# CHAPTER 3

## MATERIALS AND METHODS

The effects of heat stress and utilizing evaporative cooling equipped with tunnel ventilation on barn environment, cow comfort, reproductive performance, and milk production in dairy cows in a hot and humid climate were studied from the day of parturition to 22 week postpartum, from April 2004 to May 2005. This experiment was conducted at an experimental farm in the Veterinary Student Training Center, Faculty of Veterinary Science, Chulalongkorn University, Nakhorn pathom that is located in the central part of Thailand (latitude 13$^{o}$N and longitude 99$^{o}$E).

### Experimental animal housing

The experimental animal housing was a 20 x 10 m housing unit, divided into two parts. The tunnel ventilation barn was a 20 x 5 m housing unit, out-fitted with 5 m cooling pads at one end, at the east, and two 1.5 m, 1 hp exhaust fans at the opposite end. Fresh air was cooled as it entered the barn through the cooling pads that were kept wet with recirculating water. The tunnel ventilation barn was a tie-stall unit with individual feed troughs and a water tank to support 18 mature lactating dairy cows. This housing included an adjacent (outside) tie-stall barn (20 x 5 m) that was used to study control animals simultaneously.

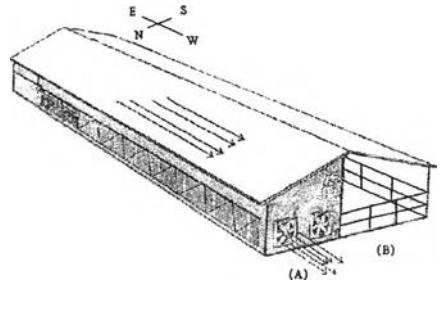


**Figure 1.** The experimental animal housing, including the tunnel ventilation barn (A) and the outside tie-stall barn (B).

Experimental animals

Thirty-six, crossbred Holstein-Friesian (93.75%HF), primiparous cows were used for this experiment. Cows were designated to calve during hot (April to June; n=12), wet (July to October; n=12) and cool (November to January; n=12) periods. Cows were assigned randomly to one of two housing treatments based upon their calving date and body weight. Cooled cows (n=18; treatment) were housed in the tunnel ventilated barn, equipped with an evaporative cooling pad system and uncooled cows (n=18; control) were housed in a naturally ventilated barn without a supplemental cooling system.

All cows were studies from parturition to 22 week postpartum, which divided into three parts:

I. First part, during first week to 12 week postpartum, all cows in both groups were studied the effect of heat stress and utilizing evaporative cooling system on barn environment, cow comfort, dry matter intake, energy balance, milk production and postpartum ovarian activity.

II. Second part, during wk 12 to 15 week postpartum, all cows were studied the effect of heat stress and utilizing evaporative cooling system on follicular development and ovulation after synchronization of oestrus.

III. Third part, during wk 15 to 22 week postpartum, all cows were studied the effect of heat stress and utilizing evaporative cooling system on conception rate and embryonic loss after synchronization of ovulation and fixed time artificial insemination.

Cows were individually fed and housed in a stanchion barn and weekly body weights were recorded. Body weight (BW) loss postpartum was calculated as the difference between the body weight measured after calving and that measured 4 week postpartum.

Cows were fed a total mixed ration (TMR), ad libitum. They were allowed continuous access to water. The TMR was composed of corn silage and concentrate. (Table 1) Daily feed intake was recorded and the diet was sampled weekly and composited monthly for analysis. Cows were milked twice daily (05.00 and 15.00 h) and the milk yield was recorded.

Sample collection

Milk samples were collected weekly during successive AM and PM milkings and were analyzed for percentage milk fat, protein, lactose and solids not fat (SNF) by Milko-Scan (Milko-Scan 133B, N.Foss Electric, Denmark).

Blood samples (20 ml) were collected twice weekly at 3-to 4-d intervals by jugular venipuncture, from 1 to 12 wk postpartum. After collection in tubes containing EDTA and no EDTA, the blood was centrifuged at 3000 x g for 10 min, for plasma and serum, respectively, were decanted and stored frozen at -20$^{o}$C until subsequent analysis.

Data collection

Environmental temperature and humidity measurement were continuously monitored and recorded at AM and PM milking in both housing units. The temperature-humidity index (THI) was calculated according to the formula:

$$THI = 0.72( C_{dry} + C_{wet} ) + 40.6$$

where $C_{dry}$ is dry bulb temperature($^{o}$C) and $C_{wet}$ is wet bulb temperature($^{o}$C)(McDowell,1972 cited by Ashutosh et al., 2001).

