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Casein hydrolyzate for drying-off lactating mammary quarters in cows with chronic mastitis

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Research Article

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Abstract

In this research communication we address the hypothesis that a single intramammary infusion of casein hydrolyzate (CH) would have a similar effect to three intramammary infusions of CH for drying-off quarters with chronic mastitis (CM) during lactation. Sixty cows with CM were selected and randomly distributed into two treatment groups: (a) three intramammary CH infusions (100 mg, 50 ml per infusion, with 24-h intervals) or (b) single intramammary CH infusion (300 mg, 50 ml). Milk samples from the treated and untreated quarters were collected for microbiological culture and somatic cell count (SCC) before and after CH infusions. Milk yield was recorded and a manual pressure index measurement was used to evaluate cessation of lactation. Of the 60 quarters selected, 43 (71.67%) had positive microbiological culture. The quarters treated with three intramammary CH infusions had higher udder pressure index than those treated with single CH infusion. However, the average milk yield and composite SCC of three functional quarters were not different among treatments. Therefore, a single infusion of CH has the potential to be used as an alternative method for drying-off mammary quarters with CM during lactation.

Chronic mastitis (CM) is generally considered for cows having high somatic cell count (SCC) >200 000 cells/ml for more than two consecutive months or by the occurrence of three or more repeated cases of clinical mastitis in the same mammary quarter during lactation (Cardozo *et al*., 2015). Cows with CM have low chance of clinical cure (Cardozo *et al*., 2015) and a high frequency of mammary tissue fibrosis, which results in permanent milk yield losses. Considering the growing concern regarding use of antimicrobials and animal welfare issues, previous studies evaluated less aggressive, non-antimicrobial alternatives for drying-off quarters during lactation, such as casein hydrolyzate (CH) (Shamay *et al*., 2002). Intramammary infusion of CH mimics the natural mammary involution that occurs at the end of lactation, through the production of peptides associated with decreased milk yield and involution of the mammary gland after drying-off (Shamay *et al*., 2003; tho Seeth *et al*., 2016).

Although previous studies evaluated the efficacy of intramammary infusion of CH at quarters drying-off with CM, the effects of the frequency of CH infusions have not yet been evaluated. Previous studies have used multiple infusions (Shamay *et al*., 2002; Shamay *et al*., 2003; Silanikove *et al*., 2005; tho Seeth *et al*., 2016), which increases the risk of injury to the teat canal and new intramammary infections, and additionally these repeated procedures are more time consuming. Thus, the aim of the present study was to evaluate the efficacy of two CH intramammary infusion protocols during lactation on composite SCC and milk yield. The hypothesis was that a single intramammary infusion of 300 mg of CH would have a similar effect as three intramammary infusions of 100 mg of CH at drying-off mammary quarters with CM during lactation.

Materials and methods

Ethics approval was obtained from the Ethical Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science, University of São Paulo (CEUA, FMVZ, USP – Brazil/SP, protocol number 1876290119).

Inclusion criteria and cases definition

Sixty Holstein and Girolando (crossbred *Bos taurus taurus* × *Bos taurus indicus*) cows (approximately 250 d in milk (DIM) and milk yield of approximately 20 kg/d) that had

only one quarter with CM (clinical and subclinical) and four functional mammary quarters were selected. Definition of clinical and subclinical CM, as well as individual cow information about parity, DIM, average milk yield before treatment, clinical mastitis history, and last composite SCC are available in online Supplementary Table S1.

Intramammary casein hydrolyzate infusion

The preparation of CH was performed according to Shamay *et al.* (2003). Selected cows were randomly distributed between two treatment groups: (a) three intramammary CH infusions (three-CH; 100 mg, 50 ml per infusion), with 24-h intervals or (b) single intramammary infusion of CH (one-CH; 300 mg, 50 ml). In both treatment groups, an internal teat sealant infusion (Sellat®, Ourofino Saúde Animal, Cravinhos, Brazil) was infused at day 7 after the last CH infusion. Prior to intramammary CH infusion, teats were immersed in a pre-milking disinfectant solution and dried after 30 s with disposable paper towels, followed by complete milking. Teat ends asepsis was performed with 70% iodized alcohol solution (70% alcohol + 2% iodine), followed by infusions of CH. At the end of the CH infusion, the quarter was immersed in a post-milking disinfectant solution.

Milk samples and analysis

Milk samples from selected quarters were collected before (day 0) and after (days 4 and 7) the CH infusions for identification of mastitis-causing pathogen and for SCC. The microbiological identification was performed by MALDI-TOF MS according to Barcelos *et al.* (2019). The SCC was determined by flow cytometry using an electronic optical counting equipment (Somacount 300®, Bentley Instruments Inc., Chaska, MN USA). Milk yield was measured by the automatic milk yield meters in each farm before the beginning of treatment (day 0) and at day 7 in order to evaluate the difference in milk production after CH infusions. The cessation of lactation was assessed using the udder pressure index (UPI) methodology, based on manual assessment of udder pressure on days 4 and 7 after the first CH infusion (Leitner *et al.*, 2007).

