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Somatic cell count and presence of aflatoxin M1 in raw milk from the farms from "Ovče Pole" region, Republic of North Macedonia

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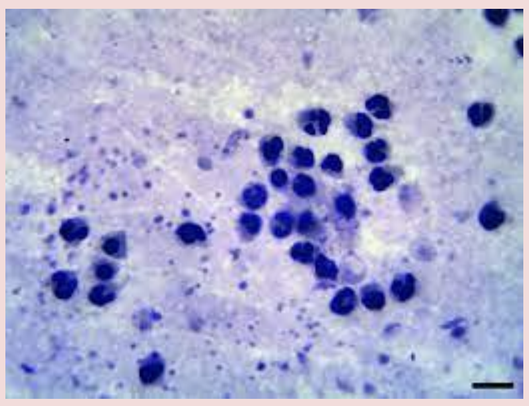
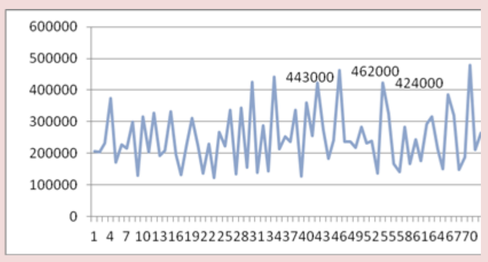
Introduction

Somatic cell count is the common method for determination of raw milk quality [1]. An increased amount of somatic cells results either from an inflammatory process due to the presence of an intramammary infection or, under non-pathological conditions, from physiological processes such as estrus or advanced stage of lactation [2,3]. Monitoring of somatic cell numbers has been simplified by automated cell counters that allow large numbers of milk samples to be evaluated quickly. Aflatoxins (AFs) are secondary metabolites produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nominus* fungi under impropriate growing and storage conditions. The most common aflatoxin with proven cancerogenic effect in raw milk is aflatoxin M1. European Community (EC) and Codex Alimentarius prescribe a limit of 50 ng/kg AFM1 in milk and 25 ng/kg for infant milk products. However, US regulation fixed the limit to a maximum of 500 ng/kg for milk and 25 ng/kg for infant milk products.





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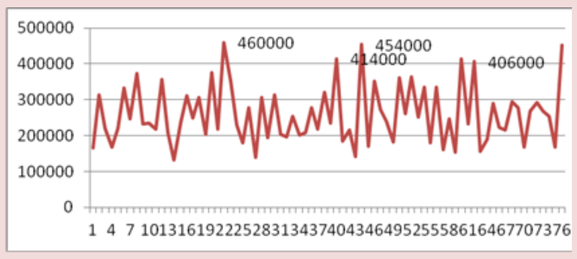

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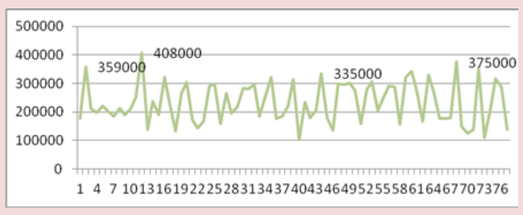

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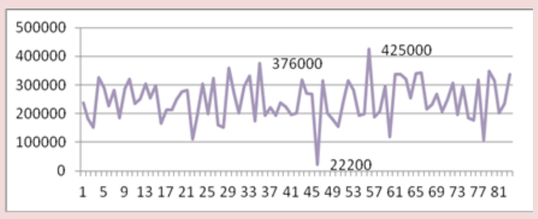

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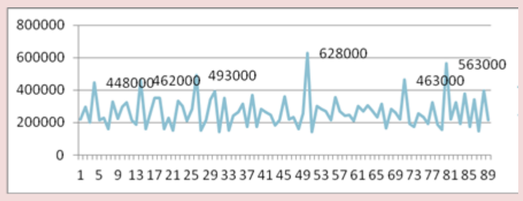

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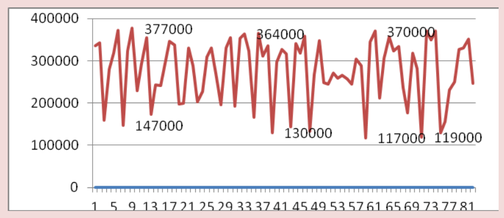

June 2018

Figure 1. Determination of somatic cells in raw milk from January till June 2018

Fig.2. Determination of number of somatic cells by automated counter.

