Effect of yeast supplementation on performance, rumination time, and rumen pH of dairy cows in commercial farm environments

V. Ambriz-Vilchis,*^{†1} N. S. Jessop,[‡] R. H. Fawcett,[§] M. Webster,[†] D. J. Shaw,[#] N. Walker,^{||} and A. I. Macrae*

*Dairy Herd Health and Productivity Service, Royal (Dick) School of Veterinary Studies and the Roslin Institute, The University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian, EH25 9RG, Scotland, UK

[†]BioSimetrics Ltd., The Cottage Scotland's Rural College Building, West Mains Road, Edinburgh, Lothian, EH9 3JG, Scotland, UK

[‡]20 Crofts Road, Lauder TD2 6QJ, Scotland, UK

[§]School of GeoScience, The University of Edinburgh, EH9 3FE, Scotland, UK

[#]Royal (Dick) School of Veterinary Studies and the Roslin Institute, The University of Edinburgh, Easter Bush Veterinary Centre, Roslin, Midlothian, EH25 9RG, Scotland, UK

^{||}AB Vista, Blenheim Road, Marlborough Business Park, Marlborough, Wiltshire, SN8 4AN, England, UK

ABSTRACT

The aims of the present study were to evaluate the effects of live yeast supplementation (Vistacell MUCL 39855, AB Vista, Marlborough, UK) on performance, rumination time, and rumen pH on dairy cows in commercial farm environments. Three trials were carried out, the trials lasted 12 (trial 1), 15 (trial 2), and 19 wk (trial 3). In each trial, 14 multiparous Holstein dairy cows were allocated to 2 groups that received (trial 1) a standard diet plus yeast, (trial 2) an acidogenic diet plus yeast, and (trial 3) grazing pasture plus yeast. Milk production, milk chemical characteristics, body weight and body condition score, rumination time, and rumen pH were monitored for each group throughout the 3 trials. No statistically significant differences were observed in any of the 3 trials for any of the recorded variables. In contrast, an effect of time (period or days in milk) on rumen pH was observed in all 3 of the trials, as time spent under the acidotic thresholds increased across the experimental periods; however the differences were not associated with live yeast supplementation. No effect of live yeast supplementation was observed in any of the 3 trials reported. Further research should include studies on animals at different stages of lactation (with emphasis on transition period and early lactation), consuming more challenging diets (higher level of inclusion of concentrates or starch), or under different environments such as grazing of succulent forages. Such studies might be required to elucidate any possible effect of live yeast supplementation of dairy cows when the rumen environment is under challenge.

Key words: yeast, rumen pH, rumination time, dairy cow, acidosis

INTRODUCTION

Modern dairy cow feeding strategies focus on meeting the energy requirements for high levels of milk production by encouraging maximum intakes of energy-dense, low-fiber diets with highly fermentable carbohydrates (Marden et al., 2008). Despite this management regimen having an immediate positive effect on milk yield, in the medium to long-term there are potentially many negative effects on dairy cow health, welfare, and performance (Zebeli et al., 2007). Ruminant nutrition has been continually searching for alternatives to enhance production, or to correct issues that potentially may reduce productivity or affect health. Direct-fed microbials (e.g., yeast) have been used as additives to improve rumen function and animal performance by increasing the capacity of fiber digestion (in vitro, in situ, and in vivo), aid in the prevention of low rumen pH, and reduce the incidence of SARA in vivo (Fonty and Chaucheyras-Durand, 2006; Bach et al., 2007). *Saccharomyces cerevisiae* is the most common yeast supplement given to dairy cows, and its use is common practice in the dairy industry. Different types of yeast products are commercially available, including live yeast products (for the purpose of this paper also referred as “yeast”) and yeast fermentation products. To be considered as live yeast, manufactured products must contain a certain level of live cells ($>1.0 \times 10^{10}$ cfu/g of DM; Fonty and Chaucheyras-Durand, 2006; Chaucheyras-Durand et al., 2008; AlZahal et al., 2014; Malekkhahi et al., 2016). It is thought that live yeast can modify the rumen environment by utilizing available oxygen and other components (Newbold et al., 1996). It also favors the number and activity of rumen cellulolytic bacteria that help reduce the level of

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¹Corresponding author: virgilio@biosimetrics.com

changes in rumen pH (Marden et al., 2008; Al Ibrahim et al., 2012) and will improve digestive function (Fonty and Chaucheyras-Durand, 2006; Chaucheyras-Durand et al., 2008, 2016; Al Ibrahim et al., 2010a).

Several studies have looked at the effect of live yeast supplementation in dairy cows on the rumen environment (Bach et al., 2007; Guedes et al., 2008; Bayat et al., 2015; Malekkhahi et al., 2016), performance (Kalmus et al., 2009; Bitencourt et al., 2011; Tristant and Moran, 2015), animal behavior (Bach et al., 2007; DeVries and Chevaux, 2014), and health (Chung et al., 2011; AlZahal et al., 2014). The review by Chaucheyras-Durand et al. (2008) looked at yeast mode of action, effects on rumen environment, effects on digestive by-products [N (NH₃ and N microbial metabolism) and CH₄ mitigation], and effects of live yeast combined with other feed additives. Desnoyers et al. (2009) carried out a quantitative analysis of the literature by performing a meta-analysis of the effects of live yeast supplementation on performance in different ruminant species. After collating data from more than 150 experiments, those authors found that live yeast supplementation had a positive effect on rumen pH, DMI, and milk yield.

Despite the quantity of research accumulated over several decades into the use of yeast as a feed additive, the results have been variable and the proposed benefits of live yeast supplementation have not always been demonstrated. For example, using 10 g/cow per day of yeast (Yea-Sacc 1026, Alltech Biotechnology Center, Nicholasville, KY), Kalmus et al. (2009) found no effect on milk yield when yeast was supplemented to early-lactation dairy cows. Chung et al. (2011) found no effect when using 2 different strains of active dried yeast on nonlactating dairy cows on BW and DMI (1×10^{10} cfu, Levucell and strain 2, Lallemand Animal Nutrition, Montreal, QC, Canada). Furthermore, those authors found contrasting results on the effect that live yeast had on rumen pH, as one of the strains reduced rumen pH compared with the control group, and the other strain was no different in the rumen pH level compared with the control group.

