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THE NUMBER OF CD 3 CELLS IN BROILER INTESTINES AFTER THE ADMINISTRATION OF AFLATOXIN AND ZEOLITES

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The density of CD3+ cells was evaluated in the lamina propria of the intestines in chickens after application of aflatoxin and two kinds of zeolites for 30 days. Analysis of CD3+ cells showed a significantly increased number of T lymphocytes after the application of both sorbents. There was no increase in the number of examined cells after the application of aflatoxin B_1.The possible role of damage to the bacterial biofilm by sorbents is discussed.

Key words: aflatoxins, zeolites, CD3 positive cells, T lymphocytes, intestine

INTRODUCTION

Aflatoxins produced by fungi of the *Aspergillus* genus, most often *Aspergillus flavus* and *Aspergillus parasiticus,* belong to the bisfuran-cumarin chemical group,. Aflatoxin B_1 (AFB_1) is the most toxic; its high toxicity occuring after bioactivation by lipid hydroperoxide-dependent mechanisms (Massey et al., 1995). At present, one of the more promising and practical approaches to prophylaxis and therapy is the use of adsorbents. Experiments with compounds having sorption properties, have been conducted in order to decrease or remove the negative effects of aflatoxin on the animal body. Selected adsorbents added to aflatoxin-contaminated feeds can detach aflatoxins during the digestive process, allowing the mycotoxin to pass harmlessly through the animal (Davidson et al., 1987; Philips et al., 1990).

Host responses to the intestinal elimination of pathogens involve an immunological reaction on the part of the gut-associated lymphoid tissues. Lymphocyte aggregates in the intra-epithelium (intra-epithelial lymphocytes - IEL) and in the lamina propria (lamina propria lymphocytes - LPL) of the intestinal wall are important components of this tissue. The IEL consists mainly of T cells, whereas the LPL contains many immunoglobulin-producing B cells (Hoggen-mueller et al., 1993).

A subpopulation of lymphocytes can be studied by using monoclonal antibodies (MoAb) and polyclonal antibodies (PoAbs) against antigens included in the systemic nomenclature CD system (cluster of differentiation) (Levkutova and Levkut, 1992; Levkut et al., 1994). MòAb techniques have shown that CD3

cells are present both in the epithelium and lamina propria of the chicken intestine (Lillehoj and Chung, 1992). At present, few antibodies can be used in formalin-fixed and paraffin-embedded tissue. A CD3 polyclonal antibody is commercially available and has been successfully employed under different pathological conditions in several animal species (Ramos-Vara et al., 1994; Levkut et al., 1995; Ševčikova, 1997).

The aim of the work was to observe the effect of aflatoxin and sorbents on the occurrence of CD3 cells in the intestine of chickens. No data concerning the quantification of immunocompetent cells after the application of adsorbents were found in the available literature.

MATERIAL AND METHODS

Animals. Thirty-six 18-day-old Arbor Acress hybrid chicks were divided into 6 groups of 6 birds each.

The animals were given AFB_1 in a physiological solution using a probe in a dose of 0.5 $\mu g.kg^{-1}$ from day 18 to 49 of age. (Table 1). The feed mixture for broilers (BR 2; Feed mixture plant Čecejovce) was supplemented with two kinds of zeolites: 0.5% naturally ground (clinoptilolite) and 0.5% thermally processed (Chepalit T). The birds were allowed access to feed and water ad libitum.

Table 1. Experimental design

	control	clinoptilolite	chepalit T	aflatoxin B1	AFB_1 + clinoptilolite	AFB_1 + chep.T
amount of substances		0.5%	0.5%	0.5 $\mu g.kg^{-1}$	0.5 $\mu g.kg^{-1}$ + 0.5%	0.5 $\mu g.kg^{-1}$ + 0.5%
mode of application		*ad libitum* in feed mixture	*ad libitum* in feed mixture	by probe	by probe + feed mixture	by probe + feed mixture

chepalit T - thermically treated clinoptilolite

Histopathological examination. Chickens were killed by cervical dislocation at the age of 49 days. Samples removed from the duodenum of each chicken were processed using a standard procedure, including fixing in 10% neutral formalin, and embedding in paraffin wax. For histological examination, sections 5 to 6 μm thich were stained with heamatoxylin-eosin.

