Effect of Feeding Slowly Fermentable Grains on Productive Variables and Amelioration of Heat Stress in Lactating Dairy Cows in a Sub-Tropical Summer

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Abstract: Feeding low-fibre and high-energy diets to dairy cows is one approach to ameliorate heat stress (HS) by reducing heat increment (HI) during digestion. However, rapidly and slowly fermentable cereal grains differ in their HI. The aim of this experiment was to quantify if feeding slowly fermentable grains ameliorated the physiological responses to HS and improved milk production (MP) in dairy cows. Holstein-Friesian lactating dairy cows were housed in shaded pens and were fed either a total mixed ration (TMR) plus wheat (TMRW), a TMR plus wheat treated with 2% of a commercial starch-binding agent (TMRB) or a TMR plus corn (TMRC) (n= 8 cows per diet) in shaded pens during summer in Queensland, Australia. Respiration rate (RR) and panting score (PS) were measured four times a day, rumen temperature (RuT) was recorded every 20 minutes, and rectal temperature (RT) and milk samples were obtained every four days. Cows fed slowly fermentable grains had higher MP than cows fed TMRW, and cows fed TMRC had lower RT than those fed TMRW and TMRB ($P < 0.001$). Rumen temperature was positively correlated with temperature-humidity index and negatively correlated with MP ($P < 0.05$). In summary, feeding TMRC ameliorated HS as indicated by lower RT and improved MP in dairy cows. Milk production was improved with starch binding agents; however, this was not associated with efficient thermoregulatory responses. Furthermore, determination of RuT enabled the prediction of changes in physiological variables and productive responses due to HS in lactating dairy cows.

Keywords: heat stress; dairy cow; rumen temperature; core body temperature; grain; fermentation

Introduction

The main objectives of the nutritional management of heat-stressed dairy cows are to increase energy and nutrient density to counteract the reduction in dry matter intake (DMI) while maintaining milk production (MP), and to reduce the heat increment (HI) of the diet to improve thermoregulation. In general, this can be achieved with low-fibre and high-energy diets containing cereal grains (Baumgard et al. 2014). However, cereal grains differ in their HI due to differences in the rate and extent of rumen fermentation (Herrera-Saldana et al. 1990, Benninghoff et al. 2015). Previous studies using grain-fed sheep have shown that differences in rumen starch fermentability among grains have an impact on the severity of HS responses (Gonzalez-Rivas et al. 2016, Gonzalez-Rivas et al. 2017) and it was observed that treating wheat with 2 % of a commercial starch-binding agent (SBA; Bioprotect, RealisticAgri, Ironbridge, UK) reduced the rate of in vitro rumen fermentation of wheat (Dunshea et al. 2012).

It is hypothesized that feeding slowly fermentable grains could reduce the amount of heat released from fermentation and digestion and this will ameliorate the physiological responses to HS and improve MP in dairy cows during summer. The aim of this study was to investigate the effect of feeding a diet based on corn grain or wheat treated with 2 % SBA on the severity of HS responses including rectal temperature (RT), respiration rate (RR) panting score (PS) and rumen temperature (RuT), and on productive responses of lactating dairy cows under naturally occurring HS conditions during summer in Queensland, Australia.

Materials and Methods

Animals and Treatments

This study was undertaken at the University of Queensland, Gatton Campus; 27.4986 °S, 153.0155 °E, 89 m elevation during summer 2015 (February to March) in the sub-tropical region of Queensland, Australia. Twenty-Four Holstein-Friesian cows ranging from first to third lactation, 635 ± 78.5 kg body weight (BW), 3.5 body condition score (BCS), 248.5 ± 64.6 days in milk (DIM), and 24.1 ± 5.5 kg milk/day, were randomly divided into three dietary treatment groups of eight cows based on parity, milk production, BW, BCS and DIM using a randomized block design. Three dietary treatment groups were; a control diet; a total mixed ration (TMR) plus crushed wheat (TMRW) and two intervention diets; a TMR plus crushed wheat treated with 2 % of SBA (Bioprotect), (TMRB) and a TMR plus crushed corn (TMRC), (Table 1). Corn and wheat grains were crushed through a roller mill to a particle size of 2-5 mm.

Before the experiment, cows were provided with an introduction period of 7 to allow a gradual access to the experimental diets to avoid acidosis. For the duration of the study (29 days), diets were offered ad libitum once a day (0800 h) with a target DMI of 20.5 kg DM per cow per day (164 kg DM per group per day) and cows had ad libitum access to water. Cows were housed on a North-South oriented feed pad divided in three pens (40 × 12 m) with one pen per treatment group each equipped with a water trough and a feed pad. Shade was provided in the feed pad and in the middle of the pens by an iron roof. No supplementary cooling was provided to the cows in the feeding and resting area. However, sprinklers and fans were used for 5 minutes before the afternoon milking.

Ambient temperature and relative humidity were obtained from an on-site weather station. Temperature-humidity index (THI) was calculated using the equation THI= (0.8 x T) + [(RH/100) x (T-14.30)] +46.4 where T is the ambient temperature (˚C) and RH is the relative humidity (%) (Mader et al. 2006).

