tree at local garden at Patna, Bihar, India. The identity of the medicinal plant was confirmed by Dr. Ramakant Pandey (Botanist), Department of Biochemistry, Patna University, Patna, Bihar, India. The collected seeds of *P. santilinus* were shade dried and were grinded to fine powder. The powder was then soaked in 70% ethanol for 48 h and finally extracted with absolute ethanol using soxhlet apparatus for 6 to 8 h and the residue was concentrated and dried at 37°C. The ethanolic extract dose was calculated after LD_{50} estimation which was 3,000 mg kg^{-1} body weight and finally made to the 1/10th dose that is 300 mg kg^{-1} body weight.

Table 1. Changes in the haematological parameters of rats exposed to Coragen (1000mgkg^{-1} body weight.) for 42 days and therapeutic effect of *P.santalinus* (300mgkg^{-1} body weight for 30 days).

Blood Parameters	Control	Coragen treated 1000 mg kg^{-1} body weight (42 days)	*P. santalinus* treated 300 mg kg^{-1} body weight (14 days)	*P. santalinus* treated 300 mg kg^{-1} body weight (30 days)
RBC (10^6/mm^3)	7.46 ± 0.49	1.64 ± 0.42	2.70 ± 0.38	3.21 ± 0.45
Hb (%)	93.36 ± 0.17	32.17 ± 4.02	53.33 ± 3.56	71.67 ± 3.78
Haematocrit (%)	272.5 ± 4.04	92.50 ± 3.27	156.8 ± 4.71	209.5 ± 3.94
MCV (fL)	362.3 ± 3.39	641.8 ± 3.19	639.8 ± 3.02	707.0 ± 3.62
MCH (pg)	127.5 ± 2.88	214.7 ± 3.10	214.8 ± 2.89	235.1 ± 2.97
MCHC (g/L)	33.77 ± 3.09	18.10 ± 2.85	4.57 ± 0.58	4.64 ± 0.76
WBC (10^3/mm^3)	7500 ± 3.78	3600 ± 3.45	5551 ± 2.64	10501 ± 3.33

Data represent mean ± SD, n=6/group, $p < 0.05$.

Experimental design

A total of 30 rats (10 male and 20 female), were randomly assigned to control and treated groups. Coragen at concentration of 1000 mg kg^{-1} body weight (5 ml/1000 g body weight suspended in 10 ml distilled water) was administered orally, once a day, for a period of 42 days. This was followed by administration of aqueous seed extract at 300 mg kg^{-1} body weight orally daily for a period of 30 days. No treatment was administered to control group and was designated as healthy control.

Haematological analysis

Rats were anaesthesized with diethyl ether and blood samples were drawn from the heart of each animal. Two blood samples were taken with or without ethylenediaminetetraacetic acid (EDTA). The one with EDTA was used for haematological analysis and the other for the preparation of serum for the biochemical assays. Blood samples with anti-coagulant EDTA were analysed for blood parameters namely red blood cell (RBC) counts, white blood cell (WBC) counts, haemoglobin percentage, haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) using an improved Neubauer's Chamber (Depth 0.1 mm, 1/400 mm^2). Haemometer was used for the measurement of haemoglobin percentage (Berberian and Enan, 1989).

Biochemical evaluation

The sera obtained from different treatments were subjected to certain biochemical analyses. Serum alkaline phosphatase (ALP; EC 3.1.3.1) activity was measured by Mod. Kind and King's method (Kind and King, 1954; Varley, 1975). The kidney function assays involved the determination of serum urea by Mod. Berthelot method (Berthelot, 1859; Fawcett and Scott, 1960), serum uric acid by Uricase/PAP method (Trinder, 1969; Fossati and Prencipe, 1980) and measurement of serum creatinine by alkaline picrate method (Bones, 1945; Toro, 1975).

Lipid peroxidation

Thiobarbituric acid reactive substances (TBARS), as a marker for LPO, were determined by the double heating method (Draper and Hadley, 1990). The principle of the method was a spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). For this purpose purpose, 2.5 ml of 100 g/L trichloroacetic acid (TCA) solution was added to 0.5 ml serum in a centrifuge tube and incubated for 15 min at 90°C. After cooling in tapwater, the mixture was centrifuged at 3000 g for 10 min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/L TBA solution in a test tube and again incubated for 15 min at 90°C. The solution was then cooled in tap water and its absorbance was measured using Thermo Scientific UV-10 (UV –Vis) spectrophotometer (USA) at 532 nm.

Statistical analysis

Results are presented as mean ± SD and total variation present in a set of data was analysed through one-way analysis of variance (ANOVA). Difference among mean values has been analysed by applying Dunnett's t-test. Calculations were performed with the Graph Pad Prism 5.0 Program (GraphPad software, Inc., San Diego, USA). The criterion for statistical significance was set at $p < 0.05$.

RESULTS

Morbidity and mortality

The rats after sub-chronic Coragen exposure (1000 mg kg^{-1} body weight) have shown signs of toxicity such as nausea, nose bleeding, bulging of eyes, lack of body co-ordination, blackening of tongue and general body weakness. However, the recovery was achieved after the administration of aqueous seed extract of *P. santilinus.*

Haematological analysis

Data of haematological parameters as studied after 14 and 21 days of Coragen exposure showed significant decrease in the erythrocyte count (RBCs), haemoglobin percentage, haematocrit percentage and leukocyte count (WBCs), but increase in the MCV, MCH, MCHC in comparison with control group (Kumar et al., 2013).

Table 1 depicts the changes in the haematological parameters of Charles Foster rats exposed to Coragen at 1000 mgkg^{-1} body weight daily for 42 days and therapeutic effect of *P. santalinus (*300 mg kg^{-1} body weight)

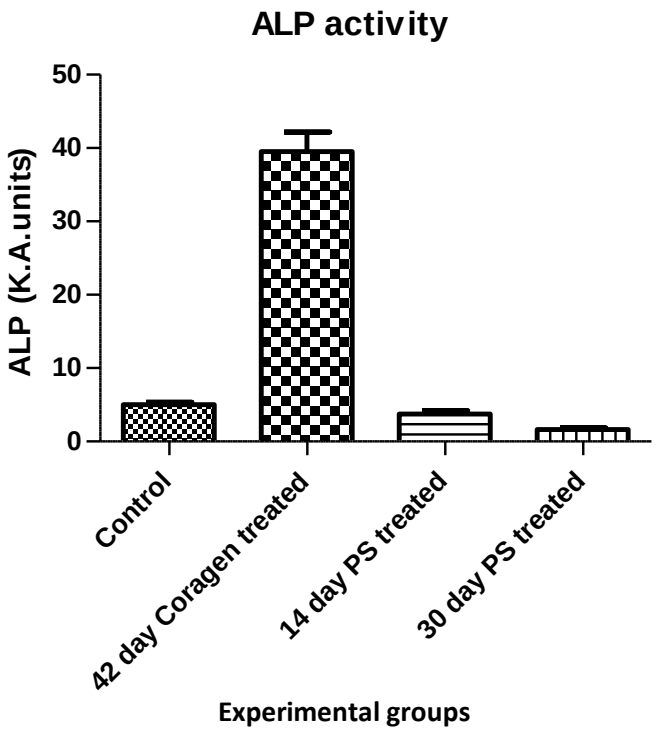

Figure 1. Effect of *P.santalinus* seed extract on serum alkaline phosphatase (ALP) after sub-chronic exposure to Coragen. Values are mean ± SD for 6 animals in each group ($p < 0.05$).

for 30 days. There was a significant increase in haemoglobin percentage, RBC count and WBC count after *P. santalinus* administration. But, the other parameters showed a somewhat different trend. *P. santalinus* administration led to an increase in haematocrit percentage as compared to that after Coragen treatment, however the value was still less than the control. There was a slight decrease in MCV level after 14-day *P. santalinus* treatment but it was followed by gradual increase after 30 days *P. santalinus* treatment. MCHC showed a decreasing trend after *P. santalinus* administration. The same trend was observed in case of MCH.

Biochemical analyses

Figures 1 to 5 depicts the effects produced on selected functional indices of rat liver and kidney, respectively, following the repeated administration of *P. santalinus* extract. *P. santalinus* administration resulted in significant decrease in serum content of alkaline phosphatase when compared with those treated with Coragen (Figure 1). The alkaline phosphatase concentration compared favorably with the control by the 30th day of administration, a trend which was sustained throughout the experimental period as is evident from the value after 30 days exposure. The administration of *P. santalinus* seed extract led to a gradual decrease in serum lipid peroxidation, as evident from decrease in MDA level. However, the result was not comparable with that of the control group (Figure 2). The urea level was gradually decreased after administration of seed extract. However, it showed a vast difference as compared to that with control (Figure 3). The other two parameters denoting kidney function, that is, serum uric acid and creatinine levels showed a similar trend. Initially, there was an increase in levels of uric acid and creatinine after seed extract administration. However, after 30-day dosage, the levels gradually decreased and were favorably comparable with the control (Figures 4 and 5).

DISCUSSION

In the present study, the protective effect of an aqueous extract of *P. santalinus* against hepatotoxicity, nephrontoxicity and oxidative damage induced by sub-chronic exposure to Coragen was undertaken. The experimental models of Coragen was previously validated as a model of other pesticide induced toxicity on some haematological parameters (Saka et al., 2011; Mongi et al., 2011; Dahamna et al., 2011; Ambali et al., 2010, 2011; Bhardwaj et al., 2010; Mansour et al., 2007; Rahman and Siddiqui, 2006; Adhikari et al., 2004; Raizada et al., 2001;

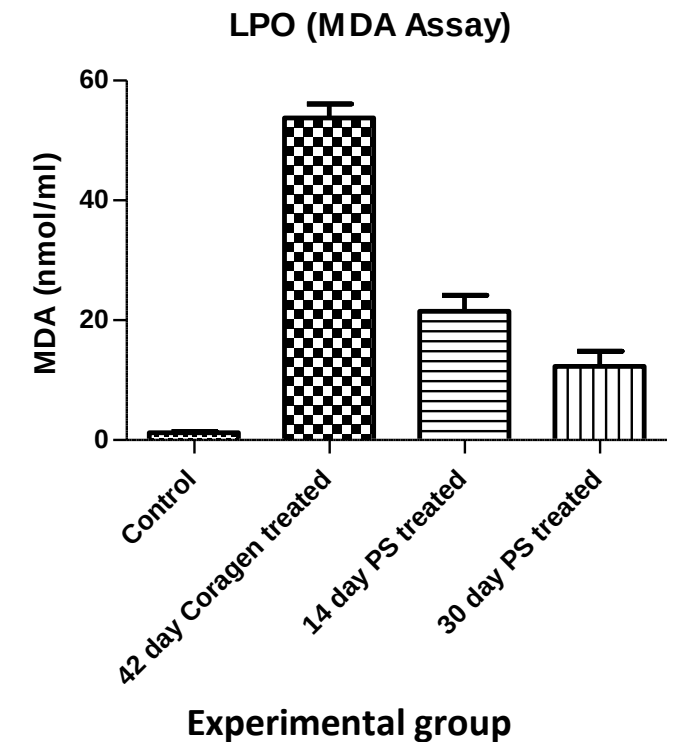

Figure 2. Effect of *P. santalinus* seed extract on lipid peroxidation after repeated dose. Values are mean ± SD for 6 animals in each group.

