Reducing rumen starch fermentation of wheat with three percent sodium hydroxide has the potential to ameliorate the effect of heat stress in grain-fed wethers^{1,2}

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ABSTRACT: Selection for high productivity has resulted in ruminants adversely affected by heat stress (HS) due to their high metabolic rate and feed intake. One mechanism to ameliorate HS is to reduce the forage-to-concentrate ratio in the diet, although the inclusion of readily fermentable grains can reduce heat tolerance. The aim of these experiments was to investigate a chemical method for reducing the rate of fermentation of wheat and its effect on improving heat tolerance in sheep. In the first experiment, fermentation kinetics and buffered rumen fluid pH variation during in vitro incubation of corn, wheat, and 3% NaOH-treated wheat grains were compared. This experiment showed that corn and 3% NaOH-treated wheat had a slower (−23 and −22%, respectively; $P < 0.001$) rate of gas production and elevated buffered rumen fluid pH ($P < 0.001$) compared with wheat. In the second experiment, 31 Merino × Poll Dorset wethers were housed in 2 climate-controlled rooms and were fed either corn grain plus forage (42.7% starch; were fed either corn grain plus forage (CD), wheat grain plus forage (WD) or 3 % NaOH-treated wheat plus forage (TWD) during 3 experimental periods: period 1 (P1), which consisted of 7 d of thermoneutral conditions (18 to 21°C and 40 to 50% relative humidity) and 1.7 times maintenance feed intake; period 2 (P2), which consisted of 7 d of HS (28 to 38°C and

30 to 50% relative humidity) and the same feed intake as in P1; and period 3 (P3), which consisted of 7 d of HS as in P2 and 2 times maintenance feed intake in a randomized control experiment. Water was offered ad libitum. The impact of HS was quantified by increases in rectal temperature, respiration rate (RR), and flank skin temperature (FT); variations in blood acid–base balance; and glucose, NEFA, and heat shock protein 70 (HSP-70) plasma concentrations. All physiological variables were elevated during HS, especially when wethers had greater feed intake (P3). Wethers fed CD had lower RR, rectal temperature, and FT than wethers fed WD ($P < 0.001$) and wethers fed TWD had lower RR and FT than wethers fed WD during HS ($P < 0.05$). There were reductions in blood CO₂, HCO₃[−], and base excess concurrent with increases in blood partial pressure of O₂ and pH during HS ($P < 0.05$). Heat stress reduced plasma NEFA and glucose concentrations whereas it increased prolactin ($P < 0.05$). Prolactin and HSP-70 plasma concentrations were greater for WD-fed wethers ($P < 0.001$) associated with Prolactin and HSP-70 plasma concentrations were greater for WD fed wethers ($P < 0.001$) during HS. These data indicate that the slow rate of rumen fermentation of CD and TWD can reduce the heat released during feed fermentation in the rumen, improving heat tolerance in sheep.

Key words: grain, heat from fermentation, heat stress, sheep, wethers

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INTRODUCTION

Heat released from digestive processes or the heat increment of feeding (**HI**) constitutes approximately 30% of the total metabolic heat production (**HP**; Webster et al., 1976) and represents an extra burden for ruminants kept at high ambient temperatures. Therefore, reducing the HI and metabolic HP can be a useful strategy to ameliorate the effects of heat stress (**HS**) in ruminants. Increasing levels of concentrates in the diet has been traditionally recommended to reduce the HI during HS (Reynolds, 2006), although the consumption of readily fermentable carbohydrates increases the risk of rumen acidosis (Nocek, 1997) in animals in which the blood acid–base balance is already compromised (West, 2003). Shifting starch digestion to the small intestine and reducing the amount of starch fermented in the rumen by either protecting starch from rumen fermentation or feeding slowly fermentable grains can improve ME utilization efficiency due to reduced energy losses as heat (Owens et al., 1986; Merchen et al., 1997; Reynolds, 2006). Our previous experiment (Gonzalez-Rivas et al., 2016) demonstrated that feeding corn, a slowly fermentable grain, is associated with improved heat tolerance in sheep due to reductions in the HI and metabolic HP during HS.

Alkaline treatment of wheat grain with 3% NaOH produces a partial hydrolysis of the grain hemicellulose and a gelatinization of the starch, slowing the attack of rumen bacteria and digestive enzymes (Berger et al., 1981). This reduces the rate of rumen starch fermentation, maintaining a favorable rumen fluid (**RF**) pH (Tománková and Homolka, 2004; Schmidt et al., 2006). However, the efficacy of modifying wheat in this manner as a strategy for managing HS in ruminants has not been investigated. The experiment described herein was undertaken to investigate the effect of feeding 3% NaOH–treated wheat to wethers under HS conditions. It was hypothesized that reducing the rumen starch fermentation of wheat with 3% NaOH would improve tolerance to HS in grain-fed wethers.

MATERIALS AND METHODS

Animal ethics approval was provided before experimentation by the Department of Economic Development, Jobs, Transport and Resources (**DEDJTR**) and The University of Melbourne Veterinary and Agricultural Sciences Animal Ethics committees Ethics number 1513585.1.

In Vitro Gas Production

The gas production (**GP**) kinetic parameters of corn, wheat, and 3% NaOH–treated wheat grains avail-

able to be used *in vivo* were quantified using an *in vitro* cumulative GP system using the technique previously described by Gonzalez-Rivas et al. (2016). Eighty-four replicates of 1 g (dry weight) of 1-mm ground corn (72.4% starch), Australian standard white (**ASW**) variety wheat (65.8% starch), and 3% NaOH–treated ASW wheat (65.6% starch) grains were weighed into 250-mL serum flasks in 4 *in vitro* fermentation runs using 7 replicates per treatment (incubation time [5, 8, and 24 h] × grain) per run. Previously, whole ASW wheat was treated with 3% NaOH according to the technique described by De Campeneere et al. (2006) adapted for laboratory conditions and *in vitro* experiments. Briefly, NaOH pellets (reagent grade, ≥98% pellets [anhydrous]; Sigma-Aldrich Corp., St. Louis, MO) were added to whole wheat at a 3% inclusion rate (3 g/100 g of grain). Wheat grain and soda pellet were mixed for 45 min with the aid of a spatula, during this period, 100 mL of water was slowly added using a 10 mL syringe. The mixture was then rested for 60 min and then was mixed for another 30 min while another 100 mL of water was slowly added. The mixture was rested for 4 h and then mixed for 15 min and left overnight. The next morning, the NaOH-treated wheat was spread on aluminum foil in a thin layer and left rest for 3 d before being ground to pass a 1-mm sieve and analyzed *in vitro*.

Rumen fluid used in the *in vitro* experiments was collected from 2 rumen-cannulated mid- to late-lactation Holstein–Friesian dairy cows from the DEDJTR, Ellinbank, Australia. The RF donor cows were fed 4 kg crushed barley grain, 5 kg oaten hay, 3 kg corn silage, 250 g mineral mix, and *ad libitum* pasture allowance, mostly perennial ryegrass, daily. Diets totaled 16 to 20 kg DM and contained approximately 18% CP and 2.51 Mcal ME/kg DM per cow per day. The collection of RF was performed using a polyvinyl chloride pipe through the rumen cannula before the morning meal. Rumen fluid from 2 donor cows was combined, and a small amount of solid rumen content was also collected from each cow to serve as the substrate during transport. During transport, the temperature of RF was kept stable at 39°C by storing the RF in glass flasks capped with a 1-way gas valve inside a portable incubator (Premium Blanket Warmer; Thermoline Scientific, Wetherill Park, NSW, Australia). Once in the laboratory, RF was squeezed and filtered through 4 layers of cheesecloth to remove feed particles. Rumen fluid pH was measured using a pH meter (Eutech pH 5+; Eutech Instruments Pte Ltd., Singapore) immediately after filtration to determine the initial pH. Then, RF was buffered with Kansas State Buffer pH 6.8 (Marten and Barnes, 1980; Ankom, 2016) at a 1:3 ratio at 39°C prior to commencing each *in vitro* fermentation run. Samples were inoculated by adding 100 mL of buffered RF into the flasks. In addition,

6 background flasks, containing only buffered RF, per incubation time per run were included. During this process, RF was under a constant flow of CO₂.

