Effects of Live Yeast Supplementation on Ruminal Parameters and Lactation Performance of Dairy Cows Fed Medium or High Levels of Dietary Concentrate

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Summary
The objectives of this study were to determine the effect of live yeast (LY) supplementation and dietary concentrate level interaction on ruminal parameters, dry matter intake (DMI), milk yield, and milk composition of lactating dairy cows. Four multiparous Holstein cows were assigned to one of four dietary treatments in a 4×4 Latin Square design in a 2×2 factorial arrangement with 21-d periods. The dietary treatments were: 1) 50% concentrate + live yeast (10 g/cow/d; 50LY), 2) 50% concentrate + no live yeast (50NLY), 3) 70% concentrate + live yeast (10 g/cow/d; 70LY), and 4) 70% concentrate + no live yeast (70NLY). A more distinct effect of the LY supplementation on ruminal pH was observed at 9 h of post-feeding, where cows that received 70NLY had the lowest ruminal pH (5.81) compared to cows that received 70LY (6.40; P< 0.05). The LY supplementation decreased the sum of ruminal isobutyrate, isovalerate, and valerate concentrations (4.3 vs. 4.6 mol/100 mol and 4.7 vs. 4.8 mol/100 mol) in both 50 and 70% concentrate diets compared to NLY (P= 0.02). Overall, the LY supplementation had only numerically higher on DMI (18.0 vs. 17.5 kg/d), milk yield (20.2 vs. 19.1 kg/d), and 3.5% fat corrected milk (19.4 vs. 18.8 kg/d) compared to NLY supplementation, respectively. The LY supplementation tended to increase (P= 0.06) milk fat yield in 50LY (0.66 kg/d) compared to 50NLY (0.62 kg/d). Similarly, the LY supplementation tended to increase (P= 0.08) solid non-fat (SNF) percentage in 50LY (9.83%) compared to 50NLY (9.63%). Although there were only numerical increases in DMI, milk yield, and 3.5% fat corrected milk with the supplementation of the LY, results indicated that the LY supplementation in the 50% concentrate diet would increase milk protein, SNF, and lactose percentages. In conclusion, ruminal pH reductions associated with feeding high dietary concentrate (70%) diets in dairy cows can be prevented with the LY supplementation.

Canlı Maya İlaresinin Orta veya Yüksek Düzeyde Konsantrte Yemle Beslenen Süt İneklerinde Ruminal Parametreler ve Laktasyon Performansını Üzerine Etkisi

Özet
Bu çalışmanın amacı, laktasyondaki süt ineklerinde canlı maya (CM) ilavesi ve rasyon konsantrte yem düzeyi etkileşimlerinin ruminal parametreler, kuru madde tüketimi (KMT), süt verimi, ve süt komposizyonunu üzerinde olan etkilerinin belirlenmesi olmuştur. Dort adet 2 ve üstü laktasyondaki Holstein süt ineği 4x4 Latin kare deneme deseni içinde 2x2 faktörül düzenlenmede 21-günlük periyotta 4 farklı muameleye rável edilmistir. Muamele grupları: 1) %50 konsantrte yem + canlı maya ilavesi (10 gr/inek/gün; 50CM), 2) %50 konsantrte yem + canlı maya ilavesi yok (50CMY), 3) %70 konsantrte yem + canlı maya ilavesi (10 gr/inek/gün; 70CM), ve 4) %70 konsantrte yem + canlı maya ilavesi yok (70CMY) olarak düzenlenmiştir. Canlı maya ilavesinin belirgin etkisi ruminal pH'da gözlenmiş olup, yemlemeden sonraki 5. saatte rumen pH'si 70CM grubundaki (5.81) meleklere 70CMY grubuna (6.40) göre önemli düzeyde olmuştur (P<0.05). Ayrıca, CM ilavesi CMY'ye göre toplam ruminal izobütrat, izovalerat ve valerat (4.3 vs. 4.6 mol/100 mol ve 4.7 vs. 4.8 mol/100 mol) konsantrasyonlarının her %50 hem de %70 konsantrte yem rasyonlarında azaltmıştır (P=0.02). Genel olarak, CM ilavesi CMY ile karşılaştırıldığında süt verim (19.4 vs. 18.8 kg/gün) ve %3.5 yağlı düzeltilmiş süt verimi (19.4 vs. 18.8 kg/gün) yönünden sadece raksamsal bir artışa sahip olmuştur. Canlı maya ilavesi, 50CM grubunda (0.66 kg/gün) 50CMY grubuna (0.62 kg/gün) göre süt verimi artışta eğilimlidir (P=0.06). Benzer şekilde, CM ilavesi süt yağsız kuru madde (YKM) yüzdesini 50CM gruba (%9.83) 50CMY gruba (%9.63) göre artırmadadır (P=0.08). Her ne kadar CM ilavesiyle KMT, süt verimi, ve %3.5 yağlı düzeltilmiş süt veriminin raksamsal artışlar gözlenmiş olsa da, sonuçlar CM ilavesinin %50 konsantrte yem rasyonunda süt proteinini, YKM ve lactoz yüzdelerini artırabildiği göstermiştir. Sonuç olarak, süt ineklerinde yüksek konsantrte yem düzeyile (%70) beslenmenin ilkişi olduğu ruminal pH düşüklükleri CM ilavesiyle önlenebilecektir.

