Effects of active dry yeast on ruminal pH characteristics and energy partitioning of finishing steers under thermoneutral or heat-stressed environment¹

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ABSTRACT: The objective of this trial was to determine the effects of supplementing active dried yeast (ADY) in the diets of finishing steers on energy and nitrogen metabolism and ruminal pH characteristics under thermoneutral (TN) or heat-stressed (HS) conditions. Eight British cross steers received 1 of 2 treatments (TRT) [either a control finishing diet (CON) or supplemented with 3 g/d of ADY] under 1 of 2 temperatures [TEMP: TN = 18 ± 0.55 °C and 20 ± 1.2% relative humidity (RH) or HS = 35 ± 0.55 °C and 42 ± 6.1% RH]. Steers were orally administered an indwelling rumen pH and temperature recording bolus. Data collection occurred for 48 consecutive hours inside 2 calorimetry chambers. Data were analyzed as a 4 × 8 Latin rectangle design with fixed effects of TRT and TEMP and random effects of steer and period. There were no TRT × TEMP interactions for metabolism or calorimetric measurements ($P \geq 0.1510$). In vivo DM digestibility (DMD) was greater for ADY-fed steers than for CON-fed steers (77.1% vs. 75.3%, respectively; $P = 0.0311$). No TRT ($P = 0.3032$) or TEMP ($P = 0.1833$) effect was observed for nitrogen retention. Energy partitioning suggested DE and ME (Mcal/kg) were greater for ADY-fed steers than for CON-fed steers ($P = 0.0097$ and $P = 0.0377$, respectively).

Steers under HS had reduced DMI but greater DMD than TN steers (77.1% vs. 75.3%, respectively; $P = 0.0316$) and greater CH₄ per unit of DM (8.53 vs. 6.47 g/kg, respectively; $P = 0.0145$). Although DE was greater for HS than TN (3.16 vs. 3.06 Mcal/kg, respectively; $P = 0.0123$), heat production energy (HE) tended to be greater for HS than TN (18.1 vs. 17.0 Mcal/d, respectively; $P = 0.0743$), resulting in a less retained energy (0.412 vs. 0.100 Mcal/kg; $P = 0.0147$). There was a tendency for an interaction of mean ruminal pH ($P = 0.1279$) where pH of ADY-fed steers was greater than pH of CON-fed steers under TN conditions (5.81 vs. 5.57, respectively), but not under HS conditions (5.37 vs. 5.41, respectively). Duration (DUR) and area under the curve (AUC) for pH > 5.6 had similar tendencies; under TN conditions, the DUR and AUC for pH > 5.6 in ADY-fed steers were greater than in CON-fed steers ($P = 0.0726$ and $P = 0.0954$, respectively), but under HS conditions, there was no difference between ADY and CON. We conclude that supplementing ADY in the diets of finishing steers improved DMD, DE, ME, and mean ruminal pH under TN conditions, but not in extreme HS conditions likely due to reduced DMI and greater HE requirements.

Key words: active dried yeast, energy partitioning, indirect calorimetry, heat stress, ruminal acidosis

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INTRODUCTION

The effects of including an active dry yeast (ADY; i.e., *Saccharomyces cerevisiae*) in the diets of dairy cattle have been extensively studied (Desnoyers et al., 2009), but its effects on beef cattle under feedlot conditions are not well defined. Improvements in DM digestibility (DMD), stabilization of ruminal pH, and increased feed efficiency are translational variables of interest in finishing cattle supplemented with ADY. The role of ADY in ruminant diets has been suggested to alter fermentative pathways from lactate to propionate by stimulating populations of lactate-utilizing and cellulolytic bacteria populations, thereby decreasing the risk of low ruminal pH and increasing ruminal digestibility (Chaucheyras et al., 1996; Lila et al., 2004). Some research in dairy cattle has even shown positive impacts when supplementing ADY during warmer months when productive functions tend to decline (Moallem et al., 2009; Salvati et al., 2015). Heat stress is a substantial drain on feed energy in beef cattle and may increase maintenance requirements by up to 18% (National Academies of Sciences, Engineering, and Medicine, NASEM, 2016). As thermal heat index increases, animal energy expenditure increases in an effort to maintain core body temperature within physiological limits. In addition, heat stress may affect feeding behavior, DMI, and cause ruminal disturbances that may lead to acute ruminal acidosis (Collier et al., 2006). During extreme weather in the United States and more commonly in subtropical latitudes, heat stress may have significant adverse impacts on beef production, reducing economic sustainability, and potentially prejudicing animal welfare. Therefore, objectives of this study were to evaluate the effects of supplemental ADY in the diets of finishing steers on the metabolism of energy and nitrogen and ruminal pH characteristics, using indirect calorimetry and indwelling rumen pH boluses, under thermoneutral (TN) or heat-stressed (HS) conditions.

MATERIALS AND METHODS

Animals, Feeding Regimens, and Dietary Treatments

Eight British crossbred steers (389 ± 30 kg, 14 mo of age) were cared for according to the approved animal use protocol (IACUC: 2016-0267) and housed individually in metabolism stalls in a climate controlled barn [18 ± 0.55 °C; $35 \pm 6\%$ relative humidity (RH)]. The Large Ruminant Nutrition System (LRNS; <http://www.nutritionmodels.com/>

[lrns.html](http://www.nutritionmodels.com/); accessed 21 January 2018; Tedeschi and Fox, 2018) was used to formulate the high-concentrate control diet (CON) using the following ingredients: cracked corn, dried distiller's grain, a medium chopped alfalfa hay (5- to 10-cm particles), and mineral supplement, as detailed in Table 1. Steers were transitioned from an ad libitum diet of Bermudagrass hay over a period of 21 d to the CON diet and further adapted for another 14 d prior to the trial. To compensate for expected drops in the voluntary intake due to extreme environmental temperature and ensure proper dosage and full consumption of feed treatment, steers were limit fed (1.5% of shrunk BW, daily DMI) at 0700 and 1700 h each day, but with unrestricted access to water. An active dry yeast (ADY) supplement (VistaCell; ABVista, Marlborough, UK) was top dressed and thoroughly hand mixed (1.5 g) at each feeding to assigned treated steers within each period

Table 1. Ingredient and chemical analysis of the basal finishing diet fed to beef steers

Items ¹	Finishing diet %
Ingredients	
Alfalfa hay, medium chop	15
Cracked corn	70
Dried distiller's grain	5.5
Molasses	6.8
Mineral supplement ²	1
Limestone	1
Urea	0.7
Chemical analysis	
DM, % of diet	82.2
CP, % DM	11.8
Soluble protein, % CP	19.1
NDF, % DM	21.7
ADF, % DM	12.4
Lignin, % DM	2.7
Crude fat, % DM	3.5
Sugar, % DM	4.6
Starch, % DM	49.2
Ash, % DM	4.9
Calcium	0.7
Phosphorus	0.4
TDN, %	82
NE _m , Mcal/kg	1.74
NE _g , Mcal/kg	1.14
peNDF, % ³	10
GE, Mcal/kg ⁴	4.16

¹Items are feed ingredients and chemical composition of diets evaluated by Cumberland Valley Analytical Services (Hagerstown, MD).

²Custom blend with 2:1 calcium to phosphorus ratio.

³peNDF is physical effective fiber; method by Penn State Particle Size separator 4-mm sieve.

⁴GE measured by bomb calorimetry.

to allow a total of 3 g/d (6×10^{10} CFU/d). Viability was ensured before and after the trial for quality control purposes by the proprietor (ABVista, Marlborough, UK).