Internal gas pressure was measured using an automated GP system containing wireless gas recording modules (Ankom RF; Ankom Technology Corp., Macedon, NY). Gas recording modules were randomly allocated to each serum flask, and all flasks were incubated in a water bath (20-L Analogue Waterbath, WB20; Ratek Instruments Pty Ltd, Boronia, VIC, Australia) at 39°C for a maximum of 24 h. The GP system measured internal gas pressure through pressure transducers located in the modules' headspace every 5 min during 24 h, and the data were stored in a computer via radio frequency transmission. Fermentation was terminated at 5, 8, and 24 h, and buffered RF pH was measured at each time point using a pH meter.

Animals and Experimental Design

Thirty-one Merino × Poll Dorset crossbred wethers (11–12 mo old, 46.3 ± 2.8 kg BW, and 30 to 40 mm of fleece cover) were selected based on BW and age. Using a randomized control experiment with 2 consecutive experiment repetitions (runs), 3 diets were randomly allocated to the wethers: a control diet, composed of 50% crushed wheat grain with 25% oats and 25% lucerne chaff (**WD**; *n* = 10), and 2 intervention diets, one composed of 50% NaOH-treated whole wheat with 25% oats and 25% lucerne chaff (**TWD**; *n* = 11) and another composed of 50% crushed corn grain with 25% oats and 25% lucerne chaff (**CD**; *n* = 10; Table 1). Grains were crushed through a roller mill to a particle size of 2 to 5 mm, and whole ASW wheat was treated with 3% NaOH according to the technique described by De Campeneere et al. (2006). Briefly, NaOH (Sodium Hydroxide Minipearl; Chem-Supply Pty Ltd, Gillman, SA, Australia) was added to the whole wheat at a 30 g/kg inclusion rate in 40-kg lots in a 50-kg capacity cement mixer. Wheat grain and soda minipearl were mixed for 45 min, during this period, 4 L of water was slowly added using a water sprayer. The mixture was then rested for 60 min and then was mixed for another 30 min while another 4 L of water was slowly added with the sprayer. The mixture was then packed in five 20-kg bags for use in this experiment. Water was available ad libitum in 8-L graduated buckets, and access to feed was provided using individual troughs.

The daily ration of feed was split into 3 equal meals fed at 0900, 1300, and 1700 h. The amount of feed offered on a daily basis as DM was calculated before each period based on BW using the following equation: feed (kg DM/d) = $W^{0.75} \times 450/1,000/ME$, in which $W^{0.75}$ is the metabolic BW, 450 is the

Table 1. Composition of experimental diets fed to wethers on a DM basis

Item	Experimental diet		
	Wheat	Corn	3% NaOH-treated wheat
Ingredient, % DM basis			
Crushed corn grain	0	50	0
Crushed wheat grain	50	0	0
Whole 3% NaOH-treated wheat	0	0	50
Oaten chaff	25	25	25
Lucerne chaff	25	25	25
Chemical composition, DM basis			
Moisture, ¹ %	9.1	10.9	11.2
Estimated ME, ¹ Mcal/kg DM	2.8	2.9	2.8
CP, ¹ %	11.6	10.1	11.8
ADF, ¹ %	15.9	16.7	16.1
NDF, ¹ %	28.3	26.7	26.4
Total starch, ² %	39.4	42.7	39.3

¹Near-infrared reflectance spectroscopy.

²Wet chemistry analysis.

maintenance energy requirement for sheep (kJ/kg $W^{0.75}$), and ME is the ME content of the diet (Liu and McMeniman, 2006). Each wether was considered an experimental unit for physiological variables and blood analysis and for feed and water intake.

Prior to the experiment, wethers were adapted to concentrate diets by increasing the grain inclusion by 50 g/d over 15 d while they were individually housed in an indoor facility. After acclimation, wethers were randomly allocated into individual metabolism crates housed in 2 climate-controlled rooms for 21 d divided into 3 experimental periods as described by Gonzalez-Rivas et al. (2016). Briefly, period 1 (**P1**) consisted of 7 d of themoneutral (**TN**) conditions (18 to 21°C and 40 to 50% relative humidity [**RH**], with a 12-h light and 12-h dark cycle) and 85% of acclimation feed intake (1.7 times maintenance), period 2 (**P2**) consisted of 7 d of HS (28 to 38°C and 30 to 50% RH, with a 12-h light and 12-h dark cycle) and the same feed intake as in P1, and period 3 (**P3**) consisted of 7 d of HS and 2 times maintenance feed intake, as measured during the acclimation period. From the first day of P2 until the last day of P3, wethers experienced cyclical daily temperature. Over 24 h, the room temperature was set to rise daily to reach a maximum of 38°C and 30% RH between 0900 and 1700 h followed by a decline of room temperature to 28°C, whereas RH was set to 50% between 1700 and 0900 h, with a 12-h light and 12-h dark cycle, during P2 and P3.

Ambient temperature in degrees Celsius and RH were measured at 10-min intervals in the climate-controlled rooms using sensors for temperature and humidity (HVAC Sensors, Schneider Electric, Ballarat, Vic, Australia) installed in one of the walls at a height of 1 m.

Table 2. In vitro gas production kinetic parameters of untreated wheat, corn, and 3% NaOH-treated wheat grains¹

Grain type	Kinetic parameter			
	y-intercept	Rate constant, mL gas/h	Time at maximum rate, h	Max _{gas} ² mL/g DM
Untreated wheat	-1.11	0.190	7.85	136.4
Corn	-2.26	0.149	8.75	126.3
3% NaOH-treated wheat	-3.03	0.145	9.19	135.3
SED	0.509	0.007	0.385	4.34
P-value	<0.001	<0.001	0.002	0.063

¹Kinetic parameters were determined using the Gompertz model: $y = A + C \exp\{-\exp[-B(X - M)]\}$, in which B is the rate of gas production (mL/h), M is the time (h) at which the maximum rate of gas production is reached, C is the maximum gas produced (maximum gas mL/g DM), A is the y-intercept, and X is the time (h) of incubation. Values are predicted means of 28 replicates per grain measured during 24 h of incubation at 39°C in buffered rumen fluid.

²Max_{gas} = Maximum gas produced.

The sensors were connected to a computer equipped with software (Datacom Systems, Southbank, Vic, Australia) and maintained by Control Tech (Controltech Oceania Pty Ltd, North Melbourne, Vic, Australia) for online data recording and control of humidifiers (Defensor Mk5; Aireven Pty Ltd, Hornsby, NSW, Australia) and heaters (Ducted Single Phase Split System Air Conditioner; Temperzone Australia Pty Ltd, Sydney, Australia). The temperature-humidity index (THI) in degrees Celsius for sheep was calculated as described by Marai et al. (2001): $THI = T - [(0.31 - 0.31 RH/100) \times (T - 14.4)]$, in which T is the dry bulb temperature (°C) and RH is the relative humidity (%). During TN conditions (P1), the average THI was 20.2 ± 0.5 . During HS conditions (P2 and P3), the mean minimum THI was 25.9 (SD 0.23) between 1700 and 0900 h whereas the mean maximum THI was 33.1 (SD 1.1) between 0900 and 1700 h.

