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Investigation of milk quality after removal of AFM1 using lactic acid bacteria and beta-glucan

Istraživanje kvalitete mlijeka nakon uklanjanja AFM1 bakterija mliječne kiseline i beta-glukana

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Summary

Contamination of milk with aflatoxin M1 (AFM1) is related to the feed for milking cows, which is contaminated with aflatoxin B1 (AFB1). Feed AFB1 converts to AFM1 by dehydrogenation. In this study, we used Lactic acid bacteria (LAB) isolated from raw milk and its products and commercial or laboratory-made beta-glucan isolated from yeast and oats to establish how these mycotoxin binders affect the quality of sterilised, long-life, 2.8% fat milk contaminated with 0.05 mg/L of AFM1. We took the content of fats, carbohydrates, sugars (lactose), and proteins, and the calculated energy values for quality parameters. The mean energy value of the milk treated with AFM1 binders ranged between 85.7% and 101.5% of the control, untreated milk, whereas the fat content ranged between 65.3% and 100.7%. The protein content ranged between 64.4% and 101.1%, carbohydrates between 83.1% and 103%, and lactose between 76.3% and 100.8%. The results indicated a good possibility of binding of AFM1 with *Lactobacilus plantarum* bacteria, and 0.01% of β-glucan from oats was 0.005% of β-glucan isolated from yeast from *Saccharomyces cerevisiae* 20. These findings suggest that milk treated with these binders can be processed further and that its treatment significantly reduces the risk of exposure through diet and the related economic damage.

Key words: milk, aflatoxin M1, mycotoxin binders, quality of milk

Sažetak

Kontaminacija mlijeka aflatoksinom M1 (AFM1) vezana je uz kotaminaciju krmiva koje služi za konzumaciju mliječnim kravama, aflatoksinom B1 (AFB1), gdje se postupkom dehidrogenacije AFB1 pretvara u AFM1. U istraživanju su kao mikofiksatori korištene bakterije mliječne kiseline (BMK), izolirane iz neprerađenih mliječnih proizvoda, beta-glukan izoliran iz kvasca i iz zobi u laboratorijskim uvijetima, te komercijalni beta-glukan iz kvasca i zobi. Cilj istraživanja bio je utvrditi kako korišteni mikofiksatori utječu na kvalitetu mlijeka. Korišteno je sterilizirano trajno mlijeko s 2,8% mliječne masti, kontaminirano s AFM1 u količini od 0,05 mg/L, te je podvrgnuto djelovanju mikofiksatora. Određivane su masti, ugljikohidrati, šećeri (laktoza) i bjelančevine. Prosječna energetska vrijednost mlijeka nakon tretiranja mikofiksatorima kretala se u rasponu od 85,7% do 101,5% u odnosu na netretirano mlijeko, a količina masti u rasponu od 65,3% do 100,7%. Vrijednosti bjelančevina kretale su se od 64,4% do 101,1% u odnosu na vrijednosti prije tretiranja mlijeka mikofiksatorima, za ugljikohidrate vrijednosti su iznosile od 83,1% do 103,0%, a za laktozu od 76,3% do 100,8%. Rezultati su ukazali na dobru mogućnost vezivanja AFM1 s bakterijama *Lactobacilus plantarum*, a 0,01% β-glukana iz zobi bilo je 0,005% β-glukan izoliranog iz kvasca iz kvasca *Saccharomyces cerevisiae* 20. Istraživanje ukazuje na to da se mlijeko nakon primjene mikofiksatora može koristiti u daljnjoj preradi, te u prehrani ljudi i/ili životinja, što u velikoj mjeri smanjuje gospodarske štete koje mogu nastupiti prilikom kontaminacije mlijeka AFM1.