Immunohistochemistry. Tissue sections for immunohistochemical examination were placed on coated slides and deparaffinized in xylene (2x10 min), 96 % benzylalcohol (2x8 to 10 min) and 70% ethyl alcohol (5 min). After inhibition of the endogenous peroxidase activity in 3% H_2O_2 the sections were washed in distilled water and digested in 0.4% pepsin in 0.01 N HCl. Sections were kept at 37°C for 30 min and washed in distilled water. Sections were incubated with the primary antibody for 14 to 16 h at 4 °C. Polyclonal rabbit anti-human T cell CD3

antibody against synthetic human CD3 epsilon chain was used in a dilution of 1:300 (Dakopatts, Glostrup, Denmark). Secondary antiserum (anti-rabbit origin, Biogenex Laboratories, San Ramon, California, USA) and peroxidase -antiperoxidase (PAP) complex were incubated at room temperature for 30 min. Biotin from secondary antiserum reacts with the avidin from the peroxidase-antiperoxidase complex. The reaction was developed using a diaminobenzidine (DAB) derivative.

Mode of evaluation of T lymphocytes. Lymphocytes in the duodenum were counted using the Meopta micrometer system, which consists of ocular and objective micrometers. Both are graduated with 100 divisions and 1 division of the objective represents 10 μm. The coefficient was calculated thus: *x* parts of the objective (x 10) are equivalent to *y* parts of the ocular micrometer. In our case 10 divisions of the objective were equivalent to 40 divisions of the ocular micrometer, 100:40 = 2.5 μm. One division of the ocular micrometer thus represented 2.5 μm. The areas of the villus epithelium and lamina propria of the duodenum, from the lamina muscularis mucosae to the point of the villi in sections equivalent to 40 divisions of the ocular micrometer were used for counting CD3 positive cells.

RESULTS AND DISCUSSION

Feed supplemented with sorbents and aflatoxin did not cause any histological changes in the intestinal mucosa of poultry. The zeolites, used in our experiments as sorbents contained the mineral clinoptilolite and can absorb various compounds such as mycotoxins, into the pores of their crystalline structure and allow them to pass harmlessly through the animal (Ledoux et al., 1999). It is known that there is a high concentration of lymphoid cells in the intestine of healthy chickens. In some instances, it has been assumed that the increased presence of T lymphocytes signifies some form of immune response (Ross, 1994).

Evaluation of CD3+ cells in the lamina propria of the intestine demonstrated significant changes of the number of these cells in the groups given the sorbents compared with the control group (Figs.1, 2 and 3). Analysis of CD3+ cells in poultry shows that there are three mutually exclusive sublineages of CD3+ cells

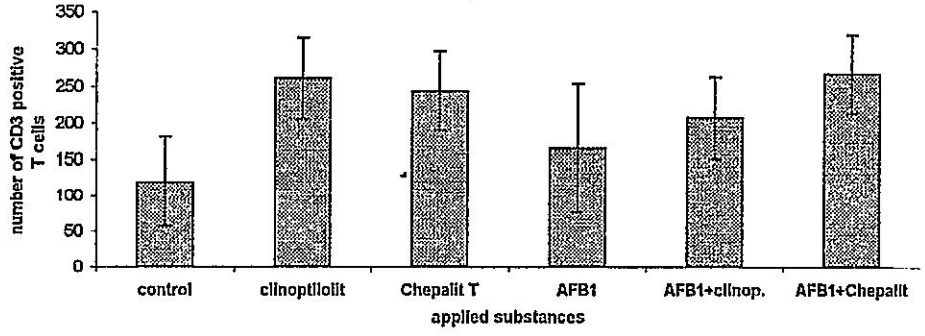

Fig.1. Number of CD3-positive cells in the duodenum of broiler chickens

recognized by the T cell receptor TCR1 (Sowder et al., 1988), TCR2 (Cihak et al., 1988) and TCR3 (Char et al., 1989). The intestine is mostly infiltrated by TCR1+ and TCR2+ cells which express CD4 or CD8 antigens (Chen et al., 1988). Haematological parameters of the peripheral blood are not changed after *per os* application of zeolites in broiler chickens (Kececi et al., 1998).

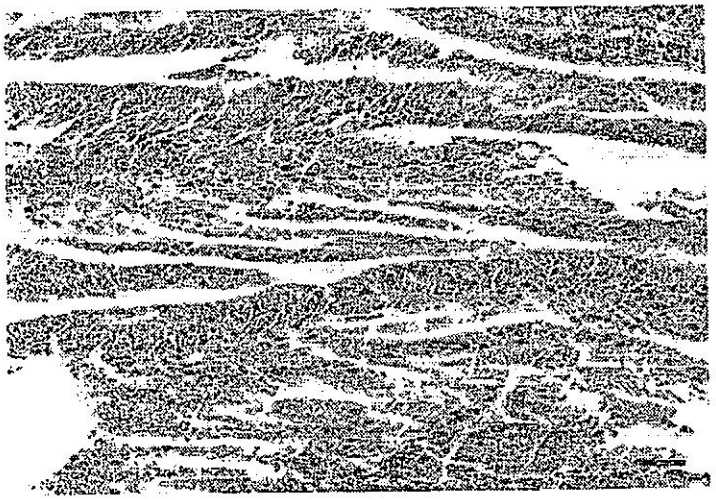

Figure 2. The duode[chickens showingùy of the intra-epithelial and lamina propria T lymphocytes. Labelling with rabbit CD 3 polyclonal antibody (bar = 5 μm).

