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EFFECTS OF HEAT STRESS AND β-CAROTENE SUPPLEMENTATION ON POSTPARTUM REPRODUCTIVE PERFORMANCE IN DAIRY COWS

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A Thesis submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Theriogenology
Department of Obstetrics Gynaecology and Reproduction
Faculty of Veterinary Science
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Thesis Title	EFFECTS OF HEAT STRESS AND β-CAROTENE SUPPLEMENTATION ON POSTPARTUM REPRODUCTIVE PERFORMANCE IN DAIRY COWS
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วินัย แก้วละมุล: ผลกระทบของความเครียดจากความร้อนและผลการเสริมเบต้า-แคโรทีนต่อประสิทธิภาพการสืบพันธุ์ในโคนม. (EFFECTS OF HEAT STRESS AND β-CAROTENE SUPPELEMTATION ON POSTPARTUM REPRODUCTIVE PERFORMANCE IN DAIRY COWS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ.น.สพ.ดร. มงคล เตชะกำพุ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ.น.สพ.ดร. ปราจีน วีรกุล, Prof. Andrew Ponter, 145. หน้า

การทดลองที่ 1 เพื่อศึกษาถึงความแตกต่างของระดับความเครียดจากความร้อนในแต่ละภาคโดยเปรียบเทียบจากค่า THI และศึกษาถึงผลกระทบของเดือนที่คลอดต่อระยะวันท้องว่างในโคนมในประเทศไทย ทำการเปรียบเทียบว่า THI ในภาคกลาง ภาคตะวันออก ภาคตะวันออกเฉียงเหนือ และภาคเหนือ พบว่ามีความแตกต่างกันของค่า THI ($P<0.001$) โดยค่า THI ของภาคเหนือต่ำกว่าของภาคอื่น ๆ ($P<0.0001$) ในทุกภาคค่า THI จะมีค่าต่ำสุดในเดือนธันวาคมและมกราคม จากการศึกษาจากข้อมูลย้อนหลังที่ได้จากกรมปศุสัตว์ เป็นข้อมูลที่เก็บบันทึกการให้นมจำนวน 13,548 ระยะการให้นมในปี พ.ศ. 2547 ถึง 2549 พบว่าเปอร์เซ็นต์ของแม่โคที่คลอดในรอบปีมีเปอร์เซ็นต์คลอดสูงที่สุดในเดือนกันยายนและตุลาคม (13.1-14.91%) และต่ำที่สุดในเดือนกุมภาพันธ์ (4.14 - 5.02%) ในแม่โคลำดับการให้นมที่ 1 จำนวน 1,962 ข้อมูล ระยะเวลาวันท้องว่าเฉลี่ย เท่ากับ 151.7 วัน โคที่คลอดในเดือนกุมภาพันธ์จะมีระยะเวลาวันท้องว่างนานที่สุด (219 ± 11 วัน) และโคที่คลอดในเดือนตุลาคมและพฤศจิกายนมีระยะเวลาวันท้องว่างสั้นที่สุด (133 ± 7 และ 133 ± 7 วัน)

การทดลองที่ 2 เพื่อศึกษาถึงผลกระทบจากความเครียดจากความร้อนต่อการทำงานของรังไข่และการเปลี่ยนแปลงของพลาสมาเมทตาบอไลน์ในช่วงหลังคลอดและผลกระทบต่ออัตราการสูญเสียตัวอ่อนในโคนมที่ให้นมครั้งแรกในฟาร์มเอกชน จำนวน 68 ตัวพบว่าแม่โคที่คลอดในเดือนที่มีค่า THI อยู่ในระดับความเครียดแบบรุนแรง (severe $78 \leq THI < 89$) มีสัดส่วนการทำงานของรังไข่ที่ผิดปกติมากกว่า ($P<0.01$) แม่โคที่คลอดในเดือนที่มีค่า THI อยู่ในระดับความเครียดแบบเล็กน้อย (mild $72 \leq THI < 78$) แม่โคที่คลอดในเดือนที่มีความเครียดอยู่ในระดับเล็กน้อยมีผลผลิตน้ำนมมากกว่า ($P<0.03$) แม่โคที่คลอดในเดือนที่มีความเครียดระดับรุนแรงมาก อย่างไรก็ตามพบว่า ระยะจากคลอดถึงดกไข่ ระยะจากคลอดถึงผสมครั้งแรก ระยะวันท้องว่าง อัตราการผสมติดในการผสมครั้งแรกหลังคลอด น้ำหนักตัว คะแนนความสมบูรณ์ของร่างกาย พลาสมาเมทตาบอไลน์ (NEFA และ IGF-1) คอร์ติซอล และอัตราการสูญเสียตัวอ่อน ไม่มีความแตกต่างกันระหว่างแม่โคที่คลอดในเดือนที่มีระดับความเครียดแบบเล็กน้อยและแบบรุนแรง

การทดลองที่ 3 เพื่อศึกษาถึงผลของการเสริม เบต้า-แคโรทีนในอาหารในช่วงแห้งนมต่อระดับของความเข้มข้นของเบต้า-แคโรทีนในเลือดแม่โค การทำงานของรังไข่ การเข้าอู่ของมดลูก และการเปลี่ยนแปลงของฮอร์โมนและพลาสมาเมดาบอไลน์หลังคลอด ผลผลิตนม คุณภาพของนมน้ำเหลือง และระดับของเบต้า-แคโรทีนและเมตาบอไลน์ในเลือด การทำงานของเอ็นไซม์ ในลูกโคหลังคลอด พบว่าการเสริมเบต้า-แคโรทีนทำให้ระดับของความเข้มข้นของเบต้า-แคโรทีนในเลือดแม่เพิ่มขึ้น ($P<0.0001$) เปอร์เซ็นต์ของนิวโทรฟิลล์ที่เก็บจากผนังคอมดลูกและมดลูกในกลุ่มที่เสริมเบต้า-แคโรทีนต่ำกว่ากลุ่มควบคุม ในวันที่ 28 หลังคลอด (คอมดลูก; กลุ่มควบคุม: 21.0 ± 3.22% กลุ่มเบต้า-แคโรทีน: 9.7 ± 3.14%, $P<0.05$ และ มดลูก; กลุ่มควบคุม: 32 ± 3.86%, กลุ่มเบต้า-แคโรทีน: 20.9 ± 3.76%, $P<0.05$) ระดับของ Hydroxyproline ในกลุ่มเบต้า-แคโรทีน (20.8 ± 1.33 µmol/L) สูงกว่ากลุ่มควบคุม (15.0 ± 1.33; $P<0.05$ µmol/L) ไม่พบความแตกต่างของการทำงานของรังไข่ พลาสมาเมทตาบอไลน์ ฮอร์โมน ขนาดมดลูก คุณภาพนมน้ำเหลือง ผลผลิตน้ำนมในแม่โค และระดับเบต้า-แคโรทีนในเลือด พลาสมาเมทตาบอไลน์ ฮอร์โมน และ การทำงานของเอ็นไซม์ ในลูกโคหลังคลอดระหว่างกลุ่มเบต้า-แคโรทีนและกลุ่มควบคุม

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ปีการศึกษา 2553 ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์ร่วม.....

ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์ร่วม.....

4875956231: MAJOR THERIOGENOLOGY
KEYWORDS: DAIRY COW/HEAT STRESS/β-CAROTENE/REPRODUCTION

WINAI KAEWLAMUN: EFFECTS OF HEAT STRESS AND β-CAROTENE SUPPLEMENTATION ON POSTPARTUM REPRODUCTIVE PERFORMANCE IN DAIRY COWS. THESIS ADVISOR: PROF. MONGKOL TECHAKUMPHU, D.V.M., Doctorate de 3e cycle, THESIS CO-ADVISOR: ASSOC. PROF. PRACHIN VIRAKUL, D.V.M., Ph.D, PROF. ANDREW PONTER, Ph.D, 145 pp.

EXP.1 The objectives were to analyses the potential impact of heat stress in different regions, determine the monthly distribution of calving throughout the year and to investigate environmental sources of variation of days open (DO) in first lactation cows. The climate data were obtained from from the 25 official provincial meteorological stations covering the 33 provinces included in the study. Reproductive data were obtained from the bureau of Biotechnology in Livestock Production, Department of Livestock Development, Ministry of Agriculture and Cooperatives. These data contained information from 13,548 lactation records collected from years 2004 to 2006. The lower mean temperature-humidity index was observed in December (72) and the highest mean in April (80). THI differed significantly between regions ($P<0.0001$), and months ($P<0.0001$). Significant interactions between region and month ($P<0.0001$) was found on THI. THI values were different among regions ($P<0.0001$). The highest frequencies of calving were observed in September and October (13.1 – 14.91%) and the lowest frequencies were observed in February (4.14 – 5.12%). The average DO in the first lactation cows was 151.70 days. Significant effects of MOC ($P<0.0001$), region ($P<0.0001$) were found on DO. February calving cows had longest DO (219 ± 11 days) while cows calving in October and November had a significantly shorter mean DO (133 ± 7 and 133 ± 7 days).

EXP. 2 The aims of this study were 1) to investigate the effect of heat stress on the resumption of ovarian activity and plasma non-esterified fatty acids (NEFA), insulin-like growth factor-1 (IGF-1) and cortisol concentrations in post partum first lactation dairy cows, and 2) to investigate the effect of heat stress on embryonic loss in first lactation dairy cows. This study was conducted in a commercial dairy farm. There were 68 first lactation cows included in the study. The proportion of normal ovarian cyclicity in mild stress ($72 \leq THI < 78$) was higher than in severe stress ($78 \leq THI < 89$) group ($P<0.01$). The interval from calving to first ovulation, interval from calving to first AI, days open and first service conception rate were not statistically different between MS and SS. BCS and body weight were unaffected by THI classification group. Plasma concentrations of NEFA, IGF-1 and cortisol, were not different between groups. Milk production was different ($P=0.03$) between MS and SS. Neither the number nor the different types of embryonic mortality were affected by heat stress.

EXP. 3 The objective was to invesrtigate whether a supplement of β-carotne given during the dry period is able to 1) increase blood concentrations of β-carotene postpartum 2) improve ovarian function and progesterone production 3) enhance uterine involution and uterine health 4) improve milk production and milk composition 5) modify hormone and metabolic status in cow 6) increase colostral IgG content 7) modify hormone, metabolic status and enzyme activity in the neonatal calf. Forty high producing Holstein cows were included in the experiment. The β-carotene supplement was given individually to the cows (1g/d β-carotene) started at drying-off until calving. The results showed that supplementation of β-carotne during the dry period increased blood concentrations of β-carotene in cows ($P<0.0001$). On day 28 postpartum the percentage of neutrophils in the BC group was lower than in the C group (cervical smear; C: 21.0 ± 3.22% vs BC: 9.7 ± 3.14%, $P<0.05$ and uterine smear; C: 32.0 ± 3.86% vs BC: 20.9 ± 3.76%, $P<0.05$). Plasma concentrations of hydroxyproline in the BC group were higher than in the C group on day 21 postpartum (BC: 20.8 ± 1.33μmol/L vs. C: 15.0 ± 1.33μmol/L; $P<0.01$). The dietary supplement of β-carotene during the dry period had no effect on ovarian activity, progesterone production, cervix and uterine horn diameters, milk production and milk composition, hormone and metabolic status in cow, colostral IgG content, hormone, metabolic status and enzyme activity in the neonatal calf.

Department: Obstetrics Gynaecology and Reproduction
Field of study: Theriogenology
Academic year: 2010

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ALP	alkaline phosphatase
ASAT	aspartate aminotransferase
BCS	body condition score
BHB	beta-hydroxybutyrate
CI	calving interval
CK	creatinine kinase
CL	corpus luteum
CP	crude protein
DMI	dry matter intake
DO	days-open
EDTA	ethylenediaminetetraacetic acid
EEM	early embryonic mortality
FCM	fat corrected milk
GH	growth hormone
GnRH	gonadotropin-releasing hormone
hCG	human chorionic gonadotropin
ICAL	intervals from calving to the first AI
IFN-τ	interferon-tau
IGF-1	insulin-like growth factor-1
IgG	immunoglobulin G
LCT	lower critical temperature
LEM	late embryonic mortality
LH	luteinizing hormone
MAI	month of insemination
MOC	months of calving
MS	mild stress
NE	non-fertilization
NEB	negative energy balance
NEFA	non-esterified fatty acids
NEL	net energy for maintenance and lactation

No. AI	number of first artificial insemination
NR45	non-return rate at 45 day
NS	no stress
PDIE	protéines digestibles dans l'intestin permises par l'énergie (intestinal digestible proteins, when fermentable energy is the limiting factor)
PDIN	protéines digestibles dans l'intestin permises par l'azote (intestinal digestible proteins, when fermentable N is the limiting factor)
PMN	polymorphonuclear
Preg	pregnancy
PSPB	pregnancy specific protein B
Rep Cat	reproductive categories
Rep Stat	reproductive status
RH	relative humidity
ROS	reactive oxygen species
SCC	somatic cell counts
SEM	standard error of the mean
SS	severe stress
TDN	total digestable nutrients
THI	temperature – humidity index
TMR	total mixed ration
TNZ	thermoneutral zone
UCT	upper critical temperature
γGT	γ-Glutamyl transferase

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

In dairy cow, reproductive efficiency has a major impact on profitability of dairy farms. A calving interval of 12-13 months or once a year calving is generally accepted as optimal to drive maximum economic benefit in non-seasonal year round calving dairy herds. Improvement in reproductive performance of dairy cattle encompasses factors associated with resumption of ovarian function, detection of estrus, and establishment and maintenance of pregnancy. Slow recovery of ovarian activity during the postpartum period is a major impediment to insemination of cows immediately after the end of the voluntary waiting period. Negative energy balance has been linked with a delay in resumption of postpartum ovulation in dairy cows. In addition to the physiological period of negative energy balance, environmental factors, such as heat stress in tropical regions, which exacerbate energy needs of dairy cows in early lactation, can further compromise energy intake and reproductive performance. Heat stress can act in more than one way to reduce fertility in lactating dairy cows. Heat Stress can reduce dry matter intake to indirectly inhibit GnRH and LH secretion from the hypothalamo-pituitary system. However, it is not clear if heat stress can also directly influence the hypothalamo-pituitary system to reduce GnRH and LH secretion. Heat stress can directly compromise the uterine environment to cause embryo loss and infertility. In heat stressed cow, it has been proposed that the oxygen-derived free radical is increased. The oxygen-derived free radical has a detrimental effect on cow health and also on reproductive performance. Dietary supplementation with antioxidant for example 'β-carotene' is of interest and may be beneficial for lactating dairy cows under heat stress. In the context of declining fertility in the dairy cows worldwide, it is important to understand how these cows resume their ovarian cyclicity in the post partum period. Evaluating factors associated

with resumption of ovarian activity, conception rate, and embryonic survival might improve our understanding of the poor reproductive efficiency in dairy cows.

In Thailand, the aproximate number of dairy cattle reported by Department of Livestock Development was 469,000 heads (DLD, 2008). These dairy cattle were raised by approximately 20,000 households. Therefore, the dairy farm in Thailand is characterized as a small-holder farm with less than 20 lactating cows. Reproductive performance in dairy cows in Thailand is poor (Aiumlamai, 2007). There were several factors implicating in the poor reproductive performance and heat stress is one of the main factors (Aiumlamai, 2007). In Thailand, where the temperature exceeds 30 ºC for several months of the year, most dairy cows are raised in conventional open-air barn, where ambient temperature and relative humidity follow those of the outside. Cows are affected by high ambient temperatures and relative humidity and this results in low reproductive performance and milk production. Additional knowledge related to the effect of heat stress on reproductive performance under Thai conditions are needed. To date, a retrospective study to investigate the effect of heat stress on reproductive performance across Thailand does not exist. Reviewing the data on the effects of heat stress on reproductive performance acquired aver a period of time may reveal useful information. Also, there has been no research to investigate the effects of heat stress on reproductive performance throughout the year. Most studies compared results between particular time periods. Therefore, a retrospective study of recorded data that aims to determine the response of cows to heat load throughout the year is needed. Additionally, some strategies to reduce the negative effects of heat stress on dairy cows, such as the utilization of cooling systems or manipulation of follicular development, have been investigated. A supplement of antioxidants for example vitamin E or β-carotene to heat stressed cows is an alternative strategy. However, the information available related to the supplementation of stressed cows with antioxidants is limited and warrants research.

1.2 Literature review

1.2.1 Heat stress and its effects on reproduction in the dairy cow

1.2.1.1 Thermoregulation in the dairy cow

Thermoregulation or temperature homeostasis refers to the process of keeping internal body temperature in a steady state, when the external temperature is changed. Cattle are neither under heat or cold stress when the effective environmental temperature is in the thermoneutral zone (TNZ; Fig. 1). Within the TNZ the highest productivity is normally achieved as their maximum genetic potential. The range of TNZ is from lower critical temperature (LCT) to upper critical temperature (UCT).

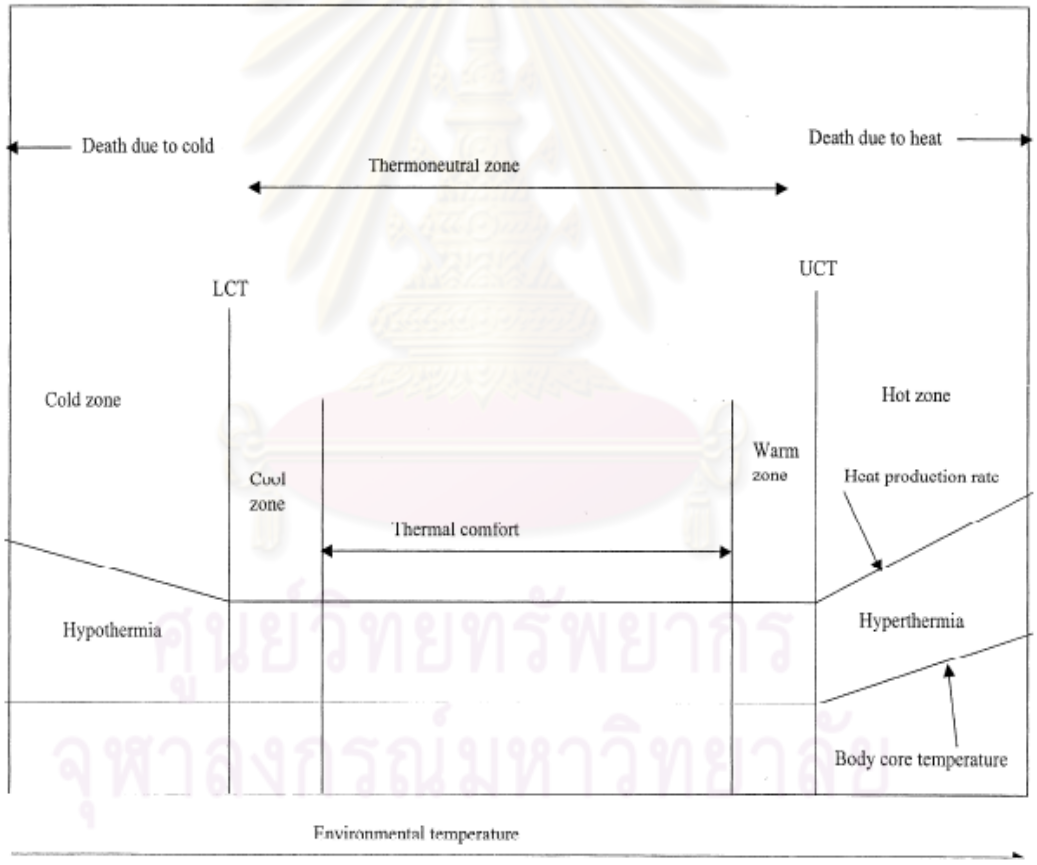


Figure 1 Schematic relationship of the animal's body core temperature, heat production and environmental temperature. LCT = lower critical temperature; UCT = upper critical temperature. (Kadsere et al., 2002)

The LCT is the ambient temperature below which the rate of heat production of a resting homeotherm increases to maintain thermal balance and the UCT is the ambient temperature above which thermoregulatory evaporative heat lost process are recruited (Igono et al., 1992). This range depends on several factors such as age, breed, feed intake, diet composition, housing conditions and behavior of an animal. Igono et al. (1992) studied the environmental profiles and effect of critical temperature on milk production of Holstein cows in a desert climate and they found that the highest milk production was measured during optimal thermal neutral periods that were characterized by ambient temperatures below 21 ºC throughout the day.

Cattle are homeotherms. They have the ability to regulate internal body temperature. This ability allows cattle to function in spite of variations in the surrounding environment. The core body temperature of cattle is normally about 39 ºC, however, body temperature fluctuates throughout the day by between 0.5 and 1.2 ºC. This is a rhythmic fluctuation, which usually reaches a peak during the early hours of the evening and drops to its lowest level early in the morning. Heat exchange between the dairy cow and its environment is the way to regulate internal body temperature. Heat is transferred between the cow and environment by radiation, conduction, convection, and evaporation process.

1.2.1.2 Heat production and heat gain

Heat production is defined as a measure of the sum total of energy transformations happening in an animal per unit time (Kadzere et al., 2002). The main source of heat production in cattle is metabolic heat. This is heat produced in the body when feed is converted by biochemical reactions to supply energy for various body functions including maintaince and production needs such as pregnancy lactation and growth. Under cold conditions, metabolic heat production can be the value in maintaining body temperature; however, under hot conditions, metabolic heat must be dissipated from the animal. Most metabolic heat is generated in the core of the animal and is transported to the animal's surrounding when its environment is cooler than the body surface. If the animal is unable to transfer sufficient heat to the environment it can accumulate in the body leading to excessive heat load and hyperthermia.

Although metabolic heat production is the major contributor to body heat load, cattle also take in additional heat from solar radiation, reflected radiation from surrounding objects and from the air itself, if air temperature is higher than the animal's body temperature. Heat production is controlled by several mechanisms. It is directly control by the nervous system (Hammel, 1968), by endocrine system, through modifications of appetite and digestive process, and indirectly by alteration of the activity of respiratory rate and protein synthesis. The heat production rate can be greatly affected by the environmental temperature through the alteration of feed intake, production and thermoregulation in an animal.

1.2.1.3 Heat dissipation mechanism

In mammals, the body temperature is maintained at a relatively constant level because of the balance that exists between heat production and heat loss. It is important to understand heat dissipation mechanisms in animals. Factors that increase heat production over basal metabolic rate include exercise of shivering, imperceptible tensing of muscles, chemical increase of metabolic rate, heat increment and disease (fever). Factors decreasing heat loss are an internal shift in blood distribution, a decrease in tissue conductance, or reduction of counter-current heat exchange. On the other hand, heat loss from the animal is enhanced by sweating, panting, a cooler environment, increased skin circulation (vasodilatation), shorter fur insulation, increased sensible water loss, increased radiation surface, and increased air movement or convection.

The animal loses heat by conduction, convection, radiation, evaporation of water, and through expired air. Heat dissipation is shifted from radiation and convection at lower ambient temperatures to vaporization at higher ambient temperatures. The amount of heat lost by skin depends partly on the temperature gradient between the skin, air and solid object. Non-evaporative heat loss declines as ambient temperatures rise above the lower critical temperature making cows more dependent on peripheral vasodilatation and water evaporation to enhance heat loss and prevent a rise in body temperature (Berman et al., 1985). However, peripheral

vasodilatation is unlikely to be a major method of increasing heat loss in cattle because of their large body mass.

1.2.1.3.1 Radiation

Heat transfer through radiation takes places in form of electromagnetic wave mainly in the infrared region. Animals continually emit and receive radiant energy from the sun and other objects in their environment. The amount of heat absorbed by an object from direct radiated heat depends not only on the temperature of the object, but also on it colour and texture. The dark surface radiates or absorbs more heat than light coloured surfaces at the same temperature. Therefore, an animal with a black coat will have an absorbance of 1 of the direct radiation, whereas, a white-coated one will have an absorbance of 0.37 and one with red fur has an absorbance of 0.65 (Cena and Monteith, 1975). Radiant heat transfer between bodies take place in both directions, and if the bodies are at different temperatures there is a net transfer of heat from the warm to the cooler body. This net heat transfer involves the loss or gain of heat by the animal through absorption or reflection of electromagnetic infrared waves. The experiments which were cited by Silanikove et al., (2000) found that cows, in experiments using artificial radiant heat load, did not respond to radiation at an ambient temperature of 7.2 ºC. However, at temperature of 21.1 and 26.7 ºC, Jersey cows had a mean heat production rate 12-14% lower with maximum radiation load. On the other hand, Holstein cows showed heat production decreases of 26% at 21.1 ºC and of 9% at 26.7 ºC. In the same experiment, Brahman cow showed little response to radiation insofar as heat production was concerned. The authors concluded that the lack of response by Brahman cows was due to their low heat production rate. Therefore, their heat dissipation requirement was not more than half that of the lactating Jersey and Holstein.

1.2.1.3.2 Evaporation

Evaporative cooling from the outer surface of cattle is significant. This method of heat dissipation is most efficient in hot and dry environments. The proportion of metabolic heat that is dissipated from an animal's body by evaporation increases with rising environmental temperatures and a decreasing temperature gradient between

animal and air. The heat required to convert water into vapor is referred to as the latent heat of vaporization. The vaporization of 1 ml of water requires 2.43 Joules and this is amount of heat lost when 1 ml of water evaporates from the skin or from the respiratory tract (Silanikove et al., 2000). Respiratory and cutaneous water losses can be separated into passive and thermoregulatory components. Passive water loss is the respiratory and and cutaneous diffusion losses, the latter constituting approximately two-thirds of passive water loss. Thermoregulatory water loss is activating by panting and sweating.

1.2.1.3.3 Conduction

Conduction is the transfer of heat due to the physical contact of the animal with a surface, air or liquid that is cooler than the animal. Conduction of heat to solid surfaces is usually minimal in standing animals as only 2% of the surface (the hooves) is in contact with the ground, however when lying-down 20-30% of the body surface may be in contact with the ground. The flow of heat by conduction depends on the temperature difference, the conductance of the media, and the area of the contact (Kadzere et al., 2002). For the high producing dairy cow it is important to know that the magnitude of conductive heat transfer depends on the nature of the materials in contact with its skin, in particular its thermal conductivity. To alleviate heat stress utilization of bedding materials with high conductance may facilitate cooling of the animal.