Ključne riječi: mlijeko, aflatoksin M1, mikofiksatori, kvaliteta mlijeka

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Introduction

Food contamination with mycotoxins, secondary products of fungi, is on the rise due to frequent and often extreme climate changes. Damaged seed becomes a good substrate for the growth of toxigenic fungi and mycotoxin production.[1,2,3] Mycotoxins are highly toxic to animals and humans,[4] to the extent that the International Agency for Research on Cancer (IARC) has classified some of them as Group 1 carcinogens for humans, including aflatoxin M_1 (AFM_1).[5] Considering that fungal contamination of food can cause great economic damage, its abatement has been gaining momentum, which also includes the prevention of mycotoxin production.[6] However, current methods used to remove mycotoxins from food vary in their efficacy, and the presence of mycotoxins is still rather common all over the world,[7] especially those produced by the *Aspergilus flavus* and the *Fusarium* species.[8,2] Contamination mostly hits cereals and bakery products, dry fruit, and milk, including milk-based infant food.[9,10] Aflatoxins find their way into milk through a carryover of aflatoxin B_1 (AFB_1) from feed given to milking cows that is transformed into aflatoxin M1 (AFM_1) by hydrogenation.[11,12,13] Research shows that 1-6% of AFB1 from feed is transformed to AFM_1 in milk.[14,15] The AFM_1 molecule resists heat treatment and does not break down during pasteurisation.[16] According to the European Commission Regulation 1881 from 2006, milk containing AFM_1 above the level 0.05 μg/kg and infant food containing over 0.025 μg/kg are considered contaminated and unsafe for consumption. Mixing unsafe with safe milk (usually to dilute it to "safe" levels) is strictly forbidden, so to reduce AFB_1 in animal feed and prevent milk contamination, the industry resorts to a variety of other methods, such as the use of additives.[17,18] Most of them are natural or synthetic adsorbents and mycotoxin binders such as zeolites and clay, but the problem is that they also bind nutrients in milk and affect its properties.[19] Some add microorganisms such as *Nannocystis exedens*, which reduces the toxicity of *Aspergillus flavus* and *Aspergillus parasiticus*.[20,21] The effectiveness of these methods is often below par, especially if timing is wrong, as the incidence of AFM_1 in milk is still quite common. To address this issue and minimise the economic damage caused by milk contamination, research has focused on the development of more efficient AFM_1 binders in milk such as lactic acid bacteria (LAB) and beta-glucans.[22] Kuharić et al.[23] reported outstanding AFM_1 binding by *Lactobacillus plantarum* bacteria, especially in the first hour milk treatment. Similar efficiency in mycotoxin binding was reported for beta-glucan obtained from yeast or oats, laboratory-made and commercial alike.[24]

However, little is still known about how these binders affect the quality of milk. The quality of milk depends on the content of macronutrients such as proteins, fats, carbohydrates, and sugar (lactose).[25] Fat or lactose content in milk, for example, determines whether it will be marketed as whole/light or lactose-free. Milk is a staple food for animals and humans, especially infants and children, but in some communities, it is also regularly consumed by adults and elderly people. Macronutrients contained in milk are important for normal growth and the development of children and elderly population health.[26] Their content seems to change with climate and the diet of the milking cows.[27] While lactose is sometimes associated with allergic reactions in some people, the benefits of milk are still considerable. It is an important source of proteins, calcium, and vitamin D.[28] Considering that the benefits of LAB and beta-glucan in removing AFM_1 from milk have already been established, the aim of our study was to see how they would affect the quality of milk, including energy values and the content of milk fats, carbohydrates, lactose, and proteins, and whether the treated milk would meet the quality requirements for human and/or animal consumption.

Materials and methods

The selected strain of BMK (*Lactobacillus plantarum*) examined the possibility of binding of aflatoxin M1 in milk by adding lyophilized cells of *Lactobacillus plantarum* and AFM1 to milk samples in which the presence of AFM1 was not detected, in the amount of 0.5 μg/L. AFM1 germ was determined immediately after contamination of AFM1 milk, and after 2 hours, after 4 hours and after 24 hours of incubation at 4°C. The binding success rate of AFM1 to BMK cells at 0 h was extremely good and averaged 80%. The binding efficiency of AFM1 with the addition of 0.01% β-glucan from oats was 65% at 0 hours, while with the addition of 0.005% β-glucan from yeast, the binding efficiency of AFM1 it was 63%.

We used commercial, sterilised (ultra-high-temperature-treated; UHT) milk with 2.8% fat, contaminated with 0.5 μg/L of AFM_1. We used lyophilised live or dead LAB cells of the *Lactobacillus plantarum* species for AFM_1 binding, as well as 5 mg/kg or 0.1 mg/kg of commercial and laboratory-made beta-glucan obtained from yeast or oats. The quality parameters – energy value and fat, carbohydrate, lactose, and protein content – were measured

