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Original Research Article

EFFECTS OF FEEDING GRAINS NATURALLY CONTAMINATED WITH *Fusarium* TOXINS ON SELECTED HAEMATOLOGICAL PARAMETERS AND LYMPHOCYTE SUBSETS IN PRIMIPAROUS SOWS

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Abstract: In a field experiment (54±1 days), 20 primiparous sows (day 89±2 of gestation) were randomly divided into two equal groups. The sows were fed diets naturally containing 0.3 mg/kg of the mycotoxin deoxynivalenol (DON) for the control group and 5.1 mg/kg DON, 0.1 mg/kg zearalenone and 21.6 mg/kg fusaric acid for the experimental group. In the control group, the concentrations of zearalenone and fusaric acid were under detection limit. The sows from the experimental group consumed significantly less feed during gestation (P=0.002), during lactation (P=0.027) and in the weaning to oestrus interval (P<0.001) than control sows. Blood samples were taken four times during the experiment (day 0, 17, 42 and 52). There were no differences in total and differential blood leukocyte count, with the exception of neutrophils on day 52, which reached 42.22±9.02% in the experimental group and 32.10±10.65% in the control group (P=0.040). Flow cytometric analysis of peripheral blood T lymphocytes with monoclonal antibodies against CD3, CD4 and CD8 revealed the percent of both $CD3^+CD4^+$ and $CD3^+CD8^+$ cells. We calculated the absolute number of $CD3^+CD4^+$ and $CD3^+CD8^+$ cells (10^9/L), which showed a decreasing trend in the experimental group, with 2.40±0.63 and 3.83±1.15, respectively, at the beginning and 1.78±0.39 and 2.74±0.89, respectively, at the end of experiment. In the control group, these values were 2.19±0.70 and 3.41±0.87 and finally 2.12±0.69 and 3.11±1.12 x 10^9/L, respectively. The obtained results suggest that feed naturally contaminated with Fusarium toxins reduces the feed intake, influences the neutrophil count, and has immunomodulatory effect on T lymphocyte numbers.

Key words: leukocytes; T lymphocytes; mycotoxins; deoxynivalenol; flow cytometry; sow

Introduction

Trichothecene mycotoxins are a group of over 200 structurally related compounds produced primarily by *Fusarium* species and related fungi. Most studies of trichothecenes have examined deoxynivalenol (DON, vomitoxin) (1). Although DON is not as toxic as other trichothecenes, such as T-2 toxin, HT-2 toxin, or fusarenon-X, it is one of the most common contaminants of wheat, corn and barley (2).

Pigs show the greatest sensitivity to DON compared to poultry, ruminants and laboratory animals. Reduced feed consumption and decreased weight gain are the principal clinical effects observed following ingestion of DON in naturally contaminated feedstuffs (1-3 mg/kg DON in feed) (1). As a result, DON is considered to be a major cause of economic losses due to reduced production performance (3).

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The ability of DON to enhance or inhibit immune function has been well established in mouse, rat and human lymphocytes (4, 5), but there are only a few reports concerning the effects of feeding grains naturally contaminated with DON to first-time pregnant sows, which represent a particularly sensitive category among pigs. Previous reports mostly investigated the feed consumption and the effects on newborn piglets (6-10) and the transfer of DON from naturally contaminated feed from the sow to the piglets (11). Nevertheless, some studies examined the effects of DON on immune response in other categories of pigs using classical immunological methods, such as lymphocyte proliferation assays and analysis of metabolic activity with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) (12-17).

Flow cytometry has become increasingly important in analysing immune responses in animals. The recent development of monoclonal antibodies (mAb) directed against cluster of differentiation (CD) and other membrane molecules of porcine leukocytes has improved phenotypic characterisation and functional analysis of various porcine leukocyte populations (18). Within a diverse panel of T lymphocyte-specific surface antigens, CD3 molecules were shown to be most potent marker for the characterization and definition of this leukocyte population (19). CD4 is a cell surface protein characteristic of T helper cells (Th), and expression of CD8 is associated with cytotoxic T lymphocytes (Tc) (20).