Anahtar sözcükler: Canlı maya, Süt ineği, Laktasyon performansı, Ruminal pH
INTRODUCTION

Yeast (*Saccharomyces cerevisiae*) and yeast by-products are used in livestock nutrition as feed additives because of their beneficial effects on animal performance. They are used mainly in high producing dairy and beef cattle rations to compensate for the ruminal fermentation disturbances associated with the feeding of high dietary concentrate diets. *Saccharomyces cerevisiae* (*S. cerevisiae*) are able to grow rapidly in the rumen and facilitate fiber digestion. Micro-nutrients found in *S. cerevisiae* also stimulate cellulolytic bacteria growth. In addition, *S. cerevisiae* also protect ruminal fermentation from lactic acid accumulation. Based on the theory proposed by Newbold et al., *S. cerevisiae* in the rumen environment can utilize the remaining dissolved oxygen and save anaerobic microorganisms from the toxic effect of oxygen.

Previous researches focusing on feeding dairy cows with yeast and yeast by-products have had variable results. Several reasons may account for these variations including composition of the ration used (forage to concentrate ratio, quality of the forage, nutrient composition of the diet etc.), amount of yeast supplemented, type and number of viable yeast, number of cows used, and lactation stage of the cows. Robinson and Erasmus summarized how nutrient composition of the diet would affect cow production variables in yeast supplemented rations. They performed a correlation analysis from the data of 22 different yeast supplemented lactation studies and found that an increase in the crude protein (CP) content of the diets supplemented with yeast was positively correlated with the yields of milk (r = 0.24), milk protein (r = 0.35), and DM (r = 0.14). However, an increase in the acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents of diets supplemented with yeast were negatively correlated with milk yields (r = -0.54 and -0.55), milk fat (r = -0.23 and -0.19) and milk protein (r = -0.53 and -0.37), as well as DM (r = -0.45 and -0.40). Similarly, Desnoyers et al. summarized the meta-analysis of yeast and yeast by-product feeding studies (n = 110) from the literature and found that yeast supplementation increased the DMI by 0.44 g/d of kg BW in dairy cows. This positive response was related to an increase of the concentrate portion in the diet not affected by the NDF and CP contents of the diet. Results from this meta-analysis also found that milk production was 1.2 g/kg of BW higher in cows supplemented with yeast compared to cows not supplemented with yeast. It was concluded that yeast supplementation in the diets of dairy cows increased the ruminal pH and VFA concentrations by 0.03 point and 2.17 mM, respectively, while reducing the lactate concentration by 0.9 mM.

