

# Rumen fermentation pattern of dairy heifers fed restricted amounts of low, medium, and high concentrate diets without and with yeast culture

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## A B S T R A C T

Restricted feeding and high concentrate diets are potential strategies for growing dairy heifers. Ruminant manipulation with additives such as *Saccharomyces cerevisiae* yeast culture (YC) has been shown to alter digestibility when added to this type of diet. An experiment was conducted to investigate the ruminal fermentation and in situ digestibility of diets with 3 different levels of forage to concentrate (F:C) fed at restricted intake without and with YC addition. Three cannulated post-pubertal Holstein heifers (age  $18.0 \pm 1.2$  months; body weight  $449.6 \pm 19.7$  kg) were fed diets consisting of corn silage as the sole forage source in a 3 period (35-day) Latin square design. Heifers were fed diets for 21 days with no YC addition, followed by 14 days where YC was added to the diet (1 g/kg as fed basis). Low (LC), medium (MC), and high (HC) concentrate diets (20, 40, and 60% concentrate) were fed once daily on a restricted basis to provide 0.22 Mcal ME/kg empty BW<sup>0.75</sup>. Rumen fluid was sampled on days 18 and 32 of each period, and rumen contents were evacuated on days 21 and 35 of each period. An in situ study was done on days 14 to 17 and on days 28 to 31. Mean ruminal pH was not different between dietary treatments and no YC effect was detected. Mean total volatile fatty acids (VFA) and ruminal ammonia-nitrogen (NH<sub>3</sub>-N) concentration was also not different among diets with different F:C. Molar proportions of acetate were decreased, and propionate were increased; while the acetate-to-propionate ratio was decreased as the concentrate level increased from LC to HC. Total VFA, propionate, and acetate as well as isoacids concentration increased, yet NH<sub>3</sub>-N concentration decreased with YC addition in all diets. From these results we conclude that feeding HC diets in restricted amounts had minimal effects on rumen fermentation rate between different F:C diets. The addition of YC modified NH<sub>3</sub>-N and volatile fatty acid concentrations in the rumen in all 3 diets in this study, presumably through alterations in end-product production and utilization.

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## 1. Introduction

One of the most useful measures of animal performance across species is feed efficiency, a direct marker of the animal's ability to be more productive. Improving the efficiency to convert units of feed to units of growth means less feed is required to raise more livestock in a given amount of time (Loerch, 1990; Hoffman et al., 2007). Another marker

of animal performance is average daily gain (ADG; Gerrard and Grant, 2003). Research suggests that a prepubertal ADG of approximately 800 g/d is appropriate for large breed dairy heifers to maximize first lactation milk yields (Zanton and Heinrichs, 2005). Adequate body size is also necessary, and an age at first calving between 23 and 24 months results in profitable milk production (Pirlo et al., 2000). Since feed costs contribute the most to raising heifers, it is logical to expect that improving feed efficiency could decrease the cost for raising dairy heifers, provided ADG is adequate.

A typical dairy heifer is fed a ration primarily derived from forages as opposed to concentrates. However, there is a large inefficiency associated with this method of feeding due to lower digestibility of most forages, greater metabolic protein

and energy requirements associated with digesting forage (Reynolds et al., 1991), and higher feed costs per unit of energy as compared to concentrates (Zanton and Heinrichs, 2007). The potential therefore exists to replace a significant proportion of the ration forage dry matter (DM) with concentrate DM, reducing some inefficiency associated with raising dairy heifers while maintaining ADG. Experiments have recently been conducted to evaluate heifer growth characteristics and nutrient utilization when fed differing forage to concentrate ratios (F:C) at restricted intakes to achieve similar ADG (Hoffman et al., 2007; Zanton and Heinrichs, 2007). In areas with limited land resources, corn silage is often the forage of choice due to its high DM yield potential per hectare. However, when limit-feeding high energy forages and diets based on corn silage, rumen fermentation and growth of the animal may be challenged.

Yeast culture (YC) has been used as an additive to ruminant diets since 1924, with various results reported in the literature. Results following addition of YC based on *Saccharomyces cerevisiae* to the diet include improved productivity in both lactating and growing animals. Though increases in productivity are generally variable, rumen fermentation rate and patterns seem to be involved in this response (Carro et al., 1992). The mode of action of YC products has not been completely described, but many hypotheses are related to changes in rumen fermentation rate and patterns. Volatile fatty acid (VFA) production, neutral detergent fiber (NDF) digestibility and disappearance, organic matter digestibility, and bacterial and protozoal populations have been enhanced by yeast supplementation (Lascano and Heinrichs, 2007). Decreases in lactic acid concentration and the lag time of DM degradation as well as stabilization of rumen pH are other observed effects (Wallace, 1996). Overall, the rumen environment typically benefits, which leads to an improved metabolic performance of the animal.

Research indicates that including live or dead YC products in adult and young ruminant diets can alter the rumen environment; however, different responses have been found depending on the diet used (Lascano and Heinrichs, 2007). In lactating dairy cows, YC has been shown to increase the nutritional value of high concentrate diets (Arambel and Kent, 1990). Therefore, the purpose of this study was to investigate the effect of YC in the rumen environment when added to limit-fed, high concentrate diets based on corn silage offered to dairy heifers.

