Impact of low light laser therapy (LLLT) on microorganisms causing subclinical and clinical bovine mastitis

Soumaya, S. A. El-Shafii[1] and Nagaty, S. F.[2]
[1]Bacteriology Department Animal Health Research Institute, Doki
[2]Animal Health Research Institute, Fayoum

Abstract

The aim of the study was to monitor the effects of radiation emitted by an alternative, non-pharmaceutical agent "infrared low laser radiation 830nm with 300mW" on the growth of microorganisms in vitro from milk of cows with elevated somatic cell count (SCC). Also, detectiom of microorganism diversification and SCC after the lead treatment in vivo by the laser.

Twenty six samples showed mastitis where five were clinically mastitic and 21 were subclinically mastitic. From those, 9 cows were chosen to be treated (two from clinically mastitic cows and 7 from subclinical mastitic cows).

The clinical mastitic cows showed swollen, hot and painful teat with no change in milk characterization and gradual decrease in milk yield. Two cows showed sudden drop in milk yield and 3 cows showed change in milk characters with gradual decrease in milk yield. The average of milk yield of tested cows was ranged from 7.8 -20L during the five days before LLLT, CMT results showed strong score 3 for the nine cows and the reading of mastit test ranged from 455-850 units.

Staphylococcus aureus (S.aureus) and *Escherichia coli* (*E.coli*)were the most predominant isolates. The two organisms were isolated as single pathogen in 30.7% and 26.9% respectively of the culture of milk samples and as co-pathen with *Strept.agalacteae* and *L.hardjo* (19.2% and 9.6% respectively). *Leptospira hardjo* was diagnosed as single pathogen in a rate of 15.3%.

Antibiotic susceptibility test revealed that *S.aureus* and *E.coli* showed multidrug resistance, where all *S.aureus* (15 isolates) resistant to tetracyclin, neomycin, chloramphenicol, amikacin, lincomycin and colistin sulfate, 14 isolates were resisitant to ampicillin, erythromycin, sulfa/trimethoprim and gentamicin, 13 isolates resistant to clindmycin, 12 isolates resistant to cephalothin and 7 isolates resistant to ernofloxacin.

On the other hand, all *E.coli* (12 isolates) resistant to tetracyclin and sulfa/trimethoprim, 11 isolates resitant to erythromycin, neomycin and chloramphenicol, 10 isolates resistant to ampicillin, amikacin and lincomycin, 9 isolates resistant to gentamycin, 8 isolates resistant to chloramphenicol and colistin sulfate, 7 isolates resistant to clindamycin and 4 isolates resistant to ernofloxacin

The increase of milk yield of each cow and normal physical character was observed during the period of LLLT and the average milk yield ranged from 6.2L up to 20L in clinical mstitic cows and 11.4L up to 17L in subclinical mstitic cows. The CMT records was decreased (score 1) and the EC decreased at the end of LLLT ranged from

330-450 units. No viable bacteria were reisolated by the end of LLLT and post LLLT (end of experiment) where the average milk yield ranged from 12 to 21L in clinical mastitic cows and from 20 to 21.4L in subclinical mastitic cows. The CMT records was negative (score 0) for both clinical and subclinical cows and the EC decreased at the end of LLLT ranged from 250 -220 units in clinical mstitic cows and from 277 to 220 in suclinical mastitic cows.

It was concluded from this study that low light laser therapy opens new perspectives in the treatment of clinical and subclinical mastitis as an alternative, non-invasive, and may be of low cost method as if compared with antibiotic treatment. Lactation was not stopped post LLLT while antibiotic therapy lactation stopped for 10-15 days post treatment to avoid antibiotic residues in milk, Also it is considered as alternative green medicine.

Key words: Low light laser therapy (LLLT), clinical and subclinical mastitis, *S.aureus, E.coli*

Introduction

Mastitis constitutes a universal disease in dairy cattle. It is recognized as the most costly disease for the producers because of the limitations imposed by the high quality milk regulation adopted by most countries and by the milk losses associated either with clinical (**de Haas *et al.*, 2002; Hortet and Seegers, 1998; Houben *et al*., 1993 and Ragala-Schultz *et al.*, 1999)** or subclinical mastitis (**Fetrow *et al.*, 1991; Hortet *et al.*, 1999**) and to mastitis treatments (**Bennedsgaard *et al.*, 2003; Hillerton, 1998**). Antibiotic tratments account for 85% of the cost in clinical mastitis. Such cost is due to the discarded milk and decreased production.