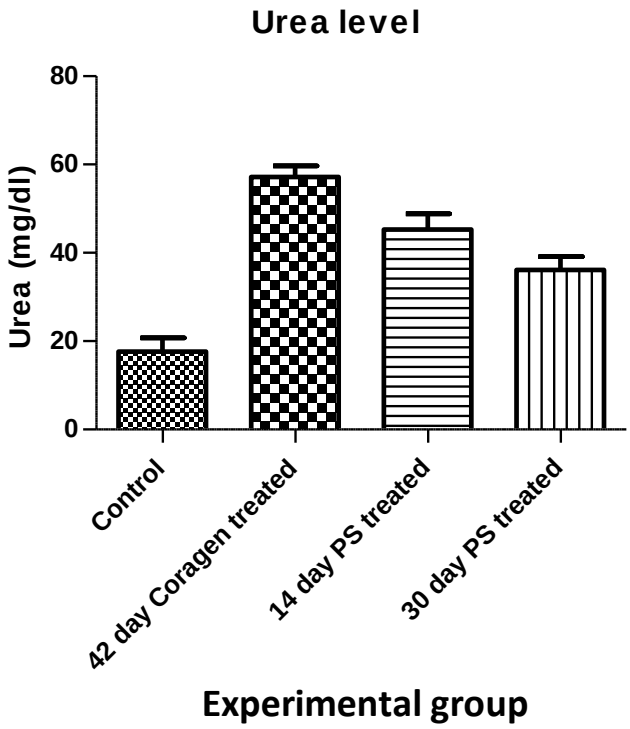

Figure 3. Effect of *P.santalinus* seed extract on serum urea after repeated dose. Values are mean ± SD with 6 animals in each group.

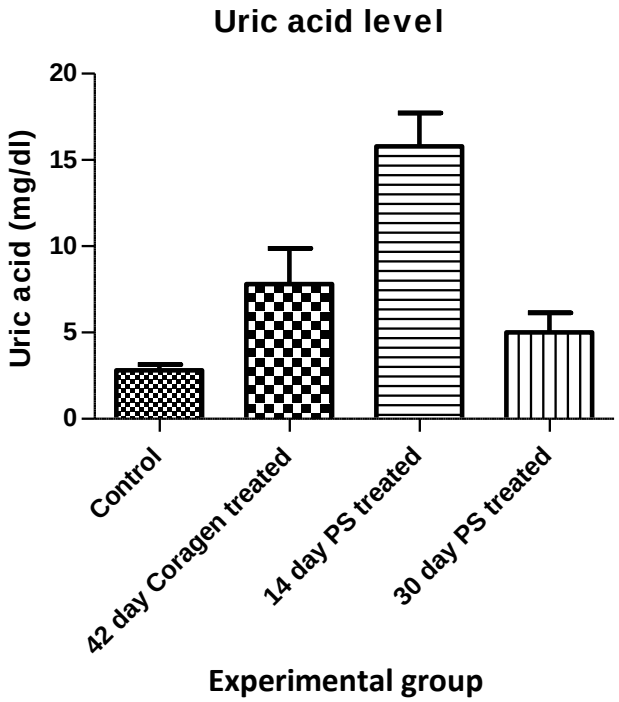

Figure 4. Effect of *P. santalinus* seed extract on serum uric acid after repeated dose. Values are mean ± SD with 6 animals in each group.

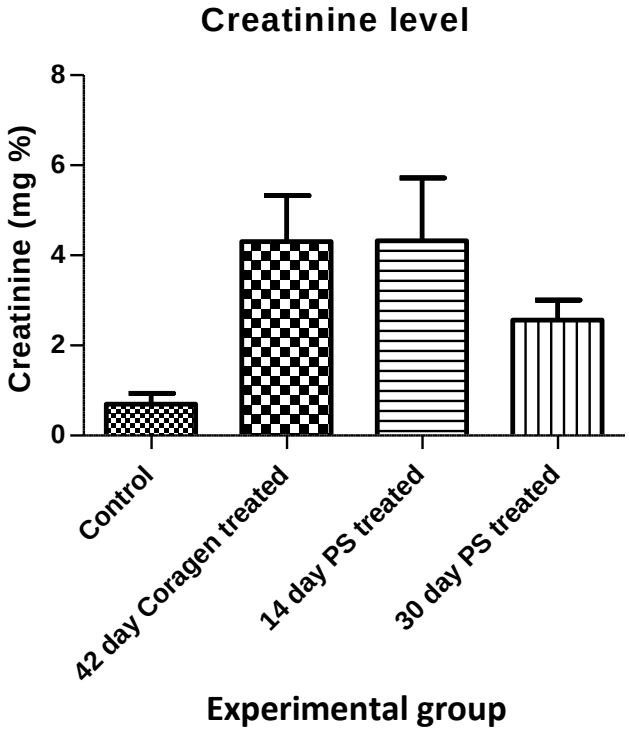

Figure 5. Effect of *P.santalinus* seed extract on serum creatinine after repeated dose. Values are mean ± SD with 6 animals in each group.

Neskovic et al., 1991). Coragen is an insecticidal preparate containing chlorantraniliprole as the active substance. The ryanodine receptor targeting insecticide, coragen, is an antranilicdiamide acting selectively on ryanodine receptors (RyRs). The compound has extremely high affinity to the open-form ryanodine receptor, a group of calcium ion channels found in skeletal and heart muscle cells. At nanomolar concentration, it locks the receptor in a half-open state, whereas, it fully closes them at micromolar concentrations. The effect of binding at nM level is that it causes release of calcium ions from calcium stores in the sarcoplasmic reticulum leading to massive muscular contractions. This is true for both mammals and insects. However, the mammalian toxicity being proposed for chlorantraniliprole is low (Lahm et al., 2007). *P. santalinus* is a highly valued woody plant, whose bark extract has a blood glucose level lowering effect in experimental animals (Varma and Vijayamma, 1991). Methanol and aqueous extracts of heartwood of PS have shown anti-hepatotoxicity in CCl_4-induced hepatotoxicity (Rane and Gramarc, 1998). Himoliv, a polyherbal Ayurvedic formulation containing *P. santalinus* as one of the ingredients has been reported to possess hepatoprotective activity (Bhattacharya et al., 2003).

Liver plays an important role in metabolism to maintain energy level and metabolic stability of the body (Guyton and Hall, 2006). It is also the site of biotransformation by which a toxic compound has been transformed in less harmful form to reduce toxicity (Hodgson, 2004). The present study has shown the therapeutic effect of aqueous extract of *P. santalinus* on liver of coragen intoxicated rats. The toxic effects of organophosphorus insecticides are to conjugate with the natural complement of enzyme in the body, thereby inactivating them. Phosphate enzymes act by hydrolyzing phosphor mono ester including 3 and 5 phosphoproteins, these may also be involved in the transfer of phosphate (Hanafy et al., 1991). Phosphatases are involved in many different processes that require mobilization of phosphate ion or dephosphorylation as part of anabolic, catabolic or transfer processes. It was found, that the increased level of phosphatases may be to indicate metabolic activity, perhaps to meet the stress induced by prolonged exposure to the pesticides. These enzyme changes are indicative of the cellular toxicity and tissue damage induced by these pesticides in the rats probably by altering the specific molecular pathways (Khalid et al., 2013). Alkaline phosphatase activity is related to hepatocyte function. An increase in its activity is due to increased synthesis in presence of increased biliary pressure (Kumar et al., 2006).

Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney, therefore, marked increase in serum urea and creatinine, as noticed in this study, confirms an indication of functional damage to the kidney (Garba et al., 2007).

Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet, whilst, since creatinine is more specific to the kidney, therefore kidney damage is the only significant factor that increases serum creatinine level (Nwanjo et al., 2005). Therefore, significant increase in urea and creatinine levels noticed in this study are a classical sign that the kidney was adversely affected by coragen administration. Under our experimental conditions, coragen had a poison effect on adult rats, increasing serum uric acid levels. Impairment in kidney function could probably occur as a result of kidney oxidative damage. In fact, uric acid in blood is the most important antioxidant (Ames et al., 1981). This compound is the end product of purine catabolism and can reduce oxidative stress by scavenging various reactive oxygen species (ROS) (Mahjoubi et al., 2008). Kidney dysfunction and nephrotoxicity induced by coragen in present investigation are consequences of oxidative stress. Treatment of coragen-intoxicated rats with aqueous extract of PS normalized the levels of urea and creatinine.

Large numbers of xenobiotics have been identified to have potential to generate free radicals in biological system (Ahmed et al., 2000; Kehrer, 1993). Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics.. Some of these free radicals interact with various tissue components, resulting in dysfunction and the question of whether oxidative stress is a major cause of injury remains equivocal. In this study, we have investigated the effects of administration of aqueous extract of PS on lipid peroxidation induced by Coragen. To our knowledge, there is no information concerning protective action of PS extract on oxidative injury induced by Coragen.

Lipid peroxidation has been extensively used as a marker of oxidative stress. Oxidative stress is an outcome of a multi-step process spanning from perturbations in the balance between the levels of oxidants/prooxidants and antioxidants (both enzymatic and non-enzymatic) to tissue damage leading to onset of several disease states and finally to apoptosis. Several factors (called as risk factors) are thought to be associated with potentiating of the impact of pesticides-induced oxidative stress in living systems and hence play crucial role in the evaluation of safety or toxicity of the pesticide concerned (Agrawal and Sharma, 2010). The increase in the levels of thiobarbituric acid-reactive substances (the marker of extent of lipid peroxidation) in serum of rats due to coragen administration produced oxidative stress due to the generation of free radicals and subsequently altered the antioxidant defense system in erythrocytes (John et al., 2001). Oxidative damage in tissues due to the formation of reactive oxygen species (ROS) can be counter-balanced by the different oxidative systems of the host. These defenses appear to be inducible by nutrients or non-nutrients in the diet (Jaiswal et al., 2013). The protective effect of *P. santalinus* seed

extract may be in part due to santalin A, B, the main constituents of *P. santalinus*. Hence, the hepatoprotective and nephroprotective activities of *P. santalinus* may be attributed to its components including alkaloid, triterpenoid, saponins and flavonoidal constituents (Metha et al., 1999; Tran et al., 2001; Vijayan et al., 2003; Xiong et al., 2003; Dhanabal et al., 2007).