## 2. Materials and methods

### 2.1. Animal, housing and diet

Three Holstein dairy heifers, each previously fitted with a 10.6 cm rumen cannula (Bar Diamond, Parma, ID, USA) under anesthesia, were housed in a mechanically ventilated and environmentally controlled tie stall barn. Animal care procedures followed the Pennsylvania State University Institutional Animal Care and Use Committee approval. Total mixed rations (TMR) contained corn silage as the sole forage source, ground corn, soybean meal and heat treated soybean meal (Table 1). Animals were fed once daily at 1000 h and no refusals were observed during the trial. Rations were mixed daily in a rotary mixer (Calan Super Data Ranger; American

Calan, Northwood, NH, USA) for approximately 5 min. Three F:C were formulated and mixed to provide 80:20, 60:40, and 40:60, designated low (LC), medium (MC), and high (HC) concentrate, respectively. Yeast culture product (YC, Yea-Sacc<sup>®</sup> 1026, Alltech, Inc., Nicholasville, KY, USA) was added at a rate of 1 g/kg/d as fed basis. This dose was selected to have a constant inclusion level of YC across treatments with different intakes. Research shows that when the amount of YC is constant regardless of the intake, the YC effect in animals with higher feed consumption tends to disappear (Lascano and Heinrichs, 2007; Robinson and Erasmus, 2007). Feed ingredients and TMR samples were collected daily and composited for every period, dried in a forced air oven (55 °C) immediately after collection, and stored for further analysis. Health conditions of experimental animals were monitored twice daily at 0830 and 2030 h. Heifers had free choice access to water and were released 1 h post-feeding for approximately 1 h daily to a paved exercise lot, except on intensive sampling days. Heifers were kept for 30 d before starting the experiment to adapt to the tie stall facility and experimental diets. Animals at 18.0 ± 1.2 months of age with 449.6 ± 19.7 kg body weight (BW) were randomly assigned to 1 of 3 treatments in a 3 × 3 Latin square design. Each of the 3

**Table 1**

Ingredient and nutrient composition of high concentrate (HC), medium concentrate (MC), and low concentrate (LC) rations fed to heifers.

Composition	Treatment			SE
	LC	MC	HC	
<i>Ingredients (%DM)</i>				
Corn silage <sup>1</sup>	80.00	60.00	40.00	.
Ground corn	5.67	29.60	47.70	.
Soybean meal (SBM)	9.47	9.94	9.00	.
Heat treated SBM	1.60	0.80	0.00	.
Sodium bicarbonate	0.35	0.35	0.35	.
High mineral mix <sup>2</sup>	0.00	1.23	2.95	.
Low mineral mix <sup>3</sup>	2.45	1.48	0.00	.
<i>Nutrients<sup>4</sup></i>				
DM %	41.93 <sup>a</sup>	50.61 <sup>b</sup>	56.51 <sup>c</sup>	1.03
CP %	12.93 <sup>b</sup>	12.38 <sup>a</sup>	13.17 <sup>b</sup>	0.10
Soluble, % of CP	43.65 <sup>b</sup>	43.65 <sup>b</sup>	33.84 <sup>a</sup>	0.76
ADF %	20.45 <sup>c</sup>	17.93 <sup>b</sup>	12.97 <sup>a</sup>	0.19
NDF %	34.18 <sup>c</sup>	30.03 <sup>b</sup>	23.23 <sup>a</sup>	0.38
NFC % <sup>5</sup>	48.05 <sup>a</sup>	53.30 <sup>b</sup>	58.48 <sup>c</sup>	0.14
TDN % <sup>6</sup>	72.13 <sup>a</sup>	75.13 <sup>b</sup>	78.63 <sup>a</sup>	0.11
ME, Mcal/kg DM <sup>7</sup>	2.63 <sup>a</sup>	2.72 <sup>b</sup>	2.84 <sup>c</sup>	0.01
Ca %	0.38	0.37	0.36	0.02
P %	0.30	0.28	0.30	0.01
Mg %	0.20	0.22	0.23	0.01
K %	1.33 <sup>c</sup>	1.12 <sup>b</sup>	1.02 <sup>a</sup>	0.03

<sup>a,b,c</sup> Means in the same row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Corn silage contained: 33.7% DM, 38.4% NDF, 23.9% ADF, 9.1% CP, 36.32% starch on DM basis.

<sup>2</sup>High mineral mix contained: 7.8% vitamin E, 2.6% vitamin ADE, 28.6% distillers corn with soluble vitamin D, 14.6% plain salt, 36.5% limestone, 2.6% magnesium oxide, 5.7% trace mineral premix, and 1.6% selenium premix on a DM basis.

<sup>3</sup>Low mineral mix contained: 7.4% vitamin E, 2.5% vitamin ADE, 28.6% distillers corn with soluble vitamin D, 13.9% plain salt, 34.8% limestone, 6.0% magnesium oxide, 5.5% trace mineral premix, and 1.5% selenium premix on a DM basis.

<sup>4</sup> $n = 6$  composite samples representing 42 samples per treatment taken daily throughout the collection periods.

<sup>5</sup>Non fibrous carbohydrates analyzed by Cumberland Valley Analytical Services Laboratory (Maugansville, MD, USA).

<sup>6</sup>Total digestible nutrients (calculated from ingredients).

<sup>7</sup>Estimated: metabolizable energy (ME) = TDN × 0.04409 × 0.82 (Lammers and Heinrichs, 2000).

periods consisted of 35 days. Heifers were fed treatment diets for 21 days with no YC addition (14 days of adaptation and 7 days of sampling period), followed by 14 days with YC addition in which 7 days were allowed for adaptation to YC and 7 days of sampling period.

Dry matter offered (g/kg BW) was determined using NRC (2001) and formulated to provide 800 g/d ADG (restrictively to attain 0.22 Mcal ME intake/kg EBW<sup>0.75</sup>). Measured BW determined the quantity of TMR received for the following 7 days; however, DM intake was not changed immediately prior to sampling, as that could have increased variation in the results. Diets were formulated to provide 2.60, 2.73 and 2.82 Mcal of metabolizable energy (ME)/kg DM for LC, MC, and HC respectively, with a fixed level of 13% crude protein (CP; Table 1), and similar daily intakes of CP, energy, minerals and vitamins (Table 2).