It is thought that the variable response to live yeast supplementation is a result of external factors such as diet or physiological stage. More often than not, studies have focused on the effect of live yeast supplementation under experimental conditions, such as the induction of SARA (AlZahal et al., 2014; Malekkhahi et al., 2016), or adverse environments, such as environmental stress (high temperatures; Salvati et al., 2015), changes in diet (from indoor feeding to out grazing; Al Ibrahim et al., 2012), or physiological challenges transition period or early lactation (Kung et al., 1997; Cakiroglu et al., 2010; Al Ibrahim et al., 2013). Furthermore, it is very

seldom that studies have looked at the effect of live yeast supplementation using noncannulated animals, or under standard commercial farm conditions. This is often because obtaining reliable data on performance and physiological parameters can only be obtained from experimental facilities at higher education institutions or research environments. With the advent of new technologies and automated systems, the recording of parameters such as rumination time and rumen pH under commercial farm environments (Rutten et al., 2013) without altering the experimental animals is possible; hence, the evaluation of the effect of live yeast supplementation in vivo can be carried out. Therefore, the aims of the present study were to evaluate the effects that live yeast supplementation might have on performance, rumination time, and rumen pH on dairy cows under commercial farm environments, specifically a standard commercial diet, an acidotic challenge, and outside grazing grass.

MATERIALS AND METHODS

Animals, Feeding and Treatments

Three trials were conducted at the University of Edinburgh at Langhill Farm, Roslin, Midlothian, Scotland, United Kingdom, during 2012 and 2013. The farm has a 240-cow Holstein milking herd. All procedures related to animals were approved by the Veterinary Ethical Review Committee of the Royal (Dick) School of Veterinary Studies of the University of Edinburgh (References: Trial 1 VERC 2011–88, Trial 2 VERC 30/12, and Trial 3 VERC 11/13).

Individual Holstein cows were unique to each trial, and cows were divided into 2 groups to facilitate management routines (e.g., milking and feeding) and to ensure similar parities and DIM between groups of cows in all 3 trials. Each group of animals was housed in contiguous pens that shared identical characteristics, such as area of feed and water troughs and cubicle or stalls with rubber mattress top-dressed with sawdust 3 times a week. Water was supplied ad libitum. Cows were milked in a 14/14 herringbone milking parlor (DeLaval, Tumba, Sweden) approximately at 0500 and 1500 h. During milking, cows received a minimum of 0.8 kg and a maximum of 6 kg of concentrate/d per cow, split into 2 equal feeds given at each milking. All of the individual cows were clearly identified with a unique number or letter by color spray (Arco Limited, Hull, UK) on both sides of the thorax or neck so they were easily viewed and recognized, and the identification was randomly allocated to each animal. Cows were given a minimum of 2 wk to adapt to the diet, facili-

Table 1. Timeline and experimental design used in trials 1, 2 and 3¹

Trial	Group	P1			P2			P3			P4			P5		
		W1	W2	W3	W1	W2	W3	W4	W1	W2	W3	W4	W1	W2	W3	W4
Trial 1	Control Treatment	PMR	PMR		PMR	PMR + Y 0.8	M	NA	PMR	PMR + Y 4.0	NA	NA	PMR	PMR	NA	NA
		PMR	PMR	M	PMR + AcCh	PMR + AcCh + Y 4.0	M	NA	PMR + AcCh	PMR + AcCh + Y 4.0	NA	NA	PMR	PMR	NA	NA
Trial 2	Group 1	PMR + AcCh	PMR + AcCh	M	PMR + AcCh	PMR + AcCh + Y 4.0	M	PMR + AcCh	PMR + AcCh	PMR + AcCh	PMR + AcCh	PMR + AcCh	PMR + AcCh	PMR + AcCh	PMR + AcCh	PMR + AcCh
		PMR + AcCh	PMR + AcCh	M	PMR + AcCh	PMR + AcCh + Y 4.0	M	PMR + AcCh	PMR + AcCh	PMR + AcCh	PMR + AcCh	PMR + AcCh	PMR + AcCh	PMR + AcCh	PMR + AcCh	PMR + AcCh
Trial 3	Group 1	PMR	PMR	M	G + PMR	G + PMR	M	G + PMR	G + PMR	G + PMR	G + PMR	G + PMR	G + PMR	G + PMR	G + PMR	G + PMR
		PMR	PMR	M	G + PMR	G + PMR	M	G + PMR	G + PMR	G + PMR	G + PMR	G + PMR	G + PMR	G + PMR	G + PMR	G + PMR

¹P = period; W = week; PMR = partial mixed ration; Y = yeast; NA = not applicable; M = measurements. Trial 1: Y 0.8 = 0.8 g of yeast/cow per day (10 billion cfu/cow per day), Y 4.0 = 4.0 g of yeast/cow per day (60 billion cfu/cow per day). Trial 2: AcCh = acidogenic challenge = 1.5 kg of wheat/cow per day. Trial 3: G = grazing.

ties, and the rumination collars. All measurements were taken in the third or fourth week after any change in diet (Table 1).

After fresh partial mixed ration (**PMR**) was delivered (once a day at approximately 0700 h), the corresponding live yeast treatment dose was administered daily to each cow in the corresponding group by top-dressing and manually mixing the yeast into the upper part of the PMR. Cows were locked in the feed trough for 15 min using the self-locking neck yoke mechanism to ensure the cows ate all of the yeast supplemented.

Trial 1. Fourteen multiparous milking cows were selected and balanced for DIM (104 ± 12 d; mean $\pm$ SEM) and parity (median lactation number = 4). The cows were then randomly allocated to 2 different groups, control (**Cx**; DIM = 103 ± 5.0 , median lactation number = 5) and treatment (**Tx**; DIM = 105 ± 4.6 , median lactation number = 4), with 7 cows in each group. At the end of the trial all of the cows were pregnant.

Cows were offered a PMR (Table 2) with additional concentrate composition = wheat 10%, barley 20%, distillers grains wheat 23%, US corn distillers grain 5%, maize 5%, Soya Hipro 3% (KW Scotland, Ayr, Scotland, UK), palm kernel 15%, cereal mix 3%, molasses 7%, limestone 1%, calcined magnesite 1%, and ruminant premix 7%) fed to yield in the milking parlor.

Table 2. Ingredient and chemical composition of the experimental partial mixed rations

Composition	Diet		
	Trial 1	Trial 2	Trial 3
Ingredient (% of DM)			
Grass silage, 1st cut	47.06	43.60	30.07
Grass silage, 2nd cut	0	15.17	0
Whole-crop wheat silage	18.82	17.06	7.80
Dairy meal ¹	14.12	18.01	10.58
Water	9.41	0	0
Maize crimped	7.06	0	0
Molasses	3.53	3.32	1.39
Wheat (ground)	0	2.84	0
Calcined magnesite	0	0	0.06
Grazing	0	0	50.11
Chemical composition			
DM (%)	56.0	40.6	31.1
CP (% DM)	16.7	16.7	15.8
NDF (% DM)	35.0	35	41
uNDF forage [% DM (kg total)]	6.9	6.5	12.7
uNDF total [% DM (kg total)]	11.3	10.3	14.8
Oil (% DM)	3.9	3.1	3.4
Sugar (% DM)	9.6	7.1	5.6
Starch (% DM)	14.3	19.8	10.2
Quick carbohydrates (% DM)	19.9	15.6	17.1
Slow carbohydrates (% DM)	38.3	42.2	41.5

¹Dairy meal = wheat 12.55%, US corn distillers grain 15.02%, rapeseed extract 15.01%, soya hulls 15.00%, Soya Hipro (KW Scotland, Ayr, Scotland, UK) = 30%, beet pulp 8.00%, Megalac (Volac International Limited, Royston, England, UK) 2.00%, mineral premix 0.20%, vitamin E 0.13%, limestone 2.00% and calcined magnesite 0.08%.

