THE EFFECT OF SODIUM BICARBONATE OR LIVE YEAST CULTURE (*Saccharomyces cerevisiae*) ON THE METABOLISM AND PRODUCTION OF LACTATING DAIRY COWS

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Masters of Science

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ABSTRACT

Recent studies have shown that feeding live yeasts to dairy cows has the ability to moderate ruminal pH. The objective of this study was to compare the effects of feeding live yeasts or sodium bicarbonate, a traditional buffer, on metabolic indices, digestibility of the total mixed ration (TMR) and milk production and composition in lactating dairy cows. Twenty-eight lactating Holstein cows (77 ± 31 days in milk, 39.4 ± 7.2 kg milk/d) were assigned to blocks by milk production, lactation number, and days in milk and randomly allocated to one of three treatments in a replicated 3 × 3 Latin square design. Treatments were supplementation of 0.021 g of *Saccharomyces cerevisiae*, strain CNCM I-1077 (containing 20×10^9 cfu/g of product) per kg of TMR dry matter (resulting in 4.2×10^8 cfu/kg TMR dry matter), sodium bicarbonate at 0.93% of TMR (dry matter basis), or no additive. Periods were 28 d in length with the last 7 d of data used for statistical analysis. Cows fed sodium bicarbonate but not yeasts consumed more dry matter than those fed the unsupplemented diet. There was no difference in milk production, 3.5% fat corrected milk, energy corrected milk, or milk components among treatments but the concentration of milk urea nitrogen was greatest for cows fed sodium bicarbonate. Feed efficiency was lower for cows fed sodium bicarbonate or yeasts when compared to those fed the unsupplemented diet. The addition of yeasts or sodium bicarbonate to the diets of lactating dairy cows did not affect the pH of ruminal fluid, feces or urine, or concentrations of serum amyloid A or haptoglobin in blood. Cows supplemented with sodium bicarbonate had lower

organic matter and dry matter digestibility of the TMR compared to other treatments. The digestibility of neutral detergent fiber was lower for cows fed sodium bicarbonate than those fed the unsupplemented TMR but similar to cows fed live yeasts. The digestion of crude protein was lower in supplemented than unsupplemented diets. The results of this experiment question the benefit of sodium bicarbonate or live yeasts in a balanced, highly digestible diet fed to lactating dairy cows.

Keywords: digestion, sodium bicarbonate, yeast

Chapter 1

INTRODUCTION

Feeding ruminants large amounts of rapidly fermentable carbohydrates results in an increase in the amounts of organic acids produced in the rumen, thus lowering ruminal pH (Nocek, 1997; Chaucheyras-Durand et al., 2008). In animals that are not adapted to high levels of fermentable carbohydrates, the concentration of lactic acid in the rumen rises to an unacceptable level because populations of lactate-utilizers, such as *Selenomonas ruminantium* and *Megasphaera elsdenii*, are low and cannot keep pace with its rapid production. As a consequence, ruminal pH can fall below an optimal level for the growth of fibrolytic bacteria (< 6.0 to 6.3) because these organisms (e.g., *Fibrobacter succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens)* are intolerant of pH below 6 (Chaucheyras-Durand et al., 2008). As the rumen pH drops below 5.0 to 5.3, *Lactobacillus* spp. become the predominant genus of bacteria in the rumen and their growth is associated with increased production of lactic acid (Nocek, 1997; Chaucheyras-Durand et al., 2008). Extremely low rumen pH can quickly lead to the lysis of gram negative bacteria, releasing lipopolysaccharides that create a systemic histamine response and cause subacute acidosis and laminitis (Gozho et al., 2007). Subacute acidosis is characterized by rumen pH of 5.5 or less and reduced feed intake. It may develop into acute acidosis,

which is characterized by severe illness, liver absesses, and decreased absorption of nutrients.

Low ruminal pH decreases the solubility of dietary protein entering the rumen. This decrease in pH can lead to the rapid destruction of peptides or amino acids required for optimal microbial growth, and may lower the amount of microbial protein that reaches the small intestine for absorption (Trenkle, 1979). A low rumen pH has also been implicated as a potential cause of milk fat depression through changes in microbial biohydrogenation of unsaturated fatty acids (Bauman and Griinari, 2000).

Acidotic conditions that occur in the rumen can be moderated through various buffering actions. First, feedstuffs have different buffering capacities based on their chemical composition. For example, alfalfa has a high buffering capacity relative to corn silage, because it contains high concentrations of calcium, potassium, organic acids, and protein (Erdman, 1988). Second, when saliva is swallowed, it buffers the rumen because it naturally contains sodium bicarbonate. Saliva production, and therefore the amount of sodium bicarbonate that reaches the rumen, is affected by the length of feed particles and its relationship to chewing time (Erdman, 1988; Staples and Lough, 1989). Additionally, mineral buffers are often added to the diets of ruminants to aid in ruminal buffering. Common examples of these include sodium sesquicarbonate (Cassida, 1988), potassium bicarbonate (Davis, 1964), and sodium bicarbonate (Erdman, 1988). Recent research suggests that feeding live yeasts to

ruminants also moderates ruminal pH (Koul et al., 1998b; Bach, 2006; Zelvyte et al., 2006).

Chapter 2

LITERATURE REVIEW

Buffers and alkalizing agents are commonly fed to dairy cattle when cattle are fed diets with high concentrations of rapidly fermentable carbohydrates because feeding such diets often results in ruminal acidosis. This can moderate drops in rumen pH and may lead to a more favorable environment for microbial growth and metabolism. As a result, ruminal digestion and animal productivity can be optimized. The stabilization of ruminal pH has also been shown to cause a change in microbial biohydrogenation, creating an increased conversion of C 18:1 into C 18:0, and thus a decreased absorption of C 18:1 fatty acids, which have been found to be a cause of milk fat depression (Bauman and Griinari, 2000).

A buffer is any material that, when present in an aqueous solution, effectively resists a change in the pH of that system through the donation or the acceptance of one or more protons. In order to be a buffer, a substance must be water-soluble, must be a weak acid, base or salt, and the pK_a must be near the physiological pH of the system that is to be buffered (Erdman, 1988). Every buffer has a pK_a, indicating the pH at which the buffering capacity is most effective. At this pH, there are an equal number of proton acceptors and proton donors so any excess acid or base can be absorbed, thereby decreasing the change in pH of the solution. In contrast, alkalizers, such as sodium carbonate, can only absorb protons, and therefore only function to increase the

pH. Sodium sesquicarbonate contains a chemical mixture of both sodium bicarbonate and sodium carbonate, leading it to function as both a buffer and an alkalizer with an average pH of 9.9 in water (Hutjens, 1998).

