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Effect of low intensity laser radiation on cow's milk microflora and somatic cell count

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Summary

The aim of the study was to monitor the effects of radiation emitted by a low energy laser on the growth of microorganisms in vitro from milk of cows with elevated SCC, microorganism diversification, and SCC after the lead treatment in vivo by the laser.

Laser irradiated microorganism cultures exhibited a weaker incidence of environmental microorganisms, especially fungi and Streptococci sp. No laser light effect was noted on S. aureus culture development. Our data shows that after laser treatment the variety of micro-organism species immediately decreases 64.28% and this indicator remains unchanged after 21 days. 21 days after completion of the therapy course the SCC decreased 20.11%. 70 days after treatment the SCC increase compared to the 21 day period increased by 20.3%, which can be associated with factors unrelated to the method of therapy. It is advisable to treat increases in SCC with low intensity laser rays conditional to environmental mastitis causative agents. Moreover, since due to the effect of laser radiation certain irradiated micro-organism cultures become more susceptible to antibiotics, it is advisable to coordinate laser therapy with antibiotic therapy.

Keywords: low level laser therapy, udder, somatic cell count, microorganisms, cows

With the expansion of ecological stock-raising, alternative methods to chemotherapy are being sought in treating livestock. Observations have been made indicating that animal tissues can interact with laser light. In human medicine therapeutics, the use of low intensity laser radiation is applied in analgesia, surgery, wound and joint treatment (16, 19, 20). In gynecology, laser rays can be applied as an assistive means in treating purulent *mastitis* (2). The effect of laser light bio-stimulation has been observed to expedite necrotic muscle regeneration (17). Laser radiation influences an increase in leukocyte activity, stimulates vascularisation, regulates local temperature and subdues symptoms of inflamation.

The efficacy of laser therapy in treating bovine *mastitis* has not been determined. In some studies no evidence for any stimulation of the healthy mammary gland or therapeutic effects on *mastitis* by low-energy laser could be found (21). Other studies indicate that the efficacy of treating bovine *mastitis* with low intensity laser radiation is comparable to antibiotic therapy (7). Studies on laboratory animals indicate that low intensity laser radiation destroys *Pseudomonas aeruginosa* and *Staphylococcus aureus* cultures, as well as induces blood-vessel regeneration (3). In the incidence of sub-clinical mastitis, somatic cell count (SCC) values increase. About 150 species of microorganisms were found to be etiological agents of *mastitis*. The main factor increasing the number of somatic cells in milk is inflammation caused by a reaction against bacteria and toxins (11). *Mastitis* treatment efficacy can be determined via bacteriologic tests of milk samples and variations in the SCC prior and post-treatment.

The aim of the study was to monitor the effects of radiation emitted by a low energy laser on the growth of microorganisms *in vitro* from milk of cows with elevated SCC, microorganism diversification and SCC after the lead treatment *in vivo* by the laser.

Material and methods

Microbiological investigation using *in vitro* laser radiation. The investigation was performed February-May 2006 on 5 dairy farms. For *in vitro* experiments, 20 Lithuanian Black/White cows (5000-6000 kg) were selected. Cows were in their 2nd-5th lactation and their bulk milk SCC was 400,000 cells/ml. The SCC was determined via Fossomatic 90 (Foss Electric, Denmark). Milk samples were obtained in compliance with aseptic requirements from final milk streams. *Mastitis* milk samples were sown under uniform conditions on McConkey (for Gram-negative bacte-

ria) (McConkey agar, Oxoid, England), Columbia blood agar medium (for staphylococci) (Oxoid, England), containing 5% sheep's blood, Edwards (for streptococci) (Oxoid, England) agars. The samples were incubated for 24-48 h at 37°C under aerobic conditions. Every 24 h the grown colony's size and color were evaluated. Grown colonies were tested with 3% hydrogen peroxide solution. To identify *Staph. aureus* we used the latex kit, Staphytect Plus Test DR 850 (Oxoid, England). Detailed identification of bacteria was peformed using API test. Sabouraud medium (for yeast and fungal) (Oxoid, England) plates were incubated for 5 days at 25°C. 20 samples from the experimental group were laser-irradiated for 1 min. at a distance of 15-20 cm from the media surface. For the control group we used milk samples obtained from the same cows as in the experimental group's 20 intact samples.