## 2.2. Feed nutrient composition

Feed ingredients and TMR were ground through a 1-mm screen using a Wiley Mill (Arthur H. Thomas, Philadelphia, PA, USA) and analyzed for DM, organic matter, ash (AOAC, 1990), acid detergent fiber, and NDF (Van Soest et al., 1991) using an ANKOM<sup>200</sup> Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY, USA) with heat treated  $\alpha$ -amylase and sodium sulfite utilized in the NDF procedure. Crude protein (AOAC, 1990) was analyzed using a Leco FP-528 Nitrogen Combustion Analyzer (Leco, St. Joseph, MI, USA), Non fibrous carbohydrates (NFC), macro and micro minerals were analyzed by the Cumberland Valley Analytical Services Laboratory (Maugansville, MD, USA). Metabolizable energy was calculated using the equation:  $TDN \times 0.04409 \times 0.82$  (Lammers and Heinrichs, 2000). Heifers were weighed weekly, with 2 measurements at 0800 and 1800 h, 2 h prior and 8 h after feeding, except the week immediately prior to intensive sampling.

**Table 2**  
Effect of feeding different levels of forage to concentrate to replacement heifers on nutrient and energy intake.

	Treatment <sup>1</sup>			SE	P-value
	LC	MC	HC		
<b>Nutrient intake<sup>2</sup></b>					
DM, kg/d	8.84 <sup>c</sup>	8.30 <sup>b</sup>	7.89 <sup>a</sup>	0.016	<0.01
CP, kg/d	1.14 <sup>b</sup>	1.03 <sup>a</sup>	1.04 <sup>a</sup>	0.002	<0.01
ADF, kg/d	1.81 <sup>c</sup>	1.49 <sup>b</sup>	1.02 <sup>a</sup>	0.005	<0.01
NDF, kg/d	3.02 <sup>c</sup>	2.49 <sup>b</sup>	1.83 <sup>a</sup>	0.007	<0.01
NFC, kg/d	4.25 <sup>a</sup>	4.42 <sup>b</sup>	4.61 <sup>c</sup>	0.008	<0.01
Ca, g/d	33.60 <sup>c</sup>	30.29 <sup>b</sup>	28.39 <sup>a</sup>	0.062	<0.01
P, g/d	26.53 <sup>c</sup>	23.03 <sup>a</sup>	23.65 <sup>b</sup>	0.045	<0.01
Mg, g/d	17.69 <sup>a</sup>	18.26 <sup>b</sup>	17.94 <sup>a</sup>	0.034	<0.01
K, g/d	117.64 <sup>a</sup>	92.96 <sup>b</sup>	80.43 <sup>a</sup>	0.237	<0.01
<b>Energy intake</b>					
TDN, kg/d <sup>3</sup>	6.38 <sup>b</sup>	6.24 <sup>a</sup>	6.20 <sup>a</sup>	0.012	<0.01
ME, Mcal/d <sup>4</sup>	23.23 <sup>b</sup>	22.54 <sup>a</sup>	22.41 <sup>a</sup>	0.042	<0.01

<sup>a,b,c</sup> Means in the same row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>HC = Heifers fed 60% concentrate diets; MC = 40% concentrate diets; LC = 20% concentrate diets.

<sup>2</sup> $n = 6$  composite samples representing 42 samples per treatment taken daily throughout the collection periods.

<sup>3</sup>Total digestible nutrients (calculated from ingredients).

<sup>4</sup>Estimated: metabolizable energy (ME) =  $TDN \times 0.04409 \times 0.82$  (Lammers and Heinrichs, 2000).

## 2.3. Rumen fluid and content samples

Rumen contents were sampled on days 18 and 32 of each period at  $-2, -1, 0, 1, 2, 4, 6, 8, 10, 12,$  and 24 h relative to the 1000 h feeding. Rumen contents were strained through 4 layers of cheesecloth, and strained fluid was collected. Rumen fluid (15 ml) was preserved into 3 ml of 25% metaphosphoric acid and 3 ml of 0.6% 2-ethyl butyric acid (internal standard), and stored at  $-20^\circ\text{C}$  until VFA and ammonia-N ( $\text{NH}_3\text{-N}$ ) analyses (Moody et al., 2007). Ruminant pH was immediately recorded with a glass electrode pH Meter (Corning M90, Corning Inc., NY, USA). Whole rumen evacuation was completed 5 h post feeding on days 21 and 35 by manual removal. Total rumen contents were weighed, a representative sample taken, and immediately returned to the respective animals. Samples were dried ( $102^\circ\text{C}$ ) to determine mass of rumen contents and rumen liquid volume. The DM turnover was calculated from the dry rumen mass divided by the DM intake (Moody et al., 2007).

## 2.4. In situ dry matter digestibility

In situ digestibility was determined on days 14 to 17 and 28 to 31 of the treatment period, using LC and HC ground samples (TMR) in heifers fed both LC and HC diets. The MC diet (50% HC: 50% LC TMR) was also measured for in situ digestibility as a standard in all heifers. The standard (MC) was used due to its similar CP percentage relative to the other two diets (in comparison to corn silage or alfalfa hay; Table 1). In situ samples were initially ground through a 2-mm screen using a Wiley Mill (Arthur H. Thomas, Philadelphia, PA, USA). Then,  $10 \times 20\text{-cm}$  ANKOM bags (pore size of  $50 \pm 15 \mu\text{m}$ ; ANKOM Technology Corporation, Fairport, NY, USA) were filled with approximately 8 g of feed and attached to cords with weights and snaps to enable attachment to the outer rim of the cannula. Bags were placed in  $39^\circ\text{C}$  distilled water for 15-min prior to insertion into the rumen, and incubated in heifers for 72, 48, 36, 24, 16, 12, 8, 4, 2, 1 and 0 h (sequentially addition) before being removed all at once 2 h post feeding (at 1200 h). Bags were separated, rinsed manually with tap water (including the 0-h bag), and put through the 2-min rinse cycle of a washing machine 3 times. Bags were then rolled, dried in a forced air oven ( $55^\circ\text{C}$ ) for 4 days, and weighed to determine DM digestibility (DMD).

