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Changes in Protein Metabolism Indicators in Dairy Cows with Naturally Occurring Mycotoxicosis before and after Administration of a Mycotoxin Deactivator

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Abstract: The aim of the study was to evaluate selected indicators of protein metabolism in cows fed with fodder contaminated with mycotoxins after application of a mycotoxin deactivator product (MDP). Experimental group (Exp.)—10 cows, fed total mixed ration (TMR) containing: 0.769 mg/kg-deoxynivalenol and 0.032 mg/kg-zearalenone TMR DM. Control group (Con.)—10 cows fed TMR without mycotoxins. In the exp. cows, the mycotoxin deactivator product (MDP) Mycofix Plus was used in the form of an additive to TMR in the amount of 10 g/head/day for 90 days. Blood was taken in Exp. group three times, before MDP administration, and on days 30 and 90 of its use. In the con. group, blood was collected once. All cows were assessed for free amino acids, total protein, albumin, globulin, and urea. Cows with mycotoxicosis (before MDP administration) showed low total protein, albumin, total-essential (TEAA) and total-non-essential amino acids (TNEAA) compared to after MDP administration ($p < 0.01$). Compared to the control group, TNEAA values were lower and TEAA higher on all study dates ($p < 0.001$). The results of our research showed the negative impact of mycotoxins on the parameters of protein metabolism in cows, and the use of MDP improved the processes of protein metabolism and improved the overall health of cows.

Keywords: amino acids; deoxynivalenol; MDP-mycotoxin deactivator product; proteins; zearalenone

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1. Introduction

Mycotoxin contamination of feed is a serious worldwide problem in animal husbandry. The widespread occurrence of fungi and the production of mycotoxins by them, both during the growth of plants and during the storage of products, makes feeding dairy cows with fodder contaminated with mycotoxins seem unavoidable. Mycotoxins, depending on the type, dose and period of consumption, negatively affect animal health to varying degrees. It is generally believed that, due to the presence of a specific forestomach microflora, ruminants are less sensitive to poisoning by fungal toxins compared to monogastric animals. Some mycotoxins are inactivated by the rumen flora, others pass through the forestomachs unchanged, and others are converted into more toxic metabolites. Studies performed both in vivo and in vitro have shown that mycotoxins, through their destructive effects on the rumen microbial environment, negatively affect fermentation, feed digestibility, use of nutrients and, consequently, all metabolic processes [1–5]. The few available studies in ruminants have shown that ingested mycotoxins can disrupt protein metabolism in their bodies. These authors showed that mycotoxins limit the use of NH3-N by rumen microorganisms

for protein synthesis and reduce the amount of absorbed protein of microorganisms in the further parts of the digestive tract and their use by cows [4]. The consequences of protein metabolism disruption in the forestomachs are significant changes in free amino acid concentrations and protein metabolism parameters in the blood. Ogunade et al. [6] showed that abnormalities in protein metabolism in cows in early lactation consuming aflatoxin B1 were mainly associated with decreased serum alanine, leucine and arginine concentrations. On the other hand, Wang et al. [5] showed that mixed mycotoxicosis (aflatoxin + zearalenone) causes the disturbance of aminoacyl-tRNA biosynthesis and the disturbance of metabolism of three amino acids: alanine, aspartate and glutamate. However, in the available literature, it was not possible to find studies evaluating the effect of Fusarium mycotoxins (DON and ZEA) on the concentration of amino acids in the blood serum of cows. Therefore, there is a need for research that would supplement the information on protein transformations, and especially the changes in the concentration of free amino acids in the blood serum of dairy cows fed with feed naturally contaminated with DON and ZEA. In order to reduce the adverse effects of mycotoxins on the body, feed additives are used to enable the administration of contaminated feed without consequences for the health of fed animals and the safety of animal products [7–9]. In practice, the most common method is to add mycotoxin deactivating agents (MDP—mycotoxin deactivator product, Anti-Mycotoxin Additive) to the feed [7]. Complex preparations can adsorb mycotoxins (glucomannan, bentonite, medical charcoal), decompose them into non-toxic compounds, supplement the environment of the forestomach with microorganisms capable of decomposing mycotoxins and enhance the detoxifying function of liver. In cows, MDP preparations have been shown to reduce the toxic effects of mycotoxins on the body, have a positive effect on metabolic parameters, increase immunity and milk yield and improve the quality of milk and meat [10]. The MDP registered in the EU for use in pig and poultry farming is Mycofix Plus 3.E. Previous studies have shown that also in dairy cows, Mycofix Plus 3.E. prevents the negative effects of mycotoxins on the body and brings beneficial health and production effects [4,8,11,12].