Physiological Variables and Blood Analysis

Individual water consumption was measured at 0900 and 1700 h, and daily feed intake (as DM) was determined by weighing the orts before the morning feeding. To calculate daily feed requirements and ADG, BW was determined using walkover scales in the morning (0800 h) at the beginning of the experiment and at the end of each period. Measurements of respiration rate (RR), rectal temperature (RT), heart rate (HR), and flank skin temperature (FT) were obtained 4 times daily at 0900, 1300, 1700, and 2100 h as described by Gonzalez-Rivas et al. (2016). Briefly, RR was determined by counting flank movements for 15 s and then converting to breaths per minute. Heart rate was determined using a stethoscope (3M Littmann Master Classic; 3M Health Care, St. Paul, MN) by counting the number of heartbeats in 15 s and then converting to beats per minute. Rectal temperature was measured using a digital thermometer (DT-01; Tollot PTY Ltd, Blacktown, NSW, Australia). Flank skin temperature was measured with an infrared thermometer (Braun Thermoscan IRT Ear Thermometer 4020; Braun GmbH, Kronberg, Germany) placed on the

left and right flank (left flank skin temperature [LFT] and right flank skin temperature [RFT], respectively) after separating the wool to expose the skin.

Blood samples were collected by jugular venipuncture into a vacutainer containing lithium heparin as an anticoagulant (Becton, Dickinson and Company, Roborough, Plymouth, UK) between 0830 and 0900 h on d 2 and 7 of each thermal period for acid-base balance determinations using a self-calibrated portable blood analyzer (epoc BGEM Blood Analysis System; Alere, Inc., Waltham, MA) as described by Gonzalez-Rivas et al. (2016). Analytical variables determined were blood pH, partial pressure of CO₂ (pCO₂), partial pressure of O₂ (pO₂), Na⁺, K⁺, Ca²⁺, Cl⁻, glucose, and hematocrit (Hct); the calculated values of bicarbonate (cHCO₃⁻), total CO₂ (cTCO₂), base excess of extracellular fluid, base excess of blood, hemoglobin (cHgb), and oxygen saturation (cSO₂); anion gap K⁺ (AGapK); and anion gap K⁺ (AGapK). Blood samples were then centrifuged at 3,000 × *g* for 10 min at 4°C, and the separated plasma was stored at -20°C. Plasma samples were analyzed for NEFA (NEFA-C kit; Wako Chemicals USA, Inc., Richmond, VA; Johnson and Peters, 1993) and heat shock protein 70 (HSP-70) concentration in plasma (SEA873Mi; Cloud-Clone Corp., Houston, TX) with a 1.29 ng/mL limit of detection. Plasma samples were sent to the University of Western Australia Institute of Agriculture, Perth, WA, Australia, for prolactin concentration determination using a double-antibody homologous RIA (Miller et al., 1995) and insulin concentration determination using a double-antibody RIA (Tindal et al., 1978). Interassay CV were 5.3, 4.3, and 7.9% for the NEFA, insulin, and prolactin assays, respectively.

Statistical Analysis

In Vitro Gas Production. In vitro GP data (mL/g DM) were analyzed using the GenStat V16 statistical package (GenStat release 16; VSN International Ltd., Hemel Hempstead, UK). The GP curves were fitted to the Gompertz model to obtain all relevant kinetic

Figure 1. Gas production curves of incubation of ground corn, untreated wheat, and 3% NaOH-treated wheat grains (NaOH-wheat). Gas production curves are representative of 24 h of incubation at 39°C in buffered rumen fluid. Curves are the result of the application of the Gompertz model to the estimated means of kinetic parameter of 28 replicates per grain after curve fitting: $y = A + C \exp\{-\exp[-B(X - M)]\}$, in which B is the rate of gas production (mL/h), M is the time (h) at which the maximum (max) rate of gas production is reached, C is the maximum gas produced (maximum gas mL/g DM), A is the y -intercept, and X is the time (h) of incubation ($P < 0.001$).

parameters: $y = A + C \exp\{-\exp[-B(X - M)]\}$, in which A is the y -intercept, B is the rate of GP (mL/h), C is the maximum gas produced (maximum gas mL/g DM), M is the time (h) at which the maximum rate of GP (C) is reached (h), and X is the time (h) of incubation.

The REML variance component analysis procedure in GenStat was used to analyze the *in vitro* GP kinetic parameters and buffered RF pH variation during fermentation. Data were analyzed using grain type (corn, wheat, or 3% NaOH-treated wheat) and incubation time (5, 8, and 24 h) for buffered RF pH variation as fixed factors. Kinetic parameters were determined at 24 h of fermentation; therefore, data were analyzed using grain type as a fixed factor. The random factors were incubation module (1 to 7) and run (1 to 4). Differences in *in vitro* GP kinetic parameters among corn, untreated wheat, and 3% NaOH-treated wheat were analyzed using the Wald test, and significance was declared if $P \leq 0.05$.

Animal Experiment. Physiological variable data were analyzed using the REML variance component analysis procedure suitable for repeated measures. Fixed factors were diet (WD, CD, and TWD), period (P1, P2, and P3), and time (0900, 1300, 1700, and 2100 h). Two- and 3-way interactions among fixed factors were examined within the model. The random factors were wethers, experiment repetition (run 1 and run 2), and experimental day, with the exception of ADG, where experimental day was not included. The Wald test was used to test significance. True differences between LFT and RFT (**Diff LFT-RFT**) were estimated by conducting a t -test.

Blood variables were analyzed using the REML variance component analysis procedure suitable for repeated measures. Fixed factors were diet (CD, WD,

and TWD), period (P1, P2, and P3), and sample day (d 2 and 7 of each period). Two- and 3-way interactions among fixed factors were examined within the model. Random factors were wethers and experimental repetition (run 1 and run 2). The Wald test was used to test significance, and the Tukey pairwise comparison test was used to determine differences among diets. Data were tested for normality using the Shapiro-Wilk test. Because HSP-70 concentration in plasma did not show a normal distribution, the Box-Cox transformation was applied for hypothesis testing.

Data were tested for nonrandom patterns of residuals and dependence of observations, and there were none. Fixed factors and interactions were statistically different at $P \leq 0.05$. Results are reported as predicted means $\pm$ pooled SED.

RESULTS

In Vitro Experiment

In Vitro Gas Production. The rate of GP was slower for 3% NaOH-treated wheat and corn than for untreated wheat (−23 and −22%, respectively; $P < 0.001$). Corn and 3% NaOH-treated wheat reached the maximum rate of GP +10 and +15% later than untreated wheat, respectively ($P = 0.001$), and there was no difference in the maximum GP at 24 h among grains ($P > 0.05$; Table 2). Figure 1 illustrates the curve of GP of untreated wheat, 3% NaOH-treated wheat, and corn. Results are reported as predicted means $\pm$ pooled SED.

Buffered Rumen Fluid pH Variation. Buffered RF pH during incubation was greater for 3% NaOH-treated wheat and corn than for untreated wheat (6.23,

Figure 2. Buffered rumen fluid pH at 5, 8, and 24 h of in vitro incubation of ground corn, untreated wheat, and 3% NaOH-treated wheat grains (NaOH-wheat). Results are estimated means and pooled SED (0.057) for the grain $\times$ incubation time effect ($P < 0.001$).

