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# Effects of dietary changes and yeast culture (*Saccharomyces cerevisiae*) on rumen microbial fermentation of Holstein heifers[1]

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**ABSTRACT:** The effects of a dietary challenge to induce digestive upsets and supplementation with yeast culture on rumen microbial fermentation were studied using 12 Holstein heifers (277 ± 28 kg of BW) fitted with a ruminal cannula, in a crossover design with 2 periods of 5 wk. In each period, after 3 wk of adaptation to a 100% forage diet, the dietary challenge consisted of increasing the amount of grain at a rate of 2.5 kg/d (as-fed basis) over a period of 4 d, until a 10:90 forage:concentrate diet was reached, and then it was maintained for 10 d. Between periods, animals were fed again the 100% forage diet without any treatment for 1 wk as a wash-out period. Treatments started the first day of each period, and they were a control diet (CL) or the same diet with addition of yeast culture (YC, Diamond V XPCLS). Digestive upsets were determined by visual observation of bloat or by a reduction in feed intake (as-fed basis) of 50% or more compared with intake on the previous day. Feed intake was determined daily at 24-h intervals during the adaptation period and daily at 2, 6, and 12 h postfeeding during the dietary challenge. Ruminal liquid samples were collected daily during the dietary challenge to determine ruminal pH at 0, 3, 6, and 12 h postfeeding, and total and individual VFA, lactic acid, ammonia-N, and rumen fluid viscosity at 0 and 6 h postfeeding. The 16s rRNA gene copies of *Streptococcus bovis* and *Megasphaera elsdenii* were determined by quantitative PCR. Foam height and strength of the rumen fluid were also determined the day after the digestive upset to evaluate potential foam production. A total of 20 cases (83.3%) of digestive upsets were recorded in both periods during the dietary challenge, all diagnosed due to a reduction in feed intake. Rumen fermentation profile at 0 h on the digestive upset day was characterized by low ruminal pH, which remained under 6.0 for 18 h, accompanied by elevated total VFA concentration and, in some cases, by elevated lactate concentration. Addition of YC during the dietary challenge did not affect the incidence (10 cases per treatment) or time (7.00 ± 0.62 d) to digestive upset. However, YC reduced ($P < 0.05$) the foam strength on the day after digestive upset, suggesting potential benefits of reducing the risk of developing bloat. The proposed dietary challenge model was successful in causing a digestive upset as indicated by reduced feed intake, but the YC addition had no significant impact on rumen fermentation.

**Key words:** digestive upset, heifer, rumen fermentation, yeast culture

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## INTRODUCTION

Digestive disorders in feedlot cattle cause from 3 to 7% of total morbidity and up to 25% of total mortality (Smith, 1998). The most critical moment is at feedlot arrival, when arriving animals are changed from a high forage to a high concentrate diet. If fermentable carbohydrate supply is increased abruptly, starch- and lactic acid-fermenting bacteria (such as *Streptococcus bovis*) respond by increasing growth rates and fermentative activities faster than lactic acid-utilizing bacteria (such as *Megasphaera elsdenii*). As a consequence, there is a nonphysiological accumulation of VFA and lactate in the rumen, resulting in less ruminal pH and acidosis, which can range from acute (when there is lactic acid accumulation) to subacute (the presence of lactic acid is not consistent; Nagaraja and Titgemeyer, 2007). In addition, the overgrowth of some bacteria (mainly *S. bovis*) causes an excessive production of mucopolysaccharides, increasing the viscosity of the ruminal fluid

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and the risk of developing frothy bloat (Cheng et al., 1998). Previous research on digestive upsets in cattle shows that the response to a dietary challenge and its recovery depend, among others, on within-animal factors (Goad et al., 1998; Brown et al., 2006; Vasconcelos and Galyean, 2008). However, response variables that could help predict individually the potential development of acidosis or bloat have not been examined simultaneously in a single study.

The use of yeast culture as a dietary supplement has been suggested as a useful tool to stabilize ruminal fermentation (Williams et al., 1991). Yeast culture products contain *Saccharomyces cerevisiae* fermentation metabolites (i.e., B vitamins, AA, organic acids) that work as stimulatory nutrients to specific fiber-digesting (Wiedmeier et al., 1987) and lactate-utilizing (Callaway and Martin, 1997) bacteria. Therefore, yeast culture addition in animals experiencing a dietary challenge with nonstructural carbohydrates could enhance the *Megasphaera elsdenii* population, preventing the negative effects of the overgrowth of starch-consuming bacteria (mainly *S. bovis*). This effect may help reduce the severity or expedite recovery from the digestive upset by avoiding the VFA and lactic acid accumulation in the rumen or the increase of ruminal fluid viscosity.

