Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism

F. C. Cowley,^{*1} D. G. Barber,[†] A. V. Houlihan,[‡] and D. P. Poppi^{*}

^{*}School of Agriculture and Food Science, Animal Studies Building 8153, and

[†]Animal Science, Agri-science Queensland, Department of Agriculture, Fisheries and Forestry, University of Queensland Gatton Campus, Lawes, Qld 4343, Australia

[‡]Innovative Food Technologies, Agri-science Queensland, Department of Agriculture, Fisheries and Forestry, Health and Food Sciences Precinct, Coopers Plains, Brisbane, Qld 4108, Australia

ABSTRACT

The effects of heat stress on dairy production can be separated into 2 distinct causes: those effects that are mediated by the reduced voluntary feed intake associated with heat stress, and the direct physiological and metabolic effects of heat stress. To distinguish between these, and identify their effect on milk protein and casein concentration, mid-lactation Holstein-Friesian cows ($n = 24$) were housed in temperature-controlled chambers and either subjected to heat stress [HS; temperature-humidity index (THI) ~ 78] or kept in a THI < 70 environment and pair-fed with heat-stressed cows (TN-R) for 7 d. A control group of cows was kept in a THI < 70 environment with ad libitum feeding (TN-AL). A subsequent recovery period (7 d), with THI < 70 and ad libitum feeding followed. Intake accounted for only part of the effects of heat stress. Heat stress reduced the milk protein concentration, casein number, and casein concentration and increased the urea concentration in milk beyond the effects of restriction of intake. Under HS, the proportion in total casein of α_{S1} -casein increased and the proportion of α_{S2} -casein decreased. Because no effect of HS on milk fat or lactose concentration was found, these effects appeared to be the result of specific downregulation of mammary protein synthesis, and not a general reduction in mammary activity. No residual effects were found of HS or TN-R on milk production or composition after THI < 70 and ad libitum intake were restored. Heat-stressed cows had elevated blood concentrations of urea and Ca, compared with TN-R and TN-AL. Cows in TN-R had higher serum nonesterified fatty acid concentrations than cows in HS. It was proposed that HS and TN-R cows may mobilize different tissues as endogenous sources of energy.

Key words: heat stress, milk protein, restricted intake, casein

INTRODUCTION

Under heat stress conditions, lactating dairy cows exhibit several physiological responses including a voluntary reduction of feed intake, an increase in maintenance requirements, a decrease in milk yield, and a decline in the quality of milk for manufacturing (NRC, 1981; Armstrong, 1994; Kadzere et al., 2002). In the past, it was accepted that beyond a temperature-humidity index (THI) of 72 (Armstrong, 1994), the physiological state and milk yield of the cow begin to be adversely affected. However, more recent analysis shows that this threshold is as low as 68 (Collier et al., 2012).

It has long been recognized that the changes in milk yield and composition from heat stress are at least partly a result of the sudden depression of intake (Hancock, 1954; Holter et al., 1997). Increasingly, research (Bandaranayaka and Holmes, 1976; Rhoads et al., 2009) indicates that changes occur in the physiological state and metabolism of cows at high ambient temperatures that are independent of the changes in feed intake. Previous studies where cows have been control-fed to match the intake of heat-stressed cows (Bandaranayaka and Holmes, 1976; Wheelock et al., 2010) have shown that DMI accounts for only part of the effect of heat stress on milk yield and that milk protein concentration in heat-stressed cows is significantly affected by factors other than intake. However, the effects of heat stress on protein composition or individual protein fractions has had limited research, particularly in controlled environments. In one study, cows provided with shade- and sprinkler-based cooling increased their mass fraction of α_{S2} -casein (Amenu et al., 2004). However, comparison of milk protein fractions in milk collected from cows in spring and summer found a decrease in combined α_S -casein mass fractions and in β -casein mass fraction and an increase in κ -casein mass fraction and serum protein fraction concentrations of milk in summer

Received June 3, 2014.

Accepted December 18, 2014.

¹Corresponding author: frances.cowley@uqconnect.edu.au

(Bernabucci and Calamari, 1998). No work is available that partitions the direct effects of heat stress on casein composition from the indirect effects of reduced DMI. The question of whether heat stress can affect protein composition after the immediate period of high THI is also an issue that has not yet been addressed, and that will affect the financial cost of heat stress to dairy farms. Ominski et al. (2002) observed a residual effect of heat stress on the milk protein and SNF concentration of milk during a 5-d recovery phase. Anecdotal reports from local dairy manufacturers suggest that the quality of milk protein may be impaired even after heat stress conditions have abated (D. G. Barber, unpublished data).

The aim of this experiment was to separate the direct effects of heat stress from the secondary effect of reduced feed intake, particularly as it relates to the production of individual caseins. It was hypothesized that (1) heat stress would have a direct effect on the production of caseins that was greater than the indirect effect of reduced intake, and (2) the direct effect of heat stress would have residual effects on the production of casein that continued beyond the period of heat stress and were longer-lasting than any residual effect of reduced intake.

MATERIALS AND METHODS

Cows and Design

Twenty-four multiparous Holstein-Friesian cows were used in a randomized, split-plot design experiment with 3 treatments and 2 replications. Twelve cows were used in each replication. At the start of the experiment, cows averaged 161 ± 4 (mean $\pm$ SD) DIM, 22.7 ± 3.4 L/d average milk yield for the week before the experiment, BCS of 4.3 ± 0.35 (1–8 scale, with 1 being emaciated and 8 being obese; Robins et al., 2003) and 509 ± 50.0 kg of BW for replication 1 and 164 ± 15 DIM, 20.8 ± 2.8 L/d, BCS of 4.0 ± 0.23 , and 493 ± 57.2 kg of BW for replication 2. All cows were of κ -casein genotype AA. The cows were stratified on β -lactoglobulin genotype (AB or BB) and milk yield (high or low) before being randomly allocated within strata to treatment groups. The cows were paired by strata so that 1 of each pair was from each β -lactoglobulin genotype and each milk yield strata. The pairs were assigned to the 6 rooms within each replication randomly.

The cows were tethered in pairs in 6 temperature-controlled rooms at Mutdapilly Research Station, Mutdapilly, Queensland (27°45'S, 152°40'E; altitude 40 m), with 2 pairs per treatment per replication, totaling 8 cows per treatment for the whole experiment. Prior to this experiment, the cows were kept in a fully grazed

milking herd at the Mutdapilly Research Station. The experiment was conducted in February and March, and the cows would have experienced high temperatures and relative humidity (RH) before this experiment commenced. In the 2 mo before the commencement of replication 1, ambient temperature and RH were a mean daily minimum of 20.4 ± 6.6 (SD)°C and $45.6 \pm 10.1\%$, respectively, and a mean daily maximum of 33.1 ± 2.8 °C and $93.4 \pm 9.8\%$, respectively. In the 2 mo before the commencement of replication 2, ambient temperature and RH were a mean daily minimum of 20.5 ± 1.9 °C and $49.4 \pm 8.1\%$, respectively, and a mean daily maximum of 32.3 ± 2.4 °C and $96.1 \pm 6.5\%$, respectively. This meant that the cows entered the experiment with some prior exposure to hot weather conditions. All procedures were reviewed and approved by the Department of Primary Industries (Queensland) Animal Ethics Committee, in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

The environmental and feeding conditions of each treatment and experimental period are described in Table 1. Each replication was preceded by a 10-d adaptation period with ad libitum feed on offer and $\text{THI} < 70$, calculated according to Jones and Hennessy (2000) and Padfield (1996), as outlined below. A 3-d covariate period continued the $\text{THI} < 70$ and ad libitum intake conditions. In the first treatment period (period 1), heat stress conditions or restricted DMI were imposed on the relevant treatment groups for 7 d. This was followed immediately by a second experimental period of 7 d (period 2) of $\text{THI} < 70$ and ad libitum intake for all treatment groups, to assess the ability of the cows to recover from the treatments imposed in period 1.

