Pesq. Vet. Bras. 33(6):826-830, junho 2013

Effects of environmental modification on mastitis occurrence and hormonal changes in Holstein cows[1]

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ABSTRACT.- Arcaro J.R.P., Matarazzo S.V., Pozzi C.R., Arcaro Junior I., Toledo L.M., Costa E.O. & Miranda M.S. 2013. **Effects of environmental modification on mastitis occurrence and hormonal changes in Holstein cows.** *Pesquisa Veterinária Brasileira 33(6):826-830.* Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz, Rodovia Jorge Amado Km 16, Ilhéus, BA, 45662-900 Brazil. E-mail: svmatarazzo@uesc.br

The purpose of this research was to evaluate the effects of evaporative cooling in freestall on mastitis occurrence, milk production, and composition, as well as cortisol, T_3 (triiodothyronine), and T_4 (thyroxin) levels in lactating dairy cows. Twenty-eight multiparous cows averaging 70 ± 10 day postpartum were used in four treatments from January to March 2003. The treatments were: *Day* (cooling from 7:00 a.m. to 7:00 p.m.); *Night* (cooling from 7:00 p.m. to 7:00 a.m.); *24-hour* (cooling 24-hour); and *Control* (no cooling). Wired cup test was used for clinical mastitis diagnosis, and the California Mastitis Test (CMT) was used to identify subclinical mastitis. Blood and milk samples were taken weekly for microbiological and hormonal analyses. The cortisol levels were higher than normal values in all treatment groups, suggesting stress conditions, but T_3 and T_4 levels remained normal in all groups. The occurrence of subclinical mastitis was lower in *Day* and *Night* groups than in *Control* and *24-hour* groups. Regarding the microbiological analyses, in all groups the isolation of *Corynebacterium* sp. from milk samples increased while negative coagulase staphylococci (CNS) declined as etiological agents of subclinical mastitis. However, in *Day* and *24-hour* groups, coagulase positive staphylococci (CPS) increased mainly *Staphylococcus aureus* (49.8% and 47.7% respectively). The *Night* group showed a decrease in subclinical mastitis occurrences. Our data indicate that all animals subjected to treatments presented high levels of cortisol, indicating a stress condition. The *Night* treatment presented a reduction in microbial isolation, suggesting a reduced susceptibility to mastitis.

INDEX TERMS: Intramammary infection, mastitis, evaporative cooling, cortisol, thyroid hormones, dairy

RESUMO.- [**Efeito da modificação ambiental sobre a ocorrência de mastite e alterações hormonais de vacas Holandesas.**] O trabalho teve como objetivo avaliar a eficiência do sistema de resfriamento adiabático evaporativo, acionado em diferentes horários, em instalação do tipo freestall e seus reflexos sobre a ocorrência de mastite, produção e composição do leite e respostas hormonais de vacas em lactação. Foram utilizadas 28 vacas em lactação (70±10 dias), multíparas, das raças Holandesa Preta e Branca e Pardo Suíça, com produção média diária de 23±2,3 kg leite/dia. O período experimental de 56 dias teve início em 20 de janeiro de 2003. Os tratamentos foram: Controle (sem resfriamento); Dia (resfriamento 7 as 19 h); Noite (resfriamento 19 às 7 h) e 24 horas (resfriamento durante 24 h). A temperatura de bulbo seco (TBS), umidade relativa do ar (UR) e a temperatura de globo negro (TGN) foram mensuradas ao longo das 24 horas. A ordenha foi realizada às 7 h e 19 h. Amostragens semanais de leite e sangue foram realizadas para análise da composição do leite (gordura, proteína, lactose e contagem de células somáticas) e deter-

[1] Received on February 25, 2013.
Accepted for publication on April 25, 2013.

