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Short communication

Casein hydrolyzate intramammary treatment improves the comfort behavior of cows induced into dry-off

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Abstract

The effectiveness of casein hydrolyzate as mean to improve the welfare of cows induced into involution was tested in high yielding cows induced into dry-off by abrupt cessation of milking. Treatment with casein hydrolysate prevented build up of udder pressure in cows induced into dry off and was clearly associated with signs (lying behavior and step numbers) that they were calmer and more comfortable than cows induced into dry off by the conventional means. We conclude that treatment with casein hydrolyzate is a viable treatment tool that can prevent the suffering associated with drying-off of high-yielding modern dairy cows.

Keywords: Behavior; Welfare; Dry-off; Mastitis

1. Introduction

Drying-off in modern dairy farming is carried out about 60 days before the expected parturition. This period is a compromise between the farmer's wish to maximize milk production and the need for a "dry-off period" sufficiently long to prevent a decline in milk production in the next lactation (Annen et al., 2004). This practice involves the abrupt cessation of milking of cows that are still producing considerable amounts of milk: 20, 40 and sometimes even 50 L day^{-1}. Such a practice results in the accumulation of massive amounts of milk in the udder, which leads to udder engorgement and milk leakage, and frequently causes noticeable agony to the cow which might scream loudly for several days. Thus, the practice, current in modern dairy farming, of drying off cows considerably impairs the welfare status of the cows.

Recently, it has been shown that the process of active involution following milk stasis in goats and cows is triggered by the induction of intensive plasmin activity, which, in turn liberates casein-derived active peptides (Shamay et al., 2002, 2003; Silanikove et al., 2006). Infusion of casein hydrolyzate (CNH), which contains active casein-derived peptides, dramatically accelerated the involution, which was completed within 3 days in goats and cows, and resulted in a marked reduction in milk yield even after the first day of treatment (Shamay et al., 2002, 2003). It has been shown, also, that within 8 h after the first application, such a CNH treatment induced marked bactericidal and bacteriostatic responses in the mammary secretion, against the main udder pathogens. This resulted from the rapid and forceful

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activation of the innate immune system and the drastic reduction in nutrient availability for bacterial growth (Silanikove et al., 2005a). Thus, treatment with CNH at drying-off was found to be very effective in eradicating bacterial infection and curing cows from both subclinical and chronic infections (Silanikove et al., 2005b), a result that is also positive from the welfare point of view.

The present study aimed to test the influence of CNH treatment on the behavior and welfare of high-yielding dairy cows induced into drying-off.

2. Materials and methods

2.1. Materials

NAFPENZAL DC (Intervet, the Netherlands), a medication for dry-period treatment was used according to the manufacturer's instructions. A prototype of the CNH hydrolyzate prepared under GLP conditions (Hy-Labs, Rehovot, Israel) was used as an adjuvant for dry cow antibiotic treatment. The solution was a pyrogen-free liquid preparation, supplied in sterile vials. The endotoxin level in the working solution was 0.48 EU mL^{-1}, according to the limulus amebocyte lysate (LAL) test, and the amount of endotoxin injected with each 10-mL dose of this solution, i.e., 0.0001 EU per kg body weight (BW), on the assumption of an average cow BW of 500 kg, was 2000 times lower than the tolerance limit of endotoxin in intrathecally administered human drugs (5 EU kg^{-1} for parenteral drugs and 0.2 EU kg^{-1} for intrathecal drugs). Each injection into a quarter contained 10 mL of CNH, with a peptide concentration of ~7 mg mL^{-1}.