Physiological Variables and Milk Sampling

Respiration rate and PS were measured 3 times a day; at 0600 h before morning milking, at 1100 h, and at 1600 h before afternoon milking. Respiration rate was measured by observing flank movements. Panting score was measured using the eight-point score scale described in Gaughan et al. (2008). Individual PS was collated for each treatment group and mean panting score (MPS) for each observation time (h) was then calculated.

Rumen temperature was recorded for 15 days using rumen boluses (RFID transmitter; Smartstock, OK, USA). One bolus per cow was orally inserted and placed in the rumen and RuT was transmitted at 20-min intervals towards two solar-powered stations; one in the feed pad and another in the milking area. Radio transmission data was converted to temperature values using a computer software (TechTrol Inc., OK, USA). Rumen temperature data was expressed as hourly and daily average for each cow. Data from one cow was not included due to zero observations. Rectal temperature was measured once every four days in the morning after milking (between 0700 and 1000 h) using a digital thermometer (DT-01, Tollot PTY, Ltd, NSW, Australia; range 32 - 41.9 ± 0.1 °C).

Cows were milked twice a day (~0600 and ~1600 h) in a herringbone milking parlor separated ~600 m from the feeding and resting area. Milk samples (30 mL) were collected twice every four days; on the afternoon before RT measurement and on the morning of RT measurement in milk preservative-containing flasks. Samples were maintained at 4 °C until sent to the laboratory (Australian Herd Recording Services, Nundah, QLD, Australia) for lactose, protein and fat percentage analysis. Milk production data of day 24/02/2015 and milk quality data of day 9/3/2015 for all cows were removed from analyses because of unusual low values.

Average daily feed intake (ADFI) as DM for each group was recorded daily by weight difference between feed offered and refusals before the morning feeding. Individual BW was obtained weekly using walk over scales to calculate average daily gain (ADG).

Statistical Analysis

GenStat V16 (GenStat release 16 VSN International Ltd., Hemel Hempstead, UK) was used for all statistical analysis. A restricted maximum likelihood (REML) analysis was used to test the effect of diet and time of observation on RR and PS. The model included diet (TMRW, TMRB, and TMRC) and time of observation (0600, 1100, and 1600 h) as fixed terms. The random terms were cow ID, lactation (1st, 2nd, and 3rd), pregnancy status (non-pregnant and pregnant) and day. For RT and milk quality the fixed terms were diet and day of sampling (S1 to S8). The random terms were cow ID, lactation, and pregnancy status. Rumen temperature pooled during 24 h was analysed as above, the fixed terms were diet, time of observation (from 0000 to 2400 h), and date. The random terms were cow ID, lactation and pregnancy status. For individual BW change and ADG the fixed term was diet and the random terms were cow ID, lactation and pregnancy status. The Wald test and the Tukey pairwise comparison were used to test significance and to determine differences respectively. Fixed factors and interaction were statistically significant at $P \leq 0.05$. Results were reported as means and pooled SED and descriptive statistics were used for ADFI.

Correlations were analysed using Pearson; RR was correlated with daily average RuT. Individual RR was correlated with PS, RuT measured at each observation time (0600, 1100, and 1600 h), and with the THI at each observation time, THI of the day before the observation (1-d lag), THI of 2 days before the observation (2-d lag), and THI of 3 days before the observation (3-d lag). Rectal temperature was correlated with RuT, and THI pooled by hour during the time of RT measurement (between 0700 and 1000 h), 1-, 2-, and 3-d lag, RuT pooled by hour of the day was correlated with THI by hour of the day, and RuT pooled by day was correlated with Av THI, 1-, 2-, and 3-d lag.

Average daily feed intake was correlated with THI pooled by day (24 h THI average), 1-, 2-, and 3-d lag. Individual MP was correlated with Av RuT, daily THI, and 1-, 2-, and 3-d lag. Milk quality and milk solids were correlated with RuT, THI of the milk-sampling day, 1-, 2-, and 3-d lag. Variables were significantly correlated when $P \leq 0.05$ and only significant correlations were reported. Rumen temperature during the time of RT measurement were subtracted from RT and then, a t-test was used to determine significant differences between RuT and RT.

Results

Thermal Conditions

Temperature-humidity index (mean ± SD) during the experiment was 72.4 ± 2.0 with Min 65.4 ± 3.5 and Max 79.0 ± 1.8, 76 % of the experimental days had average THI ≥ 72, and THI < 64 was observed on 24 % of the experimental nights at the end of the experiment. THI (mean ± SD) at observation times was 66.3 ± 3.7 at 0600 h, 75.9 ± 2.7 at 1100 h, and 77.3 ± 2.5 at 1600 h. THI (mean ± SD) during the time of RT measurement (between 0700 and 1000 h) was 70.0 ± 3.0, 72.8 ± 2.3, 74.0 ± 2.4, and 74.9 ± 2.4 for 0700, 0800, 0900 and 1000 h, respectively.

