

Effects of yeast culture on rumen microbial fermentation of Holstein heifers challenged with high-concentrate feeding¹

D. Moya*, S. Calsamiglia*, A. Ferret*, J.I. Fandiño*, and L. Castillejos[†]

*Department of Animal and Food Sciences, Autonomous University of Barcelona, Bellaterra, Spain

[†]Diamond V Europe, Assen, The Netherlands

ABSTRACT: Effects of yeast culture on rumen microbial fermentation in heifers during a feed challenge with a diet rich in rapidly fermentable carbohydrates were studied. A crossover design was performed using 12 heifers (277 ± 28 kg BW) fitted with ruminal trocars to study feed intake, ruminal pH, ruminal fermentation, ruminal fluid viscosity and foam height and strength. The feed challenge consisted of a 3 week period with a 100% forage diet and 2 additional weeks where heifers were progressively changed to a high concentrate diet by increasing grain at a rate of 2.5 kg/d and decreasing forage in same proportion until a 10:90 forage to concentrate diet was reached. Heifers were fed diets that contained 0 (control) or 14 g yeast culture (XPC_{LS}; Diamond V XPC_{LS}TM Yeast Culture, Diamond V Mills, Cedar Rapids, IA). Rumen samples were collected at 0 and 6 h post-feeding on the first and last day of the 100% forage diet, and daily during the transition into and feeding of the high concentrate diet. Ruminal pH, total and individual VFA, lactic acid, and rumen fluid viscosity were measured. Finally, foam

height and strength were determined for each heifer when digestive upset was detected. Yeast culture had no effect on ruminal fermentation during the 100% forage diet period except for an increase ($P < 0.01$) in ammonia N concentration. During the feed challenge, XPC_{LS} did not affect the incidence (83.3%) or time (7.00 ± 0.62 d) to cause a digestive upset. However, on the day after the upset, heifers supplemented with XPC_{LS} had increased ($P < 0.05$) ruminal pH and branched chain volatile fatty acids and reduced ($P < 0.05$) lactate concentration. Yeast culture reduced ($P < 0.05$) ruminal fluid viscosity 3 d prior to and 2 d after digestive upset, as well as foam strength on the day of digestive upset. Results indicate that XPC_{LS} had no effect on ruminal fermentation with a 100% forage diet and on the development of digestive upset during the feed challenge. However, XPC_{LS} contributed to a faster recovery by increasing ruminal pH and reducing lactate production. In addition, XPC_{LS} reduced the foam strength and viscosity of rumen fluid which could reduce the risk of developing bloat.

Keywords: Yeast culture, microbial fermentation, high-concentrate, acidosis, bloat

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INTRODUCTION

Receiving cattle are changed from high forage to high concentrate diets through a relatively long adaptation period that reduces growth rate. While a faster adaptation may be desirable, an abrupt change may increase the risk of digestive upsets like ruminal acidosis, which are often associated with other health problems and reduced production (Krause and Oetzel, 2006). When carbohydrate supply is increased abruptly, volatile fatty acids (VFA) and lactate in the rumen fluid increases (Owens et al., 1998), resulting in a lower pH and acidosis (Nocek, 1997). Another frequent digestive upset is bloat, which generally occurs in cattle fed diets that contain more than 50% grain and when cattle are shifted from low grain to high grain diets (Cheng and

Hironaka, 1973). With feedlot bloat, the foam-producing agents are likely related to the production of mucoproteins by microbes (Cheng and Costerton, 1975; Cheng et al., 1976). Antimicrobial feed additives could facilitate an uneventful transition to a high concentrate diet through the modulation of changes in microbial populations and their activity in the rumen (Nagaraja et al., 1995). However, the European Union legislation banned the use of antibiotics in animal feeds beginning in January of 2006 (European Union, 2003).

One suggested alternative to antimicrobial feed additives is using yeast culture as a dietary supplement (Calsamiglia et al., 2005). Yeast culture is produced by fermenting selected liquid and cereal grain raw ingredients with bakers yeast (*S. cerevisiae*) and drying the entire culture medium without destroying components associated with the yeast fermentation such as B vitamins and other fermentation products. The