within the first hour of contamination and treatment with AFM_1 binders for research has shown that these binders are the most efficient in the first hour.[29] In doing so, we followed the standard methods described by the various Official Methods of Analysis.[30-34] We used six samples for AFM_1 binding. We used for control three untreated samples of the same 2.8% fat commercial milk and three samples of milk added 0.5 μg/L AFM_1 with or without centrifugation. All the samples were analysed in triplicate and the results were expressed as mean values. The milk sample was contaminated with AFM1 so that the final concentration was 0.5 μg/kg. β-glucan was isolated from the cell wall of the yeast *Saccharomyces cerevisiae 20* for the implementation of this research, which is part of the collection of microorganisms of the Laboratory for General Microbiology and Food Microbiology, Faculty of Food Technology and Biotechnology, University of Zagreb, was used from Darvitalis (Zagreb, Croatia). High performance liquid chromatography (HLPC) and bound liquid chromatography and mass spectrometry (LC-MS/MS) were used to determine the amount of unbound AFM1.

Energy values in milk were calculated based on fat, carbohydrate, protein, water, and ash content and expressed in kcal or kJ per 100 g.

The milk fat content was defined as the content extracted with petroleum ether (Merck, Darmstadt, Germany), which includes the acid hydrolysis phase, using a Soxterm extractor SOX SE 416 (C. Gerhardt Analytical Systems, Königswinter, Germany). After the solvent evaporates and the sample cools down, the fat content is determined gravimetrically and expressed as mass fraction of grams per 100 g of sample (AOAC method No. 905.02, 2016). The lactose content was determined with a Shimadzu LC10 ADVP high-performance liquid chromatographer (HPLC) (Shimadzu Europa, Duisburg, Germany) on an amino-type column equipped with a refractive index (RI) detector, as described elsewhere.[31] The milk samples mixed with 50% acetonitrile were filtered, and lactose content determined against external standard using a calibration curve with the analyte retention times.

The protein content was determined in milk samples digested in a Kjeldatherm KT-20s block digestion unit (C. Gerhardt Analytical Systems) by titration in a Kjeltec 8400 analyser (Foss Analitcs, Hilleroed, Denmark), as described elsewhere in detail.[32] This method is based on organic matter digestion catalysed with sulphuric acid. The released ammoniac is distilled in boric acid and titrated with standard solution of hydrochloric acid. The nitrogen content is calculated from the amount of obtained ammoniac, and the protein content is calculated by multiplying the nitrogen content with the milk factor 6.38 [33] and expressed as mass fraction (g/100 g).

The carbohydrate content was calculated by deducting the content of fats, proteins, and ash from the dry matter obtained by digestion (for fat and protein determination see descriptions above). The dry matter was determined with the gravimetric method used to determine water content in food, which is defined as the mass lost to drying at 103°C for four hours and is expressed in the percentage corresponding mass fraction (g/100 g).[30-34] The ash content was determined according to the AOAC method No. 945.46[30-34] for determining ash mass fraction in milk, defined as the mass lost to ashing, expressed in percentage (% = g/100 g).

Statistical analysis

The differences between the groups were analysed with the IBM SPSS Statistics v. 25 program. Since the distribution was not normal, we used the non-parametric Kruskal-Wallis test to single out parameters with significant differences between the binder treatment groups and then the Dunn-Bonferroni test to single out groups with significant differences in these parameters. The significance was set at $p < 0.05$.

Results and discussion

The selected strain of BMK (*Lactobacillus plantarum*) examined the possibility of binding of aflatoxin M1 in milk by adding lyophilized cells of *Lactobacillus plantarum* and AFM1 to milk samples in which the presence of AFM1 was not detected, in the amount of 0.5 μg/L. AFM1 germ was determined immediately after the contamination of AFM1 milk, and after 2 hours, after 4 hours and after 24 hours of incubation at 4°C. The binding success rate of AFM1 to BMK cells at 0 h was extremely good and averaged 80%. This was also confirmed by earlier studies suggesting the ability of lactic acid bacteria used in the production of fermented dairy products as a starter culture, to reduce the amount of aflatoxin in feed for animals.[35]

The binding efficiency of AFM1 with the addition of 0.01% β-glucan from oats was 65% at 0 hours, while with the addition of 0.005% β-glucan from yeast, the binding efficiency of AFM1 was 63%, which confirms the fact that these biofixers can be used to decontaminate milk from AFM1.[36]

Tables 1 and 2 show the results of quality parameters determined in this study. Fat loss was the highest (65.3% of fat remained compared to the untreated, control milk)

when the milk was treated with live LAB cells and then centrifuged and filtered (1.70 g/100 g), which suggests that filtration further reduces the amount of fat compared to unfiltered LAB-treated milk (2.60 g/100 g). The use of dead LAB cells, in contrast, did not affect the fat content regardless of filtration, as 98.1% of the control milk content remained (Table 1).