2.2. Study layout

Twenty Israeli Holstein cows that were approaching the end of lactation (~60 days) and producing milk at 17–35 L day^{-1} were submitted to the study in two sub-experiments, each involving 10 animals. The cows were paired according to lactation, milk yield (MY), somatic cell count (SCC) and quarter bacteria isolation (Table 1). The SCC and bacterial isolation of the milk from each quarter was tested three times, separated by 1-week intervals, before drying-off. One cow of each pair was then assigned to a subgroup and after milking, according to the spin of a coin, the cows in subgroup 1 were treated with CNH and NAFPENZAL DC (C+N), whereas those in subgroup 2 were treated with NAFPENZAL DC only (N). For 1 week before treatment, the cows of both subgroups were housed together for familiarization, in an enclosed shelter that provided 10 m^2 of shaded slatted floor and 10 m^2 of concrete-surfaced yard for each animal, and in which they remained for 2 weeks. For 3 weeks, beginning 1 week before the treatment, the cows wore the leg-mounted computerized sensor. During lactation, the cows were fed typical Israeli total mixed ration (TMR) containing 65% concentrate and 35% forage (17% protein). After being dried off, the cows' diet was switched to a TMR consisting of 25% concentrate and 75% forage (12% protein). The food was offered *ad lib* in mangers located in the sheds. All the trials were carried out in the experimental dairy herd of the Agricultural Research Organization, at the Volcani Center.

2.3. Behavior and welfare-scoring features

2.3.1. Udder pressure index

An udder pressure index (UPI), on a scale that ranged from 0 (no pressure) to 3 (high pressure) was applied. The UPI was assessed according to a 0 to 3 scale on which: 0 represents no pressure; 1, medium pressure; 2, high pressure; and 3, extremely high pressure. Arbitrary UPI values were determined by pressing a finger into the tissue and assessing its resistance to pressure in two

Table 1
Lactation number, DIM, days to parturition, milk yield, cow SCC and number of quarters infected at day of drying-off in the experimental (CNH+NAFPENZAL DC) and control (NAFPENZAL DC) cows

	CNH+NAFPENZAL DC		NAFPENZAL DC	
	Mean±S.D.	Range	Mean±S.D.	Range
Lactation number	1.7±0.7	1–3	1.9±0.9	1–3
Days in milk	355.8±58.4	285–451	327.0±39.8	281–388
Days to parturition	59.3±9.8	42–70	61.2±10.6	38–75
Milk yield (kg/day)	25.1±5.3	17–32	26.4.1±5.2	21–35
SCC (×10^3)	171.6±219.9	41–704	205.8±250.6	54–900
Bacterial infection status (cow; quarters)	4;4		4;6	

n=10 for each group, the data are presented as mean±S.D.

areas, the milk cistern and the corpus ubeis, 10 cm above the teats. All measurements of UPI were carried out by the same trained person once a day at the same time, throughout experiment 2.

2.3.2. Leg-mounted sensor

A semi-commercial prototype of a leg-mounted telemetry system, the Afifarm Management System (S.A.E., Afikim, Israel), which enables monitoring and recording of accumulative records of steps, lying times and lying periods, was used to record the walking and resting activities of the cows. The sensor has data storage and transmission capabilities, and includes an integrated power source sufficient for at least 1 month of operation. Collected data were downloaded from the sensor during milking in the pre-drying-off period, and once daily at 14:30 after the cows had been induced into drying off. The accuracy of the system was established by Livshin et al. (2005).

2.4. Bacteriological examinations

Duplicate quarter foremilk samples were taken aseptically according to the International Dairy Federation (IDF) (1985) procedures, and were submitted to the laboratory within 1 h. Bacteriological analysis was performed according to accepted standards (Leitner et al., 2006).

2.5. Somatic cell count

SCC was performed with a Model Z1 Counter (Coulter Electronics Ltd., Beds, UK) according to the revised protocol of the A2B sub-group of the Mastitis Experts from the IDF (1991).

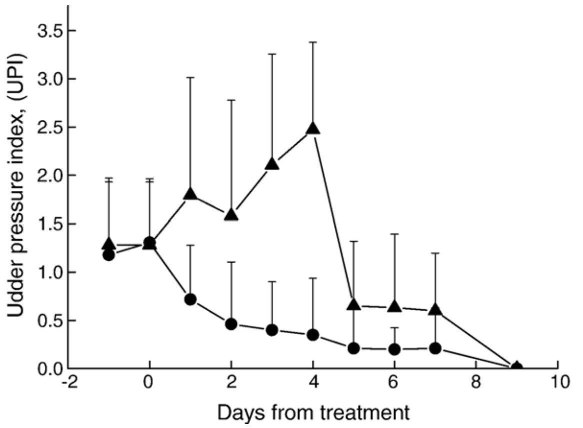

Fig. 1. Udder pressure index (UPI) of 10 experimental cows (abrupt cessation of milking + casein hydrolyzate + antibiotic dry treatment; ●) and 10 control cows dried off in the conventional way (abrupt cessation of milking + antibiotic dry treatment; ▲) before (3 days) and during the first 7 days after treatment.