Sodium Bicarbonate

Sodium bicarbonate ($NaHCO_3$), is the most widely used buffer additive in the dairy industry today, and studies describing its effects date back to the 1960's when it was shown to increase the production of milk fat (Emery and Brown, 1961; Davis et al., 1964; Emery et al., 1964). Thus, this review will focus specifically on this buffer. Traditionally, sodium bicarbonate has been fed to cows because many diets have the ability to predispose the cow to rumen acidosis (e.g., from a lack of effective fiber or excessive fermentable carbohydrates). Currently, rations for lactating dairy cows are typically supplemented with 0.75% sodium bicarbonate (DM basis) (Staples and Lough, 1989; Hutjens, 1998). The maintenance of a normal milk fat content (≥ 3.6 to 3.8%) commonly observed when cows are fed sodium bicarbonate (Hu and Murphy, 2005) could be a result of increased ruminal pH (Donker and Marx, 1980; Erdman, 1988; Okeke, 1983) or moderation in the depression of rumen pH that occurs after feeding (Erdman,1988).

It is thought that sodium bicarbonate's effects are due to its chemical buffering potential. Sodium bicarbonate has two pK_as: sodium bicarbonate can raise the pH by absorbing a proton, and becoming carbonic acid, which can then be converted to

carbon dioxide (CO_2) and water. Alternatively, it can release a proton, creating sodium hydroxide and carbonate, and effectively lowering the pH of the system. The pK_a of the reaction that most likely occurs in the rumen, where bicarbonate is turned into carbonic acid by gaining a proton, is 6.35 (Erdman, 1988). This means that when sodium bicarbonate is added to a solution, it tends to maintain the pH around this number by shifting the equilibrium via carbon dioxide production (Figure 1).

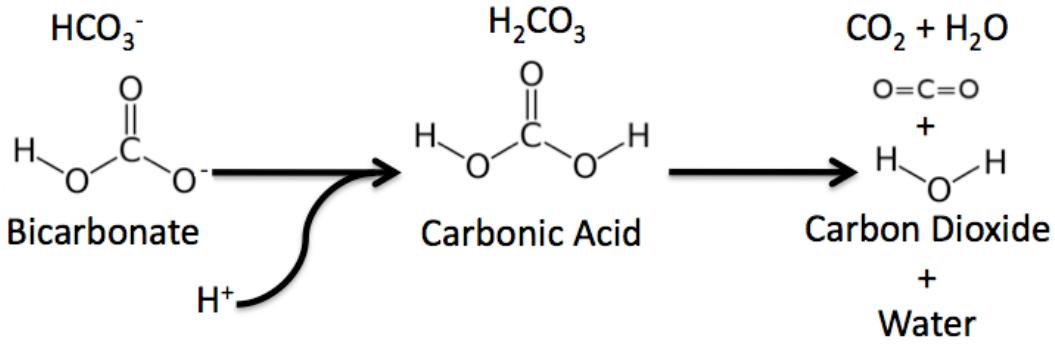

Figure 1: Chemical buffering action of sodium bicarbonate in the rumen

This hypothesis has been criticized by Russell and Chow (1993) because rumen fluid is already saturated with carbon dioxide, thus the reaction may be unable to shift, and sodium bicarbonate cannot exert its effects as a chemical buffer. These researchers hypothesized that the positive effects of feeding sodium bicarbonate to ruminants occurs because the sodium content of the compound stimulates the amount of water the animal drinks, effectively increasing the liquid dilution rate in the rumen. Russell and Chow (1993) also suggested that an increased liquid dilution rate in the rumen would mean that more starch passes through the rumen unfermented and the pH does

not drop as much. This would explain the apparent buffering effect of sodium bicarbonate. In support of this theory, Kilmer et al. (1981) reported that cows fed diets supplemented with sodium bicarbonate had a greater total output of urine, indicative of a greater water intake. However, the rate of liquid dilution in the rumen of dairy cows fed buffer increased only a 0.2% per 100 g of bicarbonate fed to the cow (Erdman, 1988), and this may not be a large enough difference to adequately explain the pH stabilization effects observed with sodium bicarbonate supplementation. Furthermore, through empirical and theoretical evidence, researchers (Kohn and Dunlap, 1998) found the bicarbonate ion to be the predominant buffering ion in the rumen.

If supplementation with sodium bicarbonate moderates the depression of ruminal pH, then there is the potential for a normal fermentation to occur in the rumen and optimal production by the cow. However, the effects of feeding sodium bicarbonate to dairy cows on intake and animal production have been somewhat variable. Some studies have found that the inclusion of dietary sodium bicarbonate increased DMI (Canale and Stokes, 1988; Donker and Marx, 1980; Rogers et al., 1985), while others did not (Staples and Lough, 1989). Additionally, some researchers report greater milk production in cows supplemented with sodium bicarbonate (Rogers et al., 1985; Staples and Lough, 1989; Thomas et al., 1984). In a recent meta-analysis by Hu and Murphy (2005) involving 30 experiments with 369 cows, animals supplemented with sodium bicarbonate had a higher DMI, higher

percentage and yield of milk fat, as well as higher ruminal pH compared to cows that were not fed sodium bicarbonate. The authors attributed the production increases to the increase in ruminal pH caused by the neutralizing action of sodium bicarbonate.

Sodium bicarbonate has been proven to be most effective in conditions where the pH of the rumen has been less than optimal. This includes but is not limited to diets with high levels of concentrate, where the buffering capacity of the feed is much lower than in high forage diets (Erdman et al., 1982; Erdman, 1988; Hu and Murphy, 2005). Moreover, both Erdman (1988) and Hu and Murphy (2005) reported that sodium bicarbonate was most effective in diets containing corn silage, rather than in diets containing alfalfa, probably due to a lower dietary buffering capacity in a corn silage based diet.

Yeast Additives

There are currently two broad categories of yeast products used in dairy rations. A "yeast culture" is a dry product composed of yeast and the media on which it was grown. There is no guarantee of live (culturable) yeasts cells in a yeast culture. In contrast, "active dried yeast" may or may not contain the culture medium in which the yeasts were grown. Most live yeast products contain extremely high numbers of organisms (~10^9 cfu/g). The yeast biomass is dried to preserve viability and activity. The most common yeast fed to dairy cows today is *Saccharomyces cerevisiae*. The use

of *S. cerevisiae* is classified in the United States with the FDA as "generally recognized as safe".

Recent evidence indicates that some yeast products can be an effective and economic alternative to using traditional mineral buffers for moderating ruminal pH in dairy cows. Feeding various yeast products has increased milk production, DMI, and milk fat content in some studies (Erasmus, 1992; Putnam et al., 1997; Sniffen et al., 2004).

Some controversy exists relative to the efficacy of yeast cultures compared to live yeasts. Koul et al. (1998b) compared the stimulatory effects of sodium bicarbonate, live yeast, or a filtered slurry of yeast cell parts containing no live yeast. Increases in ruminal pH, total bacterial counts, and total cellulolytic bacterial counts were observed in cows supplemented with sodium bicarbonate as well as in cows supplemented with active, live yeast. However, this increase was absent in the cows supplemented with the filtered slurry of yeast cell parts, indicating that the maximum stimulatory effects of yeast products occurred when the yeast cells were alive and active.

