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Effect of disodium/calcium malate or *Saccharomyces cerevisiae* supplementation on growth performance, carcass quality, ruminal fermentation products, and blood metabolites of heifers[1]

C. Carrasco,*[2] P. Medel,* A. Fuentetaja,† M. J. Ranilla,‡ and M. D. Carro§

*Imasde Agroalimentaria SL, Pozuelo de Alarcón, Madrid, Spain, 28224; †Comercial Pecuaria Segoviana SL, Coca, Segovia, Spain, 40480; ‡Departamento de Producción Animal, Universidad de León, León, Spain, 24071; and §Departamento de Producción Agraria, Universidad Politécnica de Madrid, Madrid, Spain, 28040

ABSTRACT: The aim of this study was to assess the effects of malate salts and *Saccharomyces cerevisiae* culture on growth performance, carcass quality, ruminal fermentation products, and blood metabolites in heifers raised under southern Europe practical farm conditions. A total of 108 Charolaise cross heifers (214 ± 27.3 kg BW and 6.4 ± 1.1 mo of age) were housed in 18 pens of 6 animals each and used in a 114-d feedlot study. There was a totally randomized experimental design, and 6 pens were assigned to each of the following experimental diets: a control (no supplementation), the control plus 4 g of disodium/calcium malate mixture per kilogram of concentrate (2.12 g malate/kg), and the control plus 0.15 g of *S. cerevisiae* CBS 493.94 per kilogram of concentrate (1.5×10^8 cfu/kg). The control diet consisted of wheat–barley–based pelleted concentrate (32% starch, DM basis) and full-length barley straw. Concentrate and straw were fed separately ad libitum (5% orts) in an 88:12 ratio. On Days 0, 56, and 114, ruminal fluid and blood samples were obtained from each heifer between 2 and 2.5 h after the morning feeding by ruminocentesis and tail venipuncture, respectively. Body weight, concentrate ADFI, and G:F were recorded at 28, 56, 84, and 114 d. At slaughter, hot carcass weight and yield and carcass classification were determined in 2 representative heifers per pen (12 animals per dietary treatment). Supplementation with malate salts or *S. cerevisiae* did not affect concentrate ADFI ($P = 0.98$), ADG ($P = 0.74$), or G:F ($P = 0.50$) at any time during the experiment. At slaughter, there were no differences in carcass weight ($P = 0.86$), classification ($P = 0.18$), or carcass yield ($P = 0.84$) among experimental groups. Also, there were no differences treatments on ruminal pH ($P = 0.24$), ruminal fermentation products ($P = 0.69$, $P = 0.88$, and $P = 0.93$ for total VFA, NH_3–N, and lactate, respectively), and blood metabolites ($P = 0.96$, $P = 0.82$, and $P = 0.15$ for glucose, urea N, and lactate, respectively). In conclusion, under the feeding and management conditions of this study, diet supplementation with malate salts or *S. cerevisiae* did not have any significant effects on growth performance, carcass quality, ruminal fermentation products, and blood metabolites.

Key words: beef heifers, carcass quality, growth performance, malate salt, ruminal fermentation, *Saccharomyces cerevisiae*

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INTRODUCTION

High-grain diets favor growth but could result in subacute ruminal acidosis (**SARA**), a reduction in ruminal pH below 5.8 due to VFA accumulation and insufficient buffering (Calsamiglia et al., 2012). Manipulating rumen ecology to promote lactate-uptaking microorganisms, such as *Selenomonas ruminantium* and *Megasphaera elsdenii*, has been proposed to reduce SARA (Owens et al., 1998). Malate has been reported to stimulate in vitro lactate uptake by *S. ruminantium* (Martin and Park, 1996; Martin 1998) and to increase ruminal pH and propionate production (reviewed by Carro and Ungerfeld, 2015). In contrast, information on the effects of malate on in

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[2]Corresponding author: ccarrasco@e-imasde.com

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vivo ruminal fermentation and performance of beef cattle is more limited and conflicting (Castillo et al., 2004). Yeast cultures of *Saccharomyces cerevisiae* have also been reported to stimulate lactate utilization by *S. ruminantium* and *M. elsdenii* (Callaway and Martin, 1997) and could contribute to increased rumen pH (Chaucheyras-Durand et al., 2012). As previously reviewed (Desnoyers et al., 2009; De Ondarza et al., 2010), *S. cerevisiae* supplementation to dairy cattle increases milk production, but studies on beef cattle are scarce and controversial. Inconsistences in the response to malate and *S. cerevisiae* supplementation may be explained by variations in the dose, growth rate and age of the animal, diet composition, and farming conditions, among other factors (Yoon and Stern, 1995; Carro and Ungerfeld, 2015). Our hypothesis was that dietary supplementation with malate or *S. cerevisiae* may be effective at increasing ruminal pH and improving performance in beef cattle fed high-grain diets, as animals fed these diets usually have ruminal abundance of *S. ruminantium* and *M. elsdenii* (Petri et al., 2013). This study was designed to assess the effects of malate salts and *S. cerevisiae* culture on growth performance, carcass quality, ruminal fermentation products, and blood metabolites in heifers raised under commercial farming conditions in southern Europe.