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observed benefits of supplementing yeast culture include a higher pH, increased number of cellulolytic and lactate utilizing bacteria in the rumen (Wiedmeier et al., 1987; Harrison et al., 1988; Callaway and Martin, 1997), higher DM intake (Dann et al., 2000; Lesmeister et al., 2004; Erasmus et al., 2005), improved DM and CP digestion (Wiedmeier et al., 1987; Yoon and Stern, 1996; Miller-Webster et al., 2002) and reduced acetate to propionate ratio (Harrison et al., 1988; Lynch and Martin, 2002; Erasmus et al., 2005). These effects have been attributed at least to the supply of nutrients (i.e., B vitamins, amino acids, organic acids) which stimulate the growth of specific rumen bacteria such as fiber-digesting and lactate-utilizing bacteria, which may help stabilize ruminal fermentation in high concentrate diets. Therefore, the objective of this study was to evaluate the effects of yeast culture on rumen microbial fermentation in heifers during the adaptation period to a diet rich in rapidly fermentable carbohydrates.

MATERIALS AND METHODS

In vivo cattle trial

Animal preparation. Twelve Holstein heifers (initial BW of 277 ± 28 kg), each fitted with a 1 cm i.d. plastic ruminal trocar (Divasa Farmavic SA, Vic, Spain), were individually housed in tie-stalls at the Animal and Field Experimental Farm Service of the Autonomous University of Barcelona (Spain). Ruminal fistulation was performed under local anesthesia and with full aseptic precautions one week prior to beginning the experiment. The research protocol was approved by the Campus Laboratory Animal Care Committee of the Autonomous University of Barcelona (Spain).

Experimental design. The experiment was performed using a crossover design. Each period consisted of 6 weeks, with week 1 through 3 being used for adaptation to a 100% forage diet (a mixture of 80% fescue hay and 20% alfalfa pellets) and treatments. After these three weeks, heifers were progressively changed to a high concentrate diet over 4 days to induce a digestive upset by increasing grain at a rate of 2.5 kg/d and decreasing forage in the same proportion, until a 10:90 forage to concentrate diet was reached and fed for 2 weeks. 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%), megalac (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 CP, effective fiber, mineral, and vitamin requirements of cattle (NRC, 1996) but intended to include a high proportion of non-

structural carbohydrates in the concentrate (54.3% DM). Feed was offered once a day (0900h), and was taken out of the feeders at 2100 h to force the consumption of concentrate as quickly as possible in the morning. Diet and management was designed to increase the chances of a digestive upset.

Treatments consisted of control with no supplemental yeast culture and 14 g/d of *Saccharomyces cerevisiae* based yeast culture (XPC_{LS}; Diamond V XPC_{LS}TM Yeast Culture, Diamond V Mills Inc., Cedar Rapids, IA, USA). Yeast culture treatment was offered daily at 0900 h mixed with 100 g of the same concentrate used in the experiment to guarantee consumption of the whole dose. Control heifers received the same amount of concentrate without yeast culture addition.

Confirmation of digestive upset was determined by visual observation of bloat (Paisley and Horn, 1998) and (or) a reduction in feed intake by 50% or more compared to the previous day's DMI. When a positive case was observed, this animal was switched into a 100% forage diet with no yeast culture and recovery was observed. After a minimum of 7 d wash out period, treatments were changed (cross over design) and the same protocol was repeated.

Sample Collection and Analyses. Daily DMI was measured during the adaptation period. Through the transition into and during the challenge with 10:90 forage to concentrate diet, DM intake was measured at 3, 6 and 12 h after the morning feeding. Feed DM was determined by oven drying at 105°C for 24 h. Ruminal fluid was collected at the start and end of the adaptation period, and daily after the start of the transition and during the challenge period. However, only samples collected on d -3, -2 and -1 (prior to the digestive upset), d 0 (on the day of digestive upset), and d 1, 2, and 5 (post digestive upset) were analyzed. Ruminal samples were collected 0, 2, 6 and 12 h after feeding to determine the pH (model 507, Crison Instruments SA, Barcelona, Spain). Samples collected 0 and 6 h after feeding were strained through 2 layers of cheesecloth, and 4 subsamples of the filtrate were frozen at -20°C for analyses of VFA, lactate, ammonia N, and viscosity. Additional samples of rumen fluid without being strained were collected on d 1 at 0 h to determine foaming properties.

Volatile fatty acids and lactate of ruminal fluid were analyzed through a modification of the GC method described by Richardson (1989) and Jensen (1995). To conserve the sample, 4-mL of ruminal fluid were added to 1 mL of a solution made up of 1% (wt/wt) 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. As a pre-analysis treatment, samples were thawed and centrifuged at 15,000 x g for 15 min and diluted 1:1 in distilled water.