The objective of this study was to describe the changes occurring in the rumen during a digestive upset induced by rapidly changing from a high forage diet to a high concentrate diet and to evaluate the effects of yeast culture addition on rumen microbial fermentation during this dietary challenge in heifers.

## MATERIALS AND METHODS

The research protocol was approved by the Campus Laboratory Animal Care Committee of the Universitat Autònoma de Barcelona, Spain.

### *Animals*

Twelve Holstein heifers (initial BW of 277 ± 28 kg), each fitted with a 1-cm i.d. plastic ruminal cannula (Divasa Farmavic SA, Vic, Spain), were individually housed in tie-stalls at the Servei de Granges i Camps Experimentals of the Universitat Autònoma de Barcelona, Spain. The ruminal fistulation was performed under local anesthesia and with full aseptic precautions 1 wk before the beginning of the experiment.

### *Experimental Design*

The experiment was performed using a crossover design with 6 heifers per treatment in each of the 2 periods. Each period consisted of 5 wk; the first 3 wk were used for adaptation to a 100% forage diet (a mixture of 80% fescue hay and 20% alfalfa pellets on as-fed basis) and treatments. After these 3 wk, heifers were progressively changed to a high concentrate diet over 4 d to induce a digestive upset by increasing grain at a rate of 2.5 kg/d (as-fed basis) and decreasing forage in the same proportion, until forage to concentrate ratio reached 10:90 (as-fed basis), and then the same diet was fed for 10 d. During these 2 wk, concentrate and straw were offered once a day (0900 h) in separate containers and then removed from the feeders at 2100 h, causing a 12-h fast to promote rapid consumption of concentrate the following morning. Between periods, animals were fed again the 100% forage diet without any treatment during 1 wk as a wash-out period. After the first period, animals were assigned to the opposite treatment (crossover design), and the same protocol was repeated. The concentrate consisted of (DM basis) ground barley grain (32.2%), ground corn grain (27.9%), soybean meal (11.3%), soy hulls (8.1%), wheat (7.5%), corn gluten feed (7.2%), sunflower (2.8%), calcium soaps of fatty acids (1.1%), calcium carbonate (0.5%), sodium chloride (0.5%), dicalcium phosphate (0.5%), and vitamin supplement (0.4%). The diet was formulated to meet or exceed energy, CP, and mineral requirements of cattle (NRC, 1996). The proportion of nonstructural carbohydrates in the concentrate (54.3% DM) was intentionally high. Water was available for ad libitum consumption.

Treatments consisted of a control diet (**CL**) and the same diet containing 14 g/d of *Saccharomyces cerevisiae* based yeast culture (**YC**; Diamond V $XPC_{LS}$ Yeast Culture, Diamond V Mills Inc., Cedar Rapids, IA). Yeast culture treatment was offered at 0900 h daily from the first day of each period by mixing with 100 g of the concentrate used in the experiment, which was offered separately from the daily ration to guarantee the consumption of the whole dose. Animals on the CL treatment received the same 100 g of concentrate without the addition of yeast culture.

Confirmation of digestive upset was determined by visual observation of bloat (Paisley and Horn, 1998) or by a reduction in feed intake of 50% or more compared with the intake of the previous day. Following the instructions of the Animal Care Committee, when digestive upset was observed, the affected animal was switched to a 100% forage diet with no yeast culture (wash-out period) on the following day, and recovery was monitored.

### *Sample Collection and Analyses*

Dry matter intake was measured daily at 2, 6, and 12 h postfeeding during transition and high concentrate diet feeding. Dry matter intake was not measured during the wash-out period. Feed and orts were sampled daily and composited weekly. Dry matter content of feed and orts were determined from the composite samples by oven drying at 105°C for 24 h. Ruminal fluid was collected daily at 0 and 6 h after feeding during the transition and the challenge period, strained through 2 layers of cheesecloth, and 4 subsamples of the filtrate were frozen at −20°C for analyses of VFA, lactate, ammonia-N, viscosity, and quantitative real-time

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PCR (**qPCR**) to quantify the 16s rRNA gene copies of *M. elsdenii* and *S. bovis*. To determine pH (model 507, Crison Instruments SA, Barcelona, Spain), ruminal fluid samples were collected at 0, 3, 6, and 12 h after feeding. Only the samples collected on 3, 2, and 1 d before the digestive upset; the day of the digestive upset; and 1, 2, and 5 d after the digestive upset were analyzed. Additional samples of rumen fluid were collected the day after the digestive upset at 0 h to determine its foaming properties.