6.19 and 6.12 [SED 0.033] for 3 % NaOH-treated wheat, corn and untreated wheat, respectively; $P = 0.003$). There was a reduction in pH throughout the incubation time whereby pH was greater at 5 h and lower at 24 h 6.49, 6.27 and 5.78 [SED 0.033] for 5, 8, and 24 h of incubation, respectively; $P < 0.001$). There was no interaction between grain and time. By 24 h of incubation, untreated wheat had the lowest pH and 3% NaOH-treated wheat and corn had the greatest pH values (5.68 vs 5.81 and 5.84 [SED 0.057] for untreated wheat, 3% NaOH-treated wheat, and corn, respectively; $P < 0.001$; Fig. 2).

Animal Experiment

Thermal Conditions and Physiological Variables.

Elevated THI during P2 and P3 increased water intake by approximately 60% compared with P1, from 4.4 to 7.7 and 6.9 (SED 0.40 L/d) for P1, P2, and P3, respectively ($P < 0.001$). Across experimental periods, wethers fed CD and TWD had increased water intake compared with those fed WD, whereas no differences were observed between CD-fed wethers and TWD-fed wethers (6.4, 6.6, and 6.0 [SED 0.19 L/d] for wethers fed CD, TWD, and WD, respectively; $P = 0.025$), and no interactions between experimental period and diet were present.

By design, there were no differences in DMI between P1 and P2, whereas during P3, wethers had greater DMI than during P2; wethers consumed 1,124, 1,160, and 1,316 (SED 17.4 g DM/d) during P1, P2, and P3, respectively ($P < 0.001$). Across experimental periods, wethers fed TWD had greater DMI, whereas there were no differences between those fed CD and those fed WD (1,178, 1,197, and 1,224 [SED 11.6 g DM/d] for CD, WD, and TWD, respectively; $P < 0.001$). There was a signifi-

cant interaction between diet and period ($P = 0.04$) such that wethers fed CD had lower DMI than those fed WD and TWD during P2 and wethers fed TWD had greater DMI during P3 than CD- and WD-fed wethers (Table 3). Although there was no significant effect of diet on ADG, all wethers in P3 had greater ADG than in P1 and P2 (110.6, 83.5, and 319.3 [SED 68.2 g BW/d] for P1, P2, and P3, respectively; $P < 0.001$). Greater ADG during P3 WAS associated with the increased DM and Mcal offered in that period. Diet influenced BW such that WD- and TWD-fed wethers had increased BW from P1 to P3 compared with those fed CD, whose weights did not increase during the experiment (45.1, 46.4, and 47.2 [SED 1.13 Kg BW] in P1 and 45.8, 48.6, and 48.4 [SED 1.13 Kg BW] in P3 for wethers fed CD, WD, and TWD, respectively; $P < 0.001$). However, it needs to be acknowledged that BW was measured after overnight fasting and without water restriction, which could have affected the accuracy of BW determinations.

Respiration rate increased in response to HS (P1 vs. P2), and RR was further elevated in P3 (66, 149,

Table 3. Feed intake of wethers fed 3 experimental diets on a DM basis¹

Feed intake, g DM/d	Experimental period ²		
	Period 1	Period 2	Period 3
Corn diet	1,115	1,122	1,298
Untreated wheat diet	1,133	1,167	1,292
3% NaOH-treated wheat diet	1,125	1,191	1,357

¹ P -values and SED for the effects of diet, period, and diet $\times$ period interaction were $P < 0.001$ (SED 11.6 g), $P < 0.001$ (SED 17.4 g), and $P = 0.04$ (SED 22.9 g, respectively).

²Period 1 consists of thermoneutral and restricted feeding ($1.7 \times$ maintenance), period 2 consists of heat stress and restricted feeding ($1.7 \times$ maintenance), and period 3 consists of heat stress and unrestricted feeding ($2 \times$ maintenance).

Figure 3. Relationship between respiration rate (a) and rectal temperature (b) and time of day and experimental period: period 1 (P1; thermoneutral [18 to 21°C and 40 to 50% relative humidity] and restricted feeding [$1.7 \times \text{maintenance}\{M\}$]), period 2 (P2; heat stress [28 to 38°C and 30 to 50% relative humidity and restricted feeding [$1.7 \times M$]], and period 3 (P3; heat stress and unrestricted feeding [$2 \times M$]) in wethers fed corn, wheat, or a 3% NaOH-treated wheat-based diet (NaOH-wheat). Results are means pooled across days and pooled SED for the diet $\times$ time $\times$ period interaction. The P -values for the effects of diet, period, time, diet $\times$ period, diet $\times$ time, period $\times$ time, and diet $\times$ period $\times$ time were <0.001 , <0.001 , <0.001 , <0.001 , 0.031 , <0.001 , and 0.48 , respectively, for respiration rate and <0.001 , <0.001 , <0.001 , 0.006 , 0.005 , <0.001 , and 0.59 , respectively, for rectal temperature. Max Heat = maximum room temperature between 0900 and 1700 h.

and 171 [SED 3.8 breaths/min] for P1, P2, and P3 respectively; $P < 0.001$). Respiration rate averaged across the 3 periods increased over the day, reaching the maximum during the afternoon before declining overnight (107, 138, 156, and 114 [SED 2.2 breaths/min] at 0900, 1300, 1700, and 2100 h, respectively; $P < 0.001$). Wethers fed CD had the lowest RR across all periods followed by those fed TWD and WD (120, 128, and 138 [SED 2.0 breaths/min] for wethers fed CD, TWD, and WD, respectively; $P < 0.001$). There was an interaction between diet and time ($P = 0.031$) such that wethers fed CD had lower RR at 1300, 1700, and 2100 h than those fed WD and TWD, whereas the RR of TWD-fed wethers was closer to that of those fed CD at 0900, 1300, and 2100 h (Fig. 3a). There were no differences in RR between wethers fed WD and wethers fed TWD at 1700 h. There was an interaction between diet and period ($P < 0.001$) such that there were significant differences in RR among the 3 diets during P2, with CD being lower than TWD and TWD, in turn, being lower than WD, whereas there were no dif-

ferences among diets during P1 and P3. There was an interaction between period and time ($P < 0.001$) such that RR increased to a greater extent during the hours of elevated temperature (between 0900 and 1700 h) during HS, especially during P3 (Fig. 3a).

Rectal temperature increased in response to HS (P1 vs. P2), and RT was further elevated in P3 (39.3, 39.6 ± 0.02 , and 39.7 [SED 0.02 °C] for P1, P2, and P3, respectively; $P < 0.001$). Rectal temperature averaged across the 3 periods increased over the day, reaching the maximum during the afternoon before declining overnight (39.3, 39.6, 39.7, and 39.5 [SED 0.01 °C] at 0900, 1300, 1700, and 2100 h, respectively; $P < 0.001$). Wethers fed CD had lower RT than those fed WD and TWD across all periods (39.5, 39.6, and 39.6 [SED 0.01 °C] for wethers fed CD, WD, and TWD, respectively; $P < 0.001$). There was an interaction between diet and time ($P = 0.005$) such that across periods, wethers fed CD had lower RT at 1300 and 1700 h than those fed WD and TWD, whereas TWD-fed wethers had greater RT than those fed CD and WD at 0900 and

Figure 4. Relationship between left flank skin temperature and experimental period: period 1 (P1; thermoneutral [18 to 21°C and 40 to 50% relative humidity] and restricted feeding [$1.7 \times$ maintenance {M}]), period 2 (P2; heat stress [28 to 38°C and 30 to 50% relative humidity and restricted feeding [$1.7 \times$ M]], and period 3 (P3; heat stress and unrestricted feeding [$2 \times$ M]) in wethers fed corn, wheat, or 3% NaOH-treated wheat-based diet (NaOH-wheat). Results are means pooled across days and pooled SED for the diet $\times$ period interaction. The *P*-values for the effects of diet, period, and diet $\times$ period were <0.001 , <0.001 , and <0.001 , respectively.