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minações hormonais de cortisol, tiroxina (T4) e triiodotironina (T3). Para avaliação da ocorrência de mastite clínica e subclínica foram feitos exames semanais de TAMIS (caneca de fundo preto) e *California Mastitis Test* (CMT). Foram colhidas amostras de leite de todos os quartos para identificação microbiológica dos agentes causais da mastite. O tratamento Dia diminuiu (P<0,05) a temperatura do freestall em 5,3°C às 12h e em 3,5°C às 14h em relação ao grupo Controle. A umidade relativa esteve elevada (P<0,05) às 7h no tratamento Noite e às 12h, 14h e 21h no tratamento Dia. Os maiores valores de ITU foram registrados no tratamento Noite às 12h, 14h e 21h. Não foram observadas diferenças entre os tratamentos (P>0,05) para a produção e composição do leite. Nos animais do tratamento Os níveis de cortisol mostraram-se acima (P<0,05) dos níveis normais em todos os tratamentos. Já os teores de T_3 e T_4 estiveram dentro da faixa de normalidade. Na fase pré-experimental a maior frequência de isolamento bacteriano foi para *Staphylococcus* coagulase negativa. No tratamento noite e dia, houve uma diminuição na proporção de casos positivos de mastite subclínica da fase pré-experimental em relação à última semana da fase experimental. Na última semana da fase experimental houve uma diminuição de *Staphylococcus* coagulase negativa e aumento da ocorrência de *Corynebacterium* sp.

TERMOS DE INDEXAÇÃO: Infecção intramamária, mastite, resfriamento evaporativo, hormônios tireoidianos, vacas leiteiras.

INTRODUCTION

Environmental conditions such as high temperature, humidity, and solar radiation compromise the ability of the lactating dairy cow to dissipate heat, resulting in heat stress. Also, climate variables are associated with increases in incidence of mastitis due to enhance animals' susceptibility to infections (Hammami et al. 2013).

Cooling methods should be used to alleviate the thermal stress in these conditions (Renaudeau et al. 2010). Environmental modifications for dairy facilities are generally provided by evaporative cooling such as misting or sprinklers associated to fans (Collier at al. 2006). Keister et al. (2002) verified a lower incidence of mastitis in Jersey cows housed in a freestall with a misting system compared to animals in a non-cooled freestall during the summer period in Arizona.

In general, plasma cortisol levels are increased in dairy cows during short periods of exposure to thermal stress. On the other hand, extended periods of exposure reduce these levels (Du Preez 2000). In addition, basal serum thyroxine (T_4) and triiodothyronine (T_3) concentrations decline under thermal stress. The modification of thyroid activity is accompanied by a decrease in metabolic rate, food ingestion, growth, and milk production.

The purpose of this study was to evaluate the effects of a misting system in a freestall on mastitis occurrence, milk production, and composition, as well as cortisol, T_3 and T_4 levels in dairy cows.

MATERIALS AND METHODS

The study was conducted at the Centro APTA Bovinos de Leite do Instituto de Zootecnia, located in Nova Odessa, São Paulo, Brazil at the coordinates 22°42' S and 47°18' W. The region has a subtropical climate with a hot and rainy summer. All procedures were approved by the Animal Care and Use Committee of the CAPTA Bovinos de Leite Nova Odessa, SP, Brazil.

The trial was carried out from January to March 2003. Twenty-eight multiparous cows averaging 70±10 days postpartum and 23±2.3 kg milk/day. The treatments were randomly assigned to the cows. The freestall were divided into four sections and then 1 of 4 treatments was installed: *Day* (cooling from 7:00 a.m. to 7:00 p.m.); *Night* (cooling from 7:00 p.m. to 7:00 a.m.); *24-hour* (cooling 24-hour); and *Control* (no cooling). The treatments were activated by an automatic controller. The misting system (0.3 L/h) was installed using PVC pipes with valves within a 2.8-m distance. The fans (2.5m/s) were placed about 2.5 m above the freestall bedding. The misting cycle included 5 min with 1 min of water spraying and 4 min turned off. Microclimate parameters' dry bulb temperature (DBT) and air relative humidity (RH) inside the facility were measured every 60 min over a period of 24 h using a data logger. The temperature and humidity index (THI) was calculated using the equation described previously by McDowell & Johnson (1971).

Weekly blood samples were collected from the coccygeal vein of the cows. The plasma was separated and stored at -80°C. The concentration of hormones was determined by a solid phase radioimmunoassay (RIA) using a commercial kit Coat-A-Count DPC® (DPC, Los Angeles, CA).

The animals were milked twice daily (7:00 a.m. and 7:00 p.m.). Milk samples from both milking were collected weekly to determine fat, protein, lactose, total solids (Bentley2000® Instruments Inc.), and somatic cell counts (Somacount® 300).