Lactate and VFA concentrations in ruminal fluid were analyzed using the gas chromatographic method described by Richardson et al. (1989) and Jensen et al. (1995), with the following modifications: to conserve the sample, 4 mL of ruminal fluid was added to a 1-mL solution of 1% (wt/wt) of mercuric chloride, 2% (vol/vol) orthophosphoric acid, and 0.2% (wt/wt) 4-methylvaleric acid as an internal standard in distilled water and frozen at −20°C. Samples were thawed and centrifuged at 15,000 × *g* for 15 min at 5°C and diluted 1:1 in distilled water before performing VFA analysis. Ammonia-N concentration was analyzed by spectrophotometry (Libra S21, Biochrom Analytical Instruments, Cambridge, UK) as described by Chaney and Marbach (1962).

One extraction of DNA was done from each qPCR sample by physical disruption using a bead-beating method (Mini-Beater, Biospec Products Inc., Bartlesville, OK) following the protocol described by Whitford et al. (1998) with the modifications proposed by Blanch et al. (2007). Briefly, rumen fluid (0.6 mL) was mixed with an equal volume of pH 8.0 buffered phenol solution (USB 75829, Cleveland, OH) in a 2-mL tube with 0.5 g of 0.1-mm glass beads (Ref. 11079101, Biospec Products Inc.). After adding 40 μL of 10% SDS (Sigma L4522, St. Louis, MO), tubes were shaken 3 times for 2 min on a Mini-Beater, and then spun at 11,600 × *g* for 5 min in a microfuge. The aqueous phase was transferred to a new tube, extracted with buffered phenol, and precipitated with ethanol. Samples were suspended in 100 μL of TE 0.5× buffer (Tris-EDTA buffer, Sigma T9285) and were treated with 2 μL of RNase (10 mg/mL) for 1 h at 37°C (RNase A, Roche, Sant Cugat del Vallès, Barcelona), re-extracted with phenol, precipitated with ethanol, and suspended in 50 to 200 μL of TE 0.5×. The DNA concentration was measured by spectrophotometry (NanoDrop ND-1000 Spectrophotometer, Nanodrop Technologies, Wilmington, DE). The DNA obtained was stored at −20°C in aliquots of 20 ng/μL (stock). For the qPCR, specific primers and a probe for *S. bovis* were previously designed (Blanch et al., 2007) from a 16S ribosomal RNA gene sequence available in the GeneBank database (AY442813) using the Primer Express Software (Applied Biosystems, Warrington, UK). The primers and probe sequences used were forward primer, *S. bovis* F: 5′-GATAGCTAATACCGCATAACAGCATT-3′; reverse primer, *S. bovis* R: 5′-AACGCAGGTCCATCTACTAGTGAA-3′; and probe, *S. bovis* P: 5′-TGCTCCTTTCAAGCAT-3′.

For *M. elsdenii*, previously published primers specific for a 129-bp fragment of the 16S ribosomal RNA (Ouwerkerk et al., 2002) were chosen to accomplish specific amplification, whereas a specific Taqman MGB probe (Melsprobe: 5′-ACTGGTGTTCCTCCTAATA-3′) was designed with Primer Express Software (Blanch et al., 2007). All primers were purchased from Isogen Life Science, S.L. (Barcelona, Spain) and Taqman MGB probes from Applied Biosystems. The qPCR were run in triplicate for standard curve points or in duplicate for the single extraction of each ruminal sample in a 20-μL reaction volume containing 1× TaqMan Universal PCR Master Mix (Applied Biosystems), primers at a final concentration of 900 n*M* each, 250 n*M* probe, and 100 ng of genomic DNA (5 μL of ruminal fluid samples at 20 ng/μL). The qPCR was run in the ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems) using optical grade 96-well plates with the following amplification parameters: 2 min at 50°C, 10 min at 95°C, and 40 cycles for 15 s at 95°C, and 1 min at 60°C. Reactions without DNA were used as a negative control each time. The obtained measurements were converted from nanograms of DNA per milliliter of sample to number of copies per milliliter of sample (Talbot et al., 2008). For the statistical analysis of the qPCR results, data were transformed by logarithm to obtain a normal distribution. The standard curve points were serial dilutions (1/10) from log 9.03 to log 15.03 of target copies per milliliter for *S. bovis*, and from log 7.85 to log 13.85 of target copies per milliliter for *M. elsdenii*. Whenever these probes resulted in no detection, a zero value was considered.