In the incidence of sub-clinical mastitis, somatic cell count (SCC) values increase significantly. 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 bacterial toxins (**Malinowski, 2001**)

Well-established mechanisms of action of the laser radiations have been described in the literature and several uses are being done in human medicine(**Tuner and Hode, 1999; Walker, 2002)**. It is applied in analgesia, surgery,wound and joint treatment (**Oezdemir, *et al.* 2001; Schindl, *et al*. 1999 and Simunovic Simunovic, *et al.* 2000**). In gynecology, laser rays can be applied as an assistive means in treating purulent mastitis (**Alekseenko, *et al.* 1987**). The effect of laser light bio stimulation has been observed to expedite necrotic muscle regeneration (**Podbielski, *et al.* 2006)**. Laser radiation influences an increase in leukocyte activity, stimulates vascularisation, regulates local temperature and subdues symptoms of inflamation**.**

In veterinary medicine, low laser therapy has been used by the mid1980`s. the lasers used cover the power range from 20mW-1W in the frequency range from the visible light to the infrared radiation. The most common wavelengths used are 650-

660nm in the visible spectrum and 808-830nm in the near infrared spectrum. (**Beneduci *et al.*, 2007**)

In a previous work, a low He-Ne-laser (630nm) was used to irradiate the mammary glands (**Stoffel *et al.*, 1989**). No beneficial effects of such a treatment on the affected cows. While **Beneduci, *et al.*, 2007** recorded the effecacy of 880-890nm low laser treatment caused an effective beneficial response on the cows against mastitis.

Studies on laboratory animals indicate that low intensity laser radiation destroys *Pseudomonas aeruginosa* and *Staphylococcus aureus* cultures, as well as induces blood-vessel regeneration (**Bayat, *et al.*, 2006).**

The aim of the current study was to monitor the effects of radiation emitted by an alternative, non pharmaceutical agent "infrared low laser radiation 830nm with 300mW" 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:

The present study was conducted in a farm located at Al-Fayoum government. The farm contained only 40 cows at the milking season. California mastitis test (CMT) test Electric conductivity test (ED) were applied before milking to diagnose cows with subclinical mastitis.

California Mastitis test (CMT): Leach, *et al*. (2009)

A small sample of milk (~2ml) from each quarter was collected into a plastic paddle that has four shallow cups marked A, B, C, and D. an equal amount of CMT reagent was added to the milk. In order to mix the content, the paddle was rotated and after a few seconds (~20s) the score was read. The test was performed daily to support the data obtained by a precise somatic cell counting.

Interpretation of California test:

Category	Score	Description of reaction
Negative	0	Mixture of milk and test fluid stays unchanged and can easily be shaken
Weak positive/trace	1	Mixture is slightly mucous but can still be shaken
Positive	2	With movement of the mixture an unmistakable mucous formation can be seen. It is still possible to tip a small portion of the mixture out.
Strong positive	3	Jelly-like, mucous consistency is formed and is difficult to shake the mixture. It is no longer possible to tip out and surplus liquid.

Electric Conductivity test (EC): using Mastit test (Russian) according to the manufacture manual

The cup was switched on with no milk; the LCD should display a reading from "000 to 005. The measurement cup was holed under the teat and squirt 15 ml of milk of the first fore milk directly into the cup, on/off was switched for 1-2sec. until the readout on the LCD and stabilizes at 3 digit numbers. The power switch was turned off.

Interpretation of Mastit test:

- Readings below 450 units ⟶ milk sample is of high quality and is healthy.
- Readings between 450 and 600 units ⟶ A progressively increasing incidence of subclinical infection.
- Readings over 600 units ⟶ rapid increase in the severity of infection. (This is typified by somatic cell counts rising from less than 1 million up to many millions.

CMT and EC test were applied before the first lactation each day 15 days (the end of experiment).

Bacteriological analysis:

Prior to bacteriological analysis, teats of cows were cleaned with 5% ethanol and the first three streams of fore milk were discarded. From each quarter, 15ml of milk sample were collected and stored at 2-4ºC until bacteriological analysis. The sampling was made before the morning milking. Twenty six milk samples from clinical and subclinical mastitic cows were collected for isolation and identification of different bacteria causing mastitis (the milk sample was collected from the four quarter and considered as one sample) (**Qunin *et al*., 2002** and **Cruickshank, 1975**). One hundred microliters were plated on the following plates (Oxoid): Sheep blood agar, MacConkey agar mannitol salt agar, Staph-Strept, media. The plates were incubated at 37ºC for 24-48 h. *Leptospira* Hardjo (*L.* Hardjo) was diagnosed by indirect ELISA.

Macroscopic estimation of the colonies, effectiveness of hemolysis on sheep blood agar, microscopic morphology evaluation on Gram Stained samples and biochemical characterization by oxidase test, catalase test and Staphtect (Oxoid) or by commercial API (Biomèurieux)

Antibiotic susceptibility test:

It was applied using disc diffusion method (according to **NCCLS 2002**) using different types of sensitivity discs with variable concentrations to detect the susceptibility of isolates. These discs were obtained from (Oxoid). Tetracycline (TE 30 μg), Ampicillin (AM 10 μg), Neomycin (N 30 μg), Erythromycin (E 10 μg), , Chloramphenicol (C 30 μg), Sulfa/trimethoprim (SXT 25 μg), Cephalothin (KF 30 μg), Amikacin (KA 30 μg), Clindamycin (DA 2 μg), Colistin sulfate (CT 2 μg), Gentamicin (CN 10 μg), Lincomycin (L 2 μg) and Ernofloxacin (Er 10 μg).