The trial ran from January until May 2012 and consisted of 4 periods (**P1**, **P2**, **P3**, and **P4**). Baseline measurements were taken in P1, P2, and P3 (treatment) and washout measurements were taken in P4 (Table 1). Each period lasted for 3 wk. For each period, cows had 2 wk to adapt to the diet and facilities, and all measurements were taken in the third week. The Cx group received no yeast supplementation throughout the trial. The Tx group received yeast supplementation at 2 levels. In P2, the Tx group received yeast supplementation at 0.8 g/cow per day (1×10^{10} cfu/cow per day) of yeast (Vistacell, AB Vista, Marlborough, UK) and 4.0 g/cow per day (6×10^{10} cfu/cow per day) of yeast were added in P3. The manufacturer's specification for storage, handling, and supplementation of the yeast was adhered to, and no laboratory verification was performed to assess the viability of live yeast in the product. No yeast was added during P1 and P4 (Table 1).

Trial 2. Fourteen multiparous milking cows were selected and balanced for DIM (97 ± 4.3 d) and parity (median lactation number = 3). The cows were then randomly allocated to 2 different groups, group 1 (G1; DIM 96 ± 2.7 and median lactation number = 3) and group 2 (G2; DIM 99 ± 9.2 and median lactation number = 4), with 7 cows in each group. At the end of the trial all of the cows were pregnant.

Cows were offered a PMR (Table 2), with additional concentrate fed to yield in the milking parlor. In addition, the cows were supplemented with 1.5 kg of ground wheat per cow per day. After fresh PMR was delivered, the wheat was added to it and mixed until homogeneity was obtained, the cows were then locked in the feed trough for at least 15 min using the self-locking neck yoke mechanism to ensure the cows ate the supplemented wheat.

The trial was conducted from January to May 2013 and was split into 4 periods (P1, P2, P3, and P4). Baseline measurements were recorded in P1, P2 and P3 (treatment) and washout measurements were taken in P4 (Table 1). In P1, the cows had 2 wk to adapt to the facilities and diets, and all measurements were taken in wk 3. For each of the remaining periods (P2, P3, and P4) cows had 3 wk to adapt to the diet and facilities, and all measurements were taken in the fourth week. In P2 and P3, yeast supplementation at 4.0 g/cow per day (6×10^{10} cfu/cow per day) followed a crossover experimental design. The manufacturer's specification for storage, handling, and supplementation of the yeast was adhered to, and no laboratory verification was performed to assess the viability of the live yeast product.

Trial 3. Fourteen multiparous milking cows were selected and balanced for DIM (139 ± 4.5 d) and parity (median lactation number = 4). The cows were then

randomly allocated to 2 different groups, group 1 (G1; DIM 140 ± 6.3 , median lactation number = 4)) and group 2 (G2; DIM 137 ± 6.8 , median lactation number = 4), with 7 cows in each group. At the end of the trial all of the cows were pregnant.

Cows were grazing a ryegrass (*Lolium perenne*) sward during the day and night. In addition, when the cows came in for milking in the afternoon, they were offered a buffer PMR ration (Table 2). Additional concentrate was fed to yield in the milking parlor.

The trial ran from May until September 2013 and was split into 5 periods (P1, P2, P3, P4, and P5). Baseline measurements were taken in P1 (inside), P2 (grazing), and P3 and P4 (treatment), and washout measurements were recorded in P5 (Table 1). Period 1 and P2 lasted 3 wk, cows were given 2 wk to adapt to the facilities and diets, and measurements were recorded on the third week. Periods 3 to 5 lasted 4 wk, with 3 wk of adaptation and 1 wk of measurements. In P3 and P4, yeast supplementation at 4.0 g/cow per day (6×10^{10} cfu/cow per day) was carried out following a crossover design. The manufacturer's specification for storage, handling, and supplementation of the yeast was adhered to, and no laboratory verification was performed to assess the viability of the live yeast product.

Data Collection

Milk Production and Composition. Milk yield was recorded daily throughout the entire duration of the trials, and downloaded once a week directly from the milking parlor using the automatic milk recording feature in the Alpro 5 computer program (DeLaval). During the measurement week of each period (on Monday, Wednesday, and Friday), composite milk samples (from consecutive morning and afternoon milking) were collected and analyzed for chemical characteristics (protein, butterfat and lactose) by the Cattle Information Service (Telford, UK).

BW and BCS. Once during the measurement week of each period, cow BW and BCS were recorded. Body weight was recorded using an electronic weigh scale. Body condition score was measured on a standard 1 to 5 scale (Lowman et al., 1976) by a single trained operator.

Feed Sampling and Analysis. Forage samples were taken every week and were analyzed at Bioparametrics Ltd. (Edinburgh, Scotland, UK) laboratory. Basic components of the feedstuffs, DM, ash, oil, sugar, starch, NDF, and protein, were obtained by methods of AOAC International (2012). Further analyses, which are unique to Bioparametrics Ltd. feedstuff analyses, included degradation parameters of the carbohydrates and protein performed by in vitro gas production tech-

nique (Menke and Steingass H., 1988), with modification for protein and carbohydrates (Jessop and Herrero, 1996; Palmer, 2006). The results of these analyses provide information on the degradation parameters of the carbohydrates, sugar, quickly and slowly degradable starch, fermentable NDF, and protein fractions of the feedstuffs.

Rumination Time. In all trials, a rumination collar (RC; Qwes-HR Lely Ltd., St Neots, UK) was fitted to each cow to record rumination. A tag reader was located at the exit of the milking parlor so data from the RC was downloaded and stored at least twice a day, after each milking. The output presents rumination in minutes per day. The RC has been described elsewhere (Bar and Solomon, 2010) and has been validated for use in loose-housed dairy cows in commercial environments with acceptable results (Ambriz-Vilchis et al., 2015). However, due to the poor results obtained in grazing animals (Ambriz-Vilchis et al., 2015), the data obtained from the RC in trial 3 was discarded.

