

Effects of Encapsulated Niacin on Evaporative Heat Loss and Body Temperature in Moderately Heat-Stressed Lactating Holstein Cows

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INTRODUCTION

During warm summer months milk production can decrease between 10-35% and this is a costly issue in the dairy industry (St. Pierre et al, 2003). The reduced milk yield is a result of increased body temperature induced-decline in feed intake as well as alterations in endocrine profiles, energy metabolism (Baumgard et al, 2007) and other unidentified factors (Collier et al, 2008). Increasing heat dissipation (the transfer of body heat from the core to the surface) via enhanced peripheral vasomotor function and evaporative heat loss may alleviate some of the decrease in dry matter intake and thus milk production.

Niacin, nicotinic acid, or vitamin B₃ induces vasodilatation at the skin and this increases heat loss at the periphery (Di Constanza et al, 1997). The vasodilatory effects of niacin act through prostaglandin D production by epidermal Langerhans cells (Benyo et al, 2006; Maciejewski et al, 2006) and vascular endothelial prostaglandin D₂ receptors (Cheng et al, 2006). Indeed, skin temperatures are decreased during periods of mild to severe heat stress in cows supplemented with 12, 24, or 36 g of raw niacin (Di Constanza et al, 1997). Past research evaluating niacin supplementation during heat stress has utilized raw niacin which would largely be metabolized by rumen microbes (Campbell et al, 1994). Previous research (Miller et al, 1986; Zinn et al, 1987; Santschi et al, 2005) has demonstrated that very little (3-10%) niacin or nicotinamide escape ruminal degradation. Lipid encapsulation has been used for many years to coat and protect bioactive substances from rumen degradation, with advances in the technology used more recently to protect choline. Kung et al. (2003) reported a high level of in vitro rumen protection of choline with lipid encapsulated choline (> 70%). Deuchler et al. (1998) observed increased milk choline when supplementing lipid encapsulated choline to lactating dairy cows, indicating extensive rumen bypass and intestinal release of choline. Using the same coating technology, a niacin product has been developed that allows niacin to be protected from rumen degradation (> 90%) and released in the small intestine (Balchem Corporation, New Hampton, NY, personal communication). Encapsulation technology can dramatically increase the bioavailability of compounds like niacin to the

small intestine (Deuschler et al. 1987). The effects of feeding encapsulated niacin during thermal stress have not been evaluated, but we hypothesized that if niacin is “rumen protected” then more would be bioavailable and thus produce a greater vasodilatory response. We hypothesize that this would then lead to improved heat loss in cattle fed encapsulated niacin.

Two trials were conducted with the following objectives: 1) determine if supplementing encapsulated niacin to lactating dairy cows increased free plasma niacin concentrations altered EVHL and core body temperature indices during moderate thermal stress and 2) determine if this resulted in production effects on a larger commercial dairy

MATERIALS AND METHODS

Trial One

Animals

Twelve multiparous Holstein cows producing an average of 31 kg/d (± 4.75 kg/d) and balanced for parity (2 ± 1) and stage of lactation ($\text{DIM } 145 \pm 9$) were housed in individual tie stalls in one of two environmentally controlled chambers in the William Parker Agricultural Research Center at the University of Arizona. After 4 d of adjusting to the chambers, cows entered thermoneutral (TN), which lasted 7 d and consisted of thermal neutral conditions (TN; $\text{THI} < 72$ for 24 h/d). After TN, cows entered the heat stress period (HS) which lasted for 7 d and consisted of a moderate thermal stressful environment (temperature humidity index [THI] > 72 for 12 out of 24 h/day). At the beginning of TN, six cows (3 in each chamber) were randomly assigned to receive either 0 g encapsulated niacin per cow/d (C) or 12 g encapsulated niacin per cow/d (NI; NIAHURE®; Balchem Corp, New Hampton, NY) and all cows remained on dietary treatment until the end of HS. The form in which niacin was supplemented was encapsulated and therefore only 68% by weight of pure niacin was present in each 12 g dose resulting in an actual dosage of 8.2 g of raw niacin per day. Encapsulated niacin was top dressed by suspending in molasses (50 ml) and pouring on individual feed buckets and mixed by hand. During TN, the THI pattern never exceeded 72 while during HS, the circadian temperature induced moderate heat stress (THI exceeded 72 for 12 h/d; Figure 1). Relative humidity was held constant at 18% during both periods and THI changes were achieved through computer controlled ambient temperature alterations. Milk yields were measured twice daily and sampled once a day in the morning for composition analysis conducted at Arizona DHIA Tempe, AZ. Milk fat, protein, and lactose were analyzed using AOAC approved infrared analysis (AOAC, 2000); SCC was analyzed using AOAC approved cell-staining techniques (AOAC, 2000). The International Dairy Federation and Food and Drug Administration (FDA) certified all equipment used in the analyses. Milk yield was recorded at each milking and added together for daily milk yield data. Cumulative water intake was recorded daily. Cows were fed twice/d and refusal was measured once/d.

Animal Measurements

Core body temperatures indices were recorded four times/d at the following locations: ST of both S and US areas were obtained at the rump, ST-R-S, ST-R-US shoulder, (ST-S-S,

