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Effect of feeding *Saccharomyces Cerevisiae* on performance of dairy cows during summer heat stress

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ABSTRACT

Effects of feeding a culture of *Saccharomyces cerevisiae* to lactating cows on their lactational performance during heat stress were determined. Multiparous Holstein cows ($n = 723$) calving during the summer months from two dairy farms were randomly assigned to a diet containing no yeast culture (control; $n = 361$) or 30 g/d of a *S. cerevisiae* yeast culture (YC; $n = 362$) fed from 20 to 140 d in milk (DIM). Cows were milked twice daily and the production of milk and milk components was measured every 2 weeks. Dry matter (DM) intakes from 6 pens were measured daily and pen temperature and humidity were evaluated hourly from June to November. Rectal temperature was measured in 88 cows (22/treatment/farm), once weekly, and blood was sampled from a subset of 120 cows at 58 and 100 DIM for measurements of plasma glucose, nonesterified fatty acids, 3-OH-butyrate, insulin, and urea N concentrations. Daily temperature, humidity and the temperature-humidity index in the study pens did not differ between treatments, and rectal temperature of cows in the control and YC treatments differed with days postpartum. Intake of DM was similar between diets, but cows fed YC produced 1.2 kg/d more milk, more milk true protein, solids-not-fat and lactose than that produced by control

Abbreviations: BCS, body condition score; BHBA, 3-hydroxybutyrate; DIM, days in milk; DM, dry matter; ECM, energy-corrected milk; NEFA, nonesterified fatty acids; NE_L, net energy for lactation; RH, relative humidity; SCC, somatic cell count; SCS, somatic cell count score; YC, *Saccharomyces cerevisiae* yeast culture; *S. cerevisiae*, *Saccharomyces cerevisiae*; TD, dry bulb temperature; THI, temperature-humidity index; TMR, total mixed ration.

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cows. However, energy-corrected milk yield, and concentrations of true protein, solids-not-fat and lactose did not differ between treatments. Feeding YC did not influence plasma metabolites, insulin, or body condition score of cows, but urea N concentrations were reduced. Feeding a yeast culture of *S. cerevisiae* improved yields of milk and milk components in heat-stressed multiparous Holstein cows.

1. Introduction

Microbial additives such as *Saccharomyces cerevisiae* products have been widely used in ruminant nutrition to manipulate rumen fermentation and improve animal performance. Various *S. cerevisiae* based yeast products have been shown to impact dry matter (DM) intake, rumen pH and nutrient digestibility (Callaway and Martin, 1997; Shaver and Garrett, 1997; Wohlt et al., 1998; Dann et al., 2000; Lehloenya et al., 2008). Because of its effects on rumen fermentation and nutrient digestion, some authors (e.g., Wohlt et al., 1998; Dann et al., 2000; Erasmus et al., 2005) have suggested that feeding yeast products may be most beneficial to dairy cows during late gestation and early lactation when these effects of yeast cultures might be most valuable.

Lactating dairy cows exposed to high ambient temperatures typically have reduced DM intake and lactation performance (Armstrong, 1994; Huber et al., 1994). The most comfortable environmental temperature range for dairy cattle is between 5 and 25 °C, and a temperature-humidity index (THI) above 72 usually reduces DM intake and milk production in lactating dairy cows (Armstrong, 1994). It has been suggested that fungal cultures fed to lactating dairy cows exposed to high ambient temperatures might reduce signs of heat stress, such as rectal temperature and respiration rate, and improve lactation performance (Huber et al., 1994). Recently, feeding a culture of *S. cerevisiae* to mid-lactation dairy cows during summer had no effects on yields of energy-corrected milk (ECM) and on DM intake, but improved efficiency (Schingoethe et al., 2004), which suggests that yeast culture can benefit cows subjected to heat stress.

The response in milk production to feed additives such as cultures of yeast and fungi usually ranges from 1 to 2 kg/d (Huber et al., 1994; Robinson and Garrett, 1999; Shaver and Garrett, 1997; Erasmus et al., 2005), which requires large numbers of cows to detect statistically. When fed during heat stress, a culture of *Aspergillus oryzae* improved yields of milk and reduced rectal temperature in lactating dairy cows during summer studies (Huber et al., 1994). However, the same responses have not been demonstrated with yeast culture containing *S. cerevisiae* when fed alone (Schingoethe et al., 2004) or in combination with a fungal culture (Higginbotham et al., 1994).

It was hypothesized that feeding a yeast culture would improve yields of milk and milk components of early lactation dairy cows exposed to heat stress. Objectives were to evaluate effects of feeding a culture of *S. cerevisiae* to early lactation dairy cows during heat stress on lactation performance and rectal temperature.

2. Materials and methods

2.1. Animals, housing, and feeding

Seven-hundred and twenty-three multiparous Holstein cows on two commercial dairy farms (farm 1, $n = 269$; farm 2, $n = 454$) in the San Joaquin Valley of California were blocked by lactation number and previous lactation 305 d milk yield and, within each block, randomly assigned to one of two treatments at calving. Treatments were a diet containing no yeast culture (control; $n = 361$) or 30 g/d of a culture of *S. cerevisiae* (YC; $n = 362$; A-Max XTRA, Varied Industry Co., Mason City, IA). The YC is a culture of *S. cerevisiae* yeast grown on a media of sucrose, cane molasses, and processed grain by-products that is fed to different livestock species and is the same yeast culture used in previous studies (Miller-Webster et al., 2002). Yeast culture was incorporated into the grain mix portion of the diet at 2.2 g/kg of grain

Table 1
Ingredient composition (g/kg of dry matter) of experimental diets and grain mixes.

