



## Short communication: Production performance and nutrient digestibility of lactating dairy cows fed diets with and without addition of a live-yeast supplement

G. Ferreira\*

Department of Dairy Science, Virginia Tech, Blacksburg 24061

### ABSTRACT

The objective of this study was to evaluate the use of a live-yeast product when feeding relatively high-forage diets to high-producing cows in mid lactation. Eight primiparous [ $607 \pm 43$  kg of body weight (BW) and  $130 \pm 16$  d in milk (DIM) at the beginning of the experiment] and 16 multiparous ( $706 \pm 63$  kg of BW and  $137 \pm 22$  DIM at the beginning of the experiment) Holstein cows were blocked by parity and DIM, and randomly assigned to 1 of 2 diets (control vs. yeast) for a 12-wk period according to a randomized complete block design. The formulated diets contained 50.4% corn silage, 10.4% alfalfa hay, and 39.2% concentrate. The yeast diet was formulated to provide approximately  $5.4 \times 10^{11}$  cfu/d of *Saccharomyces cerevisiae* (BeneSacc; Global Nutritech Biotechnology LLC, Richmond, VA). Total-tract nutrient digestibility was estimated using 240-h undigested neutral detergent fiber (NDF) as an internal marker. Supplementing live yeast to lactating dairy cows did not affect dry matter intake (25.0 kg/d), milk yield (38.6 kg/d), milk fat concentration (4.78%), milk fat yield (1.83 kg/d), milk protein concentration (3.09%), milk protein yield (1.18 kg/d), milk lactose concentration (4.79%), milk lactose yield (1.84 kg/d), BW gain ( $-0.05$  kg/d), or body condition score gain (0.16 units). The digestibility of dry matter was greater for the control treatment than for the yeast treatment (69.3 and 67.1%, respectively), but the digestibilities of crude protein (61.5%), NDF (40.5%), and starch (98.6%) were not affected by treatment. In conclusion, supplementation of live yeast did not affect production performance or nutrient digestibility of high-producing cows in mid lactation. The reasons for the lack of effect are not clear, but an evaluation of interactions between yeast and rumen buffer supplementation is warranted.

**Key words:** yeast, direct-fed microbial, *Saccharomyces cerevisiae*

### Short Communication

Supplementing yeast-derived products has been used as a feeding strategy to increase ruminal pH and fiber fermentation (Jiang et al., 2017; Bach et al., 2018a; Dias et al., 2018; Humer et al., 2018). These beneficial effects in the ruminal environment may lead to increased milk yields (Poppy et al., 2012; Jiang et al., 2017; Bach et al., 2018a; Dias et al., 2018). For instance, Bach et al. (2018a) reported that cows in early lactation (i.e., the first 21 DIM) supplemented with a live-yeast product produced 38.7 kg/d of milk, whereas unsupplemented cows produced 32.7 kg/d. Jiang et al. (2017) also reported greater milk yields for cows supplemented with a yeast-derived product than for unsupplemented cows (31.7 and 29.6 kg/d, respectively). Dias et al. (2018) additionally reported that cows supplemented with a yeast-derived product tended to produce more milk than unsupplemented cows (40.2 and 38.7 kg/d, respectively).

In a recent study, Ferreira et al. (2019) evaluated the use of a live-yeast product when feeding relatively low-forage diets containing rapidly fermentable carbohydrates to high-producing cows and found no differences in production performance or nutrient utilization. Based on the low NDF digestibility coefficients, the authors suggested that conditions for fiber digestion may not have been optimal and that the potential benefit of supplementing the live-yeast product may have been hindered by the rapid rate of passage of fiber particles attributed to the low concentration of forage NDF (14.3% forage NDF), the low proportion of forage in the diet ( $\sim 45\%$  forage), or their combination.

We hypothesized that adding a live-yeast product would improve production performance and nutrient digestibility when feeding relatively high-forage diets. Therefore, the objective of this study was to evaluate the use of a live-yeast product when feeding relatively high-forage diets to high-producing cows in mid-lactation.

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of

Received July 15, 2019.

Accepted August 22, 2019.