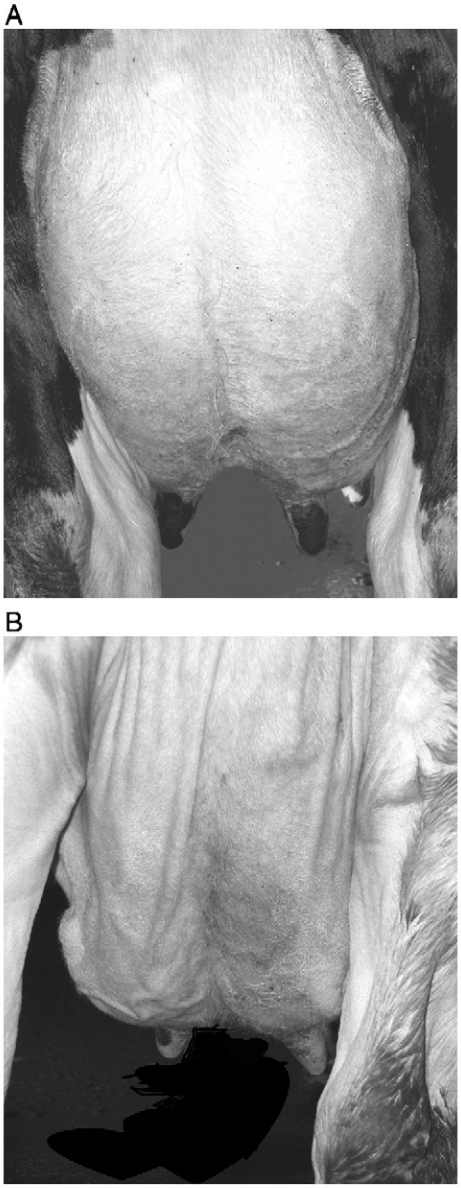

Fig. 2. Udders of two cows on day 4 post-treatment with milk yield over 30 L day^{-1} at dry-off: (A) cow treated with N (abrupt cessation of milking + antibiotic dry treatment); (B) cow treated with C + N (abrupt cessation of milking + casein hydrolyzate + antibiotic dry treatment).

2.6. Statistical analysis

The data were statistically analyzed with the Fit Model procedure for repeated measurements, within the JMP software, Version 5 (SAS Institute, Cary, NC, USA); treatment was the between-subject factor and time was the within-subject factor. Differences were considered significant at $P<0.05$. The model was:

$$Y_{ijklm} = \mu + \rho_i + \alpha_j + c_{(ij)k} + \gamma_l + \alpha\gamma_{il} + \varepsilon_{ijklm}$$

where Y_{ijklm}=the dependent variable, μ=overall mean, P_i=fixed effect of period (pre- or post-dry-off; i=1 or 2), α_j=fixed treatment effect i (i=1 or 2), $C_{(ij)k}$=random effect of cow k (k=1 to 10) within period i and treatment j; γ_l=effect of day l (l=1 to 9); $\alpha\gamma_{il}$=effect of interaction of treatment j and day l; and ε_{ijklm}=random error associated with cow k in period i and treatment j on day l.

Differences between treatments for period, or for specific days following treatment were subjected to the *t*-test by means of the Tukey–Kramer Highest Significant Difference (HSD) test.

3. Results

Data depicted in Table 1 show that there were no differences in the initial values of the indices according to which the cows were distributed between the two treatment groups. In both groups 40% of the cows were subclinically infected, most of them in one quarter, with one of the bacteria, *Streptococcus dysgalactiae*, *Staphylococcus aureus* and CNS, which were associated with increased SCC in these quarters. After treatment, only two quarters, one in each subgroup and both of them infected, were leaking milk during the first day after treatment. No interactions were found between UPI, number of steps, lying times or lying periods, on the one hand, and results classified according to sub-experiments as main independent treatment, therefore, the statistical analysis was applied to the entire data set.

