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

# F. C. Cowley,\*<sup>1</sup> 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)  $\sim 78$ ] 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  $\alpha_{s_1}$ -case in increased and the proportion of  $\alpha_{s_2}$ -case in 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  $\alpha_{S2}$ -case (Amenu et al., 2004). However, comparison of milk protein fractions in milk collected from cows in spring and summer found a decrease in combined  $\alpha_{s}$ -case mass fractions and in  $\beta$ -case mass fraction and an increase in  $\kappa$ -case n mass fraction and serum protein fraction concentrations of milk in summer

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<sup>&</sup>lt;sup>1</sup>Corresponding author: frances.cowley@uqconnect.edu.au

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(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  $\pm$  4 (mean  $\pm$  SD) DIM, 22.7  $\pm$  3.4 L/d average milk yield for the week before the experiment, BCS of  $4.3 \pm 0.35$  (1–8 scale, with 1 being emaciated and 8 being obese; Robins et al., 2003) and  $509 \pm 50.0$ kg of BW for replication 1 and 164  $\pm$  15 DIM, 20.8  $\pm$ 2.8 L/d, BCS of 4.0  $\pm$  0.23, and 493  $\pm$  57.2 kg of BW for replication 2. All cows were of  $\kappa$ -casein genotype AA. The cows were stratified on  $\beta$ -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  $\beta$ -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 temperaturecontrolled 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  $20.4 \pm 6.6 \text{ (SD)}^{\circ}\text{C}$  and 45.6 $\pm$  10.1%, respectively, and a mean daily maximum of  $33.1 \pm 2.8$ °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  $\pm$  1.9°C and 49.4  $\pm$  8.1%, respectively, and a mean daily maximum of  $32.3 \pm 2.4$ °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 THI < 70, calculated according to Jones and Hennessy (2000) and Padfield (1996), as outlined below. A 3-d covariate period continued the 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 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 THI < 70 and ad libitum intake (**TN-AL**); imposed heat stress, adequate to elicit a decrease in milk yield of 4 to 5 L (THI ~78), and ad libitum intake (**HS**); and 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  $15 \text{ m}^2$  and a volume of 40 m<sup>3</sup>. 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

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