Ammonia N concentration ($\text{NH}_3\text{-N}$) was analyzed by spectrophotometry (Libra S21, Biochrom Analytical Instruments, Cambridge, UK) as described by Chaney and Marbach (1962).

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 d 1 (a day after a positive case of digestive upset in a heifer), 50 mL of fresh rumen fluid was collected from this animal before feeding. Ruminal fluid was poured into a glass cylinder (37 mm diameter x 30 cm length) and CO_2 gas was bubbled through a bottom inlet at 60 kPa for 30s, 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 to the original fluid volume was used as an index of foam strength.

Statistical analyses

All statistical analyses were conducted using SAS (version 9.1 SAS Institute, Inc., Cary, NC). Results were analyzed using PROC MIXED for repeated measures, considering heifer as the subject with the Compound Symmetry structure, and the period as random factor. Two models were utilized:

MODEL 1: $Y_{ik} = \mu + A + H_i + T_k + (H*T)_{ik} + \varepsilon_{ik}$

MODEL 2: $Y_{jk} = \mu + A + D_j + T_k + (D*T)_{jk} + \varepsilon_{jk}$

Where:

Y = Measured parameter.

μ = Mean value.

A = Animal effect (subject).

H_i = Hour post feeding effect (fixed factor).

D_j = Day effect (fixed factor).

T_k = Treatment effect (fixed factor).

ε = Error.

Statistical differences were declared at $P < 0.05$ using a multiple comparison test (Tukey, 1953).

RESULTS AND DISCUSSION

Statistical analyses did not show interactions between the main factors in both models ($H*T$ and $D*T$). Therefore, main factors are discussed separately unless otherwise indicated and in three sections organized in chronological order: 1) effects of XPCs during the adaptation period, 2) changes occurring through the induction of the digestive upset, and 3)

effects of yeast culture on high-concentrate feeding challenge.

Adaptation period

During the adaptation period, no differences between treatments were observed for DMI (5.37 ± 0.19 kg DM), pH (6.78 ± 0.06), VFA concentration (96.3 ± 2.32 mM) and profile, and ruminal fluid viscosity (2.41 ± 0.11 centipoises (10^{-3} Pa · sec)). Ammonia N concentrations were lower ($P < 0.05$) on the last day of the adaptation period than the first adaptation day and at 6 h post-feeding than at 0 h post-feeding. Heifers supplemented with XPCs had higher ($P < 0.01$) $\text{NH}_3\text{-N}$ than control heifers (Table 1). Effects of yeast culture on $\text{NH}_3\text{-N}$ are inconsistent, with some studies reporting a decrease (Harrison et al., 1988), but others reporting no differences among treatments (Wiedmeier et al., 1987; Yoon and Stern, 1996; Erasmus et al., 2005).

Results from the adaptation period indicate that, except for the $\text{NH}_3\text{-N}$ concentration, XPCs had no effects on rumen fermentation of heifers fed a 100% forage diet. Most experiments evaluating yeast culture have been conducted with animals eating different concentrations of concentrate. Few works have studied the influence of yeast culture on 100% forage diets, reporting little effects (Olson et al., 1994a; 1994b).

Changes in rumen fermentation during the induction of the digestive upset

A total of 20 cases (83.3%) of digestive upsets were recorded in both periods. There were four cases with no signs of digestive problems after 14 days of high concentrate diet. All cases were diagnosed due to a 50% reduction in DMI (Table 2) and visual signs of bloat were not observed. Reduction in DMI after an abrupt inclusion of concentrate in the diet has been attributed to an accumulation of VFA causing an increase in osmolarity and the development of acidosis (Nocek, 1997; Owens et al., 1998; Krause and Oetzel, 2006). On average, it took 7.00 ± 0.62 d to cause a digestive upset.

Ruminal pH decreased ($P < 0.01$) after feeding as expected except on d 0, when the pH at time of feeding was lower than prior to digestive upset and increased after feeding due to the reduction in feed intake previously mentioned, becoming higher ($P < 0.05$) at 6 and 12 h post-feeding compared with the same hours of d -3, -2, and -1 (Table 3). The pH at 12 h on d 3 prior to digestive upset were between 5.62 and 5.46 (Table 3), probably due to rapid fermentation of the high-concentrate diets together with lower rumination and salivation normally associated with these type of diets (Emery and Brown, 1961; Balch, 1971), but pH recovered to above 6.0 at 0 h on d 1. However, on d 0,

pH was lower ($P < 0.05$) at 0 h than on d -3, -2, and -1 at the same hour, remaining under pH 6.0 (Table 3). Therefore, the reduction in feed intake was likely the consequence of a lack of recovery of ruminal pH, which maintained ruminal pH 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 a subacute ruminal acidosis and reduced feed intake (Owens et al., 1998). The increase in pH values post digestive upset is attributed to the change to a 100% forage diet.