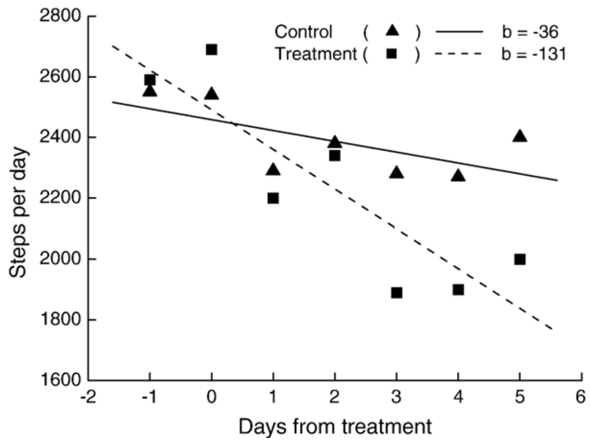

Fig. 3. Linear fit of steps per day of 10 experimental cows (abrupt cessation of milking+casein hydrolyzate+antibiotic dry treatment) and 10 control cows dried off in the conventional way (abrupt cessation of milking+antibiotic dry treatment) before (3 days) and during the first 6 days after treatment.

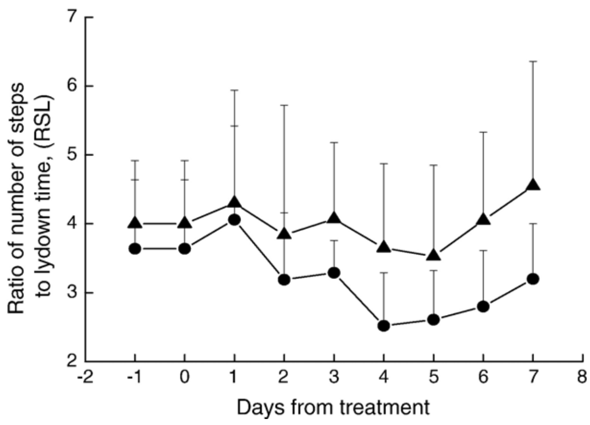

Fig. 4. The ratios of numbers of steps per day to lying down time of 10 experimental cows (abrupt cessation of milking+casein hydrolyzate+antibiotic dry treatment; ●) and 10 control cows dried off in the conventional way (abrupt cessation of milking+antibiotic dry treatment; ▲) before (3 days) and during the first 7 days after treatment.

The arbitrary values of UPI (Fig. 1) increased markedly from about 1.2 before treatment to values in the range of 1.8 to 2.5 in the cows treated only with antibiotic (N), whereas there was a sharp drop in the (C+N)-treated cows during the first 4 days after they were induced into involution. Thus, during these 4 days the UPI values in the two groups differed significantly ($P<0.01$). The UPI of the control cows did not decline until after the 5th day following dry-off induction. Fig. 2 presents photographs of the udders of two cows on day 4 after treatment; the MY of these two cows was over 30 kg day^{-1} at dry-off. The photographs in Fig. 2 illustrate the differences between a treated cow, with UPI=0 (Fig. 2B), and a control cow, with UPI=~2 (Fig. 2A).

After treatment, the number of steps per day fell at differing rates in the two groups (Fig. 3) with slopes of −36 for the N group and −131 for the C+N group, so that there were significantly ($P<0.001$) fewer steps in the C+N group than in the N group.

The average lying bout duration for the 7 days after treatment was 71±4 min for the (C+N)-treated cows and 60±7 min for the N-treated ones ($P<0.01$). The ratio between the number of steps and the duration of lying (RSL) was used as a criterion of "animal comfort" (see Discussion for justification) (Fig. 4). This ratio diminished for the (C+N)-treated cows, starting from the 2nd day after treatment, whereas it remained unchanged in the N-treated ones. As a result, the difference between the groups widened and became significant ($P<0.005$) from the 3rd day after treatment onwards.