1.2.1.3.4 Convection

Convective heat transfer occurs in response to movement of fluid or gas. When cool air comes in contact with a warm body, a layer of air surrounding the surface of the body is heated and rises moving away from the body, carrying with it heat and thereby cooling the body. On the contrary, if air temperature is greater than skin temperature, then air movement will promote the movement of heat into the animal until air temperature equals skin temperature when transfer of heat ceases. The transfer of heat during respiration is a form of convective heat transfer. The velocity of air movement affects the rate of convection and anything that resists air movement such as fur in cattle decrease the rate of heat transfer by convection.

1.2.1.4 Defining stress

The term stress is commonly used to indicate an environmental condition that is adverse to the well-being of an animal. Stress may be climatic, such as extremely low or high temperature; due to water or feed deprivation; social; due to some physiological disorder; pathogens or toxins (Scott, 1981). The term heat stress is very general and is used widely and quite loosely (Yousef, 1988). It may refer to the effects of the climate on the cow, or productive or physiologic responses by the cow (West, 2003). Lee (1965) presented a working definition of stress that is often used by physiologists in which stress denotes the magnitude of forces external to the bodily system which tend to displace that system from its resting or ground state, strain is the internal displacement from the resting or ground state brought by the application of the stress. Therefore the environmental factors external to the cow would contribute to stress. In this case, it is the heat stress. The displacement of the cow from the cow's resting state would be the response to the external stress, or heat strain (West, 2003).

The effect of hot and humid conditions in the cow's environment are thought to mediate an effect on cow body temperature. Berman et al., (1985) suggested that the ambient temperature of 25 to 26 ºC is the upper limit of ambient temperatures at which Holstein cattle may maintain stability of body temperature. The lower critical temperature was estimated for cows producing 30 kg FCM/day on the basis of calorimetric data as -16 to -37 ºC (Hamada, 1971). At or below the upper limit and above the lower critical temperature, Holstein cattle may maintain a stable body temperature. Above the upper limit or, in other words, upper critical temperature, an increase in body temperature negatively influences performance, reducing milk production and changing milk composition, and the cow enters heat stress.

Berman et al., (1985) also found that this critical temperature was apparently not modified by previous acclimatization or by milk production. This finding contradicts the conclusion of Yousef (1988) that the lower and upper limit varies with physiological state and other environment conditions. In a hot and humid area, not only temperature but also humidity affects body temperature. The combination of high relative humidity with high ambient temperature is one of the major challenges facing dairy farmers in such area. Igono and Johnson (1970) found that high

producing cows were more sensitive to heat stress and milk production declined significantly when rectal temperatures exceeded 39 ºC for more than 16 hours. In addition, Purwanto et al., (1990) found cows at high (31.6 kg/day) and medium (18.5 kg/day) milk production levels generated 48.5 and 27.3% more heat than dry cows, respectively. At a temperature 29 ºC and 40% relative humidity the milk yield of Holstein, Jersey and Brown Swiss cows was 97 93 and 98% of normal, but when relative humidity was increased to 90% yields were 69, 75, and 83% of normal (Bianca, 1965).

1.2.1.5 Assessment of heat stress in the dairy cow

Understanding when a cow enters heat stress is very useful to know when to take action to reduce heat stress in the dairy cow. Lactating dairy cows prefer ambient temperatures not more than 25 ºC. At an ambient temperature above 26 ºC, the cow can not maintain body temperature within the comfort range and enters heat stress (Kadzere et al., 2002). Body temperature has been used to assess thermal stress in cattle (Fuquay, 1981). Body temperature of dairy cattle shows great susceptibility to hot weather; therefore, it is a sensitive indicator of thermal stress (Araki et al. 1984). Although body temperature is an excellent indicator of an animal's susceptibility to heat load, this indicator is not practical when a large number of animals are monitored due to the lack of a practical device (Davis et al. 2003, Madder et al. 2006). There are viable alternative methods to use body temperature to assess animal heat load such as monitoring panting, respiration, or both (Gaughan et al. 2000, Mader et al. 2006).

The main factors influencing heat stress in the dairy cow are air temperature, relative humidity, air movement, and solar radiation (Armstrong 1994, Silanikove, 2000). To estimate the degree of heat stress affecting dairy cattle and other animals, these factors should be considered. Many heat stress indices have been developed and used to provide a weighted estimate of these factors. However, their use is limited by poor availability of data (Bohmanova et al. 2007). In early studies, black globe-humidity index which is a composite temperature used to estimate the effect of air temperature, relative humidity, wind speed and solar radiation on animals, was used (Silanikove, 2000). However, the majority of studies on heat stress in livestock have

focused mainly on temperature and humidity (Igono et al. 1985, Ravagnolo and Misztal, 2000; Ravagnolo et al., 2000; Bouraoui et al., 2002; St-Pierre et al., 2003; West, 2003; Correa-Calderon et al., 2004; García-Ispiero et al., 2007; Suadsong et al., 2008; Kaewlamun et al., 2008). This is because temperature and humidity records can be usually obtained from a meteorological station located nearby and data on solar radiation received by animal and wind speed are not publicly available.

A Temperature-Humidity Index (THI) is a single value representing the combined effects of air temperature and humidity associated with the level of thermal stress (Bohmanovo et al., 2007). This index has been developed as a weather safety index to monitor and reduce heat-stress-related losses. THI is usually classified into classes that indicate level of heat stress. Armstrong (1994) identified an index below 71 as the comfort zone, values ranging from 72 to 79 as mild stress, 80 to 89 as moderate stress and values above 90 as severe stress. There are differences in sensitivity due to ambient temperature and the amount of moisture in the air among species. Sánchez et al. (2009) reported that the onset of heat stress for milk yield varies among individual cows, and over half that variation has an additive genetic origin.

1.2.1.6 Effect of heat stress on feed consumption, milk production and economic loss

High ambient temperature affects cow dry matter intake (DMI). During hot periods, DMI was reduced compared to cool periods (West et al., 2003). Spain et al. (1998) reported that mid-lactation cows under heat stress, temperature between 24 – 33 ºC decreased feed intake by 6 to 16% when compared to thermal neutral conditions of 20 ºC. Ronchi et al. (2001) found that in heifers managed at 32 ºC and 70% relative humidity dry matter intake decreased by 23%. A study in lactating dairy cows during a cool period (average temperature 17.9 – 29.5 ºC and THI 63.8 – 76.6) and a hot period (average temperature 22.5 – 34.4 ºC and THI 72.1 – 83.6) showed that DMI and milk yield declined linearly with respective increases in air temperature or THI during the hot period (West et al., 2003). Holter et al. (1997) reported heat stress decreased intake of cows more than heifers. In lactating dairy cows DMI begins to

decline at mean daily environmental temperatures of 25 to 27 ºC and the environmental temperature at which feed consumption begins to decline is influenced by diet composition, for example, the greater proportion of roughage in the diet, the greater and the more rapid in the DMI as environmental temperature rise (Beede and Collier, 1986). There are many physiological responses to high temperature activated to maintain normal body core temperature. Reducing dry matter intake and therefore heat generated during ruminal fermentation and body metabolism aid in maintaining heat balance. Additionally, increased respiratory rates and water intake in heat stressed cows lead to concomitant reductions in DMI (Roman-Ponce et al., 1977; Mallonée et al., 1985). Attebery and Johnson (1969) measured the effect of temperature on rumen motility when feed intake was maintained at a constant level and determined if changes in rumen motility are influenced directly by environmental temperature rather than indirectly by changes in feed consumption resulting from differences in environmental temperature. They found that rates of ruminal contraction were reduced when the cows were exposed to high temperatures and rumen activity was influenced directly by high temperature rather than indirectly via feed intake. Reduction in gut motility, along with increased water intake leads to gut-fill. Warren et al. (1974) showed that the rates of passage of ingesta in steer fed forage diets during heat stress were reduced leading to gut-fill, probably depressing appetite. A direct negative effect on the appetite center of the hypothalamus may exist (De Rensis and Scaramuzzi, 2003).

As mentioned above, high ambient temperature reduces feed intake in dairy cows. Reduced feed intake results in less essential nutrients and metabolizable energy being consumed. The decline in nutrient intake has been identified as a major cause of reduced milk synthesis (Faquay, 1981). The reduction in DMI can account for 30 – 50% of the decrease in milk production when cows are heat stressed (McDowell et al., 1969; Rhoads et al., 2009). McDowell et al. (1976) studied the effect of climate on milk yield. Igono et al. (1992) investigated the effect of environmental temperature on milk production of Holstein cows in a desert climate and found that milk production declined markedly with maximum THI greater than 76, minimum THI greater than 64, or mean THI greater than 72. In the literature, West (2003) showed that milk yield and DMI exhibited significant declines when maximum THI reach 77. Estimated milk

yield reduction was 0.32 kg per unit increase in THI (Ingraham, et al., 1974) and milk yield and TDN intake declined by 1.8 and 1.4 kg for each 0.55 ºC increase in rectal temperature (West, 2003). Ravagnolo et al. (2000) estimated the effect of heat stress on milk production using THI and reported that milk yield declined by 0.2 kg per unit increase in THI when THI exceeded 72 and the authors suggested that THI can be used to estimate the effect of heat stress on production.

In dairy farming, heat stress is responsible for important economic losses. A decrease in milk production would decrease income from selling milk or milk products. There is no simple way to assess the economic impact of heat stress on a whole farm basis. St-Pierre et al. (2003) estimated the economic losses from heat stress considering all class of livestock in the United States. Nationally, heat stress results in an estimated total annual economic loss to the livestock industry that is between $1.69 and $2.36 billion.

1.2.1.7 Effects of heat stress on measured reproductive traits

The schematic description of the possible mechanisms for the effects of heat stress on reproduction in lactating dairy cows is showed in Fig. 2 and the pathways by which heat stress affects fertility are showed in Fig. 3. Heat stress impairs reproductive performance in dairy cows (Wolfenson et al., 2000; Jordan, 2003). Efforts have been made to investigate the impact of heat stress on reproductive losses. Many studies have detected seasonal differences in conception rate in dairy cows (Badinga et al., 1985, Cavestany et al., 1985, Thompson et al., 1996, Al-Katanani et al., 1999, De Rensis et al., 2002). In an early study, Ingraham et al. (1974) reported that, in Holstein dairy cows raised under a subtropical climate, conception rates for cows serviced on days with an average THI 66 was 67% as compared to 21% for cows serviced on days averaging above 78. This study was consistent with Ingraham et al. (1976) who showed that conception rates declined from 66% to 35% as the THI increased from 66 to 78 especially 2 days before AI. Ravagnolo and Misztal (2002) analyzed the reproductive data to determine the effect of heat stress on non-return rate at 45 day (NR45) in Holsteins. NR45 showed a decrease of 0.005 per unit increase in THI on the day of insemination for THI >68. Chebel et al. (2004) determined factors

associated with CR in high producing lactating Holstein cows. In their study, temperature >29 ºC was considered to be heat stress. Exposure to heat stress from -50 to -20 days before AI was associated with reduced CR from 31.3 to 23% when compared to cows not exposed to heat stress. Likewise, García-Isieto et al. (2007) reported that the decrease in conception rate was evident, from 35-33% to 21-27%, at THI values higher than 72 and they draw the conclusion from the study that climate factors (temperature and humidity, measured as THI) seem to be highly relevant for conception rate, especially during the period 3 days before to 1 day after AI. Morton et al. (2007) reported that conception rates were reduced when the cows were exposed to a high heat load form 7 days before to 7 days after service. Reductions in conception rates in hot periods are due to the combination of effects of environmental heat over prolonged time rather than on single days around the day of service. A retrospective study on the effect of environmental on conception rate in Holsteins revealed that there was a large drop in conception rates
during the summer, which coincided with high THI measures and conception rate was highest during the cool season (Huang et al., 2008).

The reduction in conception rate in heat stressed cows subsequently results in a higher DO than non-heat stressed cows. Ray et al. (1992) reported that cows calving in the spring and summer in Arizona, had the longest calving interval and required the most services per conception. In a study evaluating seasonality of DO in US Holsteins, longer DO were observed in cows calving during the months with high temperature (Oseni et al., 2003). Cows with depressed fertility resulting from heat stress always have longer DO periods. However, DO is affected by many factors other than heat stress such as management polices, herd size, production level, lactation number and AI techniques (Oseni et al., 2004[a,b]).

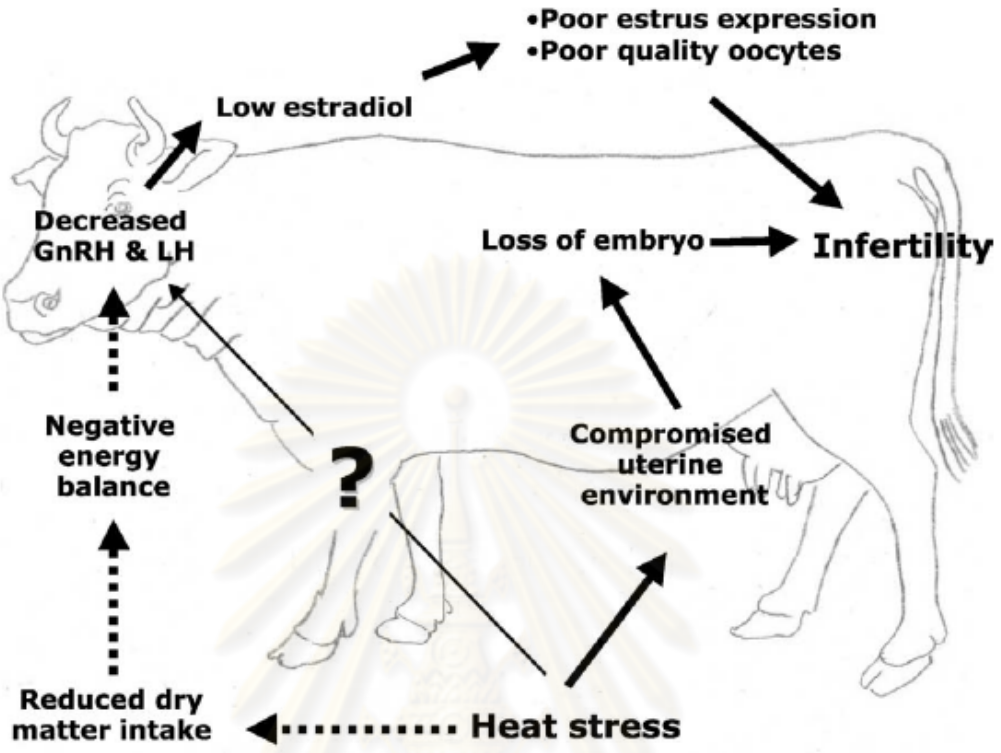


Figure 2 A schematic description of the possible mechanisms for the effect of heat stress on reproduction in the lactating dairy cow. Heat stress can act in more than one way to reduce fertility in lactating dairy cows. Heat Stress can reduce dry matter intake to indirectly inhibit GnRH and LH secretion from the hypothalamo-pituitary system (dashed lines). However, it is not clear if heat stress can also directly influence the hypothalamo-pituitary system (thin solid line) to reduce GnRH and LH secretion. Heat stress can directly compromise the uterine environment (solid lines) to cause embryo loss and infertility. (De Rensis and Scaramuzzi, 2003)

1.2.1.8 Effects of heat stress on estrus behaviour

Heat stress alters physiological and behavioural processes of estrus. In heat stressed cows, the duration and intensity of estrus were reduced (Gangwar et al., 1965; Gwazdauskas et al., 1981; Younas et al., 1993; Rodtian et al., 1996) and the incidence of anestrus and silent ovulation were increased (Gwazdauskas et al., 1981).

Wolfenson et al. (1988) reported that during the summer months in Israel, estrus behavior lasted longer (16 h) for cooled than in non-cooled Holstein cows with low BCS. The reduction in the number of mounts in hot weather compared to cool weather was observed (Pennington et al., 1985). The duration of estrus was shorter under hot climatic conditions than under cool climatic conditions, thus requiring more time spent observing estrus. High ambient temperature such as found in tropical areas has a definite and a depressing effect on the expression of estrus in cattle (Gangwar et al., 1965; De Rensis and Scaramuzzi, 2003). The poor expression of estrus signs in heat stressed cows leads to poor detection of estrus. Therefore, there is a reduction in the number on inseminations and an increase in the proportion of insemination that do not result in pregnancy (Alnimer et al., 2002; De Rensis and Scaramuzzi, 2003).

1.2.1.9 Effect of heat stress on reproductive hormones

Ovarian activity is mainly regulated by gonadotropin releasing hormone from the hypothalamus and luteinizing hormone (LH) and follicle stimulating hormone from the anterior pituitary gland. Heat stress has a detrimental effect on reproduction partly by disrupting the normal release of these hormones (Dobson et al., 2003). Some authors have studied the effects of heat stress on the secretion of these hormones. The effect of heat stress on LH concentrations in peripheral blood is inconsistent among studies. Gwazdauskas et al. (1981) and Gauthier (1986) reported unchanged concentrations, while Roman-Ponce et al. (1981) reported increased concentrations and some authors reported decreased concentrations (Madan and Johnson, 1973; Wise et al., 1988[a]) in heat stressed cows. These discrepancies may be associated with differences in sampling frequency, which varied from once a day to once every three hours and depended on whether heat stress was acute or chronic. Regarding the pattern of LH secretion in heat stressed cows, Gilad et al. (1993) found lower LH basal concentrations and lower LH amplitude in heat stress cows with low plasma oestradiol and Wise et al. (1988[a]) found lower LH pulse frequency in the heat stressed cows compared to the cows under cooling. Conflicting results have been reported regarding the preovulatory LH surge in heat stressed cows. Madan and Johnson (1973) reported a reduction in the endogenous LH surge caused by heat stress in

heifers and some authors reported that it was unchanged in cows (Gwazdauskas et al., 1981; Rosenberg et al., 1982; Gauthier, 1986). The reasons for these discrepancies are unclear. Gilad et al. (1993) have suggested that these differences are related to preovulatory estradiol levels because heat stress had no effect on tonic LH secretion or GnRH-induced LH release in cows with high concentrations of plasma estradiol and heat stress depressed LH concentrations in cows with low concentrations of plasma estradiol. De Rensis and Scaramuzzi (2003) concluded from the information available in the literature that LH levels are decreased by heat stress, that the dominant follicle develops in a low LH environment and this results in reduced estradiol secretion from the dominant follicle leading to poor expression of estrus, and hence, reduced fertility.

Gilad et al. (1993) reported lower concentrations of FSH in acute and chronic heat stressed cows which also had lower concentrations of estradiol while no alterations in concentrations of FSH were observed in cows which had normal concentrations of estradiol. Conversely, Ronchi et al. (2001) reported no differences in frequency, amplitude of FSH pulses and baseline concentrations of FSH between cows exposed and unexposed to high ambient temperatures. However, Roth et al. (2000) reported that plasma concentrations of FSH were higher in heat stressed cows than in cooled cows. It has been suggested that an increase in concentration of FSH in heat stressed cow is related to the concentration of inhibin (Roth et al., 2000; De Rensis and Scaramuzzi, 2003). Increased concentrations of FSH by heat stress may be due to decreased plasma inhibin production by compromised follicles because inhibin is an important factor in the regulation of FSH secretion and there is a negative relationship between plasma FSH and immunoreactive inhibin concentrations (Findlay, 1993; Kaneko et al., 1995, 1997). Further research related to the effect of heat stress on secretion of FSH in dairy cows is required before a conclusion can be reached.

The effect of heat stress on estradiol concentrations in peripheral blood found in the earlier studies were inconsistent, for example, Roman-Ponce et al. (1981) found unchanged, Rosenberg et al. (1982) found increased and Gwazdauskas et al. (1981) found decreased estradiol. However, most recent studies have suggested that plasma estradiol concentrations in heat stressed cows were decreased. In lactating dairy cows

which were housed in a climate controlled chamber at the ambient temperature of 29ºC and 60% relative humidity and dairy heifers which were housed at ambient temperature of 33 ºC and 60% relative humidity had a reduction in concentrations of estradiol in plasma between days 11 and 21 of estrus cycle associated with decreased follicular size (Wilson et al., 1998[a, b]). Likewise, Wolfenson et al. (1995) reported that estradiol concentrations were lower in heat stressed cows during days 4 and 8 of the first follicular wave than in non-heat stressed cows. Additionally, a pronounced reduction in concentrations of plasma estradiol in cows in late summer that had experienced chronic exposure to heat stress was observed as compared to cows in early summer that had experienced heat stress for a shorter period (Badinga et al., 1993). The longer period of exposure to heat stress may severely impair follicular function resulting in a reduction in estradiol production. The same authors also found lower estradiol concentrations in the follicular fluid of dominant follicles on day 8 of the estrus cycle in late summer compared to early summer and Wolfenson et al. (1997) reported that the estradiol concentrations in follicular fluid was low in autumn and summer and high in winter. In contrast, Badinga et al. (1994) reported that, in a field study, the concentration of estradiol was highest in the hottest month compared to the cooler months in Florida.

The studies of the effect of heat stress on plasma progesterone concentrations also report variable results. Wilson et al. (1998[a, b]) found that heat stress had no effect on plasma progesterone concentrations in lactating cows and dairy heifers when they were exposed to heat stress in a climate controlled chamber during the second half of the estrus cycle, but that luteolysis was delayed. In a study by Roth et al. (2000), plasma progesterone concentrations during the estrus cycle in cows that were exposed to heat stress or were cooled did not differ between groups and during the subsequent cycle and, plasma progesterone concentrations did not differ between previously heat stressed or cooled cows. Wolfenson et al. (1988) and Wise et al. (1988[b]) found in earlier studies that plasma progesterone concentrations were decreased in heat stressed cows. Rosenberg et al. (1982) found that plasma progesterone concentrations measured during the estrus cycle before the first insemination was higher in the winter than in the summer in multiparous, but not in primiparous cows. Jonsson et al. (1997) have reported a similar result, they showed that plasma progesterone concentrations

during the life of the second corpus luteum after calving were lower in the summer than in the winter and that THI during the first 14 days after calving was negatively correlated with progesterone production by the second corpus luteum after calving. Younas et al. (1993) measured plasma progesterone concentrations in cooled and non-cooled cows during summer and found that plasma progesterone concentrations were lower in non-cooled cows compared to cooled cows. Ronchi et al. (2001) also reported that plasma progesterone concentrations were lower in Holstein heifers under heat stress compared to thermoneutral Holstein heifers. In contrast, exposure of heifers to heat stress for 2 successive cycles has been reported to result in increased plasma progesterone concentrations on day 2 to 19 of the first cycle and on day 2 to 8 of the second cycle (Abilay et al., 1975) and Trout et al. (1998) found higher progesterone concentrations in heat stressed cows until day 19 of estrus cycle. Wise et al. (1988[a]) found that plasma progesterone in non-cooled cows tended to be higher on day 3 to 5 of the estrus cycle when compared with cooled cows. However, plasma progesterone concentrations depend on its rate of production by the CL, possible adrenal release of progesterone, the degree of haemodilution and haemoconcentration, and metabolic clearance rate. It has been suggested that the latter is related to hepatic blood flow and feed intake (Vasconcelos et al., 2003). An acute increase in hepatic blood flow occurs in following feed intake (Sangsritavong et al., 2002). Over 90% of progesterone in hepatic portal blood is metabolized during the first pass through the liver (Parr et al., 1993). Furthermore, the following factors also contribute to the plasma concentration of progesterone; the degree of hyperthermia, the type of heat exposure (acute vs. chronic), the age of the cows, their stage of lactation and the type of diet (Wolfenson et al. 2000; De Rensis and Scaramuzzi, 2003). These differences observed for the effect of heat stress on the progesterone concentrations, probably originate from uncontrolled changes in other factors that affect plasma progesterone concentrations.

1.2.1.10 Effects of heat stress on follicular development and oocyte quality

The effects of heat stress on follicular dynamics have been studied. Badinga et al. (1993) found no detectable effects of acute heat stress on the overall pattern of

growth of the first wave dominant and subordinate follicles between day 1 and 7 of estrus cycle. They also found that, at day 8, first wave dominant follicles in shaded cows were bigger and contained more fluid than first wave dominant follicles in non-shaded cows. However, Wolfenson and co-workers (Wolfenson et al. 1995) have shown that heat stress altered the growth patterns of the first and second wave dominant follicles. The size of the first wave dominant follicle decreased earlier in heat stressed cows than in controlled cows and the size of second wave dominant follicle in heat stressed cows increased earlier, in other words, the second wave preovulatory follicle emerged earlier in heat stressed cows. The number of large (≥ 10 mm) during wave 1 was increased in heat stressed cows. This response may result from depressed dominance of the large follicle, permitting one more large follicles to form (Wolfenson et al. 1995). Concerning dominance of large follicles in heat stressed cows, the results mentioned above were consistent with the reports of Wilson et al. (1998) and Roth et al. (2000) who found early emergence of the second follicular wave. The attenuation of dominance of the second wave dominant follicle was detected reflected in a medium sized follicular wave that lasted for an additional 2 days in heat stressed cows compared to cooled cows (Roth et al., 2000). In heat stressed cow, the earlier emergence, by 2 to 4 days, of preovulatory follicles prolonged the duration of dominance because the time of estrus and ovulation were not altered by the thermal state (Wolfenson et al. 1995). This may result in ovulation of older follicle which released aged oocyte. This could have consequences on fertility since the duration of dominance is negatively correlated with fertility (Mihm et al. 1994).