The aim of the study was to evaluate free amino acids and other selected indicators of protein metabolism (total protein, albumins, globulins, urea) in the blood serum of dairy cows with naturally occurring mycotoxicosis (fed with feed contaminated with DON and ZEA), before and after the application of a mycotoxin deactivator (Mycofix Plus 3E).

2. Material and Methods

2.1. Animals

The study was approved by the Local Ethics Committee at the University of Life Sciences in Lublin (No. 41/2014). The study was carried out on 20 cows from two Holstein Friesian dairy herds of similar size and the same feeding and maintenance methods. The cows were kept in the so-called free-range system, and the feeding was based on the TMR (Total Mixed Ration) system. The complete fodder constituted a proper feed ration, adjusted to the physiological period of the cows. The composition of the feed ration in the experimental farm was balanced for lactating cows with an average milk production of 20 kg. Moreover, each cow, whose milk yield exceeded 20 kg of milk, additionally received 1 kg of concentrated feed for every 2 kg of additional milk produced. The nutritional dose 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 dedicated and properly equipped milking hall.

One herd was reported as problematic because many cows in this herd had non-specific symptoms of unknown origin: decreased appetite, diarrhea, weight loss, lameness, abomasum dislocation, decreased milk yield, increased number of somatic cells in milk, and infertility. In the studied herd, the toxicological study of TMR showed the presence of 0.032 mg/kg TMR dry mass (DM) zearalenone (ZEA) and 0.769 mg/kg TMR DM deoxynivalenol (DON). The mycotoxin content was assessed by Biomin laboratory (Biomin

GmbH, Herzogenburg, Austria), based on liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) technology. The average milk yield for cows in this herd, determined in the 305-day lactation period, was 6161–6725 kg of milk with fat content of 4.27–4.83% and protein content of 3.21–3.28%. From this herd, 10 cows were selected for which the ZEA content in their blood serum was confirmed as being 14.30 ± 3.64 and, for DON, 20.92 ± 5.94 ng/mL. These cows constituted the experimental group (Exp.). The plasma concentrations of ZEA and DON were determined according to the literature data [13,14].

From the second herd of cows with no health problems, 10 clinically healthy cows were also selected as the control group (Con.). The annual milk yield in this herd, determined in the 305-day lactation, was 8 571–8 670 kg of milk with fat content of 4.51–4.84% and protein content of 3.14–3.42%.

All selected cows were in the period of 60 +/− 20 days postpartum. Cows were in their second to the fourth lactation. The animals' body condition was rated as "good" or "very good" (Body Condition Score 3.0–3.25 in Exp. cows and 3.5–4.0 in Con. cows), on a five-point scale [15]. The assessment of the general health of all cows selected for the study was carried out on the basis of a clinical examination, clinical observations and data from the interview with the owners of the cows. A reproductive system control in the herds was conducted regularly at monthly intervals by rectal examination combined with ultrasonography. Cows with no complications during parturition and no signs of inflammation were applied to the synchronization protocol of estrus and ovulation (the Presynch-Ovsynch protocol) and artificial insemination (AI) with frozen semen. The cows with uterine inflammation were properly treated and subsequently subjected to the synchronization protocol of estrus and ovulation and AI. The cows with ovarian cycle disturbances were treated individually according to the diagnosed cause. Pregnancy checking was performed routinely at around 30 to 40 days after insemination by rectal examination combined with ultrasonography. The expected date of parturition was determined by adding 280 days from the day of artificial insemination and it was also supported by the pregnancy diagnosis.

