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Assessment of selected immunological parameters in dairy cows with naturally occurring mycotoxicosis before and after the application of a mycotoxin deactivator

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Abstract

Introduction: Mycotoxins in dairy cows can cause many non-specific symptoms often resulting from immune system overreaction. The study assessed the concentration of selected cytokines and acute phase proteins (APP) in cows with natural mycotoxicosis before and after using a mycotoxin neutraliser. The cytokines were tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and interleukin 10 (IL-10), and the APP were serum amyloid A (SAA) and haptoglobin (Hp). **Material and Methods:** The research was carried out on an experimental group (Exp) of 10 herdmate Holstein-Friesian cows with mycotoxicosis. The control group (Con) was 10 healthy cows of the same breed from a different herd. Cows in the Exp group were administered the mycotoxin deactivator Mycofix for three months. Blood was drawn from Exp cows once before administering Mycofix and a second time after three months of its use. Blood was also drawn from Con cows at the same times. Serum levels of TNF-α, IL-6, IL-10, SAA and Hp were assessed using ELISA. **Results:** The concentrations of all cytokines and Hp in Exp cows were higher before treatment ($P < 0.001$) than those in Con cows. After three months of administering Mycofix, the concentrations of TNF-α and IL-6 were significantly lower than their pre-treatment levels ($P < 0.001$). The concentrations of IL-6, IL-10, and Hp were still significantly higher than those in the Con group ($P < 0.001$). In cows with mycotoxicosis, simultaneous stimulation of antagonistic processes was noted: a pro-inflammatory process in the upregulation of TNF-α and IL-6, and an anti-inflammatory one in the upregulation of IL-10. **Conclusion:** Despite the absorbent's use and the resolution of clinical symptoms in Exp cows, high levels of IL-10 and Hp and IL-6 were maintained. Assessment of the level of cytokines and APP appears to be a useful and precise tool for the evaluation and application of the appropriate dose of the mycotoxin absorbent or the evaluation of its effectiveness.

Keywords: acute phase proteins, cows, cytokine, mycotoxicosis, mycotoxin deactivator.

Introduction

In dairy cattle farming, good-quality feed and its appropriate dosage are the basic factor ensuring proper milk yield and cow health in the herd and largely determine profitability. Despite the efforts of farmers and their use of modern methods in the production of feed, the contamination of feed with mycotoxins remains a serious problem. Mycotoxins may already form at the stage of feed plant growth or during the