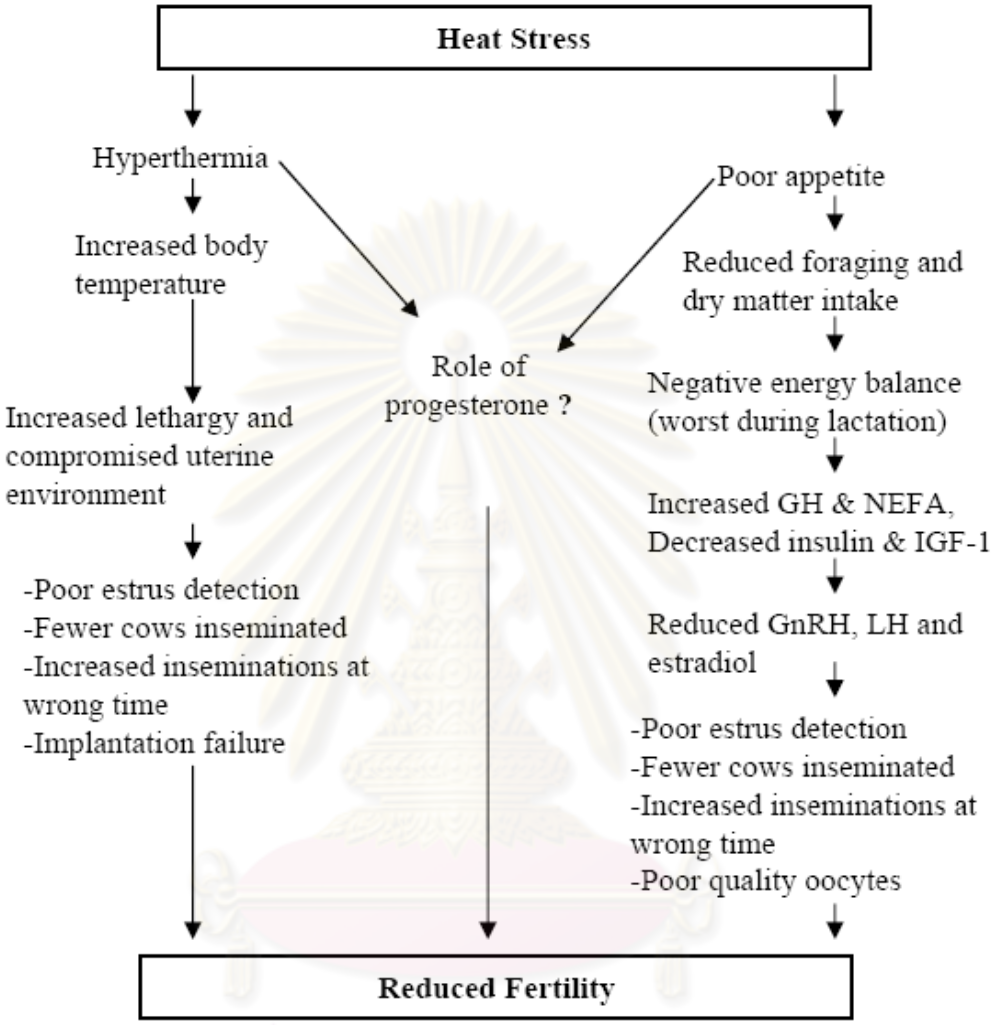
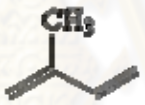


Figure 3 The two main pathways by which heat stress can affect fertility in lactating dairy cow. Hyperthermia leads to increased lethargy and a compromised uterine environment, both of which can lead to worsening infertility through poor estrus detection and embryo loss. Poor appetite leads to lower dry matter intake, thus exacerbating the effects of negative energy balance in early lactation. Negative energy balance produces lower blood concentrations of insulin and IGF-I, and higher blood concentrations of GH and NEFA, and this altered metabolic profile acting via the hypothalamo-pituitary system reduces GnRH and LH secretion, leading to reduced estradiol secretion by the dominant follicle. The consequences of reduced estradiol secretion from the dominant follicle are poor estrus detection, compromised oocyte quality, and in extreme situations, ovulatory failure. The role of

progesterone in summer infertility, if any, remains uncertain and controversial. (Adapted from De Rensis and Scaramuzzi, 2003)

1.3 β-carotene and dairy cow

β-carotene is an organic compound and is a member of family of molecules known as carotenoids. Carotenoids have a basic structure made up of isoprene units. β-carotene is made up of eight isoprene units, where the two exterior units are cyclic. More than 600 carotenoids that are synthesized by higher plants and algae and are involved in photosynthetic process have been isolated. Carotenoids are found in many plants and animals, but animals are unable to synthesize carotenoids. Carotenoids are the main group of natural pigments. Carotene, xanthophyll and lycopene are responsible for orange, yellow and red colouring, respectively.



Isoprene

all-*trans*-β-carotene

Figure 4 Chemical structure of isoprene and all-*trans*-β-carotene

β-carotene is the provitamin A and is converted to vitamin A by the specific converting enzymes. The capacity to synthesis compounds with vitamin A activity is limited to plants and microorganisms (During and Harrison, 2006). Thus, higher animals must obtain vitamin A from the diet, either by the performed vitamin or as a provitamin, carotenoids, such as β-carotene. β-carotene is quantitatively the most important carotenoid in ruminant diets (Nozière et al., 2006) and is also the main circulating carotenoid, especially the all-*trans* isoform (Yang et al., 1992).

1.3.1 β-carotene and reproduction in dairy cow

The corpus luteum (CL) is very rich in β-carotene which is responsible for its colour. The bovine CL contains about two to five times as much β-carotene as other tissues (Haliloglu et al., 2002). The high concentratons of β-carotene found in the CL have led many researchers to postulate as to the role of β-carotene in the luteal function in cattle (Table 1). In a review, Hemken and Brenel (1982) discussed the series of German studies which were conducted in the late 1970's which showed that cows fed low-carotene diets had lower conception rates, higher numbers of inseminations per conception, lower intensities of estrus, higher incidences of cystic ovary compared with cows fed high-carotene diets. Rakes et al. (1985) reported that a supplement of β-carotene 300 mg/cow/d had positive effects on reproduction. They also noted that cervix diameters for cows supplemented with β-carotene were smaller at 21 days and 28 days postpartum and the number of days from parturition to first observed estrus was lowered by β-carotene. A high incidence of retained fetal membranes was reported to be associated with lower concentrations of plasma β-carotene in dairy cows (Inaba et al, 1986; Akar and Gazioglu, 2006). Graves-Hoagland et al. (1988) suggested that a positive relationship exists between postpartum luteal function and plasma concentrations of β-carotene in cows. This was in agreement with other authors who found that β-carotene could increase progesterone production in cultured bovine luteal cells (Arikan and Rodway, 2000).

Bovine follicular fluid contains high concentrations of β-carotene and the concentration in follicular fluid seems to be proportional to corresponding concentrations in blood (Chew et al., 1984; Schweigert and Zucker, 1988; Haliloglu et al., 2002). It was proposed that the oocyte itself is under the retinoid influence within their intrafollicular growth (Gómez et al., 2006). Schweigert and Zucker (1988) found higher concentrations of vitamin A in the non-atretic follicles than in atretic follicles. They suggested that the the higher concentrations of vitamin A found in non-atretic follicles might be due to a transfer of vitamin A bound to its specific carrier protein, the retinol-binding protein-pre-albumin complex, from blood in to follicular fluid. The other possibility was that the higher concentrations might be caused by a carotene

cleaving enzyme located in follicular structures. Such a local conversion of β-carotene is found in different tissues including luteal cells. Sales et al. (2008) found the benefit of β-carotene given along with tocopherol in improving embryo quality in superovulated Holstein cows, but not in Holstein heifers.

However, many authors reported no effect of β-carotene on reproduction. Bindas et al. (1984[a]) reported that a supplementation of β-carotene 600 mg/cow/d beginning on day 30 postpartum through day 90 postpartum did not affect day to first service or service per conception in Holstein cows. This finding was consistent with Akordor et al. (1986) who found no effect of β-carotene on first service conception rate, uterine involution or milk yield. Bindas et al. (1984[b]) reported that supplementation of β-carotene had no effect on LH, progesterone, insulin, glucagon or reproductive measures. Likewise, Wang et al. (1988[a]) investigated the long term β-carotene supplementation on releasable LH in response to GnRH challenge. They found that β-carotene had no effect on basal concentrations of LH, frequency and amplitude of LH pulses or the release of LH in response to GnRH. They also noted that β-carotene had no effect on progesterone concentration. Furthermore, an adverse effect of supplementation of β-carotene in diet on fertility was reported (Folman et al., 1987).

The differences in the findings of earlier reports may be explained in part by the diversity of experimental conditions used. Such experimental differences include animal ages, stage of lactation at initiation of supplementation, length of treatment period, diet types and composition and concentration of β-carotene in basal diet (Hurley and Doane, 1989). Supplementation with β-carotene after parturition failed to demonstrate a beneficial effect of β-carotene on reproduction in dairy cows (Bindas et al., 1984[b]; Wang et al., 1988[b]). It has been accepted that plasma concentration of β-carotene decrease during the dry period in dairy cows and reach a minimum level in the first week postpartum (Rakes et al., 1985; Inaba et al., 1986; Michal et al., 1994). Recently, Kawashima et al. (2009[a]) showed, in a retrospective study, that dairy cows that went on to ovulate in the first 30 days postpartum had higher plasma β-carotene concentrations in the dry period than cows that did not go on to ovulate. Therefore, pre-parturition supplementation may be more effective than post parturition in

improving reproductive success. In experiments conducted using a supplement of β-carotene, the levels of supplement ranged from 300 to 600 mg/day (Bindas et al., 1984[a, b], Wang et al., 1988[a,b], Akordor et al., 1986). The level of supplementation may not have been optimal to enhance reproductive performance. Furthermore, Nozière et al. (2006) found that, in ruminants, degradation rates are usually higher when carotenoids are supplemented as a purified product than when provided in forages.

Table 1 The mechanism by which β-carotene may improve reproduction and immunity in daairy cows

Beneficial effect	**References**
Effects on reproduction	
Enhanced uterine involution	Rakes et al., 1985
Decreased incidence of retained fetal membranes	Inaba et al., 1986, Akar and Gazioglu, 2006
Increased progesterone production	Arikan and Rodway, 2000
Improved embryo quality	Ikeda et al., 2005, Sales et al., 2008
Effects on immunity	
Regulates membrane fluidity, gap junction communication	Chew and Park, 2004
Function as an antioxidant	Chew, 1993, Paiva and Russell, 1999, Chew and Park 2004, Sordillo and Aitken, 2009
Enhanced polymorphoneucler cell function	Tjoelker et al., 1988, Daniel et al., 1991
Enhanced/stimulate lymphocyte function	Daniel et al., 1991[b], Michal et al., 1994

1.3.2 β-carotene as its role in immune response and as an antioxidant

Independent of its role as a provitamin A, β-carotene may affect immune function. Carotenoids without vitamin A activity have been found to enhance immune function through their ability to regulate membrane fluidity, gap junction communication and as an antioxidant (Chew and Park, 2004; Table 1). *In vitro*, Daniel et al. (1991[a]) showed that β-carotene enhanced bovine blood and mammary gland phagocytic cell kill ability. Supplementation of cows with 400 mg β-carotene/cow/day from 6 week before drying-off through 2 week after drying-off showed the beneficial effect to stimulate polymorphonuclear phagocytic and bacterial killing ability. Phagocytic ability maintained after drying-off in β-carotene supplemented cows and tended to decrease after drying-off in cows fed vitamin A only (Tjoelker et al., 1988; 1990). Daniel et al. (1991[b]) also show that blood lymphocytes isolated from Holstein cows during the peripartum period and incubated with 1×10^{-9} M β-carotene had higher lymphocyte proliferation induced by concanavalin A than did an unsupplemented culture; whereas, retinol had no effect on lymphocyte proliferation. Likewise, Michal et al. (1994) reported that lymphocyte proliferation induced by mitogen stimulation was enhanced in cows supplemented with 300 or 600 mg β-carotene /cow/day before and after parturition.

The mechanisms by which carotenoids regulate immunity are not fully understood. The most widely recognized mechanism for carotenoid action is its antioxidant function (Chew, 1993). A number of studies have showed that β-carotene and others carotenoids have lipid-soluble antioxidant function (Paiva and Russell, 1999). β-carotene is one of a number of important free radical scavengers. β-carotene and others carotenoids are especially effective at quenching singlet oxygen and can prevent the subsequent formation of secondary reactive oxygen species (ROS) (Sordillo and Aitken, 2009). Chawla and Kaur (2004) reported the positive correlation between plasma β-carotene concentrations and antioxidant power measured by a ferric reducing antioxidant power assay in cows supplemented with β-carotene during the dry period and they also suggested that there is a need to supplement with β-carotene during the dry period in order to improve their plasma antioxidant vitamin

status and health after parturition. Embryo quality was improved in superovulated cows supplemented with β-carotene (Sales et al., 2008). It is possible that extracellular β-carotene in the follicular fluid and intracellular β-carotene incorporated into follicular cells and/or oocytes protects them from ROS mediated cytotoxity, thereby enhancing the developmental competent of oocytes (Ikeda et al., 2005). Retinol does not have the ability to quench single oxygen. Therefore, the uniqueness of β-carotene in bovine fertility may be relate to this ability.

1.3.3 β-carotene and neonatal calf

Calve are born premature and further morphological and functional changes are needed (Blum, 2006). Colostrum contains several components (nutrients, minerals, vitamins or provitamins (i.e. β-carotene), immunoglobulins (Ig), and cells) that influence on growth and health status in new born calves (Blum and Hammon, 2000, Zanker et al., 2001, Blum, 2006). Vitamin A and β-carotene have protective effects against infectious diseases and enhance immune system (Chew, 1987). Low plasma concentrations of vitamin A and β-carotene have been reported to be associated with the high incidence of diarrhea and mortality during the first week after birth (Lotthammer, 1979). In many species, liver stores of vitamin A are very low at birth. Kume and Toharmat (2001) suggested, based on the findings from their study, that β-carotene and vitamin A status at 6 days of age depend mainly on colostrum concentrations of β-carotene and vitamin A. Plasma concentrations of β-carotene have been shown to decrease in dairy cows during the dry period (Michal et al., 1994). This may be due to the transfer of β-carotene from blood to the colostrum or to the foetus.

It has been reported in an *in vitro* study that β-carotene enhances mitogen-induced lymphocyte proliferation and enhances bovine blood and mammary gland phagocytic cell killing ability (Bendich and Shapiro, 1986, Chew, 1993). This implies that β-carotene may increase the immunoglobulin content of colostrum. However, the mechanisms by which β-carotene or carotenoids regulate immunity are not fully understood. The importance of immunoglobulin G (IgG) levels in colostrum for calf

health is well recognized (Kume and Toharmat, 2001). Calves are born with no circulating IgG. The calf must absorb passively IgG from the colostrum.

1.4 Objectives

This thesis is comprised of 3 experiments and the objectives of each were;

Experiment I (Chapter II) Effects of region and month of calving on days-open in dairy cows in Thailand

1. To analyze seasonal and regional differences in temperature-humidity index
2. To describe the monthly distribution of calving
3. To investigate the effect of month of calving in relation to heat stress on days-open in first lactating dairy cows

Experiment II (Chapter III) Effect of heat stress on ovarian function, plasma metabolites and embryonic loss in first lactation dairy cows

4. To investigate the effect of heat stress on the resumption of ovarian activity and on plasma NEFA, IGF-1 and cortisol concentrations in postpartum first lactation dairy cows
5. To investigate the effect of heat stress on embryonic mortality in first lactation dairy cows

Experiment III (Chapter IV) Dietary supplementation with β-carotene and postpartum reproductive performance in dairy cows and calf health status at birth

6. To investigate whether a supplement of β-carotene given during the dry period was able to

6.1) increase blood concentrations of β-carotene in dairy cows

6.2) improve ovarian function and progesterone production

6.3) enhance uterine involution and improve uterine health

6.4) improve milk production and milk composition

6.5) modify hormone and metabolite status in dairy cows during the postpartum period

6.6) increase the amount of β-carotene in colostrum and increase the concentration of IgG in colostrum

6.7) modify metabolic hormone, enzyme and metabolite status in their calves at birth

CHAPTER II

Effects of region and month of calving on days-open in dairy cows in Thailand

2.1 Abstract

The objectives of this study were to analyse the potential impact of heat stress in different regions of Thailand, to determine the monthly distribution of calvings throughout the year and to investigate environmental sources of variation of days-open (DO) in first lactation dairy cows. Data included 13,548 lactation records between years 2004 to 2006. The climatic data were obtained from the provincial meteorological stations and the temperature-humidity index was also calculated. The difference between regions in THI was determined. The geographical regions in this study were Central, East, Northeast, and North. The distributions of calving by month were determined in lactations 1 to 5. The effect of month of calving (MOC) on DO was determined only in first lactation dairy cows. Fixed effect in the model included MOC, region, MOC × region. The lowest mean THI was observed in December (72) and the highest mean THI in April (80). THI differed significantly between regions (P<0.0001), and months (P<0.0001). Significant interactions between region and month (P<0.0001) were found for THI. THI values were different among regions (P<0.0001). In all regions, minimum THI values were observed in December and January and this effect was more pronounced in Northeast and North regions. The highest frequency of calving for the first lactation was observed in June (9.96%) and the lowest in February (6.63%). The highest frequencies of calving for the second (13.1%), third (14.1%), and forth (14.66%) lactation cows were observed in September and was 14.91 % for the fifth lactation cows in October. The lowest proportion of calving for second (5.02%) and forth (4.14%) lactation cows were in February and in March for the third (4.35%) and fifth (4.85%) lactation cows. The average DO in first lactation cows was 152 days. Significant effects of MOC (P<0.0001), region (P<0.0001) were found on DO. February calving cows had longest DO (219 ± 11 days) while cows calving in October and November had a

significantly shorter mean DO (133 ± 7 days). This study indicates that the high proportion of cows calving in October and November corresponds to previous breeding success in December and January which are the cool months of the year. Cows which calved during hot months had prolonged DO by several months.

2.2 Introduction

In Thailand, the introduction of extensive dairy development took place in the early 1960s. The typical Thai dairy farm is characterized as a smallholder farm with less than 20 lactating dairy cows (Hall et al., 2004). Low fertility remains one of the most important problems of Thai dairy herds to achieve optimum efficiency and economic performance (Aiumlamai, 2007). Heat stress is a major contributing factor to the low reproductive performance in lactating dairy cows worldwide (De Rensis and Scaramuzzi, 2003). In Thailand, the temperature exceeds 30 ºC for most months of the year and most cows are raised in conventional open-air barns where ambient temperature and humidity follow those observed outside. The main source of dairy cows genetic material in Thailand is temperate and subtropical countries (Koonawootrittriron et al., 2009), where the temperature and humidity are quite different from those found in Thailand. The adverse effect of heat stress on reproductive efficiency in dairy cows has been well documented and potentially includes impaired follicular development, delayed postpartum ovulation, altered intensity and estrus expression, reduced pregnancy rate and higher embryonic loss (Rodtian et al., 1996; De Rensis and Scaramuzzi, 2003; Kornmatitsuk et al., 2008). The synthetic Temperature and Humidity Index (THI), which is a function of ambient temperature and relative humidity, is widely used for measuring heat stress in lactating dairy cows. A THI value exceeding 72 indicates mild to extreme heat stress condition for lactating dairy cows (Armstrong, 1994). In high-yielding dairy cows raised in subtropical climate, the cows were said to be exposed to heat stress when temperatures were over 25 or 26 ºC (Berman et al., 1985). The impact of season of calving on subsequent reproduction has also been evaluated in many studies. Cows calving in spring and summer had the longest calving to calving intervals, they required more services per conception and presented a longer open period (Ray et al.,

1992; Jordan et al., 2002). Days-open (DO) is one of the variables which is most commonly used to measure fertility performance in dairy cattle. This is a complex trait that is affected by many factors such as season of calving, management policies, herd size, production level, lactation number and AI technique (Oseni et al., 2003). DO has become accepted as one of the best single measures of reproductive efficiency in dairy cows (Norman et al., 2002). Due to lack of information on the distribution of calving and potential heat stress on DO in dairy cows in Thailand, the present study was designed 1) to analyze regional differences in THI indexes, 2) to describe the monthly distribution of calving, and 3) to investigate the effect of month of calving in relation with heat stress on days-open in first lactating dairy cows.

2.3 Materials and methods

Data were obtained from the Bureau of Biotechnology in Livestock Production, Department of Livestock Development, Ministry of Agriculture and Cooperatives. These data contained information from 13,548 lactation records collected over the years 2004 to 2006. Data were initially edited to eliminate duplicate records. Calving intervals (CI) were calculated as the interval between two consecutive calving. Days Open (DO) was computed using the following equation; DO = CI – 280, where 280 represent the gestation period. DO was chosen to be used in the analysis in this study because we would like to place this study in practical dairy farming management and to explain the proper time to inseminate cows. Only first lactation cows with CI between 320 and 700 days and age at first calving between 720 and 1080 days were included in the final data set. This procedure resulted in 1,962 records from first lactation cows to be available for analysis. Regions were classified as Central, East, North-East,North and South (Fig. 5). However, the South was not included in the analysis since the data was available only in 1 province. The data included in the analysis were obtained from 11, 6, 6, and 8 administrative provinces in the Central, East, Northeast and North regions respectively.

Daily meteorological data were obtained over the years 2004 – 2006 from the 25 official provincial meteorological stations covering the 31 provinces included in

the study. The temperature and humidity index (THI) was calculated based on the month of calving as follows (García-Ispierto et al., 2007);

$$THI = (0.8 \times \text{Mean Temp}) + (RH/100) \times (\text{Mean Temp}-14.4) + 46.4$$

Where; Mean Temp = mean temperature (ºC), RH = relative humidity (%)

Figure 5 Map of Thailand shows the classification of the regions in this study

2.4 Statistical analyses

Data were analyzed using Statistical Analysis Systems (SAS Institute Inc., version 9.0, Cary, NC, USA). The effect of regions on THI and the impact of month of calving (MOC) on DO were analyzed by analysis of variance (Procedure GLM). The fixed effect of region, MOC, and the interaction between region and MOC were included in the model. The frequency of calving in each month was expressed as a

percentage of the total calving throughout the year. Significant differences are reported at $P<0.05$.

2.5 Results

The overall monthly means of maximum and minimum temperature, relative humidity and THI throughout the year are shown in Fig.6. Maximum temperatures were observed to range between 30 ºC in December upto 36 ºC in April. Minimum temperatures were in a range between 18 ºC (December and January) upto 24 ºC (April to September). RH ranged from 66% in December to 81% in September and THI ranged from 72 in December to 80 in April.

Mean monthly THI (Fig. 7) were different throughout year ($P<0.0001$) and the degree of monthly THI among regions were different ($P<0.0001$). For all regions, THI means were higher ($P<0.0001$) between April and September than in other months. Also, Central and Eastern regions presented very similar THI values which were especially higher ($P<0.0001$) than those observed in Northeast and North regions from October to April and during this period THI in the North region was lower than the Northeast region ($P<0.0001$). In all regions, minimum THI values were observed in December and January and this effect was more pronounced in Northeastern and Northern regions.

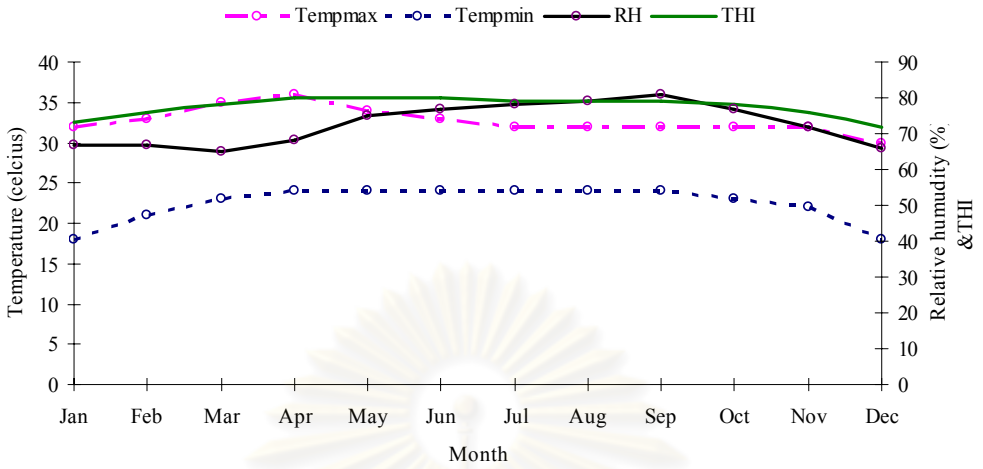


Figure 6 Monthly mean for maximum and minimum temperature, relative humidity (RH) and temperature humidity index (THI)

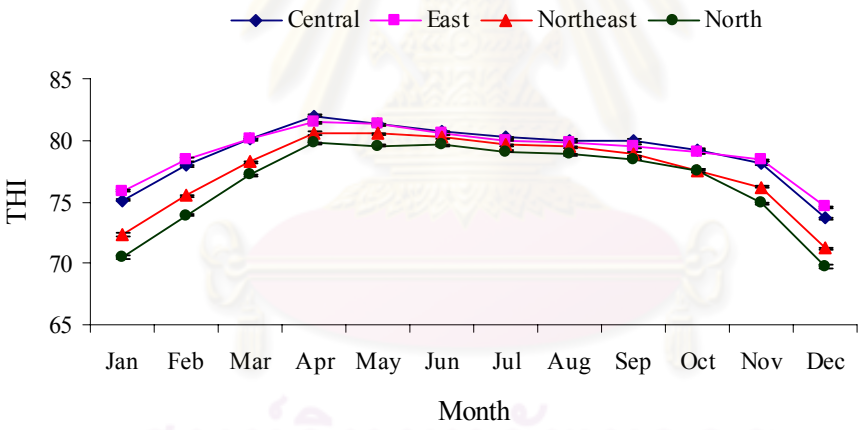


Figure 7 THI of the Central, Eastern, Northeastern, and Northern regions in pooled data from the year 2004 to 2006. LSmean ± SEM.

The frequency of calving was the lowest from December to February and then increased gradually to reach a maximum in September and October (Fig. 8). For all lactation ranks, a marked decline in the percentage of cows that calved in November was observed. The effect of the month on the frequency of calving is less pronounced in first lactation than for the other lactation ranks, the most striking differences in relation to month being observed for ranks 4 and 5.

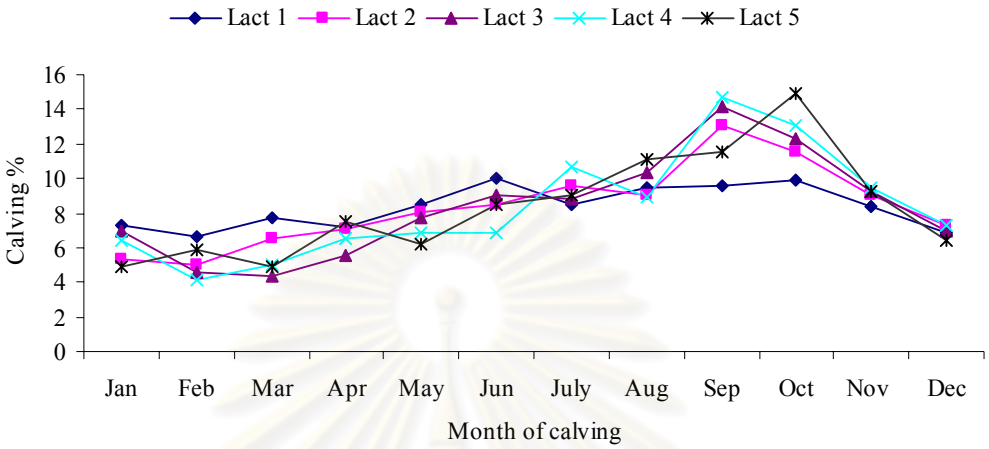


Figure 8 The percentage of calvings throughout the year in dairy cows (lactation 1 to 5), data obtained over the years 2004 to 2006.