89 ST-S-US, and tailhead ST-T-S, ST-T-US using an infrared temperature gun
 90 (Raynger[®]MX[™] model Ray MX4PU Raytek C, Santa Cruz, CA). Rectal temperatures
 91 were obtained 4 times/d using a YSI rectal thermometer (Yellow Springs Instruments,
 92 Yellow Springs, Ohio). Temperature loggers (ibutton thermochrons, Maxim Dallas
 93 Semiconductor, TX) were used as a means to record core body temperature circadian
 94 patterns. The manufacturer reports a measurable range of +15 to +46°C measuring 1/8°C
 95 increments with ±1°C accuracy. The temperature loggers were also calibrated by placing
 96 them in our laboratory using culture ovens set at 38.5 or 42°C for 24 h. The data collected
 97 was then extracted into excel files and analyzed for variance. Based on these analyses we
 98 found the variability to be $38.5 \pm 0.70^{\circ}\text{C}$ and $42 \pm 0.40^{\circ}\text{C}$. The mean offset for each
 99 thermochron button was found to be consistent from one calibration to the next and was
 100 subsequently used as a co-variate when vaginal temperature measurements were
 101 analyzed. The calibrated ibuttons were then attached to blank continuous intravaginal
 102 drug release devices (CIDR's, Pfizer Inc., New York, NY) and inserted in to the vagina
 103 of the animal on the d 4 of and removed on d 7 of P1 and P2. To measure insensible heat
 104 loss, respiration rates (**RR**) were obtained by visually counting flank movements during a
 105 15 s interval and multiplying by 4 and evaporative heat loss (**EVHL**) of the shoulder
 106 shaved (**EVHL-SH**) and unshaved (**EVHL-US**) areas were also measured four times/d
 107 using an evapometer (Delfin Technologies, LTD., Finland). Total stored heat was
 108 calculated using the formula: body temperature, °C x specific heat of tissue, (0.8°C) x
 109 body weight, kg) (Sawka and Castellani, 2007; Silanikove, 2000). These measures were
 110 then summed across days and cows and divided by total animal numbers and days in a
 111 period to obtain average total stored heat per group. Average metabolic rate (basal
 112 metabolism plus milk energy) was calculated using the formula: $70.5 \times (\text{body weight, kg})$
 113 $^{0.734} + (\text{milk yield, kg} \times 750 \text{ kcal/kg})$ (Kibler and Brody, 1944). Average total metabolic
 114 rate/kg was then calculated by summing daily averages of all cows for each period (TN
 115 and HS) and dividing by number of cows, cow weights and days.

116 Blood samples were collected into heparanized tubes using coccygeal
 117 venipuncture from individual cows at 1200 h one day prior to start of TRT, and d 1 and 7
 118 of P1 and P2. Plasma was then harvested after centrifugation and then stored at -20 °C
 119 until analysis Cows were weighed three times throughout the study, beginning, middle,
 120 and end.

121

122 ***Ambient temperature (AT), THI, RR, ST, SR, and BW***

123 Data loggers which were hard-wired into each environmental room continuously
 124 recorded ambient temperature; relative humidity and black globe temperature at 15 min
 125 intervals each day in both environmental chambers using a computer based program
 126 (PARC Control Coding[™], Copy write 2003-2008, John R. Bauer, LLC.).

127

128 ***Free Serum Niacin Concentrations***

129 Plasma harvested from blood samples obtained at 1200 h was split into two
 130 aliquots and frozen at -20°C for later analysis of niacin concentrations using the
 131 VitaFast[®] (R-Biopharm, Dharmstadt, Germany) Niacin microbiological assay.

132 ***Statistics***

Data was analyzed using ANOVA procedures of SAS (SAS, 1999). Milk yields and DMI (recorded during the acclimation period and prior to treatment or environment initiation) were included as a covariate in the analysis. Dependent variables tested were milk yield, DMI, ST (rump, shoulder, tail head, shaved and unshaved areas), EVHL, RR, core body temperature, SNF, lactose, fat, protein, SCC, and water intake. The independent variables included treatment, day, parity, time of day, room, period, and the respective interactions. The level of significance was set at $P < 0.05$ for all main effects and interactions and the LSMEANS test was conducted when significance was detected.

Trial Two

The study was conducted from August 7th to October 7th, 2007 on a 10,000 cow commercial dairy in Stanfield, Arizona. The University of Arizona's Institute of Animal Care and Use Committee approved all protocols and use of animals in the current study. Four hundred and twenty seven lactating primiparous and multiparous Holstein cows were balanced for DIM (166 ± 11), milk yield (95.90 ± 23.1 kg/d) and parity (1.73 ± 0.2) prior to start of the study and assigned to either a control (C; $n=213$) with 0 g encapsulated niacin/d or treatment (Trt; $n=214$) consisting of 12 g encapsulated niacin/d (NIASHURE™®; Balchem Corp. New Hampton, NY) in a crossover design. All cows were fed a totally mixed ration three times daily. A separate premix of encapsulated niacin was made prior to feed mixing. The diet analysis was conducted by Chandler Analytical Laboratories). Cows were fed for ad libitum intake to allow 3-5 % feed refusal daily. Cows remained on C or Trt for 30 d, and then on d 31 were assigned to the opposite group for an additional 30 d. Milk yields were individually measured three times daily and sampled once during each 30 d period for composition analysis. Individual milk samples were collected twice (once each 30 d period) and sent to the Dairy Herd Improvement Association of Arizona (DHIA; Tempe, AZ) for milk composition analysis. Milk samples were analyzed for somatic cell count, butter fat, protein, lactose and solids-non-fat [These all need AOAC method numbers]. Energy corrected milk yields were calculated using (milk, kg x $(0.327 + (\text{milk protein, \%}/100) + (12.95 \times \text{milk fat, \%}/100))$) and the 3.5% fat-corrected milk yields were calculated using (milk, kg x $(0.4255 + (16.425 \times \text{milk fat, \%}/100))$). Cows were fed three times daily and feed was pushed up every half hour. Feed was available at shaded bunks from the shade and cooler structures. There was no cooling over the feed bunks. All cows were housed in open dry-lot facilities with Saudi style shades, and pens were identical in size, location, and design. In addition, pens were located across from each other and housed with the same number of animals.

All cows were cooled using Korral Kool coolers (Korral Kool, Mesa, Arizona) which are reverse chimney evaporative cooling systems that were mounted into a conventional corral shade (Armstrong, 1993). These systems cool the environment surrounding the cow by injecting micron sized (30-65 microns @ 300 psi) water droplets into the air moving down the cooler (Ryan et al., 1992; Armstrong, 1994). Coolers are set to turn on and off depending upon ambient temperature. During the months of August and September coolers were turned on when temperature exceeded 25.6°C and off when temperature dropped below 23.9°C; however, during the month of October coolers turned

on when temperature exceeded 26.7°C and off when temperature dropped below 25.6°C. In addition, curtains are suspended from the edge of the shades in order to prevent exposure to solar radiation during late afternoons. Curtains were scheduled to come down at an ambient temperature exceeding 27.8°C and rolled up at a temperature below 27.8°C.