The 3 treatments imposed in period 1 were $\text{THI} < 70$ and ad libitum intake (**TN-AL**); imposed heat stress, adequate to elicit a decrease in milk yield of 4 to 5 L ($\text{THI} \sim 78$), and ad libitum intake (**HS**); and $\text{THI} < 70$ with intake restricted to matched cows in HS (**TN-R**). Cows in TN-R were matched with cows in HS of similar BW, and feed offered was calculated to equal the intake of their counterpart in HS the previous day (kg of DM/kg of BW) plus 5%. The treatments were randomly assigned to rooms within the animal house separately for each replication.

Housing and Temperature Control

Each room had a floor area of 15 m^2 and a volume of 40 m^3 . Exhaust airflow was $12 \text{ m}^3/\text{min}$. Temperature was controlled, but RH was that of the incoming air. Temperature and RH were recorded by a data logger (Penguin PN002, Novus Automation Inc., Porto Alegre, Brazil) every 3 min. The THI was calculated from this

Table 1. Sequence of climate and feeding conditions for treatment groups (control, TN-AL; heat-stressed, HS; restricted intake, TN-R)

Treatment group	Covariate		Period 1		Period 2	
	Climate	Feeding	Climate	Feeding	Climate	Feeding
TN-AL	THI ¹ < 70	Ad libitum	THI < 70	Ad libitum	THI < 70	Ad libitum
HS	THI < 70	Ad libitum	THI ~78	Ad libitum	THI < 70	Ad libitum
TN-R	THI < 70	Ad libitum	THI < 70	Restricted to intake of HS	THI < 70	Ad libitum

¹THI = temperature-humidity index.

information according to Jones and Hennessy (2000) for dewpoint and Padfield (1996) for water vapor pressure (WVP):

THI = dry bulb temperature + 0.36 (dewpoint temperature) + 41.2, where

dewpoint temperature = $\frac{273.3 \left(\frac{WVP}{6.107} \right)}{17.269 - \ln \left(\frac{WVP}{6.107} \right)}$,

WVP = $\ln \left(\frac{\text{actual WVP}}{610.78} \right)$,

actual WVP = $\frac{\text{saturation WVP} \times \text{relative humidity (\%)}}{100}$,

saturation WVP = $610.78 \times e^{\left[\frac{17.269 \times AT}{AT + 238.3} - 1 \right]}$,

and AT = air temperature. During the adaptation phase, covariate period, and period 2, all cows were housed in conditions targeted to a THI of < 70, with a set temperature of 21°C, and a mean observed RH of 84%, except when cows were removed for milking in the dairy twice daily for a period totaling 1 h per 24 h day. During period 1, a THI < 70 was maintained for TN-AL and TN-R; however, the THI for HS was increased to a target of 78, with a set temperature of 26°C, and a mean observed RH of 89%, except during milking time. Artificial light from 0600 to 1900 h was the sole source of illumination for all treatments.

Feed and Diet Composition

A TMR (Table 2) was fed twice daily (at 0730 and 1600 h) and formulated using the NRC diet formulation program (NRC, 2001) to provide a diet adequate to achieve 25 L of milk/d at 3.1% protein, 5.1% lactose, and 4.0% fat. An additional 1 kg of 50% barley and 50% sorghum grain mix was fed in the parlor twice daily during milking. Dietary cation-anion difference of the diet

was 9.9 mEq/100 g of DM. Samples of TMR and grain mix were collected daily with subsamples composited by period then oven-dried at 60°C for 48 h to determine DM concentration. After grinding to pass through a 1-mm screen, the subsamples were analyzed according to the Cornell Net Carbohydrate and Protein System at the DairyOne Forage Testing Laboratory (Ithaca, NY) for CP [Leco FP-528 Nitrogen/Protein Analyzer, Leco Corp., St. Joseph, MI (AOAC, 1990)], NDF [using sulfite and amylase (Van Soest et al., 1991)], ADF (Van Soest et al., 1991), starch (YSI 2700 Select Biochemistry Analyzer, YSI Life Sciences, Yellow Springs, OH), and ME content (using near infrared spectroscopy and calculations based on NRC (2001; Table 2). Drinking water was available for ad libitum consumption.

Milk Yield and Composition

Cows were milked twice daily at 0700 and 1530 h in a dairy 50 m from the animal house. Milk yields were recorded automatically by the Westfalia dairy management system (DairyPlan 5, GEA Farm Technologies, Bönen, Germany). Representative samples for milk composition analysis were collected daily during the covariate period and period 1 and on d 3, 5, and 7 of period 2 and composited by day and cow. Fourier

Table 2. Mean composition of the TMR and grain mix fed in dairy

Item	TMR	Grain mix
Ingredient (% of DM)		
Barley/sorghum grain mix ¹	10.51	100
Barley silage	74.29	
Lucerne haylage	6.58	
Soybean meal	5.32	
Forage sorghum hay	2.61	
Sodium bicarbonate	0.43	
Dicalcium phosphate	0.13	
Limestone	0.13	
Chemical composition		
DM (%)	41.4	91.6
CP (% of DM)	16.6	16.0
NDF (% of DM)	32.6	14.4
ADF (% of DM)	21.4	7.4
Starch (% of DM)	26.9	55.1
ME (MJ/kg of DM)	11.3	13.6

¹Grain mix is made of 50% barley and 50% sorghum.

transform infrared techniques (Foss Milko-Scan FT120, Hillerød, Denmark) were used to analyze milk samples for the concentrations of fat, total nitrogen, true protein, and casein. Concentrations of noncasein- and non-protein-nitrogen-containing components, whey protein and lactose, and casein number (i.e., the proportion of casein in true protein) were calculated by difference, as in Auldist et al. (2010). Urea analyses were conducted using the urea colorimetric method of Bergmeyer et al. (1985). Milk samples were also analyzed for SCC (Somacount 300, Bentley Instruments, Chaska, MN), TS (Standards Association of Australia, 1988), density (DMA 35, Anton Paar GmbH, Graz, Austria), and pH. Samples were analyzed for milk casein composition (α_{S1} -, α_{S2} -, β -, and κ -casein concentrations and proportions) using a Shimadzu LC-10 analytical reverse phase HPLC system Vydac C4 300A 3 μ m column, with a Phenomenex C4 (Butyl) guard column, with peak detection at 214 nm (Webster, 1970) gradient elution using acetonitrile/TFA (Auldist et al., 2010). Mean response coefficients were used to determine mass fractions of individual protein components relative to total casein or whey in milk samples in the manner of Groen et al. (1994).