$CD4^+$ and $CD8^+$ lymphocytes play a crucial role in immune responses. Therefore, the present study aimed to establish the effects of feedstuff containing DON, zearalenone (ZEN) and fusaric acid (FA) on two lymphocyte subpopulations, the double labelled lymphocytes $CD3^+CD4^+$ and $CD3^+CD8^+$. The Commission recommendations on the presence of DON, ZEN, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal consumption (2006) suggest that complementary and complete feedstuffs for pigs do not exceed 0.9 mg/kg DON and 0.1 mg/kg ZEN. There is no recommendation for FA. FA is probably the most widely distributed mycotoxin produced by *Fusarium* species, and according to Bacon et al. (21), FA may serve as a presumptive indicator of *Fusarium* contamination in food and grain. In addition, FA is known to have synergistic effects with other fusariotoxins. In our study, only the concentration of DON was above the recommended concentration; thus, we primarily focused on DON.

The effects of such feed on feed consumption and reproduction parameters (duration of parturition, number and weight of newborns and weanling piglets) are described elsewhere (22).

Material and methods

Experimental animals, breeding environment and feeding

The experiment was performed on a large pig farm in Slovenia with 3,600 sows, where there was a farrow-to-finish operation (22). The Veterinary Administration of the Republic of Slovenia approved realization of the experiment.

Briefly, twenty pregnant primiparous sows (Landras x Large White, day 89±2 of gestation) were randomly chosen and divided into two equal groups: the control and experimental. The sows participated in the trial 22 to 26 days before farrowing, during the lactation period (21 days) and in the period between weaning and reinsemination (5-8 days) for a total of 54±1 days.

During gestation, the sows were fed 3.5 kg/day of diet divided into two meals (6 a.m. and 1 p.m.). The feed allowance from the day of farrowing until weaning (at day 21) was 6.0 kg per sow a day. From weaning the piglets to reinsemination, the sows were fed again with 3.5 kg of feed per day, divided into two meals. To obtain relevant information about feed consumption, we collected the remaining feed and weighed it daily.

The mycotoxin content of the experimental diets

The feed was composed of 50% maize, 19% soybean meal, 8% barley, 7% beet pulp, 4.5% sunflower seed oil, 3% wheat feed, 3% dehydrated lucerne, 3% fish meal, 0.5% molasses and 2% vitamin and mineral mix. All components in the feed were the same for both groups; only the maize used in the experimental diet was naturally contaminated with DON.

The feedstuffs for both groups of sows were analysed as previously described (22). The nutritional value corresponded to all the nutritional requirements for gestating and lactating sows (23).

Sample collection

One hour after the morning feeding, sterile blood samples from sows were taken (Vacutainer systems with a luer, Vacutainer® Brand Pronto™ Holder, Becton Dickinson) from the *cranial vena cava* on days 0, 17, 42 and 52 of the experiment and transferred into tubes containing lithium (Li) heparin.

White blood cell (WBC) count

The blood samples were analysed immediately after blood collection using standard haematological equipment. Values of leukocytes (WBC) were measured by Coulter Counter ZF_6 (Coulter Electronic, UK). Differential leukocyte counts, neutrophils (Ne), eosinophils (Eo), basophils (Ba), lymphocytes (Ly), band neutrophils (Nb) and monocytes (Mo) were determined microscopically on blood smears stained by Pappenheim; 100 leukocytes were identified and differential leukocyte counts were calculated.

