

EFFECTS OF MYCOTOXIN SEQUESTERING AGENTS ADDED INTO FEED ON HEALTH, REPRODUCTION AND MILK YIELD OF DAIRY CATTLE

Michal Hulík¹, Ladislav Zeman¹

¹Department of Animal Nutrition and Forage Production, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Abstract

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Effects of mycotoxin sequestering agents in feed on health, reproduction and milk yield of dairy cattle were studied in a 5-month long experiment on 300 dairy cows divided into two groups and six subgroups. The experiment was conducted in adding a mycotoxin sequestering agent based on 1,3 and 1,6 β -glucans to standard cattle nutrition (TMR), which was regularly tested for content of important mycotoxins, in order to gain knowledge about possible positive effect of this agent on the health of dairy cattle and about possible avoidance of negative effects of mycotoxins on dairy cattle due to their structural elimination caused by the agent. The experiment's setting and conditions during it were in all aspects common and comparable within the European Union, the experiment's results should be therefore seen as relevant. Health, pregnancy rate and milk yield were carefully monitored during the experiment. Indicators of state of health (occurrence of mastitis and somatic cell count in milk) did not show any significant differences between test and control groups of dairy cows. The average milk yield of dairy cows which were fed the agent enriched feed (30.2 kg a day) was slightly lower in comparison to control groups (31 kg a day, both results with $P < 0.001$), however, fat content of milk of test groups' cows (4.02%) was considerably higher than that of control groups' cows (3.79%). The average pregnancy rate of cows which were fed the agent enriched feed also manifested considerable increase in percentage and stability (from 42.95% of control groups' cows to 62.25% of test groups' cows, the standard deviation decreased from 21.1% to 14.4% which means smaller differences among pregnancy rate of test groups' cows, hence higher stability), this increase manifested even long after the cows had been fed regular feed again.

Keywords: mycotoxins, cattle nutrition, β -glucans, pregnancy rate of cattle, milk yield of cattle

INTRODUCTION

Mycotoxins represent one of significant obstacles in intensifying agricultural production. Aside of possible contamination of consumer products, mycotoxins affect health of animals which are exposed to them in feed or environment. Secondary metabolites of moulds which cause health problems after being consumed or absorbed by animals are called mycotoxins. They are produced when molds are not under optimal conditions (WOLF, 2002; WÖHLER, 2003), such as sufficiently

high temperature, humidity and amount of carbohydrates (LEPOM *et al.*, 1990). NEDĚLNÍK and MORAVCOVÁ (2005) estimate the economic losses in livestock business caused by mycotoxins contamination of feed are worth millions of dollars worldwide.

Amongst livestock which may be exposed to negative effects of mycotoxins are dairy cattle. Contaminants of forages which are fed by cattle are with respect to climatic conditions of the Czech Republic mainly deoxynivalenol and zearalenone in grasses (SKLÁDANKA *et al.*,

2011) and mycotoxins produced by molds of genus *Aspergillus* and *Penicillium* in conserved forages. In forages (grasses and silages) the most common mycotoxins are aflatoxins, fumonisins, ochratoxin A, patulin, zearalenone and also trichothecene mycotoxins among them mainly deoxynivalenol and T-2 toxin (NEDĚLNÍK *et al.*, 2011).

Effects of aflatoxins, fumonisins, ochratoxin A and patulin on dairy cattle are not significant in normal concentrations due to their fast degradation by rumen microorganisms and fast excretion. (JOUANY and DIAZ, 2005). In terms of effects and average concentration in dairy cattle feed the most important mycotoxins are deoxynivalenol, T-2 toxin and zearalenone.

Generally speaking, the combined effect of mycotoxins in feed varies depending on many variables. Aside of kind of mycotoxin, dose, exposure time, the effect of mycotoxins on an individual animal depends mainly on its species, sex, age and state of health. On effects of mycotoxins among cattle JOUANY and DIAZ (2005) state that deoxynivalenol (known as vomitoxin) causes lowered feed intake, lowered milk yield and fat content of milk; that T-2 toxin causes rejection of feed, lesions in gastrointestinal tract, it is possible that T-2 toxin is cause of internal bleeding; that zearalenone which is almost entirely degraded in rumen to α -zearalenol affects negatively reproductive organs because of its structural similarity to estrogen, it causes decrease in pregnancy rate and disrupts hormonal equilibrium.

