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Effects of a spoilage yeast from silage on in vitro ruminal fermentation

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ABSTRACT

Feeding silages with high concentrations of yeasts from aerobic spoilage is often implicated as a cause of poor animal performance on dairies. Our objective was to determine if a commonly found spoilage yeast, isolated from silage, had the potential to alter in vitro ruminal fermentations. A single colony of *Issatchenkia orientalis*, isolated from high-moisture corn, was grown in selective medium. The yeast culture was purified and added to in vitro culture tubes containing a total mixed ration (43% concentrate, 43% corn silage, 11% alfalfa haylage, and 3% alfalfa hay on a dry matter basis), buffer, and ruminal fluid to achieve added theoretical final concentrations of 0 (CTR), 4.40 (low yeast; LY), 6.40 (medium yeast; MY), and 8.40 (high yeast; HY) $\log_{10}$ cfu of yeast/mL of in vitro fluid. Seven separate tubes were prepared for each treatment and each time point and incubated for 12 and 24 h at 39°C. At the end of the incubation period, samples were analyzed for pH, yeast number, neutral detergent fiber (NDF) digestibility, volatile fatty acids (VFA), and fatty acids (FA). We found that total viable yeast counts decreased for all treatments in in vitro incubations but were still relatively high (5.3 $\log_{10}$ cfu of yeasts/mL) for HY after 24 h of incubation. Addition of HY resulted in a lower pH and higher concentration of total VFA in culture fluid compared with other treatments. Moreover, additions of MY and HY decreased in vitro NDF digestibility compared with CTR, and the effect was greatest for HY. Overall, the biohydrogenation of dietary unsaturated FA was not altered by addition of *I. orientalis* and decreased over time with an increase in the accumulation of saturated FA, especially palmitic and stearic acids. We conclude that addition of *I. orientalis*, especially at high levels, has the potential to reduce in vitro NDF digestion and alter other aspects of ruminal fermentations.

Key words: corn silage, fiber digestibility, *Issatchenkia orientalis*, milk fat depression

INTRODUCTION

When silage is exposed to air, lactate-assimilating yeasts from *Candida*, *Pichia*, *Hansenula*, and *Endomycopsis* (Moon and Ely, 1979; Jonsson and Pahlow, 1984; McDonald et al., 1991) are often the primary initiators of aerobic spoilage (Jonsson and Pahlow, 1984). The degradation of lactic acid results in an increase in silage pH to a level that allows opportunistic bacteria (e.g., bacilli) and molds (e.g., *Aspergillus*, *Fusarium*, and *Penicillium*) to grow and further reduce silage quality (McDonald et al., 1991).

Silages that have been exposed to air can be usually characterized into 2 general categories: those that are actively spoiling (high numbers of yeasts $\geq$5.5 to 6 $\log_{10}$ cfu/g, temperatures >36–38°C, and low concentrations of fermentation acids, mainly lactic and acetic acids) and those that have spoiled and been stored for prolonged periods (e.g., the top portions of silages in bunker and pile silos). In the latter, it is not uncommon for the number of yeasts to be low or zero; Vissers et al. (2007) stated that silages with signs of aerobic deterioration are associated with high concentrations of spores of butyric acid bacteria (above 5 log/g). Both spoiled and spoiling silages pose threats to animals because they may contain undesirable microorganisms (e.g., *Listeria monocytogenes* and *Clostridium botulinum*) and mycotoxins and they may be of generally lower nutritive value due to the destruction of nutrients during spoiling (McDonald et al., 1991). The numbers of yeasts in silages vary greatly and can be affected by many different management factors, including packing density, silage DM, fermentation end-products, and the type of plastic used to seal silos (Borreani and Tabacco, 2014).

Anecdotal reports from the field suggest that problems with low intake or low milk fat tests are often reported when cows have consumed silages with high numbers of yeasts (G. D. Mechor and L. Kung Jr., personal observations). Surprisingly, only a small number of studies have evaluated the effects of feeding spoiled or spoiling silages to ruminants. Whitlock et al. (2000) reported that feeding spoiled corn silage from the surface of a bunker silo depressed nutrient digestibility and

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DM intake of steers. The spoiled silage probably had this effect because its overall nutritive value was low, with levels of NDF and ADF being 14.8 and 32.5% higher than the levels found in the nonspoiled portion of the silages, respectively. Hoffman and Ocker (1997) fed a TMR containing fresh or aerobically deteriorating high-moisture corn. Cows that were fed the TMR containing the aerobically spoiling high-moisture corn produced 3.2 kg less milk/cow per day compared with cows fed fresh silage, although DM intake was unaffected. Gerlach et al. (2013) fed spoiling silage to goats and reported negative correlations between ethyl lactate and ethanol with DMI but the strongest negative relationship with intake was from silage temperature (difference from ambient). Because spoiled silages often have high numbers of yeasts, the role that yeasts play in negatively affecting animal performance has been questioned. Ward (2011) reported that in 2010–2011, approximately 15, 15, and 9% of corn silage samples tested for yeasts contained approximately 7, 8, and 9 $\log_{10}$ cfu of yeasts/g of wet silage, respectively. Similarly, studies conducted by Dolci et al. (2011) and Borreani et al. (2013) reported total numbers of yeasts in silages as high as 8 $\log_{10}$ cfu/g. Thus, the objective of this experiment was to determine whether high numbers of a spoilage yeast isolated from silage could adversely affect ruminal in vitro fermentations.