Flow cytometry assay

For phenotyping, mAb against various cell surface molecules were used: mouse anti-pig CD3ε conjugated with fluorescein isothiocyanate (FITC), mouse anti-pig CD4a conjugated with R-phycoerythrin (R-PE) and mouse anti-pig CD8a (R-PE). mAb were purchased from BD Bioscience. Two-colour immunofluorescence analyses by flow cytometry were performed for the identification of CD4 ($CD3^-CD4^-$, $CD3^+CD4^-$, $CD3^-CD4^+$, $CD3^+CD4^+$) and CD8 ($CD3^-CD8^-$, $CD3^+CD8^-$, $CD3^-CD8^+$, $CD3^+CD8^+$). Optimal dilutions of mAb were standardized in previous experiments. Then, 100 µl of anticoagulated blood was incubated with 2.5 µl of CD3ε mAb and either 5 µl of CD4a mAb or 5 µl CD8a mAb for 20 min at room temperature in the dark. After incubation, erythrocytes were lysed with FACS Lysing Solution (Becton Dickinson). Cells were washed twice in PBS (FACSFlow, Becton Dickinson). Acquisition of data was performed using a FACSCalibur flow cytometer, and analysis was performed with CellQuest software (both Becton Dickinson). A lymphocyte gate was defined according to the position in the forward scatter/side scatter distribution, and the percentage of marked lymphocytes was determined in each sample. At least 10,000 cells were analysed for each sample.

Statistical analysis

The data obtained in the study was statistically analysed, and the significance between groups was determined by paired t-tests. The difference was considered statistically significant when $P<0.05$. Pearson's correlation was used to determine the correlation between continuous variables (consumption of feed and values of $CD3^+CD4^+$, $CD3^+CD8^+$ and neutrophils). The SPSS statistical programme (Statistical Package for Social Sciences, Version 15, 2006) was used.

Results

The chemical analysis and mycotoxin content of diets

The chemical composition and mycotoxin content of both diets are presented in Table 1.

In both feeds, aflatoxin B_1 (<0.20 µg/kg), ochratoxin A (<0.01 mg/kg), 15 A-DON (<0.05 mg/kg), nivalenol (<0.05 mg/kg), fusarenon-X (<0.05 mg/kg), DAS (<0.03 mg/kg), T-2 toxin (<0.03 mg/kg), HT-2 toxin (<0.03 mg/kg) and fumonisins B_1, B_2, and B_3 (<0.10 mg/kg) were under the detection limits.

Feed intake and body weight change of sows

Sows in the experimental group consumed statistically less feedstuff than the sows in the control group (22). The mean weight loss in the experimental group was 21.5 kg (11.2%), from 191.3±12.5 kg at the beginning of the experiment down to 169.8±9.29 kg at piglet weaning. However, in the control group the mean weight loss was only 15.5 kg (8.1%), from 192.0±12.68 kg down to 176.5±12.28 kg. However, the differences between the groups were not statistically significant.

WBC count

As demonstrated in Table 2, the *Fusarium* toxin-contaminated feed did not alter WBC count or differential leukocyte counts in general. However,

Table 1: Analysed composition and mycotoxins content of diets

Parameter (unit)	Diet	
	Control	Experimental
dry matter (g/kg)	889.7	889.8
moisture (g/kg)	110.3	110.2
crude protein (g/kg)	162.5	162.5
crude fibre (g/kg)	53.0	49.4
crude fat (g/kg)	74.0	72.0
ash (g/kg)	72.1	68.2
ME (MJ/kg dry matter)[1]	13.4	13.5
deoxynivalenol, DON (mg/kg)	0.3	5.1
zearalenone, ZEN (mg/kg)	< 0.02	0.1
fusaric acid, FA (mg/kg)	< 0.77	21.6

[1]*Metabolic Energy*

Table 2: The effects of *Fusarium* mycotoxin intoxication on the total and differential leukocyte count (mean and SD)