Experimental Design, Equipment, and Data Collection

A 4×8 Latin rectangle design was used to determine the effects of 2 feed treatments (CON and ADY) and 2 environmental temperatures (TN and HS), so that within a period each interaction of feed treatment and the temperature was replicated by 2 steers. Because we used 2 respiration chambers side-by-side, only 2 steers could be collected at one time; steers were paired together to which one would receive the TN TEMP and the other would receive the HS TEMP. After each pair completed their 48 h recording session, chambers were recalibrated, and a new pair would move in, so that in each period data recording was completed over a total of 8 d. Each period lasted 14 d where steers were adapted to their feed treatment (TRT) for 12 d before any measurements. Within each period, on day 13 for a pair of steers, a shrunk body weight (SBW) was taken prior to the morning feeding. Each steer was placed in a single stall open-circuit respiration calorimetry chamber system using a mass flow system (Flowkit model FK-500; Sable System Int., Henderson, NV) for a 48-h data collection period. Chambers were designated as either TN (18 ± 0.55 °C; $20 \pm 1.2\%$ RH) or HS (35 ± 0.55 °C; $42 \pm 6.1\%$ RH). Ambient air (baseline) and air from each chamber were sampled by a multiplexer (Respirometry Multiplexer V 2.0, Sable System Int., Henderson, NV) rotating every 2 min and measured O_2 , CO_2 , and CH_4 (FC-1B O_2 analyzer, CA-2A CO_2 analyzer and MA-10 Methane analyzer; Sable System Int., Henderson, NV). The SBW, dietary energy density, and the known dimensions of the calorimetry chambers were used to calculate the proper bank time and flow rate needed before data recording. The assumed gas concentrations of baseline ambient air ($O_2 = 20.95\%$, $CO_2 = 0.04\%$, and $CH_4 = 0.00\%$) were used to calibrate O_2 , CO_2 , and CH_4 analyzers using known gasses, SPAN, and nitrogen, before each steer entry for data collection. The measured gas was scrubbed of water vapor using fresh drierite desiccant (Hammond Drierite Co Ltd, Xenia, OH) for each 48-h collection, and the rate of O_2 , CO_2 , and CH_4 production (VO_2 , VCO_2 , and VCH_4) were determined (L/min) (Lighton, 2008). Prior to each period, an oxygen recovery trial was performed using the gravimetric nitrogen injection technique

(Cooper et al., 1991), where expected ($20.95\% \times$ volume of N) and observed VO_2 uptake were verified with recovery being no less than 100% ($O_2 < 0.001$ L/min).

Each chamber was preloaded with 4 rations of designated feed TRT, equipped with a line voltage thermostat (Ranco Enterprises, Inc., Model# ETC-111000-000), dehumidifier (Hisense USA, Model# DH-70K1SDLE), water meter (Neptune Technology Group, Inc., Model# T10-DR-075-G-F), digital HOBO temperature and humidity data loggers (Onset Computer Corporation, Model# UX100-003), and security cameras (FLIR Lorex Inc., Model# LBV1511W) for monitoring animal activity within the chambers. In addition, each chamber was equipped with a metabolism stand to allow for the collection of total urine and fecal output. After 48 h in the chambers, steers were restrained in a squeeze chute to collect approximately 1 L of rumen fluid via esophageal tubing. Animals were allowed to rest for approximately 1 h in an open pen before returning to the stalls in the climate controlled barn to begin day 1 of their next experimental period with a different diet.

Water, Feed, Fecal, and Urine Analyses

Water intake was manually recorded from the analog water meter. During each period, batch feed samples were taken and homogenized into representative samples ($n = 4$) to determine the chemical analysis of DM, NDF, ADF, lignin, CP, soluble protein, NDIN, ADIN, starch, sugars, and minerals at Cumberland Valley Analytical Services (Hagerstown, MD). Residual orts during data collection days were weighed, and a subsample was cataloged to be analyzed for DM, GE, and N to be used for calorimetry adjustments.

Total fecal output for the 48-h data collection period was weighed, homogenized, subsampled, dried, and analyzed for DM, GE, and N. Total urine collection was achieved using a large transmission funnel, and to eliminate fecal contamination, 2 non-splatter filters fitted beneath the metabolism stand and over the catch tub. The catch tub was linked to an external holding tub and vacuum pump system that remained primed to eliminate gaseous escape from the chamber. To prevent volatilization of N, 600 mL of 3 molar HCl was added to each catch tub at the beginning of each recorded steer entry. Urine was vacuumed as necessary into the external holding tub from the catch tub. Total urine was weighed and homogenized for subsampling at the end of each 48 h data collection. Urine samples were analyzed for GE and N.

All GE analyses were conducted by a bomb calorimeter (Parr adiabatic calorimeter; Parr Instruments Co., Moline, IL), and total N analysis was performed by Servi-Tech Laboratories (Amarillo, TX) using the Dumas combustion method.

Rumen Boluses

Rumen pH and temperature were recorded using a wireless, indwelling rumen bolus that communicated via radio-transmission to a base station inside the barn (smaXtec animal care, GMbH, Graz, Austria). Because manufacturer guaranteed life span was limited to 50 d, boluses were activated, calibrated, and inserted into the steers serially 1 wk before their first chamber collection days within the first period. Boluses were inserted orally using the manufacturer provided balling gun. Continuous recordings of the reticulo-ruminal pH and temperature were averaged and plotted every 10 min for the duration of the trial automatically transmitting data to the base station radio system, which stored data in the cloud for real-time monitoring. Data were serially downloaded to reflect the relative information for a given steer's stay in the calorimetry chamber. Rumen variables were chosen to reflect the time (**DUR** = duration, h/d or %/d) and magnitude (area under the curve, **AUC**) below pH thresholds of biological importance. Rumen pH variable thresholds of 5.0 and 5.6 were assigned based on work by [Nagaraja et al. \(2007\)](#) where ruminal pH below 5.0 was considered to be acutely acidotic and between 5.0 and 5.6 were considered to be subacutely acidotic. Rumen pH variables were therefore calculated as mean pH, DUR above pH 5.6 and the area above the curve (**AAC**), DUR of subacute ruminal acidosis (**SARA**; pH 5.0 to 5.6, h/d and %/d), AUC of SARA, DUR of acute ruminal acidosis (**ARA**; pH < 5.0, h/d and %/d), and AUC of ARA. Rumen temperature variables were calculated to detect changes in the normal rumen temperature above the typical heat of fermentation as a threshold of 40 °C, resulting in the variables mean rumen temperature, DUR above 40 °C (h/d and %/d), and AAC of 40 °C. The AUC and AAC variables were determined with a script that used the definite integral approach and the *rootSolve* and *Spline* functions in the CRAN package of the R ([R Core Team, 2017](#)) where y-base thresholds of pH 5.0 and 5.6 were established for rumen pH data and a y-base threshold of 40 °C was used for rumen temperature calculations.