Blood and Rumen Function Metabolites

Coccygeal arterial blood was collected from individual cows at the end of the covariate period and periods 1 and 2 of each replication, and the plasma analyzed for creatinine, creatine phosphokinase (**CPK**), total protein, Na, K, and Ca (a 10-mL sample collected in a heparinized Vacutainer; BD, Plymouth, UK), and glucose and lactate (a 5-mL sample collected in a BD Vacutainer containing 12.5 mg of NaFl and 10 mg of KO) concentrations on an Olympus AU400 automated multi-test analyzer (Olympus Optical Co. Ltd., Tokyo, Japan). Jugular venous blood was collected from individual cows at the end of the covariate period and periods 1 and 2 of each replication, and the plasma analyzed for urea concentration [**PUN**; a 10-mL sample collected in a heparinized BD Vacutainer, and PUN determined enzymatically by change in absorbance at 340 nm] and BHBA concentration [a 10-mL sample collected in a heparinized BD Vacutainer and estimated using an Olympus AU400 automatic multi-test analyzer (Olympus Optical Co. Ltd.)] and the serum analyzed for NEFA concentration (a 10-mL sample collected in a plain tube and analyzed on an Olympus AU400 automatic multi-test analyzer, Olympus Optical Co. Ltd.). Rumen fluid samples were collected orally, before morning feeding, on the last day of the covariate period and periods 1 and 2 of each replication using a stomach tube and a small vacuum pump. The first 100

mL of rumen fluid extracted was discarded to reduce contamination with saliva, and the following 100 mL was analyzed for pH immediately with a portable electronic pH meter (pH Cube, TPS Pty Ltd., Springwood, QLD, Australia), and for ammonia-N (measured by distillation, using a Büchi 321 Kjeldahl Distiller, BÜCHI Labortechnik AG, Flawil, Switzerland) and VFA profiles [prepared using an adaptation of the method of Vreman et al. (1978), using 0.9 mL of sample to which was added 0.1 mL of 1 M sulfuric acid containing the internal standard, 3-methyl-n-valeric acid (Sigma-Aldrich Pty. Ltd., Castle Hill, NSW, Australia), and analyzed by gas chromatography (GC-17 A, Shimadzu, Tokyo, Japan) using a polar capillary column (DB-FFAP 30 m $\times$ 0.53 mm $\times$ 1.0 μ m, J&W Scientific, Folsom, CA)]. For 3 consecutive days at the end of the covariate period and periods 1 and 2, a spot urine sample was collected from each animal and composited within period by cow. Urine samples from individual cows were analyzed for purine derivatives to determine microbial crude protein (**MCP**) production. Creatinine excretion was calculated according to the equation of Kolade (1994):

$$\text{creatinine excretion (mg/BW}^{0.75}\cdot\text{d)} = 9.71 + 0.81 \text{ BW}^{0.75} + 305.4 \text{ M},$$

where M = milk yield (L/kg of BW^{0.75}).

Urine output was calculated using the following equation:

$$\text{Urine output (L/d)} = [\text{CE (mg/BW}^{0.75}\cdot\text{d)} \times \text{BW}^{0.75} \text{ (kg)}] / \text{creatinine (mg/L)},$$

where CE = creatinine excretion (mg/BW^{0.75}·d).

The intestinal flow of microbial N was calculated according to the method and equations of Chen and Gomes (1995) and MCP production was calculated with the following equation:

$$\text{MCP production (g/d)} = 0.727 \times [\text{purine excretion (mmol/d)} - 0.385 \text{ BW}^{0.75}/0.85] \times 6.25.$$

Physiological Measurements

Rectal temperatures (measured by digital thermometer inserted to depth of 3 cm for 3 min duration), respiration rates (measured by counting movements of the dilatator naris lateralis muscle of the nose for 1 min), and heart rates (measured by counting heart beats by stethoscope for 1 min) were recorded at 0600, 1400, and 2100 h each day of the covariate period, and

on the first, third, fifth, and seventh days of period 2. These measurements were recorded daily at 0200, 0600, 1000, 1400, 1800, and 2200 h each day of period 1 for HS cows, and at 0600, 1400, and 2200 h for TN-AL and TN-R cows. Body weight was measured at the end of each period, including the covariate period, immediately after morning milking, by taking a static weight using Westfalia Taxatron walk-over scales (GEA Farm Technologies, Bönen, Germany).

Statistical Analyses

Results from this experiment were analyzed using a split-unit ANOVA, where the main units were the 12 rooms (2 replications $\times$ 6 rooms). Measurement of milk yields and the analysis of milk samples from individual cows were analyzed for the final day of each period. To account for the 1-d lag in DMI for TN-R cows matched to HS cows in period 1, the final day of period 1 for HS was analyzed using d 6 as the final day. Intakes of individual cows were analyzed for the mean of the period. The analysis of period means was considered more appropriate for casein composition, to smooth out the day-to-day variation observed in those variates. Within each period these data were analyzed by ANOVA using Genstat 9 (Payne et al., 2007). The model included terms for replication, rooms within replication and animals within rooms as random effects, and the β -lactoglobulin genotype and treatment and their interaction as fixed effects. Because the interaction was not significant in any relevant variates, these data are not presented here. The mean of the covariate period was used where it was significant. Least significant differences were used to compare treatment means. During replication 1, 2 cows were removed from the experiment in period 1 (1 each from TN-AL and TN-R groups) and an additional cow (from TN-AL) was removed in period 2, as a result of mastitis. The cows did not return to the experiment, and all data from these cows were discarded for the period of removal from the experiment.

RESULTS

Cows in HS experienced higher ambient temperature, RH, and THI [26.4 ± 1.5 (SD) $^{\circ}\text{C}$, $88.7 \pm 4.8\%$, and 77.6 ± 2.0 , respectively] than cows in THI < 70 ($21.0 \pm 1.2^{\circ}\text{C}$, $84.0 \pm 5.6\%$, and 69.7 ± 1.6 , respectively). No differences were found in THI between treatment groups in period 2. A diurnal pattern of variation in THI (Figure 1) was observed. The THI of HS was reduced during the time of morning milking.

Rectal temperatures and respiratory rates increased for cows in HS compared with TN-AL and TN-R (Table 3). Heart rates decreased in both HS and TN-R.

All variables returned to normal levels in period 2. No difference was observed between treatments for BW or BCS in any period (data not shown).

Dry matter intake and DMI per kilogram of BW of cows in TN-AL were both greater ($P < 0.05$) than TN-R and HS during period 1 (Table 4). Daily CP and ME intake were also greater in period 1 for TN-AL compared with TN-R and HS ($P < 0.05$; Table 4). By the end of period 1, TN-R and HS intakes had decreased to be 22% lower than TN-AL ($P < 0.01$). No differences were found in mean intakes during period 2 ($P < 0.05$). All treatment groups met requirements for the main macro-minerals of P (TN-AL: 0.36%, 0.35%; HS: 0.36%, 0.35%; TN-R: 0.36%, 0.35%), Ca (TN-AL: 0.71%, 0.66%; HS: 0.72%, 0.67%; TN-R: 0.72%, 0.66%), K (TN-AL: 1.06%, 1.17%; HS: 1.04%, 1.17%; TN-R: 1.05%, 1.17%), and Mg (TN-AL: 0.21%, 0.21%; HS: 0.21%, 0.21%; TN-R: 0.21%, 0.21%) in periods 1 and 2, respectively (NRC, 2001), and although not analyzed Se, Cu, and Co deficiencies have never been recorded in dairy diets in this region nor in the current dairy herd on the research station.