Temperature loggers (ibutton thermochrons, Maxim Dallas Semiconductor, TX) were used as a means to record and measure core body temperature. The manufacturer reports a measurable range of +15 to +46°C measuring 1/8°C increments with ±1°C accuracy. Although the manufacturer reports average accuracy for the data loggers each temperature logger was individually calibrated in our laboratories. Loggers were placed in one of two incubators set at 38.5 or 42°C for 24 hours after which they were kept at room temperature for 24 hours and then placed in the opposite incubator for 24 hours. During that period that the temperature loggers were recording data the digital temperatures for the incubator were recorded. Based on these calibrations we produced an offset for each logger which was then used as a co-variate in the statistical analysis of vaginal temperatures. Temperature loggers were then attached to blank cervical implant drug release (CIDR; Pfizer Animal Health, Kalamazoo, MI) devices and inserted into the vagina of a random sub-sample of animals (n=16; 8 primiparous and 8 multiparous cows) from each pen (n=2) with similar DIM, milk yield, and parity. The devices were inserted two weeks after being on either C or Trt during each 30 d environmental period. Data was collected from the temperature loggers for seven days after insertion. Temperature loggers were set to record and store core body temperatures every five minutes throughout the seven day insertion period; which was then downloaded onto a computer. Three temperature loggers (HOBO: H08-032-08; Onset Computer Corporation, Pocasset, MA) were placed in the north, center, and south side of both pens. Three were placed along the feed bunk and three between coolers directly behind the feed bunks, approximately 8 feet from the ground. Loggers recorded the ambient temperature and relative humidity every 15 minutes beginning one week after study began until the end of the study.

Environmental conditions outside of the barn were recorded from Arizona Meteorological Network (AZMET; <http://ag.arizona.edu/AZMET>) site which was located near (5 km) the dairy. The AZMET uses several devices to record minimums, averages, and maximums of ambient temperatures, relative humidities, dew and dew points daily.

Statistical Analyses

All data was analyzed using PROC MIXED for analysis of repeated measures and PROC GLM procedures of SAS for non continuous variables (SAS, 1999). Dependent variables tested were milk yield, DMI, core body temperatures, fat-corrected milk, energy-corrected milk, solids-not-fat (SNF), lactose, fat, somatic cell count and protein. The independent variables included trt, parity, period, sequence, and the respective interactions. The level of significance was set at $P < 0.05$ for all main effects and interactions and the LSMEANS test was conducted when significance was detected. If the higher order interactions were not significant then they were removed from the model.

RESULTS

Trial One

Plasma niacin concentrations were measured throughout the study. Prior to experiment initiation free plasma niacin levels did not differ between treatments (1.32 vs. 1.38 $\mu\text{g/mL}$), for C and NI groups, Figure 1). By the end of TN, plasma niacin concentrations were higher in animals supplemented with encapsulated niacin compared to C (1.75 vs. 1.50 $\mu\text{g/mL}$, $P < 0.03$). Plasma niacin remained elevated in NI cows compared to the controls throughout HS (1.65 vs. 1.44 $\mu\text{g/mL}$, $P < 0.03$). However, free plasma niacin did not differ between dietary groups by 3d following removal of NI, (1.50 vs 1.51 $\mu\text{g/mL}$, NS).

Environmental conditions controlled during this study were TN and mild to moderate HS (Figure 2). During TN the minimum THI was 52 and the maximum was 66 (Figure 1). During HS, the minimum THI was 67 while the maximum was 79 which resulted in an environment where the THI was greater than 72 over 14 h/d (Figure 1). Relative humidity was kept constant at 18% for both periods throughout each day (Figure 1). Add text here on feed intake, water intake, and milk yield Table 1 Take rectal temperatures and respiration rate from Table 1 and move to Table 3. Then insert here the results on milk composition (Table 2)

Milk yield did not differ between dietary groups or periods (environments; $P = 0.17$; Table 1). Dry matter intake was not affected by diet however, DMI decreased during HS (38.9 vs. 37.7 kg/d; $P < 0.05$, Table 1). Water intake tended to be higher for NI animals ($P = 0.11$) regardless of environment; however during HS C and NI fed cows had higher water intakes, respectively (40.4 vs. 52.7 and 48.6 vs. 57.7 L/d; $P < 0.01$, Table 1). Milk fat percentages did not differ between dietary groups ($P = 0.55$; Table 2) however during HS the NI group had a tendency for lower percentages (3.51 vs. 3.77 %; $P = 0.06$; Table 2). Milk protein percentages were lower for animals fed encapsulated niacin (2.84 vs. 2.93 %; $P < 0.001$; Table 2) however, during HS protein percentages were higher for both groups (C: 2.86 vs. 2.99 and NI: 2.76 vs. 2.91 %; $P < 0.001$; Table 2). Lactose was not affected by treatment or period. Solids-not-fat was lower for cows fed encapsulated niacin (8.52 vs. 8.61 %; $P < 0.01$; Table 2). Also during HS both groups had increased solids-not-fat percentages (C: 8.67 vs. 8.55 and NI: 8.59 vs. 8.44 %; $P < 0.001$). Somatic cell counts were lower for cows in the NI group (175 vs. 980 cells/mL $\times 1000$; $P < 0.001$; Table 2).