For determinations of the ruminal fluid viscosity, samples were thawed at ambient temperature, shaken, and immediately analyzed with a low viscosity adaptor UL/Y (DV-E, Brookfield Engineering Laboratories, Middleboro, MA). Foam height and strength of rumen fluid were examined according to the procedure of Pressey et al. (1963) and Min et al. (2005). On the day after digestive upset, 50 mL of fresh rumen fluid of the affected heifer was collected before feeding. Ruminal fluid was poured into a glass cylinder (37 mm diameter × 30 cm length) and $CO_2$ gas was bubbled through a bottom inlet at 60 kPa for 30 s, resulting in conversion of most of the fluid into foam. Foam height, measured as the height of foam in the cylinder, was used as a measure of potential foam production. The time for the foam column to collapse on itself to the original fluid volume was used as an index of foam strength.

### *Statistical Analyses*

Data from the experiment were analyzed as a split-split plot using the MIXED procedure (SAS Institute Inc., Cary, NC) for repeated measures when estimated R matrix is positive definite, considering heifer nested within treatment as the subject, with the unstructured covariance structure for the hours and the autoregressive covariance structure for days, according to the

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Bayesian information criterion. The model included the effects of treatment, day, hour, and all interactions, period, and sequence as fixed factors, and the effects of heifer, heifer within period, and heifer within day within period as random factors.

The statistical analysis of the effect of YC on time to develop a digestive upset, as well as results from the foam height and strength test, were also analyzed using the MIXED procedure. The model contained the treatment effect as a fixed factor, whereas heifer and heifer within period were considered as random effects. For all the statistical analyses, no effects were declared at $P \geq 0.10$, trends at $0.05 \leq P < 0.10$, and differences at $P < 0.05$, using a multiple comparison test (Tukey, 1953).

## RESULTS AND DISCUSSION

There were few interactions ($P \geq 0.10$) between time (hour or day effects) and treatment effects. Therefore, main effects are discussed separately in 2 sections, unless otherwise indicated: 1) changes in ruminal fermentation during the dietary challenge, and 2) effects of yeast culture on ruminal fermentation during the dietary challenge.

### *Changes in Ruminal Fermentation During the Dietary Challenge*

The dietary challenge model was successful in causing a total of 20 cases (83.3%) of digestive upsets in both periods: 11 of 12 heifers in the first period and 9 of 12 heifers in the second period. Visual signs of bloat were not observed; thus all cases were diagnosed due to a 50% reduction in feed intake (Table 1). Reduction of feed intake is one of the main symptoms of digestive upset (Forbes and Barrio, 1992; Owens et al., 1998). For the purpose of this study, the 50% reduction in DMI was selected as an arbitrary but objective criterion. On average, it took 7.00 ± 0.62 d from the start of dietary challenge to develop the digestive upset according to our criteria. Generally, it is well accepted that the reduction in DMI after an abrupt inclusion of concentrate in the diet is due to an accumulation of fermentation acids, causing an increase in osmolality and the development of acidosis (Nocek, 1997; Owens et al., 1998; Krause and Oetzel, 2006). In our experiment, there were 4 of 24 cases (16.7%) with no signs of digestive upset after 14 d on the high concentrate diet. Some authors (Phy and Provenza, 1998; Dohme et al., 2008) suggest that ruminants might learn from previous acidotic experiences and consequently limit their subsequent intake of a high concentrate diet. However, we found that 3 of the 4 heifers that had a digestive upset in the first period became resistant in the second period, but 1 heifer that was resistant in the first period developed a digestive upset in the second period.

There was a day × hour interaction ($P < 0.01$) in ruminal pH, where it decreased ($P < 0.05$) each day

**Table 1.** Cumulative DMI (kg) and ruminal liquid pH of animals experiencing a digestive upset (n = 20) on or around the day it appeared (0 d)