We can explain these findings according to Bueno et al.[37] and Dalie et al.,[38] who have stated that because the thermal treatment of LAB cells increases the availability of binding surface, and when it comes to binding LAB with mycotoxins, the number of the binding sites on the cell surface that are characteristic of each microorganism plays an important role. We can conclude from the above that dead LAB cells bind AFM1 in a large percentage, and that they do not bind fat molecules to themselves, but they still remain an integral part of milk.

Table 1 Mean (± SD) energy values and fat content in control milk, AFM1-contaminated milk, and contaminated milk treated with LAB or beta-glucan
Tablica 1. Srednja energetska vrijednost i sadržaj masti u kontrolnom mlijeku, AFM1 – kontaminiranom mlijeku i kontaminiranom mlijeku tretiranom s LAB ili beta – glukanom

Sample / *Uzorak*	Energy per 100 g in Kcal/kJ *Energija na 100 g u Kcal/kJ* (% of control / % *od kontrole*)	Fat in g/100 g Masti g/100 g (% of control / *% od kontrole)*
Commercial UHT 2.8% fat milk *Komercijalno UHT 2.8% masno mlijeko* (control / *kontrola*; n = 3)	239.7 ± 36.0 / 57.2 ± 8.6 (100%)	2.73 ± 0.03 (100%)
Milk + AFM_1 Mlijeko + AFM_1 (n = 3)	241.9 ± 36.3 / 57.7 ± 8.7 (100.9%)	2.77 ± 0.03 (101.4%)
Centrifuged milk + AFM_1 *Centrifugirano mlijeko + AFM_1* (n = 3)	238.1 ± 35.7 / 56.8 ± 8.5 (99.3%)	2.68 ± 0.03 (98.1%)
Mycotoxin binders / *fiksatori mikotoksina* (n = 6 for each treatment / *za svaki tretman*)		
Lyophilised live *L. plantarum* cells plus centrifugation *Liofilizirane žive L.* plantarum *stanice plus centrifugiranje*	233.4 ± 35.0 / 55.7 ± 8.4 (97.3%)	2.60 ± 0.03 (95.2%)
Lyophilised live *L. plantarum* cells plus centrifugation and filtering *Liofilizirane žive L.* plantarum *stanice plus centrifugiranje i filtracija*	163.1 ± 24.5 / 38.9 ± 5.8 (68.0%)	1.70 ± 0.02 (65.3%)
Lyophilised dead *L. plantarum* cells plus centrifugation *Liofilizirane mrtve L.* plantarum *stanice plus centrifugiranje*	233.7 ± 35.1 / 55.8 ± 8.4 (97.4%)	2.68 ± 0.03 (98.1%)
Lyophilised dead *L. plantarum* cells plus centrifugation and filtering *Liofilizirane mrtve L.* plantarum *stanice plus centrifugiranje i filtracija*	243.3 ± 36.5 / 54.5 ± 8.2 (101.5%)	2.68 ± 0.03 (98.1%)

Yeast-derived beta-glucan obtained in laboratory / *beta - glukan dobiven u laboratoriju iz kvasca*	205.5 ± 30.8 / 48.9 ± 7.3 (85.7%)	1.89 ± 0.02 (69.2%)
Oat-derived beta-glucan obtained in laboratory / *beta - glukan dobiven u laboratoriju iz zobi*	212.4 ± 31.9 / 50.5 ± 7.6 (88.6%)	2.03 ± 0.02 (74.3%)
Commercial yeast-derived beta-glucan / *komercijalni beta-glukan dobiven iz kvasca*	239.8 ± 36.0 / 57.2 ± 8.6 (100.3%)	2.70 ± 0.03 (100.7%)
Commercial oat-derived beta-glucan / *komercijalni beta glukan dobiven iz zobi*	235.8 ± 35.4 / 56.2 ± 8.4 (98.3%)	2.53 ± 0.03 (94.4%)

SD – standard deviation / *SD – standardna devijacija*

Treatment with laboratory-made beta-glucan from yeast also reduced the fat content, lowering it to 1.89 g/100 g (or 69.2% of the control content), whereas treatment with commercial beta-glucan from yeast did not affect fat content (2.70 g/100 g or 100.7% of control fat).