Rumen pH. To record rumen pH, cows were orally administered a commercially available rumen pH bolus [eCow Ltd., Devon, England, UK (trial 1), or WellCow Ltd., Roslin, Scotland, UK (trials 2 and 3)]. In all trials a bolus was administered to each cow using a bolus gun before the start of the first measurement week on P1. Prior to deployment the boluses were calibrated against known standard pH solutions (pH 4 and 7; trial 1: Osmotics, Aylsham, England, UK, and trials 2 and 3: buffer solutions were provided by WellCow Ltd.). The boluses were set to record rumen pH at 15-min intervals for the entire lifespan of the bolus's battery (up to 4 mo). The recorded data were downloaded to a personal computer for storage, collation, and further analyses.

Calculations and Statistical Analysis

Milk Production and Composition. Data on milk yield and characteristics was collated and summarized for the measurement week resulting in a data set for analyses of 7 measurements for milk yield (one per day), and 3 measurements for milk characteristics (Monday, Wednesday, and Friday).

BW and BCS. Data on BW and BCS was collated and summarized for the measurement week in each period, resulting in 1 measurement. Data on BCS was analyzed using Kruskal-Wallis one way ANOVA.

Rumination Time. Data obtained with the RC was collated to present rumination time in minutes per day, resulting in a data set for analyses of 7 measurements for rumination time (1 per day) during the measurement week in each period.

Rumen pH. The rumen boluses were set to record pH every 15 min, which resulted in 96 time points per day. Daily pH records were examined on an individual cow basis, and only days that had 96 time points were used to construct the pH database. From this data set, rumen pH was analyzed as mean rumen pH. To try to account for the dynamics of rumen pH, the time (min/d) rumen pH was below 6.2 or 5.8 pH thresholds were obtained. These rumen pH cutoff points were selected as indicators of SARA and were considered due to research evidence showing their detrimental effects on cellulolytic bacteria and fiber digestibility (pH <6.2) (Mould and Orskov, 1983; Russell and Wilson, 1996), harmful action on the animal host (pH <5.8) (Beauchemin and Penner, 2009), or both.

To analyze milk yield, milk characteristics, rumen pH, and rumination time data, a standard linear mixed effect model was used to resolve the nonindependence associated with the multiple measurements recorded per cow. In the linear mixed effect model, which cow the measurement had come from was entered as the random effect. For trial 1, the model included the fixed effects of period and treatment, and the interaction of period and treatment with cow as the random effect. For trials 2 and 3, the model included the fixed effects of period and treatment, and the interaction of period and treatment to account for the sequence on which the treatment was administered in the cross over design. Values reported are least squares means $\pm$ standard error of the mean and median for BCS.

All statistical analysis were carried out using R (R Core Team, 2013) with the linear mixed-effect analysis carried out using the 'nlme' package (version 3.1–113). Statistical significance was taken as $P < 0.05$.

RESULTS

Animals, Feeding, and Treatments

Table 2 shows the composition and chemical characteristics of the PMR fed throughout the 3 trials. The PMR was kept as consistent as practically possible for the entire duration of each of the trials to prevent major variations in the chemical characteristics of the offered rations.

Milk Production and Composition

Trial 1. No effect of treatment on mean milk yield was observed ($P > 0.05$). Least squares means for lactation performance are presented in Table 3. A statistically significant difference was observed ($P < 0.01$) between experimental periods (P1: Cx = 42, Tx = 41; P2: Cx = 38, Tx = 36; P3: Cx = 39, Tx = 41; P4: Cx

= 40, Tx = 40; $P < 0.05$; Table 3). An increase in milk yield was observed in the Tx group when cows were fed the highest dose of yeast from P2 (mean = 36 kg of milk) to P3 (mean = 41 kg of milk). However, the same increase in milk yield was also observed for Cx group during P2 (mean = 38 kg of milk) to P3 (mean = 39 kg of milk).

No statistically significant differences between the Cx and Tx groups were observed in any of the milk composition characteristics analyzed. However, a period effect was observed for all the variables analyzed ($P < 0.05$), although no interaction with treatment was observed (Table 3).

Trial 2. No effect of treatment on mean milk yield was observed (Table 4); however, a statistically significant effect of period was observed ($P < 0.01$) but no interaction of treatment $\times$ period (Table 4). No effect of yeast supplementation on any of the milk composition characteristics was observed. A period effect was observed for all the variables analyzed ($P < 0.001$); however, no interaction treatment $\times$ period was observed (Table 4).

Trial 3. No effect of yeast supplementation on mean milk yield was observed in any of the experimental groups. An effect ($P < 0.05$) of period on milk yield was observed, with no interaction period $\times$ treatment observed. No effect of yeast supplementation was observed on butterfat, milk protein, or lactose content (Table 5).

BW and BCS

Trial 1. Yeast supplementation had no effect on BW or BCS. A tendency of BW and BCS to decline (P1 to P2, Cx = -10 kg, 0.25 points of BCS; Tx = -9 kg, 0.25 points of BCS) and increase (P3 to P4, Cx = 6 kg 0.25 points of BCS; Tx = 7 kg, 0.25 points of BCS) across periods was similar and observed in both experimental groups (Table 3).

Trial 2. Yeast supplementation had no effect on BW or BCS across the experimental periods (Table 4). A tendency for BCS to decline (P1 to P3, -0.25 point of BCS for both groups) and increase (P3 to P4, G1 = 0.5 and G2 = 0.25 point of BCS) was observed in both experimental groups (Table 4).

Trial 3. Yeast supplementation had no effect on BW and BCS or change in BCS across the experimental periods (Table 5).

Rumination Time

Trial 1. No effect of yeast supplementation was observed on the amount of time (min/d) the cows spent ruminating. An effect of period on rumination time was

Table 3. Effect of yeast supplementation on milk yield, milk characteristics, rumination time, and rumen pH throughout trial 1¹