Total VFA concentration followed the opposite patterns, and was higher ($P < 0.05$) at 6 h on all days, as expected, except on d 0, when it was lower at 0 vs. 6 h post-feeding, due to the reduction in feed intake (Table 4). Post digestive upset, the total VFA concentrations were lower due to the change to a 100% forage diet. Acetate and propionate concentrations vary complementarily (Table 4). Acetate concentrations decreased ($P < 0.05$) 6 h post-feeding prior to the digestive upset, but increased ($P < 0.01$) 6 h post-feeding on d 1 and 2. Propionate concentrations increased ($P < 0.05$) 6 h post-feeding prior to digestive upset, but decreased ($P < 0.01$) 6 h post-feeding on d 2. Acetate (on d 5) and propionate (on d 1 and 2) concentrations increased ($P < 0.05$). Due to these variations, the acetate to propionate ratio decreased ($P < 0.01$) 6 h post-feeding prior to digestive disease, and increased ($P < 0.01$) 6 h post-feeding on d 2 (Table 4). These effects were due to the change to a 100% forage diet. On d 0, butyrate concentrations decreased ($P < 0.05$) 6 h post-feeding compared with 0 h (Table 5). Butyrate concentrations were also lower ($P < 0.05$) post digestive upset due to the change of diet. Branched chain VFA concentration decreased ($P < 0.05$) 6 h post-feeding on d -2 and -1 and d 1, 2 and 5. Branch chain VFA production was also lower post digestive upset at 0 and 6 h post-feeding, and was likely due to the change of diet (Table 5). Ammonia N concentration increased ($P < 0.05$) 6 h post-feeding on d -3 and -2, but decreased on d 0 ($P < 0.10$) and d 1, 2 and 5 ($P < 0.05$). In addition, $\text{NH}_3\text{-N}$ was lower at 6 h post-feeding when heifers received a 100% forage diet (Table 5).

Lactate production increased ($P < 0.05$) at 0 h on d 0 compared with the days around it, while at hour 6 post-feeding the high variation of lactate concentration did not allow detection of differences (Table 6). When heifers are switched abruptly from high-forage to a high-concentrate diet, the rumen may become severely acidotic by an overgrowth of starch-fermenting and lactate-producing bacteria (Owens et al., 1998). The increase in lactate production detected at hour 0 on d 0 may justify why ruminal liquid remained under pH 6.0 during 18 h prior to it, causing the drop of feed intake.

In addition, the excessive intake of readily fermented starch causes a rapid growth of amylolytic bacteria like *Streptococcus bovis* (Owens et al., 1998), which release mucopolysaccharides (slime). Excessive production of bacterial slime contributes to the increase in viscosity of ruminal fluid, favoring the development of bloat (Cheng et al., 1976). Ruminal fluid viscosity was analyzed to determine the potential appearance of bloat in the high-concentrate feeding challenge. At 0 h, after 12 h without feeding, ruminal fluid viscosity was higher ($P < 0.01$) than at 6 h post-feeding (Table 7), probably due to the fact that water intake was higher at 6 h than after 12 h without feed. This water intake may have affected the physical characteristics of the ruminal fluid. Ruminal fluid viscosity also increased ($P < 0.05$) at 6 h post-feeding on d 0 compared with d -3 and -2, and decreased once heifers were fed the 100% forage diet (Table 7).

The lack of clinical signs and the reduction in DMI and pH suggest that heifers were in subacute ruminal acidosis, but heifers were shifted into a 100% forage diet at the first symptom of upset. If heifers had continued with the same diet, the upset may have ended in chronic acidosis or bloat.

Effect of yeast culture during the high-concentrate feeding challenge

Average feed consumption of heifers supplemented with XPC_{LS} (6.28 ± 0.40 kg DM) was not different than control heifers (6.70 ± 0.31 kg DM). Others studies have shown that yeast culture increased DM intake in dairy cows during the transition from a pre-partum to a post-partum diet (Dann et al., 2000; Erasmus et al., 2005). Differences between forage and grain diets and the incidence of digestive upsets increased the variability of DM intake, which may have reduced the probability of observing differences in DMI due to the addition of yeast culture. However, when heifers suffered digestive upset, feed intake on d -3 and -2 tended ($P < 0.10$) to be higher in heifers supplemented with XPC_{LS} (Table 2).