	Dairy site	
	Farm 1	Farm 2
Alfalfa hay	202	235
Corn silage	84	118
Wheat silage	168	110
Grain mix	546	537
Composition of grain mix[a]		
Steam-flaked corn grain, 360 g/L	431	412
Almond hulls	156	170
Soybean meal, solvent 470 g/kg of protein	125	108
Dried corn distiller's grains	63	93
Whole upland cottonseed	125	123
Beef tallow	22	22
Animal–marine protein blend[b]	36	34
Mineral–vitamin premix[c]	42	38

[a] Culture of *S. cerevisiae* (Amax Extra, Varied Industry Co., Mason City, IA, USA) was added to grain mix fed to YC cows at 2.2 g/kg replacing steam-flaked corn.

[b] Animal–marine protein blend; Pro-Lak® (blend of marine and animal by-products; H. J. Baker & Bro., Inc., Stamford, CT, USA).

[c] Contains (dry matter basis) 55.4 g/kg Ca, 16.8 g/kg P, 90.0 g/kg Mg, 145.1 g/kg Na, 3.0 g/kg of S, 1490 mg/kg Zn, 642 mg/kg Mn, 312 mg/kg Cu, 12 mg/kg I, 42 mg/kg Co, 6.6 mg/kg Se, 95,000 IU/kg vitamin A, 900 IU/kg vitamin E, and 22,000 IU/kg vitamin D.

mix DM (Table 1), and the grain mix was fed at approximately 540 g/kg of the total mixed ration (TMR). It was expected that cows would consume approximately 30 g/d of yeast culture. At both farms, cows in both treatments were housed together during the first 20 d postpartum, and fed a common diet for the pretreatment period. At both farms, all cows received 500 mg of exogenous bovine somatotropin (Posilac®, Monsanto Co., St. Louis, MO, USA) every 14 d, starting between 90 and 96 d postpartum.

The experiment was conducted from May to December of 2004 and cows calving during the hot months of May to August were enrolled. At farm 1, cows were housed in 2 dry-lot pens (1 pen/treatment) and at farm 2 cows were housed in the same free-stall barn in 4 pens (2 pens/treatment), and pen DM intakes were measured daily in all pens throughout the study. Thus there were 3 pen replicates per treatment, but treatments and cows were, within site, switched between pens such that all cows and all treatments were exposed to all pens. Within each farm, all pens were identical in design, size, location, number of cows housed, and cows were milked and fed at similar times. All cows in the study were identified with an additional colored ear tag to facilitate identification and daily counts of cows in each study pen. The number of cows in each study pen at both farms was kept constant throughout the study. At farm 1, each study pen housed an average of 131 cows (129–134) throughout the study. In farm 2, each study pen housed an average of 159 cows (156–160) throughout the study. At farm 1, the open corrals had a shade cloth of woven polypropylene (95% shade, Windtamer Tarps Inc., Lemoore, CA, USA) that extended directly over a concrete surface behind the stanchions. The shade over the entire feedbunk was 5.0 m wide by 4.0 m high above the concrete surface. There were approximately 5 m^2 of shade cloth/cow in both pens at farm 1. Both pens were also equipped with a fixed metal framework shade located in the central area of the pens that provided an additional 5 m^2 of shade per cow. Water soakers above the feedbunk were activated when the ambient temperature reached 25 °C. At farm 2, pens were equipped with 2 rows of fans (2 fans/6 linear meters) facing the stalls, one row located in the back of the stalls, and the other row immediately above the stanchions, in the feedbunk. Fans were equipped with high pressure nozzles and both fans and nozzles were activated once ambient temperature reached 25.5 °C.

Ambient temperature and relative humidity (RH) were recorded hourly by data recorders (HOBO® H8 Pro Series Part No. H08-032-08, Onset Computer Corp., Bourne, MA, USA), operated by a computer software program (BoxCar Pro 4.0 Starter Kit, Part No.BCP4.0-ON, Onset Computer Corp., Bourne, MA, USA). Temperature and RH accuracy were within ± 0.2 °C and ± 2%, respectively. Two recorders were placed in each study pen at both farms; they were set at a height of 1.9 m from the floor and placed under the central shades in farm 1 and immediately above the stalls in farm 2. The probes

