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SANITARY-HYGIENIC AND QUALITY CHARACTERISTICS OF MILK

-ABSTRACT-

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Since Romania joined the European Union, in January 2007, a new legislation regarding the raw milk control was implemented. As part of the new legislation, a new mandatory parameter to determine the milk quality was introduced, namely, the numbers of somatic cells count, along with the total number of germs and the presence of inhibitors. Since a large part of the milk produced in Romanian farms contained more than 1 million germs/ml and 600.000 somatic cells/ml, the European Union established a deadline; by the end of 2013, to achieve the desired quality. The hygienic standards imposed are: a total number of germs/ml ≤ 100.000 and the total number of somatic cells/ml ≤ 400.000 . The new legislation also prohibited the use of antibiotics on feeding livestock without cause of disease. Analytical methods currently available for the monitoring of antibiotic residues in milk are insufficient to achieve the quality requirements.

The thesis is structured in two main parts. The general part presents the current state of knowledge regarding the raw milk quality in Romania. The experimental part is structured in four distinct parts:

1. STUDY REGARDING THE NUTRITIONAL QUALITY OF RAW MILK FROM DIFFERENT TYPES OF HOLDINGS DEPENDING ON PHYSIC-CHEMICAL PARAMETERS

The **aim** of the study was to determine the nutritional quality of raw milk produced by different production systems, based on physic-chemical parameters.

Material and methods: The research was made in collaboration with a milk processing unit. The sampling frame was provided by the collaborating unit. From this we extracted 12 sources of raw milk from which 6 are semi-intensive production systems and 6 are extensive production systems. The study period was between the 1st of January 2011 and 30 June 2012. During those 18 months a sample from each source was monthly analyzed in order to determine the physical-chemical parameters, therefore resulting 216 samples for quantitative analysis. The samples were analyzed using the *Ekomilk Total* milk analyzer.

Results: As a result of the analysis made on the two groups coming from different sources, we have identified variability and mean differences between the values of the studied indicators. The results were: density (density degrees) 27.10 - 28.46 averaging 27.78, dry matter (%) 8.11&-8.9% averaging 8.5%, fat% 3.3 - 4.0% averaging 3.6%, freezing point (C degrees) 0.55-0.56 averaging 0.55, pH 6.36 – 6.61 averaging 6.48, protein (%) 3.19% – 3.7% averaging 3.44%, temperature (C degrees) 4-6 averaging 5° C, the results are consistent with the existing literature data and standard limits allowed by applicable law.

Conclusion: although the studied groups shows significant differences in terms of hygiene, this difference was not reflected in the nutritional quality of milk, the studied parameters showed a good nutritional quality that does not directly depend on the breeding system and exploitation of the animals.

2. STUDY REGARDING THE SPECIFIC QUALITY PARAMETERS OF MILK: THE TOTAL NUMBER OF GERM, THE SOMATIC CELLS COUNT AND THE PRESENCE OF ANTIBIOTIC RESIDUES

The **aim** of the study was to establish the raw milk quality according to the most representative quality parameters: the total number of germs, the somatic cells count and the presence of the antibiotics residues, coming from two different livestock's systems and according to the new restrictive European norms.

Material and methods: for the quality parameters, 432 milk samples from farms and 432 samples from collection centers were analyzed. The sources are those mentioned in the previous chapter. Samples were collected weekly from each source, for a period 18 months. The total number of germs was determined using the SOLERIS system, the determination of the somatic cells count was made using the EKOSCOPE system and the determination of antibiotics residues was made using the BETA STAR COMBO test for β -lactam and tetracycline.

Results: Out of the total number of samples, from the total number of germs point of view, 608 samples (70.5%) were fit and 256 samples (29.2%) were unfit, having a TNG over 100.000/ml. Out of the total number of unfit samples, 29 (3.4%) samples came from farms and 227 (26.2%) samples came from milk collecting centers. In the 18 months of research, a number of 864 samples were analyzed for the somatic cells number, out of which 659 (76%) samples were fit and 208 (24%) were unfit. Out of the total number of unfit samples, 4.3% came from farms and 19.8% came from milk collecting centers. In the case of the antibiotics residues there were 626 (72.3%) negative samples.