Antibiotic susceptibility test was performed using the disc diffusion method on Muller-Hilton Agar (Oxoid, England). 15 antibiotics discs were used with determined amounts of substances (cephalothin – 30 μg, cefuroxime – 30 μg, netilmicin – 30 μg, co-trimoxazole – 25 μg, erythromycin – 15 μg, gentamycin – 10 μg, rifampicin – 30 μg, oxacillin – 1 μg, amoxycillin – 30 μg, cephalexin – 30 μg, cloxacillin – 5 μg, lincomycin – 2 μg, amoxycillin and clavulanic acid – 30 μg, neomycin – 30 μg, tetracycline – 30 μg). After incubation the zone of inhibition of growth was measured.

Microbiological investigation using *in vivo* laser radiation. For the 2nd experiment we selected 20 analagous Lithuanian Black/White production cows (5000-6000 kg). They were divided into 4 groups. Group 1 – 8 latest lactation (after 60 lactation days) cows having a SCC > 400,000/ml. Group 2 – 4 early lactation (up to 60 lactation days) cows having a SCC > 400,000/ml. Group 3 (Control) – 5 cows having a SCC up to 200,000/ml and their milk did not react with California mastitis test (CMT). Cows in all 3 groups were laser irradiated for one minute daily for 7 days after milking. The udder was irradiated regardless of which quarter was affected, 10-15 cm from the milk gland's ventral surface. Group 4 – included 3 (intact) cows having clinically healthy udders and were not laser-irradiated. Milk bacteriologic tests were performed and the SCC values were evaluated prior to, immediately after, and 21 days post laser-radiation.

Field-testing using laser-radiation. For the field test we selected 75 various-aged Lithuanian Black/White cows, milked via various methods, having at least one udder quarter that reacted with indicator CMT, a SCC > 400 000/ml, and showing no clinical signs of milk gland disorder. SCC evaluations were performed prior to treatment, immediately after, 21 days later and repeated 70 days post treatment. The SCC (thous./ml) concentration was assessed at the State Laboratory „Pieno tyrimai", using controlled cow routine test methods. Milk samples were obtained during evening milkings, from each cow's composite milk.

We used laser apparatus model STP-8, beaming a low intensity beam spectrum of infrared radiation waves (low level laser). Cows in all groups were processed with laser-radiation once daily for 7 days after milking. Udders were irradiated regardless of which quarter was infringed, 10-15 cm from the milk gland's ventral surface. Experimental data was processed via the SPSS statistical batch (SPSS for Windows 7.0, SPSS Inc.,Chicago, IL, USA, 1989-1995) with One-Way Anova Test.

Results and discussion

Affected by laser rays, microbiological cultures *in vitro* grow differently. 30% of samples influenced by laser radiation grew *Mucor* sp. whereas none were identified in the control group. *Candida* sp. were identified in 80% of samples and after irradiation – 24.5%. CNS was identified 15% more often than in those having been radiated. In the experimental group, *Enterobacter* sp. grew 10% less and *E. coli* – 15%. However, laser radiation had no influence on *Streptococcus* sp. and *S. aureus*, as both groups showed identical results – 75% and 10% samples respectively.

Some laser-radiated micro-organism cultures became more receptive to antibiotics. This can be determined via a more intensive diffusion zone which forms if the antibiotic inhibits micro-organism development. This investigation showed that laser-irradiated micro-organisms were most sensitive to netilmicin (19 mm), erithromycin (22 mm), gentamycin (20 mm), rifampicin (18 mm) and neomycin (18 mm). Amongst the micro-organism samples that were not laser-radiated, smaller antibiotic diffusion zones were determined for: netilmicin (15 mm), erithromycin (19 mm), gentamycin (19 mm), rifampicin (12 mm) and neomycin (10 mm). The micro-organisms were resistant to other antibiotics. Saprophytic and conditionally pathogenic microflora were identified in Experimental Group cows.