Variable	Group control				Group treatment				P-value		
	P1	P2	P3	P4	P1	P2, yeast (0.8 g/d)	P3, yeast (4.0 g/d)	P4	Period	Tx	Inter
Milk yield (kg/d)	42 $\pm$ 2	38 $\pm$ 2	39 $\pm$ 1	40 $\pm$ 1	41 $\pm$ 1	36 $\pm$ 1	41 $\pm$ 1	40 $\pm$ 1	<0.04	0.99	0.40
Butterfat (%)	4.23 $\pm$ 0.23	3.66 $\pm$ 0.23	3.56 $\pm$ 0.23	3.75 $\pm$ 0.23	4.22 $\pm$ 0.23	3.88 $\pm$ 0.23	3.93 $\pm$ 0.23	3.68 $\pm$ 0.23	<0.04	0.68	0.08
Milk protein (%)	3.30 $\pm$ 0.08	3.29 $\pm$ 0.08	3.35 $\pm$ 0.08	3.34 $\pm$ 0.04	3.29 $\pm$ 0.08	3.26 $\pm$ 0.08	3.25 $\pm$ 0.08	3.33 $\pm$ 0.08	<0.03	0.74	0.06
Lactose (%)	4.55 $\pm$ 0.03	4.50 $\pm$ 0.03	4.52 $\pm$ 0.03	4.54 $\pm$ 0.03	4.53 $\pm$ 0.03	4.49 $\pm$ 0.03	4.44 $\pm$ 0.03	4.52 $\pm$ 0.03	<0.03	0.42	0.20
BW (kg)	711 $\pm$ 22	701 $\pm$ 22	704 $\pm$ 22	710 $\pm$ 22	668 $\pm$ 22	659 $\pm$ 22	667 $\pm$ 22	674 $\pm$ 22	<0.03	0.23	0.85
BCS	2.75	2.50	1.75	2.25	2.75	2.50	2.00	2.25			
Rumination time (min/d)	495 $\pm$ 23	512 $\pm$ 22	480 $\pm$ 23	504 $\pm$ 23	526 $\pm$ 23	551 $\pm$ 22	528 $\pm$ 23	536 $\pm$ 23	<0.03	0.25	0.78
Rumen pH											
Mean pH	6.38 $\pm$ 0.05	6.30 $\pm$ 0.05	6.22 $\pm$ 0.05	NA	6.43 $\pm$ 0.07	6.34 $\pm$ 0.07	6.24 $\pm$ 0.07	NA	<0.01	0.67	0.15
pH <6.2 (min/d)	291 $\pm$ 114	439 $\pm$ 114	589 $\pm$ 114	NA	218 $\pm$ 151	405 $\pm$ 151	634 $\pm$ 151	NA	<0.01	0.89	0.79
pH <5.8 (min/d)	9 $\pm$ 12	6 $\pm$ 12	3 $\pm$ 12	NA	4 $\pm$ 16	9 $\pm$ 16	7 $\pm$ 16	NA	0.44	0.34	0.87

¹P = period; Tx = treatment; Inter = interaction; Mean pH = mean rumen pH/d; pH <6.2 = time pH was lower than 6.2 (min/d); pH <5.8 = time pH was lower than 5.8 (min/d); NA = not applicable. Least means square $\pm$ SEM are presented for milk yield, milk characteristics, and BW. BCS is presented as median.

Table 4. Effect of yeast supplementation on milk yield, milk characteristics, rumination time, and rumen pH throughout trial 2¹

Item	Group 1				Group 2				P-value	
	P1	P2, yeast (4.0 g/d)	P3	P4	P1	P2	P3, yeast (4.0 g/d)	P4	Period	Inter
Milk yield (kg/d)	35 ± 2	33 ± 2	31 ± 2	29 ± 2	39 ± 2	37 ± 2	34 ± 2	33 ± 2	<0.01	0.23
Butterfat (%)	4.60 ± 0.20	4.37 ± 0.27	4.72 ± 0.27	4.54 ± 0.27	4.63 ± 0.27	4.49 ± 0.27	4.80 ± 0.27	4.73 ± 0.27	<0.05	0.77
Milk protein (%)	3.41 ± 0.12	3.45 ± 0.12	3.56 ± 0.12	3.47 ± 0.12	3.34 ± 0.12	3.43 ± 0.12	3.58 ± 0.12	3.42 ± 0.12	<0.01	0.85
Lactose (%)	4.51 ± 0.05	4.44 ± 0.05	4.48 ± 0.05	4.33 ± 0.05	4.55 ± 0.05	4.50 ± 0.05	4.45 ± 0.05	4.33 ± 0.05	<0.01	0.75
BW (kg)	648 ± 12	684 ± 12	685 ± 12	695 ± 12	701 ± 12	704 ± 12	700 ± 12	707 ± 12	<0.01	0.35
BCS	2.75	2.50	1.75	2.25	2.75	2.50	2.00	2.25	<0.01	0.44
Rumination time (min/d)	406 ± 25	399 ± 25	361 ± 25	400 ± 26	435 ± 25	481 ± 25	472 ± 25	503 ± 27	<0.01	0.05
Rumen pH										
Mean pH	6.14 ± 0.06	5.99 ± 0.06	5.88 ± 0.06	NA	6.18 ± 0.06	5.93 ± 0.07	5.86 ± 0.07	NA	<0.01	0.86
pH <6.2 (min/d)	840 ± 121	1,040 ± 120	1,228 ± 120	NA	743 ± 148	1,178 ± 148	1,264 ± 147	NA	<0.01	0.88
pH <5.8 (min/d)	118 ± 123	418 ± 123	625 ± 123	NA	30 ± 151	503 ± 151	664 ± 151	NA	<0.01	0.93

¹P = period; Tx = treatment; Inter = interaction; Mean pH = mean rumen pH/d; pH <6.2 = time pH was lower than 6.2 (min/d); pH <5.8 = time pH was lower than 5.8 (min/d); NA = not applicable. Least means square ± SEM are presented for milk yield, milk characteristics, and BW. BCS is presented as median.

Table 5. Effect of yeast supplementation on milk yield, milk characteristics, and rumen pH throughout trial 3¹

Item	Group 1					Group 2					P-value	
	P1	P2	P3, yeast (4.0 g/d)	P4	P5	P1	P2	P3	P4, yeast (4.0 g/d)	P5	Period	Inter
Milk yield (kg/d)	39 ± 1.77	31 ± 1.77	28 ± 1.77	29 ± 1.77	25 ± 1.77	33 ± 1.77	28 ± 1.77	28 ± 1.77	27 ± 1.77	23 ± 1.77	<0.01	<0.10
Butterfat (%)	3.94 ± 0.18	4.33 ± 0.18	3.97 ± 0.18	4.76 ± 0.18	4.59 ± 0.18	4.39 ± 0.18	4.65 ± 0.18	4.26 ± 0.18	4.75 ± 0.18	4.72 ± 0.18	<0.01	0.31
Milk protein (%)	3.00 ± 0.08	3.20 ± 0.08	3.08 ± 0.08	3.36 ± 0.08	3.48 ± 0.08	3.18 ± 0.08	3.23 ± 0.08	3.06 ± 0.08	3.36 ± 0.08	3.52 ± 0.08	<0.01	0.15
Lactose (%)	4.26 ± 0.09	4.42 ± 0.08	4.31 ± 0.08	4.34 ± 0.08	4.07 ± 0.08	4.45 ± 0.08	4.35 ± 0.08	4.30 ± 0.08	4.25 ± 0.08	4.09 ± 0.08	<0.01	0.23
BW (kg)	663	651	649	644	678	664	649	669	660	703	<0.01	0.16
BCS	2.75	2.50	2.25	2.50	2.25	2.25	2.50	2.50	2.25	2.50		
Rumen pH												
Mean pH	6.34 ± 0.07	6.30 ± 0.07	6.22 ± 0.07	6.10 ± 0.07	6.14 ± 0.07	6.29 ± 0.07	6.23 ± 0.07	6.06 ± 0.06	5.85 ± 0.06	6.00 ± 0.06	<0.01	<0.05
pH <6.2 (min/d)	390 ± 126	490 ± 115	651 ± 117	847 ± 117	662 ± 121	548 ± 117	674 ± 105	993 ± 105	1,324 ± 107	1,005 ± 109	<0.01	<0.05
pH <5.8 (min/d)	13 ± 104	16 ± 95	107 ± 97	301 ± 97	414 ± 100	53 ± 96	16 ± 87	219 ± 87	570 ± 88	468 ± 90	<0.01	<0.05