Conclusion: Regarding the bacteriological milk quality, the milk coming from semi intensive system has a better quality than the milk coming from extensive system. This conclusion is due to the higher hygiene conditions, the automated milking systems and the precocious mastitis detecting system. The hygiene under control can be realized by implementing a strict microbiological controlling program. The percentage of non-compliant samples decreased from one year to another, with the approaching deadline set by the European Union, December 2013, the compliance rate of milk increased.

3. DETERMINATION OF TRIIODOTHYRONINE AND THYROXINE FROM PLASMA AND MILK OF LACTATING COW

The purpose of this study was to establish a method for the determination of T3 (triiodothyronine) and T4 (thyroxine) hormone concentrations in plasma, whole milk and after ethanol extraction, as well as to calculate the partition coefficient milk/plasma.

Material and methods: Ten Holstein Romanian friza milking cows were used to test the efficiency of the method. T3 and T4 were determined by an immunochemical ELISA competitive assay.

Results: The average values of T3 in plasma were 2.78 ± 1.53 ng/ml (4.27 ± 2.35 nmol/l), in whole milk 3.72 ± 1.44 ng/ml (5.71 ± 2.21 nmol/l) and in extracted milk 4.97 ± 1.67 ng/ml (7.36 ± 2.56 nmol/l). The milk/plasma partition coefficient was 2.27 ± 1.40 . The average values of T4 in plasma were 50.97 ± 7.30 ng/ml (65.60 ± 9.39 nmol/l), in whole milk 2.12 ± 0.87 ng/ml (2.73 ± 1.12 nmol/l) and in extracted milk 3.60 ± 1.15 ng/ml (4.64 ± 1.48 nmol/l) the milk/plasma partition coefficient was 0.072 ± 0.029 .

Conclusion: the milk extraction presented a good yield for triiodothyronine and thyroxine, suggesting that hormones extraction in alkaline alcohol solution is preferably used for the determination of thyroid hormones from milk. Another advantage of the extraction is the long time storage of the samples, knowing that milk is a highly perishable product. The data suggest that cow's milk can be an important source of T4 for children and infants. The values obtained are in the concentration range reported by literature data for T4 and T3 from plasma and milk.

4. IDENTIFICATION AND DETERMINATION OF FLUOROQUINOLONES FROM MILK USING A HPLC METHOD WITH FLUORESCENCE DETECTOR

The **aim** of the study was to elaborate a fast HPLC method with fluorescence detector for the screening of fluoroquinolones from milk and the development and validation of a simple method of determination of ciprofloxacin and norfloxacin from milk.

Material and methods: HPLC LaCHROM (Merck-Hitachi) system, Luna Phenomenex C18 150×4.6 column, the mobile phase was injected in gradient elution. For the analysis we used different types of commercial milk, with a fat percentage of 1.5% and 0.1%. The samples analysis was done after the protein precipitation with 20% per chloric acid.

Results: After the preparation and injection of milk samples marked with concentrations of 100 ng/ml FQ, five fluoroquinolones were separated in less than 11 minutes: norfloxacin (NFX), ofloxacin (OFL), moxifloxacin (MOX), ciprofloxacin (CFX) and enrofloxacin (EFX). At the retention time of ciprofloxacin and norfloxacin there were no matrix interference.

Conclusion: A fast screening method of five fluoroquinolones from milk in less than 11 minutes was realized, given their pharmacotoxicological potential at the repeated consumption of contaminated milk by subjects during growth period. A simple method for the determination of ciprofloxacin and norfloxacin from milk has been validated; the analytical performance of the method was verified through the major validation parameters.

Keywords: milk, quality, TGN, SCC, antibiotics, hormones, triiodothyronine, thyroxine, fluoroquinolone, HPLC.