Average Daily Feed Intake and Average Daily Gain

Average daily feed intake (Mean ± SD) per group was 170.6 ± 17.2 kg DM per day (~21.3 kg DM per cow per day) for TMRB, 168.0 ± 18.2 kg DM per day (~21.0 kg DM per cow per day) for TMRW, and 166.0 ± 18.9 kg DM per day (~20.8 kg DM per cow per day) for TMRC. Average daily feed intake was not correlated with THI ($P > 0.05$) and there was no effect of diet on ADG and BW change ($P > 0.05$; data not shown).

Milk Production and Quality

Cows fed TMRC and TMRB had greater MP than those fed TMRW ($P = 0.004$; Table 2). There was a significant effect of experimental day on MP ($P < 0.001$). There was a negative correlation between MP and THI 2-d lag in all diets (r = -0.2; $P < 0.001$) and there was a negative correlation between RuT and MP for cows fed TMRB (r = -0.43; $P < 0.001$) and TMRC (r = -0.41; $P < 0.001$).

Cows fed TMRC fed had lower milk fat percentage than cows fed TMRB and TMRW ($P = 0.049$). There was a significant effect of milk sampling day on milk protein and lactose percentage and milk solids ($P < 0.05$; Table 3). Cows fed TMRB exhibited a positive correlation between milk fat percentage and RuT (r = 0.48; $P = 0.0094$) and a negative correlation between lactose percentage and RuT (r = -0.67; $P < 0.001$). Cows fed TMRW diet exhibited a negative correlation between milk protein percentage and RuT (r = -0.5; $P = 0.005$).

Mean Panting Score and Respiration Rate

There was no significant effect of diet on MPS ($P > 0.05$). Across diets, there was a significant effect of time of observation on MPS (0.8, 1.0, and 1.2 ± 0.04 for 0600, 1100, and 1600 h respectively; $P < 0.001$; Figure 1a). There was no significant effect of diet on RR ($P > 0.05$). Across diets, there was a significant effect of time of observation on RR (57, 65, 74 ± 0.9 breaths/min for 0600, 1100 and 1600 h respectively; $P < 0.001$). Cows fed TMRC had higher RR at 1100 h than TMRB or TMRW fed cows ($P = 0.013$; Figure 1b). Panting score and RR were positively correlated in cows fed all diets (r= 0.56; $P < 0.001$). Panting scores and RR were also positively correlated with THI at the time of observation and with 1-, 2-, and 3-d lag in cows fed all diets ($P < 0.001$ for all).

Rectal Temperature

Cows fed TMRC had lower RT than those fed TMRW and TMRB (38.9 vs 39.1 and 39.1 ± 0.06 °C respectively; $P < 0.001$). Across treatments, there was a significant effect of day of observation on RT ($P <$

0.001). For cows fed TMRB there was a positive correlation between RT and THI (r = 0.26; P = 0.04), THI 1-d lag (r = 0.38; P = 0.002) and 2-d lag (r = 0.37; P = 0.003). For cows fed TMRC there was a positive correlation between RT and THI (r = 0.27; P = 0.03), THI 1-lag (r = 0.4; P = 0.001) and 2-d lag (r = 0.42; P < 0.001). For cows fed TMRW there was a positive correlation between RT and THI 1-d lag (r = 0.31; P = 0.011) and 2-d lag (r = 0.40; P < 0.001).

Rumen Temperature

There was no significant effect of diet on RuT pooled by hour of the day (P > 0.05). Figure 2 depicts the variation in RuT during the day (P < 0.001). Cows fed TMRC had higher RuT than those fed TMRB and TMRW between 0900 and 1400 h (P < 0.001). There was a negative correlation between RuT and THI by hour for cows fed TMRB (r = - 0.531; P = 0.0076). There was no significant effect of diet on RuT pooled by day (P > 0.05). Figure 3 depicts the variation in RuT during the experimental days (P < 0.001). Cows fed TMRC had higher RuT than those fed TMRB and TMRW during the initial days of the experiment. Then, Cows fed TMRW had higher RuT those fed TMRC and TMRB towards the end of the experiment (P < 0.05).

There was a positive correlation between RuT and THI of the day (r = 0.91; P < 0.001), 1-d lag (r = 0.70; P = 0.001), 2-d lag (r = 0.56; P = 0.016) and 3-d lag (r = 0.50; P = 0.033) in cows fed TMRB. There was a positive correlation between RuT and the THI of the day (r = 0.92; P < 0.001), 1-lag (r = 0.81; P < 0.001), 2-d lag (r = 0.75; P < 0.001) and 3-d lag (r = 0.74; P < 0.001) in cows fed TMRC. In cows fed TMRW, there was a positive correlation between RuT and the THI of the day (r = 0.88; P < 0.001), 1-d lag (r = 0.70; P = 0.001), 2-d lag (r = 0.75; P = 0.038). Rumen temperature was positively correlated with RR in cows fed all diets (r = 0.53; P < 0.001).