Results of the intensive research on mycotoxins also suggest that there are substances which can neutralize certain specific mycotoxins. Present-day neutralizing agents (or mycotoxin sequestering agents) function as absorbers or they neutralize mycotoxins chemically, only specific mycotoxin or group of mycotoxins can be neutralized using one agent, though (DIAZ and SMITH, 2005).

With respect to chemical properties of the most common mycotoxins in the Czech Republic, inactive biomass of *Sacharomyces cerevisiae* is used mainly. Cell wall of *Sacharomyces cerevisiae* is a rich source of 1,3 and 1,6 β -glucans which efficiently neutralize trichothecens, zearalenone by adsorption. Adsorbed mycotoxins are harmless and are excreted naturally. Inactive biomass of *Sacharomyces cerevisiae* added into cattle feed is used to prevent possible negative effects of mycotoxins (NEDĚLNÍK *et al.*, 2011).

The experiment's objective was to determine what the effect of natural level of mycotoxins on health, pregnancy rate, milk yield, parameters of milk of dairy cattle is. Using analytical methods, amounts of mycotoxins in feed at a selected farm were found and it was studied if mycotoxin sequestering agent (based on 1,3 and 1,6 β -glucans) enrichment of feed has a positive effect on dairy cattle.

MATERIAL AND METHODS

The experiment was conducted during the time between September 2009 and February 2010 in stable of Agrodužstvo Sebranice farm (Holstein dairy cows). Average milk production at the beginning of experiment was 30.1 kg of milk. Average days in milk was 150. The stable environment and management condition were stable through all the experiment. The outside temperature corresponded to season of year and latitude. Daily average temperatures ranged from 14 to 17°C in September, from 11 to 13°C in October, from 8 to 10°C in November, from 5 to 6°C in December, from -3 to 3°C in January and at the end of experiment in early February from -6 to -2°C. The stable is of modern construction of sufficient volume of air, of loose box stabling with straw bedding. Milking robots (Lelly Astronaut) are used for milking. Milk production was measured daily for each dairy cow individually. Feeding of total mixed ratio was presented to dairy cows two times per day in 12 hours period and feed was pushed 4 times per day.

The cows were divided into two groups and six subgroups, 50 cows each. The experiment was carried out in 5 phases:

- Preparation:** all subgroups were fed standard feed, all studied parameters were monitored.
- First test phase:** half of the subgroups were fed agent enriched feed, the other half were control groups, all studied parameters were monitored.
- Wash-out phases:** all subgroups were fed standard feed in order to eliminate the possible group effect, all studied parameters were monitored.
- Second test phase:** the control subgroups from the first test phase were fed agent enriched feed, hence became test subgroups, the other half were control subgroups, all studied parameters were monitored.
- Termination:** all subgroups were fed standard feed, all studied parameters were monitored.

Detailed scheme of the experiment is in the Tab. I.

The monitored parameters were milk yield and parameters of milk and somatic cell count in milk. Those parameters were monitored individually in all phases of the experiment; they were later processed by the National breeding organization. Occurrences of mastitis and pregnancy rate were recorded as well. The pregnancy rate is a result of dividing number of cows which were found pregnant 2 months after insemination using sonograph by total number of cows inseminated in given phase.

During the experiment, the cows were fed standard cattle nutrition (TMR – total mix ration Tab. II) two times per day and milked by Lelly

I: *Experimental scheme*

Parameters	Days	Experimental Groups 1			Experimental Groups 2		
Number of robots		2	3	4	5	6	7
Number of cows		50	50	50	50	50	50
Preparation phase	20	M–	M–	M–	M–	M–	M–
1 st test phase	43	M+	M+	M+	M–	M–	M–
Wash-out phase	19	M–	M–	M–	M–	M–	M–
2 nd test phase	40	M–	M–	M–	M+	M+	M+
Termination phase	39	M–	M–	M–	M–	M–	M–

Darker shade and symbol M+ indicates presence of mycotoxin sequestering agent in feed, lighter shade and symbol M– indicates its absence in feed.