Feeding live *S. cerevisiae* has been accepted as being beneficial to ruminants from a variety of different proposed mechanisms. For example, numerous authors have hypothesized that yeasts may supply nutrients for microorganisms in the rumen from within their cell bodies. B-vitamins (Nisbet and Martin, 1991; Martin and Nisbet, 1992; Callaway and Martin, 1997), amino acids (Giger-Reverdin et al., 1996; Chaucheyras et al., 1996; Nisbet and Martin, 1991) and other compounds such as malic acid (Dawson and Girard, 1997) are found in *S. cerevisiae*. These nutrients may stimulate rumen microbes. However, criticism of these theories suggest that it is impractical to actually feed the levels of compounds found stimulatory in these *in vitro* experiments. Specifically in an experiment comparing the effects of live *S. cerevisiae* with malic acid in the rumen, the live yeast stimulated bacterial numbers while the malic acid did not, indicating that malic acid was not the sole contributor to the responses observed in supplemented animals (Newbold et al., 1996).

Another mechanism for *Saccharomyces cerevisiae* in the rumen suggests that it may have oxygen scavenging abilities in the rumen, creating a more favorable environment for anaerobic bacteria, thereby stimulating their growth (Newbold et al., 1993). *Saccharomyces cerevisiae* are facultative anaerobes, but because it is more energetically favorable for them to utilize oxygen as the terminal electron acceptor, they utilize oxygen even when it is in very low concentrations, such as in the rumen. Newbold et al. (1993) reported that respiratory-deficient mutants of various *S.*

cerevisiae strains were unable to stimulate bacterial numbers *in vitro*, while wild-type parents who were able to consume oxygen were stimulatory. Likewise, the redox potential of rumen fluid was significantly lowered in the presence of *S. cerevisiae* in the rumen of sheep (Chaucheyras-Durand and Fonty, 2002) and cows (Marden, 2008).

Live *S. cerevisiae* fed to ruminants are unable to colonize and reproduce in a ruminal environment because they are washed out of the rumen via the normal rate of dilution (Kung et al., 1997; Chaucheyras-Durand, 1998). Live yeast products must be metabolically active in order to be maximally efficacious in diets for ruminants (Koul et al., 1998b), and the stimulatory effects of the yeast are greatly diminished if they are inactivated by heat. This suggests that either the metabolism of the yeast or a heat-labile component released by the yeasts is responsible for its effects on the stimulation of microorganisms, fermentation and digestion (Nocek, 1997). It has been proposed that the yeast cell contains two small molecular weight components, which have been isolated and implicated as significant factors in the stimulatory actions of yeast (Girard and Dawson, 1995). One of these small components is heat stable, and may be excreted by the yeast as a by-product of its metabolism. Contrastingly, the other component is heat-labile, found within the cell, and is only released upon cell lysis (Dawson, 1990; Chaucheyras-Durand, 2005; Beauchemin, 2006). Supporting evidence has shown that live yeasts were able to stimulate bacterial growth *in vitro*, but this stimulatory activity was minimized when the yeast cells were heat-inactivated (Chaucheyras-Durand, 2005; Girard and Dawson, 1995).

Saccharomyces cerevisiae are able to metabolize glucose anaerobically into ethanol and carbon dioxide. In contrast, at a low pH, *Streptococcus bovis* produces lactate, which can lower the pH even more. It has been reported that active yeast cells can out compete *S. bovi*s for simple sugars, such as glucose (Chaucheyras-Durand et al., 1996). Furthermore, in the presence of yeast, lactate production by *S. bovis* decreased, also leading to a higher rumen pH (Chaucheyras et al., 1995b; 1996). This competition could explain the decreased lactate production by *S. bovis* reported in the presence of *S. cerevisiae*. The decrease in the amount of lactate produced, combined with the aforementioned increase in lactate utilization could explain the increased pH that is commonly seen in animals supplemented with *S. cerevisiae*.

One of the most repeatable results observed when feeding *S. cerevisiae* to ruminants has been an increase in the total concentration of bacteria in the rumen , specifically fibrolytic bacteria (Beauchemin et al., 2006), which could be a result of a moderation of ruminal pH. For example, strains of *S. cerevisiae* have been shown to stimulate growth and activity of cellulolytics by stimulating the growth and activity of *Fibrobacter succinogenes* (Girard and Dawson, 1995; Callaway and Martin, 1997), as well as reducing the lag time for the growth of *Ruminococcus albus*, *R. flavefaciens*, and *Butyrivibrio fibrisolvens* (Dawson, 1990; Girard and Dawson, 1994, 1995; Mosoni, 2007). The addition of *S. cerevisiae* has stimulated the growth and activity of *Neocallimastix frontalis* MCH3, a cellulose-degrading fungus, by supplying thiamine

from the yeast cell body (Chaucheyras et al., 1995a; Chaucheyras-Durand and Fonty, 2001). As a result of improvements in fiber digestion in the rumen, which have been linked to an increased ruminal pH, concentrations of VFA have also been increased in animals supplemented with yeasts (Dolezal, 2005)

The remainder of this literature review will focus specifically on *S. cerevisiae* CNCM I-1077 (**SC1077**), which was selected from over 1000 different strains of yeast because it showed a maximal stabilization of rumen pH, stimulation of microbial populations, and enhancement of fiber digestion (Table 1). Feeding this strain of *S. cerevisiae* to lactating cows increased milk production in 7 out of 9 experiments. It also increased rumen pH in 2 out of 3 experiments, and digestibility of various feed components was increased in all 3 of the experiments where this parameter was measured.

Table 1: A summary of studies evaluating the effects of feeding *Saccharomyces cerevisiae* CNCM I-1077 on production and metabolism of lactating dairy cows.

Citation	Number of animals	Milk Production	Milk Fat %	DMI	pH	Anaerobic Bacteria	Cellulolytics	VFA, total mM Concentration	Digestibility
Ali Haimoud-Lekhal and Chevaux, 2002	62	+	0						
Bach et al., 2007	3	0		0	+				
Bagheri, et al., 2009	8	+	+	0	0			0	+ DM and CP digestibility
Dell'Orto et al., 2003	54	+	0						
Rihma et al., 2007	69	+	0						
Santos et al., 2006	36	0	+	0					
Schwartz and Ettle, 2002	36	+		+					+ OM and fiber digestibility
Sniffen et al., 2004	193	+	+	+					
Zelvyte et al., 2006	22	+		+	+	+	+	+	+ OM digestibility

+ = Indicates an increase in the parameter measured when cows were supplemented with live yeasts.
- = Indicates a decrease in the parameter measured when cows were supplemented with live yeasts.
0 = Indicates there was no difference in the parameter measured with yeast product supplementation.
A blank indicates the parameter was not measured.