Wired cup tests were used to diagnose clinical mastitis and California Mastitis Tests (CMT) were used to identify subclinical mastitis. Milk samples from all mammary glands were aseptically collected daily for six consecutive days in the pre-experimental phase and during the last week of the trial. The samples were cultivated in sheep blood agar, incubated under aerobic conditions at 37°C for 72 h with readings after 24, 48 and 72 h. The samples were also plated on MacConkey's agar, where they were incubated at 37°C for 48 h. The microbiological techniques used for the identification of bacteria complied with the guidelines described by Murray et al. (1999). *Staphylococci* sp. was identified based on colony morphology, mannitol salt agar, and tests of coagulase, urea, maltose, trehalose, xylose, lactose, sucrose, and raffinose. *Corynebacterium* sp. was identified based on catalase, urea, maltose, lactose, and sucrose tests (Costa et al. 1986). *Streptococcus uberis, Streptococcus agalactiae*, and *Streptococcus dysgalactiae* were identified based on catalase, esculin, and CAMP tests.

Milk yield, milk composition and, hormones were analyzed by repeated measures using PROC MIXED (SAS 2001) with a heterogeneous compound symmetry covariance structure. Parity number was adopted as a covariate for the adjustment of milk production. For meteorological variables, days were used as the observation unit. The model contained treatments, time of day and treatment by time of day interaction. The data were subjected to an analysis of variance, using the PROC MIXED for repeated measures (SAS 2001) with a heterogeneous toeplitz covariance structure. Differences were considered significant when P<0.05. The difference between the incidence of mastitis in the mammary glands evaluated in the pre-experimental phase and in the last week was performed using the Instat Graphpad Sofware with chi-square test (χ^2).

RESULTS

The environmental conditions recorded from 7:00 a.m. to 9:00 p.m. during the experimental period are shown in Table 1. The highest dry bulb temperatures (DBT) were recorded from 12:00 a.m. to 5:00 p.m. and were correlated with the lowest absolute values of relative air humidity (RH). *Day* treatment decreased the DBT by 5.3°C at midday and 3.5°C at 2:00 p.m. in relation to the *Control* group. The relative humidity was increased at 7:00 a.m. in *Night* treatment (96.5%) and at midday (70.0%), 2:00 a.m. (64.8%), and 9:00 p.m. (90.0%) in *Day* treatment. The highest values established for the THI were registered in the *Night* treatment at midday (79.3), at 2:00 p.m. (80.5), and at 5:00 p.m. (79.4). The *24-hour* treatment presented the higher THI at 9:00 p.m (77.7).

Cortisol concentration of *Control* treatment (1.1μg/dL) was increased in relation to *Night* (0.6 μg/dL) and *24-hour* (0.7μg/dL) treatment. No differences (P>0.05) were found in the levels of triiodothyronine and thyroxin among treatments. Mean values of treatments were 55.4 μg/mL and 2.9 ng/dL to T_3 and T_4 respectively (Table 2).

No differences in milk production and composition (P>0.05) were observed (Table 3). In the pre-experimental phase, 3.5% of the animals and 1.8 % of the quarters had clinical mastitis. In the last week of the experimental phase, the number of animals and quarters with clinical mastitis remained the same when compared with the pre-experimental phase. The frequency of subclinical mastitis in the pre-experimental phase in relation to the number of animals and mammary quarters was 78.5 and 65.1% respectively. There was a decrease in the percentage of animals and mammary quarters affected with subclinical mastitis with 67.8 and 40.1% in the last week of the experimental phase (Table 4).