¹P = period; Tx = treatment; Inter = interaction; Mean pH = mean rumen pH/d; pH <6.2 = time pH was w than 6.2 (min/d); pH <5.8 = time pH was lower than 5.8 (min/d). Least means square ± SEM are presented for milk yield, milk characteristics, and BW. BCS is presented as median.

observed ($P < 0.05$); however, this effect was the same for both experimental groups (Table 3; P1 to P2: Cx = 17, Tx = 25 min/d ruminating; P2 to P3: Cx = -32, Tx = -28 min/d ruminating; P3 to P4: Cx = 24, Tx = 8 min/d ruminating).

Trial 2. No effect of yeast supplementation on the amount of time (min/d) the cows spent ruminating was observed. An effect of period on rumination time was observed ($P < 0.05$; Table 4; P1 to P2: G1 = -7, G2 = 46 min/d ruminating; P2 to P3: G1 = -38, G2 = -4 min/d ruminating; P3 to P4: G1 = 39, G2 = 31 min/d ruminating).

Trial 3. As described before, due to the poor results obtained with the RC in grazing animals in previous studies (Ambriz-Vilchis et al., 2015), no data on rumination time for trial 3 was analyzed.

Rumen pH

Rumen pH data were considered suitable for further analysis if they were within normal physiological parameters (rumen pH between 5.0 and 8.0).

Trial 1. Data on rumen pH was obtained from the rumen boluses; however, incomplete data sets were obtained from some of the animals, and some of the boluses were lost across the trial. Complete data on rumen pH values per hour were obtained from 14 cows in P1, 11 cows in P2 and P3 (6 Cx, 5 Tx), and only 4 cows in P4 (2 in each group). Therefore, we decided not to analyze data from P4 due to an insufficient number of recordings. Data on rumen pH variables is therefore only presented for P1 to P3.

Table 3 shows mean values of rumen pH per group and the time rumen pH was below 6.2 and 5.8. No differences were observed in mean rumen pH values between Cx and Tx groups; however, an effect of period was observed ($P < 0.05$; Table 3). Mean rumen pH tended to decline across the experimental periods. When analyzed as time spent under the predetermined acidotic cut-off points, a statistically significant ($P < 0.05$) effect of period was observed (Table 3); however no effect of yeast supplementation on the rumen pH values analyzed was observed (Table 3).

Trial 2. Data were obtained from the rumen boluses. Although individual cow response to the acidotic challenge of 1.5 kg of ground wheat was variable, the supplementation of 1.5 kg of ground wheat/cow per day was able to produce considerable bouts of acidosis (Figure 1). The thresholds used to assess SARA were pH 5.8 and 6.2 for more than 3 h/d, which were achieved during the experimental periods when yeast was supplemented.

Complete data on rumen pH values per hour were obtained from 10 cows in P1, P2, and P3 (G1 = 6 G2 =

4) and only 3 cows in P4 (G1 = 2 and G2 = 1). Therefore, we decided not to analyze any of this data from P4, and so data on rumen pH variables is presented for P1 to P3 only.

No differences were observed on mean rumen pH values in yeast-supplemented versus nonsupplemented animals (Table 4); however, an effect of period was observed ($P < 0.05$). Mean rumen pH tended to decline across the experimental periods. When analyzing the dynamics of rumen pH, different periods of time spent under the acidotic thresholds were observed. As observed with the mean rumen pH values, the cows tended to spend more time under the acidotic thresholds across the experimental periods. An effect of period was observed ($P < 0.05$) when time spent below pH 6.2 was analyzed, although no effect of yeast supplementation was observed. Again we found significant effect of period. No effect of the sequence followed by yeast supplementation was observed on any of the rumen pH variables analyzed (Table 4).

Trial 3. Complete data on rumen pH values per hour were obtained from 13 cows in P1 and P2 (G1 = 7 G2 = 6), 12 cows in P3 (G1 = 6 and G2 = 6), and 11 cows in P4 and P5 (G1 = 6 and G2 = 5). No differences in mean rumen pH were observed as a response to yeast supplementation. A significant effect of period was obtained ($P < 0.05$; Table 5). When analyzing the dynamics of rumen pH, different periods of time spent under the acidotic thresholds were observed. Cows tended to spend more time under the acidotic threshold as trial 3 progressed; however, no statistically significant effect of yeast supplementation was obtained. An effect of period and interaction period $\times$ treatment was obtained ($P < 0.05$) (Table 5).

DISCUSSION

Several nutritional interventions have been devised to counteract SARA, including live yeast supplementation. It is hypothesized that live yeast stabilizes the rumen environment and, hence, increases rumen pH and stimulates performance (Desnoyers et al., 2009). However, the effects of live yeast supplementation on rumen pH are variable, and contrasting results are reported in the literature with either no effects, mild responses, or tenuous effects on performance, animal behavior, and rumen pH (Dehghan-Banadaky et al., 2013; DeVries and Chevaux, 2014) being reported. The aims of our study were to evaluate the effect of live yeast supplementation on performance, rumination time, and rumen pH in cows fed a standard commercial diet or an acidogenic diet and when grazing.