Treated cows:

Nine cows were chosen from 26 cows for low laser treatment, two cows shown clinical signs of mastitis, where the teat of one quarter was inflamed and painful with no drop in milk production; 3 cows showed changes in milk physical character where one showed watery yellowish milk and the other 2 cows showed bloody milk with drop in milk production with no clinical lesions in udder tissues by palpation; 2 cows showed sudden drop in milk production only and the rest of cows (2 cows) showed subclinical mastitis with apparently normal milk.

The laser apparatus:

The radiation experiment was applied by using the laser generator favorite (name of apparatus) model apparatus made by "Scientific Production Cooperation Petrolaser"

Protocol of low light laser therapy LLLT:

- The milk yield and somatic cell count (by California test) were obtained for five days before beginning of treatment at each milking.
- Two cows with clinical symptoms have been irradiated (after each milking) with active 830 nm diode laser device with output power of 300 mW by scanning around the teat and udder with a distance of ~3mm for 3min. while the other 7 cows have been irradiated by direct contact to teat and udder tissue for 3min. **Photo. (1)**
- All cows were processed with laser--radiation twice daily for consecutive five days.
- The milk yield and somatic cell count were daily recorded during treatment and for five days after LLLT.
- Reisolation of bacteria from milk samples after the end of LLLT

Effect of low laser on isolated bacteria in *vitro*:

- The isolated bacteria were inoculated on Muller Hinton broth and incubated at 37ºC over night.
    - The colony forming unit (cfu/ml) was adjusted by using McFarland tube No. 0.5 ($1*10^6$ /ml).
    - A sterile cotton swab was dipped into the Muller – Hinton broth suspension was used to streak the middle dried surface of a Muller – Hinton agar plate (15ml diameter) to avoid glass scattering of laser beam and the plates were incubated at 37ºC for 24h.
    - The incubated plates have been irradiated with active 830 nm diode laser device with output power of 300 mW for 3min. for frequent five times, recultivation was done after each time.

Results and Discussion

Twenty six out of 40 cows were infected by clinical and subclinical mastitis (5 clinal mastitic cows and 21 subclinical mastitic cows) and the rest (14 cows) were normal. Nine cows were chosen to be treated with laser. Two cows showed swollen, hot and painfull teat with no change in milk characterization and gradual decrease iin milk yield. Two cows showed sudden drop in milk yield and 3 cows showed change in milk character with gradual decrease in milk yield. The average of milk yield of experimented cows was ranged from 7.8 -20L during the five days before LLLT (**Table 1**), CMT results showed strong (score 3) for the nine cows and the reading of mastit test was ranged from 455 – 850 units **(Table 2)** which revealed that all cows suffering from subclinical mastitis.

. **Table (3)** illustrated the most predominant bacterial isolates from clinical and subclinical mastitic milk. *S.aureus* and *E.coli* were the most predominant isolates where isolated as single infection (30.7%and 26.9% respectively) and mixed infection with *Strept.agalacteae* and *L.* Hardjo (19.2% and 9.6% respectively). *Leptospira* Hardjo was detected as single infection in a rate of 15.3%. **Jemeljanovs *et al*. (2007)** reported that *Staphylococcus aureus* is a major bovine mastitis agent and primary human pathogen and from gram-positive cocci, the more frequently isolated microorganisms were *S.aureus* both in subclinically and clinically diseased cow`s udder contageous mastitis *Strept. agalacteae* were isolated (contagious mastitis).

On the other hand, *E.coli* and *L.Hardjo* were diagnosed environmental mastitis these revealed bad hygienic measure and management in the farm. **Tiwari et al. (2013)** reported that depending upon a multitude of factors such as variability in hygienic practices on farms, easy access leading to overuse of appropriate or inappropriate antibiotics at suboptimal concentrations, particularly in developing countries, mastitis is considered to be one of the most economically significant diseases for the dairy industry for backyard farmers in developing countries and high producing herds worldwide.

Antibiotic susceptibility test revealed that *S.aureus* and *E.coli* showed multidrug resistance, where 8 *S.aureus* isolates were sensitive to Ernofloxacin, 3 isolates sensitive to cephalothin and 2 isolates to clindamycin. On the other hand 8 *E.coli* isolates were sensitive to Ernofloxacin, 6 isolates to cephalothin and clindamycin, 4 isolates to chloramphenicol and colistin sulfate **(Table 4).** These results were nearly agreed with **Ranjan et al. (2010).**

This multidrug resistance of *S.aureus* and *E.coli* pointed to the misused of different antibiotic leading to difficulty in controlling clinical and subclinical mastitis.