Using in-dwelling pH probes, Bach et al. (2007) reported both an increased and stabilized pH in the rumen of lactating cows supplemented with SC1077 compared to untreated cows. Similarly, Thrune et al. (2007) measured ruminal pH of eight ruminally fistulated cows every 22 min with an in-dwelling probe. Similar to the findings of Bach et al. (2007), the mean ruminal pH was higher, and the time spent under the subacute threshold (i.e., pH < 5.6) was lower in supplemented cows. Similar results have also been reported by Chaucheyras-Durand et al. (2008) and

Marden et al. (2008). It is possible that the stimulatory effects of SC1077 on the bacterial populations in the rumen result from the moderation of ruminal pH (Zelvyte et al., 2006; Marden et al., 2008).

There are numerous hypotheses attempting to determine the way that SC1077 regulates ruminal pH. Yeast cells appear to affect the metabolic activities of some strains of bacteria and protozoa. For example, SC1077 has altered the activity of Entodiniomorphid protozoa in the rumen (Chaucheyras-Durand et al., 2008). Entodiniomorphid protozoa are able to successfully compete with amylolytic bacteria for starch (Mendoza et al., 1993). These protozoa can engulf particles of starch and ferment the starch at a slower rate than amylolytic bacteria, reducing the rapidity of fermentation in the rumen (Leng and Nolan, 1984). Furthermore, Entodiniomorphid protozoa produce acids that are not as strong as lactic acid which is normally produced by amylolytic bacteria (Williams and Coleman, 1997). Brossard (2006) reported a linear increase in the number of ruminal Entodiniomorph protozoa with increasing levels of SC1077 supplemented in the diets of sheep. However, there was no difference in ruminal pH between animals supplemented with SC1077 and those that were not, while there was a tendency for an increase in feed intake with supplementation. This suggests that the stimulatory action of *S. cerevisiae* on entodiniomorphid protozoa might not be the only mode of action of SC1077.

Changing microbial populations of that are able to utilize lactate in the rumen may be another way that SC1077 could regulate ruminal pH. The addition of SC1077 to *in vitro* rumen environments has stimulated the activity and growth of the lactate utilizer *M. elsdenii* (Chaucheyras et al., 1995b; 1996). Furthermore, when *S. ruminantium* was incubated with [C^{14}] lactate in the presence of a *S. cerevisiae* slurry, it was noted that the uptake of lactate increased almost four-fold relative to cultures where *S. cerevisiae* was absent (Nisbet and Martin, 1991). Therefore, it is possible that the increase in pH observed in animals supplemented with SC1077 is through a change in the activity and (or) numbers of lactate utilizers.

Supplementation with SC1077 has also been shown to experimentally reduce levels of ammonia in the rumen of cows (Chaucheyras-Durand and Fonty, 2001). A decreased level of ammonia could reflect an increased number of viable rumen bacteria, potentially a result of increased ruminal pH making an environment more favorable to bacterial growth (Dawson, 1990). Increased levels of bacteria would require increased amounts of amino acids and ammonia for microbial protein production and would decrease the amount of ammonia in the rumen. Moya et al. (2007) conducted an *in vitro* study examining the effect of SC1077 on nitrogen metabolism and rumen pH using different types of starch in continuous culture fermenters. The authors concluded that SC1077 improved nitrogen metabolism and limited the decrease in pH following a rapidly degradable starch challenge. Additionally, SC1077 has the ability to compete with proteolytic bacteria for

substrates for growth, limiting proteolysis, and decreasing peptidase activities (Chaucheyras-Durand et al., 2004).

Summary

Although sodium bicarbonate has traditionally been the feed additive used to moderate rumen pH, feeding live yeasts or yeast culture may be an alternative practice to accomplishing the same goal via different mechanisms. Whereas sodium bicarbonate acts a chemical buffer, yeast additives directly alter microbial metabolism, which in turn has been shown to moderate ruminal pH.

Chapter 3

OBJECTIVE

The objective of this study was to compare the effects of a traditional buffer program, utilizing sodium bicarbonate, to active dried *S. cerevisiae* CNCM I-1077 on production, ruminal fermentation, selected blood parameters, and digestion in lactating dairy cows.

Chapter 4

MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee, College of Agriculture and Natural Resources, University of Delaware and followed approved guidelines (Anon., 1998; FASS, 1999). It was conducted between March and May, 2008. Twenty-eight Holstein cows averaging 703 ± 100 kg were housed at the University of Delaware Dairy Farm in a barn with free-stalls, bedded with sand and fed individually via a system with Calan gates (American Calan, Northwood, NH).

Cows were fed a diet to meet requirements for cows with an average production of 39.4 kg of milk/d with a fat test of 3.6%, consuming 27.6 kg/d of DM and 704 kg of BW (NRC, 2001). Feed ingredients were combined using a mixer wagon (Data Ranger, American Calan, Inc. Northwood, New Hampshire, USA) to form a TMR containing 48% concentrate (Table 2), 39% corn silage, 8% alfalfa haylage, and 5% alfalfa hay on a DM basis. Cows were fed once daily, *ad libitum* at approximately 0800 h, and feed refusal was measured daily for each cow at approximately 0700 h the next morning. Cows had access to fresh water at all times. Cows were allowed to adapt to using the Calan gates for 3 wk and then were assigned to blocks based on pretreatment milk production, lactation number, and days in milk and randomly assigned to one of three treatments.

The treatments were 1) a basal TMR with no additives (**CTRL**), 2) the basal TMR containing 0.93% sodium bicarbonate on a DM basis (**BICB**), 3) the basal TMR supplemented with 0.021 grams of *S. cerevisiae* CNCM I-1077 product (Lallemand Animal Nutrition, Milwaukee, Wisconsin, USA) (20×10^9 cfu/g) per kg of TMR DM (**YEA)**. The additives were mixed into the concentrate prior to mixing with the forages to form a TMR. The numbers of viable yeast in the *S. cerevisiae* CNCM I-1077 product was determined by pour plating 10-fold serial dilutions (quarter strength Ringer's solution, Oxoid BR0052G, Oxoid Limited, Cambridge, UK) in malt extract agar (Oxoid CM0059, Oxoid Limited, Cambridge, UK). Plates were incubated aerobically for 48 to 72 h at 30°C.

The amount of TMR fed and refused was measured daily for each cow and used with the DM% of the TMR to calculate daily DM intake (DMI). Cows were weighed on two consecutive days at the beginning and completion of each period.

Cows were milked twice daily at approximately 0600 and 1600 h and milk production was recorded automatically via computer. Milk samples were taken twice on two consecutive milkings to complete a total of four milkings during week 4 of each period. Milk samples were analyzed by Dairy One Cooperative Inc. (University Park, Pennsylvania, USA) for fat, protein, lactose, and MUN using a Milkoscan System 4000 (Foss North American, Eden Prairie, Minnesota, USA). A Eurochem CL-10 reference analyzer was used for calibration of samples.