The milk yield of cows in HS at the end of period 1 was lower than cows in TN-AL and TN-R, and higher than cows in HS ($P < 0.01$; Table 5). The true protein concentration of milk from HS cows at the end of period 1 was lower than that of TN-AL, and TN-R was intermediate between the 2 other treatments ($P < 0.05$; Table 5). Fat and lactose concentrations and SCC showed no difference between treatments at the end of

Figure 1. Average diurnal patterns of temperature humidity index (THI) during thermoneutral (TNE) or heat stress (HS) conditions.

Table 3. Physiological measurements of experimental animals (control, TN-AL; heat-stressed, HS; restricted intake, TN-R)

Item/period	Treatment group			SEM	P-value
	TN-AL	HS	TN-R		
Rectal temperature (°C)					
Period 1	38.9 ^a	39.9 ^b	38.6 ^a	0.10	***
Period 2	38.6	38.6	38.5	0.10	
Respiratory rate ¹ (per min)					
Period 1	59.4 ^a	80.8 ^b	59.0 ^a	0.25	***
Period 2	59.8	57.6	58.8	0.30	
Heart rate (per min)					
Period 1	85.9 ^a	79.0 ^b	80.0 ^b	0.33	*
Period 2	86.0	82.1	83.4	0.46	

^{a,b}Different superscripts within rows indicate significant differences between treatments.¹Analyzed with covariate.* $P < 0.05$, *** $P < 0.001$.

period 1 ($P < 0.05$; Table 5). No differences were found in milk yield and gross milk composition at the end of period 2 (Table 5).

By the final day of period 1, the casein concentration of milk from HS cows was lower than TN-R and TN-AL cows ($P < 0.05$; Table 6). No effect was observed of TN-R on casein concentration in period 1 or 2. Heat stress reduced the milk casein number at the end of period 1 ($P < 0.05$), but TN-R had no effect (Table 6). Milk urea concentration of HS cows was higher than that of cows in TN-R and TN-AL at the end of period 1 ($P < 0.001$; Table 6). Noncasein N concentration was lower for cows in TN-R ($P < 0.05$), but cows in HS showed no difference from TN-AL at the end of period 1 (Table 6). Milk whey protein concentration from TN-R cows was lower than that from TN-AL cows and HS cows at the end of period 1 ($P < 0.05$; Table 6). Heat stress caused an increase in the relative proportion of α_{S1} -casein ($P < 0.05$) relative to TN-AL and a

decrease in the relative proportion of α_{S2} -casein relative to TN-AL and TN-R at the end of period 1 ($P < 0.001$; Table 7). Treatment TN-R had no effect on the mass fractions of any individual milk caseins in either period 1 or 2. All differences in milk N and casein composition were removed by the final day of period 2, when the cows were returned to $THI < 70$ and ad libitum conditions (Table 7).

Heat stress caused an increase in PUN ($P < 0.001$), plasma creatinine ($P < 0.05$), and plasma Ca ($P < 0.01$) concentrations above those of cows in TN-AL at the end of period 1 (Table 8). The HS cows also had higher concentrations of PUN ($P < 0.001$) and ($P < 0.01$) Ca and lower concentrations of serum NEFA ($P < 0.05$) than TN-R cows at the end of period 1 (Table 8). Relative to TN-AL, TN-R had no effect on any blood plasma constituents (Table 8). Heat stress and restricted intake had no effect on plasma concentrations of total protein, glucose, BHBA, L-lactate, CPK, K, or Na

Table 4. The effect of heat stress and restricted intake on mean daily DM and nutrient intakes (control, TN-AL; heat-stressed, HS; restricted intake, TN-R)

Item/period	Treatment group			SEM	P-value
	TN-AL	HS	TN-R		
DMI (kg/d)					
Period 1	19.7 ^a	16.9 ^b	17.1 ^b	0.53	*
Period 2	20.2	19.9	21.6	0.55	
DMI (g/kg BW·d)					
Period 1	40.3 ^a	33.3 ^b	33.3 ^b	0.00	**
Period 2	42.1	38.9	41.8	0.00	
ME intake (MJ/d)					
Period 1	220.4 ^a	189.5 ^b	192.2 ^b	5.82	*
Period 2	227.7	224.3	243	6.15	
CP intake (kg/d)					
Period 1	3.2 ^a	2.7 ^b	2.8 ^b	0.09	*
Period 2	3.4	3.3	3.6	0.09	

^{a,b}Different superscripts within rows indicate significant differences between treatments.* $P < 0.05$, ** $P < 0.01$.

Table 5. The effect of heat stress and restricted intake on milk composition after treatment (period 1, 7 d) and posttreatment (period 2, 7 d) periods (control, TN-AL; heat-stressed, HS; restricted intake, TN-R)

Item/period	Treatment group			SEM	P-value
	TN-AL	HS	TN-R		
Milk yield ¹ (L/d)					
Period 1	21.98 ^a	17.03 ^b	19.89 ^a	0.781	**
Period 2	21.28	19.73	19.87	0.528	
True protein ¹ (g/L)					
Period 1	34.1 ^a	33.1 ^b	33.6 ^{ab}	0.31	*
Period 2	35.9	35.5	35.5	0.26	
True protein yield ¹ (g/d)					
Period 1	759 ^a	583 ^b	624 ^b	27.7	**
Period 2	754	697	710	13.5	
Fat (g/L)					
Period 1	42.3	41.8	40.4	1.35	
Period 2	45.4	43.2	43.6	1.03	
Lactose (g/L)					
Period 1	76.4	75.9	76.5	0.59	
Period 2	51.2	49.3	48.5	0.82	
SCC, period means ($\times 1,000$ cells/mL)					
Period 1	186	134	142	66.7	
Period 2	92	128	159	44.9	

^{a-c}Different superscripts within rows indicate significant differences between treatments.

¹Analyzed with covariate.

* $P < 0.05$, ** $P < 0.01$.

in period 1 or 2 (Table 8). Treatments HS and TN-R had no effect on the proportions of rumen VFA (data not shown) or on the level of MCP production in either period (Table 8). All differences in blood metabolites or rumen function from period 1 were removed once the cows returned to THI < 70 and ad libitum conditions in period 2 (Table 8). An exception to this is the increase in plasma CPK concentration for TN-AL in period 2 ($P < 0.05$). A large SEM was present in plasma CPK

concentration for TN-AL in period 2 (9.23), indicating that this may be a function of outliers.