Surface temperatures obtained from the shoulder, rump and tail head were unaffected by NI but were altered by shaving (32.5 S vs. 31.4 US $^{\circ}\text{C}$, Table 3). All ST in both groups were higher in HS compared to TN (Table 3). Cows fed NI had higher EVHL (57.4 vs. 52.7 $\text{g/m}^2/\text{h}$, EVHL-US, $P < 0.05$, Table 3) or were numerically higher, (66.3 vs. 57.8 $\text{g/m}^2/\text{h}$, EVHL-S, $P = 0.11$) over the entire 24 h period. Furthermore, these differences became larger during peak thermal stress. The EVHL for NI fed cows were higher than C (81.1 vs. 68.2 $\text{g/m}^2/\text{h}$, $P < 0.0001$) during HS between 11:00 AM and 4:00 PM. The NI cows had lower average RT during HS compared to C fed cows (38.17 vs. 38.34 $^{\circ}\text{C}$; Table 3) and lower mean vaginal temperatures for the 72 h data collection, from d 4 thru d 7, (38.0 vs. 38.4 $^{\circ}\text{C}$; $P < 0.001$). Respiration rates tended to be higher for NI

cows compared to C fed cows during both TN and HS ($P = 0.14$; Table 3). Control and NI groups had higher respiration rates than C cows during HS, (30.6 vs. 50.8 and 32.5 vs. 54.5 bpm; $P < 0.0001$; Table 3).

Trial Two

Dry matter intakes were 26.9 kg/d for the C group and 27.0 kg/d for the Trt group and were not different, Table 4. Milk yield also did not differ for animals supplemented with encapsulated niacin or cows in the C group (37.6 vs. 37.5 kg/d $\pm$ 0.30; $P > 0.10$; Table 4). Average milk protein percentage was increased for cows supplemented with encapsulated niacin compared to the C group (3.09 vs. 3.05% $\pm$ 0.01; $P < 0.01$; Table 4). In addition, average milk fat percentage was elevated for cows supplemented with encapsulated niacin compared to the C group (3.65 vs. 3.38% $\pm$ 0.04; $P < 0.01$; Table 4). Milk yields on the day of testing for milk components were not different between the Trt and C group (38.65 vs. 38.36 kg/d $\pm$ 0.72; $P > 0.10$). Somatic cell counts were also not affected by treatment or parity (115 vs. 116 cells/mL $\times$ 1000 $\pm$ 11.76; $P > 0.10$). Solids-not-fat were not different between treatments but were different between parity (Primiparous 8.82 vs. Multiparous 8.71% $\pm$ 0.02; $P < 0.01$). Lactose also did not differ between treatments; but, there was an effect of parity (Primiparous 4.87 vs. Multiparous 4.75% $\pm$ 0.01; $P < 0.01$). The 3.5% fat-corrected milk yield was elevated for cows in the Trt group compared to cows in the C group (39.62 vs. 38.14 $\pm$ 0.38 kg/d; $P < 0.01$; Table 4). There was also a difference in 3.5% fat-corrected milk yields due to parity (primiparous: 37.15 vs. multiparous: 40.6 kg/d $\pm$ 0.4; $P < 0.01$). In addition, cows in the Trt group had increased energy-corrected milk yields compared to cows in the C group (39.51 vs. 38.26 $\pm$ 0.34 kg/d; $P < 0.01$; Table 4); this was also affected by parity (primiparous: 37.2 vs. multiparous: 40.6 $\pm$ 0.4 kg/d; $P < 0.001$). There were no parity effects on intravaginal temperatures, therefore parity groups were combined within treatment. Average core body temperatures during the hottest part of the day (1300 to 1600 h) were lower for cows in the Trt group compared to the cows in the C group (38.52 vs. 38.65°C $\pm$ 0.04; $P < 0.01$).

In this study, THI was calculated for each day throughout the study, inside the barns from the HOBO loggers or outside the barn from AZMET, (data not shown). Based on AZMET, THI was only below 72 (indicative of heat stress) four days of the 60 d study. For the months of August and September 2007, THI never decreased below 80. Inside the barns, the environment for both pens throughout the study did not differ from each other therefore both C and Trt groups experienced similar environments.

DISCUSSION

Studies have shown that both nicotinamide and nicotinic acid are synthesized in the rumen (Santschi et al., 2005) However, there is little ruminal escape of niacin to the small intestine, (Campbell et al. 2004). Absorption through the ruminal wall is also a plausible route of niacin uptake as when nicotinamide is supplemented the levels of free niacin were increased but the extent was minimal (Santschi et al., 2005). However, these investigators reported that minimal niacin escaped ruminal degradation therefore the

benefits reported when niacin has been fed are likely due to its effects in the rumen or the diffusion across the gastrointestinal wall prior to the duodenal cannula (Santschi et al., 2005). Other researchers have also found that nicotinamide is absorbed through the rumen wall at a rate of 0.98 g/h and nicotinic acid was not absorbed during a 1-hour period (Erickson et al., 1991). It has also been reported that when large quantities of niacin in their free form are present absorption through the rumen wall can occur (Rérat et al., 1958b). When nicotinamide was infused post-rationally duodenal flows of nicotinamide were not increased however, duodenal nicotinic acid concentrations were increased (Santschi et al., 2005). This suggests that there is rapid conversion of nicotinamide to nicotinic acid however; this might be occurring in the abomasum where the environment is more acidic (Santschi et al., 2005). The plasma free niacin data from this study supports the concept that encapsulation of niacin would improve rumen bypass and lead to increased blood niacin concentrations. Since the majority of niacin leaving the intestine and entering the vascular system is rapidly taken up by red blood cells (Klein et al. 1942) it is interesting that we were able to detect a significant increase in plasma niacin. Niacin in blood is stored as pyridine nucleotides Levitas et al., 1947. We did not measure blood pyridine nucleotide content in this study. It is possible that greater nicotinamide differences between supplemented and control animals existed in whole blood than we detected in plasma. Future studies will need to address this question.

The NRC (2001) estimated the synthesis and absorption of niacin from the small intestine and reported that 1804 mg/d are synthesized in the rumen with an escape of 6%. Santschi et al., (2005) reported the disappearance of niacin as nicotinamide to be 98.5% prior to the small intestine. Although the percent of actual niacin found in the encapsulated product is only 65% compared to 100% in raw niacin; the rumen stability is 80% greater in the encapsulated form resulting in the estimated bioavailability of encapsulated niacin to be 35% greater than raw niacin (Santschi et al., 2005; Deuschler et al., 1998).

Very few studies have reported plasma or serum concentrations or whole blood concentrations of niacin in cattle supplemented with niacin (Jaster et al. 1983, Campbell et al., 1994). Data from the analysis of plasma free nicotinic acid and nicotinamide in this study indicate that feeding encapsulated niacin increased plasma concentrations of niacin while cows were being supplemented.