Similar to fat, the content of proteins, carbohydrates, and lactose dropped the most when milk was treated with live LAB cells, centrifuged, and then filtered (Table 2). These findings again suggest that filtering in combination with live LAB cells affects milk quality the most.

The structure of beta glucan depends on its origin and can be linear or branched. Yeast contains branched beta glucans where glucose molecules are bound by beta - (1-3) bond, and at branching sites by beta - (1-6) bond forming longer side chains, while beta glucans originating from oats of unbranched linear structure are interconnected by a beta - (1-3) glycosidic bond. It is these differences in chain structure and branching that can affect their biological activity and binding capacity, which in this case means that beta glucans from yeast bind higher amounts of fat compared to beta glucans from oats resulting in less fat removal from milk.

The best results, in turn, just like with fat, were obtained with the treatment with dead LAB cells without filtering. Proteins dropped only 0.3%, while carbohydrates and lactose were higher than in untreated, control milk (Table 2).

The results with beta-glucan are not as consistent as with LAB, but, generally, it did not reduce the protein, carbohydrate, or lactose content, save for a slight (14.3%) decrease in protein content with laboratory-made beta-glucan from yeast.

The effect of mycotoxin binders on milk quality has been addressed in similar studies with milk contaminated with AFM_1. The binders investigated were bentonite and aluminium silicate compounds, and they did not show a significant effect on fat, protein, and lactose content.[39, 40] Research with clay showed that protein content dropped whereas the lactose content increased, which was explained by clay interference with lactose on HPLC. However, all of these studies used inorganic binders, whereas we looked into the effects of the organic ones, which clearly showed that they did not significantly affect the parameters of milk quality. The only discrepancies in quality parameters worth mentioning in the treatment with LAB are related to the use of live cells combined with filtration, which led to a major drop in the fat and lactose content and to the use of dead LAB cells, again combined with filtration that led to a minor drop in lactose content.

Our comparison of LAB vs beta-glucan treatment using the post-hoc Dunn-Bonferroni test (Table 3) showed significant differences in the quality parameters between LAB and yeast-derived commercial beta-glucan ($p < 0.05$) in favour of the latter, but these differences were still acceptable in terms of milk quality for consumption and further processing.

Table 2 Mean (± SD) protein, carbohydrate, and lactose content in control milk, AFM_1-contaminated milk, and contaminated milk treated with LAB or beta-glucan
Tablica 2. Srednja vrijednost sadržaja (± SD) proteina, ugljikohodrata i laktoze u kontrolnom mlijeku, AFM1 – kontaminiranom mlijeku i kontaminiranom mlijeku tretiranom s LAB ili beta – glukanom