Table 1. Environmental conditions measured during the experimental period

Treatment	7:00 a.m.	12:00 a.m.	2:00 p.m.	5:00 p.m.	9:00 p.m.
			DBT (°C)		
Control	21.7 ±0.25a	29.4 ± 0.24b	31.4 ± 0.24b	29.6 ± 0.28a	24.5 ± 0.19b
Day	21.5 ± 0.25a	24.1 ±0.24a	27.9 ± 0.24a	30.9 ± 0.28b	24.3 ± 0.19b
Night	21.3 ± 0.25a	29.3 ± 0.24b	31.0 ± 0.24b	30.3± 0.28ab	24.6 ± 0.19b
24-hour	21.5 ± 0.25a	29.1± 0.24b	30.8 ± 0.24b	30.1 ± 0.19b	24.9 ± 0.19b
			RH (%)		
Control	86.5±1.94a	47.5 ± 0.81a	43.1 ± 0.86a	47.5 ± 1.67a	70.0 ± 1.69a
Day	90.6±1.94a	70.0 ± 0.81b	64.8 ± 0.86b	52.0 ± 1.65b	90.0 ± 1.69b
Night	96.5±1.94b	64.6 ± 0.81c	56.1 ± 0.86c	58.0 ± 1.65c	83.5 ± 1.69c
24-hour	88.5±1.94a	59.3 ± 0.81d	51.2 ± 0.86d	53.7 ± 1.65b	75.0 ± 1.69d
			THI*		
Control	70.1± 0.26a	76.9 ± 0.26a	78.6 ± 0.26a	76.8 ± 0.26a	73.0 ± 0.26a
Day	69.9 ± 0.26a	72.2 ± 0.26b	79.2 ± 0.26b	78.4 ± 0.26b	74.1 ± 0.26b
Night	70.1 ± 0.26a	79.3 ± 0.26c	80.5 ± 0.26c	79.4 ± 0.26c	74.4 ± 0.26b
24-hour	70.0 ± 0.26a	77.5 ± 0.26d	77.3 ± 0.26d	79.4 ± 0.26c	77.7 ± 0.26d

[a,b] Means values in the same column with different letters differ by Student´s Test(P<0.05). The data are expressed as mean ±SEM. * Temperature-humidity index (THI) was calculated as [DBT – 0,55x(1-RH)x (DBT-58)].

Table 2. Levels of the hormones cortisol, T_3 and T_4 in serum plasma of dairy cows during the experimental period

Hormones	Treatments			
	Control	Day	Night	24-hour
Cortisol (μg/dl)	1.1 ± 0.13a	0.8 ± 0.13ac	0.6 ± 0.13b	0.7 ± 0.13bc
Thyroxin (T_4), μg/ml	2.7 ± 0.14a	3.1 ± 0.14a	2.8 ± 0.14a	2.9 ± 0.14a
Triiodothyronine (T_3), ng/dl	50.2 ± 5.01a	57.6 ± 5.01a	59.4 ±5.0a	54.4 ± 5.01a

[a,b] Means values in the same row with different letters differ by Student´s Test(P<0.05). The data are expressed as mean ±SEM.

Table 3. Milk production and composition of lactating cows during the experimental period

Milk Production (kg/day)	Treatments			
	Control	Day	Night	24 hour
Morning	9.4 ± 1.46a	10.1 ± 1.48a	9.9 ± 1.62a	9.3 ± 1.53a
Evening	9.3 ± 1.65a	10.1 ± 1.48a	10.1 ± 1.55a	9.5 ± 1.68a
Total	18.7 ± 3.10a	20.3 ± 2.93a	20.0 ± 3.16a	18.8 ± 3.27a
Composition (%)				
Fat	3.9 ± 0.13a	3.7 ± 0.13a	3.8 ± 0.13a	3.7 ± 0.13a
Protein	3.0 ± 0.09a	3.1 ± 0.09a	3.3 ± 0.09a	3.1 ± 0.09a
Lactose	4.3 ± 0.09a	4.6 ± 0.09a	4.6 ± 0.09a	4.6 ± 0.09a
Total solids	12. ± 0.27a	12.5 ± 0.27a	12.8 ± 0.27a	12.5 ± 0.27a

[a,b] Means values in the same column with different letters differ by Student´s Test(P<0.05). The data are expressed as mean ±SEM.

Table 4. Occurrence of clinical and subclinical mastitis (%) in the pre-experimental phase and in the last week of the experimental phase

	Cows		Quarters	
	Pre-Exp. Phase*	Last Week	Pre-Exp. Phase	Last Week
Wired cup test	1 (3.5%)	1 (3.5%)	2 (1.8%)	2(1.8%)
CMT**	22 (78.5%)	19 (67.0.%)	73 (65.1%)	45(40.1%)
TOTAL	28	28	112	112

* Pre-experimental phase = Pre-Exp Phase, ** California Mastitis Test.