\*Corresponding author: gonf@vt.edu

Virginia Tech (Blacksburg, VA). Eight primiparous ( $607 \pm 43$  kg of BW and  $130 \pm 16$  DIM at the beginning of the experiment) and 16 multiparous ( $706 \pm 63$  kg of BW and  $137 \pm 22$  DIM at the beginning of the experiment) Holstein cows were blocked by parity and DIM and randomly assigned to 1 of 2 diets (Table 1) for a 12-wk period according to a randomized complete block design with a 10-d covariate period. Cows were housed in a 24-stall pen within a freestall barn and fed once daily (1100 h) by means of a Calan gate system (American Calan Inc., Northwood, NH). Cows were trained for 2 wk before the beginning of the experiment to locate their assigned doors. Fresh water was available ad libitum in the freestall barn.

Diets were formulated to meet the requirements (NRC, 2001) for a 650-kg lactating dairy cow consuming 24 kg/d of DM and producing 35 kg/d of milk. The formulated diets contained 50.4% corn silage, 10.4% alfalfa hay, and 39.2% concentrate (Table 1). Two non-pelleted concentrates (control and yeast) were prepared at a commercial feed mill (Big Spring Mill Inc., Elliston, VA). The live-yeast product (BeneSacc; Global Nutritech Biotechnology, LLC, Richmond, VA), which contained  $4.5 \times 10^{-9}$  cfu/g of *Saccharomyces cerevisiae*,

was delivered through a premix that was prepared on-farm by mixing 7.5 kg of live-yeast product with 625 kg of soybean meal (Ferreira et al., 2019). The resulting premix was bagged and submitted to the feed mill, where it was included in the nonpelleted concentrate to provide 12 g of product for every 24 kg of DM.

Mixing and feeding was performed using a Calan Data Ranger mixer (American Calan Inc.). At feeding time, the TMR was prepared by sequentially adding the forage mix and the nonpelleted concentrate into the mixer. The TMR was delivered in quantities sufficient to allow 5 to 10% refusals. The amount of feed offered and refused was measured daily. Cows were milked twice daily (0100 and 1300 h), and milk weights were automatically recorded at each milking. The weekly averages of daily milk yields and DMI were used for statistical analysis.

All cows were weighed and weights recorded automatically after each milking session. Data from 6 consecutive milking sessions obtained from d 1 to 3 were used to obtain initial BW, and data from 6 consecutive milking sessions obtained from d 82 to 84 were used to obtain final BW. Body condition from all cows were scored (1 to 5 scale with 0.25 intervals) by 1 member of the research team and the herd manager on d 1 and 84.

Total-tract nutrient digestibility was estimated using 240-h undigested NDF (uNDF) as an internal marker and as described by Ferreira et al. (2019). Once uNDF concentrations were determined in both TMR and fecal samples, DM and nutrient digestibilities were determined using equations [1] and [2], respectively:

$$\text{DM digestibility (\%)} = 100 - \frac{\text{Dietary [uNDF]}}{\text{Fecal [uNDF]}} \times 100, [1]$$

$$\text{Nutrient digestibility (\%)} = 100 - \frac{\text{Dietary [uNDF]} \times \text{Fecal [Nutrient]}}{\text{Fecal [uNDF]} \times \text{Dietary [Nutrient]}} \times 100, [2]$$

where concentrations of uNDF are in milligrams per gram of DM and those of nutrients are in grams per gram of DM.

Samples of feed ingredients, TMR, and feed refusals were collected weekly. All samples were dried to constant weight at 55°C in a forced-air oven (Freas 645, Thermo Electron Corp., Marietta, OH) and ground to pass through the 1-mm screen of a Wiley mill (Thomas Scientific, Philadelphia, PA). Ash concentration was determined after burning samples in a furnace (Thermolyne 30400, Barnstead International, Dubuque, IA) for 3 h at 600°C (method 942.05; AOAC International, 2019). Crude protein concentration was calculated as

**Table 1.** Ingredient and chemical composition of diets (% DM basis) with or without a live-yeast additive

| Item                                      | Control | Yeast |
|---|---------|-------|
| Ingredient                                |         |       |
| Corn silage                               | 50.4    | 50.4  |
| Alfalfa hay                               | 10.4    | 10.4  |
| Corn grain                                | 9.2     | 9.2   |
| Soybean meal                              | 14.2    | 14.2  |
| Soybean hulls                             | 11.54   | 11.49 |
| Calcium salts of fatty acids <sup>1</sup> | 1.5     | 1.5   |
| Live-yeast product <sup>2</sup>           | —       | 0.05  |
| Bentonite                                 | 0.63    | 0.63  |
| Sodium bicarbonate                        | 1.04    | 1.04  |
| Salt                                      | 0.54    | 0.54  |
| Magnesium oxide                           | 0.17    | 0.17  |
| Trace mineral premix <sup>3</sup>         | 0.42    | 0.42  |
| Vitamin ADE <sup>4</sup>                  | 0.042   | 0.042 |
| Vitamin E <sup>5</sup>                    | 0.003   | 0.003 |
| Nutrient <sup>6</sup>                     |         |       |
| CP  | 13.6    | 13.5  |
| NDF                                       | 28.6    | 30.0  |
| Forage NDF                                | 18.0    | 18.0  |
| Starch                                    | 28.7    | 28.1  |