Energy Partitioning and Nitrogen Balance

Gross energy intake (**GEI**; Mcal/d) was determined by multiplying the GE of the representative diet by the kilograms of feed offered (Mcal × kg/d) minus the energy contained in the residual orts. Fecal energy (**FE**; Mcal/d) was calculated by multiplying the energy density of the feces by daily fecal output. Urinary energy (**UE**; Mcal/d) and urinary nitrogen (**UN**; g/d) were calculated by multiplying the energy (Mcal/kg) and nitrogen density (% N) of the urine by the daily urinary output (kg/d), respectively. Gaseous energy (**GASE**) was determined by multiplying the liters per day of CH₄ produced in the chamber by the density of CH₄ (0.6556 g/L at 25 °C) and its energy density (13.3 Mcal/g) to yield GASE in Mcal/d. Heat production energy (**HE**) was calculated as follows: $HE \text{ (Mcal/d)} = (3.866 \times VO_2) + (1.2 \times VCO_2) - (0.518 \times VCH_4) - (1.431 \times UN)$ ([Brouwer, 1965](#)). Final values of energy partitioning were calculated as follows: $DE = GEI - FE$; $MEI = DE - (UE + GASE)$, and retained energy (**RE**) assumed to be $RE = MEI - HE$, where ME intake (MEI) was calculated as the observed dietary ME content (Mcal/kg) multiplied by the DMI (kg/d) of diet. The NE_m (Mcal/kg) of the diet was calculated as follows: $NE_m = 1.37 \times ME - 0.138 \times ME^2 + 0.0105 \times ME^3 - 1.12$ and the partial efficiency of the use of ME for maintenance (k_m) = NE_m/ME. The NE_g (Mcal/kg) of the diet was calculated as follows: $NE_g = 1.42 \times ME - 0.174 \times ME^2 + 0.0122 \times ME^3 - 1.65$ and the partial efficiency of the use of ME for gain (k_g) = NE_g/ME. Shrunk weight gain was calculated according to [Tedeschi and Fox \(2018\)](#) where $SWG = 13.91 \times RE^{0.9116} \times SBW^{-0.6837}$. Nitrogen intake was determined by multiplying the total N (%) of the representative diet by the kilogram of feed offered (kg/d), less the total N (kg) contained in the residual orts. Fecal N was calculated as the % N of the feces (DM) by the daily fecal excretion (kg/d, DM). Dietary N retained was calculated by subtracting fecal and urinary N from N intake.

In Vitro Fermentation

As steers were removed from the calorimetry chambers, rumen fluid inoculum was obtained via esophageal tubing. Rumen fluid samples were collected in a 1-L thermos and filtered through 8 layers of cheesecloth to remove any feed particles. The in vitro gas production (**IVGP**) technique has been described previously in detail ([Tedeschi et al., 2009](#)), but briefly, approximately 200 mg of the

CON diet (ground to 2 mm) was weighed into 160-mL Wheaton bottles, containing equal sized magnetic stir bars. Samples were wetted with 2 mL of deionized water to reduce particle scattering during CO₂ flushing to maintain an oxygen reduced atmosphere. Goering and Van Soest's (1970) in vitro buffering media (14 mL) was added to each bottle under constant CO₂ flushing, sealed with a butyl rubber stopper and crimp sealed. Bottles were promptly placed in the incubation chamber to begin heating to rumen temperature (39 °C). Rumen inoculum from the treated steers was filtered through glass wool under a CO₂ atmosphere. The treatment and temperature adapted rumen fluid inoculum (4 mL) was injected into the Wheaton bottles which contained either a blank, alfalfa standard, or the representative CON diet in triplicate, respectively. Internal pressure was equilibrated across all bottles after inoculation by inserting needles into the stoppers for approximately 5 s, and pressure sensors were inserted. The pressure was recorded at 5-min intervals for 48 h plotting the fermentation profile over time for each sample. After 48 h, bottles were set in an ice bath to cease fermentation. Head space samples (1 mL) were removed and analyzed for methane concentration using the gas chromatography method (Allison et al., 1992). Final rumen fluid pH was measured, and approximately 40 mL of neutral detergent solution (ANKOM Technology, Macedon, NY) was added to each bottle of fermented feed residue. Bottles were resealed and set in the autoclave for 15 min at 121 °C. Samples were then filtered using Whatman 54 paper to collect the washed feed residue to calculate dry matter digestibility.

The kinetic analysis of the 48-h fermentation of the CON diet using treated rumen fluid was evaluated using nonlinear functions, and that with the lowest sum of square errors was selected (Schofield et al., 1994). The nonlinear fitting was performed using Gasfit (<http://www.nutritionmodels.com/gasfit.html>; accessed 21 January 2018), which executes specific R scripts to perform convergence of gas production data using the *nls* function (Chambers and Bates, 1992) and the *port* algorithm (Fox et al., 1978; Gay, 1990). Preliminary results indicated the exponential with discrete lag nonlinear function had the lowest SSE and best fit of the fermentation profiles (Williams et al., 2010). The Gasfit provides the total gas production (mL), the fermentation rate (h⁻¹), and the lag time (h).

Data analyzed from the IVGP technique included total gas production (mL), the rate of fermentation (h⁻¹), and lag time (h), apparent TDN

and ME assuming passage rate of 2, 4, 6, and 8%/h. Apparent TDN was computed using the fractional degradation rate of NDF obtained from the IVGP technique with the most likely fractional passage rate, using Eq. 1 to 6 (Tedeschi and Fox, 2018).

$$aTDN = 0.98 \times (100 - NDF - CP - EE - Ash) + dCP + dEE + dNDF - 7 \quad (1)$$

$$aCP = CP \times \left(1 - 0.004 \times \left(100 \times \frac{ADIP}{CP} \right) \right) \quad (2)$$

$$aEE = 2.25 \times (EE - 1) \quad (3)$$

$$aNDF = NDF \times \left(\frac{kd}{kd + kp} + IDNDF - IDNDF \times \frac{kd}{kd + kp} \right) \quad (4)$$

$$DE = aTDN \times 4.409 \quad (5)$$

$$ME = \frac{0.82 \times DE}{DMI} \quad (6)$$

where ADIP is acid-detergent insoluble (crude) protein, % DM; aTDN is apparent total digestible nutrients, % DM; CP is crude protein, % DM; EE is ether extract, % DM; IDNDF is intestinal digestibility of NDF (assumed to be 20%; Sniffen et al., 1992); kd is fractional degradation rate of NDF, %/h; kp is fractional passage rate of NDF, %/h, and NDF is neutral detergent fiber, % DM; DE is digestible energy, Mcal/d; ME is metabolizable energy, Mcal/kg.

Statistical Analysis

Data collected during the chamber stay (indirect calorimetry variables, rumen bolus variables, feed and water intake, and urine and fecal output variables) were analyzed according to the Latin rectangle design using the PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) using the following model:

$$Y_{ijkl} = \mu + \text{steer}_i + \text{period}_j + \text{TRT}_k + \text{TEMP}_l + \text{TRT}_k \times \text{TEMP}_l + e_{ijkl}$$

where Y_{ijkl} = response variables (heat production, respiratory quotient, methane production, GE efficiency, mean rumen temperature and pH, AUC/AAC and time spent above 5.6, subacute, acute and above 40 °C, feed intake, water intake, FE, FN, UE and UN output), μ = overall mean, steer_i = random effect of the steer within a column ($i = 1, 2, \dots, 8$

steers), period_j = random effect of the period within a row ($j = 1, 2, 3, 4$), TRT_k = the fixed effect of feed treatment ($k = \text{CON}$ or ADY), TEMP_l = the fixed effect of temperature ($l = \text{TN}$ or HS), $\text{TRT}_k \times \text{TEMP}_l$ = interaction of treatment and temperature, and e_{ijkl} = random error associated with the measurement of the i th steer within period j assigned to treatment k and temperature l .

Differences in least squares means of the in vivo analyses were declared significant when $P < 0.05$ and tendencies were discussed when $P < 0.15$.

Data collected for the IVGP technique was analyzed as a CRD. Inoculum was taken only once from each of the 8 steers at given periods so that each treatment and temperature combination was represented twice. The following model was used in the PROC Mixed of SAS (SAS Inst., Inc., Cary, NC):

$$Y_{ij} = \mu + \text{TRT}_i + \text{TEMP}_j + \text{TRT}_i \times \text{TEMP}_j + e_{ij}$$

where Y_{ij} = response variables (DMD, total gas production, fractional degradation rate, lag time, TDN, ME, CH_4 , inoculum pH, and final pH), μ = overall mean, TRT_i = fixed effect of treatment ($i = \text{CON}$ or ADY rumen fluid inoculum), TEMP_j = fixed effect of temperature ($j = \text{TN}$ or HS), $\text{TRT}_i \times \text{TEMP}_j$ = interaction of treatment and

temperature, and e_{ij} = random error associated with the measurement of the feed sample assigned to temperature j and treatment i . Differences in least squares means of the IVGP analyses were declared significant when $P < 0.05$ and tendencies were discussed when $P < 0.20$.