| | Day | | | | | | | Treatment[1] | | | *P*-value[2] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | −3 | −2 | −1 | 0 | 1 | 2 | 5 | CL | YC | SEM | H | D | H × D | T | H × T |
| DMI | | | | | | | | | | | | | | | |
| 2 h | 3.05$^{c,x}$ | 2.96$^{c,x}$ | 2.47$^{c,x}$ | 0.52$^{c,y}$ | —[3] | — | — | 1.97 | 2.55 | 0.30 | <0.01 | <0.01 | <0.01 | 0.32 | 0.67 |
| 6 h | 4.82$^{b,x}$ | 4.99$^{b,x}$ | 4.83$^{b,x}$ | 1.09$^{b,y}$ | — | — | — | 3.66 | 4.19 | | | | | | |
| 12 h | 7.00$^{a,x}$ | 7.38$^{a,x}$ | 7.18$^{a,x}$ | 2.28$^{a,y}$ | — | — | — | 5.87 | 6.04 | | | | | | |
| Ruminal pH | | | | | | | | | | | | | | | |
| 0 h | 6.67$^{a,y}$ | 6.54$^{a,y}$ | 6.47$^{a,y}$ | 5.95$^{b,z}$ | 7.01$^{a,x}$ | 7.01$^{a,x}$ | 7.02$^{a,x}$ | 6.61 | 6.72 | 0.054 | <0.01 | <0.01 | <0.01 | 0.99 | 0.12 |
| 3 h | 6.10$^{b,z}$ | 6.11$^{b,z}$ | 6.10$^{b,z}$ | 6.24$^{a,z}$ | 6.48$^{b,y}$ | 6.68$^{b,x}$ | 6.45$^{b,y}$ | 6.34 | 6.28 | | | | | | |
| 6 h | 5.93$^{c,z}$ | 5.86$^{c,z}$ | 5.76$^{c,z}$ | 6.33$^{a,y}$ | 6.40$^{bc,xy}$ | 6.54$^{c,x}$ | 6.45$^{b,xy}$ | 6.21 | 6.15 | | | | | | |
| 12 h | 5.62$^{d,z}$ | 5.61$^{d,z}$ | 5.47$^{d,z}$ | 6.06$^{b,y}$ | 6.30$^{c,x}$ | 6.28$^{d,x}$ | 6.20$^{c,xy}$ | 5.93 | 5.94 | | | | | | |

$^{a-d}$Means with different superscripts in the same column are different ($P < 0.05$).
$^{x-z}$Means with different superscripts in the same row are different ($P < 0.05$).
[1]Treatments consisted of a control diet (CL) and the same diet containing 14 g/d of *Saccharomyces cerevisiae*-based yeast culture (YC; Diamond V XPC$_{LS}$ Yeast Culture, Diamond V Mills Inc., Cedar Rapids, IA).
[2]Fixed effects were hour postfeeding (H), day (D), hour within day (H × D), treatment (T), hour within treatment (H × T), and other nonsignificant ($P > 0.10$) effects, including period, sequence, day within treatment, and hour within day within treatment.
[3]Not determined.

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after feeding, except on the day of the digestive upset when pH increased ($P < 0.01$) after feeding due to the reduction of feed intake previously mentioned (Table 1). The days before the digestive upset, the postfeeding reduction in pH was most likely due to the rapid fermentation of the high-concentrate diets together with the decreased rumination and salivation normally associated with these types of diets (Emery and Brown, 1961; Balch, 1971). The 12 h of fasting helped recover the pH back to above 6.0 at 0 h the next day. However, on the digestive upset day, pH at 0 h was less ($P < 0.05$) than the days around the upset at the same hour. Therefore, reduction in feed intake on the upset day was likely the consequence of the failure of recovery of ruminal pH, which was maintained under 6.0 for more than 18 h. Cerrato et al. (2006) indicated that the negative effects of low pH on rumen fermentation was a function of the total amount of time that pH was suboptimal, which may have caused ruminal acidosis and reduced feed intake (Owens et al., 1998). The recovery of pH after the digestive upset day is attributed to the change to a 100% forage diet.

There was a day × hour interaction ($P < 0.01$) for total VFA concentration because it was greater ($P < 0.05$) at 6 h postfeeding compared with 0 h on all days, except on the day of the digestive upset, when it was opposite, presumably due to the reduction in feed intake (Table 2). In addition, total VFA at 0 h was greater ($P < 0.05$) the day of the digestive upset than 3 and 2 d before it, which could explain the lack of pH recovery described previously. On the days after the digestive upset, total VFA concentration decreased as the diet was switched to a 100% forage diet. The hour × day interaction was also detected ($P < 0.01$) for the acetate to propionate ratio, which was less ($P < 0.05$) 6 h postfeeding than at 0 h all days, except for 1 and 2 d after the digestive upset, probably due to the change to a 100% forage diet. Lactate concentration was greater ($P < 0.01$) at 0 and 6 h postfeeding the day of the digestive upset than the days around it, probably due to the extended period of rumen pH under 6.0 previously mentioned. However, not all heifers with digestive upset had the lactate spike. Lactate concentration was greater than 1.5 m$M$ in 25% of the cases at 0 h, and 10% of the cases at 6 h, so in most cases the digestive upset occurred even without lactate being present. These data agree Owens et al. (1998) and have led to the suggestion that the role of lactic acid in the development of digestive upsets is overestimated, with the total acid load, and not lactate alone, being responsible for acidosis (Britton and Stock, 1987). There was a day × hour interaction ($P < 0.01$) for ammonia-N concentration because it was greater ($P < 0.05$) at 6 h postfeeding compared with 0 h on days previous to the digestive upset, but the opposite occurred after it. In addition, ammonia-N concentration was greater ($P < 0.01$) on days previous to the digestive upset than the days after it. These effects were likely explained by the change of diet because ammonia-N can be highly variable depending on the intake and the form of N available in the diets (Seal et al., 1992).