In a herd where the presence of mycotoxins was confirmed after the first collection of material for research (blood), the administration of the preparation Mycofix Plus 3.E (Biomin GmbH, Herzogenburg, Austria) was started. The composition of the preparation was reserved; the manufacturer only provides general information that the preparation contains specific enzymes that eliminate toxicity, adsorbents, carefully selected plants and algae extracts. Mycofix was administered at a dose of 10 g/cow per day for 3 months as an addition to the feed. A measured dose of the preparation for the appropriate number of cows was added to the fodder cart, due to which the food dose was well mixed with Mycofix, and then the TMR mixture was administered to all of the cows in the herd.

2.2. Sampling of Blood

Blood for tests from experimental cows was obtained 3 times, the first time after confirming the presence of mycotoxins in the feed (before starting Mycofix administration) and after 30 and 90 days of using the mycotoxin deactivator. From the control cows, blood was taken once to be referenced in the experimental group. Blood samples (9 mL) were collected from the external jugular vein into Vacutest clot activator tubes (Vacutest Kima srl, Arzergrande (PD), Italy). Blood samples were centrifuged at 3000 r.p.m. 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.

2.3. Mycotoxicological Tests in the Blood

The determination of ZEA and DON plasma concentrations was conducted by combined separation techniques with the use of immunoaffinity columns (Zearala-TestTM Zearalenone Testing System, G1012, VICAM, Watertown, MA, USA, and DON-TestTM

DON Testing System, VICAM, Watertown, MA, USA) and high-performance liquid chromatography (HPLC) with fluorescence detection [13,14].

2.4. Measurements of Protein Metabolism Parameters in Blood Serum

Total protein, albumin and urea were determined in the blood serum using reagents: Accent-200 Total Protein, Accent-200 Albumin, Accent-200 Urea (PZ Cormay S.A., Lomianki, Poland). The examinations analyses were performed using a BS-160 analyzer (Mindray Medical International Limited, Shenzhen, China). The concentration of globulins was calculated based on a difference between total protein and albumin. Albumin/globulin ratio was calculated from albumin and globulin concentrations.

2.5. Measurements of the Concentration of Free Amino Acids in the Blood Serum

The serum concentration of free amino acids determination was performed using ion-exchange chromatography in Ingos AAA-400 apparatus for automatic analysis of amino acids (Ingos s.r.o., Prague, Czech Republic). One milliliter of serum was added to 1 mL of 6.0% buffered sulfosalicylic acid, pH 2,9. Then, the sample was centrifuged for 15 min at 12,000 rpm using the centrifuge MPW 250 (MPW Med. Instruments, Warsaw, Poland). The obtained supernatant was used for the determination of free amino acid contents. Amino acids were separated on analytic column (Ostion LG FA, 3 mm $\times$ 200 mm). During this parathion, five lithium citrate buffers of different pH (2.9, 3.1, 3.35, 4.05, and 4.9) were used. The amino acids were derivatized with ninhydrin and their identification was performed on the basis of retention time in comparison to the standards using photocell combined with a computer. Analytical separation of acidic and alkaline amino acids was performed at 38–39 °C, while neutral amino acids were separated at 59–60 °C. The original software mikro version 1.8.0 (Ingos Corp., Prague, Czech Republic) was used for amino acid evaluation. The following essential (EAA) and non-essential (NEAA) amino acids were determined: NEAA—alanine (Ala), asparagine (Asp), aspartic acid (Asn), glycine (Gly), glutamine (Gln), glutamic acid (Glu), ornithine (Orn), serine (Ser), tyrosine (Tyr), proline (Pro) and EAA—arginine (Arg), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), valine (Val).

2.6. Statistical Analysis

All values are presented as means $\pm$ SEM. Statistical analysis was performed using the Statistica software (version 10.0). Data were found to be normally distributed, as demonstrated by Kolomogorov–Smirnov test and Lillefors correction. The obtained values were compared between cows with mycotoxicosis and healthy cows, using the non-paired Bonferroni post hoc multiple comparison test. p-value < 0.05 was considered statistically significant. Statistical differences between the results of the materials collected at different times in the group were calculated using Tukey's and Duncan's post hoc tests for a probability value of $p \leq 0.01$.