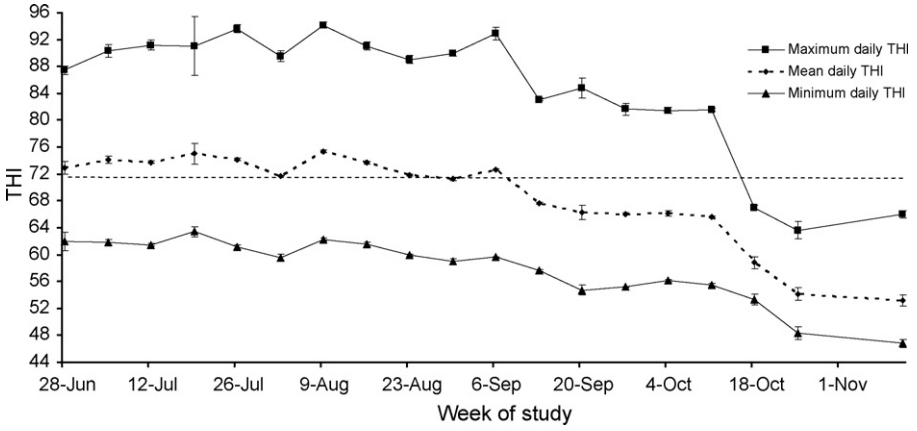

Fig. 1. Average (±S.D.) daily maximum, mean and minimum temperature and humidity index (THI; NOAA, 1976) for pens during the study. Dashed line represents THI ≥ 72, when cows are expected to experience losses of production because of heat stress (Armstrong, 1994).

recorded data from June to November, 2004. The temperature-humidity index (THI) was calculated as: THI = TD − (0.55–0.55 RH/100) (TD−58), in which TD is the dry bulb temperature in °F and RH is relative humidity expressed as a percentage (NOAA, 1976). For each 24 h period, average daily minimum, mean and maximum temperatures and humidity were determined using hourly data, and the minimum, mean and maximum THI were calculated (Fig. 1).

All diets were mixed as TMR and offered twice daily at 07:00 and 14:00 h at farm 1, and once daily at 07:00 h at farm 2. Diets were fed for 30 g refusals for each kg offered, which was measured daily in both farms. Within each farm, control and YC diets were mixed from the same ingredients in the same proportions, and were formulated to meet the metabolizable protein, net energy, mineral and vitamin requirements for lactating Holstein cows weighing 650 kg and producing 45 kg of milk containing 35 g/kg of fat and 30 g/kg of true protein when consuming 26 kg/d of DM (NRC, 2001). Grain mixes were prepared two to three times weekly at each farm and incorporated into the TMR with forages immediately prior to feeding the cows.

2.2. Measurements of milk and milk components

During the first 20 d postpartum, cows were milked four times daily and, once moved into the study pens, they were milked twice daily throughout the experimental period. Milk yields were recorded for individual cows every two weeks in an official California dairy herd improvement association test. Individual milk samples were also collected from consecutive morning and afternoon milkings, composited, and analyzed for somatic cell count (SCC), fat, true protein, lactose and solids-not-fat concentrations (Foss 303 Milk-O-Scan®; Foss Foods, Inc., Eden Prairie, MN, USA) at the dairy herd improvement association laboratory in Hanford, California. Production during the first 20 d postpartum was used as the covariate for statistical analyses of milk and milk components. The SCC was converted into a logarithmic score (SCS) according to dairy herd improvement association to normalize data for statistical analysis. The SCS is calculated using a logarithm with the base 10 of the SCC divided by 12.5. This is then divided by the logarithm on the base 10 of 2 ($SCS = [\text{Log}_{10}(SCC/12.5)/(\text{Log}_{10}\ 2)]$).

2.3. Body condition score and rectal temperatures

Cows were scored for body condition (BCS; Ferguson et al., 1994) in the first week after calving, and at 28 ± 3, 58 ± 3, and 140 ± 3 d postpartum by the same person. The BCS in the first week after calving was used as the covariate for statistical analysis of BCS.

Rectal temperatures were determined in 88 cows, 22 per treatment at each farm, once weekly from July to September, because cows were expected to be exposed to heat stress in these months. Rectal temperatures were recorded to the nearest 0.1 °C using a digital thermometer (GLA M 550, GLA Agricultural Electronics, San Luis Obispo, CA, USA) connected to a 10 cm right angle probe (M207R, GLA Agricultural Electronics, San Luis Obispo, CA, USA). The probe was inserted rectally to full depth until a stable automated reading was obtained.

2.4. Measurement of metabolites and insulin in plasma

Blood samples (8 mL) were collected from a subset of 120 cows, 30 cows per treatment per farm, at 58 ± 3 and 100 ± 3 DIM approximately 30 min after the morning feeding by puncture of the median coccygeal vein or artery into evacuated tubes containing K_2 EDTA (Vacutainer®; Becton Dickinson, Franklin Lakes, NJ, USA). Samples were immediately placed on ice and tubes were centrifuged at $2000 \times g$ for 15 min at 8 °C for plasma separation. Plasma was frozen at −25 °C and later analyzed for glucose, nonesterified fatty acids (NEFA), 3-hydroxybutyrate (BHBA), urea N, and insulin concentrations.

Plasma glucose concentration was determined by direct measurement using the YSI Model 2700 SELECT Biochemistry Analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH, USA). Measurement of plasma NEFA was completed according to Johnson and Peters (1993) using a commercial kit (NEFA C, Wako Chemicals USA Inc., Richmond, VA, USA). Urea N was assayed using a commercial kit (Urea Nitrogen Procedure No. 640, Sigma Diagnostics, St. Louis, MO). Concentration of BHBA was assayed using commercial kit (Randox Laboratories Ltd., Antrim, UK). Concentrations of insulin were analyzed by radioimmunoassay (Coat-a-Count; Diagnostic Products Corp., Los Angeles, CA) in a single assay with a coefficient of variation of 0.054, and sensitivity of 0.25 μIU/mL calculated as 2 S.D. below mean counts per minute at maximum binding.