## 2.5. Statistical analysis

All statistical analysis was conducted using the PROC MIXED procedure of SAS (2006). Least square means and standard errors were determined using the LSMEANS for nutrient content of treatments, and differences of least square means were determined using the PDIF statement. Sources of variation included design effects of period and fixed treatment effects of F:C and YC addition. Heifer was included as a random effect. All dependent variables, with the exception of ruminal repeated measurements, were analyzed as a  $3 \times 3$  Latin square design with the following model:

$$Y_{ijkd} = \mu + a_i + b_j + y_k + p_d + ya_{ik} + e_{ijkl}$$

Where  $Y_{ijkd}$  is the dependent response variable from receiving  $i$ th treatment ( $i = 1$  to 3) on the  $j$ th heifer ( $j = 1$  to

**Table 3**

Rumen fermentation parameters of Holstein heifers fed high concentrate (HC), medium concentrate (MC), and low concentrate (LC) rations with yeast culture supplementation (YC) or control (CC).

	Diet (F:C <sup>1</sup> ratio)			SE <sup>2</sup>	Yeast		SE <sup>2</sup>	Significance	
	LC	MC	HC		CC	YC		F <sup>3</sup>	YC <sup>4</sup>
	80:20	60:40	40:60						
Daily pH									
Mean	6.07	6.07	6.07	0.12	6.08	6.06	0.07	0.96	0.71
Max	7.01	7.01	7.09	0.06	7.01	7.08	0.05	0.38	0.27
Min	5.29	5.43	5.39	0.24	5.36	5.38	0.14	0.78	0.86
Total VFA (mM)	120.69	118.79	116.82	7.28	113.87	123.66	4.45	0.81	<0.01
Individual VFA, mol/100 mol									
Acetate	63.16 <sup>a</sup>	61.77 <sup>ab</sup>	59.29 <sup>b</sup>	0.91	60.98	61.83	0.7	0.04 <sup>L</sup>	0.38
Propionate	18.25 <sup>b</sup>	18.14 <sup>b</sup>	21.45 <sup>a</sup>	0.74	19.19	19.37	0.61	0.01 <sup>L</sup>	0.84
Butyrate	12.91	14.41	13.27	1.32	14.25	12.81	0.81	0.85	0.05
Valerate	2.77	2.73	2.57	0.83	2.58	2.84	0.49	0.87	0.29
Isovalerate	1.84	1.79	21.32	0.34	1.87	1.97	0.21	0.57	0.43
Isobutyrate	1.09	1.17	1.28	0.17	1.14	1.22	0.10	0.47	0.13
Isoacids <sup>5</sup>	2.93	2.96	3.42	0.51	3.01	3.19	0.31	0.53	0.31
Acetate-to-propionate ratio	3.47 <sup>b</sup>	3.44 <sup>b</sup>	2.79 <sup>a</sup>	0.13	3.23	3.24	0.11	<0.01 <sup>L</sup>	0.91
NH <sub>3</sub> N									
Mean mg/dl	4.72	4.51	4.47	0.68	5.73	3.41	0.75	0.81	<0.01
Wet rumen mass, kg <sup>6</sup>	55.80 <sup>b</sup>	48.44 <sup>b</sup>	43.07 <sup>a</sup>	2.41	48.89	49.32	1.45	0.02 <sup>L</sup>	0.61
Rumen liquid volume, l <sup>7</sup>	48.05 <sup>a</sup>	42.34 <sup>b</sup>	37.22 <sup>c</sup>	2.21	41.13	39.98	1.56	0.03 <sup>L</sup>	0.72
Rumen liquid volume, %	86.11	87.42	86.42	2.52	86.24	86.43	3.44	0.83	0.87
Dry rumen mass, kg <sup>7</sup>	7.82 <sup>b</sup>	7.10 <sup>b</sup>	6.15 <sup>a</sup>	0.28	7.02	7.03	0.16	0.01 <sup>L</sup>	0.97
DM turnover rate, per d <sup>8</sup>	0.88 <sup>b</sup>	0.81 <sup>a</sup>	0.78 <sup>a</sup>	0.01	0.82	0.82	0.01	<0.01 <sup>L</sup>	0.91
In situ DM digestion rate, %/h									
Standard (MC) <sup>9</sup>	3.73	4.05	3.57	0.29	3.83	3.78	0.18	0.66	0.87

<sup>a,b,c</sup>Means in the same row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Forage-to-concentrate ratio.

<sup>2</sup>Standard error of the main effects of diet and YC.

<sup>3</sup>Main effect of forage-to-concentrate ratio.

<sup>4</sup>Main effect of YC.

<sup>5</sup>Isobutyrate + Isovalerate.

<sup>6</sup>Determined by whole rumen evacuation.

<sup>7</sup>Samples were dried (102 °C) to determine mass of rumen contents and rumen liquid volume.

<sup>8</sup>DM turnover calculated from the dry rumen mass divided by the DM intake.

<sup>9</sup>50% HC:50% LC TMR.

<sup>L</sup>Linear effect from increasing proportion of concentrate in the diet.

3), with or without the  $k$ th YC addition ( $k = 1$  to 2) at the  $d$ th period (1 to 3);  $\mu$  is the overall mean;  $a_i$  is the fixed effect of treatment;  $b_j$  is the random effect of heifer;  $y_k$  is the fixed effect of YC (subperiod);  $p_d$  is the design effect of period;  $ya_{ik}$  is the YC  $\times$  diet interaction; and  $e_{ijk}$  is the residual error.

Because of unequally spaced rumen samples, mean daily pH of rumen fluid, NH<sub>3</sub>-N concentrations, and molar concentrations and proportions of VFA were determined by calculating the area under the response curve according to the trapezoidal rule (Shipley and Clark, 1972). All denominator degrees of freedom for  $F$ -tests were calculated according to Kenward and Roger (1997). Repeated measurements were performed for ruminal parameters such as NH<sub>3</sub>-N, VFA and pH, and the same model was used except that hour was added and the interaction with diet and YC was included. One of 5 model structures was used depending on the finite-sample corrected Akaike's information criterion value for data that best fit the model. The covariance structures were compound symmetry, heterogeneous compound symmetry, unstructured, autoregressive, and antedependence (Littell et al., 1998; Wang and Goonewardene, 2004). For each variable, the type of structure was chosen accordingly by using the smallest Akaike's information criterion value. Residual variances were assumed to be normally distributed, and all data

are presented as least squares means obtained through the LSMEANS statement. Mean separations were determined using the PDIF statement in PROC MIXED.