Copies of the 16s gene of *S. bovis* increased ($P < 0.05$) on the digestive upset day compared with 3 d before it (Table 3). This increase could be related to the increased lactate and total VFA concentration that occurred on the digestive upset day. Copies of 16s gene of *M. elsdenii* also tended to increase ($P = 0.09$) on the digestive upset day compared with 3 d before it, as expected due to increased lactic acid supply. However, this increase did not avoid the lactic acid accumulation and the development of the digestive upset, suggesting that its role as a lactic acid utilizer in the prevention of digestive disease may be overemphasized. For this reason, further research on microbial population changes during a dietary challenge is necessary.

The overgrowth of some bacteria that release mucopolysaccharides (slime), such as *S. bovis*, contributes to the increase in the viscosity of ruminal fluid, favoring the development of bloat (Cheng et al., 1998). The ruminal fluid viscosity was greater ($P < 0.01$) at 0 h than at 6 h postfeeding on the days around the digestive upset (Table 3), probably due to the differences in water intake, which should be greater postfeeding (6 h) than after 12 h of fasting (0 h). Ruminal fluid viscosity was also greater ($P < 0.05$) at 0 and 6 h on the day of the digestive upset compared with 3 and 2 d before. The increased viscosity of the ruminal fluid matched with the increased copies of the 16s rRNA gene of *S. bovis*, in agreement with Cheng et al. (1976). Because heifers were shifted to a 100% forage diet at the first symptom of upset, long-term effects of dietary challenge on the development of bloat were not observed. Once the heifers received a 100% forage diet after the digestive upset, the ruminal fluid viscosity decreased at 0 and 6 h postfeeding.

### *Effects of YC on Ruminal Fermentation During the Dietary Challenge*

The addition of YC did not affect the number of cases of digestive upsets after the dietary challenge (10 cases were recorded per treatment). The time to develop these digestive upsets after the change of diet was not affected ($P = 0.20$) by YC (CL = 7.80 d; YC = 6.20 d; SEM = 0.85).

Average DMI (kg/d) of heifers during the transition until the digestive upset day was not affected ($P = 0.32$) by treatment (Table 1). Others have shown that YC increased DMI during the transition from a prepartum to a postpartum diet (Dann et al., 2000; Erasmus et al., 2005).

From 3 d before until 5 d after the digestive upset, mean ruminal pH was not affected ($P = 0.99$) by YC addition (Table 1). The effects of YC on ruminal pH are highly variable depending on experimental condi-

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tions. Although some researchers have shown that YC maintains a more stable pH (Wiedmeier et al., 1987; Harrison et al., 1988; Callaway and Martin, 1997), others have reported no effects (Sullivan and Martin, 1999; Erasmus et al., 2005).

There was a treatment × hour postfeeding interaction ($P = 0.04$) in total VFA concentration because it was less at 0 h with YC, but the opposite was found at 6 h postfeeding (Table 2), suggesting a greater diet fermentability with YC. Therefore, the effects of YC on ruminal pH could be explained by the differences in total VFA concentration postfeeding, assuming a direct relationship between total VFA concentration and ruminal pH (Seymour et al., 2005). However, the potential increase in diet fermentability with YC did not affect the incidence or the time to cause the digestive upset. The effects of YC on total VFA concentration have been inconsistent (Yoon and Stern, 1996; Callaway and Martin, 1997; Miller-Webster et al., 2002).