Sample *Uzorak*	Proteins in g/100 g (% of control) *Proteini u g/100 g (% kontrole)*	Carbohydrates g/100 g (% of control) *Ugljikohidrati u g/100 g (% kontrole)*	Lactose in g/100 g (% of control) *Laktoza u g/100 g (% kontrole)*
Sample / *Uzorak*	3.40 ± 0.14 (100%)	4.76 ± 0.71 (100%)	4.66 ± 0.23 (100%)
Commercial UHT 2.8% fat milk *Komercijalno UHT 2.8% masno mlijeko* (control / *kontrola;* n = 3)	3.31 ± 0.13 (97.3%)	4.52 ± 0.68 (94.9%)	4.40 ± 0.22 (94.4%)
Milk + AFM_1 *Mlijeko + AFM_1* (n = 3)	3.27 ± 0.13 (96.1%)	4.90 ± 0.74 (102.9%)	4.79 ± 0.24 (102.7%)
Mycotoxin binders / *Fiksatori mikotoksina* (n = 6 for each treatment / *za svaki tretman*)			
Lyophilised live *L. plantarum* cells plus centrifugation *Liofilizirane žive L.* plantarum *stanice plus centrifugiranje*	3.44 ± 0.14 (101.1%)	4.44 ± 0.67 (93.2%)	4.35 ± 0.22 (93.3%)
Lyophilised live *L. plantarum* cells plus centrifugation and filtering *Liofilizirane žive L.* plantarum *stanice plus centrifugiranje i filtracija*	2.19 ± 0.09 (64.4%)	3.69 ± 0.55 (83.1%)	3.56 ± 0.18 (76.3%)
Lyophilised dead *L. plantarum* cells plus centrifugation *Liofilizirane mrtve L.* plantarum *stanice plus centrifugiranje*	3.43 ± 0.14 (99.7%)	4.53 ± 0.68 (102.0%)	4.40 ± 0.22 (100.8%)
Lyophilised dead *L. plantarum* cells plus centrifugation and filtering *Liofilizirane mrtve L.* plantarum *stanice plus centrifugiranje i filtracija*	3.23 ± 0.13 (93.8%)	4.35 ± 0.65 (98.6%)	4.19 ± 0.21 (89.9%)
Yeast-derived beta-glucan obtained in laboratory *Beta -glukan dobiven u laboratoriju iz kvasca*	2.95 ± 0.12 (85.7%)	5.00 ± 0.75 (112.6%)	4.88 ± 0.24 (104.7)
Oat-derived beta-glucan obtained in laboratory *Beta - glukan dobiven u laboratoriju iz zobi*	3.47 ± 0.14 (100.8%)	4.59 ± 0.69 (103.3%)	4.48 ± 0.22 (96.1%)
Commercial yeast-derived beta-glucan *Komercijalni beta-glukan dobiven iz kvasca*	3.46 ± 0.14 (100.3%)	4.75 ± 0.71 (101.7%)	4.53 ± 0.23 (102.7%)
Commercial oat-derived beta-glucan *Komercijalni beta glucan dobiven iz zobi*	3.50 ± 0.14 (98.5%)	4.83 ± 0.72 (103.4%)	4.27 ± 0.21 (96.8%)

SD – standard deviation / *SD – standardna devijacija*

Table 3 Significant differences in quality parameters between the AFM_1 binder groups
Tablica 3. Značajnost razlike u parametrima kvalitete između AFM1 fiksatora

Milk quality parameters *Parametri kvalitete mlijeka*	Kruskal-Wallis test	df	P	Compared groups *Uspoređene grupe*	P (Dunn-Bonferroni)
Energy *Energija*	34.713	5	< 0.05	1 vs 3	0.0001
				2 vs 3	0.001
				5 vs 3	0.003
				4 vs 3	0.042
Fats *Masti*	29.167	5	< 0.05	1 vs 3	0.001
				2 vs 3	0.003
				4 vs 3	0.013
Carbohydrates *Ugljikohidrati*	22.345	5	< 0.05	5 vs 4	0.0001
				5 vs 1	0.012
Lactose *Laktoza*	24.167	5	< 0.05	4 vs 3	0.007
				4 vs 1	0.004
				5 vs 1	0.016
Proteins *Proteini*	18.466	5	< 0.05	1 vs 3	0.032
				1 vs 4	0.003

1 – laboratory-made beta-glucan from yeast; 2 – laboratory-made beta-glucan from oats; 3 – commercial beta-glucan from yeast; 4 – commercial beta-glucan from oats; 5 – *L. plantarum* groups combined (mean value)
1 – beta-glukan dobiven u laboratoriju iz kvasca; 2 – beta -glukan dobiven u laboratoriju iz zobi; 3 – komercijalni beta-glukan dobiven iz kvasca; 4 – komercijalni beta glucan dobiven iz zobi; 5 – L. plantarum *kombinacija grupa (srednja vrijednost)*

Conclusion

Our findings clearly show that the treatment of AFM_1-contaminated milk with LAB and beta-glucan as mycotoxin binders (laboratory-made and commercial alike) does not affect the content of macronutrients in milk to the point that it becomes unfit for human or animal consumption. Furthermore, its quality warrants processing into a variety of milk products. The only treatment method that stands out in the reduction of macronutrients is the one with live LAB cells combined with centrifugation and filtration. However, even in this case, the quality parameters of milk were acceptable for consumption and further processing.

We believe that our findings are encouraging for the dairy industry to continue abating milk contamination with AFM_1 with the investigated mycotoxin binders and prevent economic damage caused by AFM_1. Considering that this research was done in a laboratory setting, future research should involve a much larger, industrial setting to verify the practical benefits of LAB and beta-glucan for the industry. We also point out that the results indicated a good possibility of binding of AFM1 with the bacteria *Lactobacilus plantarum*, 0.01% β-glucan from oats and with 0.005% β-glucan isolated from yeast from yeast *Saccharomyces cerevisiae 20.*

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