1300 h (Fig. 3b). There were no differences in RT between wethers fed WD and wethers fed TWD at 1700 and 2100 h. There was an interaction between diet and period ($P = 0.006$) such that across time of observation, there were differences among diets during P2 and P3, with the RT of wethers fed CD being lower than that of wethers fed WD and this, in turn, being lower than that of TWD-fed wethers (39.5, 39.6, and 39.7 [SED 0.03 °C] in P2 and 39.6, 39.7, and 39.8 [SED 0.03 °C] in P3 for CD, WD, and TWD, respectively). There were no differences in RT among diets under TN conditions. There was an interaction between period and time ($P < 0.001$) such that RT was greater during the hours of elevated temperature (between 0900 and 1700 h) during HS, especially during P3.

The effects of HS on FT were similar to the aforementioned physiological variables, in that LFT and RFT increased in response to HS (P1 vs. P2 and P3). There were no differences between LFT and RFT during P2 and P3 (38.5, 38.9, and 39.0 [SED 0.03°C] LFT and 38.4, 38.9, and 39.0 [SED 0.03°C] RFT in P1, P2, and P3, respectively; $P < 0.001$). Flank temperature averaged across the 3 periods increased over the day, reaching the maximum during the afternoon and then declining overnight (38.6, 39.0, 39.0, and 38.7 [SED 0.01 °C] LFT and 38.6, 38.9, 39.0, and 39.0 [SED 0.02 °C] RFT at 0900, 1300, 1700, and 2100 h, respectively; $P < 0.001$). Wethers fed CD and TWD had lower FT than WD-fed wethers and there were no differences in FT between CD-fed wethers and TWD-fed wethers during the experiment (38.8, 38.8, and 38.9 [SED 0.01 °C] LFT and 38.7, 38.7, and 38.8 [SED 0.01°C] RFT for wethers fed CD, TWD and WD respectively; $P < 0.001$). There

was an interaction between diet and period ($P < 0.001$; Fig. 4) such that there were differences in FT among the 3 diets during P3; CD- and TWD-fed wethers had lower FT than those fed WD but there were no differences among diets between P1 and P2. There was an interaction between period and time ($P < 0.001$) such that FT was greater during HS, especially during the hours of elevated temperature (between 0900 and 1700 h), compared with TN conditions. Across the entire experiment, LFT was greater than RFT (38.8 and 38.7 [SED 0.004 °C], respectively; $P < 0.001$). However, there were no differences among treatments or periods for the Diff LFT-RFT (0.052, 0.060, and 0.062 [SED 0.01 °C] for wethers fed CD, TWD, and WD, respectively; $P > 0.05$).

Heat stress did not significantly alter HR (P1 vs. P2), whereas HR was augmented by increased DM and Mcal offered during HS (P2 vs. P3; 86, 88, and 91 [SED 1.2 beats/min] for P1, P2, and P3, respectively; $P = 0.005$). Heart rate averaged across the 3 periods increased over the day, reaching the maximum during the afternoon before declining overnight. However, there were no differences between 1300 and 2100 h (86, 88, 91, and 89 [SED 0.6 beats/min] at 0900, 1300, 1700, and 2100 h, respectively; $P < 0.001$). Wethers fed CD and WD had lower HR than TWD-fed wethers and there were no differences between wethers fed CD and wethers fed WD during the experiment (87, 87, and 90 [SED 0.6 beats/min] for wethers fed CD, WD, and TWD, respectively; $P < 0.001$). There were no further interactions between diet and period, diet and time, or time and period (Fig. 5).

Blood Analysis. There were no differences or interactions between sample day (d 2 and 7) for any

Figure 5. Relationship between heart rate and time of day and experimental period: period 1 (P1; thermoneutral [18 to 21°C and 40 to 50% relative humidity] and restricted feeding [$1.7 \times$ maintenance {M}], period 2 (P2; heat stress [28 to 38°C and 30 to 50% relative humidity and restricted feeding [$1.7 \times$ M]], and period 3 (P3; heat stress and unrestricted feeding [$2 \times$ M]) in wethers fed corn, wheat, or 3% NaOH-treated wheat-based diet (NaOH-wheat). Results are means pooled across days and pooled SED for the diet $\times$ time $\times$ period interaction for heart rate. The *P*-values for the effects of diet, period, time, diet $\times$ period, diet $\times$ time, period $\times$ time, and diet $\times$ period $\times$ time were <0.001 , 0.005, <0.001 , 0.349, 0.214, 0.236, and 0.92, respectively. Max Heat = maximum room temperature between 0900 and 1700 h.

variable within a period nor there were interactions between diet and period; therefore, data are shown as pooled means $\pm$ SED of diet and period (Table 4). During HS (P2 and P3), blood pH increased compared with TN (P1) values (7.43, 7.45, and 7.44 [SED 0.006] for P1, P2, and P3, respectively; $P = 0.009$). Similarly, $c\text{SO}_2$ partial pressures were elevated during HS 10.2, 10.8, and 10.6 [SED 0.19] kPa for P1, P2 and P3 respectively; $P = 0.001$). Partial pressure of O_2 was greater during P2 than during P3 and P1 (5.9, 6.2, and 5.9 [SED 0.2] kPa for P1, P2 and P3 respectively; $P = 0.03$); $P = 0.03$). Conversely, $c\text{TCO}_2$, $p\text{CO}_2$, $c\text{HCO}_3^-$, and base excesses decreased during HS compared with TN conditions ($P < 0.05$). Wethers fed CD had greater values of $p\text{O}_2$ (5.5, 5.7, and 48.3 [SED 0.2] kPa for wethers fed TWD, WD and CD, respectively; $P = 0.007$) and $c\text{SO}_2$ (10.2, 10.4, and 11.0 [SED 0.27] kPa for wethers fed TWD, TW and CD, respectively; $P = 0.021$) than those fed WD and TWD.

Blood Cl^- was elevated in response to HS (105, 108, and 108 [SED 0.3 mmol/L] for P1, P2, and P3, respectively; $P < 0.001$). The Ca^{+2} values were greater during P3 than during P1 and P2 (1.33, 1.32, and 1.36 [SED 0.011 mmol/L] for P1, P2, and P3, respectively; $P = 0.002$). Wethers fed WD tended to have greater K^+ concentrations than those fed CD and TWD (4.61, 4.41, and 4.39 [SED 0.092 mmol/L] for WD, TWD, and CD, respectively; $P = 0.049$). Wethers fed TWD had lower Na^+ concentrations than those fed CD and WD (145, 146, and 146 [SED 0.3 mmol/L] for TWD, CD, and WD, respectively; $P < 0.001$). Hemoglobin, Hct, and AGapK were reduced in response to HS ($P < 0.05$), and there were no effect of diet on those variables.

Plasma glucose concentrations were lower during P3 than during P1 and P2 (3.82, 3.85, and 3.75 [SED 0.036 mmol/L] for P1, P2, and P3, respectively; $P = 0.022$). During HS, plasma NEFA concentrations were reduced compared with TN values (195, 151, and 130 [SED 10.5 $\mu\text{mol/L}$] for P1, P2, and P3, respectively; $P < 0.001$). Plasma NEFA concentrations were significantly lower for TWD-fed wethers across the experiment, whereas there was no difference between those fed CD and those fed WD (123, 164, and 189 [SED 13.2 $\mu\text{mol/L}$] for TWD, WD, and CD, respectively; $P < 0.001$).