MATERIALS AND METHODS

The study was approved by the institutional animal care committee of the Universidad de León (León, Spain) and was conducted in accordance with the Spanish guidelines for experimental animal protection (Boletín Oficial del Estado, 2013.).

Animals, Diets, and Experimental Design

A total of 108 Charolaise cross heifers with an average BW of 214 ± 27.3 kg and 6.4 ± 1.10 mo of age were used. Upon arrival to the experimental farm (Comercial Pecuaria Segoviana SL, Coca, Spain), each animal was weighed, treated for endo- and ectoparasites with ivermectin (1 mL/50 kg BW, Ivomec; Merial Laboratorios SA, Barcelona, Spain), and vaccinated against infectious bovine rhinotracheitis, parainflueza-3, and bovine viral diarrhea (2 mLper animal, Cattlemaster-4; Zoetis Spain SLU, Madrid, Spain) and enterotoxemia and carbuncle (2 mLper animal, Miloxan; Merial Laboratorios SA). A booster dose was given 3 wk later. Before starting the trial, all animals were fed an adaptation diet consisting of concentrate and straw for 7 d. Feeds were provided ad libitum (5% orts) twice a day, and fresh water was freely accessible at all times. In this period, the amount of straw and concentrate provided was recorded. Although feed refusals were not measured, a rough ratio of 70:30 concentrate-to-straw intake was estimated based on feed supply.

At the beginning of the trial, heifers were weighted and assigned to 18 pens (6 heifers per pen) according to their weight and age. The pens were 5.0 by 7.0 m and had continuous concrete floor. Barley straw was used as bedding material. There was a totally randomized experimental design, and pens were randomly allotted to 1 of the 3 experimental treatments (6 pens per treatment). The dietary treatments were a control (not supplemented; **CON**); the control plus 4 g of disodium/calcium malate mixture (**MAL**; Rumalato; NOREL SA, Madrid, Spain) per kilogram of concentrate, providing 2.12 g of malate per kilogram of concentrate; and the control plus 0.15 g of *S. cerevisiae* CBS 493.94 (**YC**; Yea-Sacc; Alltech Inc., Nicholasville, KY) per kilogram of concentrate, providing 1.5×10^8 cfu/kg of concentrate. Both additives were used at the dose recommended by the manufacturers. Experimental diets were formulated to meet or exceed the nutritional requirements of the NRC (2000) for growing/finishing heifers of 350 kg BW and 1.4 kg ADG. Ingredient and chemical composition of the concentrates fed during the adaptation and experimental periods is shown in Table 1. The composition of the concentrate was representative of those used for commercial feedlots in southern Europe, with wheat and barley grains as the main components. Concentrate and full-length barley straw were provided separately ad libitum twice daily (0700 and 1800 h), and their supply was recorded daily throughout the trial. Concentrates were pelleted to reduced feed selection and refusals. Straw refusals dropped on the floor formed part of bedding material and could not be quantified.

Productive Parameters and Carcass Characteristics

On d 28, 56, 84, and 114, animals were individually weighed and concentrate refusals in each pen were determined to calculate the following productive parameters: ADG (kg), concentrate ADFI (kg), and G:F (ADG/ADFI). In addition, feed refusals were observed daily at feeding time to adjust the amount supplied, and orts were removed and weighted when any spoilage was detected. The amount of straw provided daily was recorded, but actual intake of straw could not be determined as part of the straw was thrown to the ground by the heifers.

On d 114, animals were slaughtered at a commercial slaughterhouse (Laguna de Duero, Valladolid, Spain), and 2 representative heifers (i.e., heifers having the maximum and minimum BW were excluded) per pen were randomly selected to determine individual HCW and carcass characteristics. Individual dressing percentage was calculated as the relationship between HCW and BW at d 114 of the trial. Carcass compactness index was

Table 1. Ingredients and chemical composition of the concentrates fed over the adaptation and experimental periods