The objectives of this experiment were to test the interactions between the LY (*S. cerevisiae* NCYC R618) supplementation and dietary concentrate level on ruminal fermentation characteristics and lactation performance of lactating dairy cows.

| Nutrient | % of DM |
|----------|---------|
| OM       | 91.7    |
| CP       | 16.0    |
| NDF      | 49.9    |
| ADF      | 35.5    |
| Ash      | 8.3     |
| NE (Mcal/kg) | 1.50 |

| Ingredient                        | 50% Concentrate | 70% Concentrate |
|-----------------------------------|-----------------|-----------------|
| Corn silage                      | 43.0            | 23.0            |
| Alfalfa hay                      | 7.0             | 7.0             |
| Concentrate                      |                 |                 |
| Corn gluten feed                 | 11.0            | 15.4            |
| Wheat                             | 11.0            | 15.4            |
| Sunflower meal                   | 11.0            | 15.4            |
| Cottonseed meal                  | 6.0             | 8.4             |
| Barley                           | 5.0             | 7.0             |
| Wheat bran                       | 3.0             | 4.2             |
| Limestone                        | 1.5             | 2.1             |
| Molasses                         | 1.0             | 1.4             |
| Salt                             | 0.4             | 0.6             |
| Trace-mineral and Vitamin premix  | 0.1             | 0.1             |

*1 Contained 26.1% DM, 10.1% CP, 62.5% NDF, 45.1% ADF, 6.1% ash
2 Contained 89.5% DM, 15.2% CP, 61.2% NDF, 59.6% ADF, 7.9% ash
3 Contained 89.1% DM, 21.3% CP, 37.3% NDF, 23.7% ADF, 10.3% ash
Refused were recorded daily. Cows were fed ad-libitum, and orts were maintained at approximately 10%. Each individual feed, TMR, and orts were analyzed for DM, organic matter (OM), CP, AD, and NDF. Cows were milked a.m. and p.m., and milk yield and milk compositions were measured during the last 3 days of each period. Concentrations of milk fat, protein, lactose, and solid non-fat (SNF) were determined by an ultrasonic milk analyzer (Lactoscan SA®). Mean daily milk composition was an average of a.m. and p.m. samples using the proportion of daily production at each milking as a weighting factor.

**Ruminal Measurements**

Ruminal pH was measured during the last 2 days of each period with a special filter mounted stomach tube at 0, 3, 6, 9, and 12 h of post-feeding using a hand held pH meter (HI-8314N, Hanna Instruments, UK). The sampled rumen fluid was filtered through two layers of cheesecloth, and 10 ml of duplicate supernatants were mixed with 0.2 ml of 50% H2SO4, and then frozen at -20°C until the analyses. Volatile fatty acid (VFA) analyses of the gas chromatography (Agilent Technologies 6890N, Network GC System) conditions were as follows: rumen fluid (2 ml) was transferred into the GC vials after centrifuging at 10,000 rpm; and then 10 µl of concentrated H2SO4 was added into each of the vials before analysis with the capillary column (Stabilwax-DA®, Crossbond Carboxy-PEG for acidic compounds, 30 meter, 0.25 mm ID, 0.25 µm df, maximum program temperature of 260°C). The column temperature program was started at 100°C for 5 min, then increased by 10°C/min to 160°C for 2 min, and finally maintained at 80°C for 5 min.

**Statistical Analysis**

Intake, milk production, and composition data were analyzed by PROC GLM; ruminal pH and VFA data were analyzed by PROC MIXED procedure for repeated measures of SAS®. For ruminal pH and VFA, period and hour were used as repeated measurements. Treatment mean differences were tested using the least significant difference method after a significant F-test (P<0.05).

**RESULTS**

Effect of the LY supplementation and dietary concentrate level on post-feeding ruminal pH is presented in Fig. 1. The LY supplementation in the 50% concentrate diet numerically increased the ruminal pH of cows 3 h of post-feeding compared to NLY supplementation (6.35 vs. 5.97). A similar trend was observed after 9 h of post-feeding, where the LY supplementation in the 70% concentrate diet significantly increased the ruminal pH of cows compared to NLY supplementation (6.40 vs. 5.81; P<0.05). In addition, supplementation of the LY alone compared to NLY increased the ruminal pH numerically after 3 (6.17 vs. 5.91), 9 (6.23 vs. 5.92), and 12 h (6.45 vs. 6.23) of post-feeding (data not presented). The level of concentrate had no effect on ruminal pH, averaging 6.04, 5.99, 6.08, and 6.34 at 3, 6, 9, and 12 h of post-feeding.