MATERIALS AND METHODS

Experimental Design

Issatchenkia orientalis (formerly known as *Candida krusei*), was identified by DNA sequencing and physiological substrate profiling and is a predominant species of yeasts in high moisture corn and corn silage (Santos et al., 2011). The yeast was grown in sterile Erlenmeyer flasks containing autoclaved Sabouraud dextrose broth (Becton Dickinson, Sparks, MD) and incubated at 32°C in a shaker box (Thermo Scientific, Waltham, MA) at 120 rpm. When the optical density of the culture at 600 nm was constant (stationary phase), the cells were harvested by centrifugation at 1,077 × *g* for 10 min at 5°C. After centrifugation, the cells were washed using sterile Ringers solution and concentrated by centrifuging an additional 2 times. A pure culture of *I. orientalis* was obtained by resuspending the pellet in sterile Ringer's solution. The concentration of *I. orientalis* in the pure culture was determined by plating 1-mL aliquots of 10-fold serial dilutions on yeast and mold count Petrifilm (3M Products, St. Paul, MN).

In vitro ruminal incubations were performed according to the procedure of Goering and Van Soest (1970) with modifications. The modifications included incubations in 50-mL tubes with 0.3 g of a TMR mixed with 24 mL of mineral and buffer plus 6 mL of strained ruminal fluid. The TMR consisted of 43% concentrate, 43% corn silage, 11% alfalfa haylage, and 3% alfalfa hay on a DM basis and was balanced according to the NRC (2001) to meet the requirements of lactating dairy cows (average 72 DIM, 48 kg of milk/d, 28 kg of DMI/d, and 716 kg of BW). The ingredient composition and the nutritive value of the whole TMR are shown in Table 1. The TMR sample had been dried for 48 h at 60°C in a forced-air oven and ground through a 1-mm screen using a Cyclone Sample Mill (Udy Corp., Fort Collins, CO). Ruminal fluid was collected after morning feedings from 2 cannulated lactating Holstein cows fed the same TMR once daily and ad libitum. Ruminal fluid was pooled, strained through a double layer of cheesecloth while being gassed continuously with CO_2, and used immediately to inoculate tubes.

Yeasts were added to culture tubes to achieve a theoretical concentration of 0 (**CTR**), 4.4 (low yeast; **LY**), 6.4 (medium yeast; **MY**), and 8.4 (high yeast; **HY**) $\log_{10}$ cfu of added *I. orientalis*/mL of total in vitro fluid. The LY dose was established based on a calculation of a cow with a rumen liquid volume of 126 L consuming 30 kg of as-fed corn silage containing 5 $\log_{10}$ cfu of yeasts/g. The 4.4 $\log_{10}$ cfu/mL dose was obtained by multiplying the intake of corn silage (30 kg of fresh silage) by the concentration of yeasts (5 $\log_{10}$ cfu/g of fresh sample) and dividing this result by the rumen volume (126 L). The MY and HY doses were calculated similarly but with the assumption that the former contained 7 $\log_{10}$

Table 1. Ingredient composition and nutritive value of the whole TMR used in the in vitro fermentation

Composition	Value
Ingredient (% of DM)	
Corn silage	43.0
Alfalfa haylage	11.2
Alfalfa hay	2.7
Concentrate[1]	43.1
Nutritive value	
DM (%)	50.8
CP (% of DM)	16.0
Soluble protein (% of CP)	29.8
NE_L (Mcal/kg of DM)	1.63
ADF (% of DM)	21.5
NDF (% of DM)	33.8
Ash (% of DM)	6.5
Starch (% of DM)	28.0

[1]Concentrate composition was (DM basis) 26.2% corn hominy, 13.9% canola meal, 12.4% soybean meal, 11.8% ground corn grain, 11.3% turbo meal (extruded expelled soybean meal product), 7.0% dried citrus pulp, 3.3% ground soybean hulls, 3.2% corn distillers grains, 2.1% calcium carbonate, 2.0% blood meal, 1.6% sodium bicarbonate, 1.3% molasses cane, 0.9% salt, 0.9% saturated fat source of palmitic acid, 0.7% mineral and vitamin premix, 0.5% urea, 0.4% Rumensin (Elanco Animal Health, Greenfield, IN), 0.2% magnesium oxide, 0.2% encapsulated source of methionine and 0.1% calcium sulfate dehydrate.