RESULTS AND DISCUSSION

Digestion and Metabolism Analyses

Table 2 shows the effects of TRT and TEMP on water consumption, metabolism of the diet, dietary N, and CH_4 output. There were no significant $\text{TRT} \times \text{TEMP}$ interactions for these variables ($P \geq 0.1510$).

Treatment effects. There were no TRT effects on daily water intake (Table 2). Dietary TRT did not affect DMI ($P = 0.9786$). This result is consistent with other reports in beef cattle fed ADY supplements (McGinn et al., 2004; Zerby et al., 2011). There was a tendency for TRT to affect fecal DM excretion when expressed either as kilogram per day or as percentage of SBW basis ($P = 0.0766$). In general, ADY steers tended to excrete less fecal DM than CON steers (0.321% vs. 0.348% of SBW), indicating greater apparent DMD when receiving

Table 2. Effect of yeast treatment and environmental temperature on the water consumption and metabolism of finishing steers

Items ¹	TRT ²		TEMP ³		SEM	P value		
	CON	ADY	TN	HS		TRT	TEMP	TRT $\times$ TEMP
Water intake, kg/d	12.1	15.0	13.4	13.8	2.40	0.2514	0.8692	0.6165
Water intake, % SBW	2.51	3.09	2.76	2.84	0.444	0.2719	0.8809	0.6479
Water intake:DMI	1.78	2.25	1.89	2.13	0.370	0.2062	0.5149	0.5698
DMI, kg/d	6.77	6.77	7.10 ^a	6.43 ^b	0.197	0.9786	0.0004	0.6615
DMI, % SBW	1.41	1.40	1.47 ^a	1.34 ^b	0.045	0.8568	0.0016	0.8740
Fecal DM, kg/d	1.67	1.55	1.75 ^a	1.48 ^b	0.120	0.0766	0.0005	0.7721
Fecal DM, % SBW	0.348	0.321	0.363 ^a	0.306 ^b	0.025	0.0730	0.0007	0.8793
DMD, %	75.3 ^b	77.1 ^a	75.3 ^b	77.1 ^a	1.90	0.0311	0.0305	0.9296
Urine, kg/d	12.5	13.0	10.7 ^b	14.8 ^a	2.12	0.8252	0.0495	0.6350
Urine, % SBW	2.61	2.69	2.23 ^b	3.07 ^a	0.448	0.8387	0.0438	0.7259
Feed N, kg/d	0.128	0.128	0.134 ^a	0.122 ^b	0.004	0.9786	0.0004	0.6615
Fecal N, kg/d	0.0037	0.0038	0.0039 ^a	0.0036 ^b	0.0001	0.4578	0.0172	0.1510
Fecal N, %	2.93	2.99	2.91	3.01	0.072	0.3456	0.1238	0.1788
Urinary N, kg/d	0.0694	0.0630	0.0656	0.0668	0.004	0.2539	0.8356	0.6555
Urinary N, %	0.700	0.613	0.731	0.581	0.117	0.2887	0.0772	0.7584
N retained, %	42.1	47.0	47.8	41.3	3.65	0.3032	0.1833	0.5119
CH_4 , L/d	79.2	73.9	69.7	83.4	9.00	0.5287	0.1109	0.3458
CH_4 , g/kg DM	7.72	7.28	6.47 ^b	8.53 ^a	0.938	0.5641	0.0145	0.3557

^{a,b}Least squares means of the main effects in a row with different superscripts differ at $P < 0.05$.

¹Items are metabolism variables; SBW = shrunk body weight; N = nitrogen; DMD = dry matter digestibility.

²Treatment is control fed (CON) or control + 3 g/d of an active dried yeast (ADY).

³Temperature is thermoneutral [TN; $18 \pm 0.55^\circ\text{C}$; 20% relative humidity (RH)] or heat stressed (HS; $35 \pm 0.55^\circ\text{C}$; 42% RH).

ADY. In fact, DMD was significantly affected by TRT ($P = 0.0311$) in which DMD was greater for ADY-supplemented steers than for CON-fed steers (77.1% vs. 75.3%, respectively). This finding is supported by a meta-analysis of research conducted in dairy cattle (Desnoyers et al., 2009). This result suggested that ADY may increase available DE to steers receiving high-concentrate diets (i.e., finishing diets). There was no effect ($P = 0.8387$) of TRT on urine excretion. There was considerable variance in methane production (L/d) but no significant effects of TRT (% of DMI) ($P = 0.5519$). There was no significant effect of TRT on N intake, fecal N excretion, or urinary N excretion, and consequently, no effect on % N retained was observed ($P = 0.3032$).

Temperature effects. There were no significant TEMP effects on daily water intake (Table 2). It was expected that TEMP would increase water consumption in an effort to regulate body temperature. However, the temperature of the available water was similar to the temperature of the chamber and may have only provided minimal relief. In addition, there has been evidence that ambient temperatures $>30^{\circ}\text{C}$ have a low negative correlation with water intake (Rouda et al., 1994). There was a significant effect of TEMP on DMI (kg/d and % of SBW; $P = 0.0004$). Though steers were restricted to consuming only 1.5% of their SBW as DM, HS steers still had reduced DMI vs. TN steers (7.10 vs. 6.43 kg/d; $P < 0.0005$). This 9.4% reduction in DMI is slightly less than that predicted by the LRNS model (12.5%) for DMI adjustment due to current effective temperature index (CETI) of 35.3°C with no night time cooling (Fox et al., 2004; Tedeschi and Fox, 2018), but this is probably because animals were not consuming at their voluntary intake level.

There was also a significant effect of TEMP on fecal DM excretion and DMD ($P = 0.0007$ and $P = 0.0311$, respectively). It was expected that because steers experiencing HS had lower DMI, they would excrete significantly less DM than TN steers. Indeed, HS steers excreted significantly less fecal DM than TN steers, resulting in a 15% difference (0.306% vs. 0.363% of SBW, respectively). The net effect of decreased DMI and fecal DM excretion still resulted in significantly greater DMD for HS steers than TN steers (77.1% vs. 75.3%, respectively). High ambient temperature is known to decrease ruminal passage rate, potentially increasing the diet digestibility (Miaron and Christopherson, 1992; Bernabucci et al., 1999).

There was a significant effect of TEMP on urine excretion ($P \leq 0.0495$) where steers under HS produced more total urine than TN steers (14.8

vs. 10.7 kg/d, respectively) at a greater percentage of their SBW (3.07% vs. 2.23% of SBW, respectively). Interestingly, the urinary output of HS steers exceeded their water consumption (3.07% vs. 2.84% of SBW) indicating negative water balance. This is in contrast to what was expected as typically a retention and concentration of urine is observed under heat stress conditions (McDowell et al., 1969; Collier et al., 1982). However, due to our objectives, acclimatization to temperature was not performed prior to measurements and therefore our measurements may not yet reflect the adapted response.

Due to a decreased DMI, there was a significant effect of TEMP on N intake where HS steers consumed less N than TN steers (0.122 vs. 0.134 kg/d, respectively; $P = 0.0004$). This manifested into significantly less total N excreted in the feces for HS vs. TN steers (0.0036 vs. 0.0039 kg/d; $P = 0.0172$) with a tendency to be less concentrated (2.91 vs. 3.01% N, respectively; $P = 0.1238$). There was no TEMP effect of urinary N excretion, although as expected, there was a tendency for TN steers to produce urine with greater N concentration than HS steers (0.731% vs. 0.581%, respectively; $P = 0.0772$), likely due to the overall difference in N intake. There was no effect of TEMP on % N retained ($P = 0.1833$).