The average DO in first lactation cows was 152 days. There were also effects of the MOC ($P<0.0001$) and region ($P<0.0001$) on the length of DO. There was no interaction between MOC and region on DO ($P=0.13$). The cows which calved in February presented the longest DO (219 ± 11 days) and the cows which calved in October and November had the shortest DO (133 ± 7 days, Fig. 9). The mean DO length decreased from February to October and November and increased in cows calving from December to February. DO in first lactation cows of the Central, Eastern, Northeastern, and Northern region were 183 ± 4, 172 ± 7, 177 ± 7 and 138 ± 7 days respectively. DO for the Central, Eastern and Northeastern regions were not statistically different among groups but were higher than the DO for the cows in the Northern region ($P<0.0001$).

Fig. 10 presents the distribution of DO in first lactation cows in pooled data, and for the "extreme" months, in terms of DO mean results i.e. February vs October and November. In pooled data, the distribution shows a peak at around 71-100 days followed by a slow decline. For cows calving in October and November, the peak of relatively short DO is more marked and very few cows conceived after 190 days. On the contrary, in cows that calved in February, a lower percentage of cows were pregnant within a short DO period and percentage of cows with long DO remained

high even for the longest delay registered. For each class interval of DO between 160 days and 360 days, this percentage is relatively high and close to 10%.

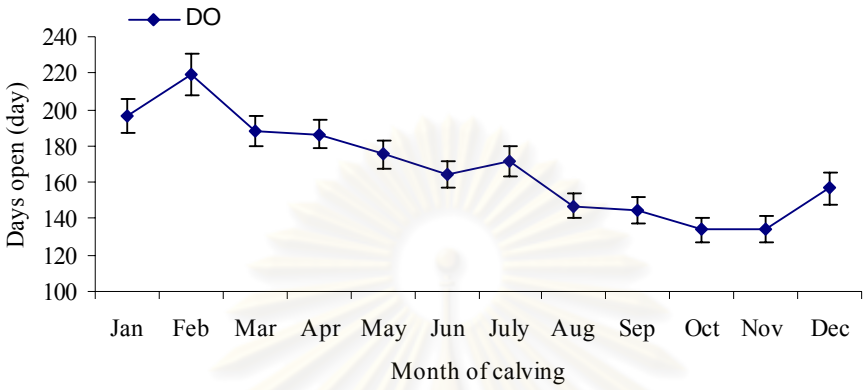


Figure 9 Least square means of days-open (DO) in first lactation cows by month of calving calculated from data over the years 2004-2006. LSmean ± SEM.

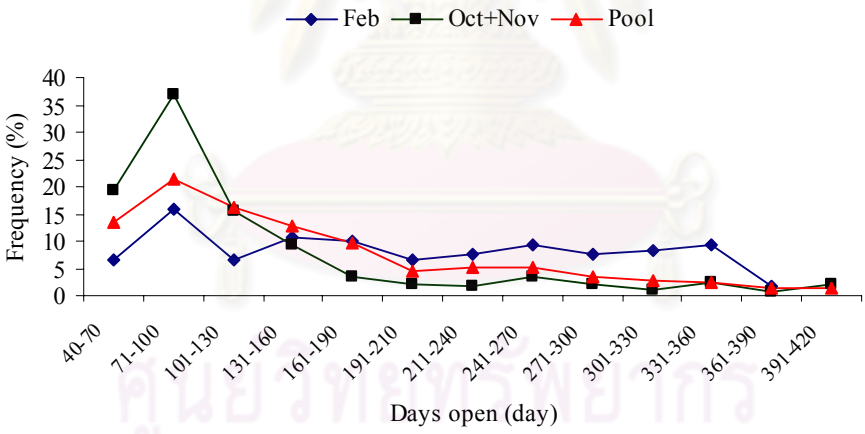


Figure 10 Distribution of days-open in first lactation cows by month of calving. Where; Pool = pooled data from every month of calving, Feb = days-open for a February calving cow representing the month of calving with highest DO, and Oct + Nov = days-open for an October and November calving cow representing the month of calving with lowest DO

2.6 Discussion

The climatic data from four regions in the present study indicated that dairy cows in Thailand are strongly exposed to heat stress throughout the year. Mean THI from October to March fell within the range of mild stress (72 to 78) and mean THI from April to September fell in the range of severe stress (>78 to 89; Armstrong, 1994). These data also show that potential heat stress is depends on regional variations, the cows being in Central and Eastern regions being more exposed than their counterparts in Northern and Northeastern regions.

In the present study the average days-open (152 d) in first lactation cows was shorter than the DO of 183 days reported by Virakul et al. (2001). However the latter DO was computed from all lactation numbers and the lower value reported here probably just illustrates and confirms the unfavourable effect of ageing or increasing rank of lactation on dairy cow fertility (Humblot et al., unpublished data). In this study the effect of MOC on the frequency of calving was less pronounced in first lactation than for other lactation ranks. Thus, the effect of MOC on DO in this study may be a slight underestimate by only including first lactation cows in the analysis. In addition to this, in our study, the data set were edited to include only cows with the calving intervals between 320 and 700 days.Thus, the extreme problem cows which had DO longer than 700 days were excluded from the study and this may have contributed to shorten DO. The present study demonstrated that MOC had a very significant effect on DO in dairy cows in relation to tropical climate changes in Thailand. These results are in agreement with those from a previous report in United States in which DO was longer for spring calving cows and shorter for fall calving cows (Oseni et al., 2003). This trend was attributed to depressed fertility during the summer, when spring calving cows are ready for rebreeding. High ambient temperature during the summer has been implicated in the reduced fertility observed in this season (Wolfenson et al., 2000). In the present study, DO were longer for February calving cows and shorter for October and November calving cows. There is a risk for cows calving in February to be rebred during the hot months. Considering that the voluntary waiting period post partum before rebreeding is about 50 days, the cows which calve in February need to be rebred in April, a time at which they are

exposed to high temperature and THI. This is in agreement with numerous studies in which a marked decrease in conception rate has been reported during the hot season (De Rensis and Scaramuzzi, 2003; García-Ispierto et al., 2007). These effects may have contributed to increase days open in dairy cows calving in February whereas cows calving in October and November reach the time for post-calving rebreeding in cool months with low mean temperature and THI (December or January). This was also observed in other studies were dairy cows had a higher conception rates during the cool season, required less services per conception (Kornmatitsuk et al., 2008) and subsequently had a shorter open period.

An intentional delay by the farmer due to poor conception in the summer period when the late cool calving cows and early summer calving cows are to be rebred is one of the possible reasons for cows with prolonged DO. However, the data to support this possibility was not available in this study. We found that DO was different between regions. The lowest DO was observed in the Northern region. This is logical in view of the fact that the mean THI of the Northern region was lower than the other regions. This indicated that the effects of heat stress on cow reproduction in this region were lower than the other regions. The conception rate was high when the cow was inseminated the during cool period (De Rensis and Scaramuzzi, 2003). The length of DO found in this study (138 days) was similar to the length of DO (131 days) reported previously for the Northern region (Punyapornwithaya and Teepatimakorn, 2004). The mean THI of the Central and Eastern region were similar throughout year but the length of DO in the Eastern region was lower than the Cental regon, although not statistically so. Even the mean THI was lower but the length of DO was higher in the Northeastern compared to the Central and Eastern regions. There are several factors implicated in the success of conception (De Rensis and Scaramuzzi, 2003, Chebal et al., 2004). Extended DO in the Northeastern region may be attributed to the insufficient feed supply especially in dry season. Most dairy farms in Thailand are characterized as smallholder farms and as an integral part of crop-livestock farming system (Hall et al., 2004). Major problems in livestock production under such a system include the insufficient feed supply for animal leading to poor nutritional status. Such a condition may increase the unfavorable effect of heat stress on fertility

(Butler, 2003, Humblot et al., 2010 unpublished) due to specific additional effects of those 2 factors on oocyte quality and early embryonic survival (Leroy et al., 2008). Considering the calving pattern from Fig. 8, it shows that the highest calving rate was obtained around September or October. These cows had to conceive around the previous December or January. The conception rate was high in December and January since they are cool months of the year resulting in most cows calving in the subsequent September and October. Calving rate in lactation number 2, 3 and 4 increased dramatically from August and reached the highest rate in September. Similar to the cows in lactation number 5 calving rate had increased dramatically from September and reached the highest rate in October. But, in first lactation cows, the pattern of calving was quite different. Calving rate in August to November was slightly changed and was lowest when compare to other lactations.

The highest calving rate in the first lactation cows occurred in June. The calving pattern in first lactation cows may partly be explained by the management. Since a lot of cows reached their expected calving date around August to October, they become a dry cow around June. Global milk production therefore decreases during this period. In order to maintain milk production, heifers are inseminated to calve in June resulting in a high calving rate of the pregnant heifers in this month. The difference between the percentage of calvings in the highest and the lowest calving month of the first lactation was low compared to the older cows and the profile of calving fluctuated slightly. This characteristic indicated that the heifers were inseminated throughout the year and the conception rates in heifer were not much different between months. However, the calving rate of the second lactation cows was highest in September corresponding to the high conception rate in the previous December or January. Therefore, it can be implied that there were some first lactation cows that reached the time for rebreeding after January and had an extended DO until December or next January.

Considering the distribution of DO, in pooled data, the highest frequency was at 71-100 days post-calving. The rate of decline from 71-100 to 191 – 210 days was steep and slow afterwards. Regarding the distribution of days-open in calving months with the longest and shortest DO, the pattern was similar to the pattern of pooled data. For February calving cows, the frequency of DO at around 40 to 130 days was lower

than in October and November calving cows and the frequency in February calving cows was greater at around 160 days than in October and November calving cows. This pattern implied that farmers inseminate cows as usual to any observed estrus. But, the conception rate in February calving cows which should be rebred in April was low and required the highest numbers of services per conception. In Thailand, Pongpiachan et al. (2003[a]) reported that the estrus detection rate and success rate of artificial insemination were high from November to January. Others found that the first AI conception rate was high in cool season of November to February compared to the hot season of March to May (Kornmatitsuk et al., 2008).

In conclusion, there were regional variations in THI which may account for the differences in the length of DO. The high proportion of cows which calved in September corresponds to the success of breeding in the previous December or January when the THI was low. Calving cows which reached the time for rebreeding in the hotter months had longer DO than calving cows which reached the time for rebreeding in the cooler months. From the present study, the suggestion is that dairy cows especially replacement heifers, should be inseminated from November to February to calve from August to November. Additionally, utilization of proper cooling systems to alleviate the effect of heat stress should be concerned. These strategies may be useful for optimizing days-open and improve dairy farming profitability in Thailand.

CHAPTER III

Effect of heat stress on ovarian function, plasma metabolites and embryonic loss in first lactation dairy cows

3.1 Abstract

The aims of this study were 1) to investigate the effect of heat stress on the resumption of ovarian activity and plasma non-esterified fatty acids (NEFA), insulin-like growth factor-1 (IGF-1) and cortisol concentrations in postpartum first lactation dairy cows, and 2) to investigate the effect of heat stress on embryonic loss in first lactation dairy cows. Sixty-eight pregnant heifers were included in the study starting from -4 weeks before expected calving date. Blood samples were collected at -4 and -2 weeks before expected calving date, and then once a week up to 12 weeks or until the first AI to determine plasma concentrations of IGF-1, NEFA and cortisol. After calving, milk samples were collected twice a week until week 12 postpartum or until the first AI. Concurrent with the blood sampling body condition score (BCS) and body weight were estimated. When the first AI was performed after calving, additional blood samples were taken on the day of AI (D 0) D 12 and D 21 for progesterone assay. In cows which did not return to estrus, blood sampling was performed once during days 30 – 35 after AI for Pregnancy Specific Protein B (PSPB) assay. Reproductive data, climate data and milk production were recorded. First lactation cows were classified into 3 groups according to the average monthly temperature – humidity index (THI). Cows were considered as not exposed (NS) to heat stress if THI <72, exposed to mild stress (MS) if 72 ≤ THI <78 and severe stress (SS) if 78 ≤ THI < 89. The initial analysis showed that the effect of month of calving (MOC) had no effect on NS and MS, therefore the data was combined in the analysis of the effect of MOC on postpartum performance. Based on progesterone measurements, a cow was considered to have normal ovarian cyclicity if ovulation occurred within 45 day postpartum followed by regular ovarian cycle. The other cows

were classified as having abnormal ovarian cyclicity. The proportion of normal ovarian cyclicity in MS was higher than in SS group ($P<0.01$). The interval from calving to first ovulation, interval from calving to first AI, days-open and first service conception rate were not statistically different between MS and SS. BCS and body weight were unaffected by THI classification group. Plasma concentrations of NEFA, IGF-1 and cortisol, were not different between groups. Milk production was different ($P=0.03$) between MS and SS. Neither the number nor the different types of embryonic mortality were affected by heat stress. This study indicated that heat stress had a negative effect on ovarian activity. However, pregnancy success was affected by factors other than heat stress. This study did not establish any detrimental effects of heat stress on embryonic mortality.

3.2 Introduction

It is likely that heat stress affects reproductive performance both by direct actions on reproduction and by indirect actions mediated through alterations in energy balance. In the dairy cow, interactions between dry matter intake, stage of lactation, milk production, energy balance and heat stress resulted in reduced LH secretion and a decreased dominant follicle diameter in the postpartum period (Jonsson et al., 1997, Ronchi et al., 2001). Since one of the main causes of anoestrus in the dairy cow is a long period of negative energy balance, any worsening of energy balance during the summer could further decrease fertility in dairy cows.

In heat stressed dairy cows there is a reduction in dry matter intake (Ronchi et al., 2001), which prolongs the period of negative energy balance. Negative energy balance alters the normal pattern of metabolites and hormones leading to decrease in plasma concentrations of insulin, glucose, and insulin-like growth factor-1 (IGF-1), and increased plasma concentrations of growth hormone (GH) and non-esterified fatty acids (NEFA) (Lucy et al., 1992, Humblot et al., 2008). All of these metabolites and metabolic hormones can affect reproduction. Metabolites and metabolic hormones acting on the hypothalamo-pituitary axis and the ovary probably mediate the inhibitory effects of negative energy balance on post partum fertility (De Rensis and Scaramuzzi, 2003).

The late dry period coincides with the last phase of fetal growth, and the nutrient requirements of the fetus increase during this period. In early lactation, energy intake is usually lower than energy requirements for maintenance and milk production, which results in negative energy balance and mobilization of body reserves, particularly of body fat. Mobilization of body fat results in elevated plasma concentrations of NEFA. It has been suggested that NEFA have a negative impact on fertility (Canfield and Butler, 1990, Jorritsma et al., 2004). In periods when NEFA concentrations are high it has been shown that they are negatively correlated with blood progesterone concentrations and that there is a decrease in *corpus luteum* weight (Spicer et al., 1990). There is also some evidence for NEFA uptake by the ovary as well as a strong correlation between the concentration of NEFA in plasma and the follicular fluid, which could explain possible harmful effects of NEFA on either granulosa cells or oocytes (Jorritsma et al., 2003, Leroy et al., 2008). The plasma concentrations of insulin, IGF-1, and glucose are decreased in summer months compared to winter months (De Rensis et al., 2002) probably because of low dry matter intake and increased negative energy balance. Insulin is required for follicle development and either beneficial (Beam and Butler, 1997; Landau et al., 2000) or unfavourable (Freret et al, 2006) effects has been reported on oocyte quality and developmental ability following fertilization. Both IGF-1 and glucose generally stimulate follicular growth and implantation and glucose is the primary fuel of the ovary (Rabiee et al., 1997). Konigsson et al. (2008) have reported that significantly higher IGF-1 levels were found in cows, in the first two weeks after calving, which resumed postpartum ovarian activity compared to cows which did not resume activity. This highlights the important role of IGF-1 as a sensitive signal between metabolism and reproduction. Glucose availability has been shown to be also directly involved in the modulation of LH secretion (Bucholtz et al., 1996). Severe hypoglycemia and a reduction in food intake have been shown to inhibit pulsatile LH secretion and prevent ovulation both in dairy and beef breeds (Grimard et al., 1995). In addition to negative effects of NEB, several studies have indicated that heat stress has detrimental effects on embryonic development (Hansen, 2002, Sartori et al., 2002, García-Ispierto et al., 2006). Heat stress affects the process of follicle development resulting in poor quality and decreased developmental competence of oocytes (Roth et al., 2000).

Cartmill et al. (2001) observed that a higher embryonic mortality (EM) rates were found in cows exposed to heat stress compared to those not exposed to heat stress.
In Thailand, where the temperature often exceeds 30 ºC for several months of the year, most dairy cows are raised in conventional open-air barns, where ambient temperature and relative humidity follow those of the outside. Cows are affected by high ambient temperature and relative humidity resulting in low reproductive performance and milk production (Aiumlamai, 2007). Under thoses conditions, additional knowledge related to the effect of heat stress on variables describing more precisely reproductive performance and information in relation with metabolic imbalance is needed.

3.3 Materials and methods

3.3.1 Animals and management

The study was conducted during January 2008 to April 2009 on a commercial dairy herd with approximately 250 lactating dairy cows in Nakhonrachasima province, Thailand. Pregnant crossbred heifers (HF $\geq$ 87.5%) that had an expected calving date (n = 73) during the period 1st January 2008 to 31st December 2008 were included in the study. At the end of experiment, five cows were culled from the herd and therefore were excluded from the study for the reasons of severe leg injury, systemic disease, emaciated or mastitis. Only healthy animals with good body condition and normal locomotion were included in the study. Artificial insemination was performed when heifers reached at least 300 kg BW and 15 months of age. The pregnant heifers were group housed and then moved to the calving pen at about 2 weeks before expected calving date. Calves were separated from their dams immediately after calving. Colostrum was obtained manually and given to the calves immediately after birth. The cows were then moved to the lactating cow pen.

The cows were grouped according to milk production level. Lactating cows were kept in free-stall barns (roofed structure with open sides, concrete-floored, central feed passageway). Cows had free access to an earth exercise area. Roughage and concentrate were given to the cows separately. The main roughage used was corn stover silage. The concentrate was formulated and mixed at the farm. The

composition of the concentrate consisted mainly of cassava pellet, soybean meal, rice bran, oil palm meal, molasses, and mineral mix. The mean chemical compositions of the concentrate were 18% CP, and 72% TDN. Throughout the experiment, the proportions of the compositions of the concentrate were adjusted according to the price but the nutritive value was maintained at the same level.

Cows were milked twice–a-day (4.30 am and 2.30 pm). Prior to milking, the cows were sprayed with water for about 3 min and then they moved to stand under electric fans which caused the water to evaporate over about 15 min before entering the milking parlour. Cows were fed four times per day; after morning milking, at about 10 a.m., after afternoon milking, and at about 7 p.m. In addition cows were sprayed with water before the feeding during the hot season. During each feeding period, the electric fans that were installed under the roof were turned on until the cows had finished feeding.

3.3.2 Reproductive management of cows

The voluntary waiting period after calving was 45 days. Detection of oestrus was performed twice a day, morning and evening, for at least 30 min. In addition, during the remainder of the day, any cows that showed oestrus behaviour were reported to the inseminators by the farm workers. Artificial insemination was performed by well trained inseminators and frozen semen used was distributed by the Department of Livestock Development. Pregnancy diagnosis was performed by a veterinarian on cows which did not return to oestrus after insemination at about 30 days post-insemination by either palpation per rectum or using an ultrasonography machine. Pregnancy was confirmed again between 60 – 90 days post-insemination using either palpation per rectum and ultrasonography machine.

3.3.3 Blood sampling

Calving was expected to take place 280 days after the last insemination date resulting in pregnancy. Blood samples were taken at -4 and -2 weeks before the expected calving date and then weekly until week 12 after calving. If a cow was

inseminated before week 12, then blood sampling was discontinued. Blood samples were collected into 9 mL EDTA coated tube (Vacutainer® EDTA). Blood samples were immediately centrifuged at 2500g for 10 min and plasma sample were kept frozen at -20 ºC until required for assay. When the first AI was performed, additional blood samples were taken on day: 0 (day of AI), 12, 21 and between 30 – 35 days after AI. The blood samples collected after AI were allowed to clot and serum was collected and kept frozen until assay.

3.3.4 Milk sampling

In order to monitor ovarian activity, milk samples were collected for progesterone assay twice a week on Tuesday and Friday until week 12 after calving or until the first AI was performed. Milk samples were obtained during afternoon milking. After the teats were cleaned and dried by wet and dry towels respectively, milk was voided 4 to 5 times from each teat before collecting a composite sample into a 20 mL plastic container. Milk samples were kept frozen until required for assay.

3.3.5 Body condition score and body weight monitoring

Body condition scores of cows were monitored at 2 weeks before expected calving date, and at 2, 4, 6, 8, 10, 12 weeks after calving. Body condition was scored as described by Ferguson et al., (1994). Concurrence with the assessment of BCS, body weight was estimated using a weigh band (Giss Marketing, Thailand). When AI was performed before 12 weeks after calving, monitoring of BCS and body weight were discontinued. Both body weight and BCS were estimated by the same veterinarian throughout the study.

3.3.6 Progesterone and plasma metabolite assay*s*

Whole-milk progesterone concentrations were measured by enzyme immunoassay using a commercial kit according to the manufacture's instructions (Ovucheck® Milk kit, Biovet, France). The inter-assay coefficient was 12 % at

1.69 ng/mL. Plasma concentrations of non-esterified fatty acids were measured by NEFA C kit which is the enzymatic colorimetric test for the *in vitro* assay of free fatty acids in serum or plasma (NEFA C, Wako Pure Chemical Industries Ltd., Osaka, Japan). Plasma concentrations of IGF-1 were measured using ACTIVE IGF-I ELISA kit (DSL-10-5600; Diagnostic Systems Laboratories, Inc., Texas, USA). Plasma concentrations of cortisol were measured using Coat-A-Count Cortisol kit (Diagnostic Product Corporation, Los Angeles, CA, USA). Plasma progesterone concentrations were analyzed using a commercial kit (Coat-A-Count progesterone®, DPC, Diagnostic Products Co., Los Angeles, CA, USA). Inter-assay coefficients of variation were 7.23% at 0.5 mmol/L, 2.31% at 113 ng/mL, 0.59% at 245.64 mmol/L and 16.4% at 5 ng/mL for NEFA, IGF-1, cortisol and plasma progesterone repectively. The concentrations of pregnancy specific protein were analyzed using a commercial kit (BOVINE PREG-TEST 29®, Biovet Inc., St-Hyacinthe, Canada). The kit is an indirect immunoenzymatic assay intended for the measurement of a placental pregnancy protein in cattle serum. For validation of the test, the values of the negative and positive quality control sample have to be ± 15% of the nominal value and in this study the measured values of the negative (0 pg/mL) and positive control (2,000 pg/mL) were 4.47 and 2,179 pg/mL, respectively.

3.3.7 Classification of ovarian activity

The criteria used to characterize ovarian activity were modified from Shrestha et al. (2004, 2005) and Stevenson and Britt, (1979). Cows with milk progesterone concentrations ≥ 3 ng/mL at least two consecutive samplings were considered to have luteal activity. Ovulation was considered to have taken place 5 days before the first increase in progesterone concentrations ≥ 3 ng/mL. The resumption of ovarian activity post partum was defined as an ovulation followed by regular ovarian cycles (approximately, 2 weeks of luteal activity and 1 week of follicular activity), before breeding. Cows were classified into 1 of 5 groups based on characteristics of their progesterone profiles.

(1) Normal resumption of ovarian cyclicity: ovulation occurred ≤ 45 days after calving, followed by regular ovarian cycles

(2) Delayed first ovulation or anovulation: first ovulation did not occur until > 45 days after calving
(3) Persistent luteal phase: ovulation occurred ≤45 days after calving, but one or more ovarian cycles had luteal activity for > 20 days
(4) Short luteal phase: ovulation occurred ≤45 days after calving, but one or more ovarian cycles (except for the first cycle) had luteal activity < 10 days
(5) Cessation of cyclicity: ovulation occurred ≤45 days after calving, but there was an absence of luteal activity for ≥ 14 days between the first and second luteal phases

3.3.8 Milk yield

Daily milk production was recorded once a week during the study. Daily milk production was the sum of milk from morning and afternoon milking on the same day.

3.3.9 Climate data and classifying climatic factors

Temperature and relative humidity in the lactating cowshed were recorded using a digital temperature and humidity recorder (Ezylog®) throughout the study. Temperature-humidity index (THI) was computed as described by Garcia-Ispierto et al. (2007) as follows:

$$THI = (0.8 \times \text{Mean Temp}) + (RH/100) \times (\text{Mean Temp} - 14.4) + 46.4$$

3.3.10 Classification of reproductive categories (Rep Cat) after the first insemination

The previous published methodology (Humblot, 2001, Grimard et al., 2006) was used to classify reproductive categories.

1. AI at the wrong time (during luteal phase): P4>1.5 ng/mL on day 0
2. Non-fertilization/Early embryonic mortality (NF/EEM): P4 < 1.5 ng/mL on day 0 and day 21, return to oestrus at regular intervals

3. Late embryonic mortality (LEM without PSPB)/prolonged luteal phase: P4 < 1.5 ng/mL on day 0 and > 1.5 ng/mL on day 21, PSPB non-detectable, non pregnant
4. Late embryonic mortality (EEL with PSPB)/prolonged luteal phase: P4 < 1.5 ng/mL on day 0 and > 1.5 ng/mL on day 21, PSPB detectable, non-pregnant
5. Pregnancy: P4 < 1.5 ng/mL on day 0 and > 1.5 ng/mL on day 21, PSPB detectable, absence of second service and/or pregnant.