Effects of the LY supplementation and dietary concentrate level on ruminal VFA concentrations are presented in Table 2. Total VFA concentration was not affected by either the LY supplementation or dietary concentrate level, averaging 102.3 mM. Neither acetate nor propionate concentrations were affected with the LY supplementation in both 50 and 70% concentrates. However ruminal acetate and propionate concentrations were decreased and increased, respectively in 70% concentrate (58.7 and 26.6 mol/100 mol; P<0.01) compared to 50% concentrate diet (61.0 and 24.0 mol/100 mol; P<0.01). The LY supplementation decreased the concentrations of otherVFAs (isobutyrate + isovalerate + valerate) in both dietary concentrate levels (4.5 vs. 4.7 mol/100 mol; P= 0.02). Although the ratio of acetate to propionate was not affected by the LY supplementation, it was decreased in the 70% concentrate diet compared to the 50% concentrate diet (2.3 vs. 2.6; P<0.01).

Live yeast supplementation alone had no significant effect on the performance of lactating dairy cows (Table 3). The LY supplementation increased DMI numerically by 0.9 kg/d only in the 50% concentrate diet. Milk yield was increased by the LY supplementation numerically across all diets by an
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of 1.0 kg/d. This was more pronounced in the 50% concentrate diet, where milk yield was 1.4 kg/d higher with the LY supplementation. Similarly, FCM and ECM yields were not affected statistically by the LY supplementation across all diets. However, FCM, ECM, protein, fat, lactose, and SNF yields were numerically higher in the LY supplemented diets in the 50% concentrate diet by 1.2, 1.4, 0.07, 0.04, 0.04, and 0.02 kg/d, respectively. Percentages of milk protein (3.33 vs. 3.26), lactose (3.64 vs. 3.41), and SNF (9.83 vs. 9.63) tended to be higher with the LY supplementation in the 50% concentrate diet (P<0.1). The DMI, milk yield, ECM, milk protein and lactose, and SNF increased significantly in the 70% concentrate diet compared to the 50% concentrate diet (P<0.05). Percentage of milk fat was decreased significantly in the 70% concentrate diet without the LY supplementation (3.07 vs. 3.74; P<0.05).

DISCUSSION

Variable DMI responses to LY supplementation have also been demonstrated in previous studies. In dairy cows starting from 4 wk pre-partum to 18 wk post-partum, Wohlt et al. 10 found that LY supplementation (10 g/cow/d S. cerevisiae,
5x10^8 cfu/g) compared to NLY supplementation increased the DMI significantly only during the first 6 wk of lactation (14.4 vs. 13.8 kg/d), but not during the entire experimental period (18.5 vs. 19.2 kg/d). Similar to our findings, Yalcin et al. found no DMI differences for dairy cows supplemented with LY (50 g/cow/d; 1.3x10^8 cfu/g) compared to NLY during mid-lactation of 25 d. In addition, Swartz et al. tested the effects of LY supplementation (5x10^19 cfu/cow/d) on DMI of lactating dairy cows (n= 306) from 7 different commercial farms and found no response. Although the LY supplementation in the present study caused a numerical increase on milk yield (averaging 1.0 kg/d) in both concentrate levels, no statistical significance was detected. Similar to DMI, previous studies of dairy cows supplemented with LY also indicated variable milk yield and milk composition results. Wohlt et al. found a significant difference on milk yield, FCM, and milk fat of cows between the 5th and 8th wk of post-partum periods when supplemented with either 10 or 20 g of LY (5x10^6 cfu/g) compared to NLY. Yalcin et al. also found a trend for higher milk fat yield of cows supplemented with 50 g/d of LY. Similarly, milk fat yield tended to be 0.04 kg/d higher with the LY compared to NLY supplementation in the 50% concentrate diet in our experiment. Shaver and Garrett also found 0.9 kg/d more milk yield response in LY supplemented (57 g/cow/d) cows (n= 585) for 30 d of mid-lactation period from 11 commercial farms. Cows on those farms were offered a TMR with 18.8% CP, 18.6% ADF, and 28.2% NDF. Nutrient content of the diet also appears to have an effect on the productivity of cows supplemented with LY. Soder and Holden tested the effects of LY supplementation (15 g/cow/d; 5x10^6 cfu/g) on the lactation performance of primi- and multiparous cows starting from 4 wk pre-partum to 13 wk post-partum. They found that LY supplementation had no effect on lactation performance and concluded that the positive response should not be related to LY itself alone. Other factors, such as lactation stage, nutrient composition of diet, type of forage fed, feeding practices, and forage to concentrate ratio may also have an effect on this response. Previous research indicated that a high proportion of dietary forage and NDF could cause a lack of response on the performance of dairy cows supplemented with LY. In the present study, the NDF and ADF contents of the diets were above the NRC minimum requirements (25-33% NDF, 17-21% ADF) for lactating cows, which might have precluded the positive lactation response to the LY supplementation. Although previous research showed that the percentage of milk protein was either decreased or unchanged with LY supplementation, we found a tendency for higher milk protein percentage of cows supplemented with the LY in the 50% concentrate diet. Similarly, percentage of milk lactose and SNF also tended to increase in cows supplemented with the LY in the 50% concentrate diet. Moallem et al. found a significant increase for milk lactose with LY supplementation (10^16 cfu/cow/4 kg DM consumed) in a diet having 60% concentrate and 16.5% CP during the hot season. In our experiment, higher percentages of lactose and SNF with the LY supplementation should be the result of greater digestible nutrient intake in the 50% concentrate diet.

