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INVESTIGATIONS CONCERNING THE EXCRETION OF ANTIBIOTIC RESIDUES IN THE MILK OF COWS TREATED WITH ANTIBIOTICS

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Abstract

The somatic cell count in the mixed milk of the 4 quarters has dropped from 1.155 million to 200 thousand within 120 hours of the last treatment. In cow 5390 somatic cell count tends to decrease after 48 hours of treatment and this can be interpreted as a success of treatment. However, 72 hours after treatment, the somatic cell count increases again, in the affected quarter causing an increase in the somatic cell count in the mixed milk of the 4 breast quarters from 565 thousand to 812 thousand somatic cells. The amount of residue excreted via milk as a percentage of the total amount applied was from 5.2% to 45.3%. In the group of cows milked 1.5 times a day, the percentage of residue excreted via milk was 17.75% compared to 27.51% in the group of cows with two milkings per day. The average milk yield of the group of cows milked twice a day was 23.55 ± 4.8 kg standard deviation and in the group of cows milked 1.5 times a day was 26.4 ± 2.59 kg.

Key words*: antibiotic, milked, somatic cells, udder.*

INTRODUCTION

The presence of antimicrobial substances in raw milk may have toxicological consequences (Dewdney et al., 1991; Currie et al., 1998; Cola et al., 2022), but also technological consequences (Molina et al., 2003).

Ideal tests are those that give positive responses as close as possible to the "Maximum Residue Limit" (MRL), defined as levels of interest. Tests giving positive results at much higher MRL are questionable. Tests giving positive results below the MRL require an excessive number of samples necessary for confirmation.

There are several screening tests. These tests were evaluated under various experimental conditions (Seymour et al., 1988; Bachmann et al., 2019; Andrew, 2000; Anika et al., 2019).

Andrew (2001) reported false-positive results of some screening tests, tests in which milk from individual cows was used. False-positive results represent losses for producers (milk may be rejected from consumption). In contrast, Bachmann et al. (2019) reported a lower incidence of false-positive results in three out of four screening tests evaluated.

The screening tests were accepted, because they met the standards for the low incidence of false-positive results but also of false-negative results (FDA, 1996). Along with these standards, there are also a few principles that must be taken into account. Those are:

- a positive result of a screening test is a presumption that the analyte (antibiotic residue) is present in the milk sample;
- the screening test does not identify a specific analyte or measure it quantitatively;
- the accepted screening tests must give positive results when the antibiotic concentration is below the safety/tolerance level; this is a false tolerance result and not a false-positive result;
- screening tests are the fastest tests for detecting residues of antibiotics in milk.

The microbial growth inhibition test uses standard cultures to test the growth of a micro-organism (e.g. *Bacillus stearotermophilus*) in solid or liquid medium. The milk sample is added to the surface of the agar and left in the medium, and if the sample contains inhibiting agents, the growth of the micro-organism is completely reduced or inhibited (Navratilova, 2008).

Microbial growth inhibition tests differ according to the type of micro-organism used, the duration of incubation and the temperature and detection levels of the residues analysed (Currie et al., 1998).

The most used commercial tests with spores of *Bacillus stearotermophilus var calidolactis* are:

Delvotest SP (DSM, Netherlands), Copan test (Copan, Italia), Charm Farm 960 Test (Charm Sciences, Inc. USA).
Tests with *Streptococcus thermophiulus* are: Valio T 101-test, Valio T 102 - test (Valio, Finland).
The most used enzyme tests are: Penzym and Penzym S (UCB Bioproducts, Belgium), and among the most used immunological tests are: Delvo- X Press β-lactum (DSM Netherlands), β star (UCB Bioproducts, Belgium), Rosa test (Charm Science, Inc USA).
Sykorova et al. (2012) compare the sensitivity of the detection of five assays for the assessment of milk aminoglycosides (gentamicin, neomycin, streptomycin, kanamycin, and spectinomycin). The sensitivity of these assays was evaluated based on the experimental determination of the detection limits (LOD). The detection limits for the STAR assay were MRL for neomycin (1.5 μg/g), gentamicin (0.10 μg/g), streptomycin (0.20 μg/g) and kanamycin (0.15 μg/g). Spectinomycin (0.20) was not detected at MRL level. Modern biotechnologies and genetic engineering represent promising solutions in the near future (Bonciu, 2020) and the discovery and application of these solutions will remain valid for the situations created by the emerging pathologies in the animal cell determined by bacteria with significant potential for the development of antibiotic resistance. On the other hand, livestock production forms an integral part of the units that practice organic farming and must contribute to the balance of agricultural production systems (Bonciu, 2022a). The management of ecological animal growth forbids the use of antibiotics, with some exceptions (Bonciu, 2022b).