II: *Composition of total mix ration TMR*

Composition of total mix ration TMR			
Ingredient	unit	fresh	dry matter
Wheat straw	kg/day	0.60	0.53
Hay grass	kg/day	1.00	0.89
Barley whole crop silage	kg/day	5.00	1.85
Corn silage	kg/day	17.50	5.67
Clover silage	kg/day	9.00	2.43
Corn cob mix	kg/day	3.00	2.01
Distillers grain corn wet	kg/day	3.30	0.83
Glycerol	kg/day	0.10	0.04
Citrus pulps	kg/day	1.20	1.09
TMR mixture	kg/day	4.80	4.36
ROBOT mixture	kg/day	5.50	4.95
Total	kg/day	51.00	24.65

III: *Mixture for TMR diets*

TMR mixture	
Nutrient	% contents
Premix	14.9
Barley	8.0
Corn	24.0
Rapeseed meal	7.0
Rapeseed whole	18.2
Soy bean meal (48% crude protein)	27.0
Oil vegetable	0.9
Total	100.0

IV: *Mixture fed in robot place*

ROBOT mixture	
Nutrient	% contents
Sugar	5.0
Barley	18.0
Corn	12.0
Wheat	17.0
Rapeseed meal	29.0
Soy bean meal (48% crude protein)	18.5
Oil vegetable	0.5
Total	100.0
M+ contained 1,3 & 1,6 β -glucans	0.4

Astronaut milking robots. Composition of, nutritional values in TMR, and composition of premix were monthly analyzed and prepared by an advisory company *Mikrop Čebín a. s.* TMR also contained concentrate named by TMR mixture its composition is in Tab. III. During milking, the cows were fed supplementary concentrate named ROBOT mixture. This ROBOT mixture contained during testing M+ period 0.4% of 1,3 and 1,6 β -glucans based mycotoxin sequestering agent. During trial period the cows were fed 20 grams of 1,3 and 1,6 β -glucans based mycotoxin sequestering agent a day. Compositions of ROBOT mixture is in the Tab. IV.

In each phase, a sample of TMR was taken in order to analyze content of mycotoxins deoxinivalenol (DON), T-2 toxin and zearalenone. To analyze the samples, method of ELISA TEST was used. Analyses were conducted in an accredited laboratory n. 1144 of Brno bureau of State Veterinary Institute in Olomouc and in accordance with unified methodic CIA ČSN EN ISO 17025:2005.

Obtained data from experiment were statistically analyzed by methods of SNEDECOR and COCHRAN (1989).

V: Analysis of mycotoxins and comparison with condition in the Czech republic

Mycotoxins	Deoxyvanilenol		T-2 toxin		Zearalenon	
Date	TMR fresh	Average CR	TMR fresh	Average CR	TMR fresh	Average CR
10 th –30 th September	328	626	36	79	92	190
1 st October–13 th November	178	449	354	53	92	108
14 th November–3 rd December	538	294	25	73	86	114
4 th December–13 th January	358	565	25	116	77	52
14 th January–22 nd February	304	433	29	53	135	101
All values are in ug/kg	---	465	---	30	---	136
EU 2006/576/EC	rec. max.: 5000		rec. max. not listed		rec. max.: 500	

Average CR = results of MVDr. Lichtenberg from the Czech republic in the same time

TMR fresh = analysis of total mix ratio fed to dairy cows during period

VI: Results on milk production and somatic cell counts

	units	(M-)	(M+)	statistical results	SE of effect (df)
Number of cows		150	150		
Average milk yield	kg/day	31	30.2	-0.8 **	0.27 (169)
ECFM (kg/d)	kg/day	33.1	33.3	0.2	0.39 (141)
Fat	%	3.79	4.02	0.23 **	0.069 (141)
Protein	%	3.55	3.57	0.02	0.015 (141)
Lactose	%	4.89	4.88	-0.01	0.019 (141)
Fat	gram/day	1176	1220	44	23.5 (141)
Protein	gram/day	1109	1086	-23 *	10.8 (141)
Lactose	gram/day	1545	1502	-43 **	16.0 (141)
SCC somatic cell counts	log units	5.01	5.06	0.05	0.043 (165)
SCC somatic cell counts	x1000/ml	101	113		

** P < 0.01; *** P < 0.001

RESULTS AND DISCUSSION

The experiment's conditions were normal in terms of mycotoxin content in TMR. As shown in Tab. V, in comparison with results from many different farms across the Czech Republic at the same time done by Mr. MVDr. Lichtenberg (State Veterinary Institute, 2009 and 2010, private notice) the mycotoxin content was nearly average. From Tab. V, it can be concluded that deoxinivalenol, T-2 toxin and zearalenone contents were sufficiently lower than levels of EU Commission Recommendation 2006/576/EC.