DISCUSSION

We observed an additional effect of heat stress on milk yield over and above its effect on feed intake, confirming previous findings by Bandaranayaka and Holmes (1976) and Baumgard and Rhoads (2012). Heat stress caused

Table 6. The effect of heat stress and restricted intake on milk nitrogen and casein composition after treatment (period 1, 7 d) and posttreatment (period 2, 7 d) periods (control, TN-AL; heat-stressed, HS; restricted intake, TN-R)

Item/period	Treatment group			SEM	P-value
	TN-AL	HS	TN-R		
Milk casein concentration ¹ (g/L)					
Period 1	28.1 ^a	26.8 ^b	27.7 ^a	0.24	*
Period 2	29.1	28.8	28.9	0.23	
Casein number ¹					
Period 1	81.80 ^a	81.05 ^b	82.64 ^a	0.15	***
Period 2	81.25	81.12	81.55	0.16	
Milk urea concentration (g/L)					
Period 1	0.32 ^b	0.38 ^a	0.30 ^b	0.016	*
Period 2	0.30	0.32	0.36	0.016	
Noncasein nitrogen ¹ (g/L)					
Period 1	1.18 ^a	1.19 ^a	1.10 ^b	0.020	*
Period 2	1.22	1.22	1.23	0.014	
Milk whey protein concentration ¹ (g/L)					
Period 1	6.34 ^a	6.39 ^a	5.91 ^b	0.086	*
Period 2	6.79	6.77	6.66	0.065	

^{a,b}Different superscripts within periods within rows indicate significant differences between treatments.

¹Analyzed with covariate.

* $P < 0.05$, *** $P < 0.001$.

Table 7. The effect of heat stress and restricted intake on mean casein mass fractions during treatment (period 1, 7 d) and posttreatment (period 2, 7 d) periods (control, TN-AL; heat-stressed, HS; restricted intake, TN-R)

Item/period	Treatment group			SEM	P-value
	TN-AL	HS	TN-R		
α_{S1} -Casein					
Period 1	36.57 ^a	38.41 ^b	37.76 ^{ab}	0.582	*
Period 2	37.01	36.16	35.76	0.375	
α_{S2} -Casein					
Period 1	13.45 ^a	11.75 ^b	14.37 ^a	0.355	*
Period 2	13.50	14.04	14.01	0.367	
β -Casein					
Period 1	38.17	38.83	37.81	0.635	
Period 2	38.65	38.84	39.13	0.411	
κ -Casein					
Period 1	10.63	11.35	10.89	0.289	
Period 2	10.84	10.97	11.11	0.335	

^{a,b}Different superscripts within periods within rows indicate significant differences between treatments.

* $P < 0.05$.

average daily milk yield to fall by approximately 20% compared with TN-AL during period 1. This decline was similar to previously reported results over a similar range of THI in cows that were gradually acclimated to increasing heat with advancing summer (Bouraoui et al., 2002; West et al., 2003). However, we observed a larger reduction in milk yield in heat-stressed animals, compared with those cows with a restricted intake only (a mean difference of 2.85 L), indicating that factors other than intake are responsible for reduced milk yield under heat stress. No residual effect was observed of heat stress on milk yield after 7 d of ad libitum feeding in THI < 70 in period 2.

We concluded that heat stress had greater influence on milk protein concentration than restriction of DMI. The decline in milk true protein concentration that we observed in heat-stressed cows was greater than in cows with a restricted intake only. In previous experiments similar to ours, concentration of protein in skim milk declined more under heat stress than with a restricted intake only (Bandaranayaka and Holmes, 1976; Rhoads et al., 2009). Because neither lactose nor fat concentrations were affected by heat stress, we concluded that the reduction in protein concentration was a result of specific downregulation of mammary protein synthesis, rather than a general reduction in milk synthesis. Bernabucci and Calamari (1998) suggested that factors other than dietary protein supply may have a negative effect on milk protein concentration in heat-stressed cows, including a decline in rumen MCP synthesis and an increase in the utilization of AA for gluconeogenesis, both of which are related to lower energy and protein intakes, but also possibly related to changes in homeostatic metabolism. Rumen MCP synthesis was not decreased by heat stress or restricted intake in our experiment, which may suggest that circulating AA

supply was unchanged. No residual effect was observed of heat stress on milk protein concentration after 7 d of ad libitum feeding in THI < 70 in period 2.

We observed that heat stress caused a decrease in casein concentration and casein number. Similar results have previously been found under imposed high temperature and humidity (Muroya et al., 1997; Nardone et al., 1997), and as season changed from spring to summer in the field (Bernabucci et al., 2002). No direct role has been found for the amount and type of energy and protein supply on casein number (Coulon et al., 1998), and we found no effect of TN-R in this experiment. No residual effect was observed of heat stress on casein concentration or casein number after cows returned to conditions of THI < 70 and ad libitum feeding in period 2. It appears, therefore, that heat stress is causing a change in the mammary synthesis of casein through factors other than changed dietary supply of substrates.

We found that heat stress was the only factor affecting individual casein mass fraction proportions (increasing α_{S1} - and decreasing α_{S2} -casein). No effect was observed of restricted intake alone on the individual casein mass fractions relative to TN-AL. α_{S2} -Casein is the major S- and P-containing milk protein. A decline in dietary S and P from reduced DMI would certainly limit microbial synthesis of the sulfur-containing AA (Kadzere et al., 2002). However, the mechanisms that would cause the differing results seen here between heat stress and restricted intake alone are open to speculation. Bernabucci and Calamari (1998) found a decrease in combined α_S -casein mass fractions and in β -casein mass fractions and an increase in κ -casein mass fraction and serum protein fraction concentrations of milk collected in summer when compared with spring (Bernabucci and Calamari, 1998). Those authors hypothesized that the decline in α_S - and β -casein in summer milk may

Table 8. Effect of heat stress and restricted intake on blood metabolites and rumen function parameters during treatment (period 1, 7 d) and posttreatment (period 2, 7 d) periods (control, TN-AL; heat-stressed, HS; restricted intake, TN-R)

Item/period	Period 1			SEM	P-value
	TN-AL	HS	TN-R		
Blood metabolite ¹ (mmol/L)					
Urea N					
Period 1	5.33 ^a	6.49 ^b	5.12 ^a	0.177	***
Period 2	5.44	5.51	6.23	0.236	
Creatinine ($\times 10^{-3}$)					
Period 1	82.5 ^a	107.5 ^b	92.8 ^{ab}	4.27	*
Period 2	90.0	97.6	95.0	2.89	
Ca					
Period 1	2.26 ^b	2.38 ^a	2.23 ^c	0.026	**
Period 2	2.25	2.38	2.38	0.023	
NEFA ²					
Period 1	0.168 ^{ab}	0.101 ^a	0.246 ^b	0.021	*
Period 2	0.093	0.104	0.113	0.013	
Total protein ($\times 10^3$)					
Period 1	73.55	78.81	74.63	1.37	
Period 2	74.6	75.8	74.7	1.94	
Glucose					
Period 1	3.28	3.08	3.24	0.067	
Period 2	3.30	3.26	3.18	0.078	
BHBA					
Period 1	0.342	0.338	0.358	0.024	
Period 2	0.375	0.450	0.458	0.021	
L-Lactate					
Period 1	1.12	1.22	0.78	0.173	
Period 2	1.04	1.12	1.12	0.149	
Creatine phosphokinase (IU/L)					
Period 1	45.4	42.5	48.0	2.88	
Period 2	62.1 ^a	39.8 ^b	45.8 ^{ab}	4.34	*
K					
Period 1	4.17	4.25	4.21	0.043	
Period 2	4.39	4.25	4.53	0.013	
Na					
Period 1	133.9	132.0	130.8	0.93	
Period 2	133.5	134.8	136.8	1.36	
Rumen function parameter					
pH					
Period 1	6.75 ^a	6.63 ^a	7.11 ^b	0.084	**
Period 2	6.94	6.79	7.03	0.059	
Rumen NH ₃ -N (mg/L)					
Period 1	115.7 ^a	148.2 ^b	125.8 ^{ab}	5.63	*
Period 2	107.4	112.9	126.1	4.54	
Microbial CP (g/d)					
Period 1	1,330	1,030	1,072	106	
Period 2	1,152	1,214	1,257	90	

^{a-c}Different superscripts within periods within rows indicate significant differences between treatments.