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improper transport of feed, but most often contaminate feed during its storage in inappropriate conditions favouring the development of mould fungi, whose metabolites are the main source of fodder contamination. The danger of feed contamination with mycotoxins is that even in low concentrations, they disturb the functioning of the body (*e.g.* decreasing appetite) (5). In livestock, mycotoxins may lead to a significant drop in productivity and cause many diseases, which inflict large economic losses. According to the literature data, the negative impact of mycotoxins is mainly immunosuppression and the associated increased morbidity and susceptibility of animals to infectious diseases, reproductive problems, and damage to internal organs, which in extreme cases may lead to death (14). Ruminants are less known for their sensitivity to the negative effects of mycotoxins than monogastric animals. Nevertheless, the consumption of feed contaminated with mycotoxins for a longer period is a threat also to ruminants and it may inhibit growth and weight gain and cause a decrease in milk production and problems in bovine reproduction (19). The observed clinical symptoms related to exposure to various types of mycotoxins are non-specific and include both metabolic and hormonal disorders, as well as inflammation caused by the reaction of the immune system (9). The mechanisms underlying these changes are not yet well understood, but it is believed that they are associated with disturbances in the rumen and intestine biota, increased permeability of the gastrointestinal mucosa, and damage to the intestinal epithelium (2). The most common mycotoxins found in dairy cow feed are zearalenol (ZEN) and deoxynivalenol (DON) (11). Zearalenol is a mycotoxin produced by fungi of the *Fusarium* genus. Chemically, ZEN is a lactone of resorcylic acid, and because of the similarity of its structure to that of natural oestrogen, its accumulation in an animal may lead to hyperoestrogenisation (17). In addition, *in vitro* and *in vivo* studies show that ZEN reduces the activity of many enzymes involved in the steroidogenesis process in animals, which may also interfere with the functioning of the reproductive system (12, 13, 46). The natural occurrence of zearalenol is most often associated with the presence of deoxynivalenol, as they are formed under similar conditions (16). Deoxynivalenol is a type B trichothecene, and thus an epoxy-sesquiterpenoid, and is commonly called vomitoxin because of its emetic effect (24). The effect of DON on the immune system, which is extremely sensitive to this type of mycotoxin, has also been studied (24). It has been shown that deoxynivalenol can have both immunosuppressive and immunostimulatory effects depending on the dose, frequency, and duration of exposure and the functioning of the immune system (33). The immunotoxic effect of DON is manifested mainly by the activation of many processes leading to apoptosis of the cells of the immune system (24). In cows, studies have shown that DON can impair the primary humoral response to specific antigens, including a reduction of the phagocytic activity of neutrophils (22). The best way to prevent mycotoxicosis is to prevent mycotoxins from being produced in the feed. However, given the widespread presence of *Fusarium* in the environment and thus the presence of mycotoxins in feed, it seems inevitable that dairy cows will sometimes be provided with feed contaminated with them. Very often, farmers do not even know that they are administering food containing fungal toxins. To counter the risk of incurring high costs related to the loss of mycotoxin-contaminated feed, it is possible to dose it with special additives that allow its use without any consequences for the condition of the animals fed (8, 20, 43). The most common practice is to add mycotoxin deactivator product (MDP) to the feed. (8). These additives have different mechanisms of action depending on the structure of the toxin they are designed to neutralise (43). They can adsorb mycotoxins, break them down into non-toxic compounds or contain microorganisms capable of decomposing them (38, 43). One of the products used in dairy cows is Mycofix Plus 3.E. Numerous studies have shown that the use of this product prevents the negative effects of mycotoxins on the body, which is beneficial to health and production (15, 20). The study aimed to assess the level of selected cytokines (TNF-α, IL-6 and IL-10) and acute phase proteins (haptoglobin (Hp) and serum amyloid A (SAA)) in cows with naturally developed mycotoxicosis before and after the administration of the mycotoxin neutraliser. By assessing selected indicators, the reaction of the dairy cows' immune system to ZEN and DON contained in the feed was demonstrated, as well as the influence of the mycotoxin neutraliser on the evaluated immunological indicators.

Material and Methods

Animals. The study was carried out on 20 cows from two Holstein-Friesian dairy herds of similar size and managed with the same feeding and maintenance methods. The cows were kept in the free-range system and fed with total mixed ration (TMR). The detailed composition of TMR is presented in Table 1. The complete fodder constituted a proper feed ration, adjusted to the physiological period of the cows. The composition of the feed ration on the experimental farm was balanced for lactating cows with an average daily milk production of 20 kg. Each cow whose milk yield exceeded 20 kg additionally received 1 kg of concentrated feed for every 2 kg of additional milk produced. The components included maize silage, haylage, hay, straw, a mixture of cereals, spent grains (brewers' grains), protein, and vitamin and mineral supplements. The mixture was given to the cows once

a day after morning milking. Mechanical milking took place twice a day in a purpose-built and properly equipped milking hall.

Health problems were reported in one herd because many cows had non-specific symptoms of unknown origin: a decreased appetite, diarrhoea, weight loss, lameness, abomasum dislocation, a decreased milk yield, an increased number of somatic cells in milk, and infertility. The toxicological study of this herd's TMR showed the presence of 32 parts per billion (ppb) ZEN and 769 ppb DON. The mycotoxin content was assessed by the Biomin laboratory (Herzogenburg, Austria) with liquid chromatography combined with tandem mass spectrometry. The average yield for cows in this herd determined in the 305-day lactation period was 6,161–6,725 kg of milk with a fat content of 4.27–4.83% and a protein content of 3.21–3.28%. Ten cows were selected for which the ZEN content in their blood serum was confirmed at 14.30 ± 3.64 and the DON content at 20.92 ± 5.94 ng/mL. These cows constituted the experimental group (Exp). The plasma concentrations of ZEA and DON were determined by combined separation techniques with the use of immunoaffinity columns and high-performance liquid chromatography (HPLC) with fluorescence detection (27, 44).