The majority of niacin absorbed across the gut wall in cattle is rapidly incorporated and stored in red blood cells (Campbell et al., 1994). However, there is measurable free niacin in blood (Campbell et al., 1994). At the start of study one, plasma niacin in NI and C fed cows did not differ. Plasma niacin concentrations in study one were in agreement with previously reported values for lactating dairy cattle (Jaster et al., 1983, Campbell et al. 1994). Supplementing encapsulated niacin increased plasma levels of free niacin during both TN and HS periods. Serum niacin concentration levels in NI fed cows returned to pre-supplementation values by 3 d post supplementation (1.51 vs. 1.50 µg/ml). Thus, feeding encapsulated niacin at a dose of 12 g/cow/d increased free plasma niacin concentration. It is possible that measuring total blood niacin would

provide greater differences in niacin concentrations between NI and C animals since the majority of niacin in blood is stored in red blood cells (Campbell et al. 1994).

No previous studies have evaluated use of supplementary dietary encapsulated niacin on evaporative heat loss and heat storage in lactating dairy cows. Given the effects of niacin on cutaneous blood flow one might predict an increase in heat loss in niacin supplemented cows subjected to heat stress. Previous studies in heat stress models have involved supplementing niacin in a raw form that is not encapsulated and have resulted in inconclusive results. Environments implemented during this study were considered thermoneutral and mild to moderate HS. The HS environment caused a 0.5° C rise in vaginal temperature in C fed cows but no change in vaginal temperature in TRT fed cows. Average RT during HS were also reduced by 0.17°C in NI compared to C fed cows. Although the HS environment altered RT and RR there was no apparent effect on milk yield of the cows. Study one was conducted in September and the cows utilized were well adapted to the summer heat in Arizona. It is possible that winter adapted cattle would have been more severely stressed.

It is also apparent that removal of the hair coat increased EVHL in both C and NI groups. This is likely due to increased airflow over the skin surface in shaved areas due to hair removal (Gebremedhin and Wu, 2001). The increases in EVHL in NI cows appears to be associated with increased EVHL, via increased sweating rate since it was most apparent in unshaved areas of the hair coat. This suggests that one effect of NI feeding is increased sweat gland activity. Vaginal probes were inserted during days 4-7 of each period to record circadian patterns of core body temperatures in study one. During TN there were no treatment differences in core body temperature patterns however during HS; NI cows had significantly lower core body temperatures than C fed cows (Figure 2). This is further supported by the increased EVHL in NI cows. The vasodilatory effects of niacin have been shown by others to act through prostaglandin D production by epidermal Langerhans cells (Benyo et al., 2006; Maciejewski et al., 2006) and vascular endothelial prostaglandin D2 receptors (Cheng et al., 2006). Niacin has also been known to induce vasodilation of the skin in humans (Gille et al., 2008); this mechanism appears to be associated with the reduction in core body temperature and increases in EVHL during HS in this study. Thus, the hypothesis that increased vasodilation induced by NI would lead to increased heat loss and lower body temperatures in HS cows seems to be validated. However, the cause of the increased EVHL (direct or indirect) in NI fed cows remains to be elucidated.

During both periods of trial one, water intake was greater for the NI cows but this difference was greatest during HS, (Table 1). This is possibly related to a numerically higher milk yield and increased insensible heat loss through increased respiratory and EVHL in the NI cows experiencing thermal stress during HS. However, since urinary water loss was not measured it is not possible to be definitive regarding the reason for increased water intake other than to state that it is associated with higher EVHL and milk yield in NI cows.

During summer months environmental conditions (such as those implemented in trial one), can cause heat stress resulting in enhanced heat storage in dairy cows. This

396 results in a reduction in dry matter intake which contributes to a decrease in milk yield.
397 However, others have recently estimated that only 50% of the loss in milk yield during
398 thermal stress is related to a reduced dry matter intake (Wheelock et al., 2006; Rhoads et
399 al., 2007). Thus, increased heat storage must also be associated with changes in whole
400 body and mammary metabolism (Rhoads et al., 2007; Wheelock et al., 2006). Therefore,
401 alleviating some or all of the increased heat storage may reduce the effects of heat stress
402 on lactating dairy cows.

403 There were no differences in DMI between dietary treatments which agrees with
404 previous nicotinic acid or nicotinamide research (Kung et al., 1980; Jaster and Ward,
405 1990). As expected, heat stress tended to decrease DMI in both C and NI cows. Milk
406 yields were numerically higher in NI compared to C however; these differences existed
407 prior to study initiation and were unexpected since the groups were balanced for parity
408 and yield during treatment assignment. As stated earlier, supplementing lactating dairy
409 cows with raw niacin has resulted in inconclusive results where some have found
410 increases (Muller et al., 1986; Drackley et al., 1998) in milk yield others have reported no
411 difference (Di Constanzo et al., 1997; Madison-Anderson et al., 1997). Milk component
412 differences found in this study were most likely attributed to the existing milk yield
413 differences prior to the start of the study or small number of animals (n=12) used in the
414 study rather than actual dietary effects.

415 A previous study did not detect a difference in rectal temperature, or respiration
416 rates in cattle fed raw niacin during thermal stress (Di Constanzo et al., 1997). However,
417 the niacin used in the study was not encapsulated, the time of day temperatures were
418 recorded varied and no other study has reported continuous circadian patterns of core
419 body temperature measurement in cattle fed encapsulated niacin. Some studies have
420 reported a decrease in ST's at different times throughout the day and have yet to be
421 consistent throughout all studies (Di Constanza et al., 1997). Also, previous studies were
422 conducted using raw or non-encapsulated products of niacin, nicotinic acid, or
423 nicotinamide (Jaster and Ward, 1990; Kung et al., 1980; Di Constanzo et al., 1997;
424 Madison-Anderson et al., 1997) Therefore, the results from Study One are unique and
425 suggest more research should be conducted on evaluating encapsulated niacin effects on
426 alleviating heat stress.