Milk yield was corrected to a 3.5% fat content where 3.5% fat corrected milk (FCM) = kg milk × (0.4255 + (16.425 × fat%/100)). Energy-corrected milk (ECM) was calculated by standardizing milk production to 3.5% milk fat and 3.2% milk protein with the formula ECM = (0.3246 × kg of milk produced daily) + (12.86 × kg of fat produced daily) + (7.04 × kg of milk protein produced daily) (Bernard, 1997). Feed efficiency was calculated by dividing the amount of FCM by the DMI. Daily intakes of starch, NDF, and CP were calculated, and DMI as a percentage of body weight was calculated by DMI/kg BW.

Silages and TMR were sampled three times each week and pooled for weekly analysis. Single samples of concentrates and hay were collected once a week for analysis. Dry matter of the feed components was determined in a forced-draft oven set at 60ºC for 48 h. The distribution of particles from the TMR was determined as described by Kononoff et al. (2003). The DM content of feeds was used for weekly adjustment of the TMR. Corn silage samples were analyzed for ADF and NDF (Goering and Van Soest, 1970) using an Ankom Fiber Analyzer and acid detergent lignin (Goering and Van Soest, 1970) was determined using the Ankom Daisy incubation system (Ankom, Inc., Macedon, NY). The digestibility of NDF was also determined on corn silage samples using the *in vitro* procedure described by Goering and Van Soest (1970) with some modifications. Those modifications included a) incubation of samples in 100 ml polycarbonate tubes each sealed with a rubber stopper

fitted with a glass tube with a rubber policeman (14-105A, Fisher Scientific, Pittsburg, PA) with a 5 mm slit to allow for venting of gas pressure, b) gentle manual swirling of the tubes at 3, 6, 9, 20 and 26 h and c) incubation for 30 h.

All other feeds were analyzed by wet chemistry methods by Cumberland Valley Analytical (Maugansville, MD). Crude protein was calculated as N × 6.25 after analyses of N (AOAC, 200) using a Leco FP-528 Nitrogen Combustion Analyzer. (Leco, St. Joseph, MI). Soluble protein was determined using the methods described by Krishnamoorthy et al. (1982). Starch was determined using the methods described by Holm et al. (1986). The ADF (AOAC, 2000) and NDF (Goering and Van Soest 1970) contents of feeds (with the exception of corn silages) were determined with the modification that Whatman 934-AH glass micro-fiber filters (Whatman, Florham Park, New Jersey, USA) with 1.5-μm particle retention were used in place of fritted glass crucibles.

Ruminal fluid (about 100 ml) was collected via vacuum pump, and feces via grab samples (~300 g) from each cow on day 28 of each period. Samples were kept on ice until pH could be measured and recorded. Fecal samples were dried in a forced-air oven at 60°C for 48 h for DM% determination. Rumen fluid was filtered through 4 layers of cheesecloth and stored at -20°C until analyzed for VFA with HPLC (Shimadzu LC-20A automated liquid chromatographic system, Shimadzu, Japan). The chromatograph was equipped with a CBM system controller, a LC-20AT

pump, a SIL-20AC autosampler, a refractive index detector (RID-10A), and a Bio-Rad Aminex Ion Exclusion HPX-87H (300×7.8mm) column was used with mobile phase (0.015N H_2SO_4 + 0.25mM EDTA) at a flow rate of 0.6 mL/min at 35°C.

On day 28 of each period, blood (~ 30 mL) was taken from the jugular vein into Vacutainer tubes (Becton Dickinson, Franklin Lakes, New Jersey) for each cow approximately 3-4 h after feeding. Serum tubes were allowed to clot at room temperature (~ 22°C), and heparinized tubes were stored on ice. Tubes were centrifuged at 1500 × *g* for 15 min at room temperature or 4°C for serum or plasma, respectively. After centrifugation, serum or plasma was transferred to a new tube and stored at -20°C until later analysis. Concentrations of haptoglobin and amyloid A were determined in the plasma and serum, respectively, using commercial ELISA kits (Tridelta Development Ltd., Greystones, Whicklow, UK). Plates were read using a Spectra MAX 190 plate reader (Molecular Devices, Sunnyvale, CA). Urine was collected via manual stimulation on day 27 of each period, and pH was immediately determined.

At the conclusion of period 3, the cows continued on their experimental diets for a fecal collection trial. Average daily intake was determined using data from the last week of period 3 and cows were fed this amount daily for 3 d to minimize orts. Fecal grab samples (~300 g) were collected daily via rectal palpation 6 h prior to feeding and 6 h after, until a total of 6 samples were collected for each cow. During

fecal collections, TMR (from each group) and any orts remaining (for each cow) were taken daily. Fecal samples and orts for individual cows and treatment TMR were pooled and dried at 60ºC in a forced-air oven for 48 h. Samples were ground through a 2-mm screen using a Cyclone Sample Mill (UDY Corp., Fort Collins, CO) and analyzed for ADF and NDF (Goering and Van Soest, 1970), N (Elementor Vario Max CN Analyzer, Elementor Americas Inc., Mt. Laurel, NJ), starch (Cumberland Valley Analytical Laboratory), and ash content (600ºC in a muffle furnace for 5 h). Indigestible NDF was used as a marker to calculate apparent digestibility from the total tract (Oba and Allen, 1999). Indigestible NDF was determined after 120 h of *in vitro* rumen incubation using the method of Goering and Van Soest (1970) with modifications. The modifications included weighing the samples into filter bags (Ankom Technology, Macedon, NY) and incubating them in buffer and ruminal fluid for 120 h using a Daisy^{II} incubator (Ankom Technology, Macedon, NY). Ruminal fluid was collected from a fistulated steer fed the control diet. After 60 h of incubation the original ruminal fluid and buffer was discarded and replaced with a fresh mixture and incubation continued for an additional 60 h.

Chapter 5

STATISTICAL ANALYSIS

Lactation data were analyzed as simultaneous 3×3 Latin squares as described by Morris (1999) using the General Linear Models procedure of SAS (SAS Institute, 1999). Means are reported as least squares means. The effects of cow, treatment, and period were used in the model where:

$Y = \mu + Period_i + Cow_j + Treatment_k + (Treatment \times Cow)_{jk} + (Treatment \times Period)_{ik} + (Period \times Cow)_{ij} + \varepsilon_{ijk}$

Where Y is the dependent variable, μ is the mean and ε is the residual error. Differences between least squares means were reported as significant when $P < 0.05$. Trends were discussed at $P < 0.10$.

Chapter 6

RESULTS

The live yeast product used in our study contained 41 × 10^9 cfu of *S. cerevisiae* CNCM I-1077 /g (data not shown). This exceeded the manufacturer's guarantee of 20 × 10^9 cfu/g of product. The nutrient composition of the TMR fed to cows during the study was generally similar among treatments (Table 4) with the exception of ash and sodium content. The sodium content of BICB (0.40%) was greater than that of CTRL and YEA (0.21 and 0.23%, respectively) and the content of ash was greater in BICB (7.6%) when compared to other treatments (6.95 and 7.10% for CTRL and YEA, respectively). The DM content of all three TMR averaged 52.65%. They averaged 16.91% CP, 21.5% ADF, 33.9% NDF, 23.38% starch and 1.68 Mcal/kg of NE_L. The mineral content of the TMR met or exceeded requirements for lactating cows (NRC, 2001). The distribution of particles of the TMR was similar among treatments throughout the study.