Protecting life and human and animal health is a constant concern of both doctors and specialists in various fields (biochemistry, biophysics, biology, etc.). Their joint efforts have led to the development of new methods of treatment based on new discoveries and technology including laser. Also Minimising mastitis and consistently producing high quality milk is a requirement for dairy farmers who wish to be competitive in the global marketplace.

Table (5) showed the increase of milk yield of each cow and its physical characterization return normal during the period of LLLT Fig. (1-9) and the average milk yield ranged from 6.2 up to 17L. The CMT records was decreased (score 1) and the EC decreased at the end of LLLT ranged from 330-450 units (**Table 6**). No reisolation of bacteria by the end of LLLT.

Table (7) showed the increase of milk yield of each cow and its physical characterization return normal for five days post LLLT (end of experiment) **Fig. (1-9)** and the average milk yield ranged from12-21.4L. The CMT records was negative (score

0) and the EC decreased at the end of LLLT ranged from 450-222 units (**Table 8**). No reisolation of bacteria by the end of experiment.

The results LLLT on microorganism in vitro revealed no growth of radiated isolates of *S.aureus* and *E.coli* on different media (sensitive to LLLT).

These result revealed the impact of LLLT on bovine mastitis where somatic cell count were reduced. **Zilaitis, *et al*. (2008)** found that it is advisable to treat increases in SCC with low intensity laser rays. Also **Beneduci *et al*. (2007)** found could treat cow mastitis after experimental adopted of low Infrared laser treatment.

Failure to reisolate microorganism from milk samples after LLLT laser in vivo and confirmed by treatment in vitro revealed the antimicrobial effect of LLLT on *S.aureus* and *E.coli* **Zilaitis, *et al*. (2008)** the variety of micro-organism species immediately decreases 64.28% and this indicator remains unchanged after 21 days but No laser light effect was noted on *S. aureus* culture development. Also, **Yueqiang, *et al.* (2014)** suggested that LLLT therapy is beneficial in decreasing the somatic cell count and improving milk nutritional quality in cows with an intramammary infection after application of LLLT on a rat model of lipopolysaccharide (LPS)-induced mastitis.

Laser phototherapy is based on laser radiation absorption by cytochromes and porphyrins, from mitochondria and cell membranes, Production of singlet oxygen leads to the formation of proton gradients across cell membranes and mitochondrial membrane. Th ese gradients of protons change the permeability of cell membranes to diff erent ions and mitochondrial membrane permeability, which leads to metabolic changes in the cell. Th ese metabolic changes induced by low power laser radiation at the cellular and subcellularlevel lead to the development of procedures for the treatment of soft and hard tissue diseases. So the use of low-power laser radiation in treating inflammations has been the focus of various researchers around the world. Pathologies they addressed were related to acute and chronic inflammation of region first phalanx, local infectious inflammation (mastitis, thrombophlebitis, abscesses, chronic sinusitis, otitis, etc.), inflammation accompanying various traumatic injuries (acute sprains, muscle and capsular rupture, hematoma), and acute and chronic tendinitis. In order to treat inflammation, laser systems with continuous or pulsed emission were used. **Petermann (1998)** used a pulsed laser system (Ppeak = 60 W/90 W, tpuls = 200 ns) for the treatment of local inflammations (arthritis and tenosynovitis) in horses, while **Stellain *et al*. (1992)** used the He-Ne laser (continuous emission) to treat temporomandibular inflammation in dogs.

It was concluded from this study that low light laser therapy opens new perspectives in the treatment of clinical and subclinical mastitis as an alternative, non-invasive, and may be of low cost method as if compared with antibiotic treatment. Lactation was not stopped post LLLT while antibiotic therapy lactation stopped for 10-15 days post treatment to avoid antibiotic residues in milk, Also it is considered as alternative green medicine.

Further studies were needed for application of LLLT as prophylactic treatment in dry season of dairy cows and on other microorgnisms.

Table (1): Records of milk yield per liter before Low laser treatment

Cow`s No.	Status of udder and milk and production	1st day	2nd day	3rd day	4th day	5th day	Average of milk yield
419	Clinical mastitis	12	10	10	8	7	9.4
62	Clinical mastitis	22	20	21	20	18	16.6
162	Sudden drop of milk	11	10	11	9	2	8.6
195	Sudden drop of milk	24	23	22	21	10	20
171	Water yellowish milk yied	11	10	7	6	3	6.14
199	Water yellowish milk yied	12	10	8	5	4	7.8
166	Bloody milk	11	9	10	8	6	8.8
523	Subclinical mastitis	18	16	17	16	15	16.4
146	Subclinical mastitis	17	15	17	16	15	16

Table (2): Records of electric conductivity test (EC) using mastit test (units)