Because there were no significant ( $P > 0.05$ ) interactions between diet and YC, only the main effect of YC and the effect of increasing the dietary concentrate are reported. In situ data were fit to the model of Orskov and McDonald (1979) for each MC TMR within heifer within period curve, with coefficients estimated in the NLIN procedure of SAS (2006), using the Marquardt compromise as the iterative method. Least squares means, standard errors, and statistical tests were then calculated by using the statistical procedures described above, with the coefficients derived from the NLIN procedure serving as the observed values. Treatment effects were considered significant when  $P < 0.05$  and trends were indicated by  $P < 0.10$ .

### 3. Results and discussion

#### 3.1. Nutrient composition

Most of the components of the rations differed between treatments as planned (Table 1). The objective of this experiment was to evaluate effect of YC on the rumen

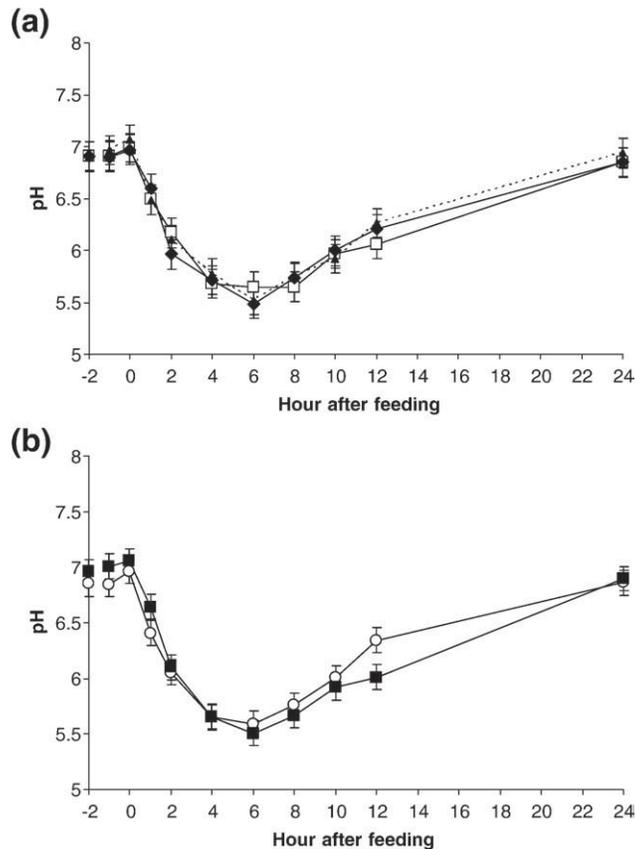
fermentation of different F:C diets. It is known that HC diets differ significantly from LC diets fed normally to dairy heifers, especially in the fibrous and non fibrous content of the diet (Moody et al., 2007). In this experiment NDF and ADF decreased as concentrate percent increased in the diets. The opposite happened with the NFC fraction of the diets. The overall protein content of the diets was similar to their formulated value of 13% CP. The ME content of MC and HC was increased to compensate for the decrease in DMI needed (Hoffman et al., 2007) to attain a similar growth of 800 g/d (NRC, 2001).

Nutrient and energy intakes of heifers fed the experimental diets are presented in Table 2. Dry matter intake decreased from LC to MC and HC diets as a direct result of higher energy density of the higher concentrate diets; hence, heifers being fed these energy dense diets consumed less DM per day to meet the necessary ME to achieve 800 g/d ADG according to NRC (2001). There were differences in the total amount of metabolizable energy consumed per day between LC, MC and HC diets (23.23, 22.54, and  $22.41 \pm 0.042$  Mcal/d respectively). This difference has been attributed to the fact that HC diets have higher energy retention ability in comparison to diets with lower concentrate levels (Reynolds et al., 1991). Thereby, net energy for maintenance and gain were similar but ME energy required to attain similar ADG increased as the level of concentrate decreased (Hoffman et al., 2007). Variation in the CP% of the diets and the different

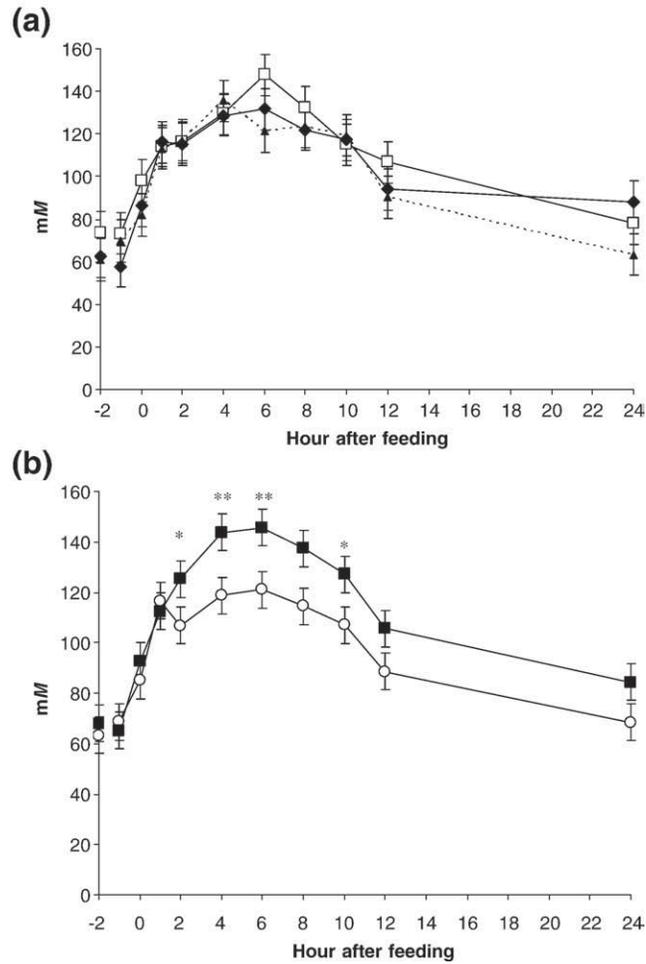
DM intake did not allow complete isonitrogenous intakes to be attained. Diets LC, MC, and HC provided 1.14, 1.03 and 1.04 kg/d of CP intake, respectively; nevertheless, provided an adequate CP intake for a target ADG of 800 g/d (NRC, 2001). Minerals such as calcium, phosphorus, and magnesium resulted in some differences among diets, due to the different intakes, but in all diets they were also within the mineral recommendations (NRC, 2001). Potassium increased as the percentage of forage increased in the diet because of the higher content of this mineral in corn silage.