MATERIALS AND METHODS

Investigations regarding the excretion of antibiotic residues in cows with clinical mastitis were made at S.C. Fenov S.R.L.
Parameters used for the characterisation of cows: registration number of the cows; lactation number; days after calving; daily average production during experiments; body weight; somatic cell count in cow's milk at the onset of the mastitis; severity of clinical symptoms (1 = slightly modified milk with small and large clots; 2 = modified milk with large clots; 3 = abnormal milk; 4 = abnormal milk plus body temperature above 39.5°C); number of quarters with subclinical mastitis; success of treatment: somatic cells below 200,000/ml at the end of the experimental period.
Milking frequency: twice a day: at 06:00 and 17:00, interval between milkings of 11 hours and 13 hours, 8 cases;
1.5 times a day: at 05:00 and 21:00 every other day and at 13:00 the next day, interval between milkings of 16 hours.

Milk sample collection

The milk samples were collected daily for the experimental period as follows:
- milk samples for each quarter of the udder;
- milk samples per cow (mixed milk from the 4 quarters).

The milk samples were kept in the refrigerator (6°C) for a maximum of 30 hours.

Medicines used

Intramammary treatments with Cobactan LC, Cefquinome 75 mg. One syringe has 8 grams and the treatment scheme was of 3 successive treatments after each milking in the affected quarter.
In order to apply the same amount of antibiotic substance over the period of time, the following scheme was applied (Table 1).

Table 1. Treatment scheme

Mastitis detection	Two milkings per day		1.5 milkings per day	
	Morning	Evening	Morning	Evening
Anamnesis	Day 1 06:00	Day 1 17:00	Day 1 06:00	Day 1 17:30
Treatments three times in 24 hours	Day 1 06:00 Day 1 17:00 Day 2 06:00	Day 1 17:00 Day 2 06:00 Day 2 17:00	Day 1 06:00 Day 1 14:30 Day 2 06:00	Day 1 17:30 Day 2 03:30 Day 2 15:00
Further milkings	06:00 17:00	17:00 06:00	21:00 13:00 05:00	05:00 21:00 13:00

Methods of analysis

1. Udder health: the somatic cell count was determined by the fluoro-opto-electronic method with the help of the SOMASCOPE MK II counter.
2. Antibiotic residue detection and quantification: identification was carried out using the EKOTEST method (EON

TRADING-USA); quantification of antibiotic residues was done by disc method.
3. Setting the waiting time: time when safety concentration is reached. For this purpose, a tolerance limit is calculated for a number of milkings per animal. This limit is the time taken for the residue concentration in milk, in the vast majority of animals, to reach safety levels (MRL).
Variance analysis: variance was used to determine which factors had a systematic influence on the waiting time.

RESULTS AND DISCUSSIONS

The status of the animals included in the experiment is presented in Table 2.
Figures 1, 2 and 3 show the evolution of somatic cell count on each quarter of the breast and on the entire mammary gland.

In Figure 1, the treatment had an effect, and the somatic cell count decreased in the milk of the affected (left anterior) breast quarter from 4.5 million to 710 thousand at the end of the 120-hour waiting period after the last treatment. The somatic cell count in the mixed milk of the 4 quarters has dropped from 1.155 million to 200 thousand within 120 hours of the last treatment.
In cow 5390 (Figure 2) the somatic cell count tends to decrease after 48 hours after treatment and this can be interpreted as a success of treatment.
However, 72 hours after treatment, the somatic cell count increases again, in the affected quarter causing an increase in the somatic cell count in the mixed milk of the 4 breast quarters from 565 thousand to 812 thousand somatic cells (Figure 3), unsuccessful treatment.