Although NLY supplementation did not cause a drastic pH reduction within 9 h of post-feeding (averaging 5.95) compared to the LY supplementation (averaging 6.13), the presence of lower ruminal pH in a sub-clinic manner over many weeks may cause significant production losses. Previous studies have found that LY supplementation is capable of enhancing ruminal pH in dairy cows due to its nature. Jouany proposed that LY may act as a balancer for the ruminal fluid redox potential and thereby maintain optimal fermenting condition for ruminal microflora, which need a high pH. Bach et al. found a significant ruminal pH enhancement (6.05 vs. 5.49) in a continuing measurement of dairy cows supplemented with 5 g/cow/d LY (10^19 cfu/g). They also found that cows supplemented with LY compared to NLY supplementation had a lesser meal interval (3.3 vs. 4.0 h). In the present study, cows fed a high concentrate diet (70%) responded better to the LY compared to NLY supplementation throughout 12 h of post-feeding, averaging 6.39 and 6.16 of ruminal pH, respectively. Koul et al. found that LY supplementation on ruminal pH is most effective after 4 h of post-feeding, and that its efficacy was equal to that of NaHCO₃. Similarly, Marden et al. tested the efficacy of both LY (5 g/cow/d; 10^10 cfu/g) and NaHCO₃ (150 g/cow/d) supplementation on ruminal pH, and found that LY (6.14) was as effective as NaHCO₃ (6.21) on enhancement of ruminal pH compared to control treatment (5.94).

Results for VFA response to LY supplementation in dairy cows has also varied in previous studies as seen for production variables. Contrary to our findings in this study, Sullivan and Martin found a higher ruminal propionate concentration in LY added to in vitro medium. In addition, Dolezel et al. detected a linear increase between the amount of LY supplemented and the total ruminal VFA concentration. Similar to our findings, Longuski et al. found that LY supplementation (56 g/cow/d) had no effect on total ruminal VFA or acetate concentrations in dairy cows. In the present study, individual ruminal VFA concentrations were affected independently by the LY supplementation and concentrate level. This may indicate that the LY supplementation alone had the potential for changing ruminal VFA production pattern without diet nutrient composition. Although we did not measure ruminal lactate concentration in the present study, the pH data supported the fact that the 70% concentrate diet supplemented with the LY could have had a lower ruminal lactate concentration. Desnoyers et al. found in the meta-analysis that LY supplementation compared to NLY in ruminant species (cattle, goats, sheep, and buffaloes) tended to decrease rumen lactic acid concentration (-0.9 mM on average).

In conclusion, 10 g/cow/d LY supplementation with 50% dietary concentrate in the present study increased DMI, milk yield, and milk fat by 0.9, 1.4, and 0.04 kg/d, respectively. Furthermore, percentages of milk protein, lactose, and SNF...
were increased with the LY supplementation in the 50% concentrate diet by 0.07, 0.11, and 0.20, respectively. It was also obvious that the LY supplementation in the 70% concentrate diet possibly controlled the ruminal pH decrease. In addition to these benefits, chemical composition of rations, stage of lactation, DMI and milk production potentials of animal’s, and viability (cfu) of the LY should be considered before determining its supplementation in dairy cow rations.

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