The aim of trial 1 was to evaluate the effect of live yeast supplementation under standard commercial

Figure 1. Circadian rumen pH dynamics of 10 cows from both experimental groups in trial 2. The horizontal red lines show the 6.2 and 5.8 rumen pH SARA threshold. Vertical solid black line shows time when feed was delivered. Each figure and color represents an individual cow. Color version available online.

conditions. No statistically significant differences were observed on any of the parameters measured between the Cx and Tx groups. These results are in line with previous studies that found no statistically significant difference for production parameters or performance between cows receiving live yeast supplementation compared with those on a control diet. Kalmus et al. (2009) using 10 g/cow per day of live yeast culture (Yea-Sacc 1026, Alltech) found no effect of yeast supplementation on milk yield, whereas using 2.5 or 10 g of live yeast (1×10^9 cfu/g, Yea-Sacc¹⁰²⁶, Alltech) Al Ibrahim et al. (2010b) found no effect on milk yield, milk fat, protein, and lactose content. Similarly, Moallem et al. (2009), when feeding 1 g of yeast (1×10^{10} cfu/g, SC47 strain, Biosaf, Lesaffre Feed Additives, Lille, France) per 4 kg of DM consumed, reported no statistically significant differences between cows fed yeast against those that were not supplemented in terms of milk fat and milk protein. Kung et al. (1997) fed 10 or 20 g of live yeast (3.5×10^9 cfu/g, Biomate Yeast Plus, Chr. Hansen Biosystems Inc., Milwaukee, WI) and found no effect of

yeast supplementation on the milk yield of mid lactation dairy cows. Similarly, when evaluating the effect of yeast (5.3 or 5.1×10^{10} cfu Cell-con, Western Yeast Company, Chillicothe, IL) on lactation performance of early-lactation dairy cows, Swartz et al. (1994) found no effect when compared with cows with no yeast supplementation.

Results on the effect of live yeast supplementation on BCS and BW are very seldom reported; however, Kalmus et al. (2009) and Al Ibrahim et al. (2010b) found no effect on BCS when yeast was supplemented to cows in early lactation, which is in line with the results found in our research. Similarly, Tristant and Moran (2015) found no effect on BCS and BW when live yeast was fed (25 g/cow per day of Yea-Sacc Farm Pak, 1.07×10^8 cfu/g, Alltech) to a group of cows of different parity and in early and mid lactation.

No differences were observed between the different doses of yeast fed; however, a slight nonsignificant increment on milk yield and butterfat were observed when the highest dose of live yeast (6×10^{10} cfu/cow

per day) was supplemented. These results are similar to those obtained by Wohlt et al. (1998), who reported better responses on milk yield and fat-corrected milk yield when doses of 10×10^{10} cfu/cow per day were fed compared with the lower dose of 5×10^{10} cfu/cow per day (20 or 10 g, respectively, of Biomate Yeast Plus, Chr. Hansen Laboratory Inc.) fed in their trial.

It is noteworthy that although no statistically significant differences between milk yields were observed, an arithmetical difference of more than 1 kg milk between groups was observed. In period 3, when cows in the Tx group were fed the highest dose of live yeast, a larger difference in milk yield was observed. These results are similar to those obtained for cows in early lactation (Kung et al., 1997; Kim et al., 2006) or mid to late lactation (Moallem et al., 2009), where a tendency on increasing milk yield was observed.

Contrasting results were reported by Ferraretto et al. (2012) when mid-lactation dairy cows were consuming contrasting diets, with 2 different doses of live yeast (30 or 60 billion cfu/cow per day, Procreatin-7, Lasaffre Feed Additives, Milwaukee, WI). Those authors found no effect ($P > 0.05$) of live yeast supplementation in any of the variables studied, although differences between the 2 doses supplemented were observed; total NDF digestibility and milk fat content were higher when 60 billion cfu/cow per day was administered.

The development of automated methods to record cow behavior facilitate the evaluation of the effect that live yeast supplementation may have on rumination time. A recent study by DeVries and Chevaux (2014) looked to investigate the effect of live yeast supplementation (1×10^{10} cfu, 0.5 g/cow per day of Levucell SC20, Lallemand Animal Nutrition) on feeding behavior of dairy cows. Those authors found that live yeast supplementation had no effect on meal pattern or rumination time; however, they reported a tendency for rumination time to increase with yeast supplementation. These results are in line with our findings, and in the Tx group rumination time tended to increase across the periods (although this was not statistically significant).

Our results showed an effect of period (or time) on rumen pH, with decreasing values of mean rumen pH and increasing time (min/d) for rumen pH spent under the SARA thresholds. These findings are difficult to compare with other studies that used more invasive techniques to measure rumen pH. Studies on the effect of live yeast supplementation on rumen environment (e.g., rumen pH) have been carried out in vitro and in vivo, only by using fistulated animals or by taking rumen fluid samples using rumenocentesis from intact animals. By their nature, these invasive techniques mean that the length of the period evaluated is short (a few days) and data sets are small (rumenocentesis is

performed only once a day). Nevertheless, using cannulated dairy cows in late lactation, Thrune et al. (2009) found that live yeast supplementation at 10^{10} cfu/cow per day (0.5 g/cow per day of Levucell SC, Lallemand Animal Nutrition) had an effect on rumen pH. In their study, yeast fed animals had higher mean rumen pH, and spent less time under the acidotic threshold (between 5.2 and 5.6) when compared with those on a control diet. Al Ibrahim et al. (2010b) analyzed the rumen fluid of dairy cows in early lactation obtained by rumenocentesis, and reported no statistically significant differences on rumen pH and other rumen fermentation parameters in yeast culture-supplemented cows when compared with nonsupplemented control cows. Similarly, Moallem et al. (2009) found no effect on rumen pH as a result of live yeast supplementation.

In our results, the observed effect of period could be explained by the stage of lactation the cows were at throughout trial 1 (mid to late lactation). A normal reduction in milk yield is expected after cows reach their peak milk yield; this reduction in milk production could affect feed intakes, which in turn would affect pH dynamics, leading to cows spending less time the under SARA thresholds.

The aim of trial 2 was to evaluate the effect of live yeast supplementation on cows under an acidotic challenge. No statistically significant effects of live yeast supplementation were observed on any of the parameters measured in the experimental groups. Response to live yeast supplementation in SARA-induced animals reported in other research studies have been similar to our results. AlZahal et al. (2014) reported no effect of live yeast supplementation (2×10^{10} cfu/g of DM, 4 g/cow per day of Biomate, AB Vista) on yield and milk characteristics in late-lactation dairy cows under an acidotic regimen; however, those authors reported an effect on FCM and lactose content in the milk of yeast-supplemented cows compared with controls. Similar results were reported by Malekkhahi et al. (2016), where no effect of live yeast supplementation (20×10^9 cfu, 10 g of Yea-Sacc 1026, Alltech) was observed.

Saliva produced during rumination is one of the elements involved in the regulation of rumen pH, and so it could be speculated that low rumen pH would result in low rumination activity. However, this was not observed in trial 2, as rumination time was not affected by live yeast supplementation.