Rectal temperatures and respiration rates were recorded twice weekly in the morning and afternoon. Electricity usage in the tunnel barn was monitored and recorded.

Analytical procedures

Plasma progesterone concentration was determined in all samples, using a solid phase RIA kit (Coat-A-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA, USA).

The concentration of nonesterified fatty acid (NEFA) was determined in weekly plasma samples by an enzymatic method, using NEFA C kits (Wako Pure Chemical Industries Ltd., Osaka, Japan).

The concentration of β-hydroxybutyrate was determined in weekly plasma samples, by using RANBUT D-3-Hydroxybutyrate kits(Randox Laboratories Ltd, Co., Antrim, UK).

The concentration of insulin-like growth factor-I (IGF-I) was determined in weekly plasma samples, by using ACTIVE IGF-I ELISA (DSL-10-5600) (Diagnostic Systems Laboratories, Inc., Texas, USA)

The concentration of cortisol was determined in weekly serum samples, by using Coat-A-Count Cortisol kit (Diagnostic Products Corporation, Los Angeles, CA, USA).

The concentrations of blood urea nitrogen (BUN) was determined in weekly serum samples, by using UREA UV Multi-Purpose Liquid Reagent (Audit Diagnostics, Cork, Ireland).

Table 1. Composition of the diet (% of DM)

| Ingredient | % of DM |
|---|---|
| Corn silage | 42.1 |
| Cassava | 10.3 |
| Soybean meal | 19.1 |
| Rice bran | 8.7 |
| Whole cottonseed | 12.8 |
| Dicalcium phosphate | 4.0 |
| Salt | 1.5 |
| Bone meal | 1.4 |
| Premix | 0.1 |
| Nutrient analysis | |
| DM (%) | 52.3 |
| CP (%) | 17.9 |
| Ca (%) | 1.7 |
| P (%) | 1.3 |
| NEL (Mcal/kg of DM) | 1.6 |

Energy balance (EB) calculations

Daily EB was calculated for the first 12 wk of lactation for all cows according to their daily milk production, DMI, weekly BW measurements, weekly milk fat tests (weighted averages derived from analysis of the a.m. and p.m. samples), and the calculated net energy ($NE_L$) value of each diet utilizing the following equations(NRC,1989):

$NE_C$ = $NE_L$ per kilogram of DM x DMI,

$NE_L$ = 0.74[milk (kilograms) x 0.4 + milk fat (kilograms) x 15],

$NE_R$ = $BW^{0.75}$ (0.08) + $NE_L$, and

EB = $NE_C$ – $NE_R$

Where $NE_C$= net energy consumed, $NE_L$= net energy for lactation, $NE_R$= net energy required, and EB= energy balance

Energy balance is expressed as mega calories of NE per day for each week and could be positive (PEB) or negative (NEB). The lowest numerical value within the actual EB profile of each cow was designated as the day of the EB nadir.

Postpartum reproductive measures

The reproductive health of all cows was monitored every week by palpation of the reproductive tract per rectum and ultrasonography. The ovaries of cows were scanned weekly by transrectal ultrasonography using a linear array, ultrasound scanner equipped with a 5.0 MHz rectal probe (Aloka SSD500, Tokyo, Japan), starting at 3 wk and continuing through to 12 wk postpartum in order to measure follicles and determine the presence of a corpus luteum. Ultrasonography also attempted to analyze the incidence of anovulation or follicular cysts.

Calving to first ovulation interval was defined by the first increase in plasma progesterone ($\geq$ 1.0 ng/ml) after calving; ovulation was assumed to have occurred 7 d before elevation of the progesterone concentration.

Synchronization of oestrus

The experiment was conducted to determine the effect of utilizing an evaporative cooling and tunnel ventilation system on follicular development, the time of ovulation and the response rates to synchronization of dairy cows which had received GnRH and $PGF_{2\alpha}$.(Part II)

The voluntary waiting period for the synchronization protocol ranges from 85 to 90 days postpartum. Cows received an initial treatment of 100 μg of gonadorelin (GnRH agonist; Fertagyl®, Intervet) by intramuscular injection followed 7 days later with 500 μg of cloprostenol ($PGF_{2\alpha}$ agonist; Estrumate®, Schering-Plough Animal Health) by intramuscular injection.

