CHAPTER 5

DISCUSSION

Climatic conditions in the present study are such that the hot season is relatively long, and generally accompanied by high relative humidity. Thus heat stress is chronic in nature and there is little relief from the heat during the evening through to the morning, and also includes intense bursts of combined heat and humidity which further depresses performance.

Environmental modifications, as described in materials and methods, led to a decrease in the ambient temperature and an increase in the relative humidity, during the day. The air temperature in the tunnel barn was up to 6 °C cooler ($P<0.05$) during the daytime than that in the outside barn, while the relative humidity increased by up to 16%. As air was drawn through the wet cooling pads, water was evaporated into the air causing the temperature to be reduced while increasing the air moisture level.

The amount of initial moisture in the outside air will directly impact on how much reduction in air temperature might be expected. Therefore, when the outside air is initially very humid (relative humidity greater than 70%), the reduction in air temperature will be minimal (less than 2.8 to 5.6°C).

The average daily minimum and maximum temperatures were 24.2 ± 0.1 and 28.4 ± 0.1°C, respectively in the evaporative, cooled tunnel, ventilated barn and 24.7 ± 0.1 and 34.4 ± 0.1°C, respectively in the outside barn, indicating less variation and more consistency in this cooling system. This evaporative, cooled tunnel, ventilated barn reduced daily fluctuation in the ambient temperature, relative humidity and THI during hot-humid weather conditions.

The upper critical temperature for heat stress to begin was between 25 and 26°C (Berman et al., 1985). Evaporative cooling reduced ($P<0.05$) afternoon barn temperature, although relative humidity increased ($P<0.05$) when compared to the barn without a supplemental cooling system. Evaporative cooling with tunnel ventilation reduced afternoon heat stress of dairy cows. High environmental relative humidity reduced the cooling capacity of the evaporative cooling, but on the days when animals were observed it reduced heat stress of the lactating dairy cows. In the present study, THI in the tunnel ventilated barn was decreased when compared to the outside barn. There was no thermoneutal zone ($THI<72$) during the study but exposure to conditions of moderate heat stress ($THI\geq79$) that occurred outside was decreased by utilizing the evaporative cooling system. This difference in environmental conditions had a dramatic effect on the physiological response of the cows, as THI was highly correlated with both rectal temperature and respiration rate. The average rectal temperature and respiration rate in the cooled cows was lower ($P<0.05$) than in the uncooled cows. West et al. (2003) found that changes in cow body temperatures were most sensitive to same day climatic factors. The parameter has the greatest influence on cows a.m. milk temperature was the current day minimum air temperature, while the cow p.m. milk temperature was most influenced by the current day mean air temperature. These results suggest that evaporative cooling and tunnel ventilation has the potential to decrease exposure to heat stress and alleviate the symptoms of heat stress.

The average daily THI in the morning and afternoon in the evaporative, cooled tunnel, ventilated barn were lower ($P<0.05$) than that in the outside barn. However, the mean THI exceeded the critical point of 72 at daytime and nighttime, suggesting that the cows were exposed continuously to conditions conducive to mild heat stress for the cooled cows and moderate heat stress for the uncooled cows.

Metabolic hormone that affected by environmental temperature is cortisol, which is reported to rise acutely (Wise et al., 1988), but decline with more chronic exposure to heat load (Abilay et al., 1975). The levels of cortisol in the present study were greater than the range seen in nonstressed animals (Arave et al., 1996).

In hot, arid conditions this system would work well and evaporative cooling has already been used very successfully to cool such dairy cows (Ryan et al., 1992), but in high humidity locations its effectiveness would be limited by the evaporation potential. In the present study the air temperature increases during the day and decreases in the evening until the next morning. As the air temperature rises during the day, the relative humidity will decrease. Accordingly during the hottest portion of the day, the outside relative humidity dropped to a level that allowed for maximum evaporation potential, making the system effective for reducing the severity of heat stress.