There was a positive correlation between RT and RuT in all diets; TMRB (r = 0.613, P = 0.001), TMRC (r = 0.428, P = 0.046), and TMRW (r = 0.552, P = 0.0026). The differences (± SED) between RuT and RT were 0.26 °C ± 0.081; P = 0.001. For TMRW and TMRB fed cows, were no differences between RT and RuT (P > 0.05) while for TMRC fed cows, there were significant differences between RT and RuT (38.7 vs 39.3 ± 0.14 °C respectively; t (6) = -8.18; P < 0.001).

Discussion

Cows in this experiment were exposed to summer conditions conducive to HS characterized by THI >72 that exceeded the limit of mild heat stress conditions (Silanikove 2000). However, the observed night-time recovery (THI < 64) might explain the amelioration in the HS responses towards the end of the experiment (Gaughan et al. 2008). It cannot be negated that this experiment was carried out late in summer, possibly, after the onset of HS and therefore the reduction of MP might already have occurred.

The primary mechanism of reduction in MP during HS is via enhanced extra-mammary glucose utilization (Wheelock et al. 2010). Therefore, provision of glucose precursors to dairy cows is crucial during HS (Baumgard and Rhoads 2012). This was demonstrated with the higher MP observed in cows fed TMRC and TMRB, because slowly fermentable grain feeding increases the amount of glucose being absorbed from the small intestine to produce lactose, the main regulator of milk volume (Liu et al. 2013, Moharrery et al. 2014). Variations in milk quality in this experiment appeared to be a consequence of HS and diet. It was observed that Holstein cows fed forage plus concentrate diets as TMR, had reduced milk production, fat, and milk protein during HS (Smith et al. 2013, Bertocchi et al. 2014). In addition, results obtained in our experiment agreed with Reynolds et al. (2001) where dairy cows fed slowly fermentable grains had reduced milk fat percentage and higher milk production because high-energy diets are associated with reduction in fat percentage due to a lower ratio acetate to propionate in the rumen (Bauman and Griinari, 2001).

Respiration rate and PS were elevated during periods of elevated THI exceeding the critical level of 80 breaths/min and MPS > 0.8 indicating critical hyperthermia (Gaughan et al. 2002, Gaughan et al. 2008, Davison et al. 2016). The positive correlation between RR, PS and RuT and between PS, RR and THI demonstrates the negative effect of high thermal load on dairy cow's physiological variables in agreement with (Sullivan et al. 2014a). Kabuga (1992) and Sullivan et al. (2014a) observed a positive correlation between RT, MPS and the average ambient temperature and THI on the day of observation and 1-d lag. Similarly, Sullivan et al. (2014b) observed a negative correlation between MP and the average THI on the day of observation and 1-, 2- and 3-d lag. These observations agreed with the data obtained in the current experiment, this delay is explained by complex physiological responses including a lengthening in nutrient digestion and utilization during HS (West 2003).

Differences on RT between cows fed TMRC and TMRW or TMRB are concurrent with the results shown previously in sheep fed slowly fermentable grain-based diets (Gonzalez-Rivas, et al. 2016, Gonzalez-Rivas, et al. 2017). These findings support the idea that slowly fermentable grain feeding can reduce HI and metabolic heat production (Reynolds 2006, Russell 2007). This may indicate an improved heat-tolerance in cows fed TMRC characterized by a low RT and high RR and PS in the morning. This phenomenon is viewed as a pre-compensatory mechanism in ruminants that increases the temperature gradient between the body and the environment favoring heat dissipation (Sullivan et al. 2014a). The temperature gradient between the rumen and the rest of the body is also necessary to drive heat exchange (Beatty et al. 2008). It was confirmed that RT was lower than RuT and both were highly correlated in all diets in this experiment as reported by

Beatty, et al. (2008), Bewley et al. (2008) and Lees et al. (2014). Significant differences between RuT and RT in TMRC indicates a large thermal gradient between the rumen and the body.

The observed variation in RuT during the day is consequence of the increase in core body temperature in response to feed intake, high ambient temperatures and HS. Mohammed, et al. (2014) observed a daily increase in RuT with a maximum 12.7 h post-feeding and a subsequent reduction until the next meal. Gaughan, et al. (2002) described that core body temperature may peak up to 5 hours after the hottest part of the day, normally between 2000 and 2200 h. These observations agree with our experiment where lower RuT was observed in the morning before feeding, then RuT had a peak around 2100 h, approximately 5 h after the peak THI (1600 h).

In summary, the results of this experiment demonstrated that feeding a corn-based TMR ameliorated HS responses and improved MP in dairy cows. Milk production was also improved with SBA treatment to wheat, and determination of RuT allowed the prediction of physiological and productive responses to HS in lactating dairy cows.

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REFERENCES

Bauman, D. E., and Griinari, J. M. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livestock Production Science, 70, 15-29.

Baumgard, L.H., Abuajamieh, M.K., Stoakes, S.K., Sanz-Fernandez, M.V., Johnson, J.S., and Rhoads, R.P., 2014. Feeding and Managing Cows to Minimize Heat Stress, Proceedings of the 23rd Tri-State Dairy Nutrition Conference, Fort Wayne, Indiana, USA, 14-16 April 2014, 61-74.