There were no differences in insulin concentrations among periods ($P > 0.05$), and insulin concentrations were not affected by diet ($P > 0.05$). However, prolactin concentrations were lower during P1 than during HS (P2 and P3; 149, 224, and 267 [SED 23.3 ng/mL] for P1, P2, and P3, respectively; $P < 0.001$). Wethers fed CD had lower plasma prolactin concentrations than those fed WD and TWD (192, 202, and 245 [SED 24.5 ng/mL] for wethers fed CD, TWD, and WD, respectively; $P = 0.049$). Similarly, HSP-70 concentrations tended to be lower in P3 than in P1 and P2 (1.85, 1.90, and 1.76 [SED 0.058 ng/mL] for P1, P2, and P3, respectively; $P = 0.051$). Wethers fed WD had increased HSP-70 concentration across the experiment than those fed CD and TWD (2.01, 1.78, and 1.72 [SED 0.085 ng/mL] for wethers fed WD, TWD, and CD, respectively; $P = 0.001$).

DISCUSSION

Data obtained from the present study confirmed the differences in in vitro fermentation kinetic pa-

Table 4. Blood analytical variables in wethers fed corn, wheat, and 3% NaOH-treated wheat-based diet and subjected to 3 experimental periods.¹ Means are predicted means pooled within period²

Blood variables ⁴	Diet				Period				Significance ³		
	Wheat	Corn	3% NaOH-treated wheat	SED ⁵	Period 1	Period 2	Period 3	SED ⁶	Diet	Period	Diet × period
Blood pH	7.43	7.45	7.44	0.008	7.43 ^a	7.45 ^b	7.44 ^{ab}	0.006	0.331	0.009	0.735
cHCO ₃ ⁻ , mmol/L	26.6 ^a	26.3 ^a	25.5 ^b	0.32	27.2 ^a	25.6 ^b	25.6 ^b	0.27	0.001	<0.001	0.169
pO ₂ , mmHg	42.8 ^a	48.3 ^b	41.5 ^a	2.05	42.0 ^a	46.4 ^b	44.2 ^{ab}	1.59	0.007	0.03	0.773
pCO ₂ , mmHg	39.9	38.5	38.3	0.83	41.5 ^a	37.2 ^b	37.9 ^b	0.76	0.27	<0.001	0.716
cSO ₂ , %	77.9 ^a	82.6 ^b	76.7 ^a	2.04	76.2 ^a	81.3 ^b	79.6 ^{ab}	1.40	0.021	0.001	0.193
cTCO ₂ , mmol/L	27.8	27.5	26.6	0.51	28.5 ^a	26.7 ^b	26.7 ^b	0.29	0.071	<0.001	0.249
BE[b], mmol/L	2.15 ^a	2.14 ^a	1.24 ^b	0.442	2.60 ^a	1.54 ^b	1.39 ^b	0.251	0.002	<0.001	0.261
BE[ecf], mmol/L	2.32 ^a	2.23 ^a	1.27 ^b	0.496	2.88 ^a	1.54 ^b	1.41 ^b	0.279	0.001	<0.001	0.253
Creatinine, mg/dL	1.08 ^a	1.07 ^a	0.98 ^b	0.419	1.04	1.04	1.05	0.016	0.026	0.54	0.483
Glucose, mmol/L	3.85	3.82	3.76	0.117	3.82 ^a	3.85 ^a	3.75 ^b	0.036	0.71	0.022	0.539
NEFA, µmol/L	164 ^a	189 ^a	123 ^b	13.2	195 ^a	151 ^b	130 ^b	10.5	<0.001	<0.001	0.104
HSP-70, ng/mL ⁷	2.01 ^a	1.78 ^b	1.72 ^b	0.085	1.85 ^a	1.90 ^a	1.76 ^b	0.058	0.001	0.051	0.950
Prolactin, ng/mL	246 ^a	192 ^b	202 ^{ab}	24.5	149 ^a	224 ^b	267 ^b	23.3	0.049	<0.001	0.695
Insulin, ng/mL	20.24	20.63	22.74	1.916	19.56	22.28	21.77	1.815	0.363	0.278	0.887
Lactate, mmol/L	1.29	1.23	0.96	0.196	1.32	1.11	1.03	0.133	0.166	0.09	0.945
Hct, %	29.1	28.7	28.8	0.41	29.7 ^a	28.7 ^b	28.2 ^b	0.41	0.633	0.002	0.913
cHgb, g/dL	9.9	9.8	9.8	0.14	10.1 ^a	9.7 ^b	9.6 ^b	0.14	0.692	0.002	0.941
AGapK, mEq/L	16.6	16.9	16.8	0.32	17.4 ^a	16.4 ^b	16.4 ^b	0.29	0.555	0.001	0.148
Na ⁺ , mmol/L	146 ^a	146 ^a	145 ^b	0.3	145	145	146	0.3	<0.001	0.411	0.856
K ⁺ , mmol/L	4.61 ^a	4.39 ^b	4.41 ^b	0.092	4.50	4.43	4.47	0.053	0.049	0.42	0.884
Ca ⁺² , mmol/L	1.34	1.33	1.33	0.011	1.33 ^a	1.32 ^a	1.36 ^b	0.011	0.431	0.002	0.823
Cl ⁻ , mmol/L	107	107	107	0.6	105 ^a	108 ^b	108 ^b	0.3	0.893	<0.001	0.610

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Period 1 consists of thermoneutral and restricted feeding ($1.7 \times$ maintenance), period 2 consists of heat stress and restricted feeding ($1.7 \times$ maintenance), and period 3 consists of heat stress and unrestricted feeding ($2 \times$ maintenance).

²Blood samples were obtained at 0900 h on d 2 and 7 of each experimental period. There were no sample day effects ($P > 0.05$); therefore, values from d 2 and 7 were pooled within period.

³There was no interaction diet $\times$ period $\times$ samples day ($P > 0.05$) for blood variables; data not shown.

⁴cHCO₃⁻ = calculated value of bicarbonate; pO₂ = partial pressure of O₂; pCO₂ = partial pressure of CO₂; cSO₂ = calculated value of oxygen saturation; cTCO₂ = calculated value of total CO₂; BE[b] = calculated value of base excess of blood; BE[ecf] = calculated value of base excess of extracellular fluid; Hct = hematocrit; cHgb = calculated value of hemoglobin; AGapK = anion gap, K⁺; HSP-70 = heat shock protein 70.

⁵Standard error of the difference for diet.

⁶Standard error of the difference for period.

⁷Values obtained after Box-Cox transformation.

parameter and physiological responses between wheat- and corn-based diets during HS previously observed (Gonzalez-Rivas et al., 2016) and showed that reducing rumen fermentation of wheat with 3% NaOH resulted in a reduction of physiological indices of HS in grain-fed wethers. In this experiment, elevated ambient temperature increased RR, RT, and FT, indicating augmented evaporative and convective cooling and efforts to dissipate excess body heat by redistribution of blood flow to the skin. Respiration rate, RT, and FT were lower in wethers fed CD and RR and FT were lower in wethers fed TWD than in those fed WD, indicating an amelioration of the HS responses in ruminants fed slowly fermentable grain diets.