The decrease in RBC count and Hb % can be correlated with the increased arterial O_2 saturation of blood, which acts indirectly as stimulus for bone marrow erythrocyte production (Zabulyte et al., 2007). The significant decrease in the RBC, haemoglobin and haematocrit may be a consequence of severe haemorrhage which results in the dilution of blood caused due to the influx of cells and fluids from body stores (Celik and Suzek, 2009; Kalender et al., 2006). In the severe anaemic condition, there is immense decrease in the number of red blood cells leading to impaired synthesis of haemoglobin due to iron deficiency or impaired production of erythrocyte due to deficiency of folic acid and vitamin B_{12} (Murray et al., 2007). The decrease in WBCs count was due to the possible reason of their getting used up while encountering a variety of inflammation injury and subsequent infections resulting due to the Coragen treatment. This decrease is possibly due to the failure or suppression or destruction of stem cells in the bone marrow, which leads to decrease in the number of leucocytes denoting marked decrease in the cellularity of bone marrow (Chandrasoma and Taylor, 1991; Uzun and Kalender, 2013).

The aqueous seed extract of *P. santalinus* normalized the haematological indices to a much greater extent. This therapeutic role may be attributed to its phytochemical constituents determining the capability of *P. santalinus* extract to repair the damaged stem cells thus leading to increased blood cell synthesis.

Conclusion

In the present scenario, we cannot check the flow of pesticides from entering it into the human food chain but can control its deleterious effect by administration of suitable antidotes. In the present study, coragen caused severe haemato-toxicity as well as hepato-renal toxicity. But *P. santalinus* played the vital role to combat the deleterious effect of coragen. Thus, *P. santalinus* is the best antidote against coragen induced toxicity.

Conflict of interest

The authors have no conflict of interest.

REFERENCES

Adhikari S, Sarkar B, Chatterjee A, Mahapatra CT, Ayyappan S (2004). Effects of Cypermethrin and Carbofuran on certain haematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). Ecotoxicol. Environ. Saf. 58(2):220-226.

Agrawal A, Sharma B (2010). Pesticides induced oxidative stress in mammalian systems: a review. Int. J. Biol. Med. Res. 1(3):90-104.

Ahmed RS, Seth V, Pasha ST, Banerjee BD (2000). Influence of dietary ginger (*Zingiber officinalis* Rosc.) on oxidative stress induced by malathion in rats. Food Chem. Toxicol. 38:443-450.

Ambali SF, Abubakar AT, Shittu M, Yaqub LS, Anafi SB, Abdullah A (2010). Chlorpyrifos induced alteration of haematological parameters in Wistar rats: Ameliorative Effect of Zinc. Res. J. Environ. Toxicol. 4(2):55-56.

Ambali SF, Ayo JO, Esievo KAN, Ojo SA (2011). Hemotoxicity induced by chronic chlorpyrifos exposure in Wistar Rats: Mitigating effect of Vit. C. Vet. Med. Int. 2011:1-7.

Ames BN, Cathcart R, Schwiers S (1981). Uric acid provides an antioxidant defense in humans against oxidant and radical caused aging and cancer; a hypothesis. Proc. Natt. Acad. Sci. USA. 78:6858-6862.

Berberian IG, Enan EE (1989). Haematological studies on white male rats exposed to some antimoulting Compounds. Bull. Environ. Contam. Toxicol. 43:60-65.

Berthelot MPE (1859). Berthelot's reaction mechanism. Report Chem. Appl. 2884.

Bhardwaj S, Srivastava MK, Kapoor U, Srivastava LP (2010). A 90 days oral toxicity of imidacloprid in female rats: morphological, biochemical and histopathological evaluations. Food Chem. Toxicol. 48(5):1185-90.

Bhattacharya D, Mukherji R, Pandit S, Das N, Sur TK (2003). Prevention of Carbon tetrachloride-induced hepatotoxicity in rats by Himoliv, a polyherbal formulation. Ind. J. Pharmacol. 35:183-185.

Bones RW, Tausky HH (1945). Colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem. 158:581-591.

Celik I, Suzek H (2009). Effects of subacute exposure of dichlorovos at sublethal dosages on erythrocytes and tissue antioxidant defense systems and lipid peroxidation in rats. Ecotoxicol. Environ. Saf. 72(3):905-908.

Celik I, Yilmaz Z, Turkoglu V (2009). Hematotoxic and hepatotoxic effects of dichlorvos at sublethal dosages in rats. Environ. Toxicol. 24:128-132.

Chandrasoma P, Taylor CR (1991). Concise Pathology 1st Ed. Appleton & Lang, San Mateo. pp. 375-421.

Copping LG, Duke SO (2007). Pest. Manag. Sci. 63:24-54.

Dahamna S, Belguet A, Bouamra D, Guendouz A, Mergham M, Harzallah D (2010) . Evaluation of the toxicity of cypermethrin pesticide on organs weight loss and some biochemical and histological parameters. Commun. Agric. Appl. Biol. Sci. 76(4):6915-21.

Dhanabal P, Kannan SE, Bhojraj S (2007). Protective and therapeutic effects of the Indian medicinal plant *Pterocarpus santalinus* on D – galactosamine – induced liver damage. Asian J. Trad. Med. 2:51-57.

Draper HH, Hadley M (1990). Oxygen Radicals in Biological Systems Part B: Oxygen Radic. Antioxid. 186:421-431.

Ebbinghaus – Kintscher V, Leummen P, Lobitz N, Schulte T, Funke C, Fischer R, Masaki T, Yabokaula N, Tohnishi M (2006). Phthalic acid diamides activate ryanodine sensitive Ca^{2+} release channels in insects. Cell Calcium 39:21-33.

Fawcett JK, Scott JE (1960). A rapid and precise method for the determination of urea. J. Chem. Pathol. 13:156.

Fossati P, Prencipe L, Berti G (1980). Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-amino-phenazone chromogenic system in direct assay of uric acid in serum and urine. Clin. Chem. 26:227-231.

Gandhi RG, Ignacimuthu S, Paulraj MG (2012) Hypoglycemic and β-cells regenerative effects of *Aegel marmelos* (L.) corr. bark extract in streptozocin-induced diabetic rats. Food Chem. Toxicol. 50(5):1667-1674.

Garba SH, Adelaiye AB, Msehelia LY (2007). Histopathological and biochemical changes in the rat kidneys following exposure to a pyrethroid-based mosquito coil. J. App. Sci. Res.3:1788-1793.

Guyton AC, Hall JE (2006). Textbook of Medical Physiology, 9th Ed. Prism Book (Pvt.) Ltd., Bangalore, India, XLIII, 1148.

Halliwell B, Gutteridge IMC. Free radicals in Biology and Medicine. 3rd ed., Oxford University Press Inc. pp. 105-245.

Hanafy MS, Arbid MS, Affify MM (1991). Biochemical and histopathological effects of the organophosphorous insecticides.