Item	Adaptation period	Experimental concentrates[1]		
		CON	MAL	YC
Ingredient, g/kg (as-fed basis)				
Sunflower seed	189	–	–	–
Olive pulp	100	–	–	–
Soybean hulls	100	–	–	–
Corn distiller's dried grains with solubles	100	–	–	–
Beet pulp	70.0	–	–	–
Rice bran	60.0	–	–	–
Animal fat	2.1	–	–	–
Wheat grain	–	282	278	282
Barley grain	–	210	210	210
Corn gluten feed	100	100	100	100
Wheat middlings	14.9	100	100	100
Sunflower meal	–	100	100	100
Palm kernel meal	50.0	55.0	55.0	55.0
Beet molasses	30.0	40.0	40.0	40.0
Alfalfa meal	100	40.0	40.0	40.0
Beet vinasse	27.9	20.0	20.0	20.0
Sepiolite	24.1	20.0	20.0	20.0
Calcium carbonate	25.4	18.0	18.0	18.0
Oleine[2]	–	10.0	10.0	10.0
Sodium chloride	7.0	2.5	2.5	2.5
Mineral/vitamin premix[3]	–	2.5	2.5	2.5
Disodium/calcium malate salts	–	–	4.00	–
Saccharomyces cerevisiae	–	–	–	0.15
Chemical composition[4]				
DM, %	ND[5]	89.3 (1.10)	89.4 (0.85)	88.6 (1.48)
OM, % of DM	–	93.8 (0.67)	93.9 (0.30)	94.0 (0.65)
CP, % of DM	–	15.2 (0.66)	15.6 (0.97)	15.9 (0.94)
NDF, % of DM	–	23.0 (2.18)	24.4 (0.47)	23.6 (2.45)
Starch, % of DM	–	31.0 (2.76)	30.9 (0.71)	31.9 (1.26)
Malate, % of DM	–	0.230	0.445	0.240

[1]CON = control (not supplemented); MAL = control plus 4 g of disodium/calcium malate mixture (Rumalato; NOREL SA, Madrid, Spain) per kilogram of concentrate, providing 2.12 g of malate per kilogram of concentrate; YC = control plus 0.15 g of *Saccharomyces cerevisiae* CBS 493.94 (Yea-Sacc 1026; Alltech Inc., Nicholasville, KY) per kilogram of concentrate, providing 1.5×10^8 cfu/kg of concentrate.

[2]A product based on refined olive soap stock (Refinación Industrial Oleícola SA, Ibros, Spain) that contained 10 g of ashes per kilogram DM and 990 g of ether extract per kilogram DM. Fatty acid profile (g/kg ether extract): 640 of C18:1, 160 of C18:2, 110 of C16:0, and 38 of C18:0.

[3]Mineral and vitamin premix contained according to the manufacturer (Comercial Pecuaria Segoviana SL, Segovia, Spain): 8,000 IU of vitamin A (retinil acetate), 1,600 IU of vitamin D_3 (cholecalciferol), 6,000 IU of vitamin E (**α**-tocopheryl acetate), 7.9 mg of Cu (cupper sulfate), 0.7 mg of Co (cobalt(II) sulfate), 0.2 mg of Se (sodium selenite), 50 mg of Zn (zinc oxide), 35 mg of Mn (manganese oxide), 438 mg of Mg (magnesium oxide), 100 mg of S (potassium sulfate), 20 mg of Fe (iron sulfate), and 0.8 mg of I (potassium iodide).

[4]Mean (SD) values of 3 composite period samples. Calculated chemical composition of control concentrate based on Fundación Española para el Desarrollo de la Nutrición Animal reference tables of feedstuffs composition (de Blas et al., 2010) was 93.5, 14.7, 22.8, and 32.0% of OM, CP, NDF, and starch, respectively (DM basis).

[5]ND = not determined. Chemical composition of adaptation concentrate was not determined. Calculated chemical composition from Fundación Española para el Desarrollo de la Nutrición Animal (de Blas et al., 2010) was 88.0, 16.9, 38.6, and 4.9% of OM, CP, NDF, and starch, respectively (DM basis).

determined as the relationship between carcass length and chest depth. Carcass length was determined on the hanged hot left midcarcass as the length from the cranial border of the first rib to the point of the pubic symphysis, and chest depth was measured from the ventral surface of the spinal canal (at fifth rib level) to the lowest point of the sternum. Leg compactness index was determined as the relationship between leg length and perimeter. Leg length was measured from the inner side of the tarsus–metatarsus joint to the point of the pubis symphysis, and leg perimeter was measured at the level of the crest of the ileum. Quality grade was determined following the European carcass grading system (Council Regulation (EC) number 1183/2006; Official Journal of the European Union, 2006.) according to the following conformation classes: superior (**S**), excellent (**E**), very good (**U**), good (**R**), fair (**O**), and poor (**P**) and degrees of fat cover: 1 (poor), 2 (slight), 3 (average), 4 (high), and 5 (very high).