¹Measured in plasma, with the exception of NEFA, which was measured in serum.

²Analyzed with covariate.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

be at least partially a result of decreased DMI. Our results indicate that DMI does not affect the proportions of casein mass fractions. Our results are in agreement with those of Amenu et al. (2004) who found that cows provided with shade- and sprinkler-based cooling increased their mass fraction of α_{S2} -casein. We found that heat stress had no residual effects on the relative proportions of individual caseins.

Our observation of increases in rectal temperature and respiratory rate indicate that cows in HS were experi-

encing moderate heat stress (Johnson and Vanjonack, 1976; Kadzere et al., 2002). The sustained high THI we recorded in the HS group would have excluded the opportunity to dissipate the accumulated heat of metabolism or maintain intake during cool periods (Kadzere et al., 2002). Although the cows were removed from the animal house for milking twice daily, these data on respiration and rectal temperatures, in conjunction with our results demonstrating reduced voluntary feed intake in HS cows, indicate that any cooling experi-

enced during this time was insufficient to dissipate their accumulated heat load. We recorded similar declines in resting heart rate in cows in HS and TN-R during period 1, indicating that this was an intake-related effect, most likely due to a slowing of metabolism (Kadzere et al., 2002). The cows used in this experiment had been grazed through summer immediately before entering the experiment. It is therefore possible that they had some level of adaptation to heat stress that may have lessened the extent of their responses to imposed high THI (Kadzere et al., 2002), even after a 10-d adaptation period and 3-d covariate period in $\text{THI} < 70$. However, our observations of rectal temperatures, respiratory rates, DMI, and milk yields demonstrate that any possible adaption was far from complete. It is also possible that pre-experimental exposure to warm and hot conditions had residual effects on the entire lactation that were not removed during the 10-d adaptation period. However, our results demonstrate that 7-d after imposed HS in period 1 ended, no residual effects were found on HS cows when compared with TN-AL cows that had been kept in $\text{THI} < 70$ for 28 d after prior exposure to hot weather. Although it is possible that the prior heat exposure had residual effects on all cows that were not alleviated by the cooler conditions, this seems unlikely given we observed that the effects of imposed HS in period 1 were completely removed after 7 d in period 2. Grazed dairy cows are most likely to experience episodes of high THI in the context of summer, where daily THI is already elevated. For this reason it was considered appropriate to test responses to HS during the summer. Although recent work (Collier et al. 2012) suggests the upper critical THI is 68, cows in TN-AL demonstrated significantly improved rectal temperatures, respiratory rates, DMI, and milk yields at $\text{THI} < 70$ compared with cows in HS.

We observed no changes in the plasma glucose concentrations of HS and TN-R cows, demonstrating the power of the glucose homeostatic regulatory system in the face of the reduction in DMI in this experiment (Bragança and Prunier, 1999). It is logical that an energy deficit would develop in the heat-stressed cow from a decrease in DMI and an increase in maintenance energy requirement as the cow increases oxygen consumption through panting and elevated rates of tissue metabolism as tissue temperatures increase (NRC, 1981). However, we did not observe the findings of similar experiments, which showed lower plasma glucose concentrations (Ronchi et al., 1997; Rhoads et al., 2009; Schwartz et al., 2009) under heat stress. In our experiment, the decline in dietary glucose may have been offset by a combination of a decrease in the lactation energy requirement, an increase in gluconeogenesis

and metabolic utilization of endogenous energy sources, as in Bernabucci and Calamari (1998), or both.

We observed that heat-stressed and intake-restricted cows had differential mobilization of endogenous energy substrates. In our experiment, TN-R cows showed a classical response to a negative energy balance of little change in PUN but elevated serum NEFA, indicating mobilization of adipose energy sources. Evidence in the literature supports our finding that body fat is mobilized with restricted intake under $\text{THI} < 70$, but not heat stress: NEFA levels were elevated in the current study only in cows treated with a restricted intake, as in Bertoni (1998), Ronchi et al. (1999), and Rhoads et al. (2009). Bertoni (1998) concluded that the hormone profile of heat-stressed cows (e.g., low glucocorticoid concentrations) would maintain a low and slow rate of lipolysis. Ronchi et al. (1999) hypothesized that lower NEFA concentrations in heat-stressed cows were a result of elevated utilization rates of NEFA for energy production in heat-stressed cows, which was supported by elevated plasma ketone concentrations. Dale and Brody (1954) identified lower blood ketone concentrations and unchanged total urine ketone bodies in heat-stressed cows compared with those with a restricted intake. However, when fasted in a thermoneutral environment, those cows rapidly developed ketosis after the second day of fasting, indicating that adipose tissues were being mobilized (Dale and Brody, 1954). Neither heat stress nor restricted intake affected plasma BHBA concentration in our experiment. The reason why restricted intake increased NEFA but not BHBA in our experiment is unclear, as elevation of ketone bodies is an expected reaction to restricted feeding where NEFA are also increased as a result of the mobilization of adipose tissues.

In comparison with cows with a restricted intake, we observed no change in plasma NEFA and an increase in PUN of heat-stressed cows. Increases in PUN can result from either reduced incorporation of rumen ammonia into MCP, or from increased catabolism of AA as gluconeogenic substrates. In this experiment, no differences were observed in either rumen ammonia concentration or MCP production between HS and TN-R, likely excluding rumen function as the source of elevated PUN. This suggests that under heat stress differential catabolism of tissues may occur, prioritizing protein rather than adipose sources for meeting the energy needs of the cow. Rhoads et al. (2009) hypothesized that metabolic adaptations to heat stress inhibit the mobilization of adipose tissues. Ronchi et al. (1999) identified no differences between heat-stressed cows and cows with restricted feeding in their rise of PUN compared with a thermoneutral environment

and unrestricted feeding, concluding this was a result of muscle catabolism. However, in light of the lower level of plasma glucose in heat-stressed cows in that experiment, it was postulated that heat-stressed cows had more limited utilization of AA for gluconeogenesis than the restricted intake group (Ronchi et al., 1999). We propose from our results and those of earlier studies (Colditz and Kellaway, 1972; Ronchi et al., 1997) that the main substrate for gluconeogenesis in heat-stressed cows is catabolized muscular tissue and free AA, hence the elevated PUN and plasma creatinine concentrations. Bertoni (1998) suggests that NEFA in heat-stressed cows is used in the oxidation of muscular tissue, which may explain the lower NEFA concentrations observed in heat-stressed cows in this study. If indeed endogenous proteins are the main source of energy substrates in the heat-stressed cow, then it is possible that EAA, which would normally be destined for milk protein synthesis, are also being scavenged for gluconeogenic purposes, reducing the precursor pool for the synthesis of milk proteins. The reduced milk protein concentration that we observed in heat-stressed cows (33.1 g/L, in contrast to 34.6 g/L in TN-AL) may indicate an imbalance of EAA as characterized by Cant et al. (2003).