### 3.2. Ruminal pH

Mean rumen pH was not affected by increasing the proportion of concentrate in the diets. Minimum pH recorded among treatments was 5.29 (Table 3) and was never maintained for more than 4 h (Fig. 1a). These results concurred with Maekawa et al. (2002), where pH did not differ between 40, 50 and 60% concentrate in rations fed to lactating dairy cows. Adaptation to HC diets seems to be an important part of feeding management in reducing their negative effect on pH. Ha et al. (1983) found that buffers (limestone,  $\text{NaHCO}_3$ , or inclusion of 10% alfalfa) had a superior effect on increasing ruminal pH during the adaptation phase than when animals were completely adapted to HC diets. Animals in the present study were adapted to their assigned diets for 10 days with increasing or decreasing proportion of



**Fig. 1.** Diurnal fluctuation of rumen pH of dairy heifers (a) fed diets containing low (LC; □), medium (MC; ◆), or high (HC; ▲) concentrate and (b) the main effect of the addition (■) or not (○) of yeast culture.



**Fig. 2.** Total volatile fatty acid (mM) concentrations the rumen of dairy heifers fed diets containing (a) low (LC; □), medium (MC; ◆), or high (HC; ▲) concentrate and (b) the main effect of the addition (■) or not (○) of yeast culture. (\*\*  $P < 0.01$  \*  $P < 0.05$ ).

concentrate. The fact that HC diets were fed restrictively at a lower intake reduces the possibility of having lower pH in comparison to ad libitum feeding systems. Causes of lower pH

can be attributed to increasing levels of starch fermentability rather than to differences in the F:C (Yang et al., 2001). Heifers were fed once daily, and the immediate decrease in ruminal

**Table 4**

Individual VFA concentration of Holstein heifers fed high concentrate (HC), medium concentrate (MC), and low concentrate (LC) rations with yeast culture supplementation (YC) or control (CC).

	Diet (F:C <sup>1</sup> ratio)			SE <sup>2</sup>	Yeast		SE <sup>2</sup>	P value	
	LC 80:20	MC 60:40	HC 40:60		CC	YY		F <sup>3</sup>	YC <sup>4</sup>
VFA concentration (mM)									
Total VFA	120.69	118.79	116.82	7.28	113.87	123.66	4.45	0.81	<0.01
Acetate	75.86 <sup>a</sup>	73.45 <sup>a</sup>	69.31 <sup>b</sup>	3.82	69.41	76.35	2.38	0.53	<0.01
Propionate	21.85 <sup>b</sup>	21.71 <sup>b</sup>	25.08 <sup>a</sup>	1.03	21.81	23.95	0.68	0.18	<0.01
Butyrate	15.95	16.81	15.46	1.22	16.24	15.91	0.78	0.63	0.14
Valerate	3.57	3.23	2.98	0.74	2.99	3.53	0.44	0.95	0.07
Isovalerate	2.17	2.18	2.48	0.37	2.13	2.43	0.21	0.37	<0.01
Isobutyrate	1.29	1.42	1.53	0.16	1.33	1.51	0.09	0.46	<0.01
Isoacids <sup>5</sup>	3.46	3.59	3.98	0.53	3.43	3.93	0.31	0.37	<0.01

<sup>a,b,c</sup> Means in the same row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Forage-to-concentrate ratio.

<sup>2</sup>Standard error of the main effects of diet and YC.

<sup>3</sup>Main effect of YC.

<sup>4</sup>Main effect of forage-to-concentrate ratio.

<sup>5</sup>Isobutyrate + Isovalerate.

pH post feeding (Fig. 1a) can be explained by the rapid consumption of readily available carbohydrates in a single meal.

Yeast culture has been shown to have a general pattern of increasing rumen pH when added to the diet of young and growing ruminants (Lascano and Heinrichs, 2007). In the present study no differences were found when YC was added to the treatment rations with respect to pH (Table 3). Fig. 1b presents the pH pattern of the addition of YC.

### 3.3. Ruminal VFA concentration

Variation of VFA throughout the day for LC, MC, and HC are presented in Table 3 and Fig. 2a. Total ruminal VFA concentrations (Table 3) and individual major VFA (acetate, propionate, and butyrate), valerate and isoacids (isobutyrate, isovalerate) concentrations did not differ between different F:C treatments (Table 4). Molar proportion of acetate in this experiment was higher in the LC diet and propionate in the HC diet (Table 3). Thus, there was a decrease in the ratio of acetate to propionate among dietary treatments (LC to HC). Whereas, acetate production is mainly due to the fermentation of structural carbohydrates by cellulolytic bacteria, propionate formation is mainly due to the fermentation of nonstructural carbohy-

drates by amylolytic bacteria (Enjalbert et al., 1999). These changes in A:P show that there is a difference in the nature of rumen fermentation when different amounts of concentrate are being fed to the animal (Table 3). It is clear from these results that there was a shift of microbial population with different F:C diets, yet fermentation was not compromised in any of the treatments.

