

# Drying-off single udder quarters of dairy cattle during lactation using a casein hydrolysate

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## Abstract

Udder quarters suffering from chronic and therapy-resistant infections represent a serious problem for dairy farms. Drying off such udder quarters during lactation would offer an opportunity to remove these single quarters from milk production without losing the whole milk yield of one cow during the current lactation. The use of a casein hydrolysate (CNH), an enzymatic fission product of casein, hence an endogenous substance, showed promising results regarding downregulation of milk secretion in udder quarters of high yielding dairy cattle in previous investigations in Israel. The objective of this study was to prove whether the infusion of CNH into the bovine mammary gland is an appropriate method to downregulate milk secretion in chronically infected udder quarters in high yielding dairy cows in Germany in order to dry off these single quarters without inducing clinical mastitis. Each treated udder quarter received six intracisternal infusions of CNH within 3 treatment days. After the last infusion of CNH on day 3, milking of the affected udder quarter was stopped. In 21 out of 24 (87.5 %) quarters a successful drying off could be achieved and only three quarters showed clinical mastitis. Furthermore, none of the animals showed a defence response or signs of pain during the intracisternal application. An increase in the average somatic cell score (SCS) during the treatment period could be revealed and 11 out of 16 conventionally milked quarters showed a decrease in milk yield. Hence, it could be shown that the infusion of CNH is an effective and gentle method for downregulating milk yield and drying off chronically infected and therapy-resistant single udder quarters during lactation.

## Introduction

The mastitis of the bovine mammary gland is a serious and widespread problem in dairy cattle herds. Most infections are caused by invasion of pathogens through the teat canal. Many affected animals show a decreased milk yield. Furthermore, these animals cause additional costs and increased workload [6,7]. Udder quarters suffering from chronic and therapy-resistant infections are potential reservoirs for infections of the mammary gland of other animals. The quality of bulk tank milk decreases due to high somatic cell counts (SCC). Hence, these quarters constitute a serious problem for dairy cattle farms.

Drying off single chronically infected udder quarters during lactation would offer an opportunity to remove these single quarters from milk

production without losing the whole milk yield of one cow due to long withdrawal periods of antibiotics, anti-inflammatory drugs and painkillers. There are different methods described in the literature regarding therapeutic cessation of milk secretion and several methods are common in the field [8,2]. Many methods have no satisfying effect and udder inflammation caused by the used substances is a major problem. Recently, casein hydrolysate (CNH), an enzymatic fission product of casein, hence an endogenous substance, was used to dry off pregnant Holstein cows at the end of lactation in Israel. A marked cessation of milk secretion in the treated quarters could be achieved in this investigation. All treated quarters resumed lactation after parturition. This suggests that a natural phenomenon was imitated and that no tissue damage was caused which would irreversibly damage the secretory function of the mammary gland [13]. These observations are related to physiological enzymatic processes which take place in the mammary tissue. Plasmin is a protease in milk and it produces heat-resistant peptides from  $\beta$ -casein. It is mainly found in the inactive form plasminogen. Nevertheless, the concentration of plasmin increases at the end of lactation because of the plasminogen activator produced by the mammary epithelia. The plasmin activator induces a conversion of plasminogen to plasmin and this process is correlated with interruption of milk removal and mammary involution [9,11,12]. It is assumed that a  $\beta$ -casein related peptide (fraction 1-28) blocks potassium channels in the apical membranes of mammary epithelia and downregulates milk secretion. By imitating the hydrolysis of Casein under laboratory conditions, several peptides are formed, inter alia, the above mentioned  $\beta$ -casein related peptide (fraction 1-28) which is known to downregulate milk secretion. By infusing CNH into the mammary gland [13], this  $\beta$ -casein related peptide was introduced into the treated quarter, inducing a reduction in lactose concentration, a loss of tight junction (TJ) integrity and an inflammatory response followed by a drying-off of mammary secretion [13,14,15]. Consequently, an artificial increase in CNH could mimic the processes described regarding the involution by cessation of milking at the end of lactation. These involution processes induced by infusing CNH took place faster and more synchronized compared to natural involution at drying off [3,5,13]. The objective of this study was to test whether or not the infusion of CNH into the bovine mammary gland is an appropriate method for downregulating milk secretion in chronically infected udder quarters in high yielding dairy

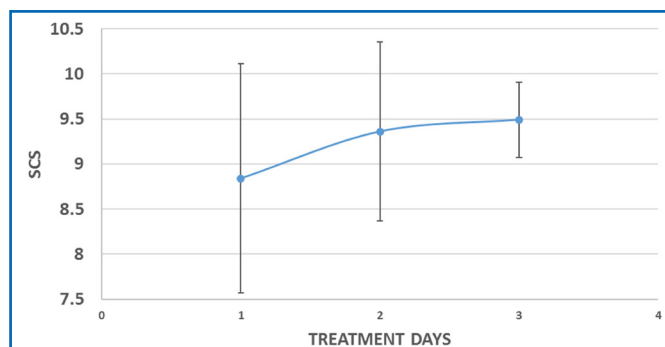
cows in Germany to dry off these single quarters without inducing clinical mastitis.