Experimental day	Group	Parameters						
		WBCx10^9/L	Ne %	Nb %	Eo %	Ly %	Mo%	Ba %
day 0	control	14.14 (3.07)	28.40 (8.42)	0.00 (0.00)	2.50 (1.35)	68.90 (7.70)	0.00 (0.00)	0.10 (0.32)
	experimental	17.54 (4.67)	30.78 (11.49)	0.00 (0.00)	2.44 (1.13)	66.44 (10.99)	0.11 (0.33)	0.22 (0.44)
	P	0.75	0.61	-	0.92	0.57	0.30	0.49
day 17	control	15.12 (4.10)	38.60 (10.28)	0.00 (0.00)	5.40 (3.02)	56.00 (10.55)	0.00 (0.00)	0.00 (0.00)
	experimental	15.41 (4.35)	36.78 (5.71)	0.00 (0.00)	4.70 (2.58)	58.00 (6.67)	0.11 (0.33)	0.00 (0.00)
	P	0.88	0.64	-	0.82	0.63	0.30	-
day 42	control	16.80 (5.44)	49.10 (9.96)	0.00 (0.00)	4.70 (2.21)	44.90 (9.74)	1.30 (1.41)	0.00 (0.00)
	experimental	15.61 (1.72)	40.67 (7.90)	0.00 (0.00)	6.89 (5.11)	52.11 (7.84)	0.33 (0.70)	0.00 (0.00)
	P	0.54	0.06	-	0.23	0.09	0.08	-
day 52	control	14.19 (4.85)	32.10 (10.65)	0.60 (0.84)	7.20 (4.49)	59.30 (10.97)	0.60 (0.84)	0.20 (0.42)
	experimental	15.48 (3.92)	42.22 (9.02)	0.20 (0.42)	4.78 (2.99)	52.33 (7.10)	0.44 (0.72)	0.11 (0.33)
	P	0.53	0.04	0.12	0.19	0.12	0.67	0.62

WBC, leukocytes; Ne, neutrophils; Eo, eosinophils; Ba, basophils; Ly, lymphocytes, Nb, band neutrophils; Mo, monocytes; the difference is statistically significant when P<0.05; experimental group: 5.1 mg/kg DON, 0.1 mg/kg ZEN and 21.6 mg/kg FA

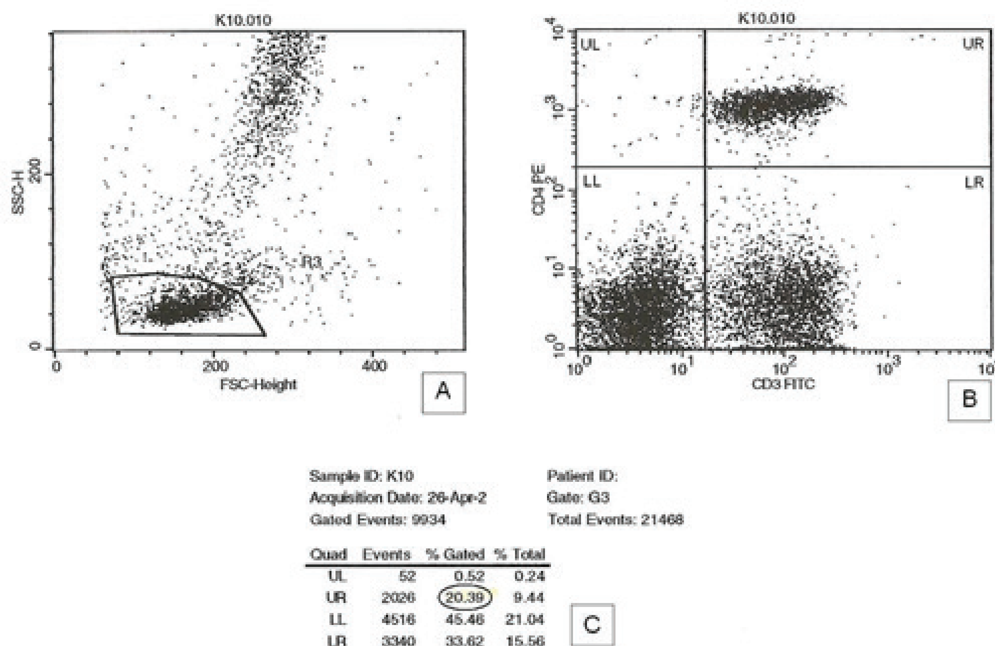

Figure 1: Dot plots of flow cytometry analysis. The lymphocytes are gated for further analysis (A). The marked lymphocytes show green (FITC) and red (PE) fluorescence (B). The percent of lymphocytes in individual quadrants (C). The percent of $CD3^+CD4^+$ lymphocytes is circled in the table below the graphics