The results of the production measurements are shown in Table 5. Cows fed BICB but not YEA consumed more DM ($P < 0.01$) and NDF ($P < 0.04$) than those fed CTRL. There was no difference in DMI between cows fed BICB and YEA. Milk production was similar among treatments (average of 38.3 kg/d). The production of 3.5% FCM and ECM was also unaffected by treatment. Milk fat content ranged between 3.19 and 3.30% and was not affected by treatment. The production of milk

fat (kg/d) was also not affected by treatment. Similar results were found for milk protein content and yield. Milk lactose content and the concentration of SCC was unaffected by treatment. However, MUN was greater for cows fed BICB than those fed CTRL and YEA. Feed efficiency was lower ($P < 0.03$) for cows fed BICB and YEA when compared to CTRL. Body weights did not differ among treatment groups.

The least squares means of the blood, urine, and fecal measurements are shown in Table 6. The addition of YEA or BICB to the diets of lactating dairy cows did not affect the pH of feces or urine, nor did it alter serum amyloid A or blood haptoglobin levels when compared to CTRL.

The least squares means of the ruminal VFA measurements are shown in Table 7. There was a trend ($P = 0.08$) for an increased molar % of acetate and isobutyrate in bicarbonate supplemented cows. The acetate to propionate ratio was similar among treatments. There were no differences among treatments in total VFA concentration or molar % of other VFA.

Nutrient digestion in the total tract of cows is shown in Table 8. Cows supplemented with BICB in their diet had lower DM and OM digestibility when compared to both CTRL and YEA. The digestibility of NDF was also lower for cows fed sodium bicarbonate than those fed the unsupplemented TMR but it was similar to cows fed live yeasts. The digestibility of CP was lower in diets from cows fed BICB

or YEA supplementation when compared to cows fed the unsupplemented diet.

Chapter 7

DISCUSSION

The dietary treatment containing sodium bicarbonate had a higher ash and sodium content relative to the other diets, and this was a direct result of the additive. Cows offered this diet consumed more DM than cows fed the other treatments but this resulted in a decrease in feed efficiency relative to cows fed the unsupplemented diet. Hu and Murphy (2005) summarized the data from 30 experiments and they also reported that DMI was greater in cows supplemented with sodium bicarbonate but this occurred only when cows were fed diets having a high proportion of corn silage as their source of forage. The authors suggested that the increase in intake was a result of neutralizing acids in the rumen. However, the increased intake may have also been a result of increased rate of rumen passage from the consumption of higher sodium as hypothesized by Russell and Chow (1993). In support of this theory, OM, DM, CP and NDF digestion were lower in cows fed sodium bicarbonate when compared to cows fed the unsupplemented diet, which could have been a result of an increased turnover of ruminal contents. In contrast to DM intake, milk production (total, 3.5% FCM or ECM) was not affected by sodium bicarbonate in the current study. Milk fat content was also unaffected by supplementation with sodium bicarbonate, which is in clear contrast with previous studies where the hallmark effect of feeding this additive to lactating cows has been an improvement in milk fat content from cows with a low milk fat test (Erdman, 1988; Hu and Murphy, 2005).

Unlike the effect of feeding sodium bicarbonate, feeding live yeasts did not affect DM intake. In a summary of 157 experiments, Desnoyers et al. (2009) reported that supplementation with live yeasts resulted in improvements in DMI (+ 0.4 kg/d over control) and milk yield (+1.2 kg/d over control) with a tendency for increased milk fat content and no effect on milk protein content. However, the data was obtained from cows fed a wide variety of products which had different feeding rates and included different strains of live yeasts that may or may not have had similar metabolic properties. Ondarza et al. (2009) conducted a meta-analysis comprised of 14 research trials where only SC1077 was used. The authors reported that cows fed SC1077 produced more 3.5% FCM and had better feed efficiencies when compared to untreated cows. However, in our study, the conversion of DM to milk was reduced with SC1077 compared to control, because there was a numerical increase in DMI without an increase in milk production.

There was a trend for an increase in the molar proportion of ruminal acetate in cows fed sodium bicarbonate, but this difference was not enough to affect the ratio of acetate to propionate (**A:P**) among treatments. The A:P is usually lower in cows fed a high grain diet versus a high forage diet and is the result of the end products of substrate fermentation. High proportions of acetate are usually indicative of ruminal fermentations from diets high in fiber, while ruminal fluid with a high proportion of propionate is often a result of fermentations from diets high in starch. The A:P in our

experiment averaged 2.61, indicative of a diet containing adequate fiber. Davis (1979) concluded that buffers such as sodium bicarbonate tend to have the greatest effect when added to diets that yield a fermentation in which the A:P is less than 2.0. Changes in rumen pH can explain up to 25% of the changes in A:P, although the effect of pH is more subtle than the effect of the diet (Russell, 1998). At a low pH, ruminal succinate can be converted to propionate at an increased rate, which can decrease the A:P. The lack of a difference in A:P among treatments in the current study could be reflective of a high ruminal pH. The molar proportions of propionate, butyrate, isovalerate, and valerate did not differ among treatments and the trend of higher isobutyrate in cows fed sodium bicarbonate is unexplainable at this time. Supplements also did not affect the concentration of total VFA. In contrast, Marden et al. (2008) reported that the addition of *S. cerevisiae* or sodium bicarbonate increased the concentration of total VFA by 14. and 10.0 m*M,* respectively, when compared to an unsupplemented diet. Lactic acid (not reported) was not detected in any of the samples in the current study but addition of live yeasts and sodium bicarbonate have both been shown to decrease its concentration in the rumen (Marden et al., 2008; Guedes et al., 2008; Koul et al., 1998a)

Low ruminal pH can be an indicator of acidosis. Rumen pH is a function of the production and absorption of VFA by the rumen microorganisms, water and saliva flow, the concentration of buffer in the saliva of the cow, and the feed acidity (Erdman, 1988). In the current experiment, rumen fluid was attained via stomach

tubing, and therefore, the values for rumen pH were high (> 7 and not different among treatments; data not reported) because of salivary contamination. Other metabolic measurements from our study suggested that cows were not under any kind of acidotic challenge. For example, urine pH is reflective of the acid-base status of the diet. A urine pH < 7.0 can be indicative of acidosis (Mellau et al., 2004). However, in our experiment, the pH of urine averaged between 8.27 and 8.34, suggesting that cows were not in acidosis. Fecal pH can be indicative of the pH in the small intestine and the colon and can affect the activity of starch-digesting enzymes, which have a pH optimum of 6.9 (Wheeler and Noller, 1977). There is an inverse relationship between starch digestion and fecal pH (Wheeler and Noller, 1977). An increased fecal pH may indicate an increase in starch utilization. Slightly elevated fecal pH, ranging in this experiment between 7.27 and 7.43, indicated adequate starch utilization and a lack of hindgut acidosis. Haptoglobin and serum amyloid A are two acute phase proteins that have been shown to be elevated during subacute ruminal acidosis, specifically as a result of inflammatory responses (Ghozo et al., 2005). Their concentrations were similar among treatments and within normal biological ranges for healthy, high producing dairy cows (Gozho et al., 2007).