Ruminal Fermentation Products and Blood Metabolites

On Days 0, 56, and 114, rumen fluid samples of each heifer were obtained between 2 and 2.5 h after morning feeding by rumenocentesis. A needle of 12.5 cm long and 1.6 mm gauge was inserted into the ventral sac of the rumen in the center of the triangle between last rib, wing of ileum, and transverse process of spine, and an aliquot (about 20 mL) of rumen fluid was obtained. The time of feed supply was adjusted to the expected time of blood and ruminal fluid sampling, and the pens of each treatment were consistently distributed across the collection period. Rumen samples were homogenized, the pH was immediately measured (Crison Basic 20 pH-meter; Crison Instruments, Barcelona, Spain), and fermentation was stopped by swirling the samples in iced water. Five milliliters of fluid were added to 5 mL of deproteinizing solution (20 g of metaphosphoric acid and 0.6 g crotonic acid/L) for VFA analysis, and 2 mL were added to 2 mL 0.5 *M* HCl for NH_3–N and lactate determinations. Samples were immediately frozen (−20°C) until analyses. In addition, blood samples (9 mL) were collected from each heifer by puncture of the median coccygeal vein into vacutainer tubes containing 143 United States Pharmacopeia units of sodium heparin. Samples were centrifuged (1,000 × *g* for 15 min at 4°C), and the plasma was immediately frozen (−20°C) until determination of glucose, urea N, and total lactate.

Analytical Procedures

Dry matter (method 934.01), ash (method 942.05), and N (method 984.13) content of concentrates were determined according to the AOAC (1999), and NDF analyses were performed according to Van Soest et al. (1991) using an ANKOM^{220} Fiber Analyzer unit (ANKOM Technology Corporation, Fairport, NY). Sodium sulfite and heat-stable amylase were used in analysis of NDF and they were expressed inclusive of residual ash. Malate content of experimental concentrates was analyzed by HPLC-UV (Callaway et al., 1997).

Concentrations of VFA, ammonia N, and total lactate in ruminal fluid were determined as described by García-Martínez et al. (2005). Plasma concentrations of glucose, urea N, and total lactate were determined by automated enzymatic methods adapted to a Cobas Integra 400 plus Analyzer (F. Hoffmann-La Roche Ltd., Basel, Switzerland).

Statistical Analysis

The experimental unit for BW, ADG, concentrate ADFI, G:F, carcass-adjusted values, HCW, and carcass yield measurements was the pen (mean data for 6 heifers). The individual animal was the experimental unit for ruminal fermentation products, blood metabolites, and carcass characteristics. Data on feed intake, growth performance, ruminal fermentation products, and blood metabolites were analyzed as repeated measures using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC). A compound symmetry was used to model the covariance structure for the repeated measures. The statistical model included diet, time (sampling day), and diet × time interaction as fixed effects and either pen or heifer as the random effect, respectively. Carcass-adjusted final BW was calculated as HCW/average dressing percent for heifers in each treatment. Carcass-adjusted ADG and G:F were calculated from carcass-adjusted final BW. Those data were analyzed using PROC GLM of SAS, and the model included diet as the main effect. Data on HCW, dressing percentage, and carcass characteristics (carcass compactness index and leg compactness index) were analyzed using PROC GLM of SAS, and the model included diet as the main effect and final BW as a covariate. Data on carcass conformation were analyzed using PROC GLIMMIX of SAS. Data are presented as least squares means. Significance was declared at $P < 0.05$, and $0.05 < P < 0.10$ values were considered to be a trend.

RESULTS

Malate content in CON concentrate was 2.30 g, and the MAL diet had 4.45 g of malate per kilogram of concentrate (Table 1). Daily average consumption of malate was 13.7, 26.3, and 14.2 g for heifers fed the CON, MAL, and YC diets, respectively.

As shown in Table 2, there were no differences among treatments on concentrate ADFI ($P = 0.98$), BW ($P = 0.95$), ADG ($P = 0.74$), or G:F ($P = 0.50$), and no treatment × time interactions were observed ($P > 0.10$). Carcass-adjusted BW, ADG, and G:F values were similar among treatments ($P = 0.94$, $P = 0.95$, and $P = 0.92$, respectively). Actual barley straw intake was not determined, but no differences among groups were detected in the amount of straw provided daily. Average straw intake over the trial was estimated to be about 120 g/kg of total DMI. As expected, concentrate ADFI and BW increased with time ($P < 0.001$).

Hot carcass weight ($P = 0.86$), dressing percentage ($P = 0.84$), carcass compactness index ($P = 0.82$), and leg compactness index ($P = 0.62$) were not affected by experimental treatments (Table 3). Carcass quality grade was also similar among experimental groups ($P = 0.18$), and fat cover grade was 3 (average) for all heifers.