Table 2. Status of the cows included in the experiment

No.	Registration number (No. of cases)	Lactation no.	Days after calving	Kg milk/day (kg)	Body weight (kg)	Quarter affected	Somatic cell count/cow x1000	Severity	Extra infected quarters	Treatment success (somatic cells below 100,000)
Cows with two milkings per day										
1	4091	3	200	22.8	652	Left anterior	800	1	1	Yes
2	5353	3	240	14,6	664	Left anterior	2100	3	1	No
3	5354	2	180	23,2	598	Right anterior	1022	2	0	Yes
4	5362	2	244	18,8	609	Right posterior	3780	3	2	Yes
5	5378	2	68	27,8	710	Left anterior	4520	2	1	Yes
6	5390	1	109	28,4	596	Left anterior	5100	4	1	No
7	5400	1	6	26,6	620	Right posterior	982	1	0	Yes
8	5830	1	66	26,2	634	Left posterior	2660	2	0	Yes
Cows with 1.5 milkings per day										
1	2231	4	1	28.8	712	Left anterior	1612	2	1	Yes
2	5851	1	82	27.6	688	Right anterior	3812	3	0	Yes
3	2239	2	61	26.4	708	Left posterior	6012	4	1	No
4	5861	1	128	22.8	640	Right anterior	1654	2	0	Yes

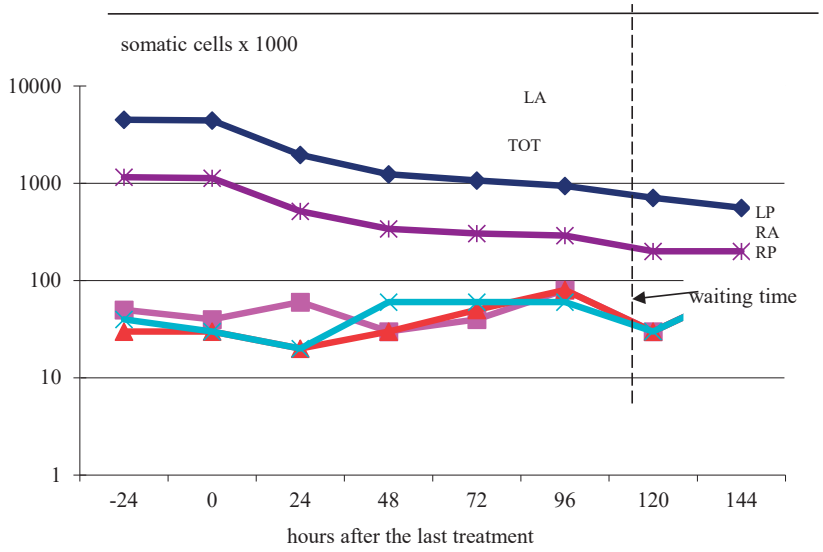

Figure 1. Evolution of the number of somatic cells in the milk of mammary quarters and from mixed milk, after treatment (cow 5378)

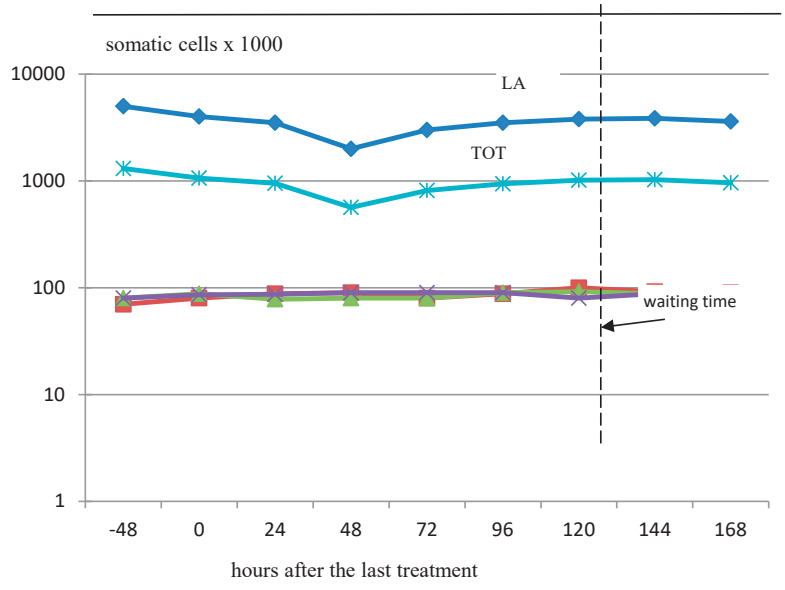

Figure 2. Evolution of the number of somatic cells in the milk of mammary quarters and from mixed milk, after treatment (cow 5390)