427
428 In study one, there were numerical increases in milk yield and feed intake in the
429 treatment group; however only 12 cows were used in the study. Although milk yield was
430 not increased, cows that had been supplemented with encapsulated niacin had increased
431 average evaporative heat loss compared to animals in the C group, and the differences
432 were more apparent during peak thermal stress. The difference was attributed to animals
433 being able to dissipate more heat if supplemented with encapsulated niacin. In study one,
434 cows in the Trt group had lowered rectal and core body temperatures compared to the C
435 group when subjected to heat stress. When under heat stress, animals on Trt had reduced
436 heat storage compared to cows in the C group which was calculated during the hottest
437 part of the day (1400 to 1700 h). Based on these results, study two was conducted to
438 evaluate whether encapsulated niacin could reduce core body temperatures and improve

production parameters during heat stress in a commercial field study with large numbers of animals.

Based on the environmental conditions from the meteorological station, the combination of temperature and humidity were elevated inducing a THI above 72 (indicative of heat stress) in Arizona from August 7th to October 7th 2007. Inside the barn, THI dropped below 72 for four days throughout the study which could be due to a drop in ambient temperature or relative humidity during the end of September and October months. Despite those four days, THI values stayed similar throughout the study providing consistent heat stress for both C and Trt groups during the study.

Energy- and 3.5% fat-corrected milk yield were increased due to an increase in milk protein and butter fat (Table 5). The reason behind the milk protein and butter fat increase cannot be explained by a dilution factor as milk yield on the day of testing for milk components was not different between NI or C groups. Drackley et al., (1998) reported a tendency for milk fat yields to be increased when fed nicotinic acid and supplemental fat. Furthermore, Harrison et al., (1995) demonstrated an increase in milk fat when raw niacin supplemented cows were also fed whole cottonseed and calcium salts of long-chain fatty acids. Others have observed a tendency for milk fat and protein yields to be increased; however, the response was attributed to increased milk yields (Di Constanzo et al., 1997). Milk yields were not increased in this study, therefore the increase in milk protein and fat percentages maybe due to interactions with the supplemental fat in the diet. The mechanism behind an increase in milk fat and protein percentages when encapsulated niacin is fed and possible interactions with other feed additives such as supplemental fat is unknown.

Vaginal temperature probes were inserted for a 7 d during both 30 d periods measuring core body temperatures. During the hottest part of the day (1300 to 1600 hrs) cows supplemented with encapsulated niacin had reduced core body temperatures on average of 0.13°C (Table 1). Therefore, the vasodilation mechanism associated with niacin and the decrease in core body temperature is in agreement with recent studies observing the same effects with either feeding raw niacin at 12, 24, and 36 g/d for three consecutive periods or 12 g of encapsulated niacin during a mild to severe heat stress (Di Constanzo et al., 1997; Study One). Research on effects of feeding encapsulated niacin during thermal stress is limited and studies utilizing raw niacin have been inconclusive. Due to differences in biological availability studies utilizing raw niacin cannot be compared to the effects of encapsulated niacin (Jaster and Ward, 1990; Kung et al., 1980; Madison-Anderson et al., 1997; Di Constanzo et al, 1997). Studies investigating encapsulated niacin at different dosage levels, physiological windows (transition and (or) breeding period), and effects on reproduction are warranted.

CONCLUSION

In a small designed study with 12 cows in environmentally controlled rooms, supplementation of encapsulated niacin (12 g/d) to thermally stressed lactating dairy cows increased EVHL and this was associated with increased water intake, decreased RT, vaginal temperatures and RR. In a large commercial herd using 427 cows the dietary supplementation of niacin (12 g/d) reduced core temperatures during the hottest part of

the day and increased fat-corrected and energy-corrected milk yield. Further research into use of niacin supplementation of lactating dairy cows during heat stress is warranted.

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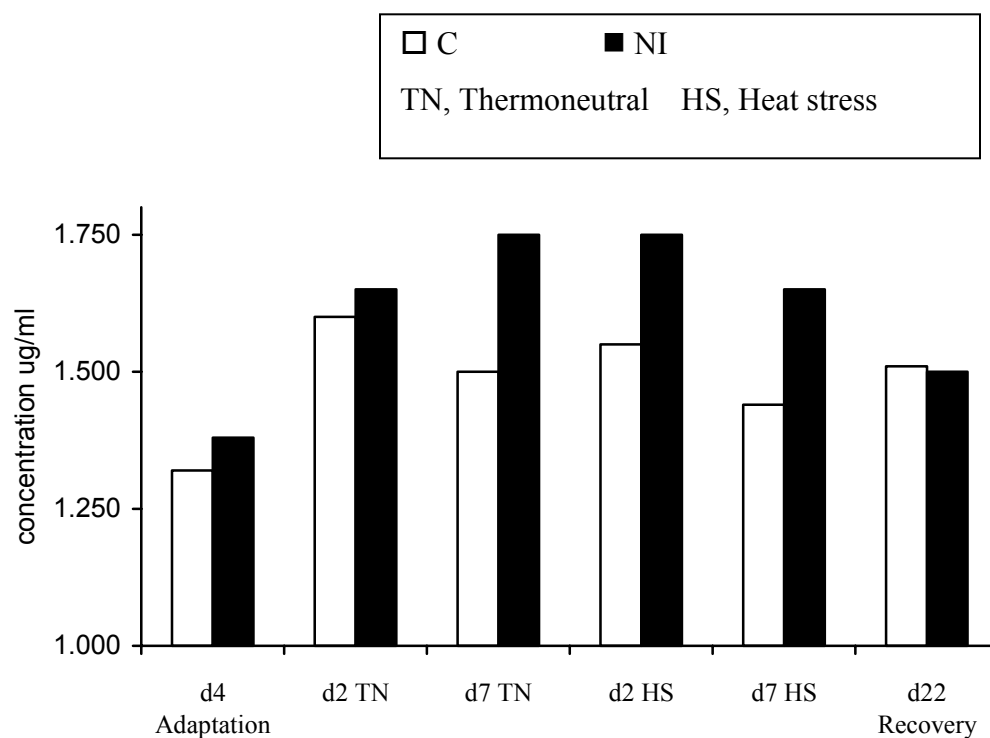
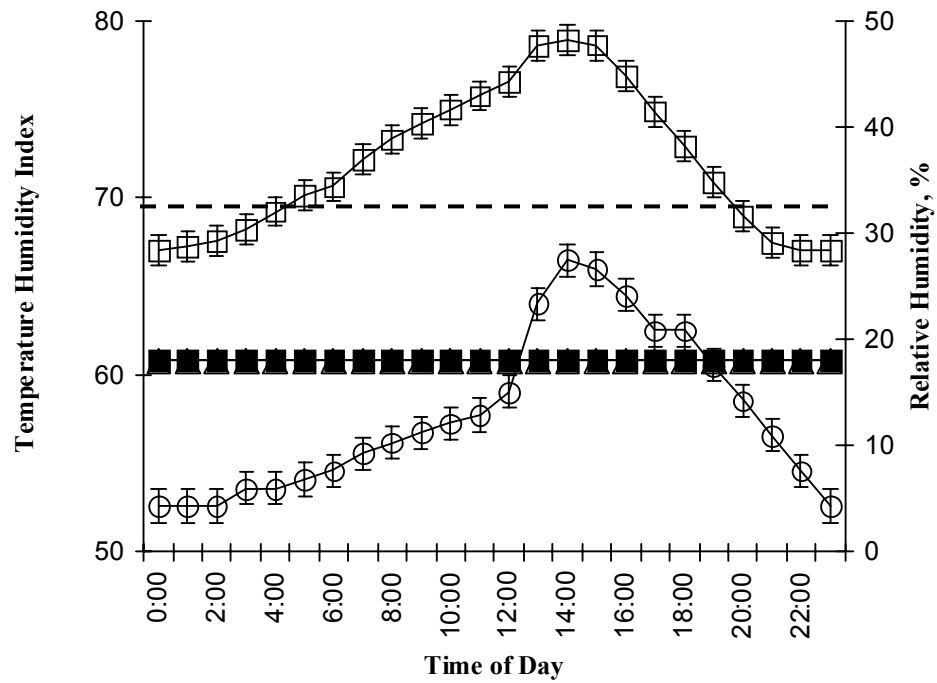