(Tamaron) in rats Indian J. Anim. Sci. 61:43-47.
Heikal TM, El-Sherbiny M, Hassan SA, Arafa A, Ghanem HZ (2012b). Antioxidant effect of Selenium on hepatotoxicity induced by Chlorpyrifos in male rats. Int. J. Pharm. Sci. 4 Suppl 4:603-609.
Heikal TM, Ghanem HZ, Soliman MS (2011). Protective effect of green tea extracts against dimethoate in duced DNA damage and oxidant, antioxidant status in male rats. Biohealth Sci. Bull. 3(1):1-11.
Heikal TM, Soliman MS (2010) Effect of fish oil supplementation on brain DNA damage and hepatic oxidant/ antioxidant status in dimethoate – treated rats. J. Egypt. Soc. Toxicol. 42:1-9.
Hodgson E (2004). A textbook of modern toxicology, 3rd Edition. John Wiley & Sons, Inc, New Jersey. pp. 203-211.
Jaiswal D, Rai DK, Mehta S, Chatterji S, Shukla S, Rai DK, Sharma G, Sharma B, Watal G (2013). Role of drumstick leaves (*Moringa oleifera*) in regulation of diabetes-induced oxidative stress. Asian Pac. J. Trop. Med. 6:426-432.
John S, Kale M, Rathore N, Bhatnagar D (2001). Protective effect of Vit. E in dimethoate and malathion induced oxidative stress in rat erythrocytes. J. Nutr. Biochem. 12:500-504.
Kalender S, Uzun FG, Durak D, demir F, Kalender Y (2010). Malathion-induced hepatotoxicity in rats: the effects of Vit. C & E. Food Chem. Toxicol. 48:633-638.
Kalender Y, Uzunhisarcikli M, Ogutcu A, Acikgoz F, Kalender S (2009). Effects of diazinon on pseudocholinesterase activity and haematological indices in rats. The protective role of Vit. E. Environ. Toxicol Pharmacol. 22(1):46-51.
Kar A, Mandal K, Singh B (2013). Environmental fate of chlorantraniliprole residues on cauliflower using QuE ChERS technique. Environ. Monit. Assess. 185(2):1255-63.
Kehrer JP (1993). Free radicals as mediator of tissue injury and disease. Crit. Rev. Toxicol. 23:21-48.
Gathwan KH, Al-Karkhi IHT, Yaseen AK (2013). Organophosphorus insecticides induced alterations in liver of Male Albino Rat. Chem. Sci. Trans. 2:S95-S98.
Kind PRH, King EJ (1954). Determination of alkaline phosphatase activity in serum. J. Clin. Pathol. 7:322.
Kirtikar KR, Basu BD (1987). Indian Medicinal Plants, 2nd Ed. Vol 1, pp. 826-827.
Krishnaveni KS, Rao S (2000). Aurone glycosides from *Pterocarpus santalinus* Linn. Chem. Pharm. Bull. 48(9):1373-1374.
Kumar SV, Sugantha C, Syamala J, Nagasudha B, Mishra SH (2006). Protective effect of root extract of *Operculina turpethum* Linn. against paracetamol induced hepatotoxicity in rats. Ind. J. Pharm. Sci. 68(1):32-35.
Kumar A, Dutta K, Najam A, Nath A, Singh JK, Ali M, Kumar R (2013). Coragen cause haematological alterations in Charles foster rats. Eur. J. Toxicol. Sci. 2013:4.
Lahm GP, Stevenson TM, Selby TP, Freudenberger JH, Cordova D, Flexner L, Bellin CA, Dubas CM, Smith BK, Hughes KA, Hollinghaus JG, Clark CE, Benner EA (2007). Rynaxypyr™: a new insecticidal anthranilic diamide that acts as a potent and selective ryanodine receptor activator. Bioorg. Med. Chem. Lett. 17:6274-6279.
Mahjoubi-Samet A, Fetoui H, Zeghai N (2008). Nephrotoxicity induced by dimethoate in adult rats and their suckling pups. Pest. Biochem. Physiol. 91:96-103.
Mansour SA, Mossa AH, Heikal TM (2007). Haematoxicity of a new natural insecticide “Spinosad” on male Albino rats. Int. J. Agric. Biol. 9(2):342-346.
Messarah M, Boumendjel A, Chouabia A, Klibet F, Abdennour C, Boulakoud MS, Feki AE (2010). Influence of thyroid dysfunction on liver lipid peroxidation and antioxidant status in experimental rats. Exp. Toxicol. Pathol. 62(3):301-310.
Metha RS, Shankar MB, Geetha M, Saluja AK (1999). Hepatoprotective activity of *Trianthema portulacastrum*. Indian Drugs 36:241-244.
Mishra A, Kumar S, Bhargava A, Sharma B, Pandey AK (2011). Studies on *in vitro* antioxidant and antistaphylococcal activities of some important medicinal plants. Cell. Mol. Biol. 57:16-25.
Mongi S, Mahfoud M, Amel B, Kamel J, Abdelfattah EF (2011). Protective effects of Vit. C against haematological and biochemical toxicity induced by deltamethrin in male Wistar rats. Ecotoxicol. Environ. Saf. 74(6):1765-9.
Murray RK, Granner DK, Mayes PA, Rodwell VW (2007). Harper’s Illustrated Biochemistry, International 26th Edition. The Mcgraw – Hill Companies, Inc. pp. 46-47, 12.
Neskovic NK, Karan VZ, Vitorovic SL, Vojinovic VD (1991). Toxic effects of bound malathion residues in rats. Biomed. Environ. Sci. 4(4):423-32.
Nwanjo HU, Okafor MC, Oze GO (2005). Changes in biochemical parameters of kidney function in rats co-administered with chloroquine and aspirin. J. Clin. Sci. 23:10-12.
Rahman MF, Siddiqui MK (2006). Hematological and Clinical Chemistry changes induced by subchronic dosing of a novel phosphorothionate (RPR-V) in Wistar male and female rats. Drug Chem. Toxicol. 29(1):95-110.
Rai DK, Sharma B (2007). Carbofuran induced oxidative stress in mammalian brain. Mol. Biotechnol. 37:66-71.
Raizada RB, Srivastava MK, Kaushal RA, Singh RP, Gupta KP (2001). Subchronic oral toxicity of a combination of insecticide (HCH) and herbicide (ISP) in male rats. J. Appl. Toxicol. 21(1):75-9.
Rane, Gramarc ND (1998). Hepatoprotective activity of *Pterocarpus marsupium* and *Butea koenex*- RoxB. Ind. J. Pharm. Sci. 5:182-184.
Saka WA, Akhigbe RE, Azeez OM, Babatunde TR (2011). Effects of pyrethroid insecticide exposure on haematological and haemostatic profiles in rats. Pak. J. Biol. Sci. 14 (22):1024-1027.
Timothy CM, Ballantyne B (2004). Pesticide Toxicology and International Regulation. England: John Wiley & Sons Ltd.
Toro G, Ackermann PG (1975). Practical Clinical Chemistry. Boston: Little Brown and Co. P 154.
Tran QI, Adnyana IK, Tezuka Y, Nagaoka T, Tran QK, Kadota S (2001). Triterpene saponins from Vietnamese ginsend (*Panax vietnamensis*) and their hepatocyteprotective activity. J. Natl. Prod. 64:456-461.
Trinder P (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor Ann. Clin. Biochem. 6:24-27.
Uzun FG, Kalender Y (2013). Chlorpyrifos induced hepatotoxic and hematologic changes in rats: The role of quercetin and catechin. Food Chem. Toxicol. 55:549-556.
Varley H (1975). Practical Clinical Biochemistry, 4th Ed. New Delhi: India. Arnold Heinman.
Varma RR, Vijayamma N (1991). Pharmacological studies on Raktchandana. J. Res. Ayurveda Sidd. 12(3):190-199.
Vijayan P, Prasant HC, Preethi Vijayaraj, Dhanaraj SA, Suresh B (2003). Hepatoprotective effect of the total alkaloid fraction of *Solanum pseudocapsicum* leaves. Pharm. Biol. 41:443-448.
Warrier PK, Nambiar VPK, Raman Kutty C (1995). Indian Medicinal Plants. Orient Longman Ltd. Vol. 4, pp. 384-387.
Xiong X, Chen W, Cui J, Yi S, Zhang Z, LiK (2003). Effects of ursolic acid on liver protection and bile secretion. Zhong Yao Cai 26:578-581.
Yoganarasimhan SN (2000). Medicinal plants of India (Tamil Nadu). Vol II, P 449.
Zabulyte D, Vleckiene S, Kalibatas J, Paltanauiciene A, Jascaniniene N, Stosik M (2007). Experimental studies on effect of sodium fluoride and nitrate on biochemical parameters in rats. Vet. Inst. Pulaury 51:79-82.

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Naturally contaminated feed with low levels of fumonisins with anti-mycotoxin additive and its impact in the immune cells and blood variables in broiler chickens

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Ninety six one-day-old broiler chickens were used to evaluate the effect of feeding naturally contaminated rations with low levels of fumonisins (FBs) and the protective effect of a commercial anti-mycotoxin additive (AMA) on circulating and intestinal immune cells, blood biochemistry, hematological variables and biomarkers of FBs exposure. Birds were assigned in three groups: Negative control (NC), positive control (PC) containing low levels (17 ppm) of FBs (FB1 + FB2) in feed, and PC with AMA at 0,2% (AMA + PC). Blood was collected and used to quantify circulating leucocytes through flow cytometry, activity of aspartate transaminase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and levels of uric acid (UA), total protein (TP), albumin (Alb), globulin (Glb), Alb:Glb ratio, total leucocytes count (TLC) and hematocrit (Ht), as well as free esphinganine to esphinogosine ratio (SA:SO). On day 3, FBs reduced circulating $CD4^+TCRV\beta1^-$ and $CD8^+CD28^-$ lymphocytes in PC; reduced B lymphocytes and increased Kul^-MHCII^+ in both PC and PC+AMA, and increased Kul^+MHCII^+ cells in PC+AMA birds. On day 7, circulating $CD4^+TCRV\beta1^+$ and $CD8^-CD28^+$ and $CD3^+$ in jejunum were increased only in PC, while $CD4^-TCRV\beta1^+$ were increased in both PC and PC+AMA birds. FBs reduced TLC and Alb:Glb in both PC and PC+AMA birds after 14 days and only in PC after 28 days, while increased Glb after 14 days in both PC and PC+AMA. On day 28, FBs increased Alb:Glb and reduced Ht only in PC birds, increasing Alb levels and GGT activity in both PC and PC+AMA birds. Serum SA:SO was increased only in PC birds on day 28. These results showed that low levels of naturally occurring FBs could induce rapid immune alterations and impaired liver function and blood homeostasis, which may reflect in a reduction in the overall birds' competence to respond to challenges. Therefore, even if the regulatory standards of FBs are met, toxicity may occur and can be detected by sensitive markers. The use of an AMA was able to alleviate most of these effects.

Key words: Fumonisins, broiler chickens, flow cytometry, immunohistochemistry, anti-mycotoxin additive.

INTRODUCTION

Fumonisins (FBs) are mycotoxins commonly produced by molds belonging to the *Fusarium* genus. Several fumonisin compounds have been already identified (Roohi et al., 2012) but FB1 (CAS number 116355-83-0)

is the most relevant from the toxicological stand point and the most studied one (Voss et al., 2007). This toxin has been related to liver and kidney damage in most species, for which the most accepted mechanism of action is through the competitive inhibition of the enzyme ceramide synthase, leading to the increase in the concentration of sphingoid bases (sphinganine (Sa) and sphingosine (So) and their phosphorylated derivatives) as well as the depletion of ceramide and complex sphingolipids (Merrill Jr et al., 2001; Voss et al., 2007).

FBs are usually identified in poultry feed once these feed are mainly based on corn, reported to be the crop most frequently exposed to mycotoxin contamination (Souza et al., 2013). Indeed, recent research shows that FBs may be found at levels as high as 77 ppm in this cereal (Schatzmayr and Streit, 2013), although levels of around 10 ppm could be considered more realistic (Grenier and Applegate, 2013). Even though several studies have been conducted concerning the toxic effects of FBs in poultry, in many cases the contamination levels are beyond those generally found in commercial feeds (Weibking et al., 1993; Kubena et al., 1995; Shetty and Bhat, 1997; Oliveira et al., 2006), although the contamination range of the toxins is very wide (Magnoli et al., 1999).

Poultry have been shown to be less sensitive to FBs than other domestic species such as swine (Diaz and Boermans, 1994). It has been mathematically estimated that to cause a 5% reduction in the growth rate of poultry, doses over 250 ppm would be necessary (Dersjant-Li et al., 2003). Immune suppression has been reported in other works at 200 ppm (Li et al., 1999). Conversely, doses as low as 10 ppm were already related to toxicity in broiler chicken (Espada et al., 1994; Del Bianchi et al., 2005). Nevertheless, European Union Commission and Food and Drug Administration (FDA) limits for FBs (expressed as the sum of FB1 and FB2) in broiler chicken feed are 20 and 50 ppm, respectively (Voss et al., 2007; Grenier and Applegate, 2013). Once FBs contamination levels may easily reach 10 to 40 ppm, depending on weather conditions (Grenier and Applegate, 2013) and as there is no consensus in literature related to safe levels of the toxin, studies are needed to understand the effects of feeding naturally contaminated grains to broiler chickens at doses close to the threshold regarded as safe for the species. One of the strategies currently available to mitigate the effect of mycotoxins in livestock is to include in feed nonnutritive ingredients, generally known as anti mycotoxin additives (AMA), with specific adsorbent and/or detoxifying properties against those compounds (Ramos et al., 1996; Varga and Tóth, 2005). However, although there are several products commercially available, studies show a variable efficacy of those additives (Swamy et al., 2002; Avantaggiato et al., 2005; Fushimi et al., 2014) and to the authors' knowledge, there are no studies assessing the benefits of AMA in feed containing relatively low levels of mycotoxins as 17 ppm of FBs.The aim of the present trial is to assess the effects of feeding broiler chickens with FBs naturally contaminated feed and its impact in the poultry's immune response, blood biochemical and hematological variables as well as to evaluate the protective effect of adding an AMA to this feed.