Yeast culture addition increased ($P < 0.05$) the pH on d 1 (Table 3), and tended to increase ($P < 0.10$) the pH at 0 h on the days around digestive upset (data not shown). These results suggest that XPC_{LS} addition contributed to the recovery of the ruminal pH after the digestive upset, and helped to maintain higher pH after 12 h without feed. However, heifers supplemented with XPC_{LS} had a tendency ($P < 0.10$) for a more abrupt post-feeding fall in pH after feeding on d -1 (statistical data not shown). Others reported that yeast culture maintains a more stable pH (Wiedmeier et al., 1987; Harrison et al., 1988; Callaway and Martin, 1997), but Sullivan and Martin (1999) and Erasmus (2005) did not observe changes in ruminal pH.

Total VFA concentration was higher ($P < 0.05$) when XPC_Ls was supplemented on d -1 (Table 4), and probably explains the more abrupt post-feeding fall in pH previously mentioned. Supplementation with yeast culture increased (Callaway and Martin, 1997; Miller-Webster et al., 2002) or had no effect (Harrison et al., 1988; Yoon and Stern, 1996) on total VFA concentration.

No effects of XPC_Ls were found on acetate and propionate concentrations, but the acetate:propionate ratio decreased ($P < 0.05$) on d 2 (Table 4). Results do not show a consistent effect of yeast culture on acetate and propionate concentrations, unlike previous reports where yeast culture reduced the acetate:propionate ratio by stimulating the concentrations of propionate at the expenses of acetate (Harrison et al., 1988; Lynch and Martin, 2002; Erasmus et al., 2005). Yeast culture reduced ($P < 0.05$) butyrate concentrations on d -1 and d 0 (Table 5), and was maintained above control heifers ($P < 0.01$) at 0 h during the days around the digestive upset (statistical data not shown). While some studies reported that supplementation of yeast culture had no effect on butyrate concentrations (Yoon and Stern, 1996), Lynch and Martin (2002) reported an increase in butyrate concentrations. Branched chain VFA (BCVFA) production increased with XPC_Ls treatment on d -1 ($P < 0.01$), and d 1 ($P < 0.05$; Table 5). Branch chain VFA increase may suggest an increase in deamination activity, which usually is accompanied with an increase in NH₃-N. However, the effects of yeast culture on NH₃-N on the days around the digestive upset were limited to a trend to be reduced ($P < 0.10$) on d -2 (Table 5).

Yeast culture reduced the lactate concentration on d 1 ($P < 0.05$) and d 2 ($P < 0.10$; Table 6). This result may be related with the better recovery of pH when XPC_Ls was supplemented. Callaway and Martin (1997) demonstrated that a sterilized filtrate of yeast culture stimulated growth of lactate utilizing ruminal bacteria *Selenomonas ruminantium* and *Megasphaera elsdenii*, but others did not find effects of yeast culture on lactate utilization (Sullivan and Martin, 1999; Lynch and Martin, 2002).

It is widely accepted that frothy bloat in cattle is caused by the development of foam in the rumen (Mangan et al., 1959; Cheng et al., 1998). Ruminal fluid viscosity and foam height and strength were parameters analyzed to determine the potential implication of XPC_Ls on the incidence of bloat. Addition of XPC_Ls reduced ($P < 0.05$) ruminal fluid viscosity on d -3 and d 2 (Table 7). Results of foam height and strength test, conducted on d 1 just before feeding, show that XPC_Ls reduced ($P < 0.05$) foam strength of the ruminal fluid (Table 8). In spite of the effects of XPC_Ls on ruminal fluid viscosity and foam strength these effects did not occur simultaneously.

Although apparent symptoms of bloat were not observed as a result of diet challenge in this study, the reduced foam strength along with reduced viscosity of rumen fluid warrant further research to investigate the potential effect of XPC_Ls on reducing the risk of bloat.

CONCLUSIONS

In these conditions, changing from a high forage to a high concentrate diet caused digestive upsets in 83% of heifers in an average of 7 d. All cases were diagnosed by a reduction in feed intake by 50% or more. The determinant factor for this upset was a previous period of low ruminal pH accompanied by an increase in total VFA production and, in some cases, by an increase in lactate concentrations. These data coupled with the increase in ruminal fluid viscosity suggest that if the feed challenge had been maintained longer, heifers may have developed chronic acidosis or bloat. The addition of XPC_Ls did not affect ruminal fermentation during the adaptation period with a 100% forage diet, except for the increase of NH₃-N concentration. During the feed challenge, the addition of XPC_Ls did not affect the incidence or the time to cause a digestive upset. However, XPC_Ls helped in the recovery after the digestive upset, with a higher ruminal pH and lower lactate production. In addition, XPC_Ls reduced foam strength and rumen fluid viscosity, which could reduce the risk of developing bloat.