Cow`s No.	1st day	2nd day	3rd day	4th day	5th day
419	600	768	788	800	800
62	550	630	700	820	800
162	490	650	699	700	768
195	670	700	760	810	850
171	450	500	690	699	810
199	510	589	600	700	790
166	478	610	700	769	830
523	455	600	620	790	850
146	509	600	710	833	814

Table (3): Type and rate of bacteria isolated from milk samples

Status of cows	Total No. of cows (26)	Single infected cows						Mixed infection			
		S.aureus		*E.coli*		*Leptospira* Hardjo*		*E.coli* + *S.aureus* + *Strep.agalacteae*		*S.aureus* + *Leptospira* Hardjo	
		No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Clinical mastitic	5	1	20	1	20	3	60	0	0	0	0
Subclinical	21	7	33.3	6	28.5	1	4.7	5	23.8	2	9.5
Total	26	8	30.7	7	26.9	4	15.3	5	19.2	2	7.6

***** Leptospira Hardjo was detected by indirect ELISA and not recorded in this study

Table (4): Antibiotic susceptibility test

Antibiotic disc*	*S.aureus* (15 isolates)		*E.coli* (12 isolates)	
	S	R	S	R
Tetracyclin TE 30µg),	0	15	0	12
Ampicillin (AM 10µg)	1	14	2	10
Neomycin (N 30µg)	0	15	1	11
Erythromycin (E 10µg)	1	14	1	11
Chloramphenicol (C 30µg)	0	15	4	8
Sulfa/trimethoprim (SXT 25µg)	1	14	0	12
Cephalothin (KF 30µg)	3	12	6	6
Amikacin (KA 30µg)	0	15	2	10
Ernofloxacin (Er 10µg)	8	7	8	4
Lincomycin (L 2µg)	0	15	2	10
Gentamicin (CN 10µg)	1	14	3	9
Colistin sulfate (CT 2µg)	0	15	4	8
Clindamycin (DA 2 µg)	2	13	6	7

Table (5): Records of milk yield during LLLT

Cow`s No,	Status of animal	1st day	2nd day	3rd day	4th day	5th day	Average
419	Clinical mastitis	7	10	10	12	14	10.6
62	Clinical mastitis	19	20	22	21	18	20
162	Sudden drop of milk	3	5	5	7	11	6.6
195	Sudden drop of milk	11	13	16	20	21	16.2
171	Water yellowish milk yied	3	4	7	7	10	6.2
199	Water yellowish milk yied	4	5	8	11	13	8.2
166	Bloody milk	6	9	10	10	14	9.8
523	Subclinical mastitis	15	16	16	18	20	17
146	Subclinical mastitis	15	15	17	19	21	11.4

Table (6): Records of electric conductivity test (EC) using mastit test (units)during LLLT

Cow`s No.	1st day	2nd day	3rd day	4th day	5th day
419	800	486	430	400	350
62	800	560	410	400	333
162	768	490	436	399	410
195	850	577	544	410	432
171	810	493	390	366	350
199	790	510	500	450	400
166	830	610	512	455	330
523	850	600	540	480	350
146	814	560	510	468	450

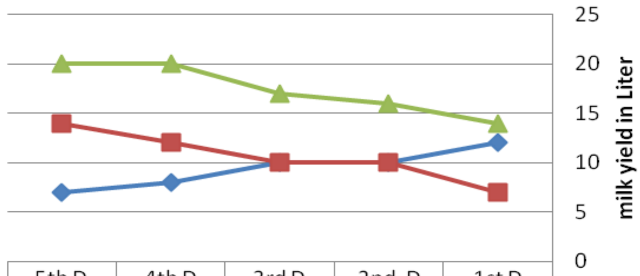
Table (7): Records milk yield after LLLT

Cow`s No.	Status of udder	Amount of milk yield (L)	Amount of milk yield (L)	Amount of milk yield (L)	Amount of milk yield (L)	Amount of milk yield (L)	Average
419	Clinical mastitis	14	16	17	20	20	17.2
62	Clinical mastitis	18	20	21	21	21	20.2
162	Sudden drop of milk	11	11	12	12	14	12
195	Sudden drop of milk	21	21	21	21	21	21
171	Water yellowish milk yied	10	11	14	14	17	13.4
199	Water yellowish milk yied	13	13	16	16	19	15.4
166	Bloody milk	14	16	18	18	20	17.2
523	Subclinical mastitis	20	20	21	22	22	21
146	Subclinical mastitis	21	20	21	22	23	21.4

Table (8): Records of electric conductivity test (EC) using mastit test (units) after LLLT