Supply of AA to the mammary gland is a function of plasma concentration and mammary blood flow (MBF; Bequette and Backwell, 1997). No difference was observed in MCP or plasma total protein concentration between any of the treatments in our experiment, which suggests that circulating AA supply may have been unaffected by HS. Despite the fact that heat stress increases peripheral blood flow as a cooling mechanism (Armstrong, 1994), in experiments with similar treatments to those in this study, MBF (Lough et al., 1990) and portal plasma flow (McGuire et al., 1989) tended to reduce during heat stress, and were better correlated with level of DMI rather than with ambient temperature. Nardone et al. (1997) proposed that a supposed reduction of MBF, as blood distribution is altered in heat stress, may decrease the supply of milk protein precursors to the mammary gland, causing a downturn in the production of casein and lactalbumin and possibly all mammary-synthesized milk constituents. This is a possible explanation for our observations of reduced milk protein and casein concentrations and casein number in HS, in comparison with TN-R and TN-AL. Although the mechanisms driving blood flow in the mammary gland are contentious (MacRae et al., 2000; Beever et al., 2001), this may be at least partially a product of the reduced heart rates that we observed in both heat-stressed (79.0 beats/min) and restricted-intake groups (80.0 beats/min) compared with TN-AL (85.9 beats/min). Cant and McBride (1995) suggest,

however, that centrally driven changes in MBF may not cause the responses in milk production that changes in blood flow initiated by local factors do. The mammary gland is also able to upregulate MBF to support protein synthesis (Cant et al., 2003).

The yield of milk protein has a low sensitivity to concentrations of single precursor AA and it may be that the utilization of AA for milk protein synthesis is fixed (Cant et al., 2003). As our results have shown that circulating plasma total protein concentrations are unaffected by heat stress, it may be that it is not delivery of AA precursors to the mammary epithelium that is changing milk protein composition under heat stress, but rather elements of the biosynthetic pathway (transcription/translation). Beyond gluconeogenic use of endogenous protein stores, there are several alternative ways in which heat stress might interfere with the synthesis of milk proteins. Although not measured in this experiment, the expression of heat shock proteins is known to increase with exposure to severe heat stress (Collier et al., 2008). They function to protect other proteins from denaturation under the effects of heat shock. The synthesis of these heat shock proteins may reduce the availability of circulating amino acids for milk protein synthesis. Other possible ways that heat stress may interfere in the synthesis of milk proteins include turnover within the mammary gland, competition for AA supply between casein and structural protein synthesis, as well as degradation of synthesized caseins within the gland (Bequette and Backwell, 1997).

CONCLUSIONS

Milk protein composition is subject to significant detrimental changes under the effects of heat stress. It is clear that heat stress has an effect on milk protein and casein production and composition that is greater than the indirect effect of reduced intake. Although reduced nutrient intake is partially responsible for some changes, under heat stress the bulk of the effects on milk protein composition and individual casein fractions were attributable to a suite of changes in physiology, nutrient metabolism, and nutrient partitioning to the mammary gland. These changes in milk protein appear to be a result of specific downregulation of mammary protein synthetic activity and are not artifacts of a general reduction in milk yield. Cows affected by heat stress or restricted intake appeared to mobilize different tissues as endogenous sources of energy. Although a week-long period of moderate heat stress caused significant changes to milk protein composition, the direct effects of heat stress had no residual effects on the production of protein or casein that continued beyond the period of heat stress.

ACKNOWLEDGMENTS

The authors thank Dairy Australia (Melbourne) for funding this work. We are grateful to Pat Pepper (Department of Agriculture, Fisheries and Forestry, Yeerongpilly, Queensland, Australia) for assistance with the design and statistical analysis of this experiment, and to Sarah Kenman (Department of Agriculture, Fisheries and Forestry, Mutdapilly, Queensland, Australia) and Natalie Spiller (Department of Agriculture, Fisheries and Forestry, Hamilton, Queensland, Australia) for their skilled technical assistance.