Baumgard, L.H., and Rhoads, R.P., 2012. Ruminant Nutrition Symposium: ruminant production and metabolic responses to heat stress, Journal of Animal Science, 90, 1855-1865.

Beatty, D.T., Barnes, A., Taylor, E., and Maloney, S.K., 2008. Do changes in feed intake or ambient temperature cause changes in cattle rumen temperature relative to core temperature?, Journal of Thermal Biology, 33, 12-19.

Benninghoff, J., Paschke-Beese, M., and Südekum, K. H. 2015. In situ and in vitro ruminal degradation of maize grain and untreated or xylose-treated wheat, barley and rye grains. Animal Feed Science and Technology, 210, 86-93.

Bertocchi, L., Vitali, A., Lacetera, N., Nardone, A., Varisco, G., and Bernabucci, U. 2014. Seasonal variations in the composition of Holstein cow's milk and temperature–humidity index relationship. Animal, 8, 667-674.

Bewley, J.M., Einstein, M.E., Grott, M.W., and Schutz, M.M., 2008. Comparison of reticular and rectal core body temperatures in lactating dairy cows, Journal of Dairy Science, 91, 4661-4672.

Davison, T.M., Jonsson, N.N., Mayer, D.G., Gaughan, J.B., Ehrlich, W.K., and McGowan, M.R., 2016. Comparison of the impact of six heat-load management strategies on thermal responses and milk production of feed-pad and pasture fed dairy cows in a subtropical environment, International Journal of Biometeorology, 50, 1961-1968.

Dunshea, F.R., Russo , V.M., Sawyer , I., and Leury, B.J., 2012. A starch-binding agent decreases the in vitro rate of fermentation of wheat, Journal of Animal Science, 90 (Suppl. 3), 158.

Gaughan, J.B., Mader, T.L., Holt, S.M., Hahn, G.L., and Young, B.A., 2002. Review of current assessment of cattle and microclimate during periods of high heat load, Animal Production in Australia, 24, 77-80.

Gaughan, J.B., Mader, T.L., Holt, S.M., and Lisle, A., 2008. A new heat load index for feedlot cattle. Faculty Papers and Publications in Animal Science. Paper 613, (University of Nebraska - Lincoln).

Gonzalez-Rivas, P.A., DiGiacomo, K., Leury, B.J., Cottrell, J.J., and Dunshea , F.R., 2017. 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, Journal of Animal Science, 95, 5547-5562.

Gonzalez-Rivas, P.A., DiGiacomo, K., Russo, V.M., Leury, B.J., Cottrell, J.J., and Dunshea, F.R., 2016. Feeding slowly fermentable grains has the potential to ameliorate heat stress in grain-fed wethers, Journal of Animal Science, 94, 2981-2991.

Herrera-Saldana, R.E., Huber, J.T., and Poore, M.H., 1990. Dry matter, crude protein, and starch degradability of five cereal grains, Journal of Dairy Science, 73, 2386-2393.

Kabuga, J.D., 1992. The influence of thermal conditions on rectal temperature, respiration rate and pulse rate of lactating Holstein-Friesian cows in the humid tropics, International Journal of Biometeorology, 36, 146-150.

Lees, A., Gaughan, J.B., Sullivan, M.L., Lees, J.C., and Lisle, A., 2014. Rumen temperature of Brahman, Angus and Charolais steers with and without access to shade, Journal of Animal Science, 92, (Suppl. 2)/ Journal of Dairy Science, 97, (Suppl. 1), 292.

Liu, H., Zhao, K., and Liu, J. 2013. Effects of Glucose Availability on Expression of the Key Genes Involved in Synthesis of Milk Fat, Lactose and Glucose Metabolism in Bovine Mammary Epithelial Cells. PLOS ONE, 8, e66092.

Mader, T.L., Davis, M.S., and Brown-Brandl, T., 2006. Environmental factors influencing heat stress in feedlot cattle, Journal of Animal Science, 84, 712-719.

Mohammed, R., Hünerberg, M., McAllister, T.A., and Beauchemin, K.A., 2014. Characterization of ruminal temperature and its relationship with ruminal pH in beef heifers fed growing and finishing diets, Journal of Animal Science, 92, 4650-4660.

Moharrery, A., Larsen, M., and Weisbjerg, M. R. (2014). Starch digestion in the rumen, small intestine, and hind gut of dairy cows – A meta-analysis. Animal Feed Science and Technology, 192, 1-14.

Reynolds, C.K., 2006. Production and metabolic effects of site of starch digestion in dairy cattle, Animal Feed Science and Technology, 130, 78-94.

Reynolds, C.K., Cammell, S.B., Humphries, D.J., Beever, D.E., Sutton, J.D., and Newbold, J.R., 2001. Effects of postrumen starch infusion on milk production and energy metabolism in dairy cows, Journal of Dairy Science, 84, 2250-2259.

Russell, J.B., 2007. Can the heat of ruminal fermentation be manipulated to decrease heat stress?, Proceedings 22nd Southwest Nutrition & Management Conference, 2007, Tempe, AZ, USA, 109-115.