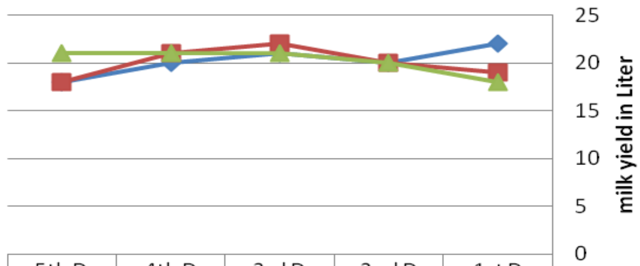
Cow`s No.	1st day	2nd day	3rd day	4th day	5th day
419	350	300	250	277	250
62	333	220	220	220	250
162	410	348	350	330	310
195	432	311	304	288	250
171	350	290	280	256	220
199	400	312	277	240	250
166	330	220	225	220	220
523	350	340	240	230	220
146	450	350	300	313	277

Fig.1: Milk yield of cow`s No. 419



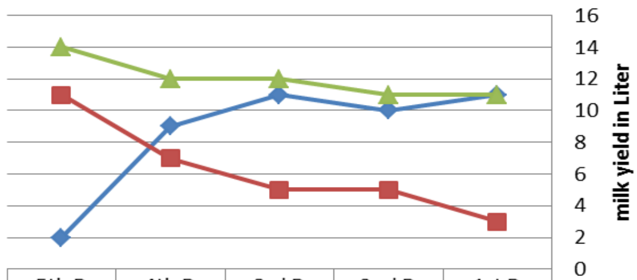
	5th D	4th D	3rd D	2nd D	1st D
before LLLT	7	8	10	10	12
during LLLT	14	12	10	10	7
after LLLT	20	20	17	16	14

Fig. 2: Milk yield of cow`s No. 62



	5th D	4th D	3rd D	2nd D	1st D
before LLLT	18	20	21	20	22
during LLLT	18	21	22	20	19
after LLLT	21	21	21	20	18

Fig. 3: Milk yield of cow`s No.162

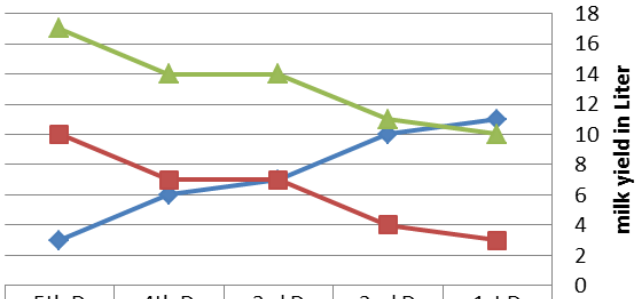


	5th D	4th D	3rd D	2nd D	1st D
before LLLT	2	9	11	10	11
during LLLT	11	7	5	5	3
after LLLT	14	12	12	11	11

Fig. 4: Milk yield of cow`s No.195

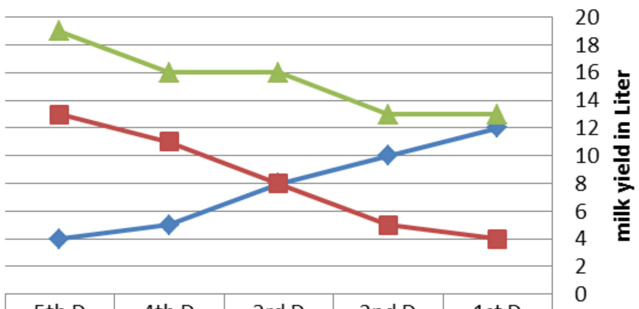
	5th D	4th D	3rd D	2nd D	1st D
before LLLT	10	21	22	23	24
during LLLT	21	20	16	13	11
after LLLT	21	21	21	21	21

Fig. 5: Milk yield of cow`s No.171



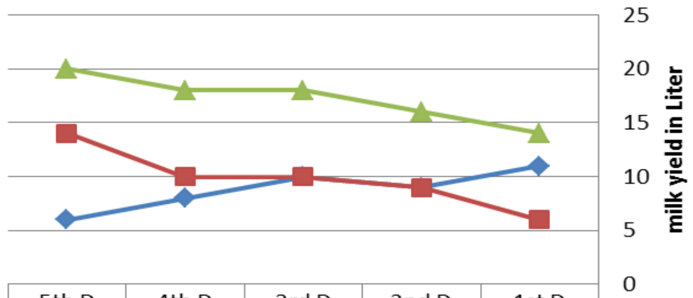
	5th D	4th D	3rd D	2nd D	1st D
before LLLT	3	6	7	10	11
during LLLT	10	7	7	4	3
after LLLT	17	14	14	11	10

Fig. 6: Milk yield of cow`s No.199



	5th D	4th D	3rd D	2nd D	1st D
before LLLT	4	5	8	10	12
during LLLT	13	11	8	5	4
after LLLT	19	16	16	13	13

Fig. 7: Milk yield of cow`s No.166



	5th D	4th D	3rd D	2nd D	1st D
before LLLT	6	8	10	9	11
during LLLT	14	10	10	9	6
after LLLT	20	18	18	16	14