Milk samples from the untreated quarters (pool of the three functional quarters) were collected at 30, 60 and 90 d after treatments to determine the composite SCC of CH treated cows. The results were compared with SCC of the four quarters obtained before to treatment.

Detailed description of sample collection procedure, MALDI-TOF MS analysis and UPI methodology are available in the online Supplementary File.

Statistical analysis

Data were analyzed using the statistical software SAS version 9.4 (SAS Institute, Cary, NC, USA). Statistical significance was defined when P-value ≤ 0.05. The adjusted means of the descriptive characteristics of the selected cows before infusion of CH, as well as the results of milk SCC were compared between treatments by Tukey test. For cessation of lactation index the PROC GLIMMIX was used. Somatic cell count ($\times 10^3$ cells/ml) was transformed to logarithmic scale according to Schukken *et al.* (2003). Analysis of milk yield data (D0 and D7) and SCC of untreated quarters (D30, D60 and D90) was performed by PROC MIXED. The model included treatment, day, their interaction, SCC log prior to treatment, CM history, DIM and parity as fixed effects and farm as random effect.

Results and discussion

Cow characteristics

Of the cows included ($n = 60$), 38 (63.33%) were Holstein and 22 (36.67%) Girolando. Parity, milk yield, SCC (composite and per quarter), clinical mastitis history and DIM before the first CH infusion were similar ($P > 0.05$) between treatment groups (details in Supplementary Table S1). Selected cows had a higher parity and advanced DIM, which are risk factors described in cases of CM. Of the 60 milk samples collected for SCC analysis prior to treatments, 11 were not evaluated because they were coagulated, and not acceptable for the analysis of flow cytometry. Thus, 25 quarters treated with three-CH and 24 quarters treated with one-CH were evaluated for SCC and were included in the study.

Frequency of mastitis causing pathogens

Of the total quarters evaluated ($n = 60$), 17 (28.33%) had negative culture before treatments. Negative culture results may occur because the number of colonies forming units is below the limit of detection of microbiological culture, there has been spontaneous bacterial cure or the causative agent is not isolated in conventional culture media. Among the positive samples, *Streptococcus uberis* had the highest frequency of isolation followed by *Staphylococcus aureus*. The ability of these pathogens to cause CM may be associated with bacterial defense mechanisms that contribute to immune system evasion.

After CH infusions (D7), of the total quarters evaluated ($n = 60$), 10 quarters (16.67%) presented bacteriological cure (three-CH = 6 mammary quarters; one-CH = 4). Although the cure rate after seven days of CH application was low, it is expected that postpartum cure rate will be higher (Silanikove *et al.*, 2005).

Evaluation of cessation of lactation using udder pressure

Mammary quarters treated with three-CH had a higher degree of udder pressure compared to those treated with one-CH ($P < 0.001$). In addition, a significant effect of CH treatment protocol on the day of evaluation was observed ($P < 0.01$, Fig. 1 and online Supplementary Fig. S2). Nevertheless, no complete quarter cessation of lactation (score 0) was observed in either treatment within 7 d of evaluation. This was not expected, since in previous studies with intramammary infusion of CH, both in chronic cases of lactation and for cows at drying-off, a complete cessation of lactation of quarter was reported in most cows (Silanikove *et al.*, 2005; tho Seeth *et al.*, 2016). Although our study did not observe a complete cessation of lactation of the mammary gland (UPI score 0), no quarter showed swelling (score 3) after treatment, which was considered a positive effect of CH infusion. Similar results were also described by Leitner *et al.* (2007), which found that CH reduced udder pressure and was associated with more comfort compared to cows that used only dry cow therapy.

Milk yield

The milk yield was measured only in five farms, totalizing 45 cows evaluated (one-CH = 21 and three-CH = 24). Milk yield data from 15 cows were not available for analyses due to the lack of farm

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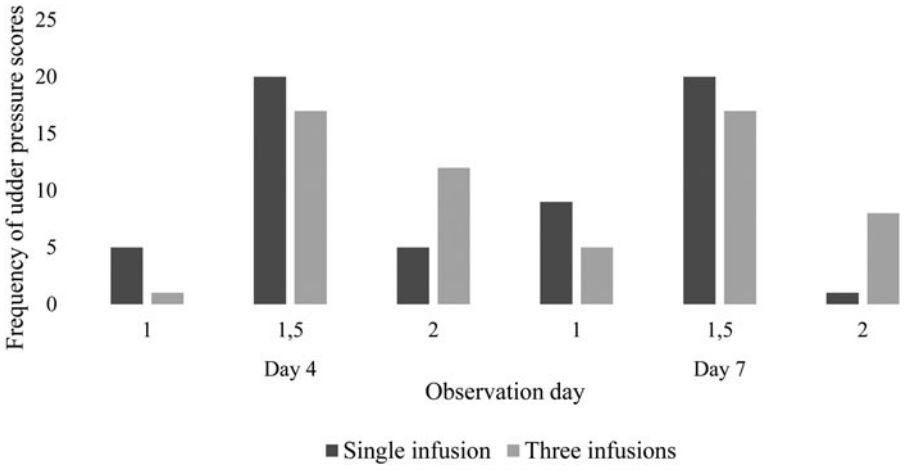