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Table 1. Effects of ammonia N concentration (mg/100mL) during the adaptation period

Item	Hours post-feeding		Treatment ¹			P-value ²	
	Hour 0	Hour 6	CON	XPC _{LS}	SEM	H	T
First adaptation day	11.26 ^a	11.60 ^a	10.70	12.16	0.80	NS	NS
Last adaptation day	7.56 ^b	5.76 ^b	5.96	7.35	1.00	0.01	0.01
SEM	1.40	1.13					

¹Treatments were yeast culture addition (XPC_{LS}) and control animals (CON).

²Main factors were hour post-feeding (H) and treatment (T).

NS: No significant differences were observed.

^{a,b}Means with different superscripts in the same column are different ($P < 0.05$).

Table 2. Feed intake (kg DM) prior to and on the day when digestive upset was declared during the sampling period

Item	Hours post-feeding			Treatment ¹			P-value ²	
	Hour 2	Hour 6	Hour 12	CON	XPC _{LS}	SEM	H	T
3 d before	3.12 ^a	4.89 ^a	7.06 ^a	4.76	5.29	0.46	0.01	0.09
2 d before	2.91 ^a	4.95 ^a	7.34 ^a	4.81	5.32	0.33	0.01	0.10
1 d before	2.39 ^a	4.75 ^a	7.10 ^a	4.93	4.56	0.93	0.01	NS
Upset day	0.52 ^b	1.09 ^b	2.28 ^b	1.18	1.42	0.26	0.01	NS
SEM	0.46	0.43	0.46					

¹Treatments were yeast culture addition (XPC_{LS}) and control animals (CON).

²Fixed factors were hour post ingestion (H), treatment (T).

NS: No significant differences were observed.

^{a,b}Means with different superscripts in the same column are different ($P < 0.05$).

Table 3. Ruminal fluid pH post feeding on the days around the digestive upset

Item	Hours post-feeding				Treatment ¹			P-value ²	
	Hour 0	Hour 3	Hour 6	Hour 12	CON	XPC _{LS}	SEM	H	T
3 d before	6.67 ^{ab}	6.11 ^c	5.93 ^b	5.62 ^b	6.03	6.14	0.08	0.01	NS
2 d before	6.53 ^b	6.11 ^c	5.86 ^b	5.60 ^b	6.02	6.03	0.09	0.01	NS
1 d before	6.46 ^b	6.08 ^c	5.75 ^b	5.46 ^b	5.95	5.93	0.10	0.01	NS
Upset day	5.92 ^c	6.21 ^{bc}	6.31 ^a	6.03 ^a	6.13	6.11	0.13	0.01	NS
1 d after	7.01 ^a	6.48 ^{ab}	6.40 ^a	6.31 ^a	6.48	6.62	0.08	0.01	0.02
2 d after	7.02 ^a	6.70 ^a	6.55 ^a	6.30 ^a	6.64	6.64	0.07	0.01	NS
5 d after	7.03 ^a	6.46 ^{ab}	6.45 ^a	6.20 ^a	6.58	6.50	0.06	0.01	0.09
SEM	0.10	0.08	0.09	0.08					

¹Treatments were yeast culture addition (XPC_{LS}) and control animals (CON).

²Fixed factors were hour post ingestion (H), treatment (T).

NS: No significant differences were observed.

^{a,b,c}Means with different superscripts in the same column are different ($P < 0.05$).