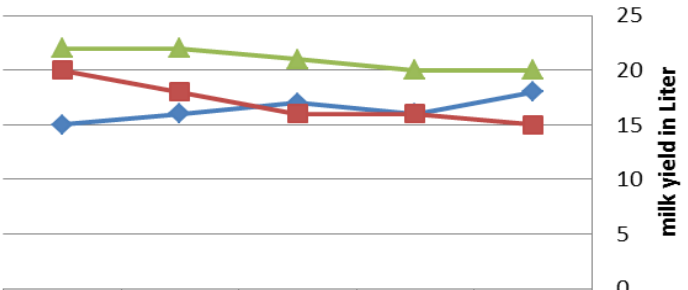


Fig. 8: Milk yield of cow`s No. 523

	5th D	4th D	3rd D	2nd D	1st D
before LLLT	15	16	17	16	18
during LLLT	20	18	16	16	15
after LLLT	22	22	21	20	20

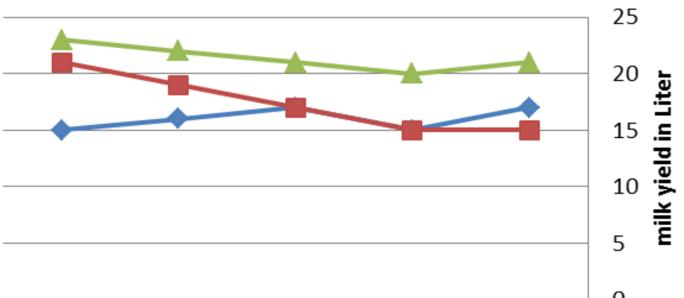


Fig. 9: Milk yield of cow`s No. 146

	5th D	4th D	3rd D	2nd D	1st D
before LLLT	15	16	17	15	17
during LLLT	21	19	17	15	15
after LLLT	23	22	21	20	21

Photo. (1): Application of LLLT on udder by direct contact using Favorite apparatus

References

Alekseenko A. V., Palianitsa S. I., Tarabanchuk V. V., Seniutovich R. V., Stoliar V. F. (1987): Treatment of suppurative mastitis using laser irradiation and continuous electric current. Vestn. Khir. Im. I. I. Grek. 139, 54-57.

Bayat M., Vasheghani M. M., Razavi N. J. (2006): Effect of low-level helium-neon laser therapy on the healing of third-degree burns in rats. Photoch. Photobiol. B., 83, 87-93.

Beneduci,A.; **Chidichimo, GNappi,M.C.;Rossi, R.; Turco, R.; Lucifora, G. and Garrafa, F. (2007):** Evaluation of the effects induced by near infrared low intensity laser radiation on bovine affected by subacute mastitis: A single-quarter foremilk sampling study. J. Animal and Vetrinary Advances, 6,(6): 761-767.

Bennedsgaard, T.W.; Enevolsen, C,; Thamsborg, S.M. and Vaarst, M. (2003): effect of mastitis treatment and somatic counts on milk yield in Danish organic dairy cows. J. Dairy Sci., 86:3174-3183.

Bjordal, J.M., Couppe, C., Chow, R.T., Tuner, J., Ljunggren, E.A.(2003): A systematic review of low level laser therapy with location-specifi c doses for painfrom chronic joint disorders. Aust. J. Physiother. 49: 107-116.

Cruickshank, R., Duguid, J. P., Marmion, B. P. and Swain, R. H. A. (1975): Medical Microbiology, 12th ed., vol. II. Churchill Livingstone, Edinburg, London and New York.

de Haas, Y.H., Barkema, H.W. and Veerkamp, R.F. (2002): The effect of pathogen-specific clinical mastitis on the lactation curve for somatic cell coun *Journal of Dairy Science* 85: 1314–1323.

Fetrow, J.,D. Mann, K. Butcher and McDani, B. (1991): Production losses from mastitis carryover from the previous lactation, J. Dairy SCI., 74: 833- 839.

Hillerton, J.E., (1998): Mastitis treatment-a welfare issue.proceeding of the British Mastitis conference 1998, Axient institute for Animal Health, Milk Dev. Council / Novartis Anim. Health, PP: 3-8.

Hoben, E.h.p.; Dijkhuzen, A.; VanArendonk, J.A.M. and Huirne R.B.M. (1993): Short-and long term production losses and repeatability of clinical mastitis in dairy cattle. J.dairy Sci., 76:2561-2578.

Hortet , P.,F. and Seegers, H. (1998): Calculated milk production losses associated with elevated somatic cell counts in dairy cows: Review and critical discussion. Vet. Res., 29 : 497-510.

Hortet , P.,F. Beaudeau, H. Seegers and Fourichon, C (1999): Reduction in milk yield associated with somatic cell counts up to 600000 cells mL-1 in French Holstein cows without clinical mastitis. Livestock prod. Sci., 61:33- 42.