Even though total VFA concentrations were similar among F:C treatments, YC addition resulted in an increased VFA concentration (Fig. 2b) which could decrease ruminal pH if the VFAs were not absorbed (Yang et al., 2004). Yeast culture did not have an effect on acetate to propionate ratio due to proportional changes in acetate and propionate concentration with the YC addition (Table 3). These results are in agreement with Mutsvangwa et al. (1992) and indicate that there was an increase in rate of rumen fermentation and bacteria population when YC was added to the different F:C diets, but not a change in the rumen fermentation pattern. In the same way, Callaway and Martin (1997) found increases in total VFA, propionate and acetate concentrations when YC filtrate (1 or 5%) was added in-vitro to *Selenomonas ruminantium* cultures, and little effect when added to *Megasphaera elsdenii* cultures. Interestingly, in this experiment, isoacid molar proportions were similar when YC was added, but molar proportion of

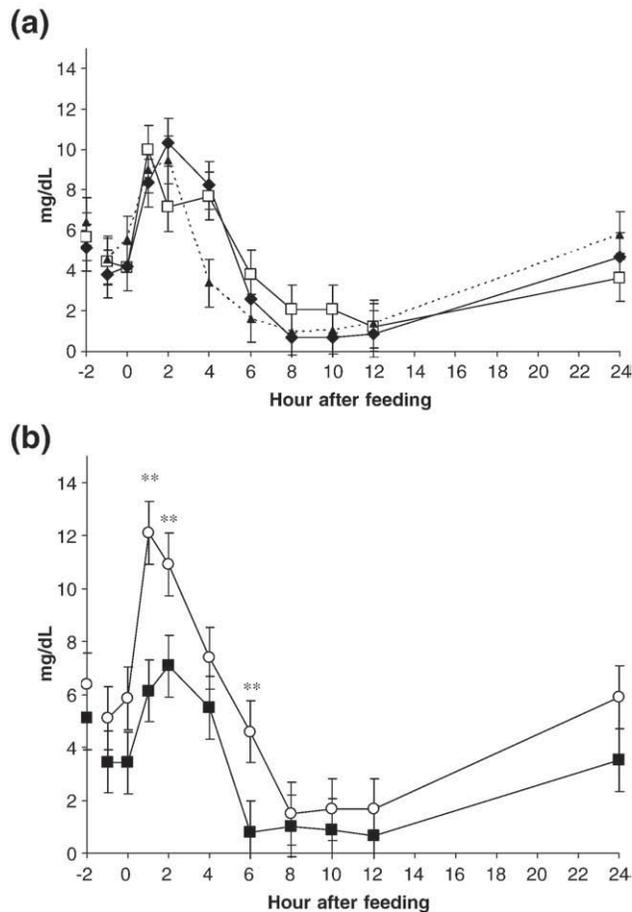


Fig. 3. Ammonia-N concentration (mg/dl) in the rumen of dairy heifers fed diets containing (a) low (LC; □), medium (MC; ◆), or high (HC; ▲) concentrate and (b) the main effect of the addition (■) or not (○) of yeast culture. (\*\*  $P < 0.01$  \*  $P < 0.05$ ).

butyrate decreased with the addition of YC. Similarly, butyrate proportions have decreased when glucose has been used as a substrate (Hino et al., 1994), and the opposite has happened when lactate has been used as the carbon source in continuous culture of *M. elsdenii* (Soto-Cruz et al., 2001). However, and in agreement with the results found in this experiment, butyrate concentration was not different when YC filtrate was added to *M. elsdenii* cultures (Callaway and Martin, 1997). All together, these results suggest that substrates or diets influenced the growth of different species of rumen microbes that are the responsible for the VFA production and pattern when YC is being used.

Published reports of the effect of YC on VFA concentrations are variable. A lack of response to supplemental YC has been attributed to low concentrate diets, low forage quality, or low feed intake (Olson et al., 1994); consequently, the variable responses in VFA production and patterns when YC has been used can be credited to dietary effects on microbial populations and numbers in the rumen (Wallace, 1996).

### 3.4. Ruminal ammonia

Ammonia concentrations were not different among F:C variations (Table 3), and there were periods where concentrations were lower than 2 mg/dl (Fig. 3a), which was the minimal NH<sub>3</sub>-N concentration recommended by Satter and Slyter (1974) for microbial growth in continuous culture. Considering that heifers were fed once a day, sources of ammonia were being exhausted by bacterial activity, enhancing microbial growth, but not compromising it. An indicator of this is the higher VFA concentrations (Fig. 2a) when NH<sub>3</sub>-N concentrations were the lowest (Fig. 3a).

Ammonia is the main source of N for microbial protein synthesis (Bach et al., 2005) and bacteria can grow with NH<sub>3</sub>-N as the sole N source (Virtanen, 1966). Rumen concentrations of NH<sub>3</sub>-N were significantly reduced by YC (Table 3). These data suggest that NH<sub>3</sub>-N concentrations may not be the limiting factor for microbial growth. But alternatively, this effect may be related to higher concentrations of cellulolytic and total bacteria (Harrison et al., 1988; Dawson et al., 1990; Williams et al., 1991), and not due to a decrease in proteolytic, amylolytic or deaminative activity of rumen microorganisms (Newbold et al., 1995; El Hassan et al., 1996). Research indicates that yeast additives either stimulate NH<sub>3</sub>-N uptake by bacteria, which allows better growth of these species in the rumen, or they stimulate the growth of cellulolytic bacteria, which could use more NH<sub>3</sub>-N to synthesize cellular nitrogenous components (Chaucheyras-Durand and Fonty, 2001). Even though, VFA molar proportions were similar without or with YC addition, the increase in VFA concentrations (Table 4) in this experiment indicates enhanced microbial activity in terms of fermentation and agrees with previous findings regarding total and cellulolytic bacteria numbers (Weidmeier et al., 1987; Dawson et al., 1990). It is noteworthy that YC has shown to have maximum stimulatory effects for 2 to 4 h with decreasing activity up to 12 h (Koul et al., 1998), indicating a necessity for frequent addition of YC to optimize effectiveness. Since yeasts are facultative aerobes and cannot easily survive in an anaerobic rumen environment; they have to be supplied continuously to maintain minimum effective concentration ( $1 \times 10^5$  CFU/g rumen contents; Jouany, 2006). The

current experiment is in agreement with the observations made by Koul et al. (1998) and Jouany (2006); in fact, YC effects seemed to decrease several hours after feeding (Fig. 3b). Furthermore, in every period where YC was not supplemented to the diet, rumen NH<sub>3</sub>-N concentrations increased (Table 3).