## Materials and Methods

**Animals:** The investigations were performed on 10 dairy farms in the federal state North-Rhine Westphalia, Germany from August 2014 to July 2015. Quarters suffering from chronic and therapy-resistant infections were chosen and presented by the farm owners for drying off. Quarters showing acute inflammatory symptoms or quarters under antibiotic treatment were not taken into account. Twenty-five udder quarters of 22 German-Holstein and 3 Simmental cows were selected for drying off. Eight cows were milked with an automated milking system; 17 cows were milked with a conventional milking system. The affected udder quarters were chosen for the following reasons: Recurrent mastitis (n=17), high SCC (n=3), insufficient milk yield (n=3), high SCC and insufficient milk yield (n=1) and teat lesions (n=1). The quarter dried off due to a fresh teat lesion received an additional antibiotic treatment during the 3 treatment days, using an intramammary applied cephalosporin because of the high infection risk.

**Casein Hydrolysate and Experimental Procedures:** The CNH was produced in the laboratory of the Department for Microbiology of the University of Applied Sciences and Arts Hannover according to the method described by Shamay et al. (2003) and kept frozen at -21 °C in 15 mL portions until use. An entire CNH portion was injected through the teat canal into the single udder quarter using a rounded plastic needle after each morning and evening milking during the treatment period. Hence, every quarter received 6 doses of CNH within 3 days. Observed defense responses or signs of pain during the intracisternal application were documented. After the final intracisternal infusion on day 3, milking of the affected udder quarter was stopped. The other quarters were milked in the usual manner. Single quarter-level milk samples were collected aseptically before every CNH treatment. If there was clinical mastitis during the following 60 days an additional milk sample was taken. The samples were sent to the Microbiology laboratory of the University of Applied Sciences and Arts Hannover for bacteriological examination according to DVG (2009) and SCC determination. The SCC was determined using SomaScope Smart (Delta Instruments, the Netherlands). Milk yield of the treated conventionally milked quarters were recorded during the 3 days of treatment using a quarter milker. On farms using an automatic milking system (Lely Astronaut®, the Netherlands) the milking times of the treated quarters were recorded and the last evening milking on day 3 was substituted by hand milking in order to determine the residual amount.

**Somatic Cell Score:** To investigate the development of SCC during the 3 treatment days, somatic cell scores (SCS) were used. For this pur-



**Figure 1: Development of SCS during the treatment period based on determined SCC of treated quarters at morning sampling. ( $\log_2(\text{SCC}/100,000) + 3$ )**

pose, the determined SCC of treated quarters was transformed to the logarithmic scale according to the formula  $\text{SCS} = \log_2(\text{SCC}/100,000) + 3$  [1,16].

## Results

Twenty-five udder quarters (11 rear left, 4 rear right, 6 front left, 4 front right) were used in the investigations. The number of quarters dried off per farm ranged from 1 to 7 with a median of 2.7 quarters per farm. One animal had to be excluded from the analysis due to suspected pericarditis and a following systemic antibiotic treatment during the 3 treatment days. Intramammary infection (IMI) was detected in 15 out of 24 quarters at treatment day 1 before drying off (Table 1).

In 21 of 24 (87.5 %) quarters successful drying off could be achieved. The udder quarter which was dried off due to a teat lesion and therefore received a local antibiotic treatment was included (83 % were successfully dried off if not including this quarter). One of these quarters showed a slight swelling during the drying off procedure, which abated quickly and the quarter could be dried off successfully. No defense responses or signs of pain during the intracisternal application were observed.

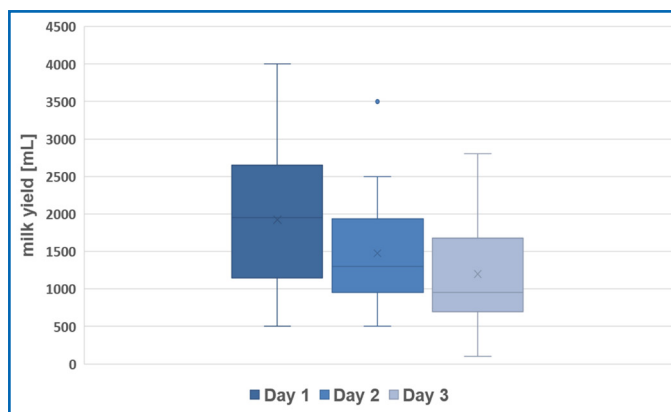
Three conventionally milked quarters showed a clinical mastitis at days 3, 5 and 9 after treatment. Hence, these quarters could not be dried off successfully. An additional milk sample of these quarters was taken. Two quarters showed an infection with *Streptococcus uberis* and one quarter a mixed infection with *Trueperella pyogenes* and *Streptococcus uberis*. Two of these quarters were bacteriologically negative when the CNH treatment started.

An increase in the average SCS during the treatment period could be revealed. The average SCS before starting the treatment with CNH at treatment day 1 was 8.84. At treatment day 3 an average SCS of 9.49 could be observed. The development of SCS during the treatment period is shown in Figure 1.