Data on rumen fermentation products and blood metabolites are shown in Tables 4 and 5, respectively. No treatment × time interaction was detected for any rumen fermentation product measured ($P > 0.10$). There

Table 2. Concentrate intake, BW, ADG, and G:F in heifers fed 1 of the following high-grain diets: control (not supplemented; CON), control plus 4 g of disodium/calcium malate mixture (MAL), or control plus 0.15 g of *Saccharomyces cerevisiae* CBS 493.94 (YC)

Item and period (days of feed)	Treatment			SEM	*P*-value		
	CON	MAL[1]	YC[2]		Treatment	Time	Treatment × time
Concentrate ADFI, kg							
d 0–28	5.68	5.71	5.65	0.139	0.98	<0.001	0.87
d 29–56	6.53	6.57	6.67				
d 57–84	6.35	6.31	6.37				
d 85–114	7.18	7.05	7.04				
BW, kg							
d 0	214	215	214	7.79	0.95	<0.001	0.85
d 28	241	242	242				
d 56	272	275	274				
d 84	298	303	300				
d 114	335	338	335				
ADG, kg							
d 0–28	0.99	0.97	1.00	0.047	0.74	<0.001	0.67
d 29–56	1.10	1.19	1.14				
d 57–84	0.93	1.01	0.95				
d 85–114	1.21	1.16	1.14				
G:F, kg/kg							
d 0–28	0.173	0.170	0.174	0.2436	0.50	<0.001	0.53
d 29–56	0.167	0.180	0.170				
d 57–84	0.143	0.159	0.149				
d 85–114	0.168	0.162	0.161				
Carcass adjusted[3]							
BW (114 d)	367	371	369	8.5	0.94	–	–
ADG (0–114 d)	1.10	1.12	1.11	0.05	0.95	–	–
G:F (0–114 d)	0.170	0.175	0.173	0.009	0.92	–	–

[1]Control plus 4 g of disodium/calcium malate mixture (Rumalato; NOREL SA, Madrid, Spain) per kilogram of concentrate, providing 2.12 g of malate per kilogram of concentrate.

[2]Control plus 0.15 g of *Saccharomyces cerevisiae* CBS 493.94 (Yea-Sacc 1026; Alltech Inc., Nicholasville, KY) per kilogram of concentrate, providing 1.5×10^8 cfu/kg of concentrate.

[3]Carcass-adjusted final BW was calculated as HCW/average dressing percent.

were no effects of dietary treatment on ruminal pH ($P = 0.24$); concentrations of NH_3–N ($P = 0.82$), lactate ($P = 0.93$), and total VFA ($P = 0.69$); and molar proportions of individual VFA ($P > 0.10$). Sampling time influenced ($P < 0.01$) all rumen fermentation products except the molar proportion of butyrate, which remained unaffected over the trial ($P = 0.49$). The lowest ruminal pH was observed at d 56 (the average value across treatments was 5.44), and the highest value was observed at the end of the trial (6.09). Evolution of lactate and total VFA concentrations over the trial was the inverse of pH, and the highest concentrations were observed on d 56. Rumen NH3–N concentrations increased over the experimental period ($P < 0.001$). Dietary treatments did not affect plasma concentrations of glucose ($P = 0.96$), lactate ($P = 0.82$), and urea N ($P = 0.15$), and no treatment × time interactions ($P > 0.10$) were detected. Urea N concentrations increased with time ($P < 0.001$), but glucose and lactate concentrations remained unchanged during the trial ($P = 0.19$ and $P = 0.27$, respectively).

DISCUSSION

This study was designed to assess the effects of malate and *S. cerevisiae* culture on growth performance, carcass quality, rumen fermentation products, and blood metabolites in heifers raised under practical farm conditions in southern Europe. The diet was typical of those fed in the practice to growing/finishing beef cattle, and both additives were supplemented at the level recommended by the manufacturer. The dietary starch supply was close to 31% DM, with wheat and barley grains being the main starch sources, which agrees with the nutritional profile of high-grain diets that induces SARA (Owens et al., 1998; Dohme et al., 2008). The relatively high basal level of malate in the CON was probably due to the presence of alfalfa meal, which is a natural source of malate (Callaway et al., 1997).

In agreement with previous studies in beef cattle supplemented daily up to 120 g of malate salts or malic acid (Martin et al., 2009; Montaño et al., 1999; Castillo

Table 3. Carcass characteristics of heifers fed 1 of the following high-grain diets: control (not supplemented; CON), control plus 4 g of disodium/calcium malate mixture (MAL), or control plus 0.15 g of *Saccharomyces cerevisiae* CBS 493.94 (YC)

	Treatment				
Item	CON	MAL[1]	YC[2]	SEM	*P*-value
HCW, kg	202	201	200	2.5	0.86
Dressing proportion, kg carcass/100 kg BW	54.9	54.5	54.3	0.69	0.84
Carcass characteristics					
Carcass compactness index[3]	1.83	1.80	1.81	0.034	0.82
Leg compactness index[4]	1.48	1.47	1.46	0.014	0.62
Quality grade (% within category)[5]	3.83	4.08	3.92	0.066	0.18

[1]Control plus 4 g of disodium/calcium malate mixture (Rumalato; NOREL SA, Madrid, Spain) per kilogram of concentrate, providing 2.12 g of malate per kilogram of concentrate.