2.5. Analyses of dietary ingredients and total mixed rations

At each farm, approximately 0.5 kg of individual dietary ingredients and grain mixes, and 1 kg of TMR were sampled weekly and dried at 55 °C for 48 h in an air-circulating oven. Dried samples were then ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA, USA) to pass a 2 mm screen, then in a cyclone mill (Udy Co., Fort Collins, CO, USA) to pass a 1 mm screen. Samples were then composited for two-month periods and analyzed for contents of DM (AOAC, 2000, Method # 925.40), ash (AOAC, 2000; method 923.03), N, fiber and minerals. The N content of samples was analyzed using an N analyzer (FP-528 Nitrogen Determinator, LECO Corporation, St. Joseph, MI, USA), and crude protein was calculated by multiplying the N content by 6.25. Neutral detergent fiber was assayed utilizing a heat stable amylase according to Van Soest et al. (1991) and with sodium sulfite, and it is expressed including the residual ash. Ether extract was analyzed according to AOAC (2000; method 7.060). Starch was determined by acidic, followed by enzymatic hydrolysis with amyloglucosidase (Sigma A3042; Sigma Chemical Co., St. Louis, MO) according to the technique described by Poore et al. (1991). The concentration of free glucose in the solution was quantified based on the glucose oxidase reaction utilizing a biochemical analyzer (YSI Model 2700 SELECT Biochemistry Analyzer, Yellow Springs Instrument Co., Inc., Yellow Springs, OH, USA). The mineral content of diets was determined at the Dairyland Laboratory (Arcadia, WI) using an inductively coupled plasma mass spectrometer (Thermo Jarrell-Ash, Franklin, MA).

2.6. Experimental design and statistical analyses

The experiment was designed as completely randomized with blocks. Within each farm, cows were blocked according to lactation number and previous lactation 305 d milk production and, within each block, randomly assigned to one of the two treatments in the first week after calving.

A sample size calculation (Win Episcope 2.0) using a one-tailed test was used to determine the number of cows required to demonstrate a statistical difference ($\alpha = 0.05$ and $\beta = 0.80$) in milk production if cows fed YC produced 1 kg/d more milk than the controls. This expected difference was based on results from previous studies (e.g., Robinson and Garrett, 1999; Shaver and Garrett, 1997). Based upon

this assessment, a minimum of 310 cows/treatment was required, assuming that average production for the study would be between 42 and 45 kg/d with a S.D. of 7 kg/d.

Lactation performance, BCS, rectal temperatures and concentrations of plasma metabolites and insulin were all analyzed by ANOVA for repeated measures (Littell et al., 2002) using the MIXED procedure of SAS (2001) using a model that included effects of treatment (control vs. YC), farm, time of measurement, interaction between treatment and farm, interaction between treatment and time of measurement, and cow nested within treatment as the random error. The covariance structures (unstructured, compound symmetry, toeplitz, and autoregressive order 1) for the repeated measures models were tested (Littell et al., 2002) and the one with the smallest Schwartz's Bayesian criterion was chosen. Measurements taken prior to initiating the treatments were used for covariate adjustment in the statistical models.

Daily DM intake, temperature, humidity and the THI were analyzed with data from 3 pens/treatment. Daily values were averaged to weekly means and analyzed by ANOVA as repeated measures (Littell et al., 2002) using the MIXED procedure of SAS (2001) with effects of treatment, week, interaction between treatment and week, and pen within treatment as the random experimental error in the model. The covariance structures (unstructured, compound symmetry, toeplitz, and autoregressive order 1) for the repeated measures models were tested (Littell et al., 2002) and the structure that best fitted the model was chosen based on the smallest Schwartz's Bayesian criterion.

Least square means and proportions are reported for all parameters evaluated. Treatment differences with $P \leq 0.05$ were considered significant, whereas tendencies to differences were accepted if $0.05 < P < 0.10$.

3. Results

3.1. Characterization of nutrient composition of dietary ingredients and diets

The grain mixes fed within each farm were similar in composition, but for unknown reasons in farm 2 the control grain mix contained a smaller concentration of aNDF and starch than the YC grain mix (Table 2).

As expected, the nutrient composition of diets was very similar within farm (Table 3). Diets were formulated to meet or exceed the nutrient requirements for multiparous Holstein cows in early lactation consuming 26 kg of DM/d and producing 45 kg of milk containing 35 g/kg of fat and 30 g/kg of true protein (NRC, 2001). However, the concentrations of aNDF in the TMR were higher than initially formulated (320 g/kg of the DM) and expected based on the composition of the ingredients of the diets. In addition to the higher fiber concentration in the TMR, rations also contained smaller concentration of calculated nonfibrous carbohydrates than initially planned, 380 g/kg of the DM.

Table 2
Nutrient composition (g/kg of dry matter ± S.D.) of dietary ingredients.