The second herd had no health problems. Ten clinically healthy cows were selected as the control group (Con). In addition, standard haematological tests comprising counts of white blood cells, red blood cells, thrombocytes, granulocytes and lymphocytes and determination of haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were performed on selected cows to confirm their general health (45). The average yield in this herd determined in the 305-day lactation was 8,571–8,670 kg of milk with a fat content of 4.51–4.14% and a protein content of 3.14–3.42%.

All selected cows were in the period of 60 +/− 20 days postpartum. The cows were in their second to fourth lactation. The animals' body condition was rated as good or very good, reflected in body condition scores (BCS) of 3.0–3.25 in Exp cows and 3.5–4.0 in Con cows on a five-point scale (37). Reproductive system monitoring in the herds was carried out regularly at monthly intervals by rectal examination combined with ultrasonography. To cows with no complications during parturition and no signs of inflammation a synchronisation protocol of oestrus and ovulation (presynch-ovsynch protocol) was applied, and artificial insemination (AI) with frozen semen was performed on those animals. Cows with uterine inflammation were properly treated and subsequently subjected to the synchronisation protocol of oestrus and ovulation and AI. Cows with ovarian cycle disturbances were treated individually according to the diagnosed cause. Pregnancy testing was performed routinely approximately 30 to 40 days after insemination by rectal examination combined with ultrasonography. The expected date of parturition was determined by adding 280 days to the day of artificial insemination and was also supported by the pregnancy diagnosis.

The administration of the Mycofix Plus 3E preparation (Biomin, Herzogenburg, Austria) was started in the herd where the presence of mycotoxins was confirmed after the first drawing of blood for testing. The composition of the preparation is not disclosed; the manufacturer only provides general information that the preparation contains specific toxin-eliminating enzymes, adsorbents, carefully selected plants and algae extracts. Mycofix was administered at a dose of 10 g/cow per day for three months as an addition to the feed. A measured dose of the preparation appropriate for the number of cows was added to the fodder cart, which mixed the TMR well with Mycofix, and then the mixture was provided to all cows in the herd.

Table 1. Composition of the total mixed (TMR) and dry mass (DM) daily feed ration for lactating cows (kilograms/cow/day)

Dose component	Amount per cow, daily	
	kg TMR	kg DM
Maize silage	25.0	8.8
Haylage	8.0	3.2
Ensiled brewer's spent grain	8.0	2.7
Wheat straw	0.8	0.7
Ensiled maize grain	2.5	1.7
Barley ground grain	1.5	1.3
Triticale ground grain	1.5	1.3
Ground rapeseeds	2.7	2.2
Extracted soyabean meal	2.0	1.7
Glycerine	0.3	0.24
Vitamin and mineral mixture	0.2	0.18
Sodium bicarbonate	0.2	0.2
Calcium carbonate	0.05	0.05
Total	52.73	24.27

Sampling of blood. Blood for tests was taken from experimental cows twice: the first time after confirming the presence of mycotoxins in the feed (before starting Mycofix administration) and the second time after three months of mixing the mycotoxin deactivator with feed. Blood was taken from the control cows at the same times. Blood samples of 9 mL were collected from the external jugular vein into Vacutest clot activator tubes (Vacutest Kima, Arzergrande, Italy). Blood samples were centrifuged at 3,000 rpm for

20 min at 4°C, and the serum was harvested and transferred to 2-mL microcentrifuge tubes and stored at −80°C until analysis.