[2]Control plus 0.15 g of *Saccharomyces cerevisiae* CBS 493.94 (Yea-Sacc 1026; Alltech Inc., Nicholasville, KY) per kilogram of concentrate, providing 1.5×10^8 cfu/kg of concentrate.

[3]Relationship between carcass length (from the cranial border of the first rib to the point of the pubic symphysis) and chest depth (from the ventral surface of the spinal canal, at fifth rib level, to the lowest point of the sternum).

[4]Relationship between leg length (from the inner side of the tarsus–metatarsus joint to the point of the pubis symphisis) and perimeter (measured at the level of the crest of the ileum).

[5]Determined according to European carcass grading system for conformation (1 = superior, 2 = excellent, 3 = very good, 4 = food, 5 = fair, and 6 = poor).

et al., 2007; Carrasco et al., 2012; Vyas et al., 2015), malate supplementation did not affect either ADFI or cattle growth performance. In contrast, Martin et al. (2009) observed that ADG and G:F significantly increased in steers receiving daily 60 or 120 g of malic acid during the 10-d step-up period. Because malate effects were not observed after 52 d of feed, Martin et al. (2009) suggested that due to the high cost of malate, supplementation would be more practical when beef cattle first arrive at the feedlot rather than throughout the finishing period. Unfortunately, the concentrate fed to heifers during the adaptation period in the present study was not supplemented with malate. Moreover, it should be noted that in the study of Martin et al. (2009), steers were fed rolled corn–based concentrates that also contained lasalocid, whereas in the current study, the concentrate was based on wheat and barley grains and did not contain ionophores. In fact, Carro and Ranilla (2003) evaluated the effects of different doses of malate on in vitro rumen fermentation of different cereal grains and observed that corn showed a greater response than wheat and barley. To our knowledge, only Martin et al. (2009) and Carrasco et al. (2012) have investigated the effects of malate supplementation on carcass characteristics of beef cattle, and the lack of effects observed in the current study agrees with their results as well as with the absence of effects on BW and ADG.

The influence of malate supplementation on rumen fermentation seems to be dose dependent (Martin et al., 2009; Carro and Ranilla, 2003) but is also affected by the type of diet and rumen microbial populations (Gómez et al., 2005; Tejido et al., 2005). The lack of effects of malate on rumen concentrations of NH_3–N, lactate, and VFA observed in the current study is consistent with other studies in which beef cattle were fed high-grain diets and supplemented daily up to 134 g of malic acid or malate salts (Montaño et al., 1999; Carrasco et al., 2012; Vyas et al., 2015). However, Liu et al. (2009) reported that total VFA concentrations in the rumen of steers linearly increased by supplementing increasing doses of malic acid (up to 210 g/d), whereas NH3–N and lactate concentrations were reduced, and Martin et al. (2009) observed that total VFA in steers tended to decrease by increasing DL-malate inclusion up to 80 g/d and L-lactate concentrations remained unchanged. These results suggest that the effective dose of malate could depend on either the *S. ruminantium* population in the rumen or its capacity of metabolizing as well as on the concentration of other metabolites of the propionate production pathway.

The reported effects of malate supplementation on ruminal pH in vivo are also inconsistent. In agreement with current results, Carrasco et al. (2012) and Vyas et al. (2015) reported no efficacy of malate supplementation in elevating ruminal pH in heifers fed barley grain–based diets. In contrast, Martin et al. (2009) and Montaño et al. (1999) observed significant increases in rumen pH in cattle fed diets based on corn and barley grains, respectively, by supplementing 80 g of malic acid daily. In the current experiment, the basal diet contained 0.23% malate and supplementation of malate salts increased it up to 0.45%, which may have not been enough to cause a positive effect on pH (supply of 26.3 g/d). Moreover, the diet in the study of Martin et al. (2009) also included lasalocid. Because ionophores can stimulate the rate of utilization of organic acids (Callaway and Martin, 1997), this could have contributed to maintaining rumen pH values. The diet fed to the animals seems to be an important factor influencing the effects of malate on ruminal pH. In fact, Foley et al. (2009) reported only a

Table 4. Rumen fermentation characteristics in heifers fed 1 of the following high-grain diets: control (not supplemented; CON), control plus 4 g of disodium/calcium malate mixture (MAL), or control plus 0.15 g of *Saccharomyces cerevisiae* CBS 493.94 (YC)