Prior to treatment, various microflora were identified in 80% of the samples. Micro-organisms were identified in some milk samples from clinically healthy cows, and in 83% of milk samples showing an increased SCC. Immediately post-treatment micro-organisms were found in 10 samples (50%). 21 days after therapy micro-organisms were identified in 40% of all samples. During this period no micro-organisms were identified in milk samples from cows that did not receive laser radiation (intact cows). No micro-organisms were identified. At 21 days post physiotherapy no micro-organisms were identified in milk samples from cows in their 1st lactation, however in other dairy cow groups micro-organisms were identified in 75% of all samples. Variations in SCC tendencies were not identified in healthy cow milk samples. Prior to treatment the dominating micro-organism species identified were: *Streptococcus* sp. – 30%, *E. coli* – 25%, CNS – 25% and *Corynebacterium bovis* was identified in 2 samples. One intact cow's samples showed different micro-organisms on day 7 and day 21 of the experiment. In post treatment, the variety of micro-organism species decreased by 60.7% and analogically remained unchanged 21 days post treatment (calculating micro-organism species respectively: 1.4 ± 0.24a 0.55 ± 0.12 and 0.5 ± 0.15b). The post

Tab. 1. Results of bacteriological research and SCC

Condition of cows	Therapy					
	Before		After 0 days		After 21 days	
	SCC 10^3/ml	Microorganisms	SCC 10^3/ml	Microorganisms	SCC 10^3/ml	Microorganisms
Milking cow (M)	605	CNS, *Ent., C. krusei*	550	*	149	CNS
M	563	*Streptococcus* sp.	950	*	1032	CNS
M	665	*KNS*, *E. coli*		*KNS*, *E. coli*	1773	CNS, *E. coli*
M	508	CNS	1817	CNS	409	CNS
M	538	*Streptococcus* sp, *E. coli*, *C. krusei*	472	*Streptococcus* sp.	506	*
M	790	CNS	5000	*	405	*
M	1387	CNS, *Streptococcus* sp., *E. coli*	33	*	45	CNS, *E. coli*
M	1315	*KNS*, *Streptococcus* sp.	1092	*Streptococcus* sp.	728	*KNS*
Fresh milk cow (F)	1016	*	705	*	528	*
F	1651	*C. bovis*	465	CNS	377	*
F	1793	*C. bovis*	915	*C. bovis*	768	*
F	576	*	469	*	395	*
Healthy (H)	63	CNS, *Streptococcus* sp., *E. coli*	269	*Streptococcus* sp.	62	CNS
H	118	CNS	533	*	196	*
H	133	*Streptococcus* sp., *E. coli*	172	CNS	160	*
H	70	CNS	49	*C. bovis*	49	*
H	50	*	30	*	35	*
Placebo (I)	24	*	28	CNS	44	*Streptococcus* sp.
I	103	*C. bovis*, *C. tropicalis*	63	*	89	*
I	197	*C. bovis*, *C. tropicalis*	133	*	108	*

Explanation: * – no growth

treatment variations in microorganism species are statistically reliable (a : b $p < 0.001$). The dominant microorganism species post-therapy were coagulase--negative staphylococcus (CNS) –50%. Post physiotherapy milk samples from infirm cows (having a SCC > 400,000/ml) showed a decreasing SCC tendency (tab. 1). As has been shown (tab. 1), the milk SCC decreased after 1 week (4.17%) (Upon completion of the therapy course).

A statistically reliable 20.11% decrease in SCC was established at 21 days post treatment. At 70 days post treatment, the average SCC differed by 3.87% from the initial concentration, however this difference is not statistically validated (tab. 2).

Contradictory reports exist about low-intensity laser light-stimulated cell proliferation (14). Laser light stimulated the growth of *Mucor* sp. in our study. The use of LLLT may modulate the activity of cells interacting with an implant (9). The modulating influence of laser light on cell growth can be explained via *in vitro* micro-organism growth and variations in resistance to antibiotics.