Legates et al. (1991) reported that in field studies air temperature had the greatest impact on physiological measurement, while radiation was second in importance, followed by vapor pressure and air movement. Increasing the air temperature reduces the temperature differential between the cows body temperature and the ambient temperature and decreases the transfer of heat to the environment. As ambient temperatures increase in the presence of low or high relative humidity, the cooling mechanisms employed by the cows shifted from a nonevaporative processes (convective, conductive, and radiation) to evaporative (sweating and panting) (Kibler and Brody, 1953 cited by West et al., 2003), this demonstrate that the percentage of cooling originating from the non evaporative processes declines as ambient temperature increases, while the evaporative process increase. As a result the uncooled cows had greater ($P<0.05$) rectal temperatures and respiration rates than the cooled cows.

In the present study, rectal temperature was positively correlated with air temperature and THI but negatively correlated with DMI. Dry matter intake was positively correlated with milk production. Milk yield and DMI exhibited a significant decline when maximum THI reached 77 (Johnson et al., 1963 cited by West, 2003). There is a significant negative correlation between THI and DMI (Holter et al., 1996; 1997), and the effect of THI is probably mediated through the effects of increasing body temperature on cow performance.

Ravagnolo et al. (2000) reported that milk yield declined by 0.2 kg per unit increase in THI when THI exceed 72 and THI can be used to estimate the effect of heat stress on production.

Mean air temperature had the greatest influence on milk yield for Holstein cows under hot conditions (Kabuga and Sarpong, 1991 cited by West et al., 2003). Maust et al. (1972) reported that the mean daily ambient temperature was highly correlated with the p.m. rectal temperature and milk yield was highly correlated with the cows p.m. rectal temperature. The elevated p.m. rectal temperatures were associated with concomitant reductions in DMI and milk yield. Uncooled cows, in the present study, had rectal temperatures exceeded $39^{o}C$ at both the a.m. and the p.m. milking, when cow temperatures should have been near their lowest and highest points, respectively. Such consistently elevated rectal temperatures, result in a significant decline in DMI and milk yield when compare with the cooled cows which had a lower rectal temperature.

McGuire et al. (1989) reported that a portion of the negative effects of heat stress on milk production could be explained by decreased nutrient intake and decreased nutrient uptake by the portal drained viscera of the cow. Blood flow which moves to peripheral tissues for cooling purposes may alter nutrient metabolism and contribute to lower milk yields during hot weather

Providing cows with supplemental shade and cooling to mitigate heat stress has been evaluated in economic terms using the increase in milk production data (Armstrong, 1994). Adding sprinklers and fan cooling increased the feed intake (7.1 to 9.2%) and milk production (7.1 to 15.8%), and decreased rectal temperature (-0.4 to -0.5 °C) and respiration rate (17.6 to 40.6 %) (Bucklin et al., 1991).

In the present study the cooling system improved cow comfort and milk production. The cooling system decreased ambient temperatures and THI. The cows with this cooling system had decreased rectal temperatures and respiration rates, and increased feed intake (30.9 %) and milk production (42.5%). In addition, an analysis of the economics in the present study showed that the rate of return on investment in cooling equipment and additional feed plus electric costs of this cooling system, showed it was profitable in hot and humid conditions.

Responses to heat stress include panting and sweating. If these are not successful in alleviating the heat load, body temperature will rise. Increased body temperature will result in reduced feed intake, higher maintenance requirements (panting) and less efficient productive ability. The higher maintenance requirements dictates that cows need to increase feed intake to maintain milk production. However, this not possible as feed intake declines when ambient temperatures exceeded 26°C. For uncooled cows in the present study, as a result of this increase in requirement and a decrease in intake, milk production may decline as much as 25-30%, and typically they mobilize body reserves and lose body weight to maintain milk production until the intake of feed can match or exceed nutritional requirements (Butler and Smith, 1989; Nebel and McGilliard, 1993), thus entering a state of negative energy balance.

Dry matter intake in uncooled cows was lower ($P<0.05$) than in cooled cows. This resulted in uncooled cows having a prolonged period of negative energy balance, postpartum body weight was greater ($P<0.05$) in cooled than uncooled cows.