Ultrasonography of the ovaries was performed once daily from the time of the initial treatment with GnRH to the time of $PGF_{2\alpha}$ treatment and then every 4-h after the $PGF_{2\alpha}$ treatment to 120 h post-treatment. All ovarian structures were measured and mapped using real time B-mode ultrasonography (Aloka SSD 500, Tokyo, Japan) with a 5.0 MHz linear array rectal transducer. Ovulation was determined by the examination of both ovaries. Confirmation of disappearance of the dominant follicle by ultrasonography was considered as ovulation.

Blood samples, used to determine hormonal concentrations, were collected after the $PGF_{2\alpha}$ treatment, every day for 3 days, by jugular venipuncture and stored in tubes containing EDTA. Blood samples were stored in an ice box until the plasma was harvested by centrifugation. Plasma samples were stored at -20°C until assayed for progesterone concentration. Progesterone concentration was quantified in the plasma by RIA (Coat-A-Count; Diagnostic Products Corporation, Los Angeles,CA).

Corpus luteum regression was defined as a cow having a plasma progesterone concentration > 1.0 ng/ml and then declining to a level <1.0 ng/ml. A cow was considered synchronized only if she met the criteria stated above for both ovulation and CL regression. The synchronization rate was defined as the percentage of cows which showed regression of the corpus luteum in addition to ovulation of a dominant follicle.

Synchronization of ovulation and fixed time artificial insemination (TAI)

This experiment was conducted to examine the effect of the modifications to barn environments by utilizing evaporative cooling and a tunnel ventilation system, on conception rates and early embryonic loss in dairy cows after synchronization of ovulation and fixed time AI. (Part III)

The voluntary waiting period after calving for the TAI breeding protocol was 120 d. At approximately 110 d postpartum, the cows received 100 μg of gonadorelin(GnRH agonist; Fertagyl®, Intervet) by intramuscular injection (Day0). Cows then received an intramuscular injection of 500 μg of cloprostenol($PGF_{2\alpha}$ agonist; Estrumate®, Schering-Plough Animal Health ) on day 7 and a second injection of 100 μg of gonadorelin on day 9. Cows were inseminated with frozen-thawed semen at a fixed time, 16-20 h after the second injection of gonadorelin.

The early establishment of pregnancy (initial conception) was defined by an elevated progesterone concentration in the plasma, $\geq$ 1.0 ng/ml, that persisted beyond d 22-24 after insemination. Ultrasonography was used to identify the embryonic vesicle and conceptus at 28, 35 and 42 d after insemination by using B-mode real time ultrasound (Aloka SSD500) and a 5 MHz linear array rectal transducer. Successful pregnancy was defined when palpable per rectum and ultrasonography 45-60 d after insemination. The presence of fluid in the uterine horn, a palpable amniotic vesicle and fetal membrane slip were considered positive indicators of pregnancy.

The conception rate (CR) was defined as the proportion of cows where early establishment of pregnancy occured. The pregnancy rate (PR) was defined as the proportion retaining a successful pregnancy.

Early embryonic death, from breeding to 18 d after insemination, was defined as cows that failed to conceive and had low plasma progesterone concentrations 22-24 d after insemination. Early embryonic death between 18 and 28 d after insemination was defined as cows that had a high plasma progesterone concentration ($\geq$ 1.0 ng/ml) during 22-24 d after insemination and failed to conceive. Early embryonic death between 28 and 35 d and 35 and 42 d after insemination was defined as a loss of conceptus between

pregnancy diagnosis by ultrasonography at 28, 35 and 42 d after insemination and the confirmation of pregnancy by palpation.

### Statistical Analyses

All statistical analyses were performed using an SPSS computer program. Data involving repeated measurements (e.g., DMI, BW, milk yield, EB, metabolite data and diameter of dominant follicle were analyzed by repeated measures ANOVA using the type of experimental housing as a fixed main effect and time (day or week depending on the data) as a repeated measure.

Single mean comparisons, including mean air temperature, humidity, THI, rectal temperature, respiration rate, day to first ovulation, day to the EB nadir, day to the EB equilibrium, nadir of NEB, body weight loss, diameter of dominant follicle and time of ovulation after synchronization of oestrus were analyzed by the Student's *t* test.

In addition, the proportion of cows which showed first ovulation within 12 week postpartum, the response rate to synchronization of ovulation, the conception rate and embryonic loss were compared between groups by Chi-square procedures.

The relationships between parameters were examined by simple correlation. *P* values of less than 0.05 were considered to be statistically significant.