Jemeljanovs A, Konosonoka IH, Dulbinskis J. (2007): Animal farming in transition – the role of animal reproduction: Mastitis symposium. Proceedings from a symposium at the St Petersburg State Academy of Veterinary Medicine, Russia January 10-12, 2007

Leach, K.A., Green, M.J., Breen, J.E., Huxley, J.N., Macaulay, R., Newton, H.T., Bradley, A.J., (2008): Use of domestic detergents in the California mastitis test for high somatic cell counts in milk. *Veterinary Record* 163:566-570

Malinowski E. (2001): Somatic cells in milk. Medycyna Wet. 2001, 57, 13-17.

NCCLS (2002): National Committee for Clinical Laboratory Standards. 2002. Performance standards for antimicrobial susceptibility testing. NCCLS approved standard M100-S12. National Committee for Clinical Laboratory Standards, Wayne, Pa.

Oezdemir F.; Birtane M. and Kokino S. (2001): The clinical efficacy of low-power laser therapy on pain and function in cervical osteoarthritis. Clin. Rheumatol. 20, 181-184.

Petermann, U. (1998): Lasertherapie in der Veterinarmedizin. Vet. Impulse, 2: 12-13.

Petersen, S.L., Botes, C., Oliveru, A., Guthrie, A.J. (1999): The effect of low level laser therapy (LLLT) on wound healing in horses. Equine Vet. J., 31: 228-231.

Podbielski M.; Otrocka-Domagaa I. and Rotkiewicz T. (2006): Effects of laser light on regenerating muscle fibres in pigs. Medycyna Wet. 62, 1158-1163.

Quinn, P.J., B.K. Markey, M.E. Carter, Donnelly, W.J.C. and Leonard,F.C. (2002): Veterinary microbiology and microbial diseases. Black Well Scientific ublications, Oxford, London.

Rajala-schultz, P.J.; GrÕhn, Y.T.;McCulloch, C.E. and Guard, C.L. (1999): effects of clinical mastitis on milk yield in dairy cows. J.Dairy Sci., 82:1213-1220.

Ranjan, R.; Gupta, M.K.; Singh, S. and Kumar, S. (2010): current trend of drug sensitivity in bovine mastitis, Veterinary Woeld,3,(1):17-20.

Schindl M., Kerschan K., Schindl A., Schon H., Heinzl H., Schindl L. (1999): Induction of complete wound healing in recalcitrant ulcers by low-intensity laser irradiation depends on ulcer cause and size. Photodermatol. Photoimmunol. Photomed.15, 18-21.

Simunovic Z., Ivankovich A. D., Depolo A. (2000): Wound healing of animal and human body sport and traffic accident injuries using low-level therapy treatment; a randomized clinical study of seventy-four patients with control group. J. Clin. Laser Med. Surgery, 18, 67-73.

Simunovic, Z., Ivankovich, A.D., Depolo, A. (2000): Wound healing of animal and human body sport and traffi c accident injuries using low-level laser therapy treatment: a randomized clinical study of seventy-four patients with control group. Journal of Clinical Laser Medicine & Surgery. 2000; 18: 6773.

Stelian, J., Gil, I., Habot, B. (1992): Improvement of pain and disability in elderly patients with osteoarthritis of the knee treated with narrow-band light therapy. J. Am. Geriatr. Soc., 1992; 40: 23-26.

Tiwari J.G, Babra C, Tiwari H.K, Williams V, Wet SD, et al. (2013): Trends Intherapeutic and Prevention Strategies for Management of Bovine Mastitis: An Overview. J Vaccines Vaccin 4:176. doi: 10.4172/2157-7560.1000176.

Tuner, J. and Hode , L. (1999): Low Level Laser Therapy: Clinical Practice and Scintific Background. Sweden AB: Prima books.

Walker M. (2002): the beneficial application of low level laser therapy. Medical Journalist report of innovative biologics. Town send Letter for Doctors and Patients.

Yueqiang WANG, Xianjing HE, Dandan HAO, Debin YU, Jianbin LIANG, Yanpeng QU, Dongbo SUN, Bin YANG, Keli YANG,Rui WU, and Jianfa WANG (2014): Low-Level Laser Therapy Attenuates LPS-Induced Rats Mastitis by Inhibiting Polymorphonuclear Neutrophil Adhesion**.** Vet Med Sci. 76 (11): 1443–1450.

Zilaitis V., Rudejevienë J., Maruðka R., Noreika A., Vorobjovas G., Japertas S. (2008): Effect of low intensity laser radiation on cow's milk microflora and somatic cell count. *Med. Welt.* 64: 49–52.

Acknowledgments: we thank **Dr. Khalid Rashwan** Green Medical Services "GMS" for providing the favorite and mastit test and for the training on the application of LLLT**.**