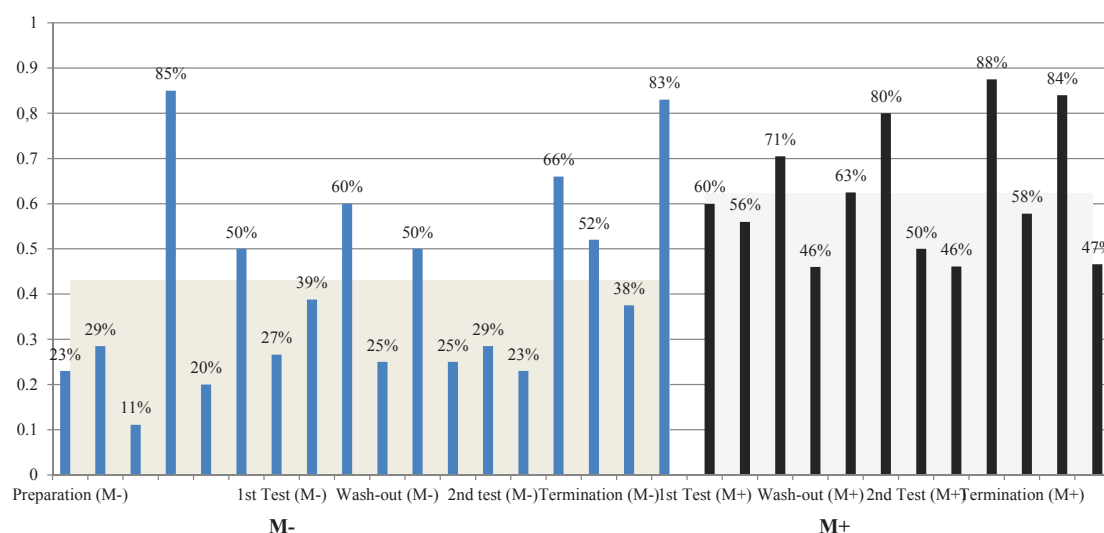
Concerning the first monitored parameter, in comparison of test and control groups of cows, the average daily milk yield of tested cows (Tab. VI) was 30.2 kg lower than that of control groups 31 kg. On the contrary, the average daily protein content in milk of test groups of cows 3.57% (1 186 grams) was higher than that of control groups of cows 3.57% (1 109 grams), so was the average daily fat content 4.02% (1 220 grams) of test groups compared to 3.59 (1 176 grams); both results P < 0.001. The raise of fat content in milk of test groups of cows can be probably attributed to raise of count of bacteria producing acetic acid, a milk fat precursor, caused by lower mycotoxin level which is a positive effect of 1,3 and 1,6 β -glucans enriched feed. This claimed causal relation can be supported by findings

of CHARMLEY *et al.* (1993) whose experiment showed substantial decrease of milk yield and milk fat content by 13% and 4% respectively of 18 cows in the middle of lactation which were fed non-toxic feed or feed with deoxinivalenol content of 2.7 or 6.4 ppm. Though statistically insignificant, the results of his experiment showed decrease of fat content in milk from 3.92 to 3.04% (P = 0.24). With our results, they can provide evidence, that high mycotoxin level in feed affects negatively the fat content in milk.

Concerning the somatic cell count in milk (Tab. VI), no clear relation between the monitored parameter and enrichment of feed by mycotoxin sequestering agent based on 1,3 and 1,6 β -glucans. Average level of SCC were during trial period stable vary from 100 000 to 113 000 CFU/ml of milk

Evaluation of occurrence of mastitis was done by dividing treated mastitis count in respective control or test group by number of days of the experiment. Amongst test groups, 237 occurrences of mastitis were treated in respective groups during 139 days of the experiment which means 1.7 treated mastitis a day. Among control groups, in respective phases of the experiment, 365 treated occurrences of mastitis occurred during 186 days, which means 1.96 treated mastitis a day. It can be supposed that agent enriched feed had a slightly positive effect

Pregnancy rates



1: Pregnancy rates in % during study

on state of health of dairy cattle in terms of mastitis occurrence.