The acidotic challenge implemented in trial 2 was able to produce SARA bouts for more than 5 h/d in all the experimental animals. However, we noted considerable differences in how individual animals coped with the acidotic challenge in terms of rumen pH levels and duration of SARA observed in individual animals. Despite similar management regimens, parity, and lac-

tation stage, individual animals showed high variation in their susceptibility to suffer SARA. This could be explained by diet characteristics, differences in eating, ruminating and sorting behavior, feed intake (which in turn will result in animal-specific diet characteristics), saliva production level, unique microbiota, differences in the absorptive capacity of the ruminal epithelia, and rate of feed passage.

The time that the cows experienced SARA was longer in duration but not lower in its level than those definitions proposed by Gozho et al. (2007; pH depression below 5.6 for more than 180 min/d) and also used by Li et al. (2012) and Khafipour et al. (2009). However, the acidotic challenge used in trial 2 was sufficient to observe the levels of SARA expected and offer a sufficient acidotic challenge for assessing the efficacy of live yeast supplementation.

Mean rumen pH declined and time spent under SARA thresholds increased throughout trial 2 in both groups. Live yeast supplementation had no effect on the rumen pH variables evaluated; in a study with nonlactating dairy cows, Chung et al. (2011) reported similar results. When evaluating 2 different live yeast strains, those authors reported that 1 strain was no different from the control group, and the other strain reduced rumen pH and extended the duration of time spent under SARA thresholds. Contrasting results are presented by AlZahal et al. (2014); using live yeast after induced SARA, those authors reported a lower time spent under the acidotic threshold pH <5.6 (treatment = 122 ± 57 min vs. control = 321 ± 53 min) for yeast-supplemented cows. Different levels and extent of episodes of SARA were observed in trial 2, and it could be that a higher acidotic challenge is required to produce a more stable and widespread SARA response in all animals, sufficient to produce clinical signs such as changes in milk quality. Such higher degrees of SARA may produce a more challenging environment in which to determine the effects of any supplementation with yeast.

The aim of trial 3 was to evaluate the effect of live yeast supplementation on performance and rumen pH in cows grazing grass. No statistically significant effect of live yeast supplementation was observed in any of the evaluated variables.

It is thought that cows consuming high-quality pastures might be at risk of suffering SARA (Kolver and de Veth, 2002; O'Grady et al., 2008). Furthermore, the change in the offered diet (from indoor feeding to grazing) may pose a risk to the animals by altering the rumen pH with negative consequences in productivity. Despite these possible risks, the effect of live yeast supplementation on production parameters in grazing animals is scarce. In our research, no effects of live yeast

supplementation on performance and milk characteristics were found. In trial 3, throughout the experimental periods, milk yield followed the well-established decline pattern of the lactation curve, with this reduction more evident from P1 (indoors PMR) to P2 (outside grazing). Live yeast supplementation was unable to counteract the decline in milk yield. Cows started trial 3 at an average of 189 DIM, when the lactation peak had passed and milk yield is normally declining. Furthermore, it was assumed that the grazing conditions are not capable of achieving the same milk yields compared with PMR-based diets in high-yielding cows. This was due to grazing animals' inability to meet the nutritional requirements imposed for high levels of milk production, as DMI was lower and the diet was of lower quality (pasture compared with PMR), which results in lower energy intakes (Stockdale, 1999).

Previous research has suggested that changing from indoor PMR feeding regimens to outside grazing poses a risk for the incidence of SARA (O'Grady et al., 2008). In the current study, a nonsignificant decline in mean rumen pH and increase in the time spent under SARA threshold was observed when cows moved from indoors to outside grazing. Similar results were reported by Al Ibrahim et al. (2012), where the authors reported differences in rumen pH values when cows were subject to 2 different management strategies: abrupt or gradual introduction to pasture. The abrupt introduction to pasture produced the lowest levels of mean rumen pH and time spent under SARA. Similar results were reported by Gasteiner et al. (2015) when measuring rumen pH in cows under a gradual transition from PMR to grazing. Those authors found a decrease in mean rumen pH when the cows were first introduced to pasture (2–7 h of grazing), but an increase in mean rumen pH when grazing time was gradually increased (Gasteiner et al., 2015). In trial 3, rumen pH continued to decline throughout the periods until P5, when a slight increment was observed. These differences could be explained by the duration of the experimental periods, which were 7 d in the reported study compared with 28 d in trial 3 in our study. Furthermore, a higher level of milk yield reported in trial 3 would require higher levels of grass intake, which may have stronger effects on rumen pH.

Decline in mean rumen pH was observed across the experimental periods, and the decline tended to reduce by P4 and P5. It could be hypothesized that for some cows, adaptation to grazing conditions could improve rumen environment hence maintaining rumen pH levels. However, other cows consistently produced low rumen pH values throughout trial 3, suggesting individual cow factors influence rumen pH dynamics.

Furthermore, the results obtained for both time under SARA and mean rumen pH might suggest a tenuous effect or challenge of grazing on rumen pH. This could be due to the quality of the offered pasture, or the high variation in individual feed intakes that is thought to occur with grazing animals (Bargo et al., 2003; Hills et al., 2015).

Live yeast supplementation had no effect on rumen pH or time spent under SARA thresholds in the current study. Similar results were obtained by Al Ibrahim et al. (2012) in early-lactation fistulated cows supplemented with live yeast (2.5×10^9 cfu/d, Yea-sacc¹⁰²⁶ TS, Alltech). However, those authors reported an effect of yeast supplementation on the time that animals spent under SARA conditions.

It is very seldom that in vivo studies look at yeast viability, and it is common practice to rely on manufacturer's indications in terms of the viability of live yeast in commercial products. From the studies referenced in this manuscript, only those by Swartz et al. (1994), Kung et al. (1997), and Bayat et al. (2015) evaluated the viability of yeast in the commercial products used. In the present study, we relied on the manufacturer's specification for storage, handling, and supplementation of the product to guarantee viability of the yeast offered. This is, therefore, one of the limitations of the present study.

CONCLUSIONS

In the present study, live yeast supplementation had no significant effect on any of the recorded variables, and no evidence for the benefits of live yeast supplementation was found. Cows fed under commercial farm environments, consuming standard diets, an acidotic challenge, or under grazing conditions did not benefit from live yeast supplementation. It might be that studies on animals at different stages of lactation (with emphasis on transition period and early lactation), consuming more challenging diets (high level of inclusion of concentrates or starch), or in different environments, such as grazing of succulent forages, are necessary to elucidate the effects of live yeast supplementation on commercial farm environments. The use of new technologies, such as rumination collars and rumen pH boluses, will aid in the study of the effects of yeast supplementation and other additives on dairy cows under standard commercial conditions.

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