Streptococcus sp., *Candida* sp. and CNS were identified in cow milk samples showing SSC > 400,000/ml. To maintain that, the most frequently isolated environmental pathogens were environmental streptococci (51.07%), fungi (20.48%), coliforms (19.87%) (10, 17). Contagious bacteria (eg. *S. aureus*, *C. bovis*, *S. agalactiae* and coagulase negative staphylococci) caused most of the mastitis infections (8). Laser irradiated microorganism cultures exhibited a weaker incidence of environmental micro-organisms, especially fungi and *Streptococci* sp. The effect of laser light on microorganisms has been observed in studies of women's cervical secretions (6). No laser light effect was noted on *S. aureus* culture development. After completion of treating cows with laser therapy, *Streptococcus* sp. and *Candida* sp. were not identified, which are environmental causative agents of mastitis, and no identified pathogens of mastitis-causing agents (*C. bovis*) were identified in milk samples from the 2 first-lactation cows. No *in vitro* laser light

Tab. 2. SCC before and after treatment with laser light

SCC thousand/ml	Treatment			
	before	after 0 days	after 21 days	after 70 days
	796.58 ± 77.98[a]	763.30 ± 97.20	636.37 ± 75.46[b]	765.80 ± 204.09

Explanation: a, b – $p \leq 0.01$

effect was noted on this type of causative agent cultures. Low-intensity laser stimulated phagocytosis, but also intracellular generation of active oxygen forms (15). A greater efficacy of laser therapy on an organism's antimicrobial reaction can be explained *in vivo*. *In vivo* laser therapy and *in vitro* radiation did not show any influence on CNS development. CNS-infected mammary tissues exhibited greater leukocyte infiltration and increased the connective tissue stroma over an uninfected control. CNS is the most frequently recovered isolate from mastitis samples, especially in first lactation and unbred heifers. Many CNS strains isolated from mastitis samples had higher protease, deoxyribonuclease (DNase), and lecitinase activity than that of CNS from normal cows (22). A clear difference of laser therapy efficacy during various stages of lactation was not noted.

Microorganisms were not identified in all milk samples having an increased SCC, nor contrarily, microflora were not identified in all milk samples showing an elevated SCC. The variation factors that could influence these SCC values and the bacteriological results are discussed (5). Our identified microflora did not correlate with SCC. In the case of sub-clinical mastitis, bacterial cultures did not yield any growth for 622 (58.8%) of these samples (13). CNS, *E. coli* and *Streptococcus* sp. were identified in milk samples from 4 healthy cows (having a SCC up to 200 000/ml). Samples with SCC less than 200 000/ml were mostly free from infection, contaminated or infected with less pathogenic bacteria. Milk samples that had less than 200 000 cells/ml were mostly (59.6%) infected with CNS or contaminated (12).

After the course of therapy bacteriological tests of cow milk samples identified CNS. Seventeen different coagulase-negative *Staphylococci* sp. (CNS) were found in 4.1% of the samples (1). The variety of CNS genus species can explain the circumstances surrounding the post-treatment micro-organism identification of this particular genus.

With successful treatment the variety of micro-organisms decreases in milk (18). Our data shows that after laser treatment the variety of micro-organism species immediately decreases 64.28% and this indicator remains unchanged after 21 days. We observed that the intact cow group SCC changes spontaneously and during the study period it had a tendency to decrease. No micro-organisms were identified (100%) in milk samples. This concurs with observations that after recovery the cell numbers retreat back to the physiological level, which takes from 7-21 days (12).

21 days after completion of the course of therapy the SCC decreased 20.11%. 70 days after treatment the SCC increase compared to the 21 day period increased by 20.3%, which can be associated with factors unrelated to method of therapy. Post-treatment SCC was associated with treatment regimen, other risk factors, and interactions among the other risk factors; but these other risk factors did not vary significantly with the treatment regimen (4).

It is advisable to treat increases in SCC with low intensity laser rays, conditional to environmental mastitis causative agents. Moreover, since due to the effect of laser radiation certain irradiated micro-organism cultures become more susceptible to antibiotics, it is advisable to coordinate laser therapy with antibiotic therapy.

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