### 3.5. Isoacids

It is well known that production of isoacids by microbes is related to potential release of amino acids (AA) and deamination from proteins in the rumen, and that this occurs when optimal rumen degradable proteins are consumed (Armentano et al., 1993; Yang et al., 2004). In the rumen, cellulolytic bacteria primarily use NH<sub>3</sub>-N while amylolytic bacteria prefer to use AA as they have a proteolytic activity. The first stage of ruminal nutrient degradation involves the attachment of bacteria to feed particles. Feed particles are attached by 70 to 80% of ruminal bacteria (Miron et al., 2001) and about 50% of these bacteria have proteolytic activity (Bach et al., 2005). The end products of this process are peptides and AA. These AA and peptides can be incorporated into microbial protein or further deaminated to VFA if energy is needed by ruminal bacteria (Bach et al., 2005). El Hassan et al. (1996) found increased content of protein in the rumen when YC was added and this could be related to the increased incorporation of NH<sub>3</sub>-N into microbial protein. The addition of YC can stimulate amylolytic bacteria that would use preferably true degradable protein, and would result in an increase in the concentration of ruminal isoacids and valerate (Dawson et al., 1990). Isoacid concentrations in the present experiment were increased with the addition of YC (Table 4). Considering that fiber digestion can be depressed when an insufficient amount of protein is supplied to the animal. It has to be taken into consideration that protein is of higher value than NH<sub>3</sub>-N for fiber digestion to be maintained (Hoover, 1986). Most cellulolytic bacteria need isoacids derived from protein deamination to enhance their growth and fiber digestion (Yang, 2002). Stimulatory effects in fiber digestion, such as supply of AA for the enhanced growth of cellulolytic bacteria, have been demonstrated with isoacids addition (Gorosito et al., 1985). Results from this experiment indicate that NH<sub>3</sub>-N concentration was lower (Fig. 3b) and the isoacids concentration was higher with YC addition. This could be one way YC favors cellulolytic bacteria activity and stimulates its growth (Hoover, 1986; Harrison et al., 1988), and fiber digestion in the rumen.

### 3.6. Rumen contents and volume

As the inclusion of concentrate increased, DM turnover was lower (Table 3). Moody et al. (2007) attributed this result to restricted intake and to the higher amount of DM intake required to attain the ME necessary to achieve ADG of 800 g/d for higher forage diets. It has been shown that when higher amounts of fiber are being fed to animals from forages, particle size is greater, and the time that these particles stay in the rumen is longer due to a reduction in the passage rate of fiber (Jung and Allen, 1995). Due to the higher DM intake of LC heifers in comparison to MC and HC, wet and dry rumen mass decreased as the proportion of concentrate included in the

diet increased (Table 3). This is opposite to what Maekawa et al. (2002) found when feeding 60:40, 50:50, and 40:60 F:C ad libitum to lactating cows; this difference could be explained by the lower DMI of cows eating the 60:40 diet in this study because of rumen fill. While in the present study, heifers fed restrictively the LC (60:40 F:C) diet had the highest DM intake (Table 2). Important to notice is that in both studies the % of liquid volume of the three different F:C diets was around 85, and rumen liquid volume (l) was proportionally equal among diets (Table 3). These results suggest that rumen volume may have a positive association with DM intake when diets are being restricted. The inclusion of YC did not have any effect on the wet or dry rumen contents or on DM turnover.

### 3.7. *In situ* dry matter digestibility

The rate of *in situ* DMD of MC (standard control) was not different among dietary treatments (Table 3). Moody et al. (2007) used corn silage as a control and found no differences in the rate of *in-situ* DMD and suggested that this effect was indicative of small differences in rumen environments between low or high concentrate diets. In this experiment the MC diet was used as a control and no differences were found among treatments nor with the addition or not of YC.

## 4. Conclusions

Feeding HC diets at restricted intakes did not have any differences on the concentration of rumen fermentation parameters. The concentrations of these fermentation parameters were similar between LC, MC, and HC diets regardless of the difference in nutrient composition of the experimental diets. Concentration of total VFA were similar among the different F:C treatments. But molar proportions of acetate and propionate increased and decreased (respectively) linearly when higher levels of concentrate were added to the diets. This resulted in a decrease in the A:P as more concentrate was added to the diet. Ruminal pH was not affected by F:C or YC supplementation. Total, wet and dry rumen contents, and DM turnover were different between all F:C treatments with no YC effect over these parameters, showing that rumen manipulation can be achieved with F:C having no major effect on fermentation rate. HC diets changed rumen fermentation towards a more propionic pattern.

There was an increase in total VFA concentrations with YC addition in all diets, but molar proportions of individual VFA (acetate, propionate), valerate and isoacids (isobutyrate, and isovalerate) were not different, suggesting an increase in the fermentation rate when YC was added but not a change in the fermentation pattern. These results are concurrent with the decreased NH<sub>3</sub>-N concentration in all treatments when YC was added, suggesting an increase in rumen microbial activity. YC enhanced the output of fermentation products at the rumen level.

In summary, these results show that dairy heifers may be fed restricted HC diets with no impact on rumen pH or ruminal ammonia concentration, decreasing the A:P and achieve similar rumen fermentation rates as LC diets. YC addition is beneficial to improve the fermentation rate in corn silage based diets across a range of F:C, suggesting an increased microbial activity and growth.

## Acknowledgements

The authors acknowledge the assistance of Maria Long and Carrie Nelson with this research as well as the financial support and yeast supplied by Alltech Inc.

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