<sup>1</sup>EnerGII (Virtus Nutrition LLC, Corcoran, CA).

<sup>2</sup>BeneSacc (Global Nutritech Biotechnology LLC, Richmond, VA).

<sup>3</sup>Contained 22.25% calcium; 7.50% magnesium; 2.75% potassium; 3.90% sulfur; 1.50% manganese; 1.50% zinc; 9,500 mg/kg iron; 2,500 mg/kg copper; 200 mg/kg iodine; 200 mg/kg cobalt; 66 mg/kg selenium; 227,273 IU/kg vitamin A; 136,364 IU/kg vitamin D<sub>3</sub>; 636 IU/kg vitamin E.

<sup>4</sup>Contained 3,500 IU/kg vitamin A; 950 IU/kg vitamin D<sub>3</sub>; 2,000 IU/kg vitamin E.

<sup>5</sup>Contained 500 IU/g of premix.

<sup>6</sup>Values are means from 12 weekly samples.

**Table 2.** Production performance of high-producing dairy cows consuming diets with or without a live-yeast additive

| Variable                  | Diet    |       |      | P-value  |                 |       |
|---------------------------|---------|-------|------|----------|-----------------|-------|
|                           | Control | Yeast | SEM  | Diet (D) | Week (W)        | D × W |
| DMI, kg/cow-day           | 24.5    | 25.5  | 1.12 | 0.47     | 0.01            | 0.01  |
| Milk yield, kg/cow-day    | 37.6    | 39.6  | 1.47 | 0.18     | 0.01            | 0.42  |
| Milk fat, %               | 4.96    | 4.59  | 0.19 | 0.19     | 0.01            | 0.70  |
| Fat yield, kg/cow-day     | 1.85    | 1.80  | 0.08 | 0.65     | 0.01            | 0.78  |
| Milk protein, %           | 3.10    | 3.07  | 0.05 | 0.66     | 0.01            | 0.17  |
| Protein yield, kg/cow-day | 1.15    | 1.20  | 0.04 | 0.30     | 0.01            | 0.69  |
| Milk lactose, %           | 4.78    | 4.80  | 0.03 | 0.64     | 0.01            | 0.07  |
| Lactose yield, kg/cow-day | 1.79    | 1.89  | 0.07 | 0.19     | 0.01            | 0.63  |
| MUN, mg/dL                | 7.63    | 8.47  | 0.30 | 0.05     | 0.01            | 0.03  |
| BW gain, kg/cow-day       | -0.04   | -0.05 | 0.07 | 0.91     | NA <sup>1</sup> | NA    |
| BCS gain, units/cow-day   | 0.19    | 0.12  | 0.06 | 0.35     | NA              | NA    |

<sup>1</sup>Not applicable.

percent N × 6.25 after combustion analysis (method 990.03; AOAC International, 2019) using a Vario El Cube CN analyzer (Elementar Americas Inc., Mount Laurel, NJ). Concentrations of NDF and ADF were determined using the Ankom<sup>200</sup> Fiber Analyzer (Ankom Technology, Macedon, NY). Sodium sulfite and α-amylase (Ankom Technology) were used for NDF analysis (Ferreira and Mertens, 2007). Concentrations of ADF and lignin were determined sequentially. After determining ADF weights, residues were incubated for 3 h in 72% sulfuric acid within a 4-L jar that was placed in a Daisy<sup>II</sup> Incubator (Ankom Technology). Starch concentration was determined using the acetate buffer method of Hall (2009) with α-amylase from *Bacillus licheniformis* (Ankom Technology) and amyloglucosidase from *Aspergillus niger* (E-AMGDF, Megazyme International, Wicklow, Ireland).

Milk samples (a.m. and p.m. milkings) were collected weekly for the determination of milk fat, true protein, lactose, and MUN concentrations with a CombiFoss FT+ Fourier transform infrared analyzer (Foss, Hillerød, Denmark) by United DHIA (Radford, VA). Yeast colony counts were determined in both concentrates, as described by Ferreira et al. (2019).