Mycotoxicological tests in the blood. Plasma concentrations of ZEA and DON were determined by combined separation techniques with the use of immunoaffinity columns (ZearalaTest WB and DONtest WB, VICAM, Watertown, MA, USA) and HPLC with fluorescence detection (27, 44).

Measurements of cytokines in blood serum. The concentrations of TNF-α, IL-6, and IL-10 in blood serum were determined using dedicated bovine enzyme-linked immunosorbent assay (ELISA) kits (USCN Life Science, Houston, TX, USA). The inter- and intra-assay coefficients of variation (CV) for all examined cytokines were <12% and <10%, respectively. All procedures were performed according to the guidelines provided by the manufacturers and methods available in the literature (21). Absorbance readings were performed on an automatic microplate reader (Asys Expert Plus; Biochrom, Cambridge, UK) at 450 nm.

Measurements of acute-phase proteins in blood serum. Measurements of the level of SAA in blood serum were performed using a commercial ELISA kit (Tridelta Development, Maynooth, Ireland). The inter- and intra-assay coefficients of variation for SAA analysis were <12.1% and <7.5%, respectively. The Hp in blood serum was determined using a commercial colorimetric assay kit (Tridelta Development). The inter- and intra-assay CVs for the Hp analysis were <5.7% and <6.3%, respectively. Procedures were performed according to the manufacturer's instructions and literature methods (41). The absorbance was read and the subsequent calculations of the final concentrations were made on the Asys Expert Plus automatic microplate reader at 450 nm, 630 nm for Hp, and 630 nm as a reference for SAA. Lyophilised bovine acute-phase serum was used as a standard and the reader was calibrated according to the European Union Concerted Action Project on the standardisation of animal APPs No. QLK5-CT-1999-0153.

Statistical analysis. All values are presented as means ± standard error of the mean. Statistical analysis was performed using Statistica software version 10.0 (StatSoft, now Tibco, Tulsa, OK, USA). Data were normally distributed, as demonstrated by the Kolomogorov–Smirnov test and the Lillefors correction. The obtained values were compared between cows with mycotoxicosis and healthy cows, using a non-paired Bonferroni post-hoc multiple comparison test. The P-value <0.05 was considered statistically significant. Statistical differences between the results for the materials collected at different times in the same group (first collection from Exp and first collection from Con *versus* second collection from Exp and second collection from Con) were calculated using Tukey's and Duncan's post hoc tests for a probability value of $P \leq 0.01$.

Results

Observations of cows with mycotoxicosis. The observations were carried out for three months from the moment the feed contamination with mycotoxins was detected. Throughout this period, the cows were fed the mycotoxin adsorbent Mycofix as an addition to the feed. In the first stage of the research, before starting the administration of the adsorbent, many non-specific symptoms were observed in experimental cows: a decreased appetite, diarrhoea, weight loss (BCS 3.0), lameness, reduced milk production at 20.2 ± 0.5 kg/head/day, and an increased number of somatic cells in milk (743 ± 50.1 × 10^3/mL). Ovarian cysts were also affecting mycointoxicated cows. After three months of Mycofix administration, most of the symptoms subsided: the appetite returned to normal, the cows ate willingly, there was no diarrhoea, the body weight increased (BCS 3.5–4.0), no lameness occurred, there was an increase in milk production (23.4 ± 1.0 kg/head/day) and a decrease in the number of somatic cells in milk (354 ± 20.3 × 10^3/mL). Control cows were healthy with BSC 4, milk yield of 28.1 ± 1.0 kg/head/day, and vastly lower somatic cell count in milk (325 ± 10.2 × 10^3/mL).