Item and sampling day	Treatment			SEM	*P*-value		
	CON	MAL[1]	YC[2]		Treatment	Time	Treatment × time
pH							
d 0	5.77	5.65	5.91	0.112	0.24	<0.001	0.57
d 56	5.58	5.32	5.43				
d 114	6.05	6.07	6.14				
NH_3–N, mg/L							
d 0	44.4	42.5	43.6	4.83	0.82	<0.001	0.88
d 56	83.9	79.8	78.6				
d 114	86.4	91.4	85.5				
Lactate, m*M*							
d 0	0.65	0.83	0.72	0.077	0.93	<0.001	0.38
d 56	1.05	0.93	1.07				
d 114	0.83	0.81	0.81				
Total VFA, m*M*							
d 0	132	139	130	7.5	0.69	<0.001	0.89
d 56	168	173	171				
d 114	143	139	134				
Individual VFA, mmol/mmol							
Acetate							
d 0	50.1	48.6	48.1	0.96	0.65	<0.001	0.57
d 56	50.2	49.4	51.1				
d 114	53.8	53.8	53.7				
Propionate							
d 0	37.6	37.4	37.2	1.03	0.86	<0.001	0.61
d 56	37.6	38.7	36.7				
d 114	31.4	31.7	32.8				
Butyrate							
d 0	8.04	9.20	9.68	0.674	0.84	0.49	0.36
d 56	8.37	8.32	8.43				
d 114	9.32	9.19	8.21				
Isobutyrate							
d 0	0.46	0.42	0.54	0.052	0.93	<0.001	0.46
d 56	0.48	0.44	0.40				
d 114	0.74	0.76	0.72				
Isovalerate							
d 0	0.50	0.46	0.54	0.145	0.55	<0.001	0.88
d 56	0.81	0.69	0.69				
d 114	1.83	1.65	1.56				
Valerate							
d 0	3.07	3.56	3.63	0.178	0.27	<0.001	0.46
d 56	1.92	1.95	1.99				
d 114	2.34	2.33	2.53				
Caproate							
d 0	0.37	0.46	0.35	1.104	0.97	0.01	0.53
d 56	0.63	0.53	0.75				
d 114	0.65	0.63	0.58				
Acetate/propionate, mol/mol							
d 0	1.40	1.34	1.38	0.079	0.67	<0.001	0.90
d 56	1.37	1.29	1.42				
d 114	1.75	1.74	1.71				

[1]Control plus 4 g of disodium/calcium malate mixture (Rumalato; NOREL SA, Madrid, Spain) per kilogram of concentrate, providing 2.12 g of malate per kilogram of concentrate.

[2]Control plus 0.15 g of *Saccharomyces cerevisiae* CBS 493.94 (Yea-Sacc 1026; Alltech Inc., Nicholasville, KY) per kilogram of concentrate, providing 1.5×10^8 cfu/kg of concentrate.

Table 5. Blood metabolites in heifers fed 1 of the following high-grain diets: control (not supplemented; CON), control plus 4 g of disodium/calcium malate mixture (MAL), or control plus 0.15 g of *Saccharomyces cerevisiae* CBS 493.94 (YC)

Item and sampling day	Treatment			SEM	*P*-value		
	CON	MAL[1]	YC[2]		Treatment	Time	Treatment × time
Glucose, mg/dL							
d 0	70.92	69.49	71.01	3.642	0.96	0.19	0.99
d 56	73.17	73.56	74.35				
d 114	74.41	75.27	75.90				
Lactate, mg/dL							
d 0	7.11	6.92	6.64	0.669	0.82	0.27	0.98
d 56	8.05	7.36	7.47				
d 114	7.50	7.28	7.38				
Urea N, mg/dL							
d 0	19.75	18.82	18.44	1.213	0.15	<0.001	0.59
d 56	25.73	22.53	22.71				
d 114	33.70	31.95	30.23				

[1]Control plus 4 g of disodium/calcium malate mixture (Rumalato; NOREL SA, Madrid, Spain) per kilogram of concentrate, providing 2.12 g of malate per kilogram of concentrate.

[2]Control plus 0.15 g of *Saccharomyces cerevisiae* CBS 493.94 (Yea-Sacc 1026; Alltech Inc., Nicholasville, KY) per kilogram of concentrate, providing 1.5×10^8 cfu/kg of concentrate.

tendency toward an overall greater pH with increasing malate supplementation in steers fed a 40:60 forage-to-concentrate ratio diet, even though the daily supply of malate ranged from 325 to 983 g/d.