The percentages of quarter milk samples without microbial isolates at the pre-experimental phase in the *Control*, *Day*, *Night*, and *24-hour* treatments were 45.9%, 40.0%, 37.2%, and 37.4%, respectively (Fig.1). *Control* treatment showed a decrease (P < 0.05) in the negative microbial isolates in the last week of experimental phase (45.9 vs. 37.7%). On the other hand, *Day* (40 vs. 51.2%) and *Night* (37.2 vs. 50.2%) treatments presented an increase in the quarter without microbial isolates. No significant difference (P>0.05) in the quarter without microbial isolates was observed in the *24-hour* treatment (37.4% vs. 43.2%).

Table 5 shows the somatic cell count and bacterial isolations of the pre-experimental phase and the last week of the experimental phase. *Day* (5.9 vs 0.0%) and *Night* (6.7 vs. 0.0%) treatments decreased (P<0.05) the frequency of

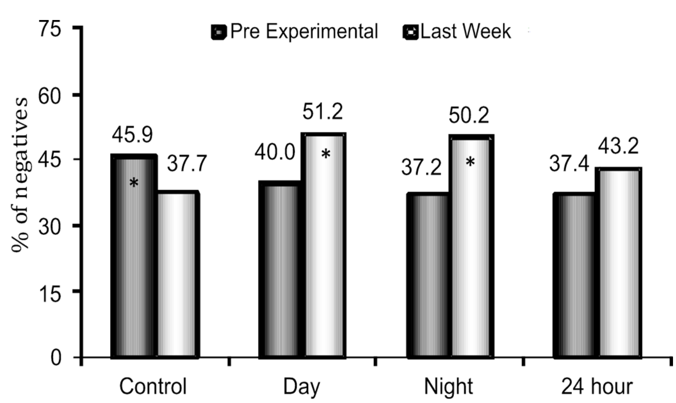

Fig.1. Percentage of mammary quarters negative for microbiological examination of dairy cows housed in freestall system with evaporative cooling systems.

Table 5. Bacterial isolation (%) from milk quarters in the pre-experimental phase and in the last week of the experimental phase

Bacterial Isolation*	Control		Day		Night		24 hour	
	Pre-Exp Phase**	Last Week	Pre-Exp Phase	Last Week	Pre-Exp Phase	Last Week	Pre-Exp Phase	Last Week
Staphylococcus spp.	3.9a	5.5a	5.9a	0.0b	6.7a	0.0b	1.6a	2.0a
Coagulase-positive *staphylococci*	17.8a	11.9a	18.8b	36.2a	10.1a	8.9a	19.6a	45.3b
S. aureus	7.9a	6.4a	5.1b	18.0a	4.2a	4.4a	11.4a	21.6b
Coagulase-negative *staphylococci*	52.4a	30.7a	56.4a	20.8a	64.4a	36.6a	59.0a	22.6a
S. uberis	3.9a	8.2a	1.7a	4.2a	0.8a	8.8a	0.8a	3.0a
S. agalactiae	0.0a	4.5a	0.0a	1.0a	0.8a	2.2a	0.0a	0.0a
S. dysgalactiae	0.0a	5.5a	0.0a	1.0a	0.0a	1.1a	0.0a	0.0a
Corynebacterium spp.	21.7a	33.3a	17.0b	35.1a	16.1a	43.5a	18.0b	42.2a
SCC (x1000/mL)***	148.7a	454.5a	100.0a	62.3a	52.1a	57.3a	96.2a	209.4a

* Bacterial isolation (n=672), ** Pre-experimental phase = Pre-Exp Phase, *** SCC= Somatic cell count express by geometric average.

Staphylococcus sp. isolates in the last week of experimental phase. Coagulase-positive staphylococci (CPS), *Staphylococcus aureus*, and *Corynebacterium* spp. isolates were increase in the *Day* (36.2, 18.8, and 35.1%) and *24-hour* treatments (45.3, 21.6, and 42.2%) in the last week of the experimental phase. There was no difference (P>0.05) for somatic cell counts between the pre-experimental and the last week of the experimental phase.

DISCUSSION

The effects of heat stress on dairy cows can be alleviated using a heat abatement system such as shades, fans, misters, and sprinklers. In our trial, *Day* treatment was efficient to reduce the freestall temperature during the hottest hours (12:00 p.m. to 2:00 p.m.). The temperature was 5.3°C lower at midday and 3.5°C lower at 2:00 p.m. in relation to the *Control* group. These results are in agreement with Smith et al. (2006) who reported a decrease of 3.1°C in dry bulb temperature using evaporative cooling in the North Mississippi, an area characterized by high temperature and humidity.