Figure 1. Serum concentrations of free niacin in animals supplemented with 0 g (C) or 12 g of encapsulated niacin (NI).

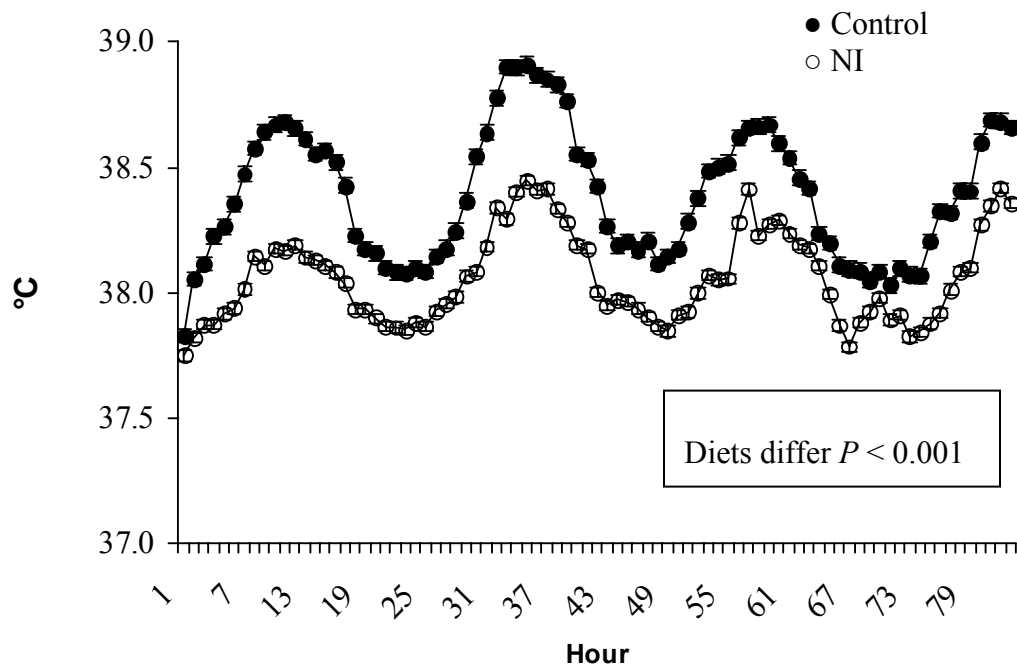


○ = THI-TN (Period 1) □ = THI- HS (Period 2)
 ■ = Relative Humidity-TN (Period 1) △ = Relative Humidity-HS (Period 2)
 ----- Line represents 72 THI

Figure 2. Twenty four hour circadian patterns for thermoneutral (TN, Period 1) and heat stressed (HS, Period 2) periods.

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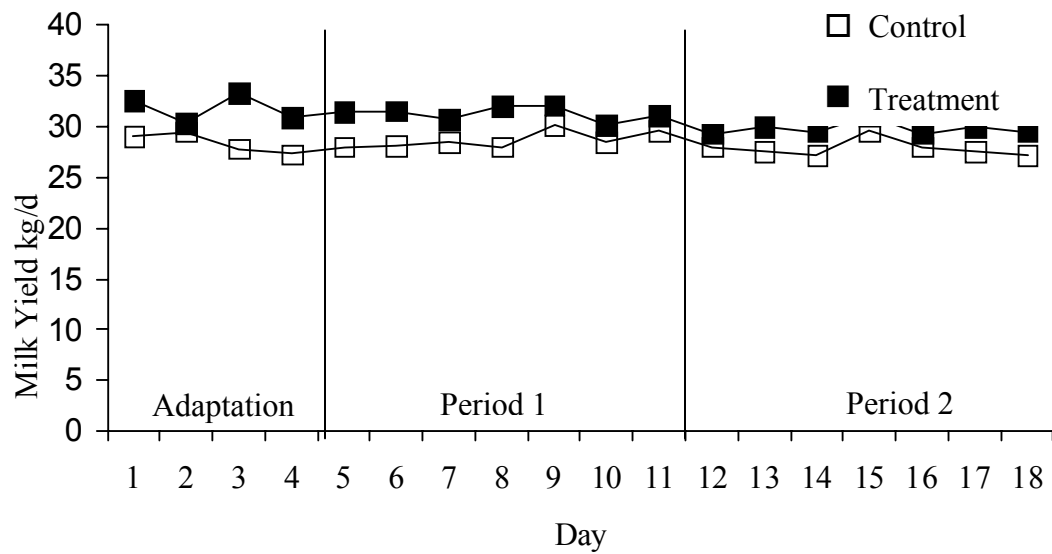