Evaluation of cytokines and APP in cows in both experimental groups. The concentration of the assessed cytokines, TNF-α, IL-6 and IL-10, and acute phase proteins, Hp and SAA, in all cows tested are shown in Fig. 1 (A–E). The presented data show that the values of all cytokines in the experimental cows in the first haematological analysis (after the diagnosis of mycotoxicosis) were significantly higher ($P < 0.001$) than those in the control cows. After three months of using the mycotoxin adsorbent Mycofix, the concentrations of all cytokines decreased markedly, but those of TNF-α and IL-6 were significantly lower than those in the first analysis ($P < 0.001$) as shown in Fig. 1 (A and B). Only the concentration of TNF-α in the second investigation was comparable to that in the control group, and the concentrations of IL-6 and IL-10 were still significantly higher than the control concentrations ($P < 0.001$) (Fig. 1C). The obtained SAA values were similar on both study dates and were close to those of the control group (Fig. 1D). The Hp values in the experimental cows were very high in both the first (905.3 ± 224.63) and the second (304.3 ± 64.38) tests; the first test values being significantly higher than the second ($P < 0.001$). These significantly exceeded the concentrations in the control group (60.53 ± 8.79 in the first test and 60 ± 5.14 in the second) ($P < 0.001$) (Fig. 1E).