Table 3: Effects of consumption of *Fusarium* toxins on blood lymphocyte subsets

Experimental day	group	Absolute number of lymphocytes $\bar{x}$ (SD) x 10^9/L	% of cells, obtained with flow cytometry $\bar{x}$ (SD)		Absolute number $\bar{x}$ (SD) x 10^9/L	
			$CD3^+CD4^+$	$CD3^+CD8^+$	$CD3^+CD4^+$	$CD3^+CD8^+$
day 0	control	9.74 (2.44)	22.47 (4.73)	35.27 (6.63)	2.19 (0.70)	3.41 (0.87)
	experim.	11.54 (3.03)	20.96 (2.53)	33.04 (4.07)	2.40 (0.63)	3.83 (1.15)
	P	0.169			0.491	0.374
day 17	control	8.33 (2.41)	22.88 (3.77)	34.38 (3.88)	1.89 (0.58)	2.87 (0.89)
	experim.	9.04 (2.91)	20.25 (1.99)	33.54 (7.90)	1.81 (0.55)	3.12 (1.20)
	P	0.570			0.755	0.608
day 42	control	7.23 (1.43)	27.59 (4.81)	38.59 (5.03)	1.96 (0.37)	2.78 (0.57)
	experim.	8.17 (1.71)	23.37 (3.70)	33.66 (4.77)	1.89 (0.42)	2.72 (0.59)
	P	0.210			0.697	0.842
day 52	control	8.20 (2.61)	26.03 (4.58)	37.57 (3.03)	2.12 (0.69)	3.11 (1.12)
	experim.	8.07 (81.98)	22.57 (3.43)	33.94 (6.17)	1.78 (0.39)	2.74 (0.89)
	P	0.907			0.222	0.440

experim., experimental group (5.1 mg/kg deoxynivalenol, 0.1 mg/kg zearalenone and 21.6 mg/kg fusaric acid; the difference is statistically significant when $P<0.05$)

at the end of the experiment, there was a higher percentage of neutrophils in the experimental group of sows consuming feed contaminated with 5.1 mg/kg DON (P=0.040) than the control group.

Pearson's correlation shows that the feedstuff consumed did not correlate with the percentage of neutrophils at day 52 in the control group (r=0.524, P=0.120) and the experimental group (r=-0.172, P=0.658).

Flow cytometry

The percentages of $CD3^+CD4^+$ and $CD3^+CD8^+$ cells were determined by flow cytometry. The model for flow cytometric analysis of the $CD3^+CD4^+$ lymphocyte subpopulation, expressed as a relative proportion of gated lymphocytes, is presented in Figure 1 (A). Diagram B shows both fluorescence intensities applied on the x- and y-axes. The percentages of marked cells in individual quadrants are shown in Figure 1 (C), under diagram A and B. For further analyses, only the values from the UR quadrants were used and compared between the groups.

The same procedure was followed to determine the percentage of $CD3^+CD8^+$ cells, and the results are given in Table 3. From these values and from the lymphocyte numbers obtained at the differential leukocyte count, the absolute numbers of $CD3^+CD4^+$ and $CD3^+CD8^+$ cells were calculated (Table 3). A notable difference between groups was observed towards the end of the experiment, when absolute numbers of $CD3^+CD4^+$ and $CD3^+CD8^+$ in the control group showed greater increases (2.12±0.69 x 10^9/L and 3.11±1.12 x 10^9/L, respectively) than the experimental group (1.78±0.39 x 10^9/L and 2.74±0.89 x 10^9/L, respectively) (P=0.222 and P=0.440, respectively).

Pearson's correlation showed that the feedstuff consumed did not correlate with the values of $CD3^+CD4^+$ cells on day 52 in the control group (r=-0.216, P=0.549) and the experimental group (r=0.143, P=0.713). Similar results were observed for $CD3^+CD8^+$ cells in the control (r=-0.279, P=0.405) and experimental group (r=0.272, P=0.479).