3. Results

3.1. Clinical Examination and Observation of Cows with Mycotoxicosis

The observations were carried out for 3 months from the moment the feed contamination with mycotoxins was detected. Throughout this period, the cows were fed with the mycotoxin adsorbent Mycofix as an addition to the feed. In the first stage of the research, before starting the administration of the adsorbent, in experimental cows, many non-specific symptoms, which were already mentioned, were observed: decreased appetite, diarrhea, weight loss (BCS-3.0), lameness, reduced milk production (20.2 $\pm$ 0.5 kg/head/day), increased number of somatic cells in milk (743 $\pm$ 50.1 $\times$ 10^3/mL), ovarian cysts were also occurring (accordingly, with earlier studies accepted for publication [16]). After one month of using Mycofix, the following outcomes were shown: an improved appetite, occasional diarrhea, an improvement in cows' mobility, a slight increase in body weight (BCS-3.2), slight increase in milk production (21.0 $\pm$ 0.5 kg/head/day) compared to before MDP

administration ($p < 0.003$) and reduced somatic cell count in milk (536 ± 30.2 × 10^3/mL) compared to before MDP administration ($p < 0.001$). After 3 months of the administration of Mycofix, most of the symptoms subsided: the appetite returned to normal, the cows ate willingly, they had no diarrhea, increased body weight (BCS-3.5-4.0), no lameness, increased milk production (23.4 ± 1.0 kg/head/day) compared to before MDP administration and after 30 days of MDP use ($p < 0.001$), a decrease in the number of somatic cells in milk (354 ± 20.3 × 10^3/mL) compared to before MDP administration and after 30 days of MDP use ($p < 0.001$). Control cows were healthy BSC-4, the milk yield (28.1 ± 1.0 kg/head/day) was higher compared to all study dates in the experimental group ($p < 0.001$), and the somatic cell count in milk (325 ± 10.2 × 10^3/mL) was lower compared to all study dates in the experimental group ($p < 0.001$) (accordingly, with earlier studies accepted for publication [16]).

3.2. Protein Metabolism Parameters

The obtained values of protein metabolism parameters (total protein, albumin, globulin, albumin/globulin ratio and urea) are presented in Table 1. The total protein concentration on the 30th and 90th day of the experiment was significantly higher compared to the values before MDP administration and to the control group ($p \leq 0.01$). The level of albumin on the 90th day of the experiment was higher compared to the values before MDP administration ($p \leq 0.01$), and similar to the control group. The lowest albumin concentration was obtained from the samples before MDP administration. The concentration of globulins on the 30th day of the experiment was higher compared to the control group ($p \leq 0.05$).

Table 1. Parameters of protein metabolism in the blood serum of cows in the control and experimental groups (Mean ± SD).

Parameters	Control Group	Experimental Group		
		Before MDP Administration	30th Day of MDP Use	90th Day of MDP Use
Total protein [g/L]	83.78 ± 2.01	80.86 ± 2.05 a	86.3 ± 2.08 *b	86.62 ± 2.15 *b
Albumin [g/L]	44.32 ± 2.11	37.88 ± 2.27 **a	42.25 ± 2.44 ab	44.0 ± 2.56 b
Globulin [g/L]	38.86 ± 4.47	40.94 ± 2.29	44.05 ± 2.73 *	41.80 ± 2.99
Albumin/Globulin ratio	1.14 ± 0.17	0.92 ± 0.17	0.95 ± 0.17	1.04 ± 0.17
Urea [mmol/L]	3.23 ± 0.70	2.45 ± 0.57 *	2.54 ± 0.55	2.81 ± 0.41

* ($p \leq 0.05$); ** ($p \leq 0.001$)—significant difference between experimental and control groups by Bonferroni-test. a, b statistically significant differences between particular dates of examinations between the experimental groups, at the level of ($p \leq 0.01$).