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618 **Figure 3.** Core body temperatures during Heat Stress (HS) from day 4 to day 7 in control
619 (C) and niacin (NI) fed animals.

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623 **Figure 4.** Temporal pattern of milk yield during adaptation, period 1 (thermoneutral) and
 624 2 (heat stress). $P = 0.14$; SEM = 0.34

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Table 1. Effect of Feeding Niashure on dry matter intake, water intake, respiration rate, and milk yield in lactating Holstein Cows.

Variable	(TN)		(HS)		Diet		Period		Diet*Period	
	C ¹	NI ²	C	NI	SEM	P	SEM	P	SEM	P
Dry Matter Intake, kg/d	39.1	38.7	38.8	36.7	1.69	0.69	1.74	0.05	1.75	0.14
Water Intake, L/d	40.4	52.7	48.6	57.7	1.01	0.11	0.68	<0.01	0.64	0.45
Milk Yield, kg/d	29.3	30.5	29.4	29.6	0.35	0.17	0.32	0.35	0.47	0.25

¹C= Control (og Niashure™)

²NI= Treatment (12g Niashure™)

Table 2. Effect of feeding Niashure on milk composition analysis.

Variable	Period 1		Period 2		Diet		Period		Diet*Period	
	C ¹	NI ²	C	NI	SEM	P	SEM	P	SEM	P
Fat, %	3.94	4.00	3.77	3.51	0.16	0.55	0.16	0.06	0.20	0.36
Protein, %	2.86	2.76	2.99	2.91	0.02	<0.001	0.02	<0.001	0.03	0.77
Lactose, %	4.68	4.66	4.69	4.69	0.03	0.66	0.03	0.62	0.04	0.75
Solids-not-fat, %	8.55	8.44	8.67	8.59	0.02	<0.01	0.02	<0.001	0.03	0.71
Somatic cell count, cells/mL x 1000	1210	249	749	101	149	<0.001	149	0.15	210	0.46

¹C= Control (0g Niashure™)

²NI = Treatment (12g Niashure™)

Table 3. Effect of feeding Niaschure on surface temperatures, evaporative heat loss (EVHL) respiration rate and rectal temperatures in lactating Holstein Cows.

Variable	Period (TN)		Period 2 (HS)		Diet		Period		Diet*Period	
	C ¹	NI ²	C	NI	SEM	P	SEM	P	SEM	P
Surface Temperature, °C										
Shoulder, shaved	31.3	30.9	34.3	34.1	0.18	0.62	0.18	<0.01	0.24	0.88
Shoulder, non-shaved	29.9	29.6	33.4	33.6	0.21	0.32	0.20	<0.001	0.26	0.23
Rump, shaved	31.4	31.3	34.5	34.5	0.16	0.85	0.17	0.51	0.23	0.74
Rump, non-shaved	30.4	30.3	33.8	33.7	0.24	0.92	0.21	<0.01	0.28	0.97
Tail head, shaved	30.5	30.7	33.4	33.7	0.19	0.18	0.19	<0.05	0.26	0.89
Tail head, non-shaved	28.4	28.5	32.8	32.6	0.28	0.93	0.26	<0.001	0.34	0.65
EVHL ³										
Shaved, g/m ² /h	23.2	18.3	92.4	114.4	5.30	0.11	5.80	<0.001	7.53	0.07
Non-shaved ⁴ , g/m ² /h	18.2	13.1	87.2	101.7	4.93	<0.05	4.79	<0.001	6.34	0.08
Respiration Rate, bpm	30.6	32.5	50.8	54.5	1.78	0.14	2.12	<0.001	2.12	0.59
Rectal Temperatures, °C	38.01	38.06	38.34	38.17	0.06	0.05	0.07	<0.001	0.08	0.07

¹C= Control (0g Niaschure™)

²NI = Treatment (12g Niaschure™)

³Closed chamber evapometer

⁴EVHL average for all 4 measurement times

Table 4. Least square means and pooled SE for milk yield, energy-corrected milk, 3.5% fat-corrected milk, milk components and core body temperatures for cows either fed a control (0g/d/cow of encapsulated niacin) or treatment (12 g/d/cow of encapsulated niacin) diet. Milk tests were taken two weeks after the beginning of the study for each monthly experimental period (n=2).

Item	C ¹	Trt ²	S.E.	P value
Milk yield, kg/d	37.53	37.61	0.31	NS
3.5% Fat-corrected Milk Yield ³ , kg/d	38.14	39.62	0.83	<0.01
Energy-corrected Milk Yield ⁴ , kg/d	38.26	39.51	0.76	<0.01
Milk fat, %	3.38	3.65	0.04	<0.01
Milk protein, %	3.05	3.09	0.01	<0.01
Somatic cell count, cells/mL x 1000	115.22	116.00	11.76	NS
Solids-not-fat, %	8.75	8.78	0.02	NS
Core body temperature, °C	38.65	38.52	0.04	<0.01

¹C= Control (0g/d/cow of encapsulated niacin)

²Trt = Treatment (12g/d/cow of encapsulated niacin)

³ 3.5% Fat-corrected Milk = milk, kg x (0.4255 + (16.425 x milk fat, %/100))

⁴ Energy-corrected Milk = milk, kg x (0.327 + (milk protein, %/100) + (12.95 x milk fat, %/100)))