The state of negative energy balance is further characterized by changes in individual metabolites, most noticeably increases in plasma non-esterfied fatty acid (NEFA) and beta hydroxybutyrate (BHB). In the present study, there was no difference in plasma NEFA concentrations between the groups. In both groups, the highest concentration was observed wk 1 postpartum and decreased dramatically until wk 6 and 7 postpartum in both cooled and uncooled cows. When compared with the value wk 1 postpartum, the concentration of NEFA decreased by 65% in cooled cows and 72% in uncooled cows. Fat mobilization in early lactation is a mechanism which dairy cows use to cope with the higher metabolic demands of lactation (Coppock et al., 1974) producing greater plasma NEFA concentrations in early lactation (i.e. wk 1) compared with the succeeding weeks, as observed by others (Staples et al., 1990).

We found that the EB of lactating cows decrease during wk1 and 2 of lactation but tended to improve during wk 3 to 12 of lactation. NEFA levels were significantly greater only during wk 1 postpartum in both groups, which may indicate that these cows had high fat mobilization during this period. Both cooled and uncooled cows improved the EB as week postpartum progressed, but cooled cows attained a more positive EB at wk 3 to 12 postpartum than uncooled cows, even though milk yield tended to increase steadily. Probably these uncooled cows were not able to compensate for their higher maintenance requirements.

In the present study, there was greater ($P<0.05$) BHB concentration in cooled than uncooled cows, but plasma BHB concentration in both groups were within the normal range. The higher BHB concentrations, indicate a shift in metabolic fuel from glucose to fatty acids which occurs when glucose supply is limited (Bell, 1995).

In the present study, IGF-I increased with weeks postpartum and is probably regulated by EB. As previously demonstrated, circulating concentrations of IGF-I decline at parturition and gradually increase over time (Schams et al., 1991 cited by Roberts et al., 1997). The magnitude of the decline and the duration of time required for IGF-I levels to return to prepartum levels are greater in animals subjected to dietary restriction (Spicer et al., 1990). Negative correlations between circulating concentrations of IGF-I and the duration of postpartum anestrus have been previously reported (Simpson et al., 1992). In the present study the energy balance was positively correlated with IGF-I ($r = 0.51$, $P<0.01$).

Inadequate dietary protein has an immediate negative impact on milk yield, but excess dietary protein takes energy to process and excrete. In addition, excessive crude protein may impair reproductive performance. Excessive crude protein results in high blood urea and ammonia levels, which reduced conception rate. But blood urea nitrogen in both groups of cows was within the normal range in the present study.

In dairy cows, negative energy balance is directly related to the postpartum interval to first ovulation, and follicle size was adversely affected by negative energy balance in early postpartum dairy cows (Buttler and Smith,1989).

Spicer et al. (1993) found that the average EB during the first 4 week of lactation was negatively correlated to the postpartum interval to first ovulation($r=-0.52$). Butler et al. (1981) found a similar correlation ($r=-0.60$), but in the present study there was an absence of a significant correlation. There was no difference in the interval to first postpartum ovulation between the cooled and the uncooled cows.

Day to the EB nadir did not differ between the groups, but day to equilibrium EB for the uncooled cows was longer than the cooled cows.

In present study, we did not find any follicular cysts in both groups of cows. However, there was anovulation during 12 week study in both cooled and uncooled cows(11.8 and 17.6%, respectively). Some recent studies have reported an incidence of anovulation in dairy cattle ranging from 18 to 29 % based on serum progesterone concentrations (Cartmilll et al., 2001b; Moreira et al., 2001; Pursley et al., 2001).

All anovular cows in this study had follicle diameters between 10 to 15 mm. The anovular condition with smaller follicles (9 to 14 mm diameter) have been reported during the early postpartum period in both lactating dairy and suckled beef cows(Williams, 1990; Schillo, 1992).

The anovular condition with smaller follicles is characterized by enhanced negative feedback effects of estradiol on GnRH secretion, from the hypothalamus(Williams, 1990 ; Schillo, 1992).

In addition, this study was designed to determine the effect of cooling cows by utilizing an evaporative cooling and tunnel ventilation system on follicular development and ovulation, in dairy cows under hot and humid climates.

Follicular dynamics of the heat stressed cows are altered when compared to control cows (Wolfenson et al., 1995). Badinga et al. (1993) reported that the first wave dominant follicle was smaller and that the subordinate follicle was larger in heat stressed cows. Heat stress has been reported to decrease growth and follicular fluid concentrations of estradiol-17β in the first wave dominant follicle (Wolfenson et al.,1997).