3.3. Free Amino Acid Concentration

The results of serum amino acids concentration in the course of the experiment are presented in Table 2. Among the non-essential amino acids (NEAA) on the 30th day of the experiment, the levels of Asn, Glu, Ser were significantly ($p \leq 0.01$) higher, while the concentration of Gln was significantly ($p \leq 0.01$) lower compared to the values before MDP administration. On the 90th day of the experiment, the concentrations of Asn, Gln, Orn were significantly higher ($p \leq 0.01$), while the concentrations of Glu, Gly, Asp were significantly lower ($p \leq 0.001$) compared to the values before MDP administration. The concentration of Asn, Orn was significantly higher compared to the control group ($p \leq 0.01$). However, the level of Glu, Gly, Ser was lower compared to the control group ($p \leq 0.01$). The total NEAA concentration in the experimental cows was significantly lower throughout the experiment compared to the control group ($p \leq 0.01$). Among the essential amino acids (EAA), only the concentration of Arg on the 90th day of the experiment was significantly higher both compared to the values before MDP administration ($p \leq 0.01$) and to the control group. The total EAA concentration in the experimental cows significantly increased with subsequent testing dates ($p < 0.01$), all obtained values were higher compared to the control group ($p < 0.001$).

Table 2. The concentration of non-essential and essential amino acid in the blood serum of cows in the control and experimental group [μmol/L] (Mean ± SD).

Amino Acids	Control Group	Experimental Group		
		Before MDP Administration	30th Day of MDP Use	90th Day of MDP Use
Non—essential amino acid (NEAA)				
Alanine (Ala)	190.9 ± 45.3	188.7 ± 20.6	197.3 ± 35.4	192.0 ± 29.5
Aspartic acid (Asn)	11.1 ± 3.9	8.6 ± 5.3 a	16.5 ± 7.7 b	45.8 ± 15.5 **c
Asparagine (Asp)	19.8 ± 7.4	24.4 ± 6.2 a	21.8 ± 4.5 ab	18.2 ± 1.6 b
Glutamine (Gln)	248.2 ± 30.7	221.8 ± 21.0 a	196.2 ± 42.4 *b	256.8 ± 14.8 c
Glutamic acid (Glu)	74.5 ± 16.4	78.9 ± 13.7 a	94.1 ± 19.1 b	57.8 ± 9.9 *c
Glycine (Gly)	554.9 ± 127.9	403.8 ± 67.0 *	461.0 ± 86.0	350.5 ± 62.3 **
Ornithine (Orn)	31.6 ± 7.2	38.3 ± 11.1 a	46.7 ± 11.8 *ab	53.8 ± 12.2 **b
Proline (Pro)	5.9 ± 2.8	4.0 ± 3.0	2.5 ± 3.8	5.5 ± 2.6
Serine (Ser)	106.2 ± 26.9	64.1 ± 13.1 **a	84.8 ± 14.9 b	71.8 ± 14.3 **
Tyrosine (Tyr)	31.6 ± 7.2	38.4 ± 5.2	42.0 ± 7.3 *	42.0 ± 10.3 *
Total NEAA	1274.5 ± 28.1	1070.9 ± 16.6 **a	1162.9 ± 23.3 **b	1094.2 ± 17.3 **c
Essential amino acids (EAA)				
Arginine (Arg)	140.6 ± 19.6	155.4 ± 17.6 a	167.3 ± 35.2 ab	197.9 ± 30.0 **b
Histidine (His)	54.4 ± 12.0	48.1 ± 6.9	45.0 ± 10.1	51.8 ± 12.2
Isoleucine (Ile)	79.6 ± 16.5	80.4 ± 12.7	84.3 ± 16.4	79.8 ± 8.4
Leucine (Leu)	85.8 ± 21.7	104.9 ± 16.8	93.7 ± 17.7	107.8 ± 11.3 *
Lysine (Lys)	74.4 ± 7.8	80 ± 13.7	95.7 ± 18.5 *	83.5 ± 19.3
Methionine (Met)	20.1 ± 2.4	17.2 ± 4.0	17.7 ± 4.4	19.4 ± 4.6
Phenylalanine (Phe)	47.4 ± 6.9	51.0 ± 5.3	46.9 ± 6.5	45.6 ± 7.4
Threonine (Thr)	62.5 ± 14.6	61.3 ± 10.2	74.7 ± 15.0	73.2 ± 8.7
Tryptophan (Trp)	12.6 ± 6.6	18.6 ± 5.6	24.7 ± 9.5 *	25.8 ± 11.2 *
Valine (Val)	180.5 ± 25.9	197.4 ± 16.2	188.5 ± 33.8	176.6 ± 32.2
Total EAA	757.8 ± 13.4	814.3 ± 10.9 **a	838.5 ± 16.7 **b	861.4 ± 14.5 **c

* ($p \leq 0.05$); ** ($p \leq 0.001$)—significant difference between experimental and control groups by Bonferroni test. a, b, c—statistically significant differences between particular dates of examinations between the experimental groups, at the level of ($p \leq 0.01$).