All variables were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Dry matter intake, milk yield, and milk component concentrations and yields were analyzed using repeated measures. The statistical model included the effects of block (random; df = 11), treatment (fixed; df = 1), block × treatment interaction (random; df = 11), week (fixed; df = 11), treatment × week interaction (fixed; df = 11), and the random residual error. Based on Akaike's criterion (Littell et al., 1996), we selected autoregressive order 1 covariance structure to test the effects of week. The statistical model for analyzing BW gain, BCS gain, DM digestibility, and nutrient digest-

ibility included the effects of block (random; df = 11), treatment (fixed; df = 1), and the random residual error. Significant differences between main effects were declared at  $P < 0.05$ .

Supplementing live yeast to lactating dairy cows did not affect DMI ( $P = 0.47$ ), milk yield ( $P = 0.18$ ), milk fat concentration ( $P = 0.19$ ), milk fat yield ( $P = 0.65$ ), milk protein concentration ( $P = 0.66$ ), milk protein yield ( $P = 0.30$ ), milk lactose concentration ( $P = 0.64$ ), or milk lactose yield ( $P = 0.19$ ; Table 2). The supplementation of live yeast to lactating dairy cows did not affect BW ( $P = 0.91$ ) or BCS gains ( $P = 0.35$ ; Table 2). Dry matter digestibility was slightly greater for the control treatment than for the yeast treatment (69.3 and 67.1%, respectively;  $P < 0.03$ ), and the digestibilities of CP ( $P = 0.36$ ), NDF ( $P = 0.40$ ), and starch ( $P = 0.41$ ) were similar between diets (Table 3).

In contrast to our hypothesis, supplementing a live-yeast product did not affect production performance or nutrient utilization. To confirm that yeast was alive throughout the experiment, we collected and cultured samples of the concentrates weekly. The average count throughout the experiment was  $1.4 \times 10^4$  cfu/g of concentrate. Compared with our previous experiment (Ferreira et al., 2019), we increased the proportion of forage substantially (i.e., from 45 to 60% forage) in this experiment in an attempt to slow down the passage rate and increase NDF digestibility. Even though NDF

**Table 3.** Dry matter and nutrient digestibility of high-producing dairy cows consuming diets with or without a live-yeast additive

| Variable  | Control | Yeast | SEM  | P-value |
|-----------|---------|-------|------|---------|
| DM, %     | 69.3    | 67.1  | 0.60 | 0.03    |
| CP, %     | 62.1    | 60.9  | 0.92 | 0.36    |
| NDF, %    | 41.4    | 39.6  | 1.47 | 0.40    |
| Starch, % | 98.7    | 98.5  | 0.13 | 0.41    |

digestibility was greater than in the previous study (40.3 vs. 36.4% NDF digestibility, respectively), supplementing the live-yeast product did not increase NDF digestibility relative to the unsupplemented treatment in the current study.

Similar to our previous study (Ferreira et al., 2019), milk yields were satisfactory for cows in mid lactation (>35 kg/d). Also, milk fat concentrations surpassed our expectations (>4.50% fat), clearly eliminating any possibility of milk fat depression. Sodium bicarbonate (~260 g/d) and magnesium oxide (~44 g/d) were included in these diets as dietary buffers (Ferreira et al., 2019). If the rumen environmental conditions were affected by the inclusion of these buffers, then the action of the supplemental yeast may have been hindered. In agreement with this possibility, after challenging high-producing cows by replacing forages with barley grain, Bach et al. (2018b) reported that rumen pH was greater for cows fed 200 g/d of sodium bicarbonate or 100 g/d of a magnesium oxide-based product compared with cows not fed any buffer.

The current and previous (Ferreira et al., 2019) studies are not the only ones to show no effects of yeast supplementation on production performance (Kung et al., 1997; Ambriz-Vilchis et al., 2017). Kung et al. (1997) performed one study with cows in early lactation and another with cows in mid lactation (75 and 120 DIM, respectively) and reported no effects on milk production when cows were supplemented with a live-yeast product. Ambriz-Vilchis et al. (2017) performed 3 studies in commercial dairy farms and reported no effects on milk production when cows were supplemented with a live-yeast product. As stated by Ambriz-Vilchis et al. (2017), the response of milk production to yeast supplementation has been variable, and the proposed benefits of supplementing yeast-derived products have not been consistently demonstrated.