	Dry matter	Ash	Crude protein	aNDF	Starch
Yeast culture	901 (±34)	41 (±0.1)	210 (±2)	115 (±5)	202 (±13)
Farm 1					
Alfalfa hay	899 (±0.1)	103 (±0.1)	200 (±5.8)	385 (±3.0)	33 (±3.1)
Corn silage	308 (±0.2)	66 (±0.1)	81 (±1.5)	439 (±5.0)	245 (±8.6)
Wheat silage	290 (±0.3)	190 (±0.3)	121 (±1.8)	512 (±6.0)	46 (±2.4)
Control grain mix	868 (±0.2)	63 (±0.1)	171 (±1.3)	293 (±3.0)	295 (±4.8)
YC[a] grain mix	871 (±0.1)	64 (±0.1)	167 (±0.1)	295 (±4.0)	295 (±33.9)
Farm 2					
Alfalfa hay	878 (±0.1)	101 (±0.1)	201 (±4.3)	377 (±3.0)	46 (±3.4)
Corn silage	293 (±0.3)	73 (±0.1)	88.9 (±1.6)	488 (±5.0)	216 (±1.1)
Wheat silage	323 (±0.1)	135 (±0.1)	123 (±0.1)	490 (±6.0)	62 (±1.3)
Control grain mix	864 (±0.4)	77 (±0.2)	176 (±2.1)	238 (±4.0)	339 (±1.1)
YC grain mix	863 (±0.2)	71 (±0.1)	168 (±1.3)	274 (±3.0)	360 (±1.3)

[a] YC: yeast culture.

Table 3
Nutrient composition (g/kg of dry matter ± S.D.) of diets.

	Dairy site			
	Farm 1		Farm 2	
	Control	YC[a]	Control	YC
Dry matter (g/kg)	487 (±30.3)	517 (±31.4)	507 (±22.2)	514 (±8.1)
Net energy for lactation[b] (MJ/kg) of DM	6.74	6.74	6.74	6.74
Crude protein	167 (±1.9)	168 (±3.7)	174 (±6.3)	173 (±5.2)
Ruminally undegradable protein[c]	68	68	69	69
aNDF	342 (±40.5)	339 (±25.6)	359 (±21.3)	374 (±11.7)
Starch	194 (±4.0)	201 (±14.0)	217 (±20.6)	208 (±12.6)
Non-fiber carbohydrates[d]	344 (±45.7)	356 (±24.8)	324 (±26.9)	305 (±16.0)
Ether extract	54.6 (±2.5)	51.2 (±4.5)	53.7 (±2.0)	5.78 (±3.2)
Ash	92 (±6.0)	86 (±6.0)	90 (±2.0)	90 (±1.0)
Ca	8.1 (±0.5)	7.9 (±0.5)	8.9 (±0.7)	8.5 (±0.1)
P	4.4 (±0.1)	4.5 (±0.1)	4.2 (±0.2)	4.1 (±0.2)
K	20.0 (±0.9)	19.4 (±0.7)	18.3 (±1.3)	18.5 (±1.6)
Mg	3.8 (±0.02)	4.0 (±0.2)	4.1 (±0.1)	4.1 (±0.2)
S	2.1 (±0.1)	2.2 (±0.01)	2.2 (±0.2)	2.1 (±0.2)
Na	4.4 (±0.2)	4.4 (±0.3)	4.6 (±0.2)	4.5 (±0.1)
Zn (mg/kg)	61.5 (±3.51)	64.3 (±1.89)	67.3 (±4.57)	66.0 (±3.16)
Cu (mg/kg)	15.0 (±0.82)	15.0 (±0.82)	15.0 (±0.82)	14.0 (±0.58)
Mn (mg/kg)	45.5 (±3.42)	46.5 (±2.08)	50.3 (±8.50)	47.8 (±1.50)

[a] YC: yeast culture.
[b] Net energy for lactation calculated according to NRC (2001) based on nutrient composition of ingredients and the average DM intake in the study (26 kg/d).
[c] Ruminally undegradable protein calculated according to NRC (2001) based on nutrient composition of ingredients and the average DM intake in the study (26 kg/d).
[d] Non-fiber carbohydrates, g/kg = 100 − (aNDF, g/kg + crude protein, g/kg + ether extract, g/kg + ash, g/kg).

3.2. Environmental temperature, humidity, THI and rectal temperature

Cows were exposed to heat stress from mid June to early September (Fig. 1), during which time the average daily mean THI in all pens in both treatments reached values above 72, which characterizes exposure to heat stress for lactating dairy cows (Armstrong, 1994). Indeed the average weekly maximum THI for each pen was above 81 from early June to the second week of October (Fig. 1). Ambient temperature, humidity and THI did not differ among study pens (Table 4). In fact, the proportion of weeks in which the study pens experienced a THI above 72 was exactly the same for both treatments.

Table 4
Rectal temperature of multiparous cows fed a culture of *S. cerevisiae* and temperature and humidity of pens housing study cows.

	Treatment			P		
	Control	YC[a]	S.E.M.	Diet	Week	Diet*Week
Cows, *n*	44	44				
Rectal temperature, °C	38.52	38.44	0.03	0.21	0.001	0.05
Pen temperature, °C						
Average daily mean	22.3	22.4	0.13	0.59	0.001	0.99
Average daily maximum	30.6	30.7	0.16	0.70	0.001	0.73
Pen relative humidity (%)						
Average daily mean	60.3	62.6	1.92	0.49	0.001	0.99
Average daily maximum	87.6	86.5	1.05	0.56	0.001	0.99
Pen THI[b]						
Average daily mean	68.5	68.8	0.11	0.19	0.001	0.98
Average daily maximum	84.7	84.7	0.37	0.94	0.001	0.82
Weeks THI > 72 (%)	84.7	84.7	–	1.00	–	–

[a] YC: yeast culture.
[b] THI: temperature-humidity index (NOAA, 1976).