MATERIALS AND METHODS

Experimental birds

Ninety six 1-day-old Cobb® 500 male broiler chickens (Granja Econômica Avícola, Paraná, Brazil) were raised in isolators at the Center for the Study of Birds Immune Response of the Microbiology and Ornitopathology laboratory from the Federal University of Paraná, Brazil. Birds were handled according to guidelines of the Canadian Council on Animal Care with all experimental procedures approved by the ethics Committee on the Use of Animals of the Federal University of Paraná, Brazil.

Experimental diets

A corn-soya bean meal based control diet was formulated to meet or exceed the nutritional specifications for broiler chickens in starter phase according to the National Research Council (NRC) (1994). The mycotoxin contaminated diets were formulated to the nutrient specifications of control diets by replacing control corn by a naturally contaminated corn with *Fusarium* mycotoxins (only FB1 at 19200 µg/kg and FB2 at 8570 µg/kg were detected). Anti-mycotoxin additive (AMA) supplemented diet was prepared with naturally contaminated corn with 0.2% of a commercial product based on clay minerals, biopolymer and enzymes (Elitox®, Impextraco N.V., Heist Op Den Berg, Belgium). Besides fumonisins (B1, B2), dietary contents of aflatoxins (B1, B2, G1, G2), deoxynivalenol, ochratoxin A, zearalenone and T-2 toxin were screened by liquid chromatography-mass spectrometry as described previously (Monbaliu et al., 2009).

Experimental design

One-day-old broiler chickens were wing banded, weighed and randomly distributed into three groups (negative control (NC); Fumonisins (PC) contaminated feed; FBs contaminated feed + AMA (AMA + PC) of 32 animals each. Birds had *ad libitum* access to feed and water throughout the entire experimental period which took 28 days from housing. A 24-h lighting program was applied throughout the trial period. Birds were evaluated daily for any clinical signs of toxicity.

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Table 1. Specificity of monoclonal antibodies[1] used in this work, with the respective cell subset recognized.

Cell surface antigen[2]	Specificity/function
$CD4^+TCRV\beta1^+$	Helper T lymphocytes important for mucosal IgA production
$CD4^+TCRV\beta1^-$	Conventional T helper lymphocytes
$CD4^-TCRV\beta1^+$	Presumptive CD8 lymphocytes/important for cytotoxic effects in mucosae
$CD8^+CD28^+$	Cytotoxic T lymphocytes, including naïve/memory cells
$CD8^+CD28^-$	Terminally activated cytotoxic lymphocytes
$CD8^-CD28^+$	Presumptive CD4 lymphocytes, including naïve and memory cells
$Kul^+MHC\ II^+$	Monocytes/macrophages
$Kul^+MHC\ II^-$	Suppressor macrophages
$Kul^-MHC\ II^+$	Other antigen presenting cells
B lymphocytes	Precursors to the plasmocytes
CD4:CD8	Ratio between these subsets that indicates their balance/associated with immunocompetence in humans

[1]References: (Hala et al., 1981; Chan et al., 1988; Cihak et al., 1991; Lahti et al., 1991; Linsley and Ledbetter, 1993; Lillehoj, 1994; Tregaskes et al., 1995; Luhtala et al., 1997; Nabeshima et al., 2002; Pawelec et al., 2004; Bridle et al., 2006; Beirão et al., 2012). [2]All primary antibodies supplied by Southern Biotechnology. CD4:CD8 is a ratio between the two cell surface antigens.

Sample collection

On days 3 and 7 of age, blood was collected from 8 birds per treatment from jugular vein using tubes with ethylenediaminetetraacetic acid (EDTA) (Becton Dickinson, New Jersey, USA) as anticoagulant to evaluate the circulating immune cells by flow cytometry. On days 7 and 14, the same procedure was undertaken and after blood collections, birds were euthanized by cervical dislocation. Samples from the jejunum of 6 animals per group were collected and processed to be further analyzed by immunohistochemistry for the $CD3^+$ cell count according to procedures described elsewhere (Pickler et al., 2012). Additionally, blood samples were also analyzed for total proteins (TP), albumin (Alb) and uric acid (UA) levels, aspartate aminotransferase (AST), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) activities using an autoanalyzer (Mindray® BS200, China). Serum globulins (Glb) were obtained by the difference between TP and Alb, and the ratio Alb:Glb was also established per treatment. Hematocrit (Ht) was quantified using the microhematocrit centrifugation method and total leucocytes count (TLC) was determined according to standard procedures (Feldman et al., 2006). On day 28, the same procedures carried out on day 14, except flow cytometric evaluations, were performed. Additionally, samples of plasma of 6 animals per group were assessed for free sphinganine-to-sphingosine ratio (SA:SO), performed according to Rauber et al. (2012). Since no differences in daily weight gain or nutrient intake were expected (Dersjant-Li et al., 2003), production parameters were not recorded.

Antibodies

The primary monoclonal anti-chicken antibodies used in this study are shown in Table 1. They were acquired from Southern Biotechnology Associates, Inc. Antibodies used to detect lymphocyte surface antigens were the following: mouse anti-chicken CD4 (specific for chicken CD4 [Mr 64 kD]), clone CT-4, isotype immunoglobulin (Ig) G1κ; anti-chicken CD8α (α chain of CD8 [34 kD]), clone CT-8, IgG1κ; anti-chicken MHC class II (chicken MHC class II [B-L] molecules), clone 2G11, IgG1κ; anti-chicken T cell receptor (TCR) αβ (Vβ1) (chicken TCR αβ [Vβ1]), clone TCR2, IgG1κ; anti-chicken CD28 (chicken CD28 [Mr 40-44 kD]), clone AV7, IgG1κ; BU1 Mouse anti-chicken (chicken B lymphocytes for specific [Mr 70-75 kD]), AV20 clone, IgG1κ; Mouse anti-chicken Monocyte/Macrophage, clone KUL01, IgG1κ. The isotypic control was performed with mouse $IgG_{1\kappa}$ isotype, clone 15H6.

Flow cytometry

Isolation of mononuclear cells from whole blood samples and staining was performed as previously described (Filho et al., 2013). Flow cytometry was performed on a FACS Calibur flow cytometer (Becton Dickinson, New Jersey, USA). Green fluorescence (FITC) was detected on the FL1 channel (530/30 nm), and orange fluorescence (PE) was detected on the FL2 channel (585/42 nm). At least 10,000 events were analyzed in the lymphocyte gate (based on forward and side scatter, including contaminating thrombocytes (Bohls et al., 2006). Data was analyzed with CellQuest Pro software (Becton Dickinson, New Jersey, USA).

Statistical analysis

The flow cytometry results were analyzed by two-way analysis of variance (ANOVA) for repeated measures with a Bonferroni *post hoc* test comparing all groups. All other data were analyzed by one way ANOVA with a Tukey *post hoc* test. TLC values were Log_{10} transformed. A significance level of $P < 0.05$ was considered for all tests. Analyses and graphs were made on GraphPad Prism 5 (GraphPad Software, Inc.).

RESULTS

Mycotoxin screening and clinical signs

Mycotoxin screening of experimental diets revealed non-

detectable levels of any mycotoxin in NC feed while the presence of FB1 (12000 µg/kg) and FB2 (5635 µg/kg) was identified in contaminated feed from both groups PC and AMA+PC, yielding for both groups a contamination level of approximately 17 ppm of FBs. No other mycotoxins were detected in contaminated feed. During the entire trial period, no abnormal behavior or clinical signs of toxicosis were detected in experimental birds.

Immune variables

Figure 1 summarizes the data obtained for each group using various cell markers. Most of the differences between groups regarding the peripheral blood leukocytes occurred on day 3 of age. On this day, statistically significant differences were found for 4 cell subsets. For the $CD4^+TCRV\beta1^-$ subset the control group was superior to PC ($P < 0.01$) with the AMA + PC group being intermediate between them (Figure 1b). The same pattern occurred for the $CD8^+CD28^-$ cells ($P < 0.01$) (Figure 1e). The control group also showed the highest percentage of circulating B lymphocytes, being statistically superior to both groups that received FBs (PC and AMA+PC) ($P < 0.001$) (Figure 1j). Groups that were challenged with FBs had the highest percentages of Kul^- MHC II^+ cells ($P < 0.01$ between NC and PC and $P < 0.05$ between NC and AMA+PC) (Figure 1i). The AMA+PC group also showed high percentages of $Kul^+MHC\ II^+$ on day 3, when it was statistically different from NC ($P < 0.05$) and PC ($P < 0.01$) groups (Figure 1g).

On day 7, three cellular subsets which were not altered on day 3, were significantly affected by treatments. The PC group showed high percentages of cells in all three cases. It was superior to NC for the $CD4^+TCRV\beta1^+$ subset ($P < 0.05$), while the AMA+PC group was close to the control group (Figure 1a). The PC group was superior to both control and AMA+PC groups for the $CD8^-CD28^+$ cellular subset ($P < 0.05$ against the control group and $P < 0.01$ for the AMA+PC group) (Figure 1f). For the $CD4^-TCRV\beta1^+$ subset of cells, both groups challenged with FBs (PC and AMA+PC) showed increased percentages of cells compared to NC group ($P < 0.05$ for both interactions) (Figure 1c). No statistically significant differences were found on day 14 between groups for the circulating cellular subsets. The $CD3^+$ cell count in the intestine was increased in the PC group compared to NC on day 7. No statistical differences were found on days 14 and 28 (Figure 1l).

Total leucocyte count was reduced in both PC and AMA+PC treated groups on day 14. However, after 28 days of exposure to contaminated feeds, AMA+PC birds showed mean TLC levels similar to both NC and PC groups, while levels from these latter differed significantly (Table 2).