**Conventional milking system:** In total, 16 quarters were milked with a conventional milking system. The average milk yield at morning milking at treatment day 1 was 1923 mL (min – max: 500 mL to 4000 mL). The development of milk yield during the treatment period in these 16 conventional milked quarters is shown in Figure 2. Eleven quarters showed a decrease in milk yield between morning milking at treatment day 1 and morning milking at treatment day 3. The average decrease in milk yield amounted to 1183 mL (61.5% reduction) (min – max: 150 mL to 3200 mL). The development of the milk yield with regard to morning milking of these 11 quarters is shown in Figure 3. Five quarters showed no decrease in milk yield between morning milking at treatment day 1 and morning milking at treatment day 3. The average milk yield

**Table 1. Bacterial results at the beginning of the investigation; IMI were detected in 15 quarters**

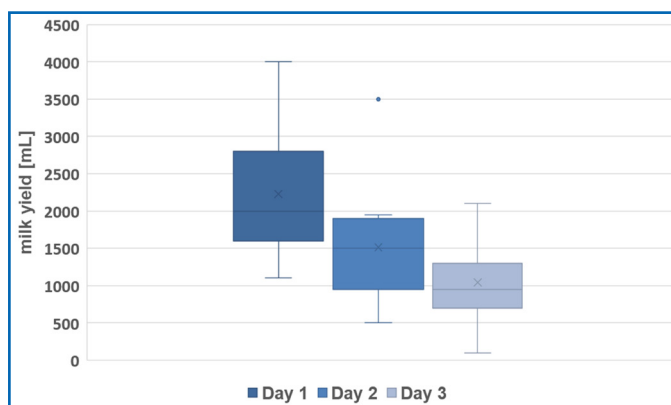
Bacterial species	IMI (n=15)
<i>Streptococcus uberis</i>	7
<i>Trueperella pyogenes</i>	2
CNS	1
Coliform bacteria	1
<i>Streptococcus dysgalactiae</i>	1
Yeasts	1
Prototheca	1
<i>Staphylococcus aureus</i>	1



**Figure 2: Development of milk yield during the treatment period in 16 conventional milked quarters based on results of morning milking**

of these quarters at morning milking at treatment day 1 was 1255 mL (min – max: 500 mL to 2500 mL).

Automated milking system: Eight quarters were milked with an automated milking system. The milking time varied during the 3 treatment days and no obvious decrease or increase in the milking time could be observed. The residual amount of 7 quarters was determined and



**Figure 3: Development of decreasing milk yield during the treatment period in 11 conventional milked quarters based on results of morning milking**

ranged from 80 mL to 2200 mL with a median of 626 mL at the evening milking on treatment day 3. Determining the residual amount of one quarter was not possible due to management reasons.

## Discussion

A successful drying off during lactation could be achieved in almost all quarters (87.5 %) and there were no differences between conventionally and automatically milked quarters. Most of the conventionally milked quarters showed a decrease in milk yield. Thus, there was no complete downregulation of milk secretion during the 3 treatment days, but the subsequent drying off (termination of milking of the affected udder quarter without any additional dry cow treatment) proceeded in a gentle way during the study procedure. These findings are similar to those of Shamay et. al (2003). An increase in SCS during the CNH treatment could be revealed as already observed by Ponchon et al. (2014).

Only three quarters showed clinical mastitis and in two of these quarters no IMI had been found before commencing with the CNH treatment. An iatrogenic origin due to contaminated application cannot

be ruled out regarding these infections. These results show that the infusion of CNH is a gentle method for drying off single udder quarters, but hygienic application is of high importance.

One quarter with a fresh teat lesion was dried off successfully with the CNH infusion, additionally using a locally applied cephalosporin due to a high infection risk. This shows that drying off quarters with fresh teat lesions is another possible indication for CNH infusion. These findings need to be verified in further studies with larger numbers of udder quarters affected by teat lesions.

The observed downregulation of milk secretion and the successful drying off in treated quarters indicates that the infusion of CNH could mimic the processes of involution by cessation of milking at the end of lactation as previously assumed by Shamay et al. (2003).

## Conclusion

The investigation revealed that the infusion of CNH is an effective and gentle method for downregulating milk yield and drying off chronically infected and therapy-resistant single udder quarters during lactation. None of the animals showed a defence response or signs of pain during intracisternal application of the CNH and only one quarter showed a slight inflammatory reaction during the drying off procedure. Further investigations should be performed to confirm these findings and to develop the applied method in order to make it accessible for practical use. Hence, it might be possible to solve an important and widespread problem of the dairy industry by using the endogenous substance CNH. Moreover, other indications such as fresh teat lesions could be found for the application of CNH in this investigation.

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## Conflict of interest

The authors declare no conflict of interest. The animal experiment was approved by the Lower Saxony State Office for Consumer Protection and Food Safety and the State Office for Nature, Environment and Consumer Affairs of North-Rhine Westphalia (AZ 84-02.04.2014.A212).

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