

## Materials and Methods

Holstein dairy cows (n=21) entering second or greater parity were enrolled in blocks of 3 during the dry period (28 days before expected calving date) and randomly assigned to 1 of 2 treatments or a control group: the BCCA group (n=7) received 384 g per day of RPBCAA (BALCHEM™) mixed with 200g of dry molasses top dressed in feed from calving to 35 days-in-milk (DIM); the BCAA plus PG (BCCAPG) group (n=7) received 384 g per day of RPBCAA mixed with 200g of dry molasses top dressed in their feed from calving to 35 DIM plus 300 ml of PG from calving until 7 DIM; the control group (n=7) received 200g of dry molasses top dressed from calving to 35 DIM. Cows were kept in tie stalls to measure daily intake and refusals. Postpartum, cows were milked 3 times a day and milk weights were recorded at each milking. Milk samples were collected from 3 consecutive milkings once a week and analyzed for fat and true protein. Blood was sampled 3 times per week 21 days before expected calving until 21 DIM. Beta-hydroxybutyric acid (BHBA) was measured cow side using a TaiDoc ketone meter (Pharmadoc, Lüdersdorf, Germany). Repeated measures ANOVA was conducted for the outcomes of BHBA, true protein as a percentage of total solids in milk, and total milk production until 35 DIM.

## Results

Beta-hydroxybutyric acid concentration (mmol/L) in least squares means (LSM) were  $0.78 \pm 0.12$ ,  $0.92 \pm 0.12$  and  $0.92 \pm 0.12$  for the BCAA, BCAAPG, and control groups, respectively. However, there was a difference in BHBA concentration (mmol/L) within the groups at 12 DIM with LSM of  $0.56 \pm 0.17$ ,  $1.26 \pm 0.17$  and  $0.93 \pm 0.17$  for BCAA, BCAAPG, and control groups, respectively (P=0.02). There was a difference between the groups true protein in milk LSM for  $5.24 \pm 0.25$ ,  $4.17 \pm 0.25$  and  $4.45 \pm 0.25$  for BCAA, BCAAPG, and control groups, respectively (P=0.01). There was no difference among the treatments for total milk production over 35 DIM.

## Significance

Branched-chain amino acid supplementation during early lactation in dairy cows may be a feasible option for effective alteration of milk yield, milk protein yield and improvement of negative energy balance in dairy cows due to its nutraceutical properties. Diets with high concentrations of BCAA have shown beneficial effect on body weight, glucose concentrations in blood and increased muscle synthesis in other species. For these reasons, supplementation of BCAA for dairy cattle could be advantageous for the industry.

# Inter- and intra-cow variability and the effect of teat-end shape on average milk flow rate, milk harvested in the first two minutes, and seconds below 2.2 lb per minute

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## Introduction

Data from electronic milk meters that measure milking characteristics such as average flow rate (AFR), amount of milk harvested in the first 2 minutes (2MIN), and seconds below 2.2 lb (1 kg) per minute flow rate (LOW) have been used as indicators of milking efficiency among cows. While these milking characteristics are valuable metrics to optimize efficiency of dairy production systems, the variability in them has not been rigorously investigated. The primary objective of this study was to describe the variability of the milking characteristics AFR (lb/min), 2MIN (lb), and LOW (sec.), both within and between cows. Our secondary objective was to investigate the influence of the explanatory variables milk yield, stage of lactation (DIM), parity, milking

time, farm, manual control mode, and teat-end shape on the aforementioned milking characteristics.

## Materials and Methods

Individual cow milking characteristics from 3,225 cow observations were recorded over a period of 30 days from 2 different dairy farms with automatic milk meters (MM27, DeLaval, Sweden). Cows were milked 3 times daily in a rotary parlor (Farm 1) or a 2 by 10 parallel parlor (Farm 2). A linear mixed-effects model was fitted to evaluate the effect of milk yield, DIM, parity, milking time, farm, and manual control mode on the outcome variables AFR, 2MIN, and LOW. To determine inter- and intra-cow variance components, variance estimates of the random effects of the null model were

used. To determine the association between teat-end shape (round = semicircular contour, pointed = cone-shaped, flat = square-shaped) and milking characteristics, a separate linear mixed-effects model of a subset of cows was fitted from Farm 2 using 136 cow observations. Model assumptions were assessed by evaluation of homoscedasticity and normality of standardized residuals. To satisfy this assumption, data of the outcome variable LOW was log transformed. Resulting estimates were subsequently back transformed.