Blood biochemical analysis, hematocrit and serum SA:SO

Total protein and Glb levels were increased in birds from AMA+PC group while the ratio Alb:Glb was reduced in both FBs fed groups on day 14. Alb levels were similar between groups at this point. On day 28, the same pattern was observed for TP while Alb levels were significantly increased in both FBs fed groups and Glb levels were not altered between groups. The ratio Alb:Glb was significantly increased in PC group when compared to NC, while values obtained for AMA+PC group were similar to levels of both NC and PC groups (Table 2). Activity of AST and UA levels were not affected by treatments. ALP activity was significantly reduced in group AMA+PC when compared to NC on day 14. At this time point, the PC group was similar to both groups. No differences were found between groups 28 days after housing. GGT mean activity was significantly increased in serum of both PC and AMA+PC groups after 28 days of exposure to experimental diets when compared to group NC. No differences were identified for this marker on day 14 (Table 2). Hematocrit was not affected by treatments on day 14. However, on day 28, Ht was significantly reduced on birds from PC group. Birds fed FBs contaminated feed and treated with AMA (AMA+PC group) showed Ht values similar to NC birds (Table 2). Serum SA:SO was significantly increased in birds from PC group at the end of the experimental period (28 days) when compared to those from the NC. The inclusion of AMA in contaminated feed (AMA+PC treated group) was able to alleviate such specific toxic effect of FBs, once mean SA:SO from this group was similar to both NC and FUM groups (Table 2).

DISCUSSION

In the present report, low doses of naturally occurring FBs were able to induce rapid immune changes in the peripheral blood leukocyte subsets of broiler chickens. Cytotoxic lymphocytes marked as $CD8^+CD28^-$ are terminally activated effector cells. These cells can be co-stimulated by IL-2 and IL-15, which are commonly reduced by FBs (Roberts et al., 2001). The presumptive reduction of the expression of such cytokines in the PC group possibly played a role in the reduction of the percentage of $CD8^+CD28^-$ cells in the current study, while the use of the AMA alleviated this effect. T lymphocytes, B lymphocytes (Waldmann, 1984) and monocytes also respond to IL-2 stimulation (Malkovsky et al., 1987). All of these cells were also affected by the presence of FBs in this study.

Fumonisins interfere with the formation of T lymphocytes directly in the thymus through an altered lipid metabolism (Martinova, 1997). In the present study,

significant differences in the amount of circulating T lymphocytes were observed in FBs exposed broiler chickens. Todorova et al. (2011) reported that lymphocytes of broiler chicken fed with FBs presented signals of a process of cell death. Li et al. (1999) reported diminished lymphocyte proliferation response to mitogens in chicks fed 200 mg FB1/kg diet. The effect of FBs on cellular proliferation and survival may explain some of the findings of the present study, such as the reduction of B-lymphocytes.

Fumonisins are reportedly toxic for chicken macrophages, leading to cellular vacuolation and nuclear disintegration (Qureshi and Hagler, 1992; Cheng et al., 2006), which is associated with a dose-dependent peritoneal macrophage death (Qureshi and Hagler, 1992), contributing to higher susceptibility to infections (Chatterjee et al., 1995). Feeding chickens with 15 ppm FBs, even lower than the 17 ppm studied here, resulted in diminished chemotaxis and phagocytosis (Cheng et al., 2006).

Among the mechanisms of FBs toxicity already discussed, the production of TNF-α is described to occur in a dose-dependent manner with relation to the toxins (Dugyala et al., 1998). This cytokine is necessary for the formation of dendritic cells, characterized by not expressing macrophage markers and being MHC II^+ (Cella et al., 1997). In the present work, these cells (Kul^- MHC II^+) were the only subset significantly increased in birds from both FBs fed groups on day 3. The production of TNF-α in the tissues, induced by FBs, is thought to collaborate to organs damage, such as liver, often reported in FBs intoxications (Dugyala et al., 1998; Tessari et al., 2006). Rauber et al. (2013) evaluated the toxicity of FB1 in broilers and reported increased relative weight of liver after 14 and 28 days of exposure to the toxin and also related it to liver damage.

The circulating $CD4^+TCRV\beta1^+$ cells followed the same trend as observed for intestinal $CD3^+$ cells in the present study. For both cellular subsets the PC group was statistically superior to the control on day 7, but not to the AMA+PC group. The $CD4^-TCRV\beta1^+$ subset was also increased on day 7 , showing that there is a preferential proliferation of cell lines dedicated to mucosal protection (Cihak et al., 1991; Lillehoj, 1994). Chickens fed 200 ppm of FBs for 21 days showed lower capacity to clear an *E. coli* infection (Li et al., 1999), for which it has already been shown that the $CD4^+TCRV\beta1^+$ subset is crucial (Filho et al., 2013). The potential damaging effects of FBs on the barrier protection of mucosae (Bouhet et al., 2004) and on the cellular membranes seems to facilitate the development of pathogenic intestinal colonization in pigs (Oswald et al., 2003), which may justify the increase in the percentage of the $CD4^+TCRV\beta1^+$ subset observed here. As observed for immune cells, blood biochemical variables were also impaired by the presence of FBs.

Andretta et al. (2012) found that TP, Alb and Glb were significantly reduced as a consequence of the presence of mycotoxins in feed. Regarding the effect of FBs some researchers have already reported an increase in TP and Alb levels (Sharma et al., 2008; Rauber et al., 2013), while in another study Alb levels were demonstrated to be significantly reduced due to the toxins (Espada et al., 1997). TP was hypothesized to be increased under FBs toxicosis due to increased levels of Alb in consequence of a higher vascular permeability (Rauber et al., 2013) or due to an increase in acute phase proteins probably caused by inflammatory processes (Espada et al., 1997; Sharma et al., 2008). “Espada et al. (1997) also suggested an inverse relationship between Alb synthesis and Glb (which may be increased in inflammatory processes).” In the present trial, Alb levels were increased only 28 days after FBs exposure while Glb levels were increased earlier. Taking into account the Alb:Glb ratio, there was an initial reduction in both FBs exposed groups at 14 days, probably caused by the presence of the toxins, and a subsequent significant increase in such ratio only in PC group, indicating that AMA was effective in alleviating the impact of the toxins in this ratio. Javed et al. (1995) also reported an increase in Alb:Glb ratio as a consequence of FBs exposure in broilers for 28 days.

Regarding enzyme activity in serum, no difference was observed for AST levels throughout the experimental period, which is in accordance to previous observations (Espada et al., 1994; Del Bianchi et al., 2005; Andretta et al., 2012). Activity of ALP was reduced in the group AMA+PC in comparison to NC at 14 days after exposure to experimental diets which was probably not influenced by the presence of FBs, once activity in PC group was similar to that of the NC group. Additionally, ALP activity in serum is expected to be increased at levels up to 54% when broilers are challenged with mycotoxins (Andretta et al., 2012). The reason for the relative reduction in ALP activity in group AMA+FUM is unclear and is probably unrelated to liver toxicity. Gamma-glutamyltransferase is another membrane bound enzyme whose activity in serum has been reported to be increased in broilers under FBs toxicosis (Espada et al., 1997; Rauber et al., 2013), probably as a consequence of bile cholestasis and duct hyperplasia (Del Bianchi et al., 2005). In the present evaluation, GGT was significantly increased in both FBs fed groups after 28 days of exposure, probably due to the action of FBs. Hematocrit was significantly reduced in birds from PC group after 28 days of exposure to experimental diets. Javed et al. (1995) also characterized such effect. The same reduction was not observed in group AMA+PC, indicating that the AMA addition could protect birds from this effect caused by contaminated diets. Total leukocyte count were also reduced in birds fed contaminated diets after 14 days of exposure, which is likely linked to the presence of FBs, having already being

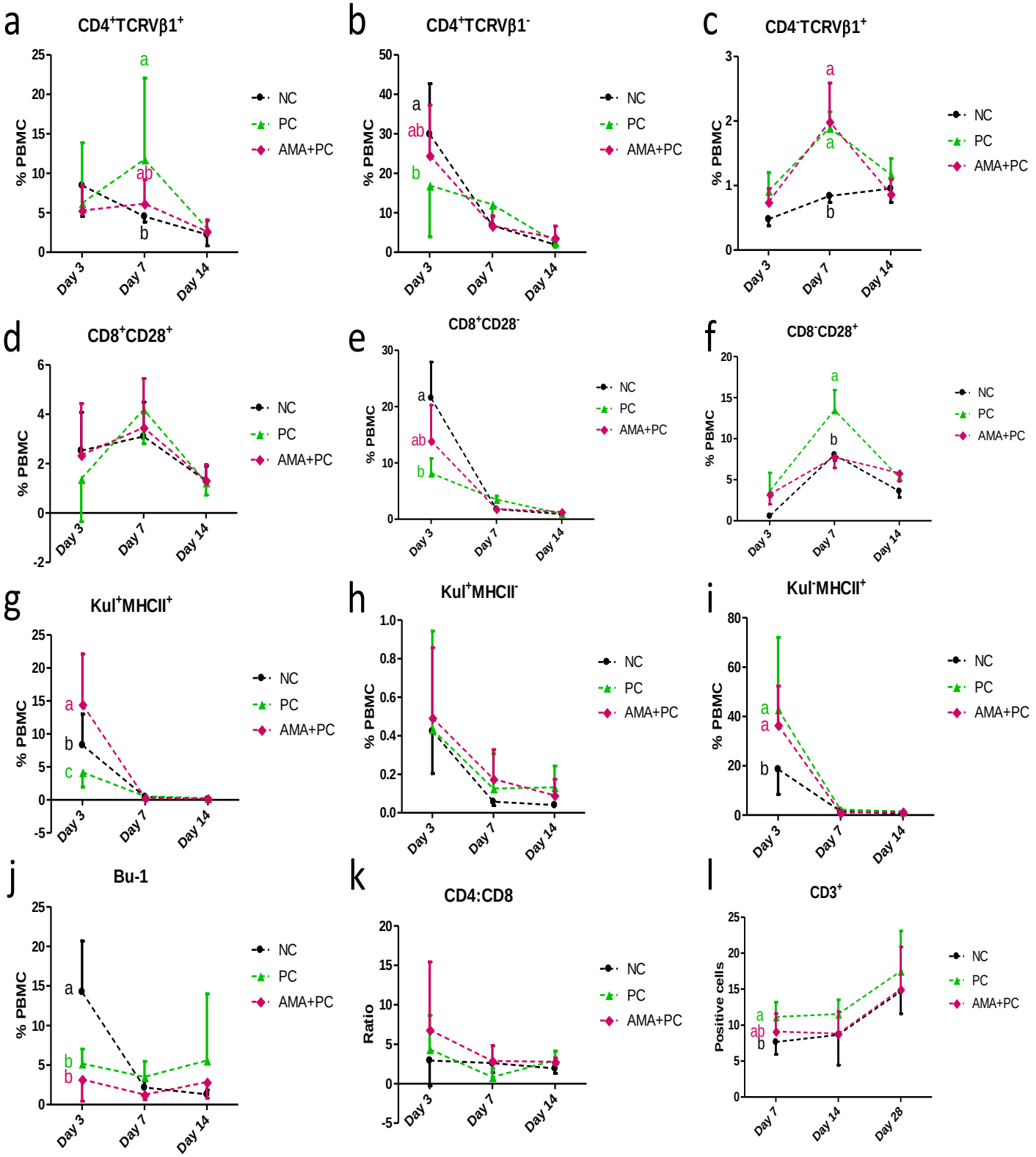

Figure 1. Percentages of cells of peripheral blood lymphocytes of chickens submitted to fumonisin challenge (PC) and treated with anti-mycotoxin additive (AMA+PC). NC is the control group. [a, b, c] Statistically significant differences ($P < 0.05$) between groups. For the CD4:CD8 variable, the y-axis indicates a ratio between these cell subsets.
Data is presented as mean±SD.