Table 4. Total VFA production (mM), acetate concentrations (mol/100mol), propionate concentrations (mol/100mol) and acetate:propionate ratio (**C2:C3**) on the days around the digestive upset during the sampling period

Item	Hours post-feeding		Treatment ¹		SEM	P-value ²	
	Hour 0	Hour 6	CON	XPC _{LS}		H	T
Total VFA, mM							
3 d before	107.2 ^{ab}	127.3 ^a	118.3	116.2	6.03	0.01	NS
2 d before	111.6 ^a	131.1 ^a	121.0	121.7	5.44	0.01	NS
1 d before	117.6 ^a	131.3 ^a	119.4	129.5	4.61	0.01	0.05
Upset day	124.8 ^a	94.12 ^b	111.1	107.9	7.30	0.01	NS
1 d after	75.70 ^c	90.99 ^b	84.78	81.92	5.14	0.01	NS
2 d after	74.62 ^c	86.35 ^b	82.85	78.11	8.68	0.01	NS
5 d after	87.78 ^{bc}	98.90 ^b	90.90	95.27	8.05	0.01	NS
SEM	4.87	5.44					
Acetate, mol/100mol							
3 d before	65.03 ^{ab}	62.25 ^b	63.01	64.27	1.43	0.01	NS
2 d before	63.56 ^b	61.24 ^b	62.69	62.11	1.30	0.03	NS
1 d before	63.33 ^b	61.10 ^b	62.14	62.28	1.17	0.04	NS
Upset day	63.96 ^b	63.02 ^b	61.84	65.14	1.95	NS	NS
1 d after	60.54 ^b	63.08 ^b	61.76	61.86	1.06	0.01	NS
2 d after	63.15 ^b	65.24 ^{ab}	64.81	63.58	1.14	0.01	NS
5 d after	69.11 ^a	68.36 ^a	68.83	68.65	0.57	NS	NS
SEM	1.18	1.02					
Propionate, mol/100mol							
3 d before	16.89 ^b	19.34 ^{bc}	18.45	17.79	0.95	0.01	NS
2 d before	17.11 ^b	19.46 ^{bc}	17.80	18.77	0.98	0.01	NS
1 d before	16.93 ^b	18.92 ^{bc}	17.15	18.70	0.62	0.04	0.09
Upset day	16.72 ^b	19.88 ^{abc}	18.70	17.90	1.54	0.07	NS
1 d after	24.09 ^a	23.79 ^a	23.86	24.02	1.37	NS	NS
2 d after	25.11 ^a	22.34 ^{ab}	23.06	24.39	1.74	0.01	NS
5 d after	17.40 ^b	17.74 ^c	17.68	17.45	0.37	NS	NS
SEM	1.03	1.01					
C2:C3							
3 d before	4.00 ^{ab}	3.31 ^{ab}	3.57	3.73	0.22	0.01	NS
2 d before	3.87 ^{ab}	3.23 ^{ab}	3.67	3.42	0.18	0.01	NS
1 d before	3.90 ^{ab}	3.29 ^{ab}	3.76	3.44	0.15	0.01	NS
Upset day	4.45 ^a	3.56 ^{ab}	3.61	4.40	0.62	NS	NS
1 d after	2.74 ^b	2.79 ^b	2.78	2.75	0.24	NS	NS
2 d after	2.64 ^b	3.01 ^{ab}	2.93	2.72	0.26	0.01	0.05
5 d after	4.01 ^{ab}	3.89 ^a	3.93	3.96	0.10	0.07	NS
SEM	0.30	0.20					

¹Treatments were yeast culture addition (XPC_{LS}) and control animals (CON).

²Fixed factors were hour post ingestion (H), treatment (T)

NS: No significant differences were observed.

^{a,b,c}Means by item with different superscripts in the same column are different ($P < 0.05$).

Table 5. Butyrate concentrations (mol/100mol), branched chain VFA production (mM) and ammonia N production (mg/100mL) on the days around the digestive upset during the sampling period