In Vitro Experiment – Effects of Grain Type on In Vitro Gas Production and Rumen Fluid pH Variation

The results presented herein agreed with previous in situ and in vitro experiments, demonstrating that wheat ferments faster than corn (Herrera-Saldana et al., 1990; Opatpatanakit et al., 1994; Lanzas et al., 2007; Gonzalez-Rivas et al., 2016). The lower in vitro fermentability of corn grain despite its large starch content compared with untreated wheat and 3% NaOH-treated wheat is explained by differences in the physical structure of the kernel rather than by its total starch content (Rooney and Pflugfelder, 1986). Slowly fermentable grains, such as corn and sorghum, contain starch granules more resistant to bacterial amylase because they contain more amylose and are surrounded by a strong zein protein matrix (Rooney and Pflugfelder, 1986;

McAllister et al., 1990; Philippeau et al., 1999a). The similarities of the maximum gas produced in our experiment indicate that the main difference among grains was the rate of fermentation and the time at which the maximum gas and, possibly, heat was produced. This indicates that corn and 3% NaOH-treated wheat yielded the same overall amount of fermentation (gas and heat) over a slower period of time than untreated wheat.

The marked differences in in vitro fermentation parameters between untreated wheat and 3% NaOH-treated wheat agree with the findings of Tománková and Homolka (2004) and Hetta et al. (2013). The reduction of in vitro fermentability of wheat after NaOH treatment is due to an increased starch granule stability and resistance to degradation under alkaline conditions: pH 11 (Lai et al., 2002). This effect is mediated by a gelatinization of the outer starch granules and a reduction of the total surface exposed to fermentation, which reduces fluctuations in the rate of fermentation (Ørskov, 1979; Roberts and Cameron, 2002).

The difference in RF pH among grains agreed with the findings of Philippeau et al. (1999b), who reported lower RF pH during in situ incubation of wheat (pH 6.26) compared with corn (pH 6.50) and with the observed elevated buffered RF pH of grains treated with NaOH (Anderson et al., 1981). This elevated RF pH may improve and modulate fiber and starch digestion (Anderson et al., 1981), and in situ and in vivo studies demonstrated that feeding 3% NaOH-treated wheat strongly decreased protein and starch rumen degradability, which was associated with lower risk of rumen acidosis compared with untreated wheat (De Campeneere et al., 2006; Schmidt et al., 2006).

Animal Experiment

Effect of Grain Type on Feed and Water Intake.

An increase in water intake is strongly associated with thermoregulation (Bianca, 1964; Bewley et al., 2008) and is driven by increased water requirements in response to the enhanced evaporative heat loss through respiration (Conrad, 1985; Silanikove, 2000; Marai et al., 2007). The results obtained in the current experiment agree with our previous observations where wethers fed CD had larger volume of water intake than those fed WD (Gonzalez-Rivas et al., 2016) and with the reported increase in water intake in ruminants fed alkali-treated grain, an increase that is possibly associated with increased Na⁺ content (Ørskov and Greenhalgh, 1977; Ørskov, 1979; Hetta et al., 2013).

The elevated DMI of wethers fed TWD compared with those fed WD and CD during HS can be explained by the slower rumen fermentation of wheat starch in TWD compared with WD that either results in a lower

HI and metabolic HP or allows greater opportunity for this heat to be dissipated to the environment without reducing feed intake. In addition, it is possible that more starch in TWD was enzymatically digested in the small intestine, promoting a better gastrointestinal environment and improving DMI, compared with CD. Therefore, the absence of differences in RR and RT between WD-fed wethers and TWD-fed wethers in P3 may be because these effects were masked by increased DMI in the TWD group when wethers were offered close to ad libitum feed intake in P3.

These results are supported by in vivo studies that demonstrated that feeding 3% NaOH-treated wheat improved ADFI and increased milk, fat, and protein yield in dairy cows compared with feeding untreated wheat (De Campeneere et al., 2006) and the evidence reported in the literature that NaOH treatment increased the amount of starch entering the small intestine (Phipps et al., 2001). Owens et al. (1986) and Reynolds (2006) suggested that by shifting starch digestion from the reticulorumen to the small intestine, improvements in the efficiency of ME utilization can be obtained due to reduced losses of energy as heat and methane.

Effect of Grain Type on Physiological Variables.

In this experiment, wethers were exposed to a severe cyclic HS (Marai et al., 2001), with mean minimum and maximum THI of 25.9 and 33.1, respectively. Among mammals, wool sheep have a reduced sweating capability compared with cattle, and like dogs, sheep rely largely on respiratory evaporative cooling (Thwaites, 1985; Marai et al., 2007). The results obtained in this experiment agreed with our previous experiment where reductions in physiological variables (RR, RT, and FT) during HS were observed in wethers fed CD compared with those fed WD (Gonzalez-Rivas et al., 2016). Furthermore, it was demonstrated that by reducing the rumen starch fermentation of wheat with 3% NaOH, RR was reduced to a level similar to that of CD during P2 and FT was reduced to a level similar to that of CD during P3, when wethers had larger feed intake, indicating improved tolerance to HS. In this experiment, a nonsignificant Diff LFT-RFT could be due to the general increase in both core body and rumen temperature at elevated DM and energy intake because changes in LFT represent changes in rumen temperature (Montanholi et al., 2008; Gonzalez-Rivas et al., 2016). This factor supports the difference in FT with our previous experiment where wethers fed a WD diet had larger Diff LFT-RFT than those fed a CD-based diet at lower feed intake (Gonzalez-Rivas et al., 2016).

There was a daily variation of HR that was mainly affected by the time of measurement and, during HS (P3), by the DM and energy intake, in agreement with our previous experiment (Gonzalez-Rivas et al., 2016).

Those findings support the conclusions of Webster et al. (1975) and Mader et al. (1997) that HR was more indicative of ADFI than of heat load. In addition, TWD-fed wethers had greater HR, which is associated with the aforementioned larger feed intake of TWD compared with CD and WD, especially during HS, when the wethers were offered close to ad libitum feed intake in P3.

In general, the elevated physiological variables observed in P3 compared with P2 in all diets demonstrated that feed intake directly increases HP from fermentation and digestive processes (Webster et al., 1975) and, as a consequence, the metabolic HP and physiological variables during HS (Gonzalez-Rivas et al., 2016). Large feed intake reduces the ability to maintain normal body temperature and is detrimental to heat tolerance (Mader et al., 1997) because it was demonstrated that restricting the amount of feed offered to beef cattle can improve physiological responses to HS (Mader et al., 2002; Davis et al., 2003).

Effect of Grain Type on Blood Variables. Although blood acid-base balance variables were within the physiological range for the species in both TN and HS experimental periods (Radostits et al., 2000; Jackson and Cockcroft, 2002; Peiró et al., 2010), variations in blood pH were observed due to both diet and HS, as a consequence of increases in RR for thermoregulation purposes. Increases in blood pH and pO_2 and reductions in pCO_2 represent a stage of respiratory alkalosis, which is rapidly compensated for through urinary excretion of HCO_3^- to adjust the blood pH toward normality (Schneider et al., 1988; Sanchez et al., 1994; Odongo et al., 2006), whereas the lower concentration of HCO_3^- and base excess in blood represents the initial stage of the compensated metabolic acidosis, after which a reduction in blood pH is expected (Sanchez et al., 1994; Kadzere et al., 2002; Srikanthakumar et al., 2003). The observed variation in blood pH, pCO_2 , and bases excess during HS were in agreement with variations reported in sheep by Srikanthakumar et al. (2003), Odongo et al. (2006), and Chauhan et al. (2015).