3.4 Data management and analysis

The average monthly THI in year 2008 ranged from 70.22 to 79.82. To determine the effect of heat stress during the months of calving on ovarian activity and plasma metabolites in postpartum cows, the calendar months were classified into three groups based on the average monthly THI according to the degree of heat stress described by Armstrong, (1994). Cows calving in a month when the average THI was < 72 were classified as no stress (NS), cows calving in a month when the average THI was between 72 to 78 were classified as mild stress (MS) and cows calving in a month when the average THI was > 78 were classified as severe stress (SS). The month that was classified as the NS was December. The months that were classified as the MS were January, February, March, November, and the months that were classified as the SS were April, May, June, July, August, September and October. The initial analysis was performed and the results showed that there were not different between NS and MS in all variables. Therefore, it was decided to combine the NS data with MS in the analysis.

The data obtained from the cows in MS and SS months were compared. There were very few cases of persistent corpus luteum and cases of cessation of ovarian activity and it was decided to combine them with delayed resumption of ovarian activity (classified as abnormal ovarian activity) in the analysis. The statistical analysis was performed using SAS® software (Version 9.1; SAS Institute, Cary, NC, USA). The proportion of normal and abnormal ovarian activities and first service

conception rates were compared between groups using Chi-square test. The interval from calving to first AI and days-open were analyzed using the GLM procedure. The repeated measured of BCS, body weight, IGF-1, NEFA and cortisol were analyzed using the Mixed procedure.

Samples were collected from 53 cows after calving to investigate the effect of heat stress on EM. No cow was inseminated at the wrong time and the other reproductive categories (Repcat) were classified according to Humblot (2001) into four groups as the following; 1 = NF/EEM, 2 = LEM without PSPB, 3 = LEM with PSPB, and 4 = pregnant. Reproductive status (Rep Stat) after the first insemination was classified as pregnant and non-pregnant cows. The reproductive status was binary outcome (1 = pregnant and 0 = non-pregnant). Serum concentrations of progesterone at day 12 and 21, and serum concentrations of PSPB measured in one sample taken between days 30 to 35 were analyzed by ANOVA (ANOVA Proc) to determine the difference in concentrations between reproductive categories. The intervals from calving to the first AI (ICAI) were calculated and were then classified into three categories; 1) ICAI < 50 days 2) ICAI 50 – 70 days, and 3) ICAI > 70 days. Mean ± standard error of the mean (SEM) for progesterone concentrations at different stages, PSPB concentrations and ICAI were calculated. Plasma progesterone concentrations at day 12 post-AI were compared within groups (NS, MS, and SS) between pregnant and non-pregnant to first AI post-calving using GLM procedure of SAS.

Reproductive categories (Rep Cat) and reproductive status (Rep Stat) were compared between cows calving in cool and hot seasons. The average monthly THI was used to classified the cows into three groups in order to determine the degree of heat stress of the month of insemination (MAI) on reproductive categories and pregnant status after the first calving. The cows were classified into three groups according to Armstrong, (1994) as followings; 1) No stress (NS); cows were inseminated in the month when THI was lower than 72, 2) Mild stress (MS); cows were inseminated in the month when THI 72 to 78, and 3) Severe stress (SS); cows were inseminated when THI >78 to 89. Categorical variables (MOC, MAI, ICAI, Rep Cat, and Rep Stat) were analyzed by Chi-square test (SAS FREQ procedure).

3.5 Results

Average monthly temperature, relative humidity and average THI are shown in Table 2. Sixty-eight cows were included in the study, of which, there were 13 cows in the MS and 55 cows in the SS group. The types of ovarian activity of each individual cow are shown in Table 3. The proportion of normal ovarian activity in MS was higher ($P<0.01$) than in SS. The interval from calving to first ovulation was 31.8 ± 5.66 d in MS and 35.7 ± 3.03 d in SS ($P=0.54$). The intervals from calving to first AI were 127.2 ± 20.38 days and 93.2 ± 5.36 days ($P=0.11$) in MS and SS respectively. First service conception rates were 41.7% and 50.0% in MS and SS, respectively and they were not different ($P=0.75$). Days open were 145.5 ± 38.30 d for group MS and 132.8 ± 10.24 d for group SS and were not statistically different ($P=0.5$).

Plasma NEFA were not statistically different between groups ($P>0.05$; Fig. 11). However, they were affected by time postpartum ($P<0.0001$), with the highest concentrations observed 1 week after calving in both groups. The concentrations tended to decrease after calving throughout the study. There was no interaction between group and time on the plasma concentrations of NEFA.

Plasma concentrations of IGF-1 (Fig.12) were not different between groups ($P>0.05$). The plasma concentrations of IGF-1 were affected by time ($P<0.0001$) but there was no interaction between time and group ($P>0.05$).

Plasma concentrations of cortisol (Fig. 13) were not different between groups ($P>0.05$). The concentrations of cortisol initially decreased and then increased during the study ($P<0.01$). An interaction between group and time was not detected ($P>0.05$). Plasma concentrations of cortisol increased before calving and then decreased until week 2 post calving. Cortisol remained relatively stable from week 2 to the end of study.

Table 2 Monthly temperature, relative humidity (RH) and Temperature – Humidity Index (THI) during the study. LSmean ± SEM.

Month/Year	Mean temp(ºC)	RH (%)	Mean THI
Jan 2008	24.45 ± 0.27	66.35 ± 1.01	72.65 ± 0.39
Feb 2008	24.47 ± 0.28	63.82 ± 1.04	72.42 ± 0.40
Mar 2008	28.3 ± 0.27	59.64 ± 1.01	77.41 ± 0.39
Apr 2008	29.23 ± 0.28	68.5 ± 1.02	79.82± 0.40
May 2008	27.96 ± 0.27	77.96 ± 1.01	79.25 ± 0.39
Jun 2008	28.57 ± 0.28	74.8 ± 1.02	79.82 ± 0.40
Jul 2008	28.11 ± 0.27	75.16 ± 1.01	79.16 ± 0.39
Aug 2008	27.87 ± 0.27	77.09 ± 1.01	79.04 ± 0.39
Sep 2008	27.17 ± 0.28	80.06 ± 1.02	78.38 ± 0.40
Oct 2008	27.04 ± 0.27	82.83 ± 1.01	78.48 ± 0.39
Nov 2008	27.74 ± 0.28	75.53 ± 1.02	74.10 ± 0.40
Dec 2008	22.57 ± 0.27	70.64 ± 1.01	70.22 ± 0.39
Jan 2009	22.13 ± 0.29	64.09 ± 0.98	69.08 ± 0.41
Feb 2009	27.51 ± 0.31	62.57 ± 1.03	76.61 ± 0.43
Mar 2009	27.92 ± 0.29	71.09 ± 0.98	78.30 ± 0.41
Apr 2009	29.08 ± 0.30	72.56 ± 1.00	80.13 ± 0.42

Neither body weight nor BCS (Fig. 14 and 15) were affected by the period of calving ($P>0.05$). However, both parameters changed during the study ($P<0.001$), decreasing around calving and slightly increasing from week 3 throughout the rest of the study.

There was a difference in milk production (Fig. 16) measured once per week between MS and SS ($P=0.03$) and milk production was influenced by time postpartum ($P<0.0001$). There was no interaction between the period of calving (MS or SS) and time postpartum on milk production.

Table 3 The distribution of calving incidence and types of ovarian activities by month of calving in first lactation dairy cows in year 2008.

Month	Calving (n)	Normal[1]	Delayed[2]	PCL[3]	Cessation[4]
Mild stress					
Jan 2008	1	1	-	-	-
Feb 2008	1	-	1	-	-
Mar 2008	4	4	-	-	-
Nov 2008	1	1	-	-	-
Dec 2008	6	5	-	-	1
Sub-total	13	11	1	0	1
Severe stress					
Apr 2008	10	8	2	-	-
May 2008	4	2	2	-	-
Jun 2008	9	4	5	-	-
Jul 2008	9	4	5	-	-
Aug 2008	9	4	4	1	-
Sep 2008	11	2	7	2	-
Oct 2008	3	1	2	-	-
Sub-total	55	25	27	3	-
Total	**68**	**36**	**28**	**3**	**1**

[1]Normal resumption of ovarian cyclicity: ovulation occurred ≤ 45 days after calving, followed by regular ovarian cycles,

[2]Delayed first ovulation or anovulation: first ovulation did not occur until > 45 days after calving

[3]Persistent luteal phase: ovulation occurred ≤45 days after calving, but one or more ovarian cycles had luteal activity for > 20 days.

[4]Cessation of cyclicity: ovulation occurred ≤45 days after calving, but there was an absence of luteal activity for ≥ 14 days between the first and second luteal phases

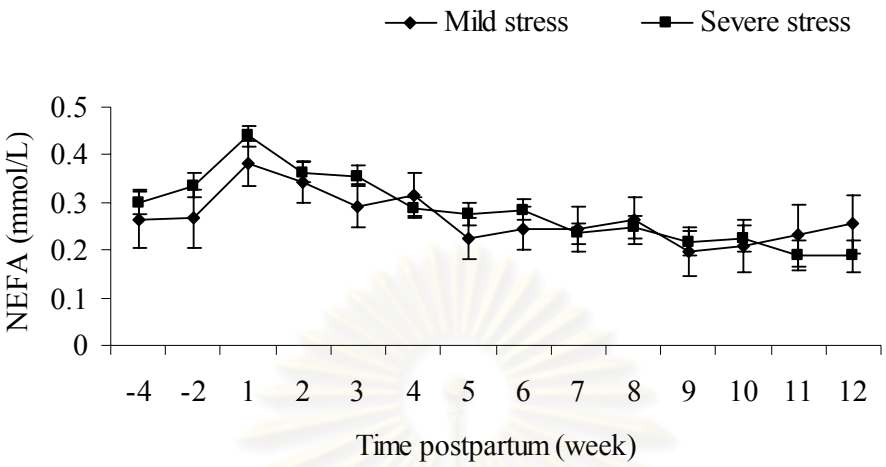


Figure 11 Plasma concentrations of non-esterified fatty acids (NEFA) from 4 weeks before to 12 weeks after calving in first lactation dairy cows that calved either during a mild (THI 72-78, n= 13) or severe (THI >78-89, n=55) stress period of the year

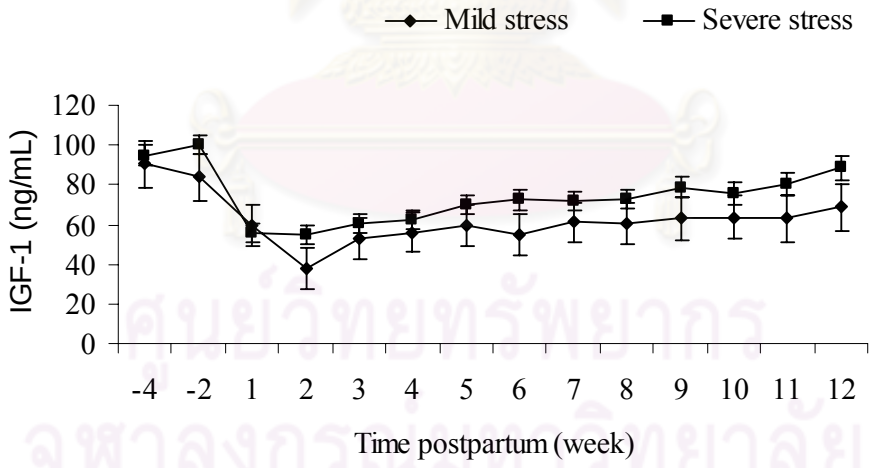


Figure 12 Plasma concentrations of insulin-like growth factor-1 (IGF-1) from 4 weeks before to 12 weeks after calving in first lactation dairy cows that calved either during a mild (THI 72-78, n= 13) or severe (THI >78-89, n=55) stress period of the year

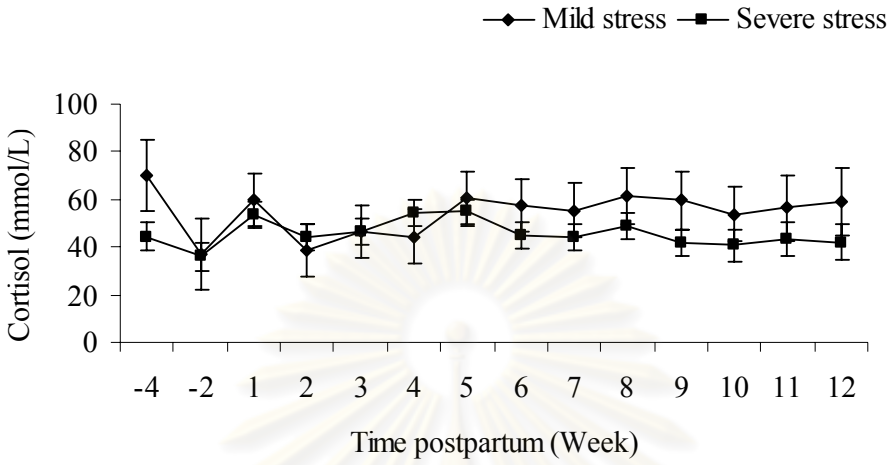


Figure 13 Plasma concentrations cortisol from 4 weeks before to 12 weeks after calving in first lactation dairy cows that calved either during a mild (THI 72-78, n=13) or severe (THI >78-89, n= 55) stress period of the year

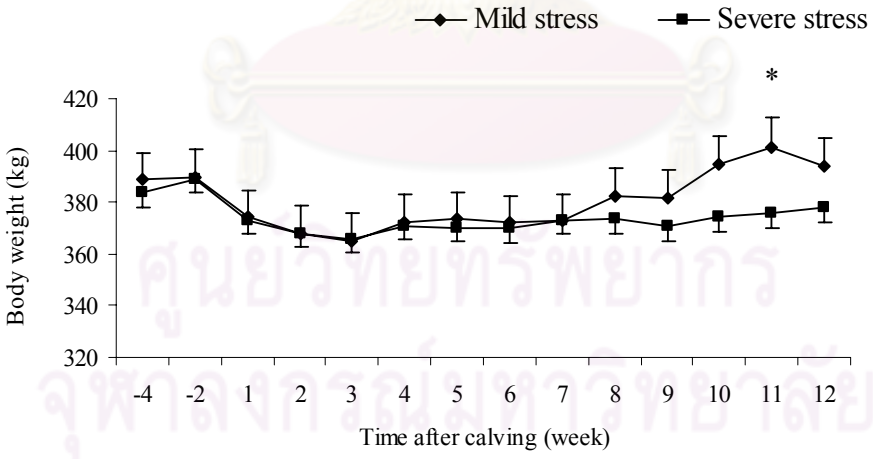


Figure 14 Body weight from 4 weeks before to 12 weeks after calving in first lactation dairy cows that calved either during a mild (THI 72-78, n=13) or severe (THI >78-89, n=55) stress period of the year. *P<0.05

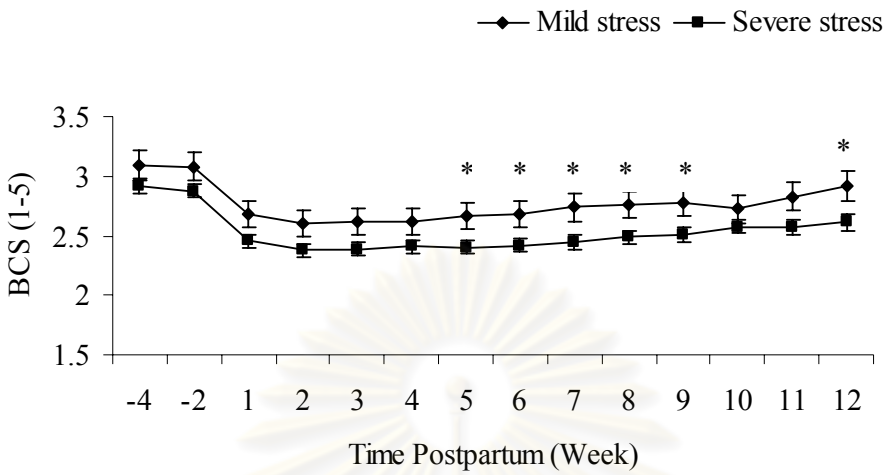


Figure 15 Body condition score (BCS) from 4 weeks before to 12 weeks after calving in first lactation dairy cows that calved either during a mild (THI 72-78, n=13) or severe (THI >78-89, n=55) stress period of the year. (*P<0.05)

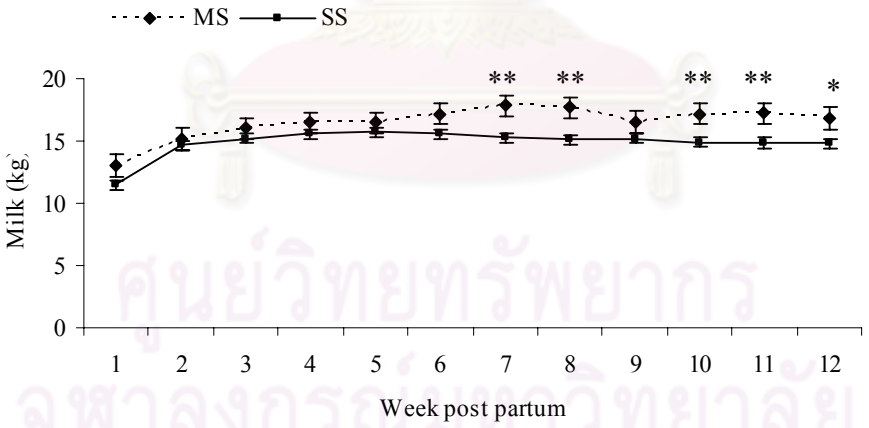


Figure 16 Weekly measures of milk production in first lactation dairy cows that calved either during a mild (THI 72-78, n=13) or severe (THI >78-89, n=55) stress period of the year. The difference between NS and SS, P=0.03, **P<0.01, *P<0.05

In the 54 cows studied in the present study, there were 28.6% NF/EEL, 7.4% LEM without PSPB, 12.9% LEM with PSPB and 50% pregnant (Table 4). The

concentrations of progesterone at different stages and PSPB of each type of reproductive category are shown in Fig. 17. There was no difference in plasma concentrations of progesterone at day 12 among the groups. On day 21 after insemination, plasma concentrations of progesterone of cows with LEM without PSPB, LEM with PSPB and pregnant cows were not significantly different but they were significantly higher than cows with NF/EEL (P<0.0001) (Fig 12). The intervals between the first and the second insemination (Fig. 18) were not different between groups.

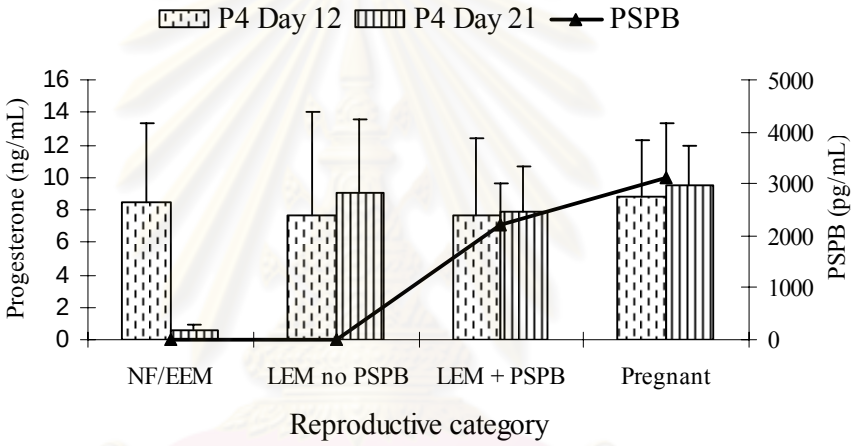


Figure 17 Plasma concentrations of progesterone on day 12 and day 21 after the first insemination and plasma concentrations of PSPB at day 30-35 for each reproductive category after the first insemination in first lactation dairy cows. LSmean ± SEM.
NE/EEM = Non-fertilization/Early Embryonic Mortality (n = 15); LEM no PSPB = Late Embryonic Mortality and no-detectable PSPB in blood (n = 4); LEM + PSPB = Late Embryonic Mortality and detectable PSPB in blood (n = 7); Pregnant = cows that were pregnant to first service (n = 27)

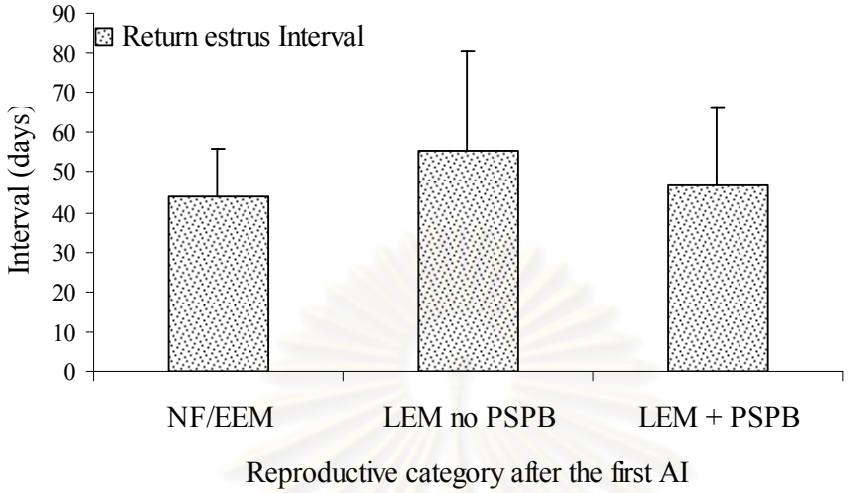


Figure 18 The intervals from the first insemination to the second insemination in first lactation dairy cows that failed to become pregnant after the first insemination. LSmean ± SEM. NF/EEM = Non-fertilization/Early Embryonic Mortality (n = 15); LEM no PSPB = Late Embryonic Mortality and not-detectable PSPB in blood (n = 4); LEM + PSPB = Late Embryonic Mortality and detectable PSPB in blood (n = 7);

The degree of heat stress, mild or severe, in the month of calving did not affect the types of reproductive categories (*P*=0.38). There were 11 (20.4%) cows inseminated in the months with no stress, 15 (27.8%) cows inseminated in the months with mild stress and 28 (51.9%) cows inseminated in the months with severe stress. Neither reproductive categories (*P*=0.50) nor pregnancy status (*P*=0.38) after the first insemination were affected by the degree of stress in the month of insemination (Table 4). There were no differences in plasma progesterone concentrations within groups between pregnant and non-pregnant cows at day 12 post-AI (Table 4).

There were no relationships between ICIA categories and Rep Cat (*P*=0.31) and ICIA and Rep Stat (pregnant or not pregnant, *P*=0.99) after the first insemination.

3.6 Discussion

One of the most important factors affecting dairy cow production and reproductive efficiency in Thailand is the climate. The genetic make-up of dairy cows

in Thailand originates from temperate and subtropical areas such as European countries, for instance, the Netherlands where temperature and humidity are lower than in Thailand.

Table 4 Mean THI, number of first AI cows (No. AI) and number of cows in different types of reproductive categories by month of insemination (MAI) in first lactation cows

MAI	THI[1]	No. AI	Reproductive categories			
			NF/EEM[2]	LEM with PSPB[3]	LEM no PSPB[4]	Preg[5]
No stress	**<72**					
Dec 2008	70.22	8	-	1	1	6
Jan 2009	69.08	3	2	-	-	1
Sub-total		**11**	**2**	**1**	**1**	**7**
Mild stress	**72-78**					
Nov 2008	74.10	7	1	1	-	5
Feb 2009	76.61	8	2	1	2	3
Sub-total		**15**	**3**	**2**	**2**	**8**
Severe stress	**78 -89**					
May 2008	79.25	2	-	-	-	2
Jun 2008	79.82	6	1	1	-	4
Jul 2008	79.16	4	2	1	-	1
Aug 2008	79.04	6	3	-	-	3
Sep 2008	78.38	5	2	1	1	1
Oct 2008	78.48	5	3	1	-	1
Sub-total		**28**	**11**	**4**	**1**	**12**
Total		**54**	**16**	**7**	**4**	**27**
Overall %		**100**	**28.6**	**13**	**7.4**	**50**

[1]THI = Temperature-Humidity Index

[2]NF/EEM = Non-fertilization/Early Embryonic Mortality (n=16);

[3]LEM + PSPB = Late Embryonic Mortality and detectable PSPB in blood (n=7);

[4]LEM no PSPB = Late Embryonic Mortality and not-detectable PSPB in blood (n=4);

[5]Preg = cows that became pregnant to first service (n=27)

Table 5 Plasma progesterone concentrations at d 12 post insemination compared within groups (NS, MS, and SS) between pregnant and non-pregnant to first service after calving in dairy cows. LSmeans ± SEM

AI month	n	P4 d 12[1]	*P* value
NS[2]			
Pregnant	7	8.16 ± 1.11	0.54
Non-pregnant	4	6.86 ± 1.69	
MS[3]			
Pregnant	8	7.33 ± 1.57	0.53
Non-pregnant	7	8.81 ± 1.68	
SS[4]			
Pregnant	12	10.13 ± 1.28	0.26
Non-pregnant	16	8.03 ± 1.28	

[1] Plasma progesterone concentrations at d 12 post AI,

[2] Non stress; AI in month with THI < 72

[3] Mild stress; AI in month with 72 < THI ≤ 78,

[4] Severe stress; AI in month with THI > 78

Generally, the seasons in Thailand can be classified as; winter (cool and dry), summer (hot and dry) and rainy season (hot and humid). Several studies have been conducted to investigate the effects of season on reproduction in dairy cows under the environmental conditions present in Thailand (Rodtian et al., 1996, Pongpiachan et al., 2003a, b, Kornmatitisuk et al., 2008, Kornmatitsuk et al., 2009). The classification system for season varies slightly between studies. Rodtian et al. (1996) classified cool and hot seasons from November to February and June to August, respectively. Kornmatitsuk et al. (2008) classified cool and hot seasons from November to February and March to May, respectively. Pongpiachan et al. (2003) classified three seasons as cool-dry (October to February), hot-dry (March to May) and hot-wet (June to September). In the present study, the seasons, or more specifically; the effect of heat stress, were classified by the level of heat stress that could affect dairy cows. The impact of heat stress on cows was classified by using the THI index which takes into

account both the effect of temperature and humidity. The mean monthly THI of the month of calving during the study was classified into three levels of heat stress, no, mild and severe. Cows in this study were likely affected by heat stress throughout the year.