In present study a new wave dominant follicle emerged earlier in both groups. It has been reported (Wolfenson et al.,1995) that heat stress caused earlier emergence of the dominant follicle in the second follicular wave. Initiation of heat stress on day 11 of the oestrous cycle decreased the size of the second wave dominant follicle in heifers (Wilson et al., 1998a) and circulating concentrations of estradiol-17β in heifers and cows (Wilson et al., 1998a ;1998b). In the present study the size of the largest ovulatory follicle at the time of $PGF_{2\alpha}$ treatment on cooled cows tended to be greater than for uncooled cows, but there was no difference in the maximal size of the largest ovulatory follicle and growth rate after luteolysis in both groups.

The time from giving $PGF_{2\alpha}$ to oestrus or ovulation is influenced by the oestrus cycle and the follicular developmental stage in the follicular wave at the time of the $PGF_{2\alpha}$ treatment (Stevenson et al., 1998). In our experiment, follicular development at the time of $PGF_{2\alpha}$ treatment was expected to be similar in both groups because the dominant follicle was synchronized by the GnRH injection and there was no difference in growth rate after luteolysis, therefore the time from $PGF_{2\alpha}$ to ovulation did not differ between the cooled and the uncooled cows. However, the response rate for the synchronization of ovulation in cooled cows tended to be greater than in the uncooled cows (82.4 and 52.9, respectively; P=0.08).

The exposure of lactating cows to heat stress has been shown to cause a decrease in follicular growth and to reduce concentrations of serum estradiol (Wilson et al., 1998b). In the present study the cooled cows had a greater ($P < 0.05$) average diameter of the largest ovulatory follicle than the uncooled cows. Heat stress inhibited follicular growth and dominance during the preovulatory period. Abnormal ovarian function in heat stressed cows was manifested as a decrease in the proestrus rise in estradiol, and the smaller size of the second wave dominant follicle (Wilson et al., 1998b). Circulating estradiol concentration during the preovulatory period is necessary to produce an LH surge and ovulation. In addition, a reduced estradiol peak may also alter aspects of the LH surge that could account for some types of anovulation in lactating cows (Wiltbank et al., 2002). A reduction of the endogenous LH surge by heat stress was reported in heifers (Madan and Johnson, 1973). It has been suggested that these differences are related to preovulatory estradiol levels because the amplitude of tonic LH pulses and GnRH-induced preovulatory plasma LH surges are decreased in cows with low plasma concentrations of estradiol but not in cows with high plasma concentrations of estradiol (Gilad et al., 1993) Therefore, the synchronization rate in the uncooled cows tended to lower than in the cooled cows. In addition, the ovulation rate in response to a second injection GnRH of Ovsynch was reported to be between 87%(Vasconcelos et al., 1999) and 91% (Moreira et al., 2001) in cycling cows. Therefore a second GnRH injection after the $PGF_{2\alpha}$ treatment might be used to improve the ovulation rate in dairy cows under heat stress.

Heat stress can compromise reproductive performance by decreasing the expression of oestrus behavior, altering follicular development, affecting oocyte competence, inhibiting embryonic development due to a reduced synchronization of ovulation response, lowering fertilization rates, and reducing embryo quality.

In the present study the modification of the barn environment and fixed TAI in lactating dairy cows in a hot and humid climate resulted in higher initial conception rates compared to cows housed in a barn without a supplemental cooling system (43.8 and 23.5%, respectively). The results indicate that this method has the potential to attenuate some of the detrimental effects of heat stress on embryo survival during this period.

Although, initial conception rates in cooled cows was higher than uncooled cows, it was still compromised by heat stress as the conception rate decreased further in both groups, indicating that embryo mortality may still have occurred after that. This was probably due to environmental heat stress (Putney et al., 1989a), which causes maternal body temperature to rise leading to the impairment of embryo survival, or this cooling was not sufficiently cool to protect embryos from direct effect of high temperatures, or early embryos during this period might be sensitive to elevated temperature.