There was no effect of TRT or TEMP detected for methane production (L/d), although there was a tendency for HS steers to produce more CH_4 than TN (83.4 vs. 69.7 L/d, respectively; $P = 0.1109$). When CH_4 production was normalized to gram per kilogram of DMI, a significant effect of TEMP was observed where steers that experienced HS produced greater CH_4 than TN steers (8.53 vs. 6.47 g/kg DMI, respectively; $P = 0.0145$). The greater CH_4 output per kilogram of intake is likely due to the slower ruminal passage rate previously suggested to contribute to the greater DMD of HS steers. By this logic, with ADY-fed steers having greater total tract DMD but similar CH_4 production as CON-fed steers, it could be suggested that ADY may influence postruminal digestibility rather than ruminal digestibility.

Indirect Calorimetry

Table 3 shows the results of the indirect calorimetry analysis. There were no significant TRT $\times$ TEMP interactions ($P \geq 0.2589$). The RQ of CO_2/O_2 was above 1.0 for all steers indicative of probable lipogenesis (Ferrannini, 1988) regardless of TRT or TEMP. This is also validated by the SWG and the k_g (mean = 0.79, based on regression), indicative that the composition of gain from RE was

Table 3. Effect of treatment and temperature on energy metabolism of finishing steers using indirect calorimetry

Items ¹	TRT ²		TEMP ³		SEM	P-value		
	CON	ADY	TN	HS		TRT	TEMP	TRT × TEMP
RQ	1.11	1.10	1.11	1.09	0.009	0.6295	0.0734	0.4324
GEI, Mcal/d	28.1	28.1	29.5 ^a	26.7 ^b	0.819	0.9797	0.0004	0.6604
FE, Mcal/d	7.44 ^a	6.77 ^b	7.75 ^a	6.45 ^b	0.513	0.0320	0.0003	0.5638
DE, Mcal/d	20.7	21.4	21.8 ^a	20.3 ^b	0.946	0.1886	0.0096	0.8239
DE, Mcal/kg DM	3.06 ^b	3.16 ^a	3.06 ^b	3.16 ^a	0.082	0.0097	0.0123	0.6485
DE, % GE	73.6 ^b	76.0 ^a	73.7 ^b	75.9 ^a	1.96	0.0094	0.0136	0.6543
UE, Mcal/d	0.882	0.896	0.912	0.866	0.061	0.8279	0.4941	0.8978
GASE, Mcal/d	0.690	0.644	0.607	0.727	0.078	0.5287	0.1109	0.3458
GASE, % GE	2.47	2.33	2.07 ^b	2.73 ^a	0.300	0.5644	0.0147	0.3550
ME, Mcal/d	18.9	19.8	19.9	18.8	1.00	0.1687	0.0991	0.9802
ME, Mcal/kg DM	2.80 ^b	2.93 ^a	2.80 ^b	2.92 ^a	1.03	0.0281	0.0347	0.6054
ME, % DE	91.4	92.5	91.5	92.5	1.48	0.3360	0.3591	0.6967
HE, Mcal/d	17.5	17.6	17.0	18.1	0.663	0.8852	0.0743	0.2589
HE, % GE	62.6	62.9	57.5 ^b	67.9 ^b	1.68	0.8834	<0.0001	0.3668
RE, Mcal/d ⁴	1.43	2.22	2.95 ^a	0.695 ^b	0.884	0.3166	0.0091	0.3696
RE, Mcal/kg DM	0.199	0.313	0.412 ^a	0.100 ^b	0.131	0.3355	0.0147	0.3297
RE, % ME	6.42	9.95	14.3 ^a	2.04 ^b	4.56	0.3855	0.0062	0.4731
SWG, kg/d	0.123	0.186	0.251 ^a	0.058 ^b	0.073	0.3208	0.0054	0.4040

^{a,b}Least squares means within a row with different superscripts differ at $P < 0.05$.

¹Items are variables representative of the net energy system for beef cattle, indirect calorimetry procedures and combustion analysis. RQ = respiratory quotient (CO_2/O_2); GEI = GE intake (total feed energy); DE = digestible energy [GEI – fecal energy (FE)]; ME = metabolizable energy [DE – urinary energy (UE) and methane energy (GASE)]; RE = retained energy [ME – heat production energy (HE)]. Shrunken weight gain (SBW) = $13.91 \times \text{RE}^{0.9116} \times \text{SBW}^{-0.683}$.

²Treatment is control fed (CON) or control + 3 g/d of an active dried yeast (ADY).

³Temperature is thermoneutral [TN; $18 \pm 0.55^\circ\text{C}$; 20% relative humidity (RH)] or heat stressed (HS; $35 \pm 0.55^\circ\text{C}$; 42% RH).

likely due to accumulation of much more fat than protein (Tedeschi et al., 2004, 2010; Chizzotti et al., 2008; Marcondes et al., 2013), likely influenced by the maturity of the steers in this trial. Tedeschi and Fox (2018) summarize the data from Tedeschi et al. (2004), Chizzotti et al. (2008), Tedeschi et al. (2010), and Marcondes et al. (2013) reveal that the efficiency of use of ME for growth is inversely related to the percentage of RE in the form of protein, and at zero protein deposition, k_g is between approximately 0.6 and 0.8, depending on the ME concentration of the diet. There was no significant effect of TRT or TEMP on RQ, although there was a tendency for an effect of TEMP where TN steers had greater RQ than HS (1.11 vs. 1.09, respectively; $P = 0.07$), which may indicate that TN steers were more efficient at depositing energy as fat than HS steers. The indirect calorimetry technique consistently yields greater RE values than the comparative slaughter technique when animals are fed at production levels (Johnson et al., 1997). Likewise, comparative slaughter techniques may inflate the HE of cattle in production settings. Due to the inability to effectively separate energy used to support maintenance from that used to support growth requirements at different levels of energy intake,

either technique may result in errors between predicted and observed k_g , which is also affected by dietary energy concentrations and composition of the gain. Tedeschi and Fox (2018) discussed the needs for the next generation of growth models to highlight a more integrated system to better predict NE_m requirements and k_g at different levels of MEI. This is illustrated in Fig. 1 where the HE and RE of steers (kcal/kg MBW) are plotted against MEI with a line indicating the threshold for NE_m with adjustments for no physical activity ($\text{NE}_m = 70$ kcal/kg MBW) according to NASEM (2016). Using the regression of RE on MEI, the k_m and ME of the diet, the predicted NE_m is greater for both TN and HS compared with expected values (99.0 and 114.9 vs. 70 kcal/kg^{0.75} of SBW; NASEM, 2016). However, when regressing log HE on MEI, the antilog of the intercept multiplied by the k_m resulted in values closer to this threshold (75.8 vs. 103.3 kcal/kg MBW of NE_m for TN and HS steers, respectively), indicating the nonlinear regression of HE on MEI may be more precise at predicting NE_m than regressions from RE (Chizzotti et al., 2008). Deviations in the observed performance from predicted dietary NE_m and NE_g contents may also be due to the inherent errors when using the assumed DE to ME ratio of

Figure 1. Regression of heat energy (HE) and retained energy (RE) on ME intake (MEI) of steers using indirect calorimetry. (A) Under thermoneutral [18 ± 0.55 °C; 20% relative humidity (RH)] or (B) heat-stressed (35 ± 0.55 °C; 42% RH) conditions. The NE_m of 70 kcal/kg MBW recommended by NASEM (2016) is provided as a reference threshold.