Fig. 1. Frequency of udder pressure scores of the mammary quarters treated with one or three intramammary casein hydrolyzate infusions for days 4 and 7 after treatment. Udder pressure scores range from 0 (no pressure, lactation has ceased) to 3 (significant pressure, full lactation). For a full statistical evaluation refer to online Supplementary Fig. S2.

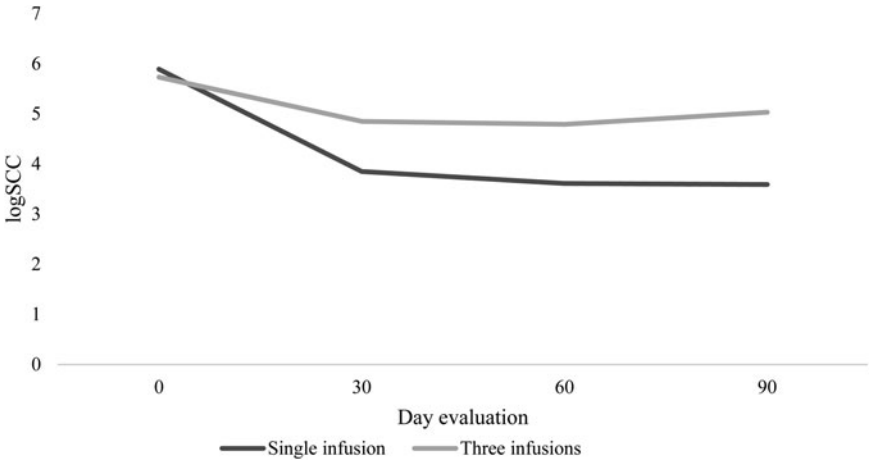

Fig. 2. Effect of casein hydrolyzate infusion in a single mammary quarter with chronic mastitis on log SCC in milk sampled from the other healthy quarters. For a full statistical evaluation refer to online Supplementary Fig. S3.

production records. Milk yield of cows treated with one-CH decreased from 21.65 to 18.77 kg, while that of cows treated with three-CH decreased from 21.14 to 17.09 kg. The average milk yield was only affected by DIM and evaluation day (both $P > 0.001$). On average, the difference in milk yield before (D0) and after treatment (D7) was 2.9 kg (13.45% of pre-infusion milk yield). The decrease of total milk yield was greater than that reported in other studies that evaluated the loss of cow's milk yield when a quarter was dried off with CH infusions. While in the Silanikove *et al.* (2005) study, the total cow's milk yield was not affected after drying-off a quarter, Leitner *et al.* (2012) reported a reduction of approximately 9% in milk yield 30 d after mammary quarter drying-off. However, the milk yield reduction was lower than that reported by Skarbye *et al.* (2018) who, when evaluating the abrupt quarter drying-off with subclinical mastitis, observed an average reduction of 4.1 kg of milk. This result may suggest improved comfort conditions for CH-infused cows, since there was no increase in udder pressure after the interruption of the quarter milking.

SCC of untreated mammary quarters

Of all cows selected ($n = 60$), 28 remained in lactation for at least 90 d after CH infusions and were evaluated for SCC of composite milk samples of untreated quarters on days 30, 60 and 90. Thirty-two cows were not included, in which 16 were dried-off by proximity of calving, 10 were dried-off for other reasons (reproductive problems and low milk yield) and six were voluntary culled by herd owners from the herd. There was no difference of SCC of composite milk samples between CH treatment protocols at any of the evaluated days ($P > 0.05$). For cows treated with three-CH, SCC was significantly lower only at day 60 ($P < 0.05$), which is similar to the results described by Silanikove *et al.* (2005), while cows treated with one-CH had significantly lower SCC on days 30, 60 and 90 ($P < 0.01$ or better) when compared to the results of SCC evaluated before CH infusion (Fig. 2 and online Supplementary Fig. S3). Our results should be interpreted carefully because we did not have a control group. Moreover, future studies should evaluate the effect of CH in chronic clinical cases and chronic subclinical cases separately, also testing single doses below 300 mg of CH and postpartum effects in the subsequent lactation.

In conclusion, the protocol with one-CH has the potential to be used as an alternative method for drying-off mammary quarters with CM during lactation, but further studies are needed considering that intramammary therapy with CH constitutes an unapproved use in commercial dairy farms.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029921000467

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