Silanikove, N. (2000). Effects of heat stress on the welfare of extensively managed domestic ruminants. Livestock Production Science, 67, 1-18.

Smith, D. L., Smith, T., Rude, B. J., and Ward, S. H. 2013. Short communication: Comparison of the effects of heat stress on milk and component yields and somatic cell score in Holstein and Jersey cows. Journal of Dairy Science, 96, 3028-3033.

Sullivan, M.L., Gaughan, J.B., Son, N., J., L., and Lees, A.M., 2014a. Correlation between mean panting score and temperature humidity index in lactating dairy cows in a sub-tropical summer, Journal of Animal Science 92, (Suppl. 2)/ Journal of Dairy Science, 97, (Suppl. 1), 293.

Sullivan, M.L., Gaughan, J.B., Son, N., J., L., and Lees, A.M., 2014b. Correlation between milk production, days in milk, and temperature humidity index in lactating dairy cows in a sub-tropical summer, Journal of Animal Science 92, (Suppl. 2)/ Journal of Dairy Science, 97, (Suppl. 1), 293.

West, J.W., 2003. Effects of heat-stress on production in dairy cattle, Journal of Dairy Science, 86, 2131-2144.

Wheelock, J.B., Rhoads, R.P., VanBaale, M.J., Sanders, S.R., and Baumgard, L.H., 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows, Journal of Dairy Science, 93, 644-655.

Table 1 Composition of experimental diets fed to dairy cows on a DM basis

Item	Experimental diet		
	TMRB	TMRC	TMRW
Ingredient, % DM basis			
Lucerne hayilage UQ	20.7	20.7	20.7
Barley silage	35.8	35.8	35.8
Barley straw	4.4	4.4	4.4
Crushed 2 % Bioprotect-treated wheat	27.0	-	-
Crushed corn grain	-	27.0	-
Crushed wheat grain	-	-	27.0
Canola meal	4.6	4.6	4.6
Soybean meal	4.6	4.6	4.6
Heat mineral mix[1]	3.0	3.0	3.0
Chemical composition, DM basis[2]			
Moisture, %	57.3	56.0	54.3
MP, %	18.2	18.7	18.2
NDF, %	31.2	30.8	31.2
ADF, %	18.7	18.1	18.7
NFC, %	40.2	40.3	40.2
Starch, %	27.8	27.7	27.8
Sugar, %	2.6	2.4	2.6
Lignin, %	4.8	4.6	4.8

[1] Heat mineral mix includes CP 32.1 %, Ca 7.57 %, P 2.74 %, Co 25.5 %, and provides Vit. A 126.7 IU, Vit. D 25.3 mg, Mg 3.52 mg, Fe 1,892 mg, Vit. E 1267 IU per kg

[2]MP = Metabolizable protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; NFC = non-fiber carbohydrates.

Table 2 Variation in milk production and composition in Holstein-Friesian dairy cows fed TMRB, TMRC, and TMRW during the experiment. Means are predicted means pooled by diet

	Experimental diet				
Item	TMRB	TMRC	TMRW	SED	Significance
Fat, %	4.9[b]	4.3[a]	5.0[b]	0.30	0.049
Lactose, %	4.9	5.1	5.0	0.06	0.086
Protein, %	3.7	3.5	3.7	0.18	0.42
Milk Production, Kg	20.0[a]	20.6[a]	19.4[b]	0.36	0.004
Milk Solids[1], Kg	1.7	1.6	1.7	0.15	0.87

[a–b] Means within a row with different superscripts differ ($P < 0.05$)
[1] Milk Solids (kg) = kg Milk × (% Protein + % Fat).

Table 3 Variation in milk production and composition in Holstein-Friesian dairy cows fed TMRB, TMRC, and TMRW during the experiment. Means are predicted means pooled by sample day

	Sample day								
Item	**1**	**2**	**3**	**4**	**5**	**7**	**8**	SED	Significance
THI Average[1]	72.7	74.5	72.2	74.7	75.1	72.3	68.2		
Fat, %	4.6	4.7	4.6	4.5	4.8	5.0	4.8	0.18	0.095
Lactose, %	5.0[a]	5.0[a]	5.0[a]	5.0[a]	5.0[a]	4.9[b]	4.9[b]	0.03	0.001
Protein, %	3.5[a]	3.6[b]	3.5[a]	3.7[c]	3.8[d]	3.8[d]	3.8[d]	0.04	< 0.001
Milk Production, Kg	23.3[a]	22.2[a]	20.1[b]	19.6[cb]	18.6[cb]	18.6[cb]	20.4[b]	0.60	< 0.001
Milk Solids, Kg	1.8[a]	1.8[a]	1.6[b]	1.6[b]	1.6[b]	1.6[b]	1.7[ab]	0.06	< 0.001

[a–d] Means within a row with different superscripts differ ($P < 0.05$)

[21] THI average = average temperature humidity index of the sampling day.