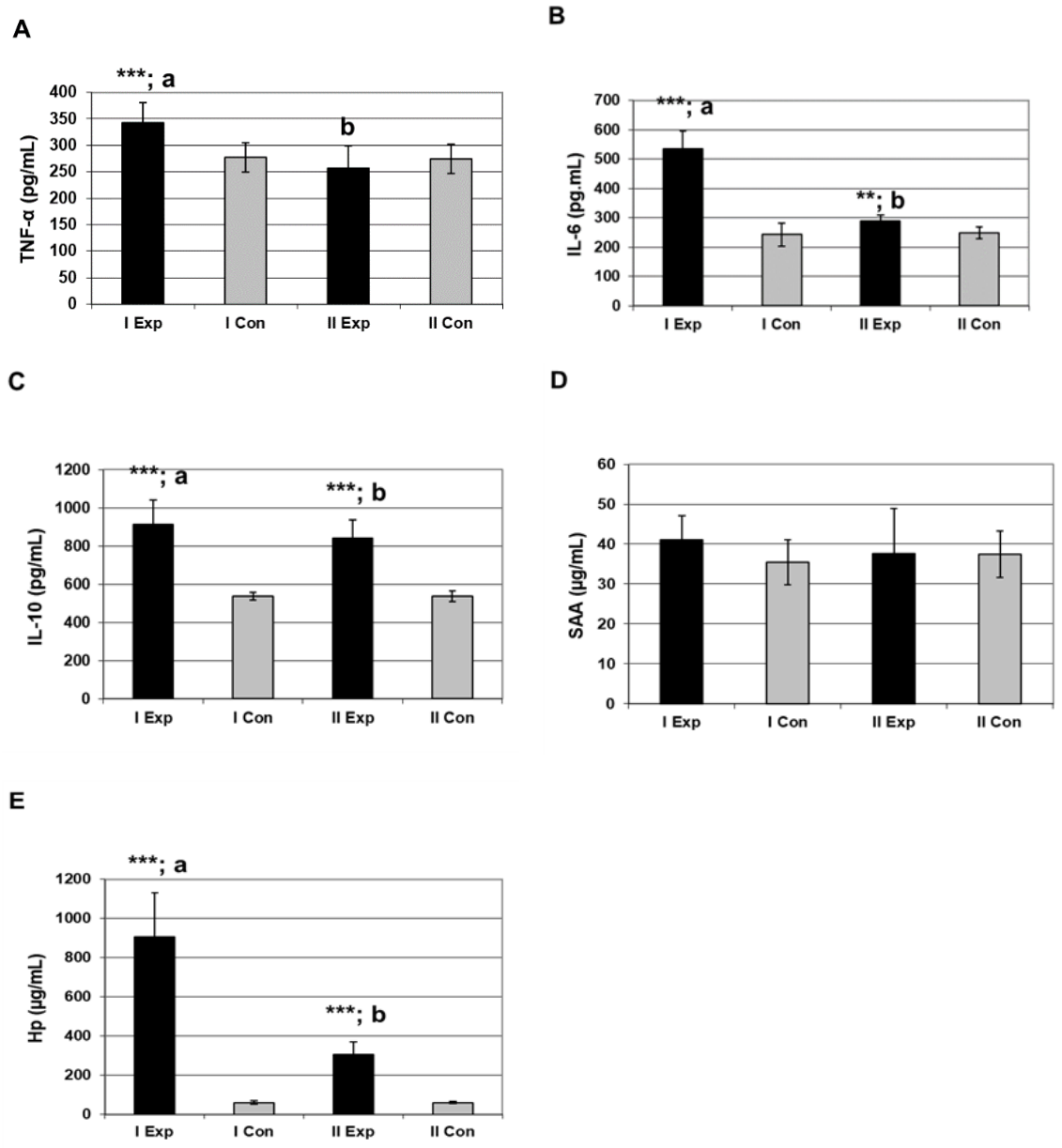


Fig. 1. Levels of tumour necrosis factor (TNF-α) (A), interleukin 6 (IL-6) (B), interleukin 10 (IL-10) (C), serum amyloid A (SAA) (D) and haptoglobin (Hp) (E) in the blood serum of experimental cows with mycotoxicosis (Exp) and control cows (Con) in good health

* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$ – statistical differences between Exp cows and Con cows

a and b – statistical differences between the results for the material collected at different times in the same group (I Exp. *versus* II Exp.), $P < 0.01$. There were no significant differences in the control cows

Discussion

In this research, the concentrations of the TNF-α cytokine, the IL-6 and IL-10 interleukins, and the SAA and Hp APPs were assessed. These indices were analysed in the blood serum of dairy cows suffering naturally acquired mycotoxicosis caused by the consumption of food contaminated with ZEN and DON. The cows for the experimental group were selected from a herd in which various non-specific disease symptoms were observed, such as a decreased appetite, diarrhoea, progressive weight loss, a decreasing milk yield, an increased number of somatic cells in milk, lameness and decreased fertility. These symptoms appeared and gradually intensified, and treatment of symptoms and attempts to adjust the nutritional dose did not bring the expected improvement in the efficiency or health of the cows in the herd. It was recommended to test for the presence of mycotoxins in feed, and the positive test result was something of a surprise, as no link between these non-specific symptoms and mycotoxicosis had been made beforehand. It is generally known that ruminants are more resistant to mycotoxins than other animals because the microbiota of their rumen decompose and deactivate mycotoxins quite effectively, and thus protect them from mycotoxicosis (26, 47). Using their antibacterial, antiprotozoal and antifungal properties,

various mycotoxins are able to quantify and modify the rumen bacterial flora (7, 10, 29). It has been documented that exposing animals to fusarium mycotoxins such as ZEN and DON, even at low doses, adversely affects the stability of the gastrointestinal biocenosis, which is an important indicator of animal health (35). The modified rumen microbiota are not able to ensure proper breakdown and use of the food ration or to properly deactivate the mycotoxins that enter further parts of the gastrointestinal tract and are absorbed into blood as in monogastric animals (7, 28). Therefore, the initially observed disease symptoms differ from typical mycotoxin poisoning and resemble malnutrition with accompanying dysbacteriosis, leading to acidosis, slowing down the digestive processes of feed with the consequences of weight loss and mild diarrhoea with undigested fibre in the stool. Long-term consumption of fodder with mycotoxins leads to a decrease in milk production and an increase in the number of somatic cells in milk. There are also cases of laminitis and infectious diseases (6, 12). The reproductive performance of cows in sick herds also decreases as a result of significant metabolic and hormonal changes and the occurrence of a negative energy balance in animals (46).