Consistent with that observed in the rumen, plasma concentrations of lactate, glucose, and urea N were not affected by the inclusion of malate, which is in agreement with the results of other studies (Martin et al., 2009; Montaño et al., 1999; Carrasco et al., 2012) in which beef cattle were supplemented with malic acid or malate salts at up to 120 g/d. In contrast, Castillo et al. (2007) reported that supplementing malic acid or a mixture of disodium and calcium malate at 4 g/kg of concentrate (DM basis) significantly lowered plasma L-lactate concentrations compared with unsupplemented bulls. Similarly, Hernández et al. (2011) reported that supplementing the diet of finishing bull calves with disodium/calcium malate salts (4 g/kg DM diet) lowered plasma concentrations of L-lactate, urea N, and creatinine compared with unsupplemented animals. As previously discussed, the inconsistent results reported in the literature for the effects of malate supplementation on ruminal fermentation, plasma metabolites, or ruminant performance can be attributed to differences in the diet (i.e., forage:concentrate ratio, forage type, cereal grains, etc.), malate dose, chemical form in which malate was fed (i.e., free acid vs. salts), and characteristics of experimental animals such as physiological state, level of production, ruminal populations, etc. (Carro and Ungerfeld, 2015). Results indicate that greater levels of malate than those used in the current study are necessary to detect significant effects on in vivo rumen fermentation products and growth performance.

Yeast cultures can be supplied live and dried or dead with its culture media being a source of nutrients with prebiotic effect. In this study, a dried live *S. cerevisae* culture was supplied, and its daily supply in ad libitum feeding system provided a continued flow of yeast, as *S. cerevisiae* is viable for only 24 to 30 h in the rumen (Kung et al., 1997; Durand-Chaucheyras et al., 1998). In agreement with previous studies (Vyas et al., 2014a,b), ADFI of heifers was unaffected by *S. cerevisiae*. In contrast, Mutsvangwa et al. (1992) reported an increase in ADFI with *S. cerevisia***]** supplementation, and Lascano et al. (2009) suggested that *S. cerevisiae* inclusion in the diet tended to require less DMI to maintain growth compared with nonsupplemented heifers on a restricted feed supply management. Despite these effects on ADFI, Mutsvangwa et al. (1992), Lascano et al. (2009), and Vyas et al. (2014a) did not observe a positive effect of supplementation on growth or feed efficiency, which is in agreement with the current results. Accordingly to growth performance results, we did not find any effect of on carcass characteristics, which is consistent with previous results in steers (Mir and Mir, 1994) and lambs (Issakowicz et al., 2013).

Saccharomyces cerevisiae has been reported to stimulate lactate utilization by *M. elsdenii* and *S. ruminantium* (Callaway and Martin, 1997) and to reduce the incidence of SARA by increasing daily average ruminal pH and decreasing rumen lactic acid concentration (Desnoyers et al., 2009; Vyas et al., 2014a). In contrast, and in agreement with the current results, others (Mir and Mir, 1994; Lascano and Heinrichs, 2009; Moya et al., 2009; Vyas et al., 2014b) reported no influence of *S. cerevisiae* supplementation on ruminal pH with dif-

ferent diets and levels of inclusion. Reported effects of *S. cerevisiae* on rumen fermentation pattern are inconsistent and seem to be influenced by diet characteristics and the yeast strain, among other factors (Carro et al., 1992; Chaucheyras-Durand et al., 2012). In accordance with the results of other studies in beef cattle (Mir and Mir, 1994; Moya et al., 2009), supplementation did not influence the VFA profile in the current study.

The lack of effect of YC on plasma levels of glucose, lactate, and urea N is in agreement with the results of Stella et al. (2007) and Yalçm et al. (2011) in dairy goats and cows, respectively. Lascano et al. (2012) observed that glucose concentrations tended to quadratically increase with increasing doses of *S. cerevisiae* (*S. cerevisiae* CBS 493.94; 1×10^{10} to 5×10^{10} cfu/d) in dairy heifers fed low-starch diets (16.7% DM). The greatest response on glucose concentration was obtained at 3×10^{10} cfu/d, which is 30-fold the daily dosage used in the current study.

The decrease in rumen pH is usually due to increased production of VFA and lactate. In the current study, rumen pH was negatively correlated with VFA concentrations ($R2 = 0.78$; all data included) but showed a poor correlation with lactate ($R2 = 0.08$). In addition, lactate concentrations were low (≤1 m*M*), which is indicative of SARA (Beauchemin and Penner, 2009). It has to be noted that ruminal fluid was obtained by rumenocentesis and that metabolites and pH in the obtained fluid may not be representative of the rumen conditions as a whole. However, Duffield et al. (2004) concluded that rumenocentesis was the most accurate field technique for ruminal sampling compared with an oral stomach tube, as rumenocentesis values were highly correlated to direct sampling through a cannula.

In conclusion, under the feeding and management conditions of this study, supplementation of either malate salts (2.12 g malate/kg of concentrate) or a live *S. cerevisiae* culture (1.5×10^8 cfu/kg of concentrate) had no effect on growth performance, carcass quality, ruminal fermentation products, and blood metabolites in feedlot heifers.

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