Table 2. Blood (biochemical, hematological, and SA:SO) variables (means±S.D.) measured in different treatments after feeding broilers with naturally contaminated FBs (PC and PC+FUM) and control (NC) diets for 14 and 28 days.

Blood variable	14 days			28days		
	NC	PC	AMA+PC	NC	PC	AMA+PC
TP (g/dl)	2.17 ± 0.2^{B}	2.43 ± 0.13^{AB}	2.57 ± 0.3^{A}	2.47 ± 0.15^{b}	2.65 ± 0.23^{ab}	2.75 ± 0.26^{a}
Alb (g/dl)	1.27 ± 0.14^{a}	1.35 ± 0.08^{a}	1.42 ± 0.2^{a}	1.44 ± 0.1^{B}	1.63 ± 0.13^{A}	1.67 ± 0.16^{A}
Glb (g/dl)	0.9 ± 0.08^{B}	1.08 ± 0.09^{A}	1.15 ± 0.11^{A}	1.03 ± 0.08	1.03 ± 0.12	1.09 ± 0.13
Alb:Glbratio	1.41 ± 0.11^{A}	1.27 ± 0.12^{B}	1.23 ± 0.1^{B}	$1.41 + 0.1^{a}$	1.6 ± 0.15^{b}	1.54 ± 0.13^{ab}
AST (U)	174.08 ± 28.43^{a}	152.54 ± 27.9^{a}	145.54 ± 21.46^{a}	196 ± 15.25^{a}	214.24 ± 22.76^{a}	209.7 ± 26.97^{a}
GGT (U)	4.38 ± 1.63^{a}	5.54 ± 1.32^{a}	3.7 ± 1.93^{a}	5.43 ± 0.86^{B}	8.15 ± 1.58^{A}	7.94 ± 2.39^{A}
ALP (Ux1000)	6.29 ± 1.74^{A}	5.69 ± 1.24^{AB}	4.18 ± 0.96^{B}	2.5 ± 0.38^{a}	2.53 ± 0.64^{a}	2.43 ± 0.48^{a}
UA(mg/dl)	5.32 ± 1.39^{a}	5.29 ± 2.14^{a}	6.14 ± 2.61^{a}	7.08 ± 1.87^{a}	5.88 ± 3.25^{a}	4.33 ± 1.37^{a}
Ht (%)	30.4 ± 2.08^{a}	31.72 ± 2.22^{a}	31.13 ± 2.95^{a}	33 ± 1.61^{A}	29.88 ± 2.54^{B}	34.72 ± 2.82^{A}
TLC (Log_{10}/µl)	4.16 ± 0.13^{A}	3.78 ± 0.19^{B}	3.74 ± 0.23^{B}	4.03 ± 0.17^{A}	3.68 ± 0.35^{B}	3.84 ± 0.23^{AB}
SA:SO	-	-	-	1.12 ± 0.61^{B}	2.01 ± 0.25^{A}	1.55 ± 0.54^{AB}

Means bearing a similar letter within a row at the same sampling point do not differ significantly as determined by one-way ANOVA and Tukey's test (a,b $P \leq 0.05$), (A,B $P \leq 0.01$).

being described by Javed et al. (1995). After 28 days of exposure, birds from PC group remained with low TLC means in comparison to NC birds while birds from AMA+PC showed TLC means similar to the latter, indicating a protective effect of AMA when added to contaminated diets.

Although already reported in literature, the cause of white and red blood cells reduction due to FBs intake is not fully understood and may be a consequence of indirect cell toxicity caused by the accumulation of sphingoid bases as well as the depletion of ceramide and complex sphingolipids. The inhibition of sphingolipid biosynthesis is a direct effect of FBs, which leads to the elevation of free sphingoid bases in serum. Such elevation has been proposed to be a functional biomarker for exposure to FBs (Piva et al., 2005). Indeed, increased serum levels of sphingoid bases and SA:SO in broilers exposed to FBs at levels starting from 80 mg of FBs/kg of diet have already been reported (Henry et al., 2000; Rauber et al., 2013). In the present evaluation, birds from PC group showed increased serum levels of SA:SO in comparison to those fed the control diet, even though the contamination level was much lower than those predicted to induce differences in this ratio. Birds fed FBs contaminated ration treated with AMA showed SA:SO levels similar to both NC and PC groups, indicating a protective effect of the product as viewed through this specific biomarker. These results suggest that levels of 17 ppm of FBs can also induce specific signs of toxicity in broiler chickens.

The protective effect observed in birds from AMA+PC group in the present trial may be linked to the capacity of the product to bind and inactivate FBs in the gastrointestinal tract of the birds, according to the AMA's declared composition. Jard et al. (2011) have extensively reviewed the mechanisms of action and efficacy of several materials used to decrease availability of toxins by adsorption and stated that clay minerals have shown variable efficacy when tested *in vivo* against toxins other than aflatoxin B1. The authors suggested that organic binders would be more effective against other toxins like FBs and that combination of both clay minerals and organic binders may be more effective tools to counteract mycotoxin problems *in vivo*. It has been shown that a standard mixture of adsorbing agents based on carbon and aluminosilicates was able to reduce the bioaccessibility of FB1 and other toxins in a specific *in vitro* gastrointestinal model (Avantaggiato et al., 2007). Results obtained

in the current evaluation for the SA/SO ratio in AMA+PC birds may also suggest a lower bioavailability of FBs, once this biomarker has been directly linked to FBs absorption (Solfrizzo et al., 2001).

Conclusion

Feeding broiler chickens with 17 ppm FBs naturally contaminated ration during 28 days from hatch significantly altered the profile of circulating leucocytes after three and seven days since provision of contaminated feed, as well as increased the number of $CD3^+$ cells in jejunum after 7 days. The addition of an AMA was able to revert FBs induced alterations for most of the studied cells, alleviating the impact of the toxins. After 14 and 28 days, FBs contaminated diets altered levels of Alb and Glb and Alb:Glb, as well as increased GGT serum activity (28 days), decreased TLC (14 and 28 days) and Ht values (28 days), and led to an increase in the SA:SO. Most of these effects were partially reverted by the addition of an AMA to contaminated diets. These findings suggest that FBs may be toxic for broilers at dosages as low as 17 ppm, altering the functions of different systems. Even though it was not expected that such low doses could affect the production parameters, the use of sensitive markers was able to demonstrate the toxic effects of low doses of FBs. The addition of a commercial AMA was able to alleviate toxicity of naturally contaminated diets in broiler chickens. Since this level of feed contamination is presently considered to be below the limit threshold by regulating agencies, the use of AMA may be recommended even in feed that has been tested for FB and approved for animal consumption.

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Conflict of interest

The first author is affiliated to Impextraco Latin America.

REFERENCES

Andretta I, Kipper M, Lehnen CR, Lovatto PA (2012). Meta-analysis of the relationship of mycotoxins with biochemical and hematological parameters in broilers. Poult. Sci. 91:376-82.

Avantaggiato G, Havenaar R, Visconti A (2007). Assessment of the multi-mycotoxin-binding efficacy of a carbon/aluminosilicate-based product in an in vitro gastrointestinal model. J. Agric. Food Chem. 55: 4810-4819.

Avantaggiato G, Solfrizzo M, Visconti A (2005). Recent advances on the use of adsorbent materials for detoxification of Fusarium mycotoxins. Food Addit. Contam. 22:379-388.

Beirão BCB, Fávaro Jr C, Nakao LS, Caron LF, Zanata SM, Mercadante AF (2012). Flow cytometric immune profiling of specific-pathogen-free chickens before and after infectious challenges. Vet. Immunol. Immunopathol. 145:32-41.

Bohls RL, Smith R, Ferro PJ, Silvy NJ, Li Z, Collisson EW (2006). The use of flow cytometry to discriminate avian lymphocytes from contaminating thrombocytes. Dev. Comp. Immunol. 30:843-850.

Bouhet S, Hourcade E, Loiseau N, Fikry A, Martinez S, Roselli M, Galtier P, Mengheri E, Oswald IP (2004). The mycotoxin fumonisin B1 alters the proliferation and the barrier function of porcine intestinal epithelial cells. Toxicol. Sci. 77:165-171.

Bridle BW, Julian R, Shewen PE, Vaillancourt JP, Kaushik AK (2006). T lymphocyte subpopulations diverge in commercially raised chickens. Can. J. Vet. Res. 70:183-190.

Cella M, Sallusto F, Lanzavecchia A (1997). Origin, maturation and antigen presenting function of dendritic cells. Curr. Opin. Immunol. 9:10–16.

Chan MM, Chen CL, Ager LL, Cooper MD (1988). Identification of the avian homologues of mammalian CD4 and CD8 antigens. J. Immunol. 140:2133-2138.

Chatterjee D, Mukherjee SK, Dey A (1995). Nuclear disintegration in chicken peritoneal macrophages exposed to fumonisin B1 from Indian maize. Lett. Appl. Microbiol. 20:184-185.

Cheng Y-H, Ding S-T, Chang M-H (2006). Effect of fumonisins on macrophage immune functions and gene expression of cytokines in broilers. Arch. Anim. Nutr. 60:267–276.

Cihak J, Hoffmann-Fezer G, Ziegler-Heibrock HW, Stein H, Kaspers B, Chen CH, Cooper MD, Losch U (1991). T cells expressing the V beta 1 T-cell receptor are required for IgA production in the chicken. Proc. Natl. Acad. Sci. USA 88:10951-10955.

Del Bianchi M, Oliveira CA, Albuquerque R, Guerra JL, Correa B (2005). Effects of prolonged oral administration of aflatoxin B1 and fumonisin B1 in broiler chickens. Poult. Sci. 84:1835-1840.

Dersjant-Li Y, Verstegen MW, Gerrits WJJ (2003). The impact of low concentrations of aflatoxin, deoxynivalenol or fumonisin in diets on growing pigs and poultry. Nutr. Res. Rev. 16:223–39.

Diaz GJ, Boermans HJ (1994). Fumonisin toxicosis in domestic animals: a review. Vet. Hum. Toxicol. 36:548.