Most of the described symptoms were observed in the herd from which the experimental cows were chosen. The three-month observation of the herd showed that the administration of Mycofix was associated with a gradual improvement in the health of the cows in the herd and with the gradual disappearance of the symptoms described. After this time, the number of health disorders in the cows was comparable to the number in the herd from which the control cows were picked. The assessed immunological parameters in the first haematological analysis of cows with mycotoxicosis showed that the concentration of all the evaluated cytokines and Hp in the experimental cows was very high and significantly higher than that of the control cows. Undoubtedly, it was a reaction related to the influence of mycotoxins on the lymphatic tissue of the gastrointestinal tract and possibly also on immunologically competent cells in the entire organism of the cow. The effect of mycotoxins is supported by the parameters of the experimental cows exceeding those of the control cows by as much as fifteen times (see Hp results). This suggests the existence of a very strong body immune response caused by the stimulation of the immune system, with simultaneous pro-inflammatory processes (quantitative increases in TNF-α and IL-6) and anti-inflammatory processes (an increase in IL-10 concentration). Studies by other authors carried out in pigs after administration of even low doses of ZEN also showed a simultaneous significant increase in the concentration of pro-inflammatory (IL-4) and anti-inflammatory (IL-10) interleukins in the lymphoid tissue derived from intestinal Peyer's patches (30). These antagonistic processes often act simultaneously as pro-inflammatory defence mechanisms that protect the animal against pathogens and anti-inflammatory mechanisms that prevent the body's own tissues from harming themselves through overactivity of the immune system. The increase in the amount of anti-inflammatory IL-10 may result from the increasing number of TCD4+Foxp3+ and TCD8+CD25+ regulatory lymphocytes, which have suppressor functions that inhibit inflammatory responses (3, 4, 18). By secreting IL-10, these lymphocytes function protectively for body tissues because of the inhibitory effect on immunocompetent effector cells, which weakens autoimmune reactions (32, 39). In the event of triggering regulatory processes, as we observed in our research, the tolerance of the immune system to a strong stimulant – mycotoxins – increases, thanks to which it is possible for the bovine immune system to maintain homeostasis. Otherwise, prolonged exposure to fungal toxins could lead to self-damage in tissues and organs (40). It is not known how long the cows studied were exposed to mycotoxins. However, observing the chronicity of the clinical symptoms described above and the specificity of changes in the assessed immunological parameters, we can assume that the consumption of mycotoxins by cows lasted for quite a long time. The activation of regulatory processes initiated protectively as a result of an acute inflammatory reaction, especially the very high level of Hp and normal SAA in experimental cows, proves an ongoing chronic inflammatory process to be in operation. Acute phase proteins characteristic of cattle such as SAA and Hp are mainly produced by hepatocytes in the liver in response to pro-inflammatory cytokines (*e.g.* IL-6 and TNF-α) and glucocorticoids (42). Serum amyloid A is an apolipoprotein that appears up to 48 h after the event triggering the inflammation, *e.g.* after infection, as a first-line response protein, and its secretion is dependent on IL-1 and/or TNF-α (34). Haptoglobin, however, is a second-line protein, secretion of which is regulated by IL-6, and its high level is characteristic of long and less intense inflammatory processes (34). This would explain the results of our research, in which in experimental cows the increase in TNF-α was not accompanied by an increase in SAA, but an increase in IL-6 coexisted with high levels of Hp, which is characteristic of chronic inflammatory processes. This is important because it can be concluded that the cows had been exposed to mycotoxins for a long time; unfortunately, with only the present results as any basis, we were not able to determine the duration of exposure to effects of the mycotoxins more precisely.

After three months of administering Mycofix, pro-inflammatory processes were significantly suppressed, which was documented by a decrease in the TNF-α and IL-6 values. In contrast, the level of IL-10, despite its decrease, was still much higher than the level in the control group. This may indicate ongoing anti-inflammatory processes that protect against