Table 5
Effect of feeding a culture of *S. cerevisiae* on lactation performance of multiparous cows during heat stress.

	Diet			P		
	Control	YC[a]	S.E.M.	Diet	Week	Diet*Week
Cows, *n*	361	362				
Group DM intake (kg/d)	26.0	25.8	1.02	0.91	0.01	0.98
Milk (kg/d)	42.2	43.4	0.37	0.02	0.001	0.14
ECM[b] (kg/d)	38.4	38.9	0.31	0.20	0.001	0.34
Milk fat						
in (g/kg)	35.8	34.8	0.21	0.001	0.001	0.58
Yield (kg/d)	1.51	1.50	0.01	0.84	0.001	0.52
Milk protein						
in (g/kg)	28.3	28.1	0.10	0.13	0.001	0.33
Yield (kg/d)	1.19	1.22	0.01	0.05	0.001	0.19
Milk solids-not-fat						
in (g/kg)	85.9	85.9	0.20	0.80	0.001	0.02
Yield (kg/d)	3.58	3.68	0.03	0.05	0.001	0.36
Milk lactose						
in (g/kg)	48.3	48.4	0.10	0.67	0.001	0.37
Yield (kg/d)	2.02	2.07	0.02	0.04	0.001	0.38
Net energy for lactation in milk						
in (MJ/kg)	2.85	2.81	0.01	0.01	0.01	0.63
in (MJ/d)	120.3	122.1	1.0	0.21	0.01	0.34
SCS[c]	2.65	2.70	0.09	0.69	0.001	0.66
Body condition score	2.89	2.91	0.01	0.32	0.001	0.29

[a] YC: yeast culture.
[b] ECM: energy-corrected milk.
[c] SCS: somatic cell count score calculated according to the following formula: SCS = [Log_{10} (somatic cell count/12.5)/(Log_{10} 2)].

These data indicate that cows in both treatments were exposed to similar degrees of heat stress and it occurred on at least one day in 85% of the weeks in the study.

Treatment did not have an overall effect on rectal temperature of cows (Table 4). Nonetheless, an interaction (P=0.05) between treatment and week postpartum was observed for rectal temperature. Control cows had slightly higher (0.2 °C) rectal temperature after 72 d postpartum than YC cows, but temperatures were similar earlier in lactation.

3.3. Lactation performance

Intake of DM averaged 25.9 kg/d and did not differ (Table 5) for control and YC. Yield of milk was 1.2 kg/d higher (P=0.02) for YC compared with control cows (Table 5), and the response was consistent throughout the 120 d treatment period (Fig. 2). However, production ECM was similar (P=0.20) for both treatments. The lack of response to feeding a culture of *S. cerevisiae* on production of ECM was because of a reduction (P<0.001) in the concentration of milk fat when cows were fed YC compared with control. Although the concentration of fat was reduced by feeding YC, the treatment had no impact on concentrations of other milk components, as well as the SCS. In fact, because of higher milk yield, daily production of true protein, solids-not-fat, and lactose increased in YC compared with control cows. The lower concentration of fat in the milk of cows fed YC resulted in reduced (P<0.01) concentration of net energy for lactation (NE_L) in milk compared with that of control cows. The similar (P=0.21) daily NE_L output in milk between treatments, which averaged 121.1 MJ, suggests that these cows shifted the use of NE_L relative to milk component synthesis.

3.4. Blood metabolites, insulin, and body condition score

Concentrations of glucose, NEFA and BHBA in plasma of dairy cows during heat stress were not influenced (P>0.10) by feeding YC (Table 6). Similarly, concentrations of insulin were not affected

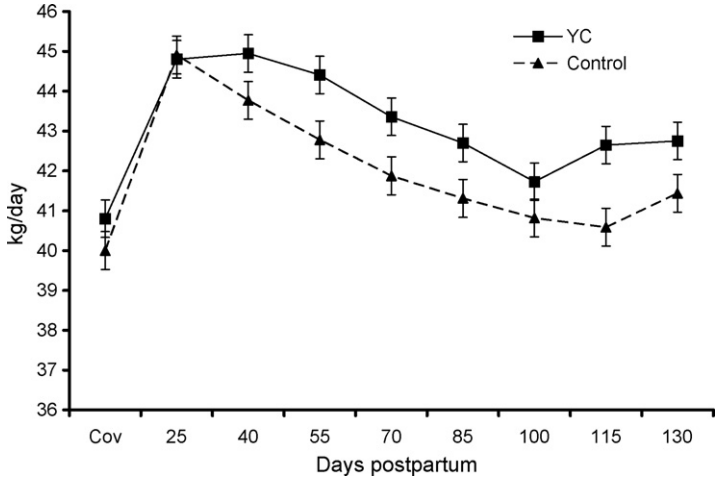

Fig. 2. Effect of feeding a culture of *S. cerevisiae* (YC) on yields of milk (least square means and S.E.M. are depicted; Cov, covariate period). Control: 42.2 kg/d; YC: 43.4 kg/d. Effects, diet (P=0.02), day postpartum (P<0.001) and interaction between diet and day postpartum (P=0.14).