Climatic factors that may influence the degree of heat stress include: temperature, humidity, radiation and wind (Gwazduaskas, 1985). The upper critical temperature for heat stress begins between 25 and 26°C (Berman et al., 1985). When environment temperatures exceed 30°C the day after insemination, pregnancy rates were adversely affected in lactating dairy cows than in heifers (Thatcher and Collier, 1986). Maternal hyperthermia is detrimental to embryonic development and survival(Gordon et al., 1987; Monty and Racowsky, 1987; Ulberg and Sheenan,1973). The oocyte and early cleavage stage embryo are the most sensitive to heat stress, while embryos that are 3 d or older are more tolerant (Ealy et al., 1993; Putney et al., 1988). Conception rates decline from 61 to 45% when rectal temperature 12 h, post breeding, increased by 1°C (Ulberg and Burfening, 1967). Furthermore, cattle with a rectal temperature of 40°C, as a result of exposure to 32.2 °C ambient temperatures for 72 h after inseminating, had conception rates of 0%, compared with a conception rate of 48% when rectal temperature was 38.5°C, for cows in an ambient temperature of 21.1 °C (Ulberg and Burfening, 1967). Given that exposure to conditions of high environmental temperature and humidity has been shown to elevate rectal and uterine temperatures (Gwazdauskas et al.,1973)

Cartmill et al. (2001a) reported that whenever the THI was $\geq$ 72, fewer cows were detected in oestrus and conception rates were lower. Similarly, Ingraham et al. (1976) reported that conception rates declined from 66 to 35% when the THI increased from 68 to 78 the second day before breeding.

In the present study, the evaporative cooling system reduced (P<0.05) afternoon barn temperature, however the relative humidity increased (P<0.05) as compared to the outside barn. High environmental relative humidity reduced the cooling capacity of the evaporative cooling, however on the days when animals were observed it reduced heat stress of the lactating dairy cows. The exposure to conditions of moderate heat stress (THI≥79) that occurred in the outside barn decreased when utilizing the evaporative cooling system, however the THI level was still above 72. For cows housed in the tunnel ventilated barn, the average rectal temperatures were lower ($P<0.05$) than for the cows housed in the outside barn and average respiration rates for the cooled cows were also lower ($P<0.05$) than for the uncooled cows. These results suggest that evaporative cooling and tunnel ventilation has the potential to decrease exposure to heat stress and alleviate the symptoms of heat stress.

The modified environment had been used to reduce the effect of heat stress, however, this approach has not eliminated all problems. Timed AI might be particularly effective during heat stress periods because of the decreased incidence of missed oestrus, but heat stress has also a direct effect on the development of the embryo. Conception rates have ranged between 31 to 42 % in cows following the Ovsynch protocol (Pursley et al., 1997a; 1997b; Fricke and Wiltbank, 1999; Tenhagen et al., 2001; Moreira et al., 2001; Pursley et al., 2001). However, conception rates in heat-stressed cows following the Ovsynch protocol were lower than in non stressed cows.

Timed AI programs based on follicular recruitment by synchronized ovulation have been developed (Pursley et al., 1995). Although TAI programs have been shown to improve the pregnancy rates of cows suffering from heat stress, increased subsequent embryonic losses have sometimes occurred. Cartmill et al. (2001) showed that submission rates and pregnancy rates between d 27 and 30 were enhanced when a TAI program was used. However, the advantage was lost between d 40 and 50 due to increased embryonic mortality in the cows bred using TAI.

In the present study, it was hypothesized that a cooling system and Timed AI would improve fertility in dairy cows under heat stress. In fact, this cooling improved the percentage of the embryonic survival during first 2 week after AI, resulting in an increased early establishment of pregnancy (initial conception) in the cooled cows but it did not increase pregnancy rates 45 to 70 d after breeding.

Ryan et al. (1993) found that a high percentage of embryonic loss occurred between d 7 and 14. In the present study we found that a high percentage of embryonic loss occurred before d 18 in the uncooled cows, but an increased rate of embryonic loss occurred between d 18 and 28 in the cooled cows. This delayed embryonic loss may due to improved heat stress management through the use of this cooling system as such environmental adaptations have been used elsewhere to improve fertility of cattle during summer heat stress (Berman and Wolfenson, 1992).