Increases in plasma Cl^- were previously observed in response to HS in sheep (Srikanthakumar et al., 2003; Odongo et al., 2006), goats (Hamzaoui et al., 2013), and dairy cows (Calamari et al., 2007). The collecting ducts of nephrons increase HCO_3^- excretion in response to alkalosis through active tubular H^+ resorption and exchange of Cl^- for intracellular HCO_3^- (Klein, 2013). Therefore, increased Cl^- in plasma is associated with a mild compensated metabolic acidosis and urinary HCO_3^- excretion to normalize blood pH during HS (Odongo et al., 2006; Calamari et al., 2007).

Reductions in plasma Ca^{+2} concentration during HS were reported in lactating dairy cows (West et al., 1991) and laying hens (Mahmoud et al., 1996) and was

associated with respiratory alkalosis and an exhaustion of CO_2 during panting (Sanchez et al., 1994). However, in the current experiment, there was an increase in free Ca^{+2} concentration during P3. A reduction in AGapK ($AGapK = Na^+ + K^+ - Cl^- - HCO_3^-$) associated with a reduction in Hct and plasma protein was observed in heat-stressed sheep fed high-grain diets (Odongo et al., 2006), in agreement with our findings. In our current experiment, the reduction of AGapK can be a consequence of the observed increased plasma Cl^- concentration and reduced Hct. A reduction in cHgb and Hct during HS compared with TN conditions was also observed in our previous experiment (Gonzalez-Rivas et al., 2016) and in heat-stressed Polwarth sheep (Gomes da Silva et al., 1992), which can be explained by blood dilution as consequence of increased water consumption during HS and can be associated with a reduction in O_2 -carrying capacity explained by reductions in pO_2 and SO_2 during HS.

There was no improvement of acid-base balance due to diet during HS. Elevated saturation of blood with O_2 (greater pO_2) was observed in wethers fed CD. Similarly, Omani sheep had greater pO_2 in the blood than Merino sheep, and this was also associated with reduced RR and possibly a deeper respiratory pattern resulting in greater efficiency to saturate the blood with O_2 during HS, suggesting that Omani sheep were less heat stressed than Merino sheep (Srikanthakumar et al., 2003).

Reduction in blood glucose concentration due to HS were reported in dairy cows by Settivari et al. (2007) and Wheelock et al. (2010) and in sheep by Achmadi et al. (1993) and Srikanthakumar et al. (2003) and in our previous experiment (Gonzalez-Rivas et al., 2016), supporting the hypothesis that ruminants exposed to high ambient temperatures rely on glucose as an energy source (Sano et al., 1983; Wheelock et al., 2010). The absence of increased plasma NEFA concentration during HS is a common response of heat-stressed dairy cows and sheep, contrasting the traditional glucose-sparing mechanism of animals under negative energy balance (Sano et al., 1983; Rhoads et al., 2009; Wheelock et al., 2010), partially because the reduction of lipid mobilization is an efficient mechanism to minimize the metabolic HP (Habeeb et al., 1992; O'Brien et al., 2010). The difference in NEFA values observed between P2 and P3 can be attributed to increased feed intake and HS. In cattle, plasma NEFA concentrations decrease as feed and energy intake increase (Holmes and Lambourne, 1970). Nevertheless, basal (TN) and HS plasma NEFA concentrations in all diets were within the reference interval ($<500 \mu\text{mol/L}$), suggesting that wethers were near or in positive energy balance (Russel et al., 1967; Bowden, 1971). The absence of diet or experimental period effect in plasma insulin concentration could be explained by the aforementioned positive energy balance.

Differences in rumen starch availability can explain differences in plasma NEFA concentration between among diets. It was previously observed that ewes fed corn-based diets differing in grain processing (whole vs. ground or extruded) had plasma NEFA concentrations negatively associated with rumen starch fermentation (Landau et al., 1992). Hence, the elevated basal levels (P1) of NEFA in CD wethers could indicate insufficient energy absorption and the failure of blood glucose and VFA to supply energy (Annison, 1960) concurrent with an increase of fat tissue mobilization to meet energy requirements (Dunshea et al., 1988). In contrast, the data presented here indicate an improved energy balance in wethers fed WD and TWD associated with the data presented here indicate improved energy balance in wethers fed WD and TWD. This improved energy balance was associated with elevated DMI observed in those animals during the experiment. Similar results were observed by Argov-Argaman et al. (2012), where nonpregnant heifers fed a forage-based diet had elevated NEFA concentration in plasma compared with those fed grain (corn and barley) supplemented diets. Differences between wethers fed TWD and wethers fed WD during P1 and P2 are supported by the finding of Demeterova and Vajda (2000), who described a lower NEFA concentration in dairy cows feed diets containing 3% NaOH-treated wheat compared with those fed a diet containing untreated wheat.

Prolactin concentration is greater in heat-stressed sheep and dairy cows compared with animals kept at TN conditions (Hooley et al., 1979; Bell et al., 1989; Ronchi et al., 2001; DiGiacomo et al., 2012), being prolactin concentration negatively associated with heat tolerance (Scharf et al., 2010). In previous experiments, a positive correlation between prolactin concentration and RT was observed in ruminant species such as sheep (Hill and Alliston, 1981) and goats (Sergent et al., 1985) and in heat-intolerant beef cattle (Scharf et al., 2010). This indicates that elevated prolactin could support thermoregulation at high environmental temperatures (Alamer, 2011). Further explanation can be provided by the findings of Kataria and Kataria (2011), who found a nearly 5-fold rise in plasma prolactin concentration associated with stress that originated from different pathologies such as *Haemonchus contortus* infestation, pneumonia, and enterotoxaemia in Marwari sheep. These stress responses can explain the increase in plasma prolactin during HS (P2 and P3) and the lower plasma prolactin concentrations in wethers fed CD. This lower plasma prolactin concentration in CD-fed wethers was associated with lower RT than in those fed wheat-based diets.

Reduced expression of HSP-70 has been associated with thermotolerance in beef and dairy cattle, goats, and buffalos (Hansen, 2004; Lacetera et al., 2006; Meza-

Herrera et al., 2006; Kumar et al., 2015). In contrast, elevated concentration of HSP-70 was observed in mononuclear cells of Pelibuey sheep compared in Suffolk sheep, and this was associated with improved thermoregulation (Romero et al., 2013). Therefore, the association between thermotolerance and HSP-70 is unclear. The increased plasma HSP-70 concentration in WD wethers can be explained by heat shock protein responses to external stressors such as management, disease, intestinal damage, hypoxia, oxidative stress (Shapiro et al., 1986; Iwaki et al., 1993; Kiang and Tsokos, 1998), or ruminal acidosis (Rabbani et al., 2009). Furthermore, the reduction in plasma HSP-70 concentration during P3 across diets can be associated with acclimation to elevated ambient temperatures, as was previously observed in beef cattle by Gaughan et al. (2013). From these findings, differences in basal (P1) HSP-70 plasma concentrations among diets may indicate a basal cellular damage or stress level other than HS including high-grain feeding, which explains the elevated physiological responses of wethers fed WD during HS. Consequently, in the absence of further interactions, lower plasma HSP-70 concentration in CD and TWD wethers during the experiment was likely due to their better thermoregulation mechanisms, which reduced the level of stress that triggers the release or expression of HSP-70.

Collectively, these data support the hypothesis that by reducing the rate of starch fermentation in the rumen, the heat from fermentation, the HI, and metabolic HP can be reduced and this will benefit ruminant animals during HS conditions. Starch-based diets with either corn or 3% NaOH-treated wheat fed to wethers resulted in physiological advantages compared with those fed untreated wheat-based diets. An additional explanation for differences in physiological variables under HS could include a shift in the proportion of postrumen starch digestion among grain sources.

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