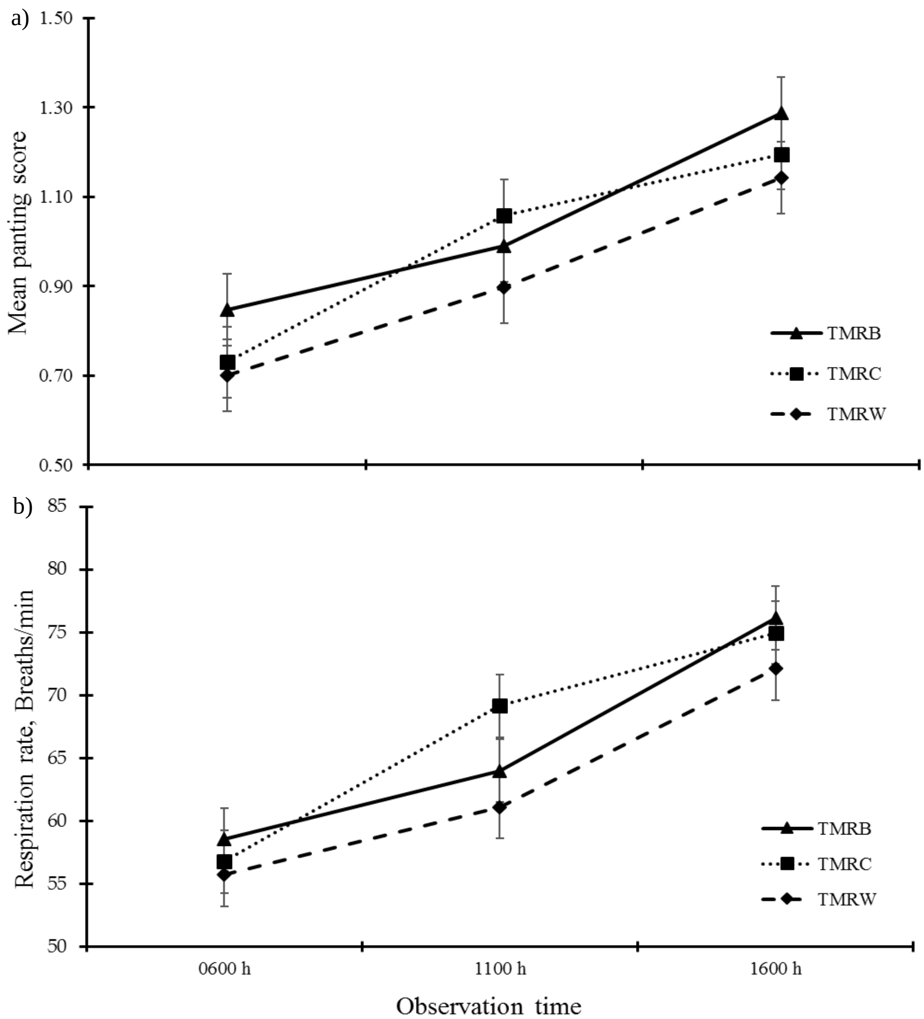

Figure 1 Relationship between mean panting score (a), respiration rate (b) and observation time in Holstein-Friesian dairy cows fed TMRB, TMRC, and TMRW. Results are means pooled across days and pooled SED for the interaction diet × observation time. *P*-values for the effects of diet, observation time and diet × observation time were 0.19, < 0.001, and 0.24 respectively for panting score, and 0.27, < 0.001, and 0.013 respectively for respiration rate.