### Results

Inter-cow differences accounted for 82% of the variance in AFR and 2MIN, while intra-cow variance accounted for 18% of the variance. The variance in LOW was 31% attributed to inter-cow differences, whereas intra-cow variance accounted for 69%. Mean ( $\pm$  SD) AFR (lb/min) was  $6.9 \pm 1.7$  (7.0, 0.2 – 25.7 (median, range)). Mean 2MIN (lb) was  $15.5 \pm 4.8$  (15.5, 0.1 – 38). Mean LOW (sec) was  $14.9 \pm 16.4$  (11.0, 0 – 446).

We observed the highest AFR in cows between 241 to 300 DIM (6.6, 0.1 (LSM, SE)). Milk yield, stage of lactation, parity, milking time, farm, and manual control mode had an effect on AFR ( $P < 0.02$ ). 2MIN was highest in 2nd-lactation

cows during the last trimester of lactation (13.5, 0.2 (LSM, SE)). In addition, parity, milking time, milk yield, and DIM had an effect on 2MIN ( $P < 0.001$ ). Primiparous cows spent less time in LOW (20.7, 20.1 to 21.3 (LSM, 95CI)) than cows in lactation 2 (21.6, 21.0 to 22.3 (LSM, 95CI)), 3 and greater (22.8, 22.1-23.5 (LSM, 95CI);  $P < 0.001$ ). AFR (lb/min  $\pm$  SE) was increased by  $1.9 \pm 0.5$  ( $P < 0.001$ ), 2MIN (lb  $\pm$  SE) was increased by  $4.5 \pm 1.3$  ( $P = 0.001$ ), and LOW was decreased by 24% ( $P = 0.10$ ) in cows with flat teat-end shape, compared with cows with pointed teat-end shape.

### Significance

While most of the unexplained variability in AFR and 2MIN was associated with differences between cows, the majority of the variability in LOW could be explained by differences within cows. As a consequence, AFR and 2MIN represent valuable metrics for optimizing efficiency of dairy production and understanding the dynamics between cow and milking machine, while LOW can be considered a good tool for evaluating the dynamics between milking routine and cow. Consideration of differences in milking parameters in cows with different teat-end shapes will help to improve parlor efficiency and udder health.

## Milk microbiome assessed through 16S rRNA sequencing during antimicrobial treatment of mastitis -- a randomized clinical trial and longitudinal follow up

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### Introduction

Clinical mastitis affects 20 to 30% of all dairy cows at least once throughout lactation (Hertl et al, 2014). Mastitis has been reported to be responsible for as much as 80% of all antimicrobials used in dairy farms in Wisconsin (Pol and Ruegg, 2007) and accounts for 16.5% of all diseases identified in dairy cattle the United States (USDA NAHMS Dairy, 2007). Gram-negative bacteria and culture-negative cases represent more than 50% of mastitis diagnosis in New York (Schukken et al, 2009). Contrasting evidence has been presented regarding the beneficial effects of treating cows with clinical mastitis caused by gram-negative pathogens (Schukken et al, 2011; Suojala et al, 2013), whereas treatment of culture-negative

cases is not currently recommended. There is increasing concern regarding the use of antimicrobials in food animals and the possible implications in human health, such as antibiotic resistance in pathogens, highlighting the importance of judicious use of antimicrobials in production animals. Specific aims were to: 1) evaluate the effect of antibiotics on cure of clinical mastitis; 2) use high throughput sequencing to assess the microbiome of milk samples from a mastitic quarter and compare it to the microbiome of ipsilateral healthy quarter of cows diagnosed with clinical mastitis caused by either a Gram-negative pathogen or with no bacterial growth on conventional aerobic culture; and 3) evaluate the over-time effect of prolonged antibiotic therapy on the microbiome profile of mastitic milk.