The intervals from calving to first ovulation were not affected by climate in the present experiment. This finding was similar to the results of Suadsong et al. (2008) who found that the use of an evaporative cooling system to cool cows did not reduce the interval from calving to the onset of ovarian activity compared to un-cooled cows. This study showed that cows calving during the mild stress period had a higher proportion of normal ovarian activity than cows calving during the severe stress condition. This finding is in agreement with a previous report (Kornmatitsuk et al., 2008). However, the other reproductive parameters (calving to first AI interval, first service conception rate, and days open) were not different between cows calving in mild or severe stress conditions. The mechanisms by which heat stress affects ovarian activity are not clear. It has been suggested that the effects of heat stress on ovarian activity may be through an alteration in the balance of reproductive hormones leading to compromised folliculogenesis and ovulation (De Rensis and Scaramuzzi, 2003). López-Gatius et al. (2005) reported that ovulation failure was 3.9 times higher in cows inseminated during a warm period compared to a cool period in Spain. Suadsong et al. (2008) found that the ovulation rates and the interval from calving to the onset of ovarian activity were not different between cooled cows (evaporative cooling) or un-cooled cows. The ultimate goal of reproductive management is to obtain a pregnant cow as soon as possible after the voluntary waiting period. It has been reported that there are several factors implicated in the failure of conception in dairy cows (Chebel et al., 2004). In the current study the data indicate that ovulation in both groups occurred at about one month postpartum but the first inseminations were performed about three months postpartum. Even if average monthly THI were lower in the mild stress period, it was still higher than the mean critical value of THI 72 (Igono et al., 1992). Thus dairy cows were exposed to heat stress during both periods. The long delay between mean first ovulation and date of first AI may be due to the fact that in heat stressed cows the development of follicles has been shown to be affected resulting in lower oestradiol production. The intensity of oestrus signs was

decreased and the percentage of cows which were not observed in oestrus was higher in the hot season compared to cool season (Rodtain et al., 1996).

After calving, cows usually suffer from a period of negative energy balance (NEB) when energy required is higher than that consumed. NEB extends for approximately 10 to 12 weeks post partum (Butler, 2003) and NEB during the first 3 to 4 weeks is highly correlated with the time of first ovulation in dairy cows (Butler and Smith 1989). Under field conditions, the evaluation of the instantaneous energy status of individual dairy cows is not practical. BCS is a useful tool to asses the nutritional status of the cow which is related to long-term energy status. In this study the dramatic decrease in body weight and BCS occurred immediately postpartum and then body weight and BCS were gradually increased throughout the study but remained lower compared to the body weight and BCS before calving. A decrease in dry matter intake has been observed in peripartum cows especially under heat stress (Suadsong et al., 2008). Kornmatitsuk et al. (2008) found that BCS was affected by the heat stress condition and resulted in reduced reproductive performance in postpartum dairy cows in central Thailand. When a cow is in NEB, body reserves are mobilized to provide adequate energy for maintenance and production. The main body reserve which is mobilized is lipid and eventually muscle tissue. Mobilization of the lipid results in an increase circulating NEFA.

When considering both the BCS and plasma concentrations of NEFA in the present study, the nutritional management used in the farm appeared to be good. Both profiles followed the normal physiological picture and were in the acceptable range. The pregnant heifers were managed to have an adequate body condition score of 3 at calving (Mulligan et al. 2006). It has been reported that the normal range in plasma concentrations of NEFA is 0.2 to 0.5 mmol/L (Robert et al., 1981) and the concentrations of NEFA in the present study were in this range. The decrease in dry matter intake during the early postpartum period was associated with a decrease in plasma metabolic hormones such as IGF-1 which is generally, involved in stimulating follicular growth and implantation (De Rensis and Scaramuzzi, 2003). However, plasma concentrations of IGF-1 in this study were not different between heat stress groups. This is logical in view of the fact that the nutritional status of the cows based on the BCS and NEFA were not different between groups.

Cortisol is an important hormone released from the adrenal glands. Cortisol is involved in several processes such as the response to stress and the control of glucose metabolism. Plasma concentrations of cortisol increase during milking and feeding (Gorewit et al., 1992; Wagner and Oxenreider, 1972). When an animal is fasting or plasma glucose concentrations are low, cortisol causes an increase in circulating glucose by increasing the rate of gluconeogenesis (Carbonaro et al., 1992, Samuelsson et al., 1996, Ward et al., 1992, Baidoo et al., 1992). Several studies have reported that heat stress has a negative effect on milk production through a decrease in diet intake (Faquay, 1981, McDowell et al., 1969; Rhoads et al., 2009).

In the present study there were statistically different in BCS and body weight between MS and SS and these parameters were better in MS after 6 weeks postpartum. Also, good body condition was associated with higher milk production in MS than in SS cows. The increase in milk production led to an increase in cortisol concentrations in MS compared to SS as showed in Fig. 13 showing that due to their production and possible higher metabolism, MS cows suffered at least as much as SS cows from high external temperatures.

It is generally accepted among researchers, veterinarians, bovine practitioners, and dairy farm producers that heat stress affects both production and reproduction in dairy cows raised under Thai climatic conditions. However, except for the proportion of cows with normal resumption of ovarian activity, the other reproductive parameters were not statistically different between cows calving during mild stress and severe stress conditions. When considering the nutritional parameters measured in this study, the nutritional status of dairy cows was managed correctly. This study was conducted in a commercial dairy farm where intensive management was employed. Corn stover silage and concentrate were fed to the cows throughout the year. Good quality feed together with the feeding system and cooling methods used in the farm may have enhanced cow feed intake and therefore ensured that each cow had an adequate nutritional level. It has been reported that the combination of soaking or sprinkling with water and forced ventilation has the potential to reduce body temperature or heat stress in dairy cows (Flamenbaum et al., 1986) leading to an increase in the amount of feed consummed (Suadsong et al., 2008). Tillard et al. (2008) investigated post-calving factors affecting fertility in Holstein dairy cows in tropical and sub-tropical

conditions and found that the nutritional imbalance strongly affected fertility. Pongpiachan et al. (2003[a]) reported that the utilization of forced ventilation by electric fans and water splinklers were effective enough to alleviate the effect of heat stress on reproduction in Friesian cows. The effects of heat stress on reproduction in our dairy cows were probably reduced due to the utilization of a cooling system (as described previously) and the intake of an adequate amount of diet of adequate nutritional value. Thus, the expected difference in reproductive performance was not observed in this study.

Both under temperate and tropical conditions, it has been reported that early embryonic loss is the major problem contributing to pregnancy failure in dairy cows. There are several factors that are implicated in embryonic loss (Santos et al., 2004). It has been proposed that heat stress is the factor that has the greatest impact on pregnancy rate in dairy cows (Sartori et al., 2002). To our knowledge, only a few studies aiming to investigate the effect of heat stress on embryonic loss in Thailand exist. Suadsong et al. (2001) investigated the effects of heat stress on embryonic loss by using the combination of plasma progesterone concentrations measured on day 22 post-AI and ultrasonography performed on day 27, 34 and 42 post-AI. They found that the highest conception rate (54.5%) and the lowest embryonic loss (18.2%) were observed at 42 days post-AI in December. The ranges of embryonic mortality at 22 days post-AI in cows inseminated between March and September were 65 to 80%. They also found that a high incidence of embryo mortality occurred before 27 days post AI (50-100%).

The effects of heat stress during the month of insemination on embryonic loss were determined in this study. Unlike Suadsong et al. (2001), the present study found that the degree of heat stress in the month of calving and the month of insemination had no effect on the different types of embryonic mortality and on the success of pregnancy to first AI. A study conducted under the climate conditions observed in Saudi Arabia showed that the embryo viability was markedly decreased from 59% at day 7 to 27% at day 14 after AI in the hot season and that on the contrary such a decrease in embryo viability was not observed during the cool season (Ryan et al., 1993). Regardless of the effects of heat stress on embryonic mortality, the frequencies of NF/EEM fell within the range of previous studies (20.5 to 43.6%, Humblot, 2001).

The incidences of LEM (with and without PSPB) in this study (20.8%) were slightly higher than the range reported in previous studies performed mainly under temperate conditions (8 to 17.5%, Humblot, 2001). But the incidences of LEM in this study were lower than the incidences of embryonic/foetal loss (25.2%) observed in a low fertility farm managed under temperate conditions (Grimard et al., 2006). In many other studies undertaken in dairy cattle, fertilization rate has been reported to be high ranging from 55 to 100% with most values over or close to 90% (Sartori et al., 2002). Most of embryonic mortality occurs during early embryonic development before the embryo reachs the blastocyst stage (Humblot, 2001, Silke et al., 2002). The combination of progesterone and pregnancy specific protein measurements used in this study did not allow us to differentiate between non-fertilization and early embryonic mortality. To evaluate the fertilization rate, previous studies mostly used the techniques of uterine flushing to recover the embryo or ova (Breuel et al., 1993, Ahmad et al., 1995 Sartori et al., 2002). This technique is not practical in a field study. Measurement of specific substances produced by fertilized oocytes released into the blood circulation could be a good alternative to determine the occurrence of fertilization. However, to date, there is no practical technique reliable and suitable for field studies or even for research purposes.

Progesterone measurements following insemination allows the identification of two types of embryonic mortality. Non-fertilization or early embryonic mortality is associated with the occurrence of luteolysis within 24 days post-AI whereas late embryonic mortality occurring at or more than 16 d after insemination is associated with prolonged *corpus luteum* function, high circulating progesterone and a return to oestrus after 24 days (Humblot, 1988, 2001). In the present study, the intervals between the first and second inseminations in cows which were classified as having NF/EEM were longer than the regular interval of normal oestrus cycle (21-24 d). As in previous studies from temperate countries, these data indicated the failure to detect a cow's return to oestrus or defaults in oestrus expression (Peralta et al., 2005). Non-pregnant cows with low progesterone concentrations on day 21 after insemination may express a late return to oestrus due to poor quality heat detection and/or silent oestrus. The absence of progesterone means that these cows may be classified as

LEM. Thus, as reported before, progesterone measurements were necessary to estimate in field studies the respective incidence of EEM and LEM.

Several studies have reported that the increase in progesterone concentrations during the first days following fertilization / after insemination is associated with fertility in dairy cows (Stronge et al., 2005, Mann et al., 2006, Demetrio et al., 2007). Detrimental effects of heat stress on follicular development have been reported and this induced a decrease in progesterone production after ovulation (De Rensis and Scaramuzzi, 2003). In the present study concentrations of progesterone in blood were not statistically different by 12 days post-AI between pregnant and non-pregnant cows exposed to MS or SS, and progesterone concentrations were higher in pregnant cows in both groups.

PSPB is a trophoblastic protein, secreted by binucleated cells, found in the dam's maternal circulation (Reimers et al., 1985). In some pregnant cows, blood concentration of PSPB can be detected as early as day 15 after insemination. The blood concentrations of PSPB are most of the time (in >95% of the cases) detectable (over 0.5 ng/mL) and can reach 2 to 3 ng/mL within day 35. However, high individual variations were observed when measuring peripheral concentrations of PSPB during early pregnancy (Humblot et al., 1988; Humblot, 2001). In the case of EEM, embryonic mortality occurs before day 16 after insemination. Therefore this protein is usually undetectable. In contrast, in the case of LEM associated with maintained *corpus luteum* function, PSPB concentrations in blood can be detected but the mean concentrations are usually lower than those found in pregnant heifers or cows. The combination of progesterone and PSPB measurements have been used to study the respective frequencies of different types of embryonic mortality and to relate them with factors which may influence fertility at specific stages of pregnancy (Humblot, 2001).

In this study, LEM was further classified into: LEM with PSPB and LEM without PSPB. The reason for this classification was to determine the incidence of both which may be related to different mechanisms. LEM occurs after the regression of the *corpus luteum* is stopped and luteal cells continue to produce progesterone. One of the most important substances secreted by the conceptus that is involved in the maintenance of corpus luteum function is IFN-τ (Igwebuike, 2006, Green et al.,

2010). IFN-τ is produced by mononucleated cells between day 10 and day 24 of gestation and it has been shown that IFN-τ has anti-luteolytic properties (Igwebuike, 2006) while PSPB (the isoforms measured here) is produced and secreted by binucleated cells (Humblot et al., 1988). In the case of LEM without PSPB, even if the *corpus luteum* is actively maintained by IFN-τ, the early placenta does not function well. This may be interpreted in the way the two types of cells function within the trophoblast (mononucleate and binucleate) and are regulated by different processes. To date, this topic related to the LEM with or without secretion of PSPB has not been studied in a way precisely related to abnormal expression of different genes and more precise analysis of cell function of both mono- and binucleated cells or structural development of the placenta. The relationship between such LEM events and the differential secretion of the different proteins from the PSPB/PAG family would be also of some interest but is not easy to approach due to the relatively low number of LEM cases and the phenotypic variation associated with these cases (different delays for luteolysis and return to oestrus).

Additionally, the classification of LEM by the criteria used in this study may confound the different situations and to overestimate the incidence of LEM. Effectively in some cows or heifers not inseminated, *corpus luteum* function has been shown to persist by studying progesterone profiles before AI. This situation may also explain why cows inseminated but unfertilized may express prolonged luteal function without PSPB. This may lead to confusion between the two different situations. The level of over-estimation of the frequency of LEM is dependent on the incidence of such cases. There are, at the present time, no tools or methods available to differentiate between these events. Thus, further investigation on this topic is warranted.

The effect of heat stress on the EM rates between groups (NS, MS, and SS) was not different in this study. The range of THI in May during the study was from 69.08 to 79.25. The lack of difference in frequency of EM may be due to the fact that the conditions induced by MS are detrimental to embryonic development and are negative enough to induce a rise in the frequency of EM. Alternatively, it can be hypothesized that cows under MS expressed better their milk potential and had a higher metabolic activity leading to similar internal temperatures.

Additionally, the methods used in this farm to cool down the cows before milking throughout the year and before feeding in the hot season were probably effective enough to limit the unfavourable impact of more severe heat stress on EM. Thus, together with good nutrition, the frequency of EM in NS and MS were not different from SS.

In conclusion, the effects of heat stress on reproductive performance were not obviously seen in this study. However, the proportion of normal ovarian activity was higher in cows that calved in the MS compare to SS. This indicated that heat stress has a detrimental effect on follicle development and ovarian function. But the success of pregnancy depends on many subsequent events and embryo loss can occur at any time. This study was conducted in a large dairy farm with a good management. Preventive cooling methods were employed and are thought to be the factor which meant that there was no effect of heat stress on reproductive performance and embryonic loss in this study. Most dairy farms in Thailand are characterized as smallholder farms where the reproductive and nutritional management varies and is different from the intensive management used on large dairy farms. The effect of heat stress may be more pronounced in smallholder farms. Thus, the study of the effect of heat stress under the management conditions of smallholder dairy farms is needed.

CHAPTER IV

DIETARY SUPPLEMENTATION WITH ß-CAROTENE AND POSTPARTUM PERFORMANCE IN DAIRY COWS AND CALF HEALTH STATUS AT BIRTH

The study in this chapter is divided into 3 experiments which were conducted using the same experimental animals.

4.1 Effects of dietary supplementation of β-carotene given to dairy cows during the dry-period on postpartum ovarian activity, progesterone and uterine health in dairy cows

4.1.1 Abstract

β-carotene is the main natural precursor of vitamin A and plays an important role in reproductive efficiency and immune function in dairy cows. The objective of this study was to investigate whether a supplement of β-carotene given during the dry period is able to 1) increase blood concentrations of β-carotene postpartum, 2) improve ovarian function and progesterone production, and 3) enhance uterine involution and uterine health. This study was conducted using 40 Holstein cows. On the day of drying-off, cows were allocated to one of two dietary treatments: control diet (C, n=20) or control diet plus 1g/d β-carotene (BC, n=20). The β-carotene supplement was given individually to the cows until calving. Blood samples were obtained regularly before and after calving from the cows to measure the concentrations of β-carotene. The diameters of the cervix and uterine horns were measured regularly using ultrasonography. Endometrial cytology samples were acquired from the cervix and uterus to determine uterine health. Milk samples were obtained three times per week for progesterone assay. Additional blood samples were taken on the day of calving, 7 and 21 days postpartum to determine the plasma

concentrations of amino acids. Blood concentrations of β-carotene were not different before the start of the experiment (C, 3.03 ± 0.22mg/L vs. BC, 3.12 ± 0.22mg/L, $P>0.05$). Blood concentrations of β-carotene in the BC group peaked (7.45 ± 0.24 mg/L) 1 month after drying-off while the concentrations in the C group remained constant. β-carotene concentrations then decreased in both groups. The difference in blood concentrations of β-carotene between groups became significant 2 weeks after the start of the supplement until 2 weeks postpartum. There was no significant difference in the interval from calving to ovulation between groups (C, 27.8 ± 3.46d vs. BC, 35.8 ± 3.55d, $P>0.05$). The dietary supplement of β-carotene during the dry period had no effect on ovarian activity, progesterone production, cervix and uterine horn diameters. Plasma concentrations of hydroxyproline in the BC group were higher than in the C group on day 21 postpartum (BC: 20.8 ± 1.33μmol/L vs. C: 15.0 ± 1.33μmol/L; $P<0.01$). On day 28 postpartum the percentage of neutrophils in the BC group was lower than in the C group (cervical smear; C: 21.0 ± 3.22% vs BC: 9.7 ± 3.14%, $P<0.05$ and uterine smear; C: 32.0 ± 3.86% vs BC: 20.9 ± 3.76%, $P<0.05$). In the present experiment a dietary supplement of β-carotene during the dry period had no effect on ovarian activity postpartum. However, due to effects of β-carotene on hydroxyproline profiles and their potential relationship with uterine function we speculate that uterine involution may have been more complete and that uterine inflammation may have been reduced in cows which received the β-carotene compared to controls.

4.1.2 Introduction

β-carotene is the principal natural precursor of vitamin A in cattle and it is mainly provided by forages. β-carotene is either absorbed intact or metabolized in the intestinal mucosa and the resulting retinol is absorbed. β-carotene is transported with fat in the lymphatic system and temporarily stored in the liver. Besides being a vitamin A precursor β-carotene plays a role in reproductive efficiency in cows. Low circulating β-carotene has been associated with prolonged oestrus, delayed ovulation, a reduction in the intensity of the signs of oestrus, low conception rate and low

progesterone concentrations (Hemken and Bremel, 1982, Rakes et al., 1985, Arikan and Rodway, 2000). Some authors have reported a beneficial effect on reproduction (e.g., enhanced uterine involution (Rakes et al., 1985), shortened calving to first oestrus interval (Rakes et al., 1985), and improved luteal progesterone production (Arikan and Rodway, 2000), while others have reported no effect of beta-carotene on reproductive performance (Bindas et al, 1984[a,b], Akordor et al., 1986, Wang et al., 1988).

There is increasing evidence that β-carotene functions as an antioxidant by quenching singlet oxygen and scavenging the peroxyl radical, in contrast to retinol, which does not have this capacity (Ikeda et al., 2005). β-carotene is an antioxidant which has been implicated in immune function in dairy cows (Chew, 1987). The antioxidant system in the cow is sufficiently robust to cope with the production of free radicals under normal physiological conditions. However, during the periparturient period, dairy cows undergo substantial metabolic and physiological adaptations shifting from pregnancy to lactation. During this period, the production of free radicals usually exceeds the capacity of the body's antioxidant system and oxidative stress develops (Bernabucci et al., 2002, Castillo et al., 2005). Some diseases including mastitis, metritis and retained foetal membranes commonly occur in the early postpartum period (Miller et al., 1993, Kankofer, 2002). A β-carotene supplement given to dairy cows during the peripartutient period enhanced lymphocyte proliferation induced by mitogen stimulation (Michal et al., 1994). Moreover, Akar and Gazioglu (Akar and Gazioglu, 2006) showed that dairy cows suffering from retained placenta were more likely to have low blood β-carotene concentrations than healthy cows. Data from a retrospective study indicated that plasma β-carotene concentrations during the dry period were greater in dairy cows that ovulated within the first 30 d postpartum than the concentrations in cows that did not ovulate during the first 30 d postpartum (Kawashima et al., 2009[a]).

Therefore, in an effort to resolve conflicting evidence concerning the effects of β-carotene supplementation of dairy cows during the dry period on health and reproduction the present study was designed to investigate whether a supplement of β-carotene given during the dry period was able to 1) increase blood concentrations of β-carotene postpartum, 2) improve ovarian function and progesterone production, and

3) in the event of endometritis, enhance uterine involution and improve uterine health. However, the last objective could not be addressed since there were no spontaneous cases of metritis.

4.1.3 Materials and methods

The present study was carried out according to French legislation on animal experimentation (code rural: articles R 214-87 to R214-94) in line with the European Convention for the Protection of Vertebrates used for Experimental and other Scientific Purposes (European Directive 86/609). The scientist in charge of the experiments was licensed to perform experiments on animals.

4.1.3.1 Animals and management

Forty high-producing Holstein, primiparous and multiparous, cows were used in the experiment (average annual milk production of 10,000kg). On the day of drying-off, which was determined as being 60 days before the presumed date of calving, cows were allocated to one of two dietary treatments: control diet (control group, C: n=20) or control diet plus 1g/cow/d β-carotene (β-carotene group, BC: n=20, Rovimix® β-Carotene containing 10% β-carotene; DSM Nutritional Products Ltd., Paris, France). The criteria used to form the groups were: live weight, body condition score, age, milk production level over the first 100 days of the previous lactation, expected calving date, and blood β-carotene concentration. The dry period lasted two months. Cows were group-housed in a barn on straw and received total mixed rations (TMR), formulated to meet average requirements for maintenance and production (Hoden et al., 1988). Three different diets based on maize silage were formulated (Table 6) and fed to the cows depending on their requirements (first and second month of the dry-period and lactation). The dry-period diets were given in fixed quantities per cow while the lactation diet was given *ad libitum*. The diets contained a vitamin and mineral mix which covered vitamin A requirements. Cows had free access to water and salt licks. One cow in the BC group was culled towards the end of the experiment due to an accident unrelated to the protocol.

Table 6 Composition and nutritional value of the diets given to dairy cows during the dry-period and during lactation.

	Dry period		Lactation
	1st month	2nd month	
Diet composition	(kg DM/cow/d)	(kg DM/cow/d)	(% DM)
Maize silage	5.2	5.9	38.74
Brewers grains	-	-	6.44
Sugar beet pulp	-	-	9.26
Molasses	-	-	0.84
Orange peel	0.6	-	-
Rapeseed meal	1.4	0.85	18.04
Grass hay	2.6	2.6	14.33
Barley	-	-	6.92
Mineral and vitamin mix*	0.2	0.2	1
Urea	-	-	3.62
Salt licks	ad lib	ad lib	ad lib
$CaCO_3$	0.02	-	0.12
MgCl	-	0.05	-
Sodium bicarbonate	-	-	0.4
Nutritional values	(/cow/day)	(/cow/day)	(/kg DM)
NE_L (Mcal)	14.11	10.88	1.632
PDIN (g)	703	500	106
PDIE (g)	755	546	101
Calcium (g)	52.3	22.0	7.6
Phosphorus (g)	38.8	24.2	4
Starch (%)	-	-	16.8
Crude fiber (%)	-	-	19.3
β-carotene (mg)	42.3	41.8	2.35

* Mineral and vitamin mix contained 240g Ca, 35g P, 40g Na, 50g Mg, 1g Cu, 3.6g Zn, 3.6g Mn, 66mg I, 22mg Co, 20mg Se, 400,000 IU vitamin A, 66,700 IU vitamin D3, and 1200 IU vitamin E per kg as fed (Centralys, Trappes, France).

NEL = Net energy for lactation
PDIN = true intestinal digestible protein, when fermentable N is the limiting factor.
PDIE = true intestinal digestible protein, when fermentable energy is the limiting factor.
(INRA, 1989)

4.1.3.2 Measurement of β-carotene concentrations in the diets given to the cows

The rations fed during the experiment were sampled each month (500 g) and immediately frozen (-20 ºC). At the end of the experiment, samples were freeze-dried. A pooled sample of each of the three TMR and the rapeseed meal was analysed for β-carotene. The β-carotene levels were measured by HLPC at the Analytical Research Center, DSM Nutritional Products Ltd, Basel, Switzerland.

4.1.3.3 Supplementation with β-carotene and measurement of blood β-carotene concentrations

The cows which were allocated to the β-carotene group were supplemented individually with 1g/cow/d of β-carotene (purified-encapsulated β-carotene) starting from the day of drying-off until calving. The β-carotene supplement was top-dressed once-a-day onto 500g rapeseed meal while the cows in control group only received the rapeseed meal. Cows were restrained in a neck lock stanchion during the distribution of the rapeseed meal with or without β-carotene. Blood concentrations of β-carotene were measured after caudal venipuncture every two weeks before calving and weekly after calving for 10 weeks. The concentrations of β-carotene in blood were measured as described previously (Kawashima et al., 2009a) after one-step protein denaturation and extraction of β-carotene into organic solvent using the iEx™ assay system. The concentration of β-carotene was then measured using a photometer (iCheck™; BioAnalyt GmbH, Germany).

4.1.3.4 Blood sampling

Blood samples were obtained at -8, -6, -4, -2 weeks before calving, at calving and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks after calving by caudal venipuncture before the morning feed. Blood was collected into 9 mL heparin-coated tube and placed immediately in ice-cooled water. Blood concentrations of β-carotene were measured and the sample was then centrifuged for 10 min at 4 ºC and 2,500 rpm (Eppendorf centrifuge 5702 R). Plasma was collected and frozen (-20ºC) until required for metabolite and hormone assay.

4.1.3.5 Milk sampling, milk progesterone assay and ovarian activity

Milk samples for progesterone assay were collected 3 times per week at afternoon milking starting the 1st wk postpartum and continuing until the 10th wk postpartum. Composite milk samples were collected in 20 mL plastic containers containing a preservative tablet (Bronopol/Natamycin; Microtabs, Control Systems, Inc, D&F U.S.A.) and were frozen (-20 ºC) until required for analysis. Whole-milk progesterone concentrations were measured by enzyme immunoassay using a commercial kit (Ovucheck® Milk kit, Biovet, France). The coefficient of variation was 12% at 1.69 ng/mL.

