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Effect of dietary metabolizable protein level and live yeasts on ruminal fermentation and nitrogen utilization in lactating dairy cows on a high red clover silage diet

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ABSTRACT

The objective of this study was to determine the differences in nitrogen (N) use and rumen function in lactating dairy cows fed red clover-based diets with two levels of metabolizable protein (MP) and live yeasts. Eight rumen-fistulated Holstein dairy cows were used in a duplicated 4 × 4 Latin square design, with a 2 × 2 factorial arrangement of treatments. Isoenergetic diets, highly (HD) or moderately (MD) deficient in MP [−14% or −5% less than requirements, respectively], were fed with or without live yeast supplement (10 g/d of *Saccharomyces cerevisiae* (2×10^{10} cfu/g). Total mixed ration (60:40 red clover silage:concentrate barley based) was fed 12 times daily. Under the conditions prevailing in this study, yeast supplementation had limited effects. The dry matter (DM) intake was 1.1 kg greater in cows fed MD vs. HD diets. Given that milk production was not different between treatments, this lead to a tendency to improved milk efficiency for cows fed HD. The concentration of isoacids in the ruminal fluid was reduced by yeast supplementation ($P \leq 0.02$). Ruminal fluid lactate concentration was increased in the HD diet compared with the MD diet (0.14 vs 0.12 m*M*, SEM = 0.01; $P = 0.02$), whereas that of ammonia-N (NH_3-N) was decreased in HD compared with MD diet (2.5 vs 4.8 mg/dL, SEM = 0.6; $P = 0.02$). Apparent digestibility of N (664 and 616 g/kg, SEM = 17; $P < 0.001$) and urinary N excretion (264 and 163 g N/d, SEM = 9; $P < 0.001$) were greater in the MD than in the HD diet. Apparent digestibility of DM, organic matter, neutral detergent fiber (NDF) and acid detergent fiber were not different among diets. Yeast supplementation tended to reduce total tract apparent digestibility of NDF (565 vs 589 g/kg, SEM = 28; $P = 0.09$). The diet HD reduced plasma urea concentration by 28% compared with MD. Average microbial counts, protozoa counts,

Abbreviations: ADF, acid detergent fiber expressed inclusive of residual ash; BW, body weight; CP, crude protein; DM, dry matter; HD, highly deficient in metabolizable protein; MD, moderately deficient in metabolizable protein; MP, metabolizable protein; MUN, milk urea nitrogen; N, nitrogen; aNDF, neutral detergent fiber assayed with sodium sulfite and with a heat-stable α-amylase, and expressed inclusive of residual ash; NE_L, net energy of lactation; NFC, non-fiber carbohydrates; NH_3-N, ammonia-N; RDP, rumen degradable protein; RUP, rumen undegradable protein; SC, *Saccharomyces cerevisiae*.

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total viable bacteria and cellulolytic bacteria were not affected by treatments. Yeast supplementation tended to decrease in situ effective DM degradability of corn and decreased that of barley DM, without affecting DM degradability of timothy-dominated hay. In conclusion, the present study showed that diets designed to reduce MP supply from 0.95 to 0.86 of requirements improved N efficiency and consequently contributed to the decrease in N excreted to the environment. Under these dietary conditions, there were no advantages of supplementing with yeasts.

1. Introduction

With growing concern in North America and Europe regarding the negative impact of nitrogen (N) released into the environment, it is appropriate that farmers optimise the protein content of their cows' diet i.e. decrease the supply in protein without affecting dairy cows performances, with the additional advantage of reducing diet cost. Several manuscripts reported no significant benefit in term of milk yield to formulate dairy diets beyond 170 g of CP/kg of DM (Broderick, 2003; Olmos Colmenero and Broderick, 2006). Furthermore, Lee et al. (2015) reported that cows fed a deficient MP diet (137 g of CP/kg of DM) supplemented with rumen protected methionine and/or lysine had a similar milk yield than cows fed adequate MP diet (155 g of CP/kg of DM).