The main objective of the misting system is to decrease the air temperature. However, the system efficiency is highly related with relative humidity (RH). High environmental temperature and RH (higher than 70%) may limit the performance of the confined cows because of a reduction in heat dissipation. This restriction of thermal changes will provide an increase in body temperature due to the storage of heat (Davis 2001). RH values higher than 70% were found in all treatments at 7:00 a.m. and at 9 p.m. in the *Day, Night*, and *24-hour* treatments. The high RH observed in these situations is probably associated with the large volume of steam (from breathing) and urine produced by the animals that cannot be removed from the building. Thus, the air changes should be improved to move the warm and humid air away from the facilities.

According to Hahn (1985), THI values of 70 or less are considered comfortable, 71–78 mild stress, 79–83 moderate, and values greater than 83 as severe stress with lactating cows being unable to maintain their thermoregulatory mechanisms or normal body temperature. The highest values found for the THI in the *Night* treatment suggested that it is required to provide an effective cooling system during the hottest hours of the day in order to help cows dissipate more heat into the environment.

Cortisol concentrations were high in all treatment groups. According to Smith (1993), the normal levels for bovine cortisol are around 0.35g/dL. There is evidence that cortisol, together with other hormones, influences the metabolism of the mammary gland. On the other hand, T_3 and T_4 levels were within the normal ranges, which in cows are 41-170 ng/dl for T_3 and 3.6-8.9mg/ml for T_4. These results are similar to those observed by Avendaño-Reyes et al. (2010) who found lower concentrations of T_3 and T_4 in the months when the temperature and the air humidity were higher, although they remained within the normal range.

In the pre-experimental phase, 3.5% of the animals presented clinical mastitis being this percentage higher than the 3.0% that is accepted internationally (Reneau 1993). To know the clinical mastitis levels allows us to make inferences on subclinical mastitis rates. Pearson et al. (1971) verified a proportion of 1:14 between the cases of clinical mastitis for subclinical mastitis. In relation to mammary quarters, it was expected that this proportion would be higher due the cows present more than one quarter with mastitis. In our results, were found the proportion of 1:22 (animals) and 1:36 (mammary quarters) cases of clinical mastitis for subclinical mastitis. The increase of mammary quarters with negative CMT can be attributed to the spontaneous cure that results from the efficiency between udder defense mechanisms and the inflammatory process.

The increase in the quarter without microbial isolates in *Day* (40 vs. 51.2%) and *Night* (37.2 vs. 50.2%) treatments can be attributed to animal resistance. Heat stress promotes acute and chronic changes in plasma cortisol levels, which when increased leads to the suppression of immune response and a decrease in the number of circulating leukocytes (Granner 1994). Cortisol levels were higher in the *Control* treatment, which can partly explain the great number of positive cases in the experimental phase.

The increase in the number of CPS isolates, especially *Staphylococcus aureus*, in the *Day* and *24-hour* treatments could be due to the decrease in animal resistance. According to Sutra (1990), some *Staphylococcus aureus* strains cause depression of the immune system and a consequently weak cellular response to infection in the mammary gland. Consistent with this, an increase in the number of *Corynebacterium* spp. isolates was observed in the quarter milk samples in all treatments.

The contagious mastitis occurs in different forms that depend on the microorganism involved, pathogenicity, and the ability to prevent tissue invasion. The host response is related to the resistance of the mammary gland (Blood & Radostits 1991). On the other hand, heat stress increases susceptibility to infection by decreasing animal resistance, or increasing exposure to pathogenic agents by promoting conditions that enhance their growth and proliferation in the environment (Whitaker et al. 2004).

CONCLUSIONS

Our data indicate that all the animals subjected to treatments presented high levels of cortisol, indicating a stress condition.

The *Night* treatment showed a reduction in microbial isolation, suggesting a reduced susceptibility to mastitis. In contrast, the animals subjected to the *24-hour* treatment had the highest occurrence of *Staphphylococcus aureus* and therefore, the worst results.

Acknowledgment.- The study was supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP, Process nº 01/11093-2).

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