autoimmune reactions. It is difficult to explain what the cause of the maintenance of anti-inflammatory processes was; it is possible that despite mycotoxins' limited absorption, they were still affecting the bovine organism. It is also possible that the effect of Mycofix was insufficient or that the dose of this preparation used in our research (10g/cow/day) turned out to be too low, not fully protecting against the penetration of mycotoxins from the gastrointestinal tract into the blood. The limited inactivation of mycotoxins in the rumen and the passage of their small amounts to the distal parts of the gastrointestinal tract may no longer cause any symptoms, but may allow them to affect the gastrointestinal immune system and gut-associated lymphoid tissue. Such an explanation is very probable, because after three months of administration of Mycofix, an elevated value of IL-6, and especially a high concentration of Hp, was maintained, which indicated a chronic, mildly intensified inflammatory process. It is also possible that longer time or a prolonged period of use of the mycotoxin deactivator is required for IL-10 and Hp levels to decrease. It also cannot be ruled out that the increase in the level of cytokines and APP in cows in our studies was a consequence of other comorbidities, because, according to the literature data, these biomarkers are non-specific and may also increase in many other disease entities' courses (3, 4, 42). In the studies cited, the increase in the level of cytokines only coincided with some disease entities, and only for some may they be markers. Our research confirmed that the increase in the level of cytokines and Hp coexisted with mycotoxicosis in cows. However, the authors want to draw attention to the emergence of various non-specific symptoms in the cows with mycotoxicosis, which are unlikely to be caused by mycotoxins. We can assume that too strongly activated nonspecific immune mechanisms are responsible for at least some of these symptoms. There are studies that have unequivocally shown that cytokines actively participate in and can even cause many diseases, both metabolic and non-metabolic (25, 36). It has been shown that there are close correlations between the increase in IL-6 concentration and disturbance of lipid and protein metabolism, abnormal fatty acid metabolism and even the occurrence of oxidative stress. Dysfunctions in one or all of these processes can lead to metabolic diseases, liver damage and liver failure (25). Similarly, as a pro-inflammatory cytokine, TNF-α in humans is involved in the pathogenesis of many metabolic diseases, mainly chronic ones such as diabetes, contributing to insulin resistance and disorders of lipid metabolism (36). In cows with fatty liver syndrome, a correlation was also described between the increase in TNF-α and insulin resistance as well as disturbance of lipid metabolism (1, 31). In addition, IL-6 and TNF-α can stimulate the breakdown of adipose tissue in the body by lowering feed consumption, inducing insulin resistance and directly initiating lipolysis (23).

Taking into account the above information, it should be emphasised that the level of cytokines and APP in cows during mycotoxicosis is an important descriptor of the state and reactivity of the immune system, and even the intensity of the ongoing inflammatory reaction. However, the value of the studied indicators as markers of mycotoxicosis in cows is limited, mainly because their levels also increase in many other disease entities. In the presented study, there are also too few data to clearly indicate that the increase in the concentration of cytokines and APP may be the cause of medical conditions in cows with mycotoxicosis. Only the close relationship between mycotoxicosis, an increase in cytokines and Hp and the emergence of non-specific clinical symptoms can be observed with certainty. On the other hand, the presented research clearly revealed that despite the administration of the mycotoxin absorbent in the amount recommended by the producer and the recovery of the cows to full health, the levels of IL-10, Hp and IL-6 were still significantly higher than those in the control group. Therefore, the determination of the level of cytokines and APP seems to be a very useful and precise tool for the assessment and application of an appropriate dose of the absorbent or the evaluation of its effectiveness.

Our research shows that the assessment of the level of cytokines and APP in cows with mycotoxicosis ably elucidates the state and reactivity of the immune system and the intensity of any ongoing inflammatory reaction. Simultaneous stimulation of antagonistic pro-inflammatory and anti-inflammatory processes has been shown, and the processes serve to protect the animal against pathogens and prevent autoimmune reactions. A close relationship has also been demonstrated between mycotoxicosis, an increase in the level of cytokines and Hp and the emerging non-specific clinical symptoms. However, the data are inadequate to indicate that upregulation of cytokines and APP provoke diseases in cows with mycotoxicosis. Cytokine and APP level assays may be treated as precise techniques for the assignment of the appropriate dose of mycotoxin absorbent or the investigation of how well it alleviates mycotoxicosis, because despite its use and the disappearance of clinical symptoms in the tested cows, high levels of IL-10, Hp and IL-6 were maintained.

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Animal Rights Statement: The authors declare that the experiments on animals were conducted in accordance with the laws and regulations of the local

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