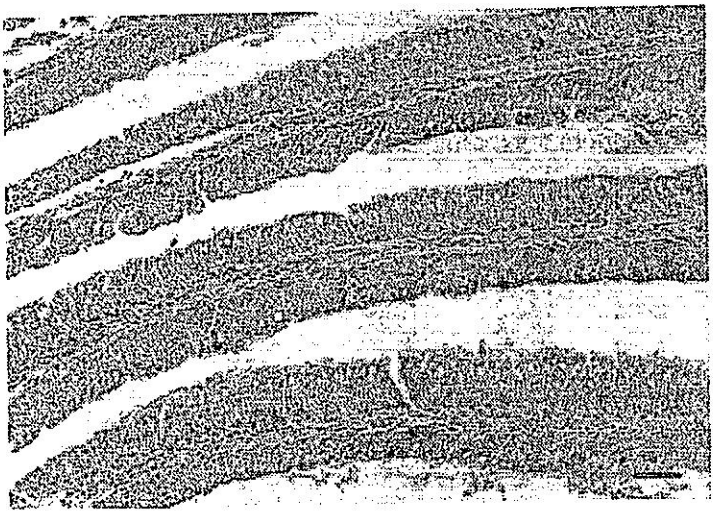

Figure 3 The duodenum of control chickens with the intra-epithelial and lamina propria T lymphocytes. labelling by rabbit CD3 polyclonal antibody (bar = 5 μm).

The structure of inorganic sorbents (Shah et al., 1996) suggests that they will not be immunogenic in the digestive tract. Birds consume little water in comparison to mammals. Using dehydrated sorbents causes hydration of these substances in the digestive tract. Absorption of water into the crystalline structure of sorbents in the lumen of the intestine of poultry suggests that it might affect the circulatory system of water - filled channels in the mucosae (Stryer, 1988). It is questionable as to what degree a bacterial biofilm could be impaired, followed by the structure of cellular components. Changes affecting both the resorption capability and the structure of immunocompetent cells could be partially related to the differences in the utilization of nutrients in the digestive tract after the application of sorbents (Pond and Yen, 1982).

The application of feed with aflatoxins did not cause any significant changes in the number of CD3+ cells compared to the control group (Fig. 1). Aflatoxin B_1 has proved to be a potent hepatotoxic and carcinogenic agent (Newberne, 1973). Moreover, previous studies in chickens have demonstrated changes in haematological parameters in peripheral blood after the ingestion of low and repeated doses of aflatoxin. A dose-related decrease in the activity of B and T cells has been observed in mice (Reddy et al., 1987). Aflatoxin B_1, however, is effective in the organism only after bioactivation in the liver. It impairs DNA-dependent RNA polymerase activity and inhibits RNA and protein synthesis (Corner, 1991). Therefore, it may be supposed that changes were not observed in the number of mucous lymphocytes after the long-term application of aflatoxin B_1.

In conclusion it can be stated that long-term peroral administration of two kinds of sorbents caused an increase in the number of CD3+ cells in the lamina propria in the chicken duodenum. Peroral administration of aflatoxin B_1 did not cause significant changes in the number of CD3+ lymphocytes. The increase in the number of observed cells could be connected with the effect on the circulating system of water-filled channels in the mucosae and the subsequent composition of cellular components.

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BROJ CD 3 ĆELIJA U CREVIMA BROJLERA POSLE APLIKACIJE AFLATOKSINA I ZEOLITA

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SADRŽAJ

U ovom radu su izneti rezultati ispitivanja zastupljenosti CD 3+ ćelija u lamini propriji creva brojlerskih pilića posle aplikacije aflatoksina i dve vrste zeolita tokom 30 dana. Analiza prisustva CD 3+ ćelija je pokazala da se njihov broj značajno povećava posle aplikacije obe vrste adsorbenata. Posle aplikacije aflatoksina B 1 nije bilo povećanja broja ispitivanih ćelija. U radu se razmatra moguće oštećenje bakterijskog biofilma posle aplikacije adsorbenata.