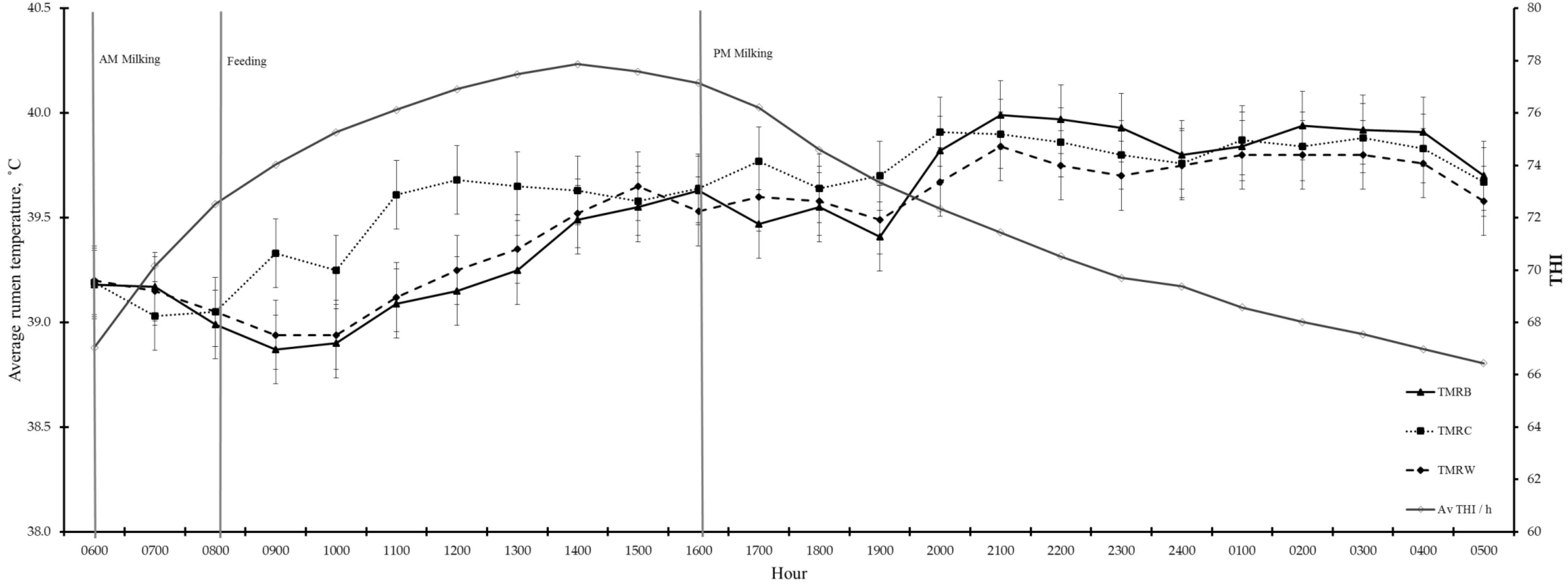

Figure 2 Relationship between rumen temperature and temperature humidity index (THI) during the day (Av THI /h) in Holstein-Friesian dairy cows fed TMRB, TMRC, and TMRW. Results are estimated means per hour and pooled SED for the interaction diet × hour. *P*-values for the effects of diet, hour, and diet × hour were 0.61, < 0.001, and < 0.001 respectively.

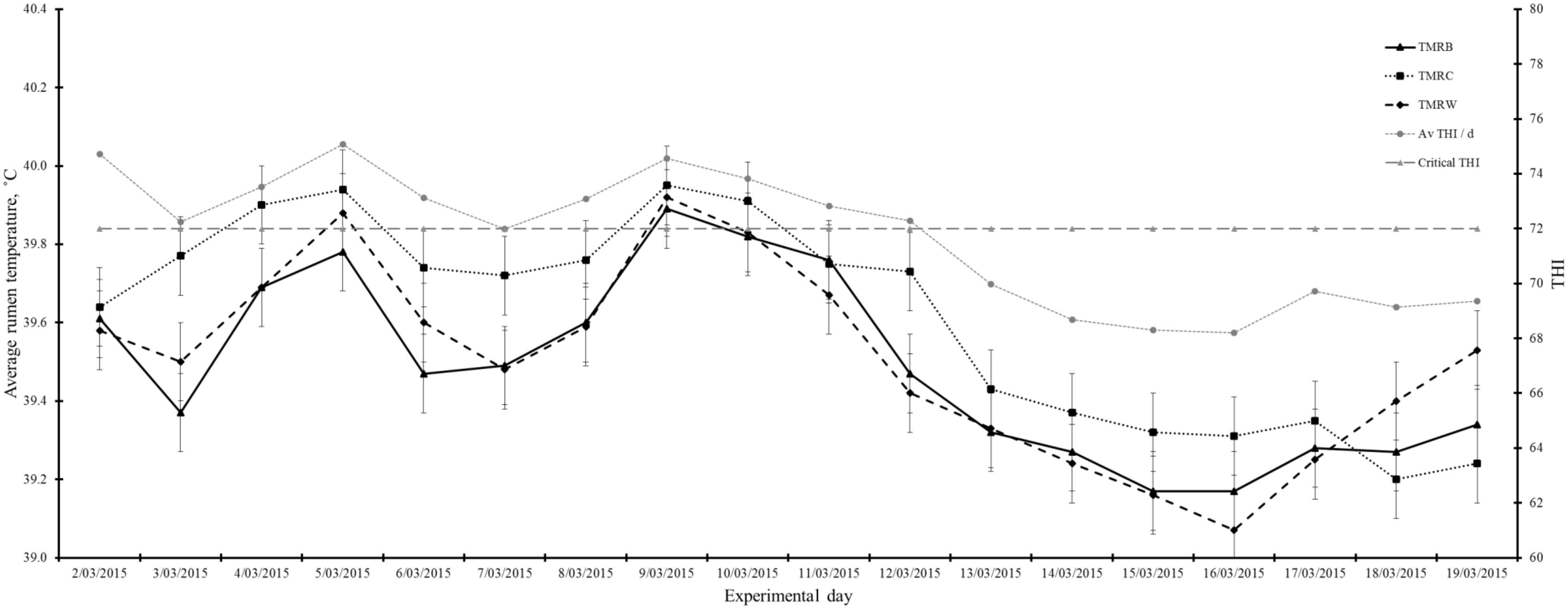

Figure 3 Relationship between rumen temperature and temperature humidity index (THI) during the experimental days (THI / d and critical THI) in Holstein-Friesian dairy cows fed TMRB, TMRC, and TMRW. Results are estimated means per hour and pooled SED for the interaction diet × experimental day. *P*-values for the effects of diet, experimental day, and diet × experimental day were 0.62, < 0.001, and 0.003 respectively.