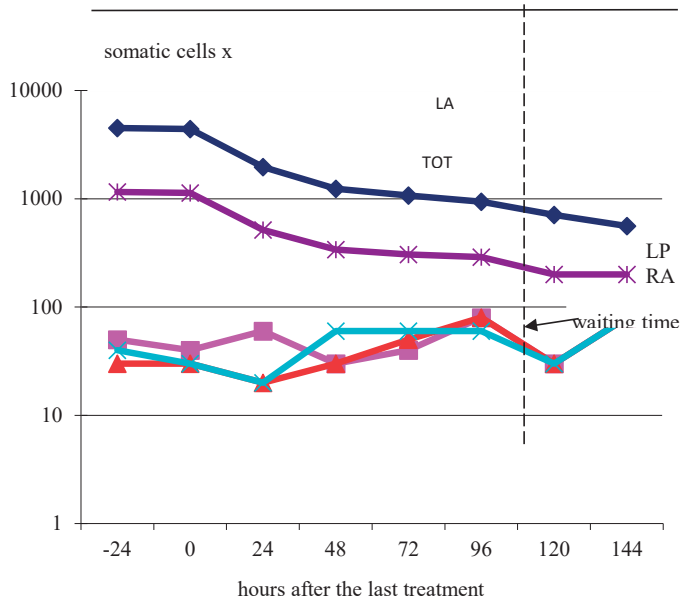

Figure 3. Evolution of the number of somatic cells in the milk of mammary quarters and from mixed milk, after treatment (cow 5378)

Table 3. Residue excretion of Cefquinome via milk

Cow registration number	Milk production (kg)	Cefquinome excreted	
		mg	% of dose applied
4091	22.8	70	30.2
5353	14.6	12	5.2
5354	23.2	32	38.2
5362	18.8	102	45.3
5378	27.8	40	20.8
5390	28.4	60	32.4
5400	26.6	55	27.8
5830	26.2	48	20.2
Average with two milkings/day ± Standard deviation	23.55 ± 4.8	52.38 ± 26.88	27.51 ± 12.3
Coefficient of variation	20.3	51.3	44.7
2231	28.8	42	17.3
5851	27.6	25	14.7
2239	26.4	65	28.0
5861	22.8	32	11.0
Average of 1.5 milkings per day ± Standard deviation	26.4 ± 2.59	41 ± 17.45	17.75 ± 41.1
Coefficient of variation %	9.8	42	41.1

In cow 5353 the treatment was not successful. Somatic cell count tends to decrease. However, the number of somatic cells in the mixed milk increases from 727 thousand to 1,650 million at 72 hours after treatment due to an increase in the number of somatic cells from another breast quarter. Table 3 shows the amount of Cefquinome excreted per each cow and milking group (with different periods between milkings). The amount of residue excreted via milk as a percentage of the total amount applied was from 5.2% to 45.3%.

In the group of cows milked 1.5 times a day, the percentage of residue excreted via milk was 17.75% compared to 27.51% in the group of cows with two milkings per day.

The average milk yield of the group of cows milked twice a day was 23.55 ± 4.8 kg standard deviation and in the group of cows milked 1.5 times a day was 26.4 ± 2.59 kg standard deviation.

Quantitatively, Cefquinome excreted via milk was 52.38 mg in the group of cows milked twice daily and 41 grams in the other group of cows. In cows with two milkings, the excretion of Cefquinome residue was 7.8% higher than in cows with 1.5 milkings per day.

CONCLUSIONS

The sensitivity of the ECOTEST method guarantees the rapid detection of antibiotic residues in cow's milk.

Milk from S.C. Fenov S.R.L. poses no health risks to consumers.

Some components of milk, after the treatment of severe mastitis, influence the test for the detection of antibiotic residues. These include: somatic cells, lactoferrin, lysozyme, free fatty acids or sodium.

The use of antibiotic overdoses for the treatment of sick animals must be associated with the detection of antibiotic residues after the waiting period.

It is recommended to test milk from treated animals on the first day after the waiting period to detect milk with antibiotic residues from animals with severe diseases or overdoses.

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