Ovarian activity was estimated using milk progesterone concentrations. Cows with milk progesterone concentrations ≥3 ng/mL for at least two consecutive samples were considered to have luteal activity. Ovulation was considered to have taken place five days before the first increase in progesterone ≥3 ng/mL. The interval from calving to ovulation was calculated. To determine ovarian activity, the post partum period was divided into two sections; from calving to day 50 and from day 51 to the end of experiment. The criteria used to classify the ovarian activity were adapted from (Shrestha et al., 2005) and the ovarian activity during each time period was classified as:

1) Normal cycle; ovulation occurred and was followed by regular cycles with approximately 2 weeks of luteal phase and 1 week of follicular phase

2) Anovulation; no luteal activity (progesterone <3 ng/mL)

3) Irregular cycle; ovulation occurred and was followed by an ovarian cycle with a luteal phase of <10 days or absence of luteal phase for at least 14 days between cycles
4) Persistent corpus luteum; ovulation occurred and was followed by a luteal cycle of >20 days.

The normal cycle progesterone profiles were used to calculate the area under the curve by AUC = $\Sigma (C_t + C_{t+1})/2 \times dt$, where C_t is the concentration at time t, C_{t+1} the concentration at time t + 1 and dt is the time (days) between samples taken at t and t + 1 after subtracting the cut-off level for luteal activity (3 ng/mL) (Rhoads et al., 2009). The area under the curve was considered to be total progesterone production.

4.1.3.6 Endometrial cytology of the cervix and uterus

Endometrial samples for cytological examination were collected using a Cytobrush® Plus GT (Medscand Medical, USA) modified especially for this study (see below). Endometrial cervical and uterine cytology samples were taken starting at 8 days postpartum. The interval between samples was 10 days. An artificial insemination gun (AI gun: IMV, Technology, L'Aigle, France) without its plunger was used to introduce the cytobrush into the uterus. The handle of the cytobrush was shortened to about 8 cm and treaded into an AI gun. The cytobrush and AI gun were fixed together with surgical tape (Adhéroplaste Fibranne®; BSN Medical, Vibraye, France). The cytobrush and AI gun combination was covered by a breeding sheath (Alcyon, France) from which the plastic insert was removed before use. The sampling instrument was then covered by a plastic sanitary sheath (IMV Technologies, France) to protect the cytobrush from vaginal contamination. The vulva was cleaned with clean water and then with povidone-iodine (Vétédine savon, Vétoquinol, Lure, France) and dried with a paper towel. The sleeved arm of the experimenter was lubricated and introduced into the rectum to facilitate the passage of instrument through the vagina and cervix. The instrument was passed through the vagina. When the tip of the instrument was at the external os of the cervix, the plastic sanitary sheath was punctured and the instrument was manipulated through the cervix and into the base of the uterine horn where the breeding sheath was retracted to expose the cytobrush. Uterine cytology samples were collected by rotating the cytobrush while in

contact with the uterine wall. The cytobrush was retracted into the breeding sheath prior to removal from the uterus. An endo-cervical cytology sample from the cervix also was collected. The cytobrush was rotated to obtain the cellular material from the adjacent endometrium when it was in the middle of the cervix. The cytobrush was rolled on a clean glass microscope slide and allowed to air-dry.

The cytology slides were stained with May-Grünwald-Giemsa staining using an automated slide stainer (Aerospray; Wescor, Kitvia, Labarthe Inard, France). Each slide was examined using 1000x magnification after assessment of the homogeneity of the slide under a 400x magnification. From each slide, 200 cells were counted and: the number of epithelial cells, neutrophils, eosinophils, basophils, lymphocytes and monocytes/macophages were noted. Large epithelial cell membranes and ruptured cells were not included. All slides were examined by the same person.

4.1.3.7 Measurement of cervical and uterine horn diameters

Immediately after taking the endometrial samples, an ultrasonographic scanner equipped with 6.0MHz linear rectal transducer (Échographe Aquila, Pie Médical, The Netherlands) was used to determine the diameters of the uterine horn and cervix. Cervical diameters were measured by placing the transducer over the middle of the cervix. To measure the uterine horn diameters, the transducer was placed 10cm cranially to the bifurcation of the uterus. Built in machine calipers were activated and used to measure the distance from serosa to serosa and this was considered to correspond to the diameter.

4.1.3.8 Blood sampling and measurement of plasma amino acids

Blood samples were taken on the day of calving, day 7 and day 21 postpartum, all by caudal venepuncture into 9mL heparinized tubes before the morning feed. The samples were chilled and then centrifuged (Eppendorf centrifuge 5702R) at 2 000 g for 10 min at 4 ºC. Plasma was harvested and frozen (-20 ºC) until required for the analysis. Plasma amino acid concentrations were quantified as described in (Neveux et al., 2003). Briefly, plasma was deproteinated (with sulfosalicylic acid, 30mg/mL)

and protein-free amino acid concentrations were determined in the supernatent by ion-exchange chromatography with ninhydrin detection using an AminoTac JLC-500V analyzer (Jeol, Tokyo, Japan).

4.1.4 Statistical analysis

Statistical analysis was performed with either an ANOVA using a general linear model or with the MIXED procedure of the SAS Software for repeated measures, including a random female effect. The effects of treatment on the types of ovarian activities were examined for differences using the Chi-square test. Results are presented as LSmeans ± standard error of the mean (SEM). Significant differences are reported at $P<0.05$.

4.1.5 Results

There were no physiological differences between cows in the C and BC groups before the start of the experiment (Table 7). Blood β-carotene concentrations during the dry and postpartum period are shown in Fig. 19. There was no significant difference between the two treatment groups before the start of the dietary β-carotene supplementation. In the BC group, blood concentrations of β-carotene increased gradually and reached a peak 1 month after drying-off and then decreased ($P<0.001$) until week 2 postpartum. In the C group, blood concentrations of β-carotene remained constant for the first month after drying-off and then decreased significantly until calving ($P<0.05$) and reached a nadir concentration at week 2 postpartum.

Table 7 Measurements made in cows to form group before starting the experiment. LSMean ± SEM.

Parameter	Control group (n = 20)	β-carotene group (n = 20)	*P*-value
Milk production (kg)	11300 ± 707	11010 ± 707	0.77
Lactation No.	1.95 ± 0.26	1.85 ± 0.26	0.78
Body weight (kg)	710.3 ± 17.92	714.2 ± 17.92	0.87
BCS[1] (scale 1-5)	2.26 ± 0.17	2.21 ± 0.17	0.84
β-carotene (mg/L)	3.03 ± 0.22	3.12 ± 0.22	0.77

[1]BCS = body condition score

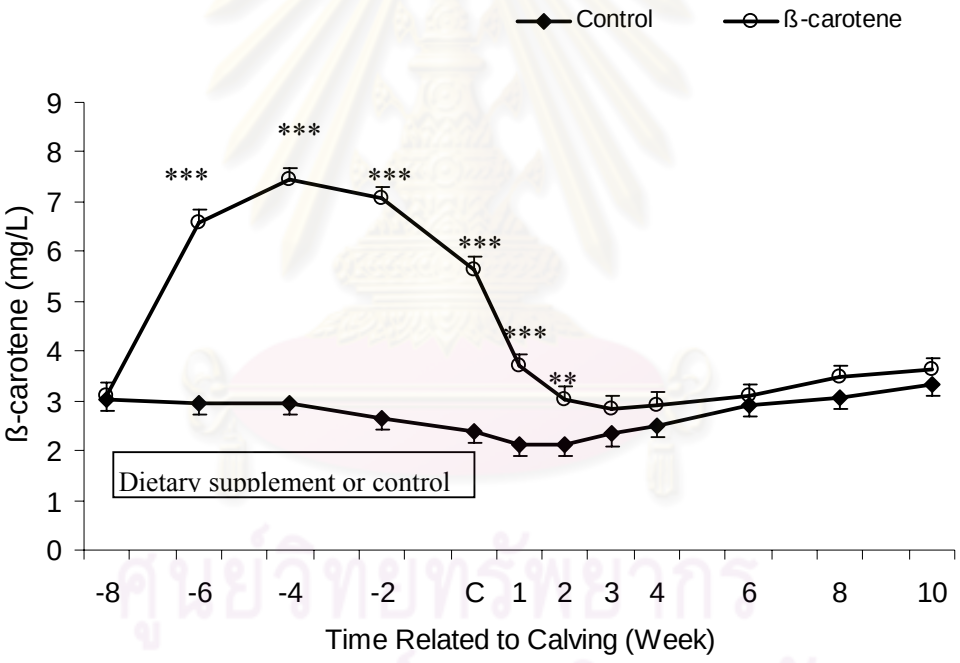


Figure 19 Blood β-carotene concentrations in Holstein cows given either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=20) starting 8 wks before calving until calving. LSMean ± SEM. Significant difference between dietary treatments, *** $P<0.001$, ** $P<0.01$. C = Calving

There was no significant difference in the interval from calving to ovulation between groups (C, 27.8 ± 3.46d vs. BC, 35.8 ± 3.55d; $P>0.05$). The different types of ovarian activity observed are shown in Table 8. The dietary supplement of β-

carotene had no effect on postpartum ovarian activity during the periods studied (0-50d and 51-77d). Progesterone production during normal cycles, determined by the calculation of the area under the curve of normal progesterone profiles, was not different between treatments (C, 416.6 ± 39.85 ng.d per mL vs. BC, 381.0 ± 43.05 ng.d per mL; $P>0.05$).

The diameters of the cervix and uterine horns were not different between groups (Fig. 20). The diameter of cervix and uterine horns decreased rapidly during the first month post partum and remained constant from day 40 post partum onwards.

Table 8 Number of Holstein cows expressing different types of ovarian activity postpartum when given either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=19) starting 8 wks before calving until calving. Types of ovarian activity are expressed from calving to 50 days postpartum and from 51 to 77 days postpartum.

Type of ovarian activity	Treatment			
	Control		β-carotene	
	≤ 50 d postpartum	51-77 d postpartum	≤ 50 d postpartum	51-77 d postpartum
Normal	7/20 (35.0%)	14/20 (70.0%)	6/19 (31.6%)	13/19 (68.4%)
Anovulation	6/20 (30.0%)	1/20 (5.0%)	7/19 (36.8%)	2/19 (10.5%)
Irregular cycle	3/20 (15.0%)	2/20 (10.0%)	2/19 (10.5%)	0/19 (0%)
Persistent *corpus luteum*	4/20 (20.0%)	3/20 (15.0%)	4/19 (21.1%)	4/19 (21.1%)

Plasma concentrations of amino acids are shown in Table 9. Plasma concentrations of hydroxyproline were affected by treatment on day 21 postpartum (BC: 20.8 ± 1.33 μmol/L vs. C: 15.0 ± 1.33 μmol/L; $P<0.01$). The other plasma amino acids were not affected by treatment.

The percentage of neutrophils in the endometrial smear from both cervix and uterus are shown in Table 10. At 28 days postpartum the percentage of neutrophils in the BC group was lower than in the C group (cervical smear; C: 21.0 ± 3.22% vs. BC:

9.7 ± 3.14%; $P<0.05$ and uterine smear; C: 32.0 ± 3.86% vs. BC: 20.9 ± 3.76%; $P<0.05$).

4.1.6 Discussion

In the present study, we showed that a supplement of β-carotene given during the dry period increased circulating concentrations of β-carotene which is in agreement with previous reports (Kawashima et al., 2009[b]). However, the possibility of comparing the blood concentrations of β-carotene in our study with other reports is limited because there were differences in the levels and duration of supplementation, in the physiological status of the animals, in the form of β-carotene used, in the composition of the diets and in the breeds of cattle studied.

In the present study, a purified-encapsulated form of β-carotene was used and it resulted in circulating concentrations of β-carotene which were above the recommendation of 3 mg/L necessary to cover dairy cow requirements (Frye et al., 1991).

The supplement of β-carotene in the present study had no effect on the length of the interval from calving to first ovulation. Previously, Kawashima et al. (2009[a]) showed in a retrospective study that dairy cows that went on to ovulate in the first thirty days post partum had higher β-carotene concentrations during the dry period than cows that did not go on to ovulate. This finding led to further experiments which aimed to examine the effect of a supplement of β-carotene, given during the close-up dry period, on ovulation at the first follicular wave postpartum. Kawashima's group showed that a supplement of β-carotene might enhance the occurrence of ovulation at the first follicle wave postpartum (Kawashima et al., 2009[b]). There is evidence in the literature that ovulation occurred earlier after the onset of oestrus in β-carotene supplemented cows compared to controls (Wang et al., 1982). However, in the present experiment due to the method used to estimate ovulation (presence of P4 in milk) we are unable to confirm or refute previous results. Monitoring of blood progesterone profiles to determine the ovulation in dairy cows has been widely useded (Shrestha et al., 2005). However there is a large variation in timeing between ovulation and the postovulatory progesterone rise above the cut-off level for luteal activity (Roelofs et

al., 2006, Starbuck et al., 2006). While monitoring of ovulation by frequent ultrasonic examination, for example at 4 h or 6 h interval, give more accurately in timing of ovulation. In the present study the use of ultrasonic examination was not practical. Since the experiment lasted for a long time and oestrus occured spontaneously without hormonal intervention.

High concentrations of β-carotene are present in the ovary and especially in *corpus lutea*. This led to speculation that β-carotene may play a role in *corpus luteum* function and progesterone production. Inaba et al. (1986) found that cows with ovarian cysts had significantly lower circulating β-carotene concentrations than cows without cysts. However, no relationship between the concentration of β-carotene in plasma and in cyst fluid was observed (Haliloglu et al., 2008). In our study, we were unable to show a relationship between ovarian activity and supplemental β-carotene. In addition, total progesterone production in cows with normal ovarian cycles was not different between treatment groups. Our findings support a previous report in which ovarian activity and plasma progesterone were unaffected by β-carotene supplementation (Wang et al., 1988[a]). However, Graves-Hoagland et al. (1988) found that progesterone production by luteal tissue was positively related to plasma concentrations of β-carotene.

Uterine involution was complete at about 30 to 40 days postpartum and we found no differences in the rate of involution as judged by changes in uterine horn and cervical diameters. The absence of a β-carotene effect in our study is in agreement with Wang et al. (1988[a]) but contrasts with Rakes et al. (1985), who found that the diameter of the cervix in β-carotene supplemented animals was smaller than that of control animals measured at 21 and 28 d postpartum. However, in the latter experiment the supplementation of 300mg/cow/d was started on the day of calving and continued to 100d postpartum, compared to the present study when β-carotene supplements began during the dry period and finished at calving. Also the methods used to measure cervix diameter were different between the studies; Rakes et al. used transrectal palpation whereas we used ultrasonography (Rakes et al., 1985).

The increase in uterine weight throughout pregnancy is accompanied by collagen deposition. Collagen is a fibrous protein whose molecule consists of three polypeptide chains which contain significant amounts of the amino acids; glycine,

proline and hydroxyproline (Sarges et al., 1998). Collagen is involved both in placental development and in the uterine involution processes. The collagen accumulated in the cotyledons during gestation accounts for 20 to 25% of endometrium dry matter (Abribat et al., 1992). After calving, collagen is degraded under the action of collagenase culminating in the appearance of free glycine and hydroxylproline in blood. Hydroxyproline is not found in feedstuffs and is unique to collagen. It accounts for 9 to 10% of the collagen amino acid residues and has not been found in other mammalian proteins except elastin (about 1%). Circulating concentrations of hydroxyproline and glycine increase during the first week following calving (Abribat et al., 1992). The solubility of the collagen in the uterus and blood concentrations of hydroxyproline and glycine are related to uterine involution and can be used as indicators of its speed and completeness (Abribat et al., 1992).

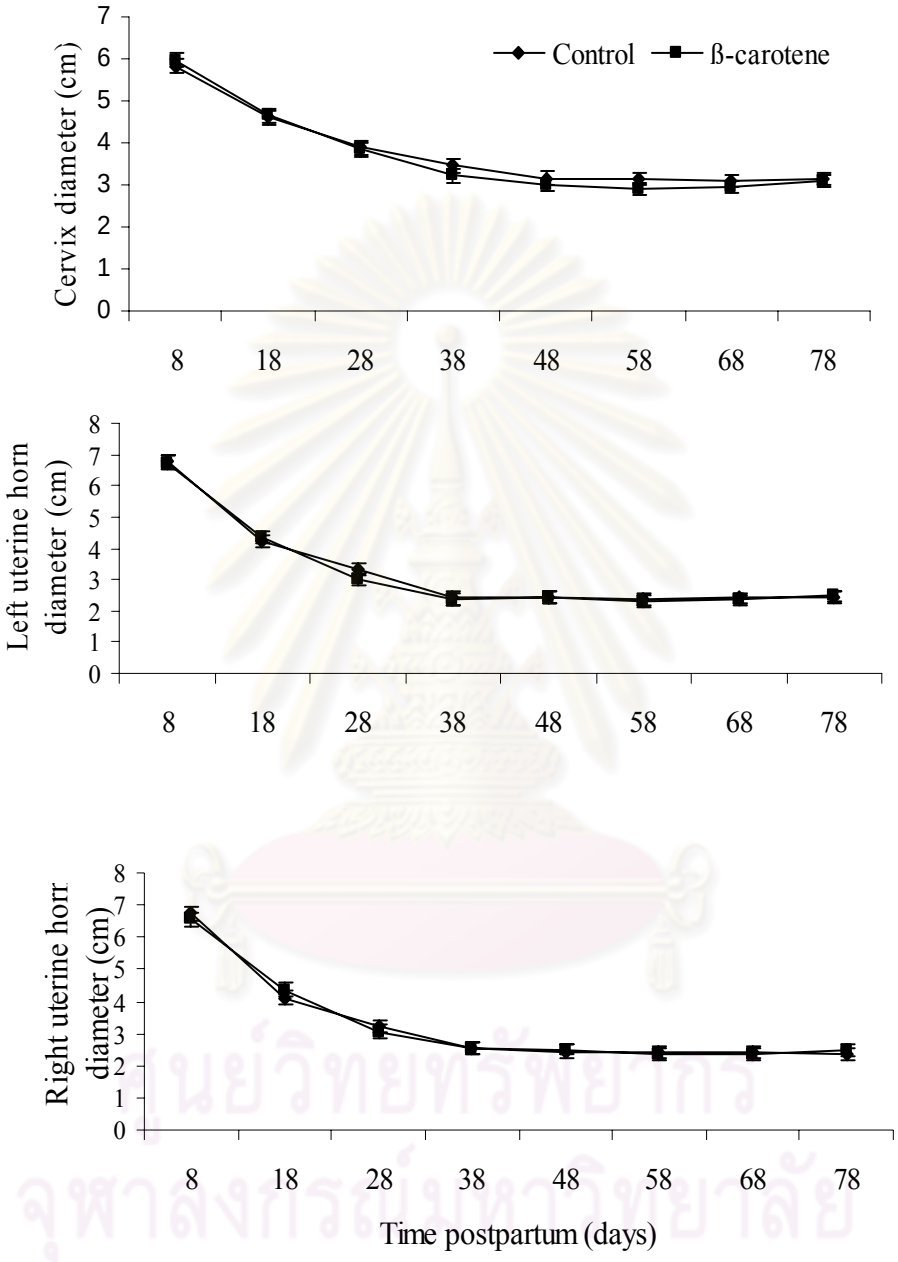


Figure 20 Diameters of the cervix and the uterine horns measured by ultrasound starting 8 days postpartum every 10 days in cows receiving either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=20) starting 8 wks before calving until calving. LSMean ± SEM

Table 9 Plasma amino acid concentrations (μmol/L) measured on the day of calving, day 7 and day 21 postpartum in cows receiving either: a control diet (C, n=20) or a control diet plus 1g/d β-carotene (BC, n=20) starting 8 wks before calving until calving. LSmean ± SEM

Amino acid	Group	Plasma Concentration (μmol/mL)			
		Day 0 (calving)	Day 7	Day 21	SEM
Glycine	C	514.02	653.88	624.12	39.06
	BC	602.26	606.42	613.03	
Hydroxyproline	C	15.82	20.25	14.95 [a]	1.33
	BC	16.46	21.33	20.75 [b]	
Threonine	C	56.78	86.52	115.85	6.35
	BC	58.04	82.56	97.52	
Valine	C	142.79	203.49	260.88	11.65
	BC	145.66	211.22	231.95	
Methionine	C	25.38	26.46	22.36	1.37
	BC	21.78	22.36	22.05	
Leucine	C	114.94	127.16	130.27	7.22
	BC	109.71	135.22	124.54	
Histidine	C	53.28	61.04	43.02	2.91
	BC	53.43	56.03	49.47	
Lysine	C	53.59	90.79	90.47	3.74
	BC	58.11	84.83	83.28	
Taurine	C	38.18	47.58	46.64	3.36
	BC	36.50	41.25	47.78	
Serine	C	109.14	112.38	110.89	6.60
	BC	105.98	93.98	105.03	
Asparagine	C	21.40	36.04	44.49	1.85
	BC	20.65	30.58	37.40	
Glutamate	C	55.08	51.88	55.84	2.66
	BC	54.00	54.27	55.70	
Glutamine	C	297.58	233.48	213.09	13.03
	BC	256.51	208.94	212.51	
Alanine	C	251.90	215.13	266.06	12.14
	BC	234.46	207.08	247.64	
Citrulline	C	70.86	64.97	87.08	4.23
	BC	63.08	62.08	93.68	
Isoleucine	C	86.07	124.78	147.12	7.81
	BC	87.08	129.08	127.69	
Tyrosine	C	33.06	40.83	51.64	2.30
	BC	28.15	39.01	45.67	
Phenylalanine	C	46.60	57.60	48.20	2.18
	BC	43.79	58.87	48.68	
Ornitine	C	14.25	22.96	33.09	1.42
	BC	15.41	21.37	31.41	
Arginine	C	39.38	62.21	61.94	2.88
	BC	40.84	51.38	59.11	
Proline	C	61.99	79.93	89.61	3.63
	BC	60.13	73.16	79.16	

SEM: pooled standard error of the least square mean for each amino acid
[a, b] concentrations of hydroxyproline at day 21 postpartum were different; $P<0.01$

Table 10 Percentage of neutrophils in smears taken from the cervix and the uterus starting 8 days postpartum every 10 days in cows receiving either: a control diet (C, n=20) or a control diet plus 1g/d β-carotene (BC, n=20) starting 8 wks before calving until calving. LSmean ± SEM

Section of reproductive tract	Days post partum	Percentage of neutrophils	
		Control	β-carotene
Cervix	8	20.31 ± 3.30	24.28 ± 3.14
	18	16 .85 ± 3.30	23.35 ± 3.14
	28	20.97 ± 3.22[a]	9.65 ± 3.14[b]
	38	6.86 ± 3.22	6.05 ± 3.21
	48	9.05 ± 3.22	4.05 ± 3.38
	58	8.84 ± 3.22	3.99 ± 3.21
	68	6.10 ± 3.30	8.21 ± 3.39
	78	3.19 ± 3.50	4.46 ± 3.50
Uterus	8	32.17 ± 3.96	31.67 ± 3.76
	18	35.28 ± 3.86	37.32 ± 3.76
	28	31.95 ± 3.86[a]	20.92 ± 3.76[b]
	38	18.50 ± 3.86	16.29 ± 3.94
	48	16.72 ± 4.05	7.57 ± 4.16
	58	9.48 ± 4.05	8.50 ± 4.18
	68	8.41 ± 3.95	5.78 ± 4.61
	78	4.27 ± 4.45	5.48 ± 4.48

[a, b] Percentages of neutrophils within a row with different superscripts differ, $P<0.05$.

In the situation of delayed uterine involution, the catabolism of uterine collagen is slow and the concentrations of hydroxyproline and glycine in blood remain low (Abribat et al., 1992). We found in the present study that the blood concentrations of hydroxyproline in the BC group were significantly higher than those

in the C group. This indicates that collagen catabolism was greater in BC compared to C. Hydroxyproline has been used to study calcium metabolism in periparturient hypocalcaemic dairy cows (Evans et al., 1976, Goff and Horst, 1998). In calcium deficient cows, bone resorption occurs to release the skeletal calcium into the blood circulation to compensate for calcium deficiency. This process results in raised blood hydroxyproline (Goff and Horst, 1998). However, clinical hypocalcaemia was not observed in this study and hypocalcaemia usually occurs within a few days postpartum. Thus, we suggest that the circulating concentrations of hydroxyproline in this study might have been a result of the catabolism of uterine collagen during involution.

A series of samples taken every ten days in this study showed that the percentage of polymorphonuclear leucocytes (PMN) in the uterus and cervix in the BC group was lower than that in the C group at 28 days postpartum. In dairy cows, it is common for the dilatation of the cervix during the calving process to allow the entry of bacteria into the uterus (Sheldon et al., 2002, Gautam et al., 2010). These bacteria can trigger the cow's defence mechanisms. As a consequence, PMN are attracted and penetrate the uterus. Without intervention, cows may recover from bacteria infection within a short time period. Clinically, the diagnosis of endometritis is usually performed 3 weeks postpartum due to concern about the over-diagnosis of uterine infection (Gautam et al., 2010). As mentioned above, our results show that an indicator of bacterial infection clearance, during the first 3 weeks postpartum, occurred more rapidly in cows supplemented with β-carotene compared to controls. The mechanisms by which carotenoids regulate immunity are not fully understood. In laboratory studies (Daniel et al., 1991[a,b]), β-carotene enhanced bovine blood and mammary gland phagocytic cell kill ability. Supplementation of cows with 400mg β-carotene/cow/d from 6 weeks before until 2 weeks after drying-off produced a beneficial effect in cows by stimulating polymorphonuclear phagocytic and bacterial killing ability. Phagocytic ability was maintained after drying-off in β-carotene supplemented cows and tended to decrease after drying-off in cows fed only vitamin A (Tjoelker et al., 1988, 1990). However, the phagocytic ability of PMN was not verified in the present study. The difference between groups in blood concentrations

of β-carotene was observed until the second week postpartum. This difference may have helped PMN to clear the bacteria from uterus and cervix during the spontaneous recovery period.

It is possible that by stopping the β-carotene supplement on the day of calving we may have reduced the positive effect of the supplement on an indicator of uterine involution (hydroxyproline) and neutrophil percentages. It is interesting that even though the treatment stopped at calving there was still a positive carry-over effect which was observed 3 weeks later. The effect that we observed may have been more pronounced if the β-carotene supplement was continued throughout the first weeks postpartum.