Red clover has a greater concentration of undegradable protein compared with many forages owing to its content in polyphenol oxidases which inhibit plant proteases (Broderick et al., 2000) and less non-protein N than alfalfa silage (Owens et al., 1999). We therefore hypothesized a greater efficiency of protein utilization with high red clover diets containing 170 g/kg as opposed to 190 g/kg CP. In support of this characteristic of red clover, Benchaar et al. (2015) recently reported that N retained was 2-fold greater for cows fed red clover than corn silage when both silages constituted 0.57 of the TMR on a dry matter (DM) basis. Additionally, milk yield was increased by 2 kg/d in cows fed red clover silage diet compared with cows fed corn silage diet. Legume silages allow greater DMI than grass silages (Dewhurst et al., 2003). However among legumes, red clover provides lower consumption results when compared to alfalfa. Live *Saccharomyces cerevisiae* (SC) supplementation has often shown benefit to rumen metabolism, digestion and milk production (Desnoyers et al., 2009). Positive effect of SC on DM and NDF digestibility was reported in several studies (Arcos-García et al., 2000; Bagheri et al., 2009; Bitencourt et al., 2011; Ding et al., 2014). Early work by Giger-Reverdin et al. (1996) reported improved overall performance of goats when SC was supplemented to adequate-N diet and this improvement was even enhanced with low-N diet. In a study by Putnam et al. (1997), live SC increased RUP in cows fed CP deficient diets, through increased DMI. Because reduction of dietary protein was often accompanied by a reduction in feed intake (Sinclair et al., 2014), and given the characteristics of SC reported above, we hypothesized that DMI as well as DM and fiber digestibility of red clover could be enhanced by adding live yeasts.

The objective of this experiment was to determine differences in N use and rumen function in dairy cows on a diet rich in red clover silage, with two levels of metabolizable protein (MP) and supplemented or not with SC.

2. Material and methods

2.1. Animals, diets and experimental periods

Eight rumen-fistulated Holstein dairy cows (678 kg BW; SD = 66) were used in a duplicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments. The first factor was the amount of MP supply which was either moderately or highly deficient (−129 g of MP/d [MD] or −353 g of MP/d [HD]; NRC, 2001). These diets provided 188 and 168 g/kg of CP, respectively. Metabolizable protein deficiency was estimated based on the difference between theoretical MP requirements and the actual MP supply calculated from DMI and nutrient composition of the diets minus MP exported in milk. *Saccharomyces cerevisiae* (0 g [**SC**−] or 10 g/d; 2×10^{10} organisms/g; LEVUCELL® SC [**SC+**]) constituted the other factor. One week following calving, cows were introduced to experimental diets. A TMR distributed every other hour in 12 equal meals (Ankom Technology, Fairport, NY) was offered daily to cows on an individual basis for ad libitum consumption. One kg (on as-is basis) of long mature timothy-dominated hay was fed once in the morning to prevent ruminal disturbances. Feed consumption was recorded daily. Silage DM was analyzed weekly to adjust TMR offered (to about 0.10 daily orts). Cows were housed in tie stalls and milked twice daily at 12-h intervals. The TMR contained 0.60 red clover silage and 0.40 concentrate on a DM basis (Table 1). The main difference between the MD and the HD diets was the substitution of soybean meal for ground corn. The diets were formulated to meet the requirements of energy, minerals and vitamins for a cow producing 36 kg of milk/d (NRC, 2001). The experiment started 1 week after calving date and lasted 18 weeks. The first 3 weeks allowed for transition to experimental diets. Ruminal fluid sampling, pH recording and the in situ study were performed during week 4, whereas a total tract digestibility study and collection of performance and blood data were performed during week 5. After this sequence (week 1–5), only yeast treatments were switched between cows within each MP supply and 2 wks were allowed for adaptation (weeks 6 and 7) before rumen sampling and digestibility studies were repeated (weeks 8 and 9). At week 10,