4. Discussion

The general knowledge of animal mycotoxicosis proves that ruminants are the animals most resistant to mycotoxins because the forestomach microbiota quite efficiently decompose and inactivate them, and therefore protect their hosts [17,18]. However, not all fungal metabolites are affected by rumen microbial enzymes and various mycotoxins, using their antimicrobial, antiprotozoal and antifungal effects, are able to quantitatively reduce and modify the rumen microbiota [19,20]. It has been documented that exposure of animals to fusarium mycotoxins (ZEN, DON), even at low doses, adversely affects the stability of the gastrointestinal biocenosis, which is an important indicator of animal health [21]. The modified rumen microbiota is unable to ensure neither the proper distribution and use of the ration, nor the appropriate deactivation of mycotoxins, which penetrate into the further parts of the digestive tract and are absorbed into the blood as in monogastric animals [19,22]. Therefore, the initially observed symptoms differ from typical mycotoxin poisoning and resemble malnutrition with accompanying dysbacteriosis, leading to acidosis, slowing down the processes of digesting feed—weight loss and mild diarrhea with undigested fiber in the feces. Long-term consumption of feed with mycotoxins, leads to a decrease in milk production with an increase in the number of somatic cells in milk, animals with laminitis and infectious diseases also appear [23–25]. The reproductive performance of cows in diseased herds also decreases, as a result of significant metabolic and hormonal changes and the occurrence of a negative energy balance in animals [26]. Most of the described symptoms were observed in the herd from which the experimental cows were selected. A three-month observation of the herd showed that the administration of Mycofix was

associated with a gradual improvement in the health of cows in the herd and a gradual disappearance of the above-mentioned disease symptoms. After this time (3 months), the number of health issues in the cows was comparable to the herd from which the control cows originated.

Protein metabolism indicators evaluated before MDP administration in cows with mycotoxicosis showed that the concentration of total protein, albumin, albumin/globulin ratio, urea and total-non-essential amino acids (TNEAA) in experimental cows was lower (albumin, urea and TNEAA were significantly so) compared to control cows. Presumably, it was a reaction related to the impact of mycotoxins contained in the feed, which contributed to limiting the absorption of protein products. Mycotoxins lead to this mainly through a weakened appetite and lower intake of feed rations but also through a reduction in the amount of protein produced by microorganisms, which can result from modification of the rumen bacterial flora [19,22]. Under the influence of Fusarium mycotoxins in the rumen, there is a reduced utilization of available amino acids for the synthesis of bacterial proteins [2]. The consequence of limiting the amount of absorbed protein was a decrease in the value of the assessed parameters of protein metabolism in the blood serum of the examined cows. The confirmation of our hypothesis is provided by the results of further studies, following the introduction of a mycotoxin adsorbent (MDP) into the feed ration. After using Mycofix for three months, there was an insignificant increase in most of the described indicators, and only in the case of albumin was the increase statistically significant, which were comparable to the control group. The observed increase in the concentration of total protein was mainly due to the increase in the concentration of albumin and, to a lesser extent, globulins. Similar changes in the level of total protein and urea in cows with mycotoxicosis after the use of MDP were demonstrated by other authors [11]. Jovaisiene et al. [8], also after the application of Mycofix for mycotoxicosis in cows, showed an increase in albumin compared to values before MDP administration as well as to cows of the control group. The applied preparation, by reducing the activity of mycotoxins in the rumen and their absorption in the further parts of the digestive tract, presumably caused the gradual return of the normal microenvironment of the digestive tract, increased intake, better distribution and absorption of feed, and consequently the improvement of protein metabolism indicators observed in our study [4].