0.82 rather than being directly measured (Galyean et al., 2016). The impact of inconsistent use of DE-to-ME and ME-to-NE efficiency calculations was illustrated by Tedeschi and Fox (2018) where they compare the relationships of ME-to-DE ratio and NE_m -to-ME ratio vs. DE intake using empirical equations recommended by NRC (1996, 2000, 2001), Galyean et al. (2016), and NASEM (2016). In summary, the steers in this study exhibited NE_m values that were greater than the NASEM (2016) recommended maintenance energy requirement, likely due to major differences in the k_m and k_g of steers fed at production levels vs. fasted or maintenance levels, calorimetry technique, physical activity, and dietary factors.

Treatment effects. Because there was no difference in DMI between TRT, the GEI was also not different ($P = 0.9797$). However, there was a significant effect of TRT on FE in which ADY steers excreted roughly 10% less FE than CON steers (6.77 vs. 7.44 Mcal/d, respectively; $P = 0.0320$). There was

no effect of TRT on DE (Mcal/d; $P = 0.19$); however, the conversion of GE to DE was significantly greater for ADY-fed steers vs. CON-fed steers (76% vs. 73.6% of GE). There was no significant effect of TRT on UE ($P = 0.8279$) or GASE ($P = 0.5287$) in megacalories per day. Losses of GASE as a % of GE were not significantly different ($P = 0.5644$). Therefore, there was no significant effect of TRT on megacalories per day of ME ($P = 0.1687$), and the conversion of ME to DE was similar for CON and ADY (91.4% and 92.5%, respectively; $P = 0.3360$). As anticipated, these values are much higher than the used ME:DE conversion of 0.82 (NASEM, 2016). Studies conducted by Hales et al. (2012, 2013) feeding Jersey steers dry-rolled or steam-flaked corn and different levels of wet distiller's grain plus solubles have also observed greater ME:DE ratios (91.9% to 96%) than that predicted by the NASEM (2016) equations. Tedeschi and Fox (2018) provide a very good discussion of this matter and suggest that because the current models predicting TDN and DE

are not discounted for diet type or level of intake, true ME may be underestimated, especially for high-concentrate diets. In fact, when we computed the NE_m of the diet based on observed ME from steers we calculated a 7.4% greater NE_m than what was predicted by the feed analysis; 1.88 Mcal/kg for TN steers vs. the predicted 1.75 Mcal/kg. Heat production was not affected by TRT in either Mcal/d ($P = 0.8852$) or as a % of GE ($P = 0.8843$). Overall, there was no difference of RE between CON- and ADY-fed steers (1.43 vs. 2.22 Mcal/kg, respectively; $P = 0.3166$), and RE:ME was neither different (6.42% vs. 9.95%, respectively; $P = 0.3208$).

Temperature effects. Differences in DMI influenced GEI in which there was a significant reduction GEI of HS steers vs. TN steers (29.5 vs. 26.7 Mcal/d, respectively; $P = 0.0004$). Temperature also had a significant effect on FE excretion where HS steers excreted roughly 17% less FE than TN steers (6.45 vs. 7.75 Mcal/d, respectively; $P = 0.0003$) and probably a result of the greater DMD of HS steers vs. TN steers. This resulted in a significant effect of TEMP on DE availability where TN retained more total energy than HS (21.8 vs. 20.3 Mcal/d; $P = 0.0096$) primarily due to greater GEI, but interestingly, the conversion of GE to DE was greater for HS than for TN (75.9% vs. 73.7% of GE, respectively; $P = 0.0123$). Greater conversion of GE to DE may again corroborate the theory of a reduction in passage rate and thus a greater opportunity for digestion as was observed in the differences of DMD.

Although the HS steers produced more urine, there was no significant effect of TEMP on UE ($P = 0.4941$) indicating urine was more dilute of energy than TN. There was a tendency for HS steers to produce more GASE than steers in TN conditions (0.727 vs. 0.607 Mcal/d, respectively; $P = 0.1109$) and convert significantly more GE to GASE (2.73% vs. 2.07% of GE, respectively; $P = 0.0147$). Regardless of the dietary restriction imposed, the conversion of GE to CH_4 for steers under TN conditions was on the lower end of the 2006 Intergovernmental Panel on Climate Change inventory report ($3.0 \pm 1.0\%$ of GE for feedlot cattle consuming a 90% concentrate diet), whereas those under HS were much closer to this value.

As expected, there was a tendency for the remaining ME to be greater for steers in TN conditions than HS conditions (19.9 vs. 18.8 Mcal/d, respectively; $P = 0.0991$); however, ME was greater for steers under HS than for those in TN conditions when expressed as megacalories per kilogram of DM (2.92 vs. 2.80 Mcal/kg DM; $P = 0.0347$). The conversion of DE to ME was similar ($P = 0.3591$)

for steers under TN and HS conditions indicating that effects of TEMP on UE and GASE losses are minor when steers are consuming a restricted amount of finishing diet.

Heat production energy tended to be affected by TEMP when expressed as megacalories per day ($P = 0.0743$). When HE was expressed as a percentage of GE intake, there was a significant effect of TEMP where HS steers lost more HE than TN steers (67.9% vs. 57.5%, respectively; $P < 0.0001$) possibly due to increased respiration in attempt to maintain core body temperature. This equates to roughly an 18.1% increase in maintenance energy costs for heat dissipation and is consistent with the heat stress adjustment factor (HSF) of 1.18 for open-mouth panting (NASEM, 2016) although panting was not analyzed. Calculating the CETI of the environment within the HS chamber ($35 \pm 0.55^\circ\text{C}$ and $42 \pm 6.1\%$ RH), assuming a wind speed and sunlight exposure of 0, respectively, HSF would range from 1.12 to 1.24 with a mean of 1.19 (Tedeschi and Fox, 2018). Using the CETI to account for NE_m requirements under HS conditions slightly overpredicted individual requirements, but offers greater control of differing environmental variables than the panting index, which only designates 2 adjustments, 1.07 or 1.18 times NE_m . Overall, steers under TN conditions retained 2.25 Mcal/d more than steers experiencing HS ($P = 0.0091$), as well as per kilogram of DM (0.412 vs. 0.100 Mcal/kg DM, respectively; $P = 0.0147$) and a greater conversion of ME to RE (14.3% vs. 2.04%, respectively; $P < 0.0062$). Using equations reported by Tedeschi and Fox (2018), the calculated SWG was roughly 76.9% less for steers under HS conditions vs. TN conditions (0.058 vs. 0.251 kg/d, respectively; $P = 0.0054$).