The exact reasons for the general lack of response in milk production and milk fat content from cows fed sodium bicarbonate or live yeasts in the current study are unknown. However, a lack of response may be related to the fact that the composition of the diet has been shown to interact with responses to either live yeasts

or sodium bicarbonate. For example, Davis (1979) noted that feeding sodium bicarbonate was most effective in diets that were low in either total fiber or effective fiber. Robinson and Erasmus (2009) and Desnoyers et al. (2009), reported the production response to feeding live yeasts was diminished as dietary NDF or ADF increased because more effective fiber stimulates the natural production of sodium bicarbonate from chewing. The concentrations of dietary NDF and ADF in our study were about 34% and 21%, respectively, which were both considered "high" by the standards of Robinson and Erasmus (2009). Furthermore, the sizes of feed particles in our diets were within accepted guidelines for supplying adequate effective fiber to lactating cows (Heinrichs and Kononoff, 2002). For SC1077, the digestibility of corn silage has been reported to affect the effect of this live yeast on NDF-D. Guedes (2008) reported that SC1077 improved the in situ 36 h NDF digestion (NDF-D) of corn silages with low (20-30% NDF-D) but not high (35 to 45% NDF-D) potential for digestion. Although we did not measure the 36 h NDF-D of our corn silage, it was a brown mid rib hybrid, which is known to have a high NDF-D (Ebling and Kung, 2004). Furthermore the NDF-D of the diets in the total tract in our study ranged from 45.2 to 49.9% indicating that our diets were highly digestible.

The level of starch in the diet may also have had an effect on the results of our study. For example, sodium bicarbonate has been ineffective in affecting milk production and composition when cows were fed diets low in starch (Davis, 1979; Erdman, 1988; Hu and Murphy, 2005) and our diets contained about 23% starch

which was moderate. There is conflicting evidence to support the fact that the level of starch in a diet affects the response to feeding live yeasts. For example, the stimulatory effect of SC1077 on milk fat percentage was only observed when cows were fed a diet high in starch (Santos et al., 2006). However, data from a recent meta-analysis by Robinson and Erasmus (2009) found no relationship between the level of concentrate in the diet and response to live yeasts.

Chapter 8

CONCLUSIONS

Cows in the current study were fed a well-balanced diet with moderate levels of starch and adequate concentrations of fiber and effective fiber. Overall, the general metabolic measurements from blood and the rumen suggested that the cows in this study were not in subacute or acute acidosis and the levels of milk fat were not considered severely depressed (Hurley, 2009; Pennington, 2009) for Holstein cows. Thus, the addition of sodium bicarbonate or *S. cerevisiae* CNCM I-1077 had no effects on the production of milk, milk fat or milk protein contents. The results of this experiment question the necessity of sodium bicarbonate or *S. cerevisiae* CNCM I-1077 in a balanced, highly digestible diet that is fed to lactating dairy cows.

Table 2. Ingredient composition (% of DM) of the concentrate fed to lactating cows

Ingredient	%
Superflake corn	22.71
Soy hulls	19.56
Wheat middlings	16.01
Soybean meal	15.35
Amino plus[1]	14.48
Sugar	2.49
Limestone	2.49
Fat	1.78
Megalac[2]	1.36
Distillers dried grains	1.01
Salt	0.93
Urea	0.66
Dynamate[3]	0.60
Magox/magnesium	0.25
Trace minerals	0.094
ADE Vitamin premix	0.066
Selenium premix	0.065
Pennfield Quadra[4]	0.044
Organic selenium	0.033
Niacin, 99%	0.029

[1]A blend of essential amino acids including lysine, glycine, and methionine.
[2]A rumen bypass fat with a NE_L of 5.20 Mcal/kg.
[3]Contains 22% S, 18% K, 11% Mg, 0.1% Fe, and 0.0005% Pb.
[4]A mineral blend from Pennfield Feeds, Lancaster, PA, containing Ca, Mg, Mn, and other supplements.

Table 3. Assignment of cows to treatments

Cow	Lactation #	Avg. Milk (kg/d)	DIM	Avg. Intake (DMB)	Body Weight (kg)	Body Condition Score
Group 1[1]						
660	4	32.1	103	50.0	766	2.50
676	5	28.9	68	54.4	808	3.00
742	3	47.0	34	50.5	670	2.25
747	3	36.2	61	49.5	798	3.25
758	3	44.8	44	53.6	759	3.00
769	2	50.6	87	60.1	712	2.00
781	2	38.8	88	41.3	624	2.75
801	2	47.7	46	49.4	635	2.50
815	1	31.3	123	37.9	513	2.50
Group 2[2]						
499	8	31.3	135	34.7	626	2.25
737	3	53.6	87	67.5	872	2.75
752	3	34.0	91	54.5	795	2.75
755	3	31.7	47	61.5	740	3.00
761	3	32.7	76	50.0	735	2.00
768	2	34.4	108	52.8	827	3.25
771	2	46.9	39	49.0	732	2.25
787	2	44.6	38	43.8	616	2.75
820	1	33.3	91	45.6	671	3.00
829	1	36.0	64	40.6	532	3.00
Group 3[3]						
698	3	45.8	119	68.1	832	2.75
729	3	50.2	82	58.9	845	2.75
744	3	43.2	89	54.4	674	3.00
762	2	32.6	142	51.7	605	2.50
775	2	44.2	42	58.3	799	2.75
778	2	40.5	74	46.3	646	2.50
783	2	43.5	34	43.8	736	2.50
839	1	31.2	98	40.5	558	3.00
841	1	35.7	47	41.1	581	2.50

[1]Cows (n = 9) were fed an untreated (CTRL) TMR for period 1, a TMR containing 0.021 grams of the *S. cerevisiae* CNCM I-1077 product (20×10^9 cfu/g) per kg of TMR DM (YEA) for period 2, and a TMR containing 9.43 grams of sodium bicarbonate per kg of TMR DM (BICB) for period 3.
[2]Cows (n = 9) were fed YEA for period 1, BICB for period 2, and CTRL for period 3
[3]Cows (n = 10) were fed BICB for period 1, CTRL for period 2, and YEA for period 3