No effects of YC were found on other fermentative variables including acetate and propionate proportions as well as ammonia-N concentration (Table 2). Reported effects of YC on ruminal fermentation patterns are not consistent. For example, Harrison et al. (1988) found that YC reduced the acetate:propionate ratio by stimulating the proportion of propionate at the expense of acetate, whereas Robinson and Garrett (1999) reported the opposite. There was no effect ($P = 0.48$) of YC on lactate concentration, in agreement with Sullivan and Martin (1999) and Lynch and Martin (2002). Callaway and Martin (1997) demonstrated that a sterilized filtrate of yeast culture stimulated growth of lactate utilizing ruminal bacteria like *M. elsdenii*. Under the present experimental conditions, YC tended to interact ($P = 0.10$) with the hour postfeeding on copies of the 16s rRNA gene of *S. bovis* (Table 3) because it was greater at 0 h than at 6 h postfeeding on CL animals (0 h = 11.58, 6 h = 11.41 logarithm of target copies/mL of ruminal liquid), but the opposite occurred with YC (0 h = 11.89, 6 h = 11.94 logarithm of target copies/mL of ruminal liquid). There is no clear hypothesis to justify these changes. For this reason, further in vivo microbiological studies would be needed to clarify the effects of YC on microbial population structure.

Frothy bloat in cattle is caused by the entrapment of gas in ruminal fluid (Mangan, 1959; Cheng et al., 1998). The ruminal fluid viscosity and the foam height and strength were analyzed to determine the potential implication of YC in the prevention of bloat. Addition of YC did not affect ($P = 0.57$) the ruminal liquid viscosity (Table 3). However, YC reduced ($P < 0.05$) the foam strength of the ruminal liquid (Table 4). Although symptoms of bloat were not observed in this study, the reduced foam strength of rumen fluid suggests the potential effect of YC on reducing the risk of bloat. Further research is warranted to investigate the effect of YC on rumen fermentation under challenging dietary conditions.

**Table 2.** Total VFA concentration (m*M*), acetate to propionate ratio, and lactate (m*M*) and ammonia-N (mg/100 mL) concentrations of animals experiencing a digestive upset (n = 20) on or around the day it appeared (0 d)

| | Day | | | | | | Treatment[1] | | | *P*-value[2] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | −3 | −2 | −1 | 0 | 1 | 2 | 5 | CL | YC | SEM | H | D | H × D | T | H × T |
| Total VFA | | | | | | | | | | | | | | | |
| 0 h | $107.3^{b,x}$ | $111.3^{b,x}$ | $117.6^{b,wx}$ | $125.0^{a,w}$ | $76.3^{b,z}$ | $75.9^{b,z}$ | $89.6^{b,y}$ | 102.1 | 98.0 | 4.67 | <0.01 | <0.01 | <0.01 | 0.96 | 0.04 |
| 6 h | $127.4^{a,w}$ | $130.8^{a,w}$ | $131.3^{a,w}$ | $94.3^{b,xy}$ | $91.6^{a,xy}$ | $87.6^{a,y}$ | $100.2^{a,x}$ | 106.5 | 110.9 | | | | | | |
| Acetate:propionate | | | | | | | | | | | | | | | |
| 0 h | $3.97^{a,w}$ | $3.87^{a,w}$ | $3.90^{a,w}$ | $4.46^{a,w}$ | $2.78^{x}$ | $2.69^{b,x}$ | $3.97^{w}$ | 3.62 | 3.71 | 0.22 | <0.01 | <0.01 | <0.01 | 0.81 | 0.56 |
| 6 h | $3.29^{b,xy}$ | $3.23^{b,xy}$ | $3.29^{b,xy}$ | $3.57^{b,wx}$ | $2.83^{y}$ | $3.07^{a,xy}$ | $3.85^{w}$ | 3.30 | 3.32 | | | | | | |
| Lactate | | | | | | | | | | | | | | | |
| 0 h | $0.99^{x}$ | $1.01^{x}$ | $1.21^{x}$ | $10.3^{a,w}$ | $1.06^{x}$ | $0.89^{x}$ | $0.96^{x}$ | 2.01 | 2.61 | 1.43 | 0.15 | <0.01 | <0.01 | 0.48 | 0.50 |
| 6 h | $1.07^{x}$ | $1.20^{x}$ | $1.19^{x}$ | $5.34^{b,w}$ | $1.16^{x}$ | $1.10^{x}$ | $1.03^{x}$ | 1.12 | 2.28 | | | | | | |
| Ammonia-N | | | | | | | | | | | | | | | |
| 0 h | $8.45^{b,xy}$ | $9.21^{b,x}$ | $8.40^{b,xy}$ | $13.0^{a,w}$ | $6.12^{yz}$ | $5.22^{z}$ | $7.76^{a,xy}$ | 8.67 | 7.84 | 1.11 | 0.76 | <0.01 | <0.01 | 0.54 | 0.80 |
| 6 h | $13.6^{a,w}$ | $13.3^{a,w}$ | $10.7^{a,wx}$ | $9.85^{b,x}$ | $4.33^{y}$ | $3.35^{y}$ | $4.85^{b,y}$ | 8.71 | 8.28 | | | | | | |

[a,b]Means with different superscripts in the same column are different ($P < 0.05$).