Rumen Parameters

Feed treatment and environmental temperature effects on rumen parameters are shown in Table 4. There was a tendency for an interaction of TRT and TEMP on mean ruminal pH ($P = 0.1279$; Table 4; Fig. 2). Under TN conditions, ADY steers tended to have greater mean pH than CON steers (5.81 vs. 5.57, respectively), but under HS conditions, mean ruminal pH was much lower and not different between CON and ADY (5.42 vs. 5.37, respectively). Theoretically, if HS resulted in the suggested slower ruminal passage rate, decreased acid clearance could have led to overall lower mean pH in the rumen. The results of mean pH in a TN environment indicate that supplementing ADY promotes higher

Table 4. Effects of treatment and temperature on rumen pH characteristics of finishing steers

Items ¹	TRT ²		TEMP ³		SEM	P value		
	CON	ADY	TN	HS		TRT	TEMP	TRT × TEMP
Mean pH	5.50	5.59	5.69 ^a	5.39 ^b	0.16	0.2901	0.0029	0.1279
pH variation	0.11	0.13	0.13	0.11	0.02	0.3328	0.3500	0.5440
DUR pH > 5.6, min/d	658	686	741	603	167	0.8093	0.2407	0.0726
DUR of SARA, min/d	450	376	432	394	101	0.5519	0.7604	0.5925
DUR of ARA, min/d	277	314	195 ^b	396 ^a	141	0.6816	0.0381	0.1353
AUC pH > 5.6, h	8.36	13.1	13.2	8.32	3.3	0.0976	0.0923	0.0954
AUC in SARA, h	18.1	17.8	20.8 ^b	15.2 ^a	3.2	0.8482	0.0028	0.2997
AUC in ARA, h	2.75	2.62	0.70 ^b	4.67 ^a	1.72	0.9255	0.0079	0.8862
Mean temperature, °C	40.04	40.01	39.33 ^b	40.73 ^a	0.15	0.7616	<0.0001	0.6289
Temperature variation, °C	0.463	0.417	0.241 ^b	0.638 ^a	0.076	0.5831	0.0001	0.9342
DUR > 40 °C, min/d	624	591	91 ^b	1,124 ^a	65	0.5422	<0.0001	0.4778
AUC > 40 °C/h	21.9	20.2	0.6 ^b	41.5 ^a	4.9	0.7222	<0.0001	0.7641

^{a,b}Least squares means within a row with different superscripts differ at $P < 0.05$.

¹Items are variables derived from indwelling ruminal pH and temperature boluses; DUR = duration, SARA = subacute ruminal acidosis (pH = 5.0 to 5.6); ARA = acute ruminal acidosis (pH < 5.0); AUC = area under the curve (pH units × time, h).

²Treatment is control fed (CON) or control + 3 g/d of an active dried yeast (ADY).

³Temperature is thermoneutral [TN; 18 ± 0.55 °C; 20% relative humidity (RH)] or heat stressed (HS; 35 ± 0.55 °C; 42% RH).

Figure 2. Representation of the mean diurnal fluctuation of ruminal pH (A) and ruminal temperature (B) over 48 h consuming a finishing diet. Treatments were control diet (CON) or control diet + 3 g/d of an active dried yeast (ADY). Temperatures were thermoneutral [TN; 18 ± 0.55 °C; 20% relative humidity (RH)] or heat stressed (HS; 35 ± 0.55 °C; 42% RH). Interaction of TRT and red arrows indicate feeding events. The mean pH represents the average of 8 replications per treatment combination, respectively over 48 h at 10-min intervals.

mean pH, notably above the SARA threshold. Evaluations made on the effects of feeding ADY and its role in modulating ruminal pH suggest shifting fermentation pathways from lactate to propionate (Desnoyers et al., 2009; Humer et al., 2018); however, it would seem that under extreme HS conditions, this effect may be lost. There was no significant effect of TRT ($P = 0.3328$) or TEMP ($P = 0.3500$) on the daily ruminal pH variation. There were no effects of TRT on mean ruminal

temperature, temperature variation, DUR > 40 °C or AUC > 40 °C, however, as expected TEMP significantly affected these variables (Table 4; Fig. 2B). Mean ruminal temperature and temperature variation were much greater for steers in HS than in TN conditions (40.73 ± 0.638 vs. 39.33 ± 0.241 °C, respectively; $P < 0.0001$). The DUR and AUC > 40 °C was also much greater for steers in HS than in TN conditions (1,124 vs. 91 min/d and 41.5 vs. 0.6 °C/h, respectively; $P < 0.0001$). Time spent

above 40 °C for the TN observations was likely due to the heat of fermentation while the steers undergoing HS were simply unable to properly dissipate heat resulting in severe heat loading.

There was a tendency for an interaction of TRT and TEMP on DUR > pH 5.6 ($P = 0.0726$; Fig. 3A). Steers supplemented with ADY spent greater DUR > pH 5.6 than CON-fed steers under TN conditions (863 vs. 619 min/d, respectively), but under HS conditions, this was reversed (509 vs. 698 min/d, respectively). The DUR of time spent in SARA range (Table 4) was not affected by TRT (450 vs. 376 min/d for CON and ADY, respectively) or TEMP (432 vs. 394 min/d for TN and HS, respectively). However, there was a tendency for a significant interaction of TRT and TEMP on DUR of time spent in ARA range ($P = 0.1353$; Fig. 3B) in which steers experiencing HS and supplemented ADY spent more time in ARA range than CON (485 vs. 307 min/d, respectively). For TN conditions and CON diet, however, steers spent greater DUR in ARA range than ADY-supplemented steers (246 vs. 143 min/d, respectively; Fig. 3B). The main effect of TEMP on DUR of ARA was significant ($P = 0.0079$) where steers experiencing HS spent twice as much time in ARA than steers in TN conditions (396 vs. 195 min/d, respectively).

There was a tendency for interaction of TRT $\times$ TEMP for AUC > pH 5.6 ($P = 0.0954$). The AUC > pH 5.6 indicated that under TN conditions, ADY-fed steers had greater AUC above pH 5.6 compared with CON under TN conditions (17.9 vs. 8.39 h/d, respectively; Fig. 2A), but there was no difference between TRT under HS conditions (8.31 vs. 8.34 h/d, respectively). The main effects (Table 4)

also revealed tendencies for TRT and TEMP effects in which ADY-fed steers had greater AUC > pH 5.6 than CON-fed steers (13.1 vs. 8.36 h/d, respectively; $P = 0.0976$) and steers under TN conditions had greater AUC > pH 5.6 than those under HS conditions (13.2 vs. 8.32 h/d, respectively; $P = 0.0923$). There was a significant effect of TEMP on the AUC in the SARA range in which TN steers had greater AUC than HS steers (20.8 vs. 15.2 h/d, respectively; $P = 0.0028$). In addition, there was a significant effect of TEMP on AUC in ARA range, where the AUC of steers under HS was nearly 7 times greater than steers under TN conditions (4.67 vs. 0.70 h/d, respectively; $P < 0.0079$).

The effect of rumen temperature greatly affects ruminal pH and the duration and magnitude of subclinical and acute ruminal acidosis. We believe it is likely due to reduced passage rate and increased ruminal digestibility, but the risk of acidosis could be compounded by sudden shifts in the rumen microbiome. There has been evidence of microbial population shifts under heat stress conditions in dairy heifers, notably the genus *Streptococcus* (Uyeno et al., 2010), which is accepted as the major culprit of the onset of ruminal lactic acidosis. Research on the growth and metabolism of *S. bovis* isolated from the rumen has indicated that peak growth occurs when media is maintained at a pH of 5.0 to 6.2 and at a temperature of 39 °C (Russell and Hino, 1985; Yuwono and Kokugan, 2008). However, peak enzyme production seems to occur between 40 and 44 °C (Bailey, 1959). Therefore, it could be that the interaction of decreasing pH and increasing temperature of the rumen creates exceptionally favorable conditions for *S. bovis* and other

Figure 3. Interaction of treatment and temperature on ruminal pH variables; (A) duration of time spent above pH 5.6 (min/d) and (B) duration of time spent in acute ruminal acidosis range, pH < 5.0 (min/d). Treatments were control diet (CON) or control diet + 3 g/d of an active dried yeast (ADY). Temperatures were thermoneutral (TN; 18 $\pm$ 0.55 °C; 20% RH) or heat stressed (HS; 35 $\pm$ 0.55 °C; 42% RH) indicated by the broken lines. Red arrows indicate feeding events.

lactic acid producers to proliferate and outcompete pH-sensitive bacteria.

In Vitro Gas Production Technique

The IVGP technique provided valuable information into the fermentative capacity of the adapted rumen fluid inoculant (Table 5; Fig. 4).