Our own research in cows consuming feed contaminated with DON and ZEN also showed changes in free amino acid concentration. In these cows, prior to MDP administration, the values of total non-essential amino acid (TNEAA), including serine (Ser) and glycine (Gly), were significantly lower compared to the values obtained in control cows. On the other hand, on the 90th day of MDP use, the concentration of these amino acids was even lower compared to the values before MDP administration and to the control group. A significant simultaneous decrease in the concentration of Ser and Gly may result from the combined metabolism of both amino acids (serine is a precursor of glycine) and the possibility of mutual conversion, which would mean that a decrease in one of them may also result in a decrease in the other [27,28]. Glycine is an amino acid used during detoxification processes in the liver, it combines with deoxycholic acid and, in this way, participates in the proper circulation of bile acids and enhances bile secretion [28]. The reduced Gly concentration seen in our study seems to confirm the possibility of using it to improve the circulation of bile acids and, at the same time, to improve the detoxifying function of the liver. This was clearly evident after 3 months of using Mycofix, which by inhibiting the negative effects of mycotoxins and due to the plant ingredients it contains, has the ability to improve the functional state of the liver [8,12]. After 90 days of MDP use, increases in glutamine (Gln) and decreases in glutamic acid (Glu) were also observed. These amino acids are also interdependent and metabolically related. In ruminants, amino acids absorbed from the gut are transported to the liver, where they are converted to Glu. In the course of further metabolic processes, Glu is converted to Gln, resulting in a decrease in glutamic acid levels and an increase in glutamine levels in the blood [27]. Such changes were observed in the presented research. Glutamine is crucial for the proper functioning of

immune system cells and has antioxidant properties [29]. Some authors believe that the increase in Gln improves the function of the immune system and enhances antioxidant processes, leading to a reduction in inflammation [30]. Our earlier research [16] in the same cows confirmed the presence of a strong inflammatory process during mycotoxicosis and its regression after using Mycofix. The reduction in inflammatory parameters in conjunction with the increase in glutamine levels seems to confirm the involvement of this amino acid in the suppression of inflammatory processes, as has been suggested by a study conducted by Nemati et al. [29]. These authors also showed that the feeding of rumen-protected glutamine (rumen-protected Gln) led to an improvement in the general health of animals and an increase in the parameters of protein metabolism [29].

In our research, in cows with mycotoxicosis, the concentration of asparagine (Asn), ornithine (Orn) and arginine (Arg) was higher compared to control cows and constantly increased after the application of Mycofix. The reason for an increase in the levels of these amino acids and what mechanisms influenced this is difficult to explain. It is possible that the increase in the level of these amino acids is the result of increased neutralization of ammonium ions (NH4+), formed in excess in the rumen as a result of abnormal degradation of the feed (mainly protein), due to the destructive effect of mycotoxins on the rumen microbiota [5,31]. Studies by some authors confirm a significant increase in ammonia concentration in rumen contents of cows with mycotoxicosis [2]. Ammonium ions have strong alkalizing properties, making them toxic and therefore they cannot be accumulated in the body in high concentrations; they must be converted to urea in the liver and excreted in this form. In the occurring urea cycle and in the transformations preceding this cycle, glutamic acid (Glu) and aspartic acid (Asp) are used, from which asparagine (Asn), ornithine (Orn) and arginine (Arg) are formed as a result of the occurring transformations [27]. This may explain the results of our own research in the form of reduced concentrations of Glu and Asp and an increase in Asn, Orn and Arg. It is possible that the results of this study, especially regarding free amino acid levels, were influenced by other mechanisms not addressed in the discussion, but this is a very complex issue and difficult to explain completely.

5. Conclusions

The present results showed many non-specific symptoms: decreased appetite, diarrhea, weight loss, lameness, increased number of somatic cells in milk and ovarian cysts in dairy cows with natural feed mycotoxicosis. In addition, in these cows, it showed low total protein, albumin, total-essential (TEAA) and total-non-essential amino acids (TNEAA) in the serum of cows. After 3 months of using the mycotoxin adsorbent, the disease symptoms and the mentioned parameters of protein metabolism were improved significantly. Based on the present results, we can conclude that Mycofix is an effective preparation that inhibits the action of mycotoxins in the described scope of our research.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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