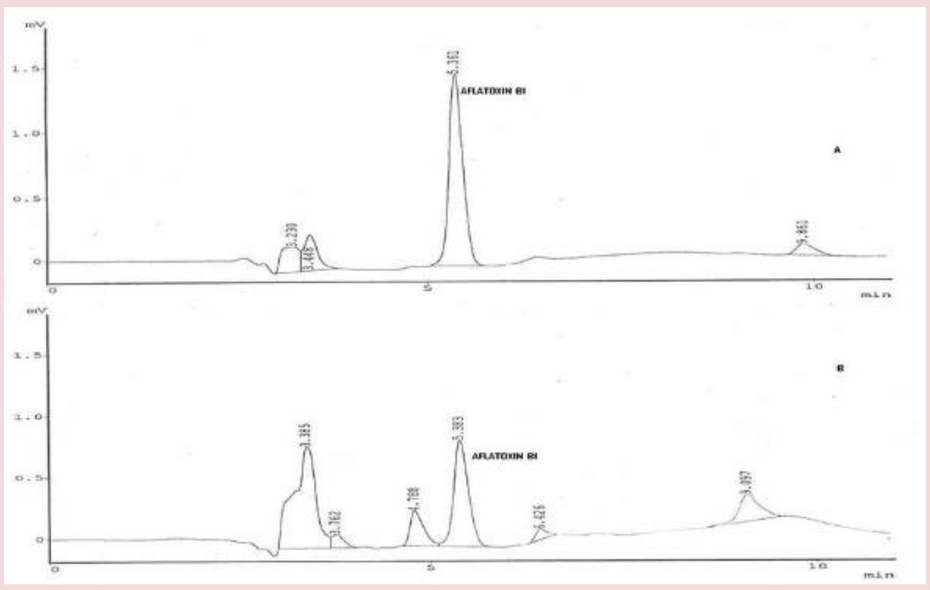

Fig.3. Determination of aflatoxin M1 in raw milk by HPLC (A-standard and B- sample of raw milk)

Materials and Methods

A 60 samples of raw milk from the farm in the region of "Ovče Pole" were the subject of the presence of aflatoxin M1 and somatic cell counts. All samples were stored at 2-8ºC and tested for 24 hours. Some samples that we were not able to analyze within 24 hours were stored at -20ºC. For determining the number of somatic cells, 478 samples of raw milk from producers from the "Ovče Pole" region were analyzed. Samples are taken and delivered in sterile plastic cups with a volume of 50 ml canned by Adizol (Sigma-Aldrich vol. 25 ml). After taking, they were transported at a temperature of 4ºC in the laboratory for testing the quality of raw milk. Before somatic cell counting, the samples are heated to 40ºC and analyzed twice on a Fossomatic 5000 (FossElectric, Denmark). The somatic cell counting procedure was performed in accordance with the accredited method ISO 17025-FVM-SOP-398 according to references from ISO 13366-2: 2006. ELISA equipment Immunoscreen AFM1 (Tecna, s.r.l, Trieste, Italy) and HPLC equipped by fluorescence detector (Waters Alliance 2695) were applied for determination of Aflatoxin M1 in 60 samples of raw milk. All standard controls were duplicated on a 96-well plate coated with anti-AFM1 antibodies. After colorization, using the appropriate chromogen, the samples were weighed using a microplate Bio-Rad Model 680 (Philadelphia, USA) photometer set at 450 nm. The measured absorption was inversely proportional to the AFM1 concentration in the sample and the measured apparatus ranging from 5 to 250 ng/kg.