REFERENCES

- Amenu, B., T. Cowan, and H. Deeth. 2004. Impact of forage quality and heat stress on milk composition and cheddar cheese yield. Pages 5–8 in *Proc. Anim. Prod. in Aust.: Proc. 25th Biennial Conf. of the Aust. Soc. Anim. Prod.*, Melbourne, Australia.
- AOAC. 1990. *Official Methods of Analysis*. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.
- Armstrong, D. V. 1994. Heat stress interaction with shade and cooling. *J. Dairy Sci.* 77:2044–2050.
- Auld, M. J., C. Grainger, A. V. Houlihan, J. J. Mayes, and R. P. W. Williams. 2010. Composition, coagulation properties, and cheese-making potential of milk from cows undergoing extended lactations in a pasture-based dairying system. *J. Dairy Sci.* 93:1401–1411.
- Bandaranayake, D. D., and C. W. Holmes. 1976. Changes in the composition of milk and rumen contents in cows exposed to a high ambient temperature with controlled feeding. *Trop. Anim. Health Prod.* 8:38–46.
- Baumgard, L. H., and R. P. Rhoads. 2012. Ruminant production and metabolic responses to heat stress. *J. Anim. Sci.* 90:1855–1865.
- Beever, D. E., J. D. Sutton, and C. K. Reynolds. 2001. Increasing the protein content of cow's milk. *Aust. J. Dairy Technol.* 56:138–149.
- Bequette, B. J., and F. R. C. Backwell. 1997. Amino acid supply and metabolism by the ruminant mammary gland. *Proc. Nutr. Soc.* 56:593–605.
- Bergmeyer, H. U., J. Bergmeyer, and M. Grassl. 1985. *Methods of Enzymatic Analysis—Metabolites 3: Lipids, Amino Acids and Related Compounds*. 3rd ed. Wiley-Blackwell, Weinheim, Germany.
- Bernabucci, U., and L. Calamari. 1998. Effects of heat stress on bovine milk yield and composition. *Zootec. Nutr. Anim.* 24:247–257.
- Bernabucci, U., N. Lacetera, B. Ronchi, and A. Nardone. 2002. Effects of the hot season on milk protein fractions in Holstein cows. *Anim. Res.* 51:25–33.
- Bertoni, G. 1998. Effects of heat stress on endocrine-metabolic and reproductive status of the dairy cows. *Zootec. Nutr. Anim.* 24:273–282.
- Bouraoui, R., M. Lahmar, A. Majdoub, M. Djemali, and R. Belyea. 2002. The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate. *Anim. Res.* 51:479–491.
- Cant, J. P., R. Berthiaume, H. Lapierre, P. H. Luimes, B. W. McBride, and D. Pacheco. 2003. Responses of the bovine mammary glands to absorptive supply of single amino acids. *Can. J. Anim. Sci.* 83:341–355.
- Cant, J. P., and B. W. McBride. 1995. Mathematical analysis of the relationship between blood flow and uptake of nutrients in the mammary glands of a lactating cow. *J. Dairy Res.* 62:405–422.
- Chen, X. B., and M. J. Gomes. 1995. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives: An Overview of the Technical Details. International Feed Resources Unit, Rowett Research Institute, Aberdeen, UK.
- Colditz, P. J., and R. C. Kellaway. 1972. The effect of diet and heat stress on feed intake, growth, and nitrogen metabolism in Friesian, F₁ Brahman × Friesian and Brahman heifers. *Aust. J. Agric. Res.* 23:717–725.
- Collier, R. J., J. L. Collier, R. P. Rhoads, and L. H. Baumgard. 2008. Invited review: Genes involved in the bovine heat stress response. *J. Dairy Sci.* 91:445–454.
- Collier, R. J., L. W. Hall, S. Rungruang, and R. B. Zimbleman. 2012. Quantifying heat stress and its impact on metabolism and performance. Pages 74–84 in *Proc. 23rd Annual Florida Ruminant Nutrition Symposium*, Gainesville. University of Florida, Gainesville.
- Coulon, J. B., C. Hurtaud, B. Remond, and R. Verite. 1998. Factors contributing to variation in the proportion of casein in cow's milk true protein: A review of recent INRA experiments. *J. Dairy Res.* 65:375–387.
- Dale, H. E. and S. Brody. 1954. *Environmental Physiology and Shelter Engineering with Special Reference to Domestic Animals*. 30. Thermal stress and acid-base balance in dairy cattle. *Res. Bull. Miss. Agric. Exp. Stn.*
- de Bragança, M. M. d., and A. Prunier. 1999. Effects of low feed intake and hot environment on plasma profiles of glucose, nonesterified fatty acids, insulin, glucagon, and IGF-1 in lactating sows. *Domest. Anim. Endocrinol.* 16:89–101.
- Groen, A. F., R. van der Vegt, M. A. J. S. van Boekel, O. L. A. M. de Rouw, and H. Vos. 1994. Case study on individual animal variation in milk protein composition as estimated by high-pressure liquid chromatography. *Neth. Milk Dairy J.* 48:201–212.
- Hancock, J. 1954. The direct influence of climate on milk production. *Dairy Sci. Abstr.* 16:90–102.
- Holter, J. B., J. W. West, and M. L. McGilliard. 1997. Predicting ad libitum dry matter intake and yield of Holstein cows. *J. Dairy Sci.* 80:2188–2199.
- Johnson, H. D., and W. J. Vanjonack. 1976. Effects of environmental and other stressors on blood hormone patterns in lactating animals. *J. Dairy Sci.* 59:1603–1617.
- Jones, R. N., and K. J. Hennessy. 2000. Climate change impacts in the Hunter Valley. Page 23 in *CSIRO Atmospheric Research*, CSIRO, Aspendale, Victoria, Australia.
- Kadzere, C. T., M. R. Murphy, N. Silanikove, and E. Maltz. 2002. Heat stress in lactating dairy cows: A review. *Livest. Prod. Sci.* 77:59–91.
- Kolade, M. M. 1994. Renal excretion of purine derivatives in cattle as a measure of microbial N flow to the duodenum. PhD thesis. University of Queensland, St Lucia, Australia.
- Lough, D. S., D. L. Beede, and C. J. Wilcox. 1990. Effects of feed intake and thermal stress on mammary blood flow and other physiological measurements in lactating dairy cows. *J. Dairy Sci.* 73:325–332.
- MacRae, J. C., J. B. Bequette, and L. A. Crompton. 2000. Synthesis of milk protein and opportunities for nutritional manipulation. in *BSAS Occ. Publ. Vol. 25. Br. Soc. Anim. Sci.*, Edinburgh, UK.
- McGuire, M. A., D. K. Beede, M. A. DeLorenzo, C. J. Wilcox, G. B. Huntington, C. K. Reynolds, and R. J. Collier. 1989. Effects of thermal stress and level of feed intake on portal plasma flow and net fluxes of metabolites in lactating Holstein cows. *J. Anim. Sci.* 67:1050–1060.
- Muroya, S., F. Terada, and S. Shioya. 1997. Influence of heat stress on distribution of nitrogen in milk. *Anim. Sci. Tech.* 68:297–300.
- Nardone, A., N. Lacetera, U. Bernabucci, and B. Ronchi. 1997. Composition of colostrum from dairy heifers exposed to high air temperatures during late pregnancy and the early postpartum period. *J. Dairy Sci.* 80:838–844.
- NRC. 1981. *Effect of environment on nutrient requirements of domestic animals*. 6th rev. ed. Natl. Acad. Press, Washington, DC.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Ominski, K. H., A. D. Kennedy, K. M. Wittenberg, and S. A. M. Nis. 2002. Physiological and production responses to feeding schedule in lactating dairy cows exposed to short-term, moderate heat stress. *J. Dairy Sci.* 85:730–737.
- Padfield, T. 1996. Equations describing the physical properties of moist air. *Conservation Physics*. Accessed Nov. 11, 2008. <http://www.conservationphysics.org/atmcalc/atmoclc2.pdf>.

- Payne, R. W., S. A. Harding, D. A. Murray, D. M. Soutar, D. B. Baird, S. J. Welham, A. F. Kane, A. R. Gilmour, R. Thompson, R. Webster, and G. T. Wilson. 2007. The Guide to Gnstat Release 8, Part 2: Statistics. VSN International, Hemel Hempstead, UK.
- Rhoads, M. L., R. P. Rhoads, M. J. VanBaale, R. J. Collier, S. R. Sanders, W. J. Weber, B. A. Crooker, and L. H. Baumgard. 2009. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. *J. Dairy Sci.* 92:1986–1997.
- Robins, C., A. Stockdale, J. Crosby, and J. Morton. 2003. The Condition Magician: Body Condition Scoring in Dairy Herds. 2nd ed. Dept. Nat. Res. Environ., Melbourne, Victoria, Australia.
- Ronchi, B., U. Bernabucci, N. Lacetera, and A. Nardone. 1997. Effects of heat stress on metabolic-nutritional status of Holstein cows. *Zootec. Nutr. Anim.* 23:3–15.
- Ronchi, B., U. Bernabucci, N. Lacetera, A. Verini Supplizi, and A. Nardone. 1999. Distinct and common effects of heat stress and restricted feeding on metabolic status of Holstein heifers. *Zootec. Nutr. Anim.* 25:11–20.
- Shwartz, G., M. L. Rhoads, M. J. VanBaale, R. P. Rhoads, and L. H. Baumgard. 2009. Effects of a supplemental yeast culture on heat-stressed lactating Holstein cows. *J. Dairy Sci.* 92:935–942.
- Standards Association of Australia. 1988. AS 2300.1.1 General Methods and Principles—Determination of Total Solids and Moisture. Australian Standard of Chemical and Physical Testing for the Dairying Industry.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Vreman, H. J., J. A. Dowling, R. A. Raubach, and M. W. Weiner. 1978. Determination of acetate in biological material by vacuum micro-distillation and gas chromatography. *Anal. Chem.* 50:1138–1141.
- Webster, G. C. 1970. Comparison of direct spectrophotometric methods for the measurement of protein concentration. *Biochim. Biophys. Acta* 207:371–373.
- West, J. W., B. G. Mullinix, and J. K. Bernard. 2003. Effects of hot, humid weather on milk temperature, dry matter intake, and milk yield of lactating dairy cows. *J. Dairy Sci.* 86:232–242.
- Wheelock, J. B., R. P. Rhoads, M. J. VanBaale, S. R. Sanders, and L. H. Baumgard. 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. *J. Dairy Sci.* 93:644–655.