Table 4. Average composition (DM basis, ± SEM) of experimental diets

Item	CTRL[1]	±	YEA[2]	±	BICB[3]	±
DM, %	52.14	1.37	52.41	1.97	53.40	0.98
CP, %	17.06	0.34	16.68	0.58	16.99	0.78
SP[4], %	38.05	4.64	37.83	3.11	35.22	2.17
NE_L, Mcal/kg	1.69	0.01	1.69	0.01	1.67	0.02
ADF, %	21.48	1.08	21.70	0.89	21.33	1.00
NDF, %	33.93	1.40	34.13	1.25	33.63	1.24
Ash, %	6.95	0.34	7.10	0.30	7.60	0.52
Starch, %	23.46	1.33	23.06	1.35	23.61	4.71
NFC[5], %	38.82	1.38	38.78	1.21	38.68	1.58
Ca, %	0.94	0.06	0.96	0.06	0.97	0.04
P, %	0.37	0.02	0.37	0.02	0.37	0.03
Mg, %	0.34	0.02	0.33	0.02	0.34	0.02
K, %	1.49	0.10	1.47	0.10	1.50	0.11
Na, %	0.21	0.02	0.23	0.09	0.40	0.13
Fe, ppm	326	57.26	346	60.68	351	111.23
Mn, ppm	84	8.18	84	10.25	85	6.87
Zn, ppm	113	13.38	115	16.85	116	14.53
Cu, ppm	19	3.70	18	2.25	18	2.02
Particle Size Distribution (%)						
> 1.91 cm	4	1	4	1	4	1
0.79 to 1.91 cm	42	3	41	3	39	2
0.18 to 0.79 cm	41	3	42	4	42	4
< 0.18 cm	12	4	13	5	14	4

[1]Untreated (control) TMR.
[2]TMR containing 0.021 grams of the *S. cerevisiae* CNCM I-1077 product (20 $\times 10^9$ cfu/g) per kg of TMR DM (Resulting in 4.2 $\times 10^8$ cfu/kg TMR DM)
[3]TMR containing 9.43 g of sodium bicarbonate per kg of TMR DM
[4]Soluble protein.
[5]Non fiber carbohydrate.

Table 5. Effect of feeding sodium bicarbonate or live yeasts on intake and production of lactating cows (Data are presented as least-squares means)

Item	CTRL[1]	YEA[2]	BICB[3]	SEM	*P*-Value
DMI, kg/d	24.78[b]	25.41[a,b]	26.52[a]	1.80	0.01
DMI, %BW	3.46[b]	3.56[a,b]	3.70[a]	0.01	0.27
NDF intake, kg/d	8.34[b]	8.40[b]	8.77[a]	0.68	0.04
NDF intake, % BW	1.17[b]	1.18[a,b]	1.23[a]	0.10	0.06
CP intake, kg/d	4.22	4.33	4.32	0.34	0.41
Starch intake, kg/d	5.81	5.85	5.87	0.42	0.91
Milk, kg/d	38.83	37.58	38.43	2.31	0.31
Milk fat,					
%	3.22	3.19	3.30	0.18	0.25
kg/d	1.22	1.19	1.26	0.10	0.22
Milk protein,					
%	2.89	2.89	2.89	0.05	0.69
kg/d	1.12	1.08	1.11	0.08	0.30
3.5% FCM, kg/d	36.65	35.32	36.99	2.42	0.18
ECM[4], kg/d	36.27	35.29	36.62	2.42	0.33
FCM/DMI	1.50[a]	1.41[b]	1.42[b]	0.69	0.03
MUN, mg/dl	11.14[b]	12.31[b]	14.87[a]	0.77	<0.01
Milk lactose, %	4.69	4.65	4.67	0.05	0.31
SCC (× 1000/mL)	412	406	395	101	0.86
BW, kg	723	716	718	20	0.99

[a,b] Means in rows with unlike superscripts differ.

[1]Untreated (control) TMR.

[2]TMR containing 0.021 g of the *S. cerevisiae* CNCM I-1077 product (20×10^9 cfu/g) per kg of TMR DM (4.2×10^8 cfu/kg TMR DM).

[3]TMR containing 9.43 g of sodium bicarbonate per kg of TMR DM.

[4]Energy Corrected milk

Table 6. Blood and pH measurements (Data are presented as least-squares means)

Item	CTRL[1]	YEA[2]	BICB[3]	SEM	*P*-Value
Urine pH	8.29	8.27	8.34	0.06	0.34
Fecal pH	7.43	7.27	7.30	0.31	0.43
SAA[4], μg/ml	163.0	166.0	134.4	26.8	0.28
Blood haptoglobin, (mg/ml)	0.23	0.22	0.25	0.21	0.95

[a,b]Means in rows with unlike superscripts differ ($P < 0.05$).
[1]Untreated (control) TMR.
[2]TMR containing 0.021 g of the *S. cerevisiae* CNCM I-1077 product (20×10^9 cfu/g) per kg of TMR DM (4.2×10^8 cfu/kg TMR DM).
[3]TMR containing 9.43 grams of sodium bicarbonate per kg of TMR dry matter
[4]Serum amyloid A.

Table 7. Ruminal VFA (Data are presented as least-squares means)

Item	CTRL[1]	YEA[2]	BICB[3]	SEM	*P* value
VFA, molar %					
Acetate	60.6	61.0	62.4	0.7	0.08
Propionate	23.8	23.5	22.6	0.6	0.19
Butyrate	12.2	12.1	11.5	0.5	0.36
Isobutyrate	0.4	0.4	0.5	<0.1	0.08
Valerate	1.6	1.6	1.4	0.1	0.15
Isovalerate	1.3	1.3	1.3	0.1	0.88
Acetate:Propionate	2.6	2.7	2.8	0.1	0.17
TVFA[4], mM	60.3	63.5	62.3	9.6	0.92

[a,b]Means in rows with unlike superscripts differ ($P < 0.05$).
[1]Untreated (control) TMR.
[2]TMR containing 0.021 g of the *S. cerevisiae* CNCM I-1077 product (20×10^9 cfu/g) per kg of TMR dry matter (4.2×10^8 cfu/kg TMR DM).
[3]TMR containing 9.43 g of sodium bicarbonate per kg of TMR DM.
[4]Total VFA.

Table 8. Digestibility of the TMR (% DM basis) (Data are presented as least-squares means)

Item	CTRL[1]	YEA[2]	BICB[3]	SEM	*P*-Value
OM	70.5[a]	70.3[a]	67.6[b]	0.70	0.01
DM	68.6[a]	68.1[a]	66.1[b]	0.70	0.03
NDF	49.9[a]	47.0[ab]	45.2[b]	1.62	0.10
ADF	50.5	52.5	45.5	2.54	0.13
Starch	98.1	98.3	98.0	0.17	0.38
CP	65.8[a]	60.3[b]	59.6[b]	1.49	0.01

[a,b]Means in rows with unlike superscripts differ ($P < 0.05$).

[1]Untreated (control) TMR.

[2]TMR containing 0.021 g of the *S. cerevisiae* CNCM I-1077 product (20×10^9 cfu/g) per kg of TMR DM (4.2×10^8 cfu/kg TMR DM).

[3]TMR containing 9.43 grams of sodium bicarbonate per kg of TMR DM.

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