In conclusion, under the conditions of this experiment, supplementation of a live yeast did not affect production performance or nutrient digestibility by dairy cows in mid lactation. The reasons for the lack of effect are not clear, but an evaluation of interactions between yeast and rumen buffers supplementations is warranted.

## ACKNOWLEDGMENTS

We are grateful to Virginia Tech undergraduate students Garret Fleming, Claudia Bollinger, Sarah Thomas, and Becky Wilkins (Department of Dairy Science) for their assistance feeding the cows and col-

lecting, processing, and analyzing the samples. This project was funded by Global Nutritech Biotechnology LLC (Richmond, VA).

## REFERENCES

- Ambriz-Vilchis, V., N. S. Jessop, R. H. Fawcett, M. Webster, D. J. Shaw, N. Walker, and A. I. Macrae. 2017. Effect of yeast supplementation on performance, rumination time, and rumen pH of dairy cows in commercial farm environments. *J. Dairy Sci.* 100:5449–5461.
- AOAC International. 2019. Official Methods of Analysis. 21st ed. AOAC International, Rockville, MD.
- Bach, A., I. Guasch, G. Elcoso, F. Chaucheyras-Durand, M. Castex, F. Fàbregas, E. Garcia-Fruitos, and A. Aris. 2018a. Changes in gene expression in the rumen and colon epithelia during the dry period through lactation of dairy cows and effects of live yeast supplementation. *J. Dairy Sci.* 101:2631–2640.
- Bach, A., I. Guasch, G. Elcoso, J. Duclos, and H. Khelil-Arfa. 2018b. Modulation of rumen pH by sodium bicarbonate and a blend of different sources of magnesium oxide in lactating dairy cows submitted to a concentrate challenge. *J. Dairy Sci.* 101:9777–9788.
- Dias, A. L. G., J. A. Freitas, B. Micai, R. A. Azevedo, L. F. Greco, and J. E. P. Santos. 2018. Effects of supplementing yeast culture to diets differing in starch content on performance and feeding behavior of dairy cows. *J. Dairy Sci.* 101:186–200.
- Ferreira, G., and D. R. Mertens. 2007. Measuring detergent fibre and insoluble protein in corn silage using crucibles or filter bags. *Anim. Feed Sci. Technol.* 133:335–340.
- Ferreira, G., E. S. Richardson, C. L. Teets, and V. Akay. 2019. Production performance and nutrient digestibility of lactating dairy cows fed low-forage diets with and without the addition of a live-yeast supplement. *J. Dairy Sci.* 102:6174–6179.
- Hall, M. B. 2009. Determination of starch, including maltooligosaccharides, in animal feeds: Comparison of methods and a method recommended for AOAC collaborative study. *J. AOAC Int.* 92:42–49.
- Humer, E., I. Kröger, V. Neubauer, K. Schedle, N. Reisinger, and Q. Zebeli. 2018. Supplementing phytogetic compounds or autolyzed yeast modulates ruminal biogenic amines and plasma metabolome in dry cows experiencing subacute ruminal acidosis. *J. Dairy Sci.* 101:9559–9574.
- Jiang, Y., I. M. Ogunade, K. G. Arriola, M. Qi, D. Vyas, C. R. Staples, and A. T. Adesogan. 2017. Effects of the dose and viability of *Saccharomyces cerevisiae*. 2. Ruminal fermentation, performance of lactating dairy cows, and correlations between ruminal bacteria abundance and performance measures. *J. Dairy Sci.* 100:8102–8118.
- Kung, L., E. M. Kreck, R. S. Tung, A. O. Hession, A. C. Sheperd, M. A. Cohen, H. E. Swain, and J. A. Z. Leedle. 1997. Effects of a live yeast culture and enzymes on in vitro ruminal fermentation and milk production of dairy cows. *J. Dairy Sci.* 80:2045–2051.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS® System for Mixed Models. SAS Institute Inc., Cary, NC.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academies Press, Washington, DC.
- Poppy, G. D., A. R. Rabiee, I. J. Lean, W. K. Sanchez, K. L. Dorton, and P. S. Morley. 2012. A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of *Saccharomyces cerevisiae* on milk production of lactating dairy cows. *J. Dairy Sci.* 95:6027–6041.

## ORCID

G. Ferreira  <https://orcid.org/0000-0002-8254-8090>