Item	Hours post-feeding		Treatment ¹		SEM	P-value ²	
	Hour 0	Hour 6	CON	XPC _L s		H	T
Butyrate, mol/100mol							
3 d before	14.09 ^a	14.34 ^a	14.77	13.66	0.64	NS	0.06
2 d before	15.32 ^a	15.28 ^a	15.64	14.97	1.13	NS	NS
1 d before	15.78 ^a	16.27 ^a	17.01	15.04	0.88	NS	0.02
Upset day	15.64 ^a	12.74 ^a	15.71	12.67	1.22	0.03	0.03
1 d after	10.49 ^b	9.02 ^b	10.00	9.51	0.64	0.08	NS
2 d after	7.78 ^b	8.51 ^b	8.31	7.98	0.53	NS	NS
5 d after	9.42 ^b	10.15 ^b	9.87	9.70	0.52	0.07	NS
SEM	0.72	0.80					
BCVFA, mM							
3 d before	1.79 ^{ab}	1.61 ^a	1.63	1.77	0.13	NS	NS
2 d before	1.86 ^a	1.65 ^a	1.71	1.79	0.14	0.05	NS
1 d before	1.95 ^a	1.68 ^a	1.64	2.00	0.14	0.01	0.01
Upset day	1.72 ^{ab}	1.39 ^a	1.39	1.72	0.21	0.06	0.09
1 d after	1.56 ^{abc}	0.92 ^b	1.15	1.33	0.09	0.01	0.04
2 d after	1.20 ^c	0.79 ^b	1.00	0.98	0.08	0.01	NS
5 d after	1.39 ^{bc}	0.94 ^b	1.06	1.27	0.20	0.01	0.06
SEM	0.13	0.14					
Ammonia N, mg/100mL							
3 d before	8.26 ^{bc}	13.07 ^a	11.83	9.49	1.80	0.03	NS
2 d before	9.02 ^{ab}	13.09 ^a	12.84	9.28	1.30	0.05	0.08
1 d before	8.41 ^{bc}	10.70 ^a	9.45	9.67	1.20	NS	NS
Upset day	13.39 ^a	10.28 ^a	11.79	11.89	1.67	0.06	NS
1 d after	6.03 ^{bc}	4.24 ^b	4.73	5.53	0.94	0.03	NS
2 d after	5.15 ^c	3.28 ^b	4.43	4.00	0.83	0.01	NS
5 d after	7.63 ^{bc}	4.72 ^b	5.90	6.44	1.39	0.01	NS
SEM	1.02	1.50					

¹Treatments were yeast culture addition (XPC_Ls) and control animals (CON).

²Fixed factors were hour post ingestion (H), treatment (T).

NS: No significant differences were observed.

^{a,b,c}Means by item with different superscripts in the same column are different ($P < 0.05$).

Table 6. Lactate concentration production (mM) on the days around the digestive upset during the sampling period

Item	Hours post-feeding		Treatment ¹		SEM	P-value ²	
	Hour 0	Hour 6	CON	XPCLs		H	T
3 d before	1.00 ^b	1.08	0.99	1.09	0.08	NS	NS
2 d before	1.03 ^b	1.22	1.19	1.06	0.12	NS	NS
1 d before	1.21 ^b	1.20	1.06	1.34	0.17	NS	NS
Upset day	10.16 ^a	5.25	4.48	10.92	4.43	NS	NS
1 d after	1.07 ^b	1.17	1.25	0.98	0.13	NS	0.03
2 d after	0.90 ^b	1.10	1.11	0.90	0.09	0.08	0.09
5 d after	0.96 ^b	1.03	0.91	1.07	0.08	NS	NS
SEM	1.86	1.25					

¹Treatments were yeast culture addition (XPCLs) and control animals (CON).

²Fixed factors were hour post ingestion (H), treatment (T).

NS: No significant differences were observed.

^{a,b}Means by item with different superscripts in the same column are different ($P < 0.05$).

Table 7. Ruminal fluid viscosity (centipoises, cP) on the days around the digestive upset during the sampling period

Item	Hours post-feeding		Treatment ¹		SEM	P-value ²	
	Hour 0	Hour 6	CON	XPCLs		H	T
3 d before	7.30 ^{ab}	3.88 ^{bcd}	6.27	4.92	0.73	0.01	0.05
2 d before	8.04 ^{ab}	4.58 ^{bc}	6.74	5.88	0.80	0.01	NS
1 d before	8.59 ^{ab}	4.85 ^{ab}	6.78	6.67	0.92	0.01	NS
Upset day	10.51 ^a	6.80 ^a	8.03	9.28	1.30	0.01	NS
1 d after	6.51 ^{bc}	2.58 ^{cd}	4.76	4.34	0.53	0.01	NS
2 d after	4.04 ^c	2.09 ^d	3.37	2.77	0.21	0.01	0.03
5 d after	3.79 ^c	1.93 ^d	2.86	2.86	0.47	0.01	NS
SEM	0.78	0.47					

¹Treatments were yeast culture addition (XPCLs) and control animals (CON).

²Fixed factors were hour post ingestion (H), treatment (T).

NS: No significant differences were observed.

^{a,b,c,d}Means by item with different superscripts in the same column are different ($P < 0.05$).

Table 8. Foam height and strength for each treatment

Item	Treatment ¹		SEM	P-value ²
	Control	XPCLs		Treatment
Foam height, cm	17.85	16.16	2.00	NS
Foam strength, min	32.26	12.08	5.99	0.03

¹Treatments were yeast culture addition (XPCLs) and control animals (CON).

²Fixed factors were treatment.

NS: No significant differences were observed.