Dugyala RR, Sharma RP, Tsunoda M, Riley RT (1998). Tumor Necrosis Factor-α as a Contributor in Fumonisin B1 Toxicity. J. Pharmacol. Exp. Ther. 285:317-324.

Espada Y, Ruiz de Gopegui R, Cuadradas C, Cabañes FJ (1994). Fumonisin mycotoxicosis in broilers. Weights and serum chemistry modifications. Avian Dis. 38:45460.

Espada Y, Ruiz de Gopegui R, Cuadradas C, Cabañes FJ (1997). Fumonisin mycotoxicosis in broilers: plasma proteins and coagulation modifications. Avian Dis. 41:73-9.

Feldman BF, Zinkl JG, Schalm OW (2006). Schalm`s veterinary hematology. 5th ed. Wiley-Blackwell, Iowa.

Filho TF, Fávaro Jr C, Ingberman M, Beirão BCB, Inoue A, Gomes L, Caron LF (2013). Effect of Spray Escherichia coli Vaccine on the Immunity of Poultry. Avian Dis. 57:671-676.

Fushimi Y, Takagi M, Hasunuma H, Uno S, Kokushi E, Watanabe U, Liu J, Marey MA, Miyamoto A, Otoi T (2014). Application of mycotoxin adsorbent to cattle feed contaminated with zearalenone: urinary zearalenone excretion and association with anti-Müllerian hormone. World Mycotoxin J. 7:367-378.

Grenier B, Applegate TJ (2013). Modulation of intestinal functions following mycotoxin ingestion: meta-analysis of published experiments in animals. Toxins (Basel). 5:396-430.

Hala K, Boyd R, Wick G (1981). Chicken major histocompatibility complex and disease. Scand. J. Immunol. 14:607-616.

Henry MH, Wyatt RD, Fletchert OJ (2000). The toxicity of purified fumonisin B1 in broiler chicks. Poult. Sci. 79:1378-1384.

Jard G, Liboz T, Mathieu F, Guyonvarc'h A, Lebrihi A (2011). Review of mycotoxin reduction in food and feed: from prevention in the field to detoxification by adsorption or transformation. Food Addit. Contam. Part A 28:1590-1609.

Javed T, Dombrink-Kurtzman M , Richard JL, Bennett G, Cote LM,

Buck WB (1995). Serohematologic alterations in broiler chicks on feed amended with *Fusarium proliferatum* culture material or fumonisin B1 and moniliformin. J. Vet. Diagnostic Investig. 7:520-526.
Kubena LF, Edrington TS, Kamps-Holtzapple C, Harvey RB, Elissalde MH, Rottinghaus GE (1995). Effects of feeding fumonisin B1 present in fusarium moniliforme culture material and aflatoxin singly and in combination to turkey poults. Poult. Sci. 74:1295-1303.
Lahti JM, Chen CL, Tjoelker LW, Pickel JM, Schat KA, Calnek BW, Thompson CB, Cooper MD (1991). Two distinct alpha beta T-cell lineages can be distinguished by the differential usage of T-cell receptor V beta gene segments. Proc. Natl. Acad. Sci. USA 88:10956-10960.
Li YC, Ledoux DR, Bermudez AJ, Fritsche KL, Rottinghaus GE (1999). Effects of fumonisin B1 on selected immune responses in broiler chicks. Poult. Sci. 78:1275-1282.
Lillehoj HS (1994). Analysis of *Eimeria acervulina*-induced changes in the intestinal T lymphocyte subpopulations in two chicken strains showing different levels of susceptibility to coccidiosis. Res. Vet. Sci. 56:1-7.
Linsley PS, Ledbetter JA (1993). The role of the CD28 receptor during T cell responses to antigen. Annu. Rev. Immunol. 11:191-212.
Luhtala M, Tregaskes CA, Young JR, Vainio O (1997). Polymorphism of chicken CD8-alpha, but not CD8-beta. Immunogenetics 46:396-401.
Magnoli CE, Saenz MA, Chiacchiera SM, Dalcero AM (1999). Natural occurrence of *Fusarium* species and fumonisin-production by toxigenic strains isolated from poultry feeds in Argentina. Mycopathologia 145:35-41.
Malkovsky M, Loveland B, North M, Asherson GL, Gao L, Ward P, Fiers W (1987). Recombinant interleukin-2 directly augments the cytotoxicity of human monocytes. Nature 325:262-265.
Martinova EA (1997). Functional disruption of the immune system by mycotoxin fumonisin B1. Toxicon 35:497.
Merrill Jr AH, Sullards MC, Wang E, Voss KA, Riley RT (2001). Sphingolipid metabolism: roles in signal transduction and disruption by fumonisins. Environ. Health Perspect. 109:283.
Nabeshima S, Murata M, Kikuchi K, Ikematsu H, Kashiwagi S, Hayashi J (2002). A reduction in the number of peripheral CD28+CD8+T cells in the acute phase of influenza. Clin. Exp. Immunol. 128:339-346.
Oliveira GR, Ribeiro JM, Fraga ME, Cavaglieri LR, Direito GM, Keller KM, Dalcero AM, Rosa C a (2006). Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. Mycopathologia 162:355-62.
Oswald IP, Desautels C, Laffitte J, Fournout S, Peres SY, Odin M, Le Bars P, Le Bars J, Fairbrother JM (2003). Mycotoxin fumonisin B1 increases intestinal colonization by pathogenic Escherichia coli in pigs. Appl. Environ. Microbiol. 69:5870-5874.
Pawelec G, Akbar A, Caruso C, Effros R, Grubeck-Loebenstein B, Wikby A (2004). Is immunosenescence infectious? Trends Immunol. 25:406-410.
Piva A, Casadei G, Pagliuca G, Cabassi E, Galvano F, Solfrizzo M, Riley RT, Diaz DE (2005). Activated carbon does not prevent the toxicity of culture material containing fumonisinB1 when fed to weanling piglets. J. Anim. Sci. 83:1939-1947.
Qureshi MA, Hagler WM (1992). Effect of Fumonisin-B1 Exposure on Chicken Macrophage Functions in vitro. Poult. Sci. 71:104-112.
Ramos A-J, Fink-Gremmels J, Hernández E (1996). Prevention of toxic effects of mycotoxins by means of nonnutritive adsorbent compounds. J. Food Prot. 59:631-641.
Rauber RH, Dilkin P, Mallmann AO, Marchioro A, Mallmann CA, Borsoi A, Nascimento VP (2012). Individual and combined effects of Salmonella Typhimurium lipopolysaccharide and fumonisin B1 in broiler chickens. Poult. Sci. 91:2785-2791.
Rauber RH, Oliveira MS, Mallmann AO, Dilkin P, Mallmann CA, Giacomini LZ, Nascimento VP (2013). Effects of fumonisin B1 on selected biological responses and performance of broiler chickens. Pesqui. Vet. Bras. 33:1081-1086.
Roberts AI, Lee L, Schwarz E, Groh V, Spies T, Ebert EC, Jabri B (2001). Cutting Edge: NKG2D Receptors Induced by IL-15 Costimulate CD28-Negative Effector CTL in the Tissue Microenvironment. J. Immunol. 167:5527-5530.
Roohi S, Gholampour Azizi I, Hashemi M (2012). Fumonisin contamination based on flour quality used in bakeries and confectioneries in Qaemshahr (city of the Northern Iran). Afr. J. Microbiol. Res. 6:1815-1818.
Schatzmayr G, Streit E (2013). Global occurrence of mycotoxins in the food and feed chain: facts and figures. World Mycotoxin J. 6:213-222.
Sharma D, Asrani RK, Ledoux DR, Jindal N, Rottinghaus GE, Gupta VK (2008). Individual and combined effects of fumonisin B1 and moniliformin on clinicopathological and cell-mediated immune response in Japanese quail. Poult. Sci. 87:1039-51.
Shetty P, Bhat R (1997). Natural occurrence of fumonisin B1 and its co-occurrence with aflatoxin B1 in Indian sorghum, maize, and poultry feeds. J. Agric. Food Chem. 2170-2173.
Solfrizzo M, Visconti A, Avantaggiato G, Torres A, Chulze S (2001). *In vitro* and in vivo studies to assess the effectiveness of cholestyramine as a binding agent for fumonisins. Mycopathologia 151:147-153.
Souza M de LM de, Sulyok M, Freitas-Silva O, Costa SS, Brabet C, Machinski Junior M, Sekiyama BL, Vargas EA, Krska R, Schuhmacher R (2013). Co-occurrence of mycotoxins in maize and poultry feeds from Brazil by liquid chromatography/tandem mass spectrometry. Sci. World J. 2013.
Swamy H, Smith TK, MacDonald EJ, Boermans HJ, Squires EJ (2002). Effects of feeding a blend of grains naturally contaminated with Fusarium mycotoxins on swine performance, brain regional neurochemistry, and serum chemistry and the efficacy of a polymeric glucomannan mycotoxin adsorbent. J. Anim. Sci. 80:3257-3267.
Tessari ENC, Oliveira CAF, Cardoso ALSP, Ledoux DR, Rottinghaus GE (2006). Effects of aflatoxin B1 and fumonisin B1 on body weight, antibody titres and histology of broiler chicks. Br. Poult. Sci. 47:357–364.
Todorova KS, Kril AI, Dimitrov PS, Gardeva EG, Toshkova RA, Tasheva YR, Petrichev MH, Russev RV (2011). Effect of fumonisin B1 on lymphatic organs in broiler chickens-pathomorphology. Bull. Vet. Inst. Pulawy 55:801-805.
Tregaskes CA, Kong FK, Paramithiotis E, Chen CL, Ratcliffe MJ, Davison TF, Young JR (1995). Identification and analysis of the expression of CD8 alpha beta and CD8 alpha alpha isoforms in chickens reveals a major TCR-gamma delta CD8 alpha beta subset of intestinal intraepithelial lymphocytes. J. Immunol. 154: 4485–4494.
Varga J, Tóth B (2005). Novel strategies to control mycotoxins in feeds: A review. Acta Vet. Hung. 53:189-203.
Voss K, Smith GW, Haschek WM (2007). Fumonisins: Toxicokinetics, mechanism of action and toxicity. Anim. Feed Sci. Technol. 137:299-325.
Waldmann TA (1984). Expression of interleukin 2 receptors on activated human B cells. J. Exp. Med. 160:1450-1466.
Weibking TS, Ledoux DR, Bermudez AJ, Turk JR, Rottinghaus GE, Wang E, Merrill AH (1993). Effects of feeding *Fusarium moniliforme* culture material, containing known levels of fumonisin B1, on the young broiler chick. Poult. Sci. 72:456-466.

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