The first objective of the present study was confirmed, since a dry period β-carotene supplement increased postpartum blood concentrations of β-carotene. We were unable to show that a β-carotene supplement pre-partum improved ovarian function and progesterone production postpartum. Since there were no spontaneous cases of endometritis, it was not possible to investigate the last objective of our study which was to test whether a β-carotene supplement could improve uterine health (involution and inflammation). However, β-carotene increased an indictor of uterine involution (blood hydroxyproline) and had a positive effect on the percentage of polymorphoneuclear leucocytes in both the uterus and cervix compared to control cows. Dietary supplementation with β-carotene to dairy cows prior to calving can have positive effects on reproduction and immunity. The relatively modest effect observed postpartum in this experiment may be due to the fact that the supplement was stopped on the day of calving. The present experiment, using forty cows, has highlighted some potentially interesting pathways for future studies. We believe that it would be worthwhile and informative to test the robustness of our findings by conducting a large, controlled, multi-location study.

4.2 Effects of a dietary supplement of β-carotene given during the dry period on milk production and circulating hormones and metabolites in dairy cows

4.2.1 Abstract

The objective of this study was to investigate whether a supplement of β-carotene given during the dry period is able to 1) improve milk production and milk composition and 2) modify hormone and metabolic status in dairy cows during the dry and postpartum periods. This study was conducted using 40 Holstein, primiparous and multiparous cows. On the day of drying-off, cows were allocated to one of two dietary treatments: control diet (n=20) or control diet plus 1g/d β-carotene (n=20). The β-carotene supplement was given individually to the cows until calving. Blood samples were obtained regularly and the concentrations of β-carotene in blood and circulating metabolites and hormones in plasma were measured. Live weight and body condition score (BCS) were monitored once a month. Daily milk production was recorded after calving. Milk composition was measured every 15 days. The dietary supplement of β-carotene increased blood concentrations of β-carotene during the dry period and although the difference decreased postpartum β-carotene concentrations remained higher compared to the control group. Live weight, BCS, milk production and composition: milk protein, milk fat, milk urea and somatic cell count, were unaffected by treatment (P>0.05). Plasma concentrations of insulin, insulin-like growth factor-1, glucose, non-esterified fatty acids and urea were unaffected by dietary supplementation with β-carotene (P>0.05). In conclusion, supplementation with β-carotene during the dry period increased blood concentrations of β-carotene but had no effect on performance or hormone and metabolic status.

4.2.2 Introduction

In the dairy cow, the peripartum period is a stressful time due to the dramatic physiological and metabolic adaptations required during the changed from pregnancy to lactation. Energy demand is increased due to the need to meet the requirements for rapid foetal growth and milk production. During this period, cow immunity is suppressed leading to an increase in susceptibility to a number of diseases. It has been

reported that neutrophil function before parturition is impaired (Kimura et al., 2002) and this has been linked to mammary gland and uterine infection. The changes in metabolism associated with rapid foetal growth, parturition and initiation of lactation results in increased production of free radicals. When the production of free radicals exceeds the antioxidant defence mechanisms present in the body, the cow is in oxidative stress. Oxidative stress in periparturient cows may be a contributory factor for disease susceptibility. Independent of its role as provitamin A, β-carotene may affect immune function. β-carotene has been found to enhance immune function through its ability to regulate membrane fluidity, gap junction communication and as an antioxidant (Chew and Park, 2004, Tjoelker et al., 1988, Tjoelker et al., 1990). *In vitro* studies showed that β-carotene enhanced bovine blood and mammary gland phagocytic cell killing ability and enhanced lymphocyte proliferation induced by a mitogen (Daniel et al., 1991[a,b], Michal et al., 1994).

β-carotene is a crucial member of a group of molecules which are free radical scavengers. β-carotene and other carotenoids are especially effective at quenching singlet oxygen and can prevent the subsequent formation of secondary reactive oxygen species (ROS) (Sordillo et al., 2009). Chawla and Kaur, (2004) reported a positive correlation between plasma β-carotene concentrations and antioxidant power measured by a ferric reducing antioxidant power assay in cows supplemented with β-carotene during the dry period. They also suggested that there is a need to give cows a β-carotene supplement during the dry period in order to improve their plasma antioxidant status and health after parturition and to improve milk production and quality due to reduced mammary gland infection. Negative energy balance commonly occurs in the early postpartum period and reaches a maximum during the first or second week postpartum (Butler and Smith, 1989, McNamara et al., 2003). Similarly, plasma concentrations of β-carotene and vitamin E in non-supplemented cows decrease throughout the dry period and reach their lowest levels during the first week postpartum (Michal et al., 1994, Calderón et al., 2007). Thus, the decrease in plasma concentrations of β-carotene could be associated with the occurrence of metabolic and infectious diseases during the peripartum in dairy cows. Additionally, the concentrations of several metabolites and metabolic hormones are modified by the adaptations which occur around calving. However, there have been few reports on the

effect of supplemental β-carotene on milk production and quality and on blood metabolites in peripartum cows. Therefore, this study aimed to investigate whether a supplement of β-carotene given during the dry period is able to 1) improve milk production and milk composition and 2) modify hormone and metabolic status in dairy cows during the postpartum period.

4.2.3 Material and methods

4.2.3.1 Animals, management, blood sampling and measurement of blood concentrations of β-carotene

This experiment was conducted using the same animals as in experiment 4.1. Plasma samples that were taken at -8, -6, -4, -2 weeks before calving, at calving and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks after calving were used to determine the plasma concentrations of insulin, IGF-1, glucose, NEFA and urea. Blood concentrations of β-carotene measured as described in experiment 1 were also used to for analysis in this experiment.

4.2.3.2 Milk yield and milk compositions

The average individual daily milk yields (milk collected in the evening and the following morning) were measured with milk meters (MM15, DeLaval Inc., Elancourt, France) connected to Alpro (Alpro, DeLaval Inc., France). Milk composition was measured individually once every two weeks on a composite mixture of milk from successive evening and morning milkings. The samples were kept at room temperature with a preservative (Bronopol, Lanxess, Langenfeld, Germany) until analysis. Samples were sent to the laboratory of the Milk Recording Organisation (Syndicat Interdépartemental de l'Elevage, Le Mée, France) to determine milk fat, protein and urea concentrations by infrared spectrophotometry (MilkoScan 6000, Foss Electric, Nanterre, France). Somatic cell counts (SCC) were evaluated by flow cytometric measurement (Fossomatic 5000, Foss Electric, Nanterre, France).

4.2.3.3 Body weight and body condition score

Body weight and BCS were recorded on the day of drying-off, 1 month after drying-off, just after calving, 1 and 2 months postpartum. Body condition score was evaluated by the same person on a scale of 1-5 with a 0.25 increment where 1 represents extremely thin or emaciated cows and 5 represents extremely fat or obese cows (Ferguson et al., 1994).

4.2.3.4 Insulin and Insulin-like growth factor–1

Insulin and IGF-1 were analysed by radioimmunoassays respectively based on porcine insulin (Insulin-CT®, CIS Bio International, Gif-sur-Yvette, France) and human recombinant IGF-1 (IGF-1-RIACT®, CIS Bio International, Gif-sur-Yvette, France). IGF-BPs were removed following the manufacturer's instructions. Intra-assay coefficients of variation were 4.8 % at 153.8 pmol/l and 3.8 % at 57.45 ng/ml for insulin and IGF-1, respectively.

4.2.3.5 Measurement of blood metabolites

Plasma samples were analysed by photometric methods for glucose (Glucose-RTU®, BioMérieux, Lyon, France), NEFA (NEFA C®, Wako Chemicals, Neuss, Germany), and urea (Urea-kit S®, BioMérieux, Lyon, France). Inter-assay coefficients of variation were 4.3 % at 3.47 mmol/l, 12.65 % at 0.31 mmol/l, 8.3 % at 2.99 mmol/l, for glucose, NEFA, and urea, respectively.

4.2.4 Statistical analyses

Statistical analysis was performed using SAS (Version 9.1; SAS Institute, Cary, NC, USA). The parameters measured in this study were repeated measures over time on individual cows. The MIXED procedure was used to determine the difference between treatment groups including a random female effect and contrast statement

was used in the model. Results are presented as LSmeans ± standard error of the mean (SEM). Significant differences are reported at $P<0.05$.

4.2.5 Results

Neither live weight nor BCS were modified ($P>0.05$) by dietary treatment (Fig. 21) however both parameters increased to a peak 1 month prior to calving and then decreased during the rest of the study ($P<0.001$). During the first four days postpartum daily milk production was not recorded. Milk production per day was recorded starting on the 5th day postpartum. An average milk yield per day over 100 DIM was not affected by treatment (BC= 40 ± 6.1 kg/d, C= 39 ± 7.2 kg/d; $P>0.05$). Milk production was influenced by time after calving ($P<0.0001$) but there was no interaction between treatment and time ($P>0.05$).

Neither insulin nor IGF-1 (Fig. 22) differed between treatments. Plasma concentrations of insulin increased slightly during the first month of the dry period and then drastically decreased at calving. The lowest blood concentrations of insulin were observed at calving in the BC group and at 2 weeks postpartum in control group. Similarly, plasma concentrations of IGF-1 increased slightly during the first month after drying-off and then decreased and reached a minimum at 2 weeks postpartum. Thereafter IGF-1 increased gradually throughout the rest of the study.

The dietary supplement of β-carotene did not affect ($P>0.05$) blood concentrations of glucose, NEFA, and urea (Fig. 23). Plasma concentrations of these metabolites were affected by time postpartum ($P<0.0001$) but there was no interaction between time and treatment ($P>0.05$).

Milk composition (protein, fat and urea; Fig. 24) and SCC (Fig. 25) were not affected by treatment ($P>0.05$). Milk protein and fat were influenced by time postpartum ($P<0.0001$) while urea and SCC were unaffected. There was no interaction between treatment and time on milk composition ($P>0.05$).

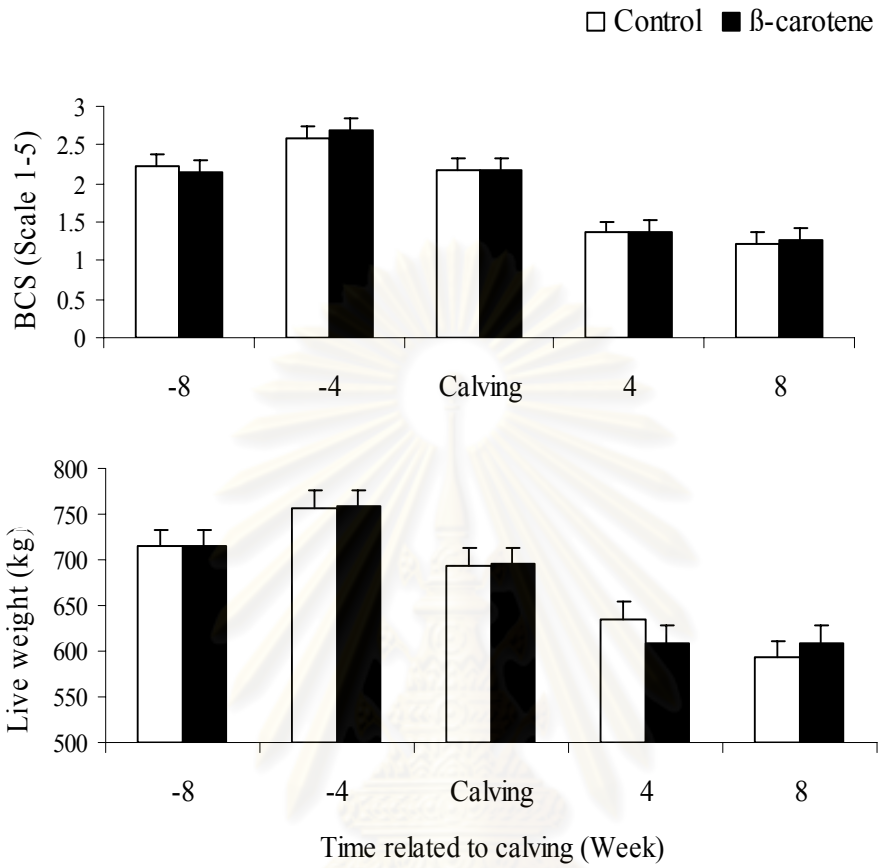


Figure 21 The evolution of live weight and body condition score (BCS) in dairy cows given either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=20) starting 8 wks before calving until calving. LSMean ± SEM.

4.2.6 Discussion

The present experiment a β-carotene supplement had no effect on milk production as shown by others (Bindas et al., 1984[b], Wang et al., 1988[a,b], Rakss et al., 1985). In contrast, a study conducted with heat stressed cows showed that cumulative milk yield increased by 6 to 11% in β-carotene supplemented compared to non-supplemented cows (Aréchiga et al., 1998). Moreover, Oldham et al. (1991) found

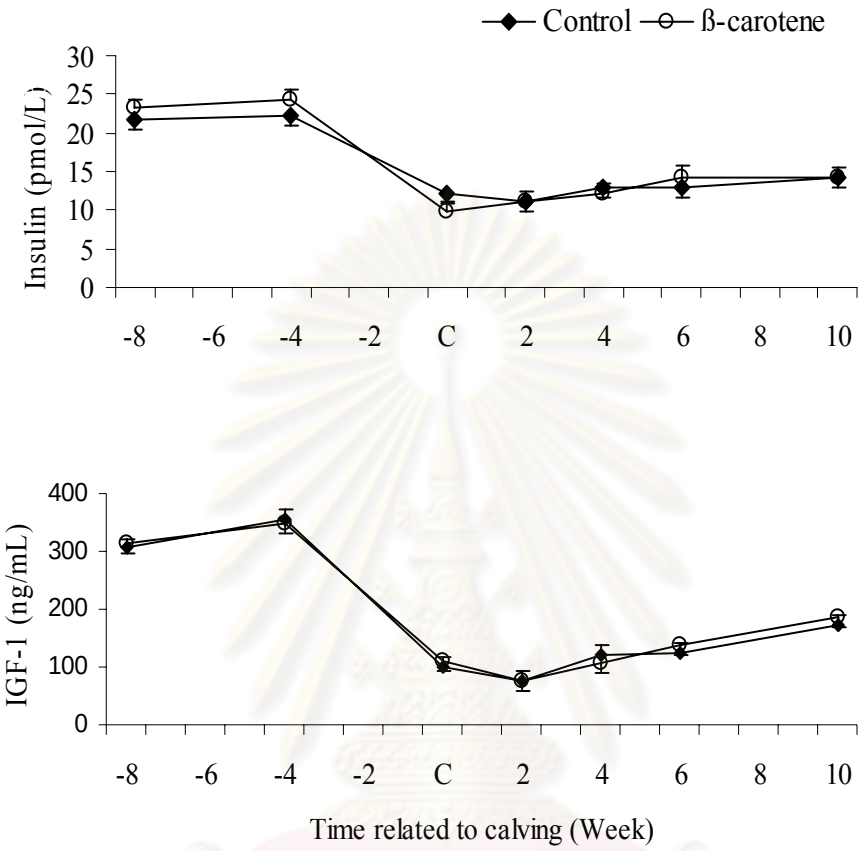


Figure 22 Plasma concentration of insulin and insulin-like growth factor-1 in dairy cows given either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=20) starting 8 wks before calving until calving. LSMean ± SEM. C=calving

that a supplement of β-carotene during the dry period and early lactation could increase milk yield in non-heat stressed dairy cows. Although they found a positive effect of β-carotene on milk production, they were unable to explain the result and they concluded that the effect of β-carotene on milk production warranted additional research. Differences in β-carotene concentrations in the control diet, the initial blood concentration of β-carotene, the level of supplementation, the timing of supplementation, the duration of supplementation may have contributed to the lack of consistency in responses to β-carotene supplementation previously reported.

In this study, the evolution in plasma glucose is in agreement with a previous report (Ingvartsen et al., 2003). What is commonly seen is that glucose concentrations remain stable or increase slightly during the prepartum period, rise at calving, and then decrease immediately after calving (Santos et al., 2001, Ingvartsen et al., 2006). The increase in plasma glucose at calving reflects an increase in gluconeogenesis which is in response to calving stress. The decrease immediately after calving may be associated with the modest increase in dry matter intake concomitant with the very high uptake of circulating glucose by the mammary gland for lactose synthesis (Wathes et al., 2007).

The changes in the plasma metabolites; NEFA, and hormones; IGF-1 and insulin together with the changes in live weight and BCS reflect the nutritional status of the cows. These variables confirm the very large energy demands of cows during the last month of pregnancy. The energy supply from feed intake could not meet the requirements for maintenance and rapid foetal growth. Therefore, body energy reserves, mainly in the form of body fat, are mobilized to provide the energy and to cover requirements. This mechanism resulted in an increase in NEFA, and a decrease in insulin and IGF-1 and the loss of body condition. In our study, there was no effect of a dietary supplement of β-carotene on milk protein content which is consistent with previous work (Rakss et al., 1985). Recently, de Ondarza et al. (2009) also reported that a supplement of β-carotene had no effect on milk protein and SCC but did increase milk fat compared to non-supplemented cows. Hino et al. (1993) found, in an *in vitro* study, that β-carotene plus α-tocopherol increased the growth of cellulolytic bacteria cultured in fat-supplemented media and increased cellulose digestion. The increase in fibre digestion in the rumen may explain why milk fat increased after β-carotene supplementation. Another possibility is that β-carotene supplementation is associated with altered rumen bio-hydrogenation as has been observed for another antioxidant, vitamin E (Bell et al., 2006). A supplement of vitamin E when given with a linseed supplement (rich in C18:3n-3) altered rumen bio-hydrogenation and resulted in an increase in the production of vaccenic acid (*trans*-11 C18:1) and a decrease in the production of *trans*-10 C18:1 in the rumen (Pottier et al., 2006). Since *trans*-10 C18:1 has been associated with milk fat depression (Bauman and Griinari, 2001) this may explain why a dietary antioxidant supplement can in some situations increase

milk fat level. A potentially interesting effect of β-carotene supplementation could be to increase rumenic acid in milk since vaccenic acid is its precursor in the mammary gland. It has been shown that rumenic acid is able to reduce the incidence and the growth of tumours, prevent diabetes and atherosclerosis and enhance immune function (Belury, 2002). In the present study, although the blood concentrations of β-carotene postpartum were different between treatments the BC cows were no longer receiving the dietary β-carotene supplement therefore it is unlikely that ruminal microbe growth and cellulolytic bacteria function would be affected. In the studies where the supplement of β-carotene increased milk production, cows also received β-carotene during the postpartum period (Aréchiga et al., 1998, Oldhan et al., 1991, de Ondarza et al., 2009).

β-carotene functions as an antioxidant and may enhance immunity. This role is of interest in the fight against infection. Several studies have been conducted to determine the effect of β-carotene on cow immunity or udder health (Chawla and Kaur, 2004, Oldhan et al., 1991, LeBlance et al., 2004, Spears and Weiss, 2008). Some studies showed positive effects on udder health while others did not. Somatic cell count in cow milk has been used as an indicator of udder health and milk quality and can be related to the response of cellular immunity to pathogens. Rakes et al. (1985), for example, showed that a daily supplement of 300 mg β-carotene lowered SCC in milk. Others reported that the incidence of clinical mastitis was lower in β-carotene supplemented compared to non-supplemented cows (Kawashima et al., 2009[b]). We, however, found no effect of a supplement of β-carotene on SCC. Although in the present experiment β-carotene remained higher in BC cows compared to C cows postpartum, even though the supplementation was stopped at calving, the difference was probably not large enough to affect SCC. Other studies have shown that β-carotene was ineffective in lowering somatic cell numbers and protecting against mastitis (Bindas et al., 1984[a,b], Oldhan et al., 1991, LeBlance et al., 2004).

The present study showed that a dietary supplement of β-carotene in a purified form given to dairy cows can escape degradation in the rumen and increase circulating concentrations in dairy cows.

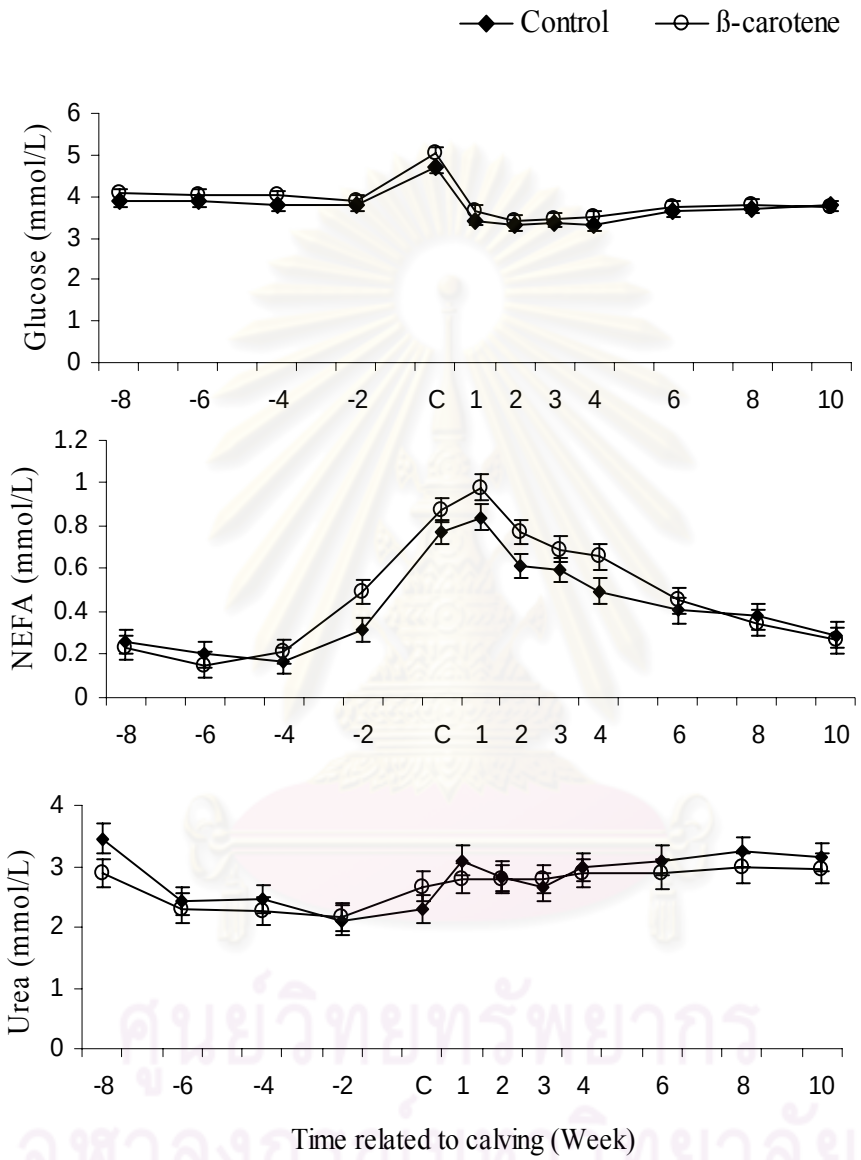


Figure 23 Plasma concentrations of glucose, non-esterified fatty acids (NEFA), and urea in dairy cows given either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=20) starting 8 wks before calving until calving. LSMean ± SEM. C=calving

Although in the literature β-carotene has been shown to have specific effects on metabolism and production in dairy cows the parameters that we measured in the present experiment were unable to shed light on the physiological functions that require β-carotene. Although the β-carotene supplement was stopped the day of calving, blood concentrations remained higher in the treated cows compared to the controls during the postpartum period. There was no effect of β-carotene on milk production and metabolism after the supplement was stopped.

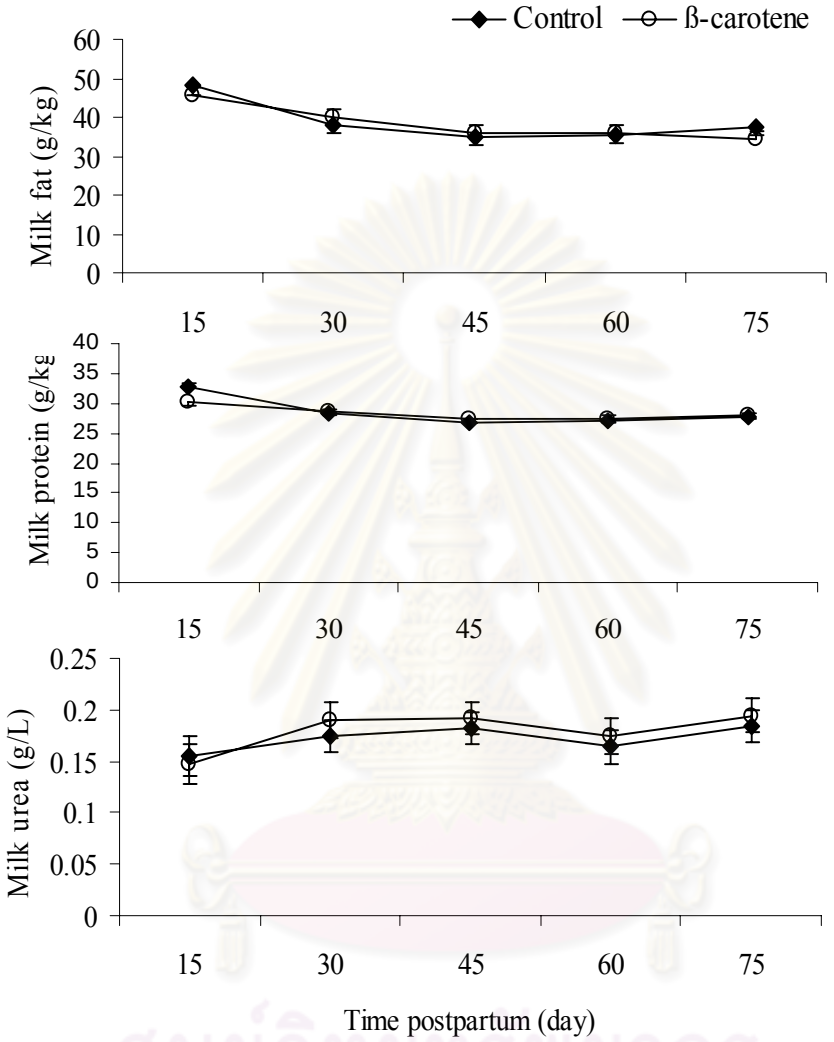


Figure 24 Milk composition (milk fat, protein lactose, and urea) in dairy cows given either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=20) starting 8 wks before calving until calving. The samples were taken every 15 days post partum and lasted for 10 weeks. LSMean ± SEM