[w–z]Means with different superscripts in the same row are different ($P < 0.05$).

[1]Treatments consisted of a control diet (CL) and the same diet containing 14 g/d of *Saccharomyces cerevisiae*-based yeast culture (YC; Diamond V XPC$_{LS}$ Yeast Culture, Diamond V Mills Inc., Cedar Rapids, IA).

[2]Fixed effects were hour postfeeding (H), day (D), hour within day (H × D), treatment (T), hour within treatment (H × T), and other nonsignificant ($P > 0.10$) effects, including period, sequence, day within treatment, and hour within day within treatment.

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**Table 3.** Copies of 16S rRNA gene of *Streptococcus bovis* and *Megasphaera elsdenii* (logarithm of target copies/mL of ruminal fluid; each sample was run in duplicate, n = 20) and ruminal fluid viscosity (centipoises, n = 20) of animals experiencing a digestive upset on or around the day it appeared (0 d)

| | Day | | | | | | Treatment[1] | | | *P*-value[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | −3 | −2 | −1 | 0 | 1 | 2 | 5 | CL | YC | SEM | H | D | H × D | T | H × T |
| *S. bovis* | 11.5[y] | —[3] | — | 11.9[x] | — | — | — | 11.5 | 11.9 | 0.29 | 0.38 | 0.05 | 0.99 | 0.22 | 0.10 |
| *M. elsdenii* | 3.07 | — | — | 4.75 | — | — | — | 4.36 | 3.45 | 1.01 | 0.77 | 0.09 | 0.70 | 0.56 | 0.94 |
| Viscosity | | | | | | | | | | | | | | | |
| 0 h | 7.29[a,x] | 7.95[a,x] | 8.53[a,wx] | 10.3[a,w] | 6.56[a,x] | 4.11[a,xy] | 3.84[a,y] | 7.17 | 6.71 | 0.56 | <0.01 | <0.01 | 0.09 | 0.57 | 0.44 |
| 6 h | 3.90[b,xy] | 4.49[b,x] | 4.79[b,x] | 6.61[b,w] | 2.66[b,yz] | 2.12[b,z] | 1.99[b,z] | 3.82 | 3.76 | | | | | | |

[a,b]Means with different superscripts in the same column are different ($P < 0.05$).
[w–z]Means with different superscripts in the same row are different ($P < 0.05$).
[1]Treatments consisted of a control diet (CL) and the same diet containing 14 g/d of *Saccharomyces cerevisiae*-based yeast culture (YC; Diamond V XPC$_{LS}$ Yeast Culture, Diamond V Mills Inc., Cedar Rapids, IA).
[2]Fixed effects were hour postfeeding (H), day (D), hour within day (H × D), treatment (T), hour within treatment (H × T), and other nonsignificant ($P > 0.10$) effects, including period, sequence, day within treatment, and hour within day within treatment.
[3]Not determined.

**Table 4.** Foam height and strength of ruminal fluid from animals experiencing a digestive upset

| | Treatment[1] | | | |
|---|---|---|---|---|
| Item | CL | YC | SEM | *P*-value |
| Foam height, cm | 17.8 | 16.6 | 1.9 | 0.54 |
| Foam strength, min | 32.3 | 12.1 | 5.9 | 0.02 |

[1]Treatments were the ruminal fluid from heifers with yeast culture addition (YC, n = 10; Diamond V XPC$_{LS}$ Yeast Culture, Diamond V Mills Inc., Cedar Rapids, IA) and from control animals (CL, n = 10), collected on the day after digestive upset before feeding.

## *Conclusions*

Within the conditions of this study, the dietary challenge caused digestive upsets in 83% of the heifers after an average of 7 d. All cases were diagnosed by a reduction in feed intake. The determinant factor for this upset was a previous period of low ruminal pH, accompanied by an increase in total VFA concentration and, in some cases, by an increase in lactate concentration. The addition of YC did not affect the incidence or the time to cause the digestive upset. However, YC reduced the foam strength, suggesting potential benefits in reducing the risk of developing bloat.

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