The last monitored parameter was pregnancy rate. The pregnancy rate is a result of dividing number of cows in respective group which were found pregnant 2 months after insemination using sonograph by total number of cows inseminated in given phase. This characteristic ranged from 11 to 85%. These values are shown in a graph in the Fig. 1. In the left half of the graph, pregnancy rate values corresponding to control groups in all phases of the experiment. In the right half of the graph, pregnancy rate values corresponding to control groups in all phases of the experiment except the preparation phase, since it started with the preparation phase when all six subgroups were fed standard feed, average pregnancy rate thereof is labeled Preparation (M-). The experiment continued with the first test phase when a half of subgroups were fed agent enriched feed, average pregnancy rate thereof is labeled 1st Test (M+), the other half was fed normal feed, average pregnancy rate thereof is labeled 1st Test (M-). The experiment continued with the wash out phase when all six subgroups were fed standard feed, average pregnancy rate of subgroups which were fed agent enriched feed is labeled Wash-out (M+), average pregnancy rate of the other half of groups is labeled Wash-out (M-). In the second test phase, the control subgroups from the first test phase were fed agent enriched feed, hence test subgroups, average pregnancy rate thereof is labeled 2nd Test (M+), the other half were control subgroups, average pregnancy rate thereof is labeled 2nd Test (M-). In the terminal

phase, all subgroups were fed standard feed, average pregnancy rate of subgroups which were fed agent enriched feed is labeled Terminal (M+), and average pregnancy rate of the other half of subgroups is labeled Terminal (M-). From this graph, a clear increase in pregnancy rate and a significant decrease in variability of pregnancy rate are visible in comparison of M- and M+ groups.

Overall, the average pregnancy rate of test groups (M+), cows fed 1,3 and 1,6 β -glucans based agent enriched feed, was $62.25 \pm 14.4\%$ compared to $42.95 \pm 21.1\%$ of control groups (M-), the decrease in variability is shown in significantly lower standard deviation. Difference this significant can have a direct positive effect on economics of dairy cattle husbandry. This effect was probably caused by neutralization of zearalenone and other mycotoxins by the mycotoxin sequestering agent which may have protected reproductive organs from negative influence of mycotoxins. This claimed causal relation can be supported by findings of various authors (ROSSI *et al.*, 2009) describes that zearalenone and aflatoxins have direct negative impact on reproduction of dairy cattle due to dysfunction of ovaries. During his experiments in Hungary on Holstein cattle, GYULA *et al.* (1995) found out that occurrence of T-2 toxin and zearalenone in feed caused lowered ovaries function which resulted in lower pregnancy rates. In Turkey on 300 Holstein cows, OZSOY *et al.* (2005) found direct impact of aflatoxin B1 in feed on worsening laminitis occurrences and infertility of dairy cattle.

SUMMARY

Mycotoxins in feed have a significant negative impact on dairy cattle in terms of various characteristics, hence affects the economics of dairy cattle husbandry substantially. On the market, substances which

can neutralize mycotoxins, mycotoxin sequestering agents, are emerging. This article's objective was to assess effects of one mycotoxin sequestering agent on dairy cattle. The experiment was conducted in adding a mycotoxin sequestering agent based on 1,3 and 1,6 β -glucans to standard cattle nutrition (TMR), which was regularly tested for content of important mycotoxins, in order to gain knowledge about possible positive effect of this agent on the health of dairy cattle and about possible avoidance of negative effects of mycotoxins on dairy cattle due to their structural elimination caused by the agent. The experiment's setting and conditions during it were in all aspects common and comparable within the European Union, the experiment's results should be therefore seen as relevant. Health, pregnancy rate and milk yield were carefully monitored during the experiment. Indicators of state of health (occurrence of mastitis and somatic cell count in milk) did not show any significant differences between test and control groups of dairy cows. The average milk yield of dairy cows which were fed the agent enriched feed (30.2 kg a day) was slightly lower in comparison to control groups (31 kg a day, both results with $P < 0.001$), however, fat content of milk of test groups' cows (4.02%) was considerably higher than that of control groups' cows (3.79%). The average pregnancy rate of cows which were fed the agent enriched feed also manifested considerable increase in percentage and stability (from 42.95% of control groups' cows to 62.25% of test groups' cows, the standard deviation decreased from 21.1% to 14.4% which means smaller differences among pregnancy rate of test groups' cows, hence higher stability), this increase manifested even long after the cows had been fed regular feed again. These results can serve as a solid foundation for further research.

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Contact information

Michal Hulík: mhulik@alltech.com
Ladislav Zeman: zeman@mendelu.cz