Treatment effects. Inoculum collected from CON- and ADY-treated steers had similar pH, likely due to being taken in the fasted state (6.23 vs. 6.28, respectively; $P = 0.6991$). However, after 48 h of in vitro fermentation, there was a tendency for those feeds inoculated with ADY-adapted rumen fluid to have lower final pH than CON-adapted rumen fluid (6.12 vs. 6.23, respectively; $P = 0.0986$), possibly indicating greater accumulation of fermentation end-products. There were no differences observed for total gas production ($P = 0.4420$), the rate of fermentation ($P = 0.2320$), or lag time ($P = 0.9806$) between TRT. Numerically, ADY inoculum resulted in greater NDFd over CON, but due to variation between fermentation batches, this was not significant (46.4% vs. 36.4%, respectively; $P = 0.5448$), still, the means agree with the DMD observed in the steer's metabolism where ADY resulted in greater digestion than CON feed TRT. Although feed TRT did not result in significant

differences in CH_4 production, the trends were similar to the TRT means of CH_4 obtained from the calorimetry chambers for ADY and CON (8.7 vs. 18.8 g/kg DM, respectively; $P = 0.2684$), whereas the IVGP technique resulted in larger values than in vivo due to the complete fermentation of the diet. Although the numerical differences are suggestive, the variation between fermentations was larger than expected. The IVGP technique is known to be subject to variation especially when different rumen fluid donors are utilized, but this may be overcome with greater replication (Tedeschi and Fox, 2018). Replication in this study, however, was limited due to timing and logistics of obtaining rumen fluid from noncannulated animals.

Temperature effects. Similar to the mean pH obtained from rumen boluses, the inoculum was also significantly affected by TEMP where TN steers had greater pH than HS steers (6.70 vs. 6.16, respectively; $P = 0.0339$). The final pH after 48 h fermentation was not affected by TEMP ($P = 0.3910$). There was no effect TEMP on the fermentation pattern of the in vitro feed samples; total gas production, the rate of fermentation, and lag time of fermentation were similar ($P \geq 0.2580$). There were no significant effects of TEMP on NDFd ($P = 0.62$) although the means were greater for TN than ADY (45.5% vs. 37.3%, respectively).

Table 5. In vitro fermentation of a finishing diet using donor rumen fluid inoculum from treatment and temperature adapted steers

Items ¹	TRT ²		TEMP ³		SEM	P value		
	CON	ADY	TN	HS		TRT	TEMP	TRT × TEMP
Inoculum pH	6.23	6.28	6.59 ^a	5.91 ^b	0.087	0.6991	0.0116	0.6473
Final pH	6.23	6.12	6.20	6.15	0.063	0.0986	0.3910	0.6681
Total gas, mL	25.1	27.1	24.5	27.6	4.57	0.4420	0.2580	0.6946
Rate of fermentation, h ⁻¹	11.9	16.7	15.8	12.8	2.31	0.2320	0.4189	0.5541
Lag time, h	0.524	0.520	0.586	0.458	0.195	0.9806	0.4607	0.2629
NDFd, %	36.4	46.4	45.5	37.3	12.0	0.5448	0.6165	0.3988
TDN, %								
kp 2%/h	80.8	80.9	80.4	81.3	0.436	0.7869	0.1743	0.7244
kp 4%/h	79.2	79.3	79.2	79.3	0.323	0.8726	0.7655	0.1692
kp 6%/h	78.2	78.2	78.1	78.3	0.466	0.8752	0.8189	0.1651
ME, Mcal/kg								
kp 2%/h	2.92	2.92	2.91	2.94	0.019	0.7944	0.1861	0.7944
kp 4%/h	2.86	2.87	2.86	2.87	0.017	0.9093	0.7177	0.1411
kp 6%/h	2.83	2.83	2.83	2.83	0.017	0.9052	0.9052	0.1474
CH_4 , g/L gas	0.143	0.069	0.110	0.102	0.033	0.2106	0.8712	0.8866
CH_4 , g/kg DM	18.8	8.70	13.7	13.8	5.28	0.2684	0.9855	0.9445

^{a,b}Least squares means within a row with different superscripts differ at $P < 0.05$.

¹Items are variables that represent the rumen fluid inoculum or its fermentation effects of a finishing diet. Inoculum is initial pH from the donor steer. Final pH is after 48 h of fermentation in vitro. Total gas production from fermentation of 0.2 g of the finishing diet. NDFd = NDF digestibility. ME is shown with passage rates (kp) of 2, 4, and 6%/h.

²Treatment is control fed (CON) or control + 3 g/d of an active dried yeast (ADY).

³Temperature is thermoneutral [TN; $18 \pm 0.55^\circ\text{C}$; 20% relative humidity (RH)] or heat stressed (HS; $35 \pm 0.55^\circ\text{C}$; 42% RH).

Figure 4. Interaction of treatment (TRT) and temperature (TEMP) on the predicted ME and comparison of predicted ME by the in vitro gas production technique (IVGP) and the direct measure ME of steers consuming a finishing diet. Treatments were control diet (CON) or control diet + 3 g/d of an active dried yeast (ADY). Temperatures were thermoneutral (TN; 18 ± 0.55 °C; 20% RH) or heat stressed (HS; 35 ± 0.55 °C; 42% RH). Contrasts of TN and HS (red) are included to denote possible effect of TEMP on passage rate and accuracy of IVGP prediction.

Interactions. There was a tendency for a TRT $\times$ TEMP interaction regarding the ME estimations at a passage rate of 4 and 6%/h ($P \leq 0.1474$; Table 5) where inoculum taken from steers adapted to the CON diet tended to result in greater in vitro ME under HS than TN, but inoculum from ADY-adapted steers was greater under TN than HS. This trend was in contrast to the in vivo derived values for ME and worthy of further investigation. Interestingly, this interaction was not observed when using passage rate of 2%/h. When we compared the observed ME of the steers with that predicted from the IVGP technique using 2, 4, 6, and 8%/h fractional passage rates, the IVGP predicted ME at 2 and 4%/h was closer to the observed mean values for the HS animals (2.93 Mcal/kg), whereas the predicted ME at 6%/h was closer to TN animals (2.85 Mcal/kg), confirming our hypothesis that ruminal escape may have been significantly reduced with increased heat load (Fig. 4). Previous research using a similar HS environment reported ruminal passage rate to be closer to 4 ± 0.02 %/h in dairy cattle as well (Bernabucci et al., 1999). Moreover, although it was not an objective of the research we may conclude that if the expected ruminal passage rate is carefully considered, the IVGP technique may be able to accurately estimate the biological value of a diet for animals under HS, even when using rumen inoculum from non-HS steers.

CONCLUSION

Under TN conditions, supplemental ADY in the diets of finishing steers under feedlot conditions may improve DMD, DE, ME, and possibly RE without affecting DMI. In addition, supplemental ADY may

significantly increase mean ruminal pH, above the SARA threshold under thermoneutral conditions. However, heat stress remains an environmental risk to finishing cattle by significantly affecting feed energy efficiency, methane production, and acute ruminal acidosis. There have been reports that live yeast supplemented to dairy cows during the hot season improved DMI, ruminal pH, and productivity (Moallem et al., 2009; Salvati et al., 2015). However, the dietary characteristics of lactation diets are much different from typical finishing diets of beef cattle regarding energy density and minimum effective fiber. Under HS conditions, we could not detect a significant benefit of ADY for feed efficiency or ruminal pH, which we propose may be due to the antagonism between slower ruminal passage rate and increased digestibility. It may be that supplemental ADY optimizes fermentation characteristics within a certain scope of energy density, effective fiber, and ruminal temperature. Future work should focus on the long-term feeding of ADY at different energy densities and combine rumen parameters with performance traits of beef cattle.

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