These results may indicate that cows were successfully induced to ovulate and subsequently conceive but had a reduced ability to maintain pregnancy. Previous studies also showed high (9.3 to 16.8 %) pregnancy losses between 28 and 56 d after AI (Vasconcelos et al., 1997; Santos et al., 2001).

Sugiyama et al. (2003) demonstrated that *in vitro* heat stress during the critical stage of early embryo development significantly increase the incidence of early embryonic mortility. These results indicate that increased maternal body temperatures adversely affects embryo quality and the conception rate. An increase in maternal body temperature may result in an increase in the ambient temperature of oocytes, zygotes and embryos in the oviduct or the uterus. At temperature increase of 0.5^{o}C above the basal body temperature has been associated with a decreased pregnancy rate (Gwazdauskas et al., 1973).

There was an evidence that fertilization rates following AI was compromised by heat stress (Sartori et al., 2000). The proportion of oocytes classified as morphologically normal and the rate of blastocyst development following in vitro fertilization was lower in the summer than in the winter (Rocha et al., 1998).

Near oestrus, the oocyte appears sensitive to damage since the exposure of superovulated cows to heat stress for 10 h, beginning at the onset of oestrus, had no effect on fertilization rates but reduced the proportion of normal embryos recovered on day 7 after oestrus (Putney et al., 1989b).

The possible mechanisms by which heat stress could compromise oocyte competence is by the disruption in patterns of folliculogenesis which could lead to the ovulation of an aged oocyte, with a lowered potential for fertilization (Mihm et al., 1999).

Oocyte competence in Holstein cattle declines during the summer. This result suggests that damage to the oocyte is one cause of reduced fertility during the summer (Al-katanani et al., 2002).

Although bovine blastocysts were protected from exposure to elevated temperatures inside their housing unit, the cooled cows went outside, without a supplement cooling system for milking at morning and afternoon, for approximately 30 min. Rectal temperatures and respiration rates were seen to increase similar to that of uncooled cows. The evidence suggests that elevated body temperature have a direct effect on the development of embryos and increases sensitivity to heat shock (Arechiga et al., 1995). If embryos are susceptible to heat stress during milking periods this may be enough to detrimentally affect the development of these embryos.

Cows that are exposed to conditions of high environmental temperature and humidity have been shown to have elevated rectal and uterine temperatures (Gwazdauskas et al., 1973). The findings of this study suggest that cooling dairy cows during hot weather periods is desirable not only while in the housing unit but also in the holding and milking areas if improved pregnancy rates are required.

Conclusion

The exposure of dairy cows to elevate temperatures have a variety of effects, including decreased fertility, depressed appetite, and decreased milk production, all of which contribute to the goal of decreasing the production of metabolic heat in order to maintain thermoneutrality.

This study showed significant advantages for the evaporative cooled and tunnel ventilated barn can be used to reduce heat stress of dairy cows housed in hot and humid climates. The combined effect of higher milk production and increasing lactation persistency, with minimal costs could improve the financial status of dairy operations. The benefit demonstrated increased income over costs.

Respiration rates and rectal temperatures, which affect both milk production and reproduction, were reduced by this environmental modification. These results suggest that the evaporative cooling and tunnel ventilation system has the potential to decrease the exposure to heat stress, alleviate the symptoms of heat stress and improve milk production and metabolic efficiency during early lactation. This modification of the barn environment can reduce some of the detrimental effects of heat stress on follicular development and can improve the response rate to the synchronization of ovulation in dairy cows in hot and humid climates.

The implementation of evaporative cooling and tunnel ventilation systems for dairy cows in hot and humid climates increased the percentage of cows that initialy established a pregnancy and increased successful pregnancy while decreasing early embryonic mortality, if these cows were sufficiently cooled after breeding. It appears that modification of environment need to be developed further to improve reproductive performance of dairy cows in hot and humid climates.

The finding that the cooling of cows did not alleviate all the effects of heat stress on pregnancy rates suggests that the degree of cooling was not sufficient to prevent the adverse effects of heat stress. It is also possible that the cooling of dairy cows needs to be done not only in the housing unit but also in the holding and milking areas to improve pregnancy rates.

However additional research is needed to determine the effects of tunnel ventilation and evaporative cooling system on postpartum reproductive performance and production when compared to conventional methods of cow cooling in the tropical area.