4. Discussion

It is well established that the beginning of the dry period (Rajala-Schultz et al., 2005) and the period around parturition (Sordillo, 2005) are associated with increased risk of acquiring intramammary infection (IMI). The increased risk around the start of the dry period in dairy cows is closely related to milk yield at drying off (Rajala-Schultz et al., 2005), and may also be related to the slow activation of the innate immune system (Silanikove et al., 2005a). It was calculated that for every 5 kg increase in milk yield above 12.5 kg day^{-1} at dry-off, new IMI caused by environmental bacteria increased by 77% (Rajala-Schultz et al., 2005), therefore, in Israel, most cows are at high risk of acquiring IMI at drying-off, since they are still producing above 20 L day^{-1}.

In the present study, the contrasts in UPI findings and cow behavior, between the N and the C+N treatments can be attributed to the precipitous decline in mammary secretion that followed the treatment of the glands with CNH (Shamay et al., 2002, 2003). Shamay et al. (2002, 2003) found that the decline in mammary secretion could exceed 50% of the pretreatment milk yield on the first day after treatment with CNH. The present data indicate that a single treatment with CNH was sufficient to prevent the increase in udder pressure, and even to reduce it below the normal pre-milking level. To achieve success (by preventing udder inflation in this case) with a single treatment is very important from a practical point of view, because the expectation of success with a single application should considerably simplify the procedures and minimize the effect on the workload in a given farm, and, therefore, increase the likelihood that farmers would adopt this treatment.

Rest and activity are fundamental and complementary components of animal behavior. In dairy cows, lying behavior (LB) reflects rumination activity as well as resting, and it may be affected by the daily routine (feeding, milking) or individual temperament, and is often considered as an indicator of cow comfort when different housing environments are compared (Metz, 1985; Singh et al., 1993; Ketelaar de Lauwere et al., 1999; Sonck et al., 1999; Fregonesi and Leaver, 2001, 2002; Horning et al., 2001). The LB data may be related to physiological conditions such as estrus (Phillips and Schofield, 1990; Brehme et al., 2004) or to health problems (Galindo and Broom, 2002).

A recently developed leg-mounted sensor which enables monitoring, recording and transmission of animal activity (expressed as steps) and LB, with minimal disturbance to other free-animal behaviors was found to provide a useful indication of cow's welfare (Livshin et al., 2005). We took advantage of the availability of this equipment to check whether CNH treatment modified the cow's behavior.

Intuitively, one would expect to find an inverse relationship between number of steps and LB, and this was indeed the case in the present study (data not shown). However, some of a cow's behavior may be altered by farm management practices in ways that are not related to the experimental questions. For instance, during lactation, the cows were walked from their yard to the milking parlor (about 300 m) three times daily, whereas after the drying off treatment they were walked only once a day. This inevitably entailed a reduction in the number of daily steps by the dry cows in a manner that was not related to the treatments. We found that analyzing the changes in number of steps over time (as in Fig. 3) was more informative than just presenting the actual number of steps over time, in showing that the reduction in number of steps was more significant in the CNH-treated cows than in the controls because of the considerable "background noise" associated with the former. It is known that the ratio between two random variables is a well-defined random variable (Cedilnik et al., 2006). Thus, another way to overcome such experimental "noise" is to present the weighted measurements of the two behaviors. In the present case, we found that the ratio between number of steps and lying-down time was particularly effective (i.e., by contrasting the differences between treatments on a day to day basis; Fig. 4) in demonstrating that the CNH treatment changed cow behavior relative to that of the controls. Thus, this ratio may be considered as an "animal comfort" index.

All in all, the activity and resting behavior of cows treated with CNH was clearly associated with signs that they were calmer and more comfortable than cows induced into dry off by the conventional means. In consistent, Osterman and Redbo (2001) have shown by analyzing LB that milking three times a day in comparison to twice daily contributes to increased comfort in high-producing dairy cows partly due to reduced udder pressure.

"Refinement means any decrease in the incidence or severity of inhumane procedures applied to those animals which still have to be used" (Russel and Burch, 1959, reprinted 1992; p. 64). Hitherto, the imposed compromise to cows' welfare in the course of drying-off in modern dairy farming appears largely to have been ignored in the relevant literature and by public opinion leaders in this sector, most likely because of the overwhelming impact of economic considerations. In the present communication, we present a viable treatment tool that can prevent the suffering associated with the drying-off of high-yielding cows.

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