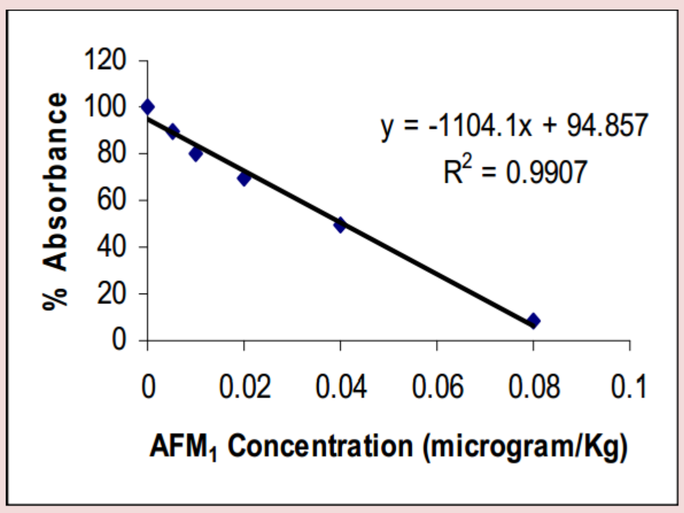

Fig.4. A sample enzyme-linked immunosorbent assay calibration curve

Results and discussion

According to the results obtained from 482 samples taken once per month, 462 samples meet the National and EU standards for the total number of somatic cells as a parameter for milk quality. The measured average values showed that the highest value of somatic cells count was 277743.90 scc/m in June 2018 and the lowest measured average value was detected for March 2018 (233701.3 scc/m). Furthermore, from 482 samples collected in the "Ovče Pole" region, 95.8% met the criteria prescribed in the milk and dairy products regulative of 2016 where the maximum number of somatic cells can be 400,000 cfu/ml in raw cow's milk and are also satisfied and EU milk quality standards. Identification of area-specific and farm-specific risk factors was crucial in cow mastitis control programs. From the results above, it can be concluded that the raw milk had good quality (in relation to somatic cell count) and selected exclusively healthy head of cattle had a low percentage of mastitis and good control of the mammary gland.

Determination of the amount of aflatoxin M1 was determinated in 60 samples of raw milk. In two samples a concentration higher than 0.05 ng/kg was detected by ELISA method. The amount of aflatoxin A1M1 in those two samples were additionally analyzed by HPLC with fluorescent detector, as a confirmation method. AFM1 concentrations in both samples (3.3%) exceeded the maximum permissible levels, and the highest detected concentration was 0.58 ng/ kg, which is 0.08 ng/kg above the permissible limit.

Conclusion

The results presented in this research showed that the quality of raw milk in relation to the somatic cell counts and the presence of aflatoxin is at a satisfactory level. Monitoring of somatic cell numbers has been simplified by automated cell counters that allow large numbers of milk samples to be evaluated quickly. Somatic cells tend to be higher in afternoon milking's, which undoubtedly occurs because of the shorter milking interval and lesser fluid milk dilution of sloughed epithelial cells. Therefore increased frequency of milking (three or four times/day) may slightly elevate.

The previous Aflatoxin crisis due to high AFM1 contamination of maize in 2013 has increased the awareness of the food safety risk managers; induced regulatory measures, research, and innovation activities; and reinforced the consciousness of the food business operators. Consequently, they have implemented strict monitoring and regular control along the feed and food chain utilizing the availability of rapid and less expensive detection kits. This self-control and corrective measures at dairy farms resulted in the slow decrease of AFM1 contamination.

Reference

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3. Bogalho, F., Duarte, S., Cardoso, M., Almeida, A., Cabeças, R., Lino, C., et al. (2018). Exposure assessment of Portuguese infants to aflatoxin M1 in breast milk and maternal social-demographical and food consumption determinants. Food Control 90, 140–145. doi: 10.1016/j.foodcont.2018.02.043