Table 6
Effect of feeding a culture of *S. cerevisiae* on concentrations of metabolites in plasma in multiparous cows during heat stress.

	Diet			P		
	Control	YC[a]	S.E.M.	Diet	Period[b]	Diet*Period
Cows, number	60	60	–	–	–	–
Glucose (mg/dL)	59.4	59.8	0.55	0.64	0.51	0.47
NEFA[c] (mEq/L)	0.137	0.124	0.008	0.24	0.001	0.52
BHBA[d] (mg/dL)	4.6	4.2	0.6	0.18	0.15	0.45
Insulin (μIU/mL)	3.1	3.3	0.4	0.68	0.10	0.58
Urea N[e] (mg/dL)	14.3	12.8	0.6	0.05	0.40	0.35

[a] YC: yeast culture.
[b] Period: day postpartum (58 or 100 d postpartum) when blood samples were collected for analyses of metabolite concentrations.
[c] NEFA: nonesterified fatty acids.
[d] BHBA: 3-OH-butyrate.
[e] N: nitrogen.

(P=0.68) by diet, although it tended (P=0.10) to increase with day postpartum. In agreement with blood energy metabolites and insulin, treatment had no effect (P=0.32) on the BCS of dairy cows throughout the study (Table 5). Although concentrations of energy metabolites did not differ between treatments, feeding YC reduced (P=0.05) concentrations of urea N in plasma.

4. Discussion

Environmental and nutritional methods to alleviate heat stress usually improve lactation performance of dairy cows (Armstrong, 1994; Huber et al., 1994). Cows in the current study were exposed to heat stress, and only minor effects of treatment were observed for rectal temperature. The slight reduction in rectal temperature in cows fed YC after 72 d postpartum might suggest that feeding a culture of *S. cerevisiae* positively impacted the rectal temperatures of cows at the time when DM intake is usually highest.

It has been suggested that fungal cultures such as *A. oryzae*, when fed to lactating dairy cows exposed to high ambient temperatures, might improve signs of heat stress such as rectal temperature and respiration rate (Huber et al., 1994). In several studies reviewed by Huber et al. (1994), fungal

cultures decreased rectal temperature and respiration rate in cows exposed to heat stress, but the authors indicated that the mechanism was not clear. Observations from the current study indicate that rectal temperatures of dairy cows during heat stress can be altered by feeding a culture of *S. cerevisiae*.

Intake of DM was similar for control and YC. Addition of cultures of *S. cerevisiae* to the diet of dairy cows has had mixed effects on DM intake of dairy cows. When fed during the transition period, a culture of *S. cerevisiae* failed to increase DM intake pre- and postpartum (Robinson and Garrett, 1999). However, transition Jersey cows fed a culture of *S. cerevisiae* consumed more DM pre- and postpartum (Dann et al., 2000). Mid-lactation dairy cows fed a culture of *S. cerevisiae* during summer had DM intake similar to that of cows not fed yeast culture (Schingoethe et al., 2004).

Benefits of feeding yeast cultures on lactation performance of dairy cows have been previously demonstrated in several studies. In a field study with 11 commercial dairy farms, Shaver and Garrett (1997) reported that milk yield increased in 8 of the 11 farms that fed a culture of *S. cerevisiae*. In the current study, the lack of a treatment by farm interaction for yields of milk and milk components indicates that cows fed YC had improved performance compared with controls in both farms. In some studies, addition of a culture of *S. cerevisiae* did not improve yields of milk and milk components (Robinson and Garrett, 1999; Dann et al., 2000; Schingoethe et al., 2004) and, when fed with a fungal culture (Higginbotham et al., 1994), the combination yeast and fungal cultures did not improve lactation performance of dairy cows. Because milk response to feeding a culture of *S. cerevisiae* usually ranges between 1 and 2 kg/d (Huber et al., 1994; Robinson and Garrett, 1999; Shaver and Garrett, 1997), the existing literature on feeding of yeasts and yeast cultures is characterized by repeated numerical improvements in milk production without statistical support.

In spite of the minor effects on rectal temperature, the response to feeding a culture of *S. cerevisiae* in cows exposed to heat stress was similar to results observed previously by others when cows were in thermoneutral conditions (Shaver and Garrett, 1997; Wohlt et al., 1998). The decrease in milk fat concentration is likely the result of higher milk yield as the production of fat was similar between the two treatments. Feeding YC might alter rumen fermentation and increase fiber and DM digestibility with subsequent increased supply of absorbed nutrients for milk synthesis. Increased mammary secretion of protein might be due to increased synthesis of microbial protein and increased supply of metabolizable protein to the intestine of cows. Changes in the flow of microbial N to the duodenum of cows have been demonstrated when a culture of *S. cerevisiae* was added to the diet (Erasmus et al., 1992), but this has not been a consistent response (Putnam et al., 1997) although it has seldom been measured. Changes in rumen fermentation *in vivo* have been observed in cows fed yeast culture, as demonstrated by the increased molar concentration of propionate (Erasmus et al., 2005). In steers fed diets high in aNDF, addition of yeast culture did not alter the duodenal flow of N compounds (Lehloenya et al., 2008), although steers supplemented with yeast culture tended to consume less dietary N, which is expected to mask any potential benefit on flow of non-ammonia N to the duodenum. Another explanation for increased synthesis of milk true protein is a possible change in the profile of intestinally absorbed amino acids. When a culture of *S. cerevisiae* was fed to dairy cows, the duodenal flow of methionine increased from 41 to 58 g/d (Erasmus et al., 1992), which could favor synthesis of milk protein if methionine was a limiting amino acid in the diet (Schwab et al., 1992).