Discussion

The comparison between the control group and the experimental group confirmed previous results that DON reduces feed consumption. Notably, in addition to DON, ZEN (0.1 mg/kg) and FA (21.6 mg/kg) were present in the diet for the experimental group, and consequently, they may have had an effect on the results as well. In many trials, the FA concentrations were not analysed, although synergistic interactions between FA and DON have been reported. FA was shown to increase the toxicity of DON in starter pigs (24). FA concentration, however, is seldom determined in swine feeds due to its low toxicity when consumed in the absence of other toxins (25, 26).

The percentage of $CD3^+CD4^+$ and $CD3^+CD8^+$ cells, representing the Th and Tc, obtained with flow cytometry and their calculated absolute numbers indicated that the feed may have immunomodulatory effects and suppress the immune response of sows in the perinatal period. Recently, Ferrari et al. (27) used flow cytometric analysis to characterize and quantify the lymphocyte subsets $CD3^-CD8^+$, $CD4^+CD8^-$, $CD4^-CD8^+$, $CD8^{high}$, $CD4^+CD8^+$ and TCRγ/δ+ in 8-week-old pigs that received 0.5 mg/kg DON in the first week and 1 mg/kg for the next 5 weeks. Although increased mean absolute values for Tc ($CD4^-CD8^+$, $CD8^{high}$) were observed in the control group in the last experimental weeks, the DON treatment did not significantly influence the levels of lymphocyte subpopulations, which is similar to the findings in our study.

Mycotoxins, DON among them, are believed to be one of the most immunosuppressive factors in animal diets. For the vast majority of mycotoxicoses, which are chronic, the signs of disease are generally subtle and unspecific. Thus, it is difficult to establish a cause-effect relationship to contaminated feedstuffs (3). Our experiment had similar results: the reduced feed consumption was the only clinically observed difference between the groups. However, the analysis of WBCs showed that towards the end of the experiment, sows that consumed 5.1 mg/kg DON per feed had a significantly increased relative number of neutrophils in their blood. The increased number of neutrophils occurs in the early stage of bacterial infection, which could lead to three conclusions: the sows from the experimental group are more susceptible than those from the control group and defend themselves from subclinical infections, this is their prolonged reaction to parturition, or DON influences the secretion of immune system mediators, which increase the number of neutrophils. An increased number of neutrophils

was also by reported Rotter et al. (12) in pigs that consumed feed with 0.75-3 mg/kg DON for 29 days. Other authors did not find these results (14).

A recent report (28) found that low concentrations of DON can alter the immune functions of pig polymorphonuclear cells, the first line of defence against infection, which suggests the involvement of p38 mitogen-activated protein kinase in the signal transduction pathway. These immunosuppressive effects of DON may have implications for humans and/or animals when eating contaminated food/feed.

The absolute numbers of both the $CD3^+CD4^+$ and $CD3^+CD8^+$ subsets towards the end of the experiment increased more obviously in the control group. These changes might be caused by damage of macrophages or T regulatory cell activities. Chen et al. (29), working with pigs fed DON, described the decreased mRNA expression levels of IFN-γ, TNF-α and IL-2, which could be a possible explanation for the decreased number of Tc ($CD3^+CD8^+$) cells in the experimental group.

The characteristics of porcine lymphocytes forced us to take into consideration not only Th lymphocytes ($CD3^+CD4^+$) and Tc ($CD3^+CD8^+$) cells but also the double positive cells $CD4^+CD8^+$ cells. These cells can be found in extrathymic sites in healthy pigs, while in humans and mice, this population is found only in some physiological disorders (30). Porcine $CD4^+CD8^+$ double positive lymphocytes were shown to increase gradually with age (30-55% by 3 years of age). Cells could proliferate in response to stimulation with recall viral antigen, consistent with the hypothesis that this population in swine includes memory/effector T cells (18, 31, 32). This cell function obscurity is acceptable in our experiment because the sows were young, and the focus of our study was not to define the exact number of Th and Tc cells but to establish the differences between groups.

Dabrowski et al. (33) studied the in vivo effect of low doses of ZEN and DON, administered individually or in combination, on immune system function based on the subpopulations of $CD4^+8^-$, $CD4^+8^+$ and $CD4^-8^+$ lymphocytes in the peripheral blood of pigs. The results revealed that long-term exposure to low doses of ZEN, DON and ZEN+DON disrupted linear proliferation of $CD4^+8^+$ cells. Co-contamination of feed with both mycotoxins had a stronger effect on the immune system and led to a transient decrease in the percentage of $CD4^+8^+$ lymphocytes in week 5 of exposure. Another study (34) also suggested that prolonged exposure to low doses of DON can change the proportions of immunocompetent cells (a shift towards humoural immunity), without affecting their overall counts.

In experiments with *Fusarium* mycotoxins on animal models, it is important to evaluate correlation between described changes and decreased feed consumption. By using Pearson's correlation, we demonstrated that the results for the absolute number of $CD3^+CD4^+$ and $CD3^+CD8^+$ cells and the percentage of neutrophils at the end of the experiment did not have any correlation with sow consumption.

In conclusion, the results of this study could be useful for further elucidation of the cellular basis of immune responses to *Fusarium* toxins in pigs and consequently in humans, since pigs are used as animal models for human diseases. In addition, humans, as well as animals, are exposed to mycotoxins by consumption of contaminated grains.

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UČINEK KRME, NARAVNO KONTAMINIRANE S TOKSINI PLESNI *Fusarium* sp., NA IZBRANE HEMATOLOŠKE PARAMETRE IN LIMFOCITNE PODVRSTE PRVIČ BREJIH SVINJ

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Povzetek: V nadzorovanem poskusu, ki je na veliki farmi prašičev trajal 54±1 dan, smo 20 mladic (breje 89±2 dni) naključno razdelili v dve enaki skupini. Živali so zauživale krmo, ki je bila v kontrolni skupini naravno kontaminirana z mikotoksinom deoksinivalenolom v koncentraciji 0,3 mg/kg krme. Krma za poskusno skupino je vsebovala 5,1 mg/kg deoksinivalenola, 0,1 mg/kg zearalenona in 21,6 mg/kg fuzarne kisline. Mladice iz poskusne skupine so v obdobju brejosti (P = 0,002), laktacije (P = 0,027) in v času od odstavitve pujskov do ponovne osemenitve (P < 0.001) zaužile statistično značilno manjšo količino krme kot živali iz kontrolne skupine. Med poskusom smo mladicam štirikrat odvzeli kri (dan 0, 17, 42 in 52). V skupnem številu levkocitov in diferencialni krvni sliki med skupinama ni bilo statistično značilnih razlik, razen v številu nevtrofilcev 52. dan poskusa, ko je bila vrednost v poskusni skupini 42.22±9.02 % in v kontrolni skupini 32.10±10.65 % (P = 0.040). S pretočno citometrijo smo določili delež limfocitov T in njihovih podvrst, celic T pomagalk ($CD3^+CD4^+$) in citotoksičnih celic T ($CD3^+CD8^+$), ki smo jih označili z monoklonskimi protitelesi. Iz dobljenih podatkov smo izračunali absolutno število $CD3^+$ $CD4^+$ in $CD3^+$ $CD8^+$ (10^9/L), ki se je v poskusni skupini zmanjševalo od 2.40±0.63 in 3.83±1.15 na začetku poskusa do 1.78±0.39 in 2.74±0.89 na koncu poskusa. Vrednosti v kontrolni skupini so bile na začetku poskusa 2.19±0.70 in 3.41±0.87 ter na koncu 2.12±0.69 in 3.11±1.12 x 10^9/L. Rezultati kažejo, da zauživanje krme, naravno kontaminirane s toksini plesni vrste *Fusarium*, vpliva na ješčnost živali, število nevtrofilcev in ima imunomodulatorni učinek na število limfocitov T.

Ključne besede: levkociti; limfociti T; mikotoksini; deoksinivalenol; pretočna citometrija; prašiči