The similar production of milk fat, but increased production of milk and milk true protein might indicate that changes in rumen fermentation as a result of feeding YC increased the supply of glucogenic and aminogenic (Erasmus et al., 1992; Erasmus et al., 2005), but not lipogenic substrates. Similar to our results, Shaver and Garrett (1997) also observed that the addition of a culture of *S. cerevisiae* to the diet of lactating dairy cows increased milk production by 0.9 kg/d, but decreased milk fat concentration from 36.5 to 35.5 g/kg, with no effect on milk fat yield. In 7 of 11 farms, milk fat concentration was numerically less for cows fed yeast culture compared with controls, and in only 3 of 11 farms, cows fed yeast culture had greater milk fat content than that of unsupplemented cows (Shaver and Garrett, 1997). In the same study (Shaver and Garrett, 1997), milk crude protein content was less for cows fed yeast culture, although the difference was small (31.5 vs. 31.3 g/kg), and varied with farm. Nonetheless, cows fed yeast culture produced 30 g/d more milk crude protein than cows fed the unsupplemented diet.

Feeding a culture of *S. cerevisiae* does not seem to increase the energy concentration of the ration when fed to early lactation cows (Robinson and Garrett, 1999; Dann et al., 2000; Erasmus et al., 2005). However, an *in vitro* study indicated that the incorporation of a culture of *S. cerevisiae* altered microbial fermentation and increased digestibility of DM and crude protein (Miller-Webster et al., 2002), which has been suggested to favor microbial growth in some (Erasmus et al., 1992), but not all *in vivo* studies (Putnam et al., 1997; Lehloenya et al., 2008). In some studies, total digestive tract digestibility of crude protein and fiber increased when cows were supplemented with *S. cerevisiae* (Wohlt et al., 1998). Furthermore, *in vitro* studies in which *S. cerevisae* was supplemented to the culture media suggest changes in the rumen microbial population that might favor a more stable rumen environment (Callaway and Martin, 1997). These responses have also been demonstrated with calves *in vivo* (Koul et al., 1998). Therefore, improvements in animal performance because of the addition of a culture of *S. cerevisiae* are likely the result of increased rumen digestibility of DM and the fibrous fractions of the diet, changes in the supply of metabolizable protein, and improved stability of rumen pH, which might favor small increases in DM intake and supply of energy for milk synthesis.

Concentrations of glucose, NEFA, BHBA and insulin were similar between treatments. The cows were sampled at 58 and 100 DIM, when they had been fed the experimental diets for at least 5 weeks. In the current study, DM intake was similar for both treatments, which would not favor improvements in energy status unless energy concentration of the diet was altered by YC. Corroborating with lack of changes in energy metabolites in plasma, BCS was not affected by treatments. Others have observed similar results when cows were fed a culture of *S. cerevisae* during late gestation and early lactation (Robinson and Garrett, 1999; Dann et al., 2000; Erasmus et al., 2005), suggesting that feeding this culture of *S. cerevisiae* did not improve energy status of these dairy cows in which DM intake remained the same. On the other hand, the concentration of urea N in plasma was reduced by feeding YC, which suggests that protein utilization might have been improved with the addition of a culture of *S. cerevisiae.* In fact, intake of DM and protein were similar between treatments, but protein secretion in milk was improved with YC, which would result in improved protein utilization and the observed reduction in urea N concentration in blood plasma. Feeding a culture of *S. cerevisiae* generally reduces the concentration of ammonia N in the rumen (Erasmus et al., 2005; Lehloenya et al., 2008), although differences are not always significant.

The increased synthesis of milk and solids-not-fat in milk suggests that the supply of nutrients for milk synthesis was altered; however, energy output in milk did not increase with feeding of YC. The lack of changes in milk NE_L output was caused by the lower NE_L concentration in milk of cows fed YC. In three studies in which energy concentration of the diet was evaluated based on DM intake, energy output in milk, and body weight change with Holstein (Robinson and Garrett, 1999; Erasmus et al., 2005) and Jersey cows (Dann et al., 2000) in early lactation, addition of a culture of *S. cerevisiae* did not influence the calculated NE_L content of rations. Therefore, the similar secretion of energy associated with similar DM, and likely energy intake might explain the lack of effects of feeding a culture of *S. cerevisiae* on concentrations of glucose, NEFA, BHBA and insulin in plasma and BCS of early lactation dairy cows.

5. Conclusions

Few studies have examined effects of *S. cerevisiae* culture as nutritional supplements to heat-stressed dairy cows. In the current experiment, lactating dairy cows exposed to heat stress and supplemented with 30 g/d of a culture of *S. cerevisiae* had increased yields of milk and milk components, but reduced concentrations of milk fat. Supplemental yeast culture had minor effects on rectal temperature, and did not influence BCS and concentrations of metabolites in plasma. Results indicate that supplemental yeast culture improved lactation performance of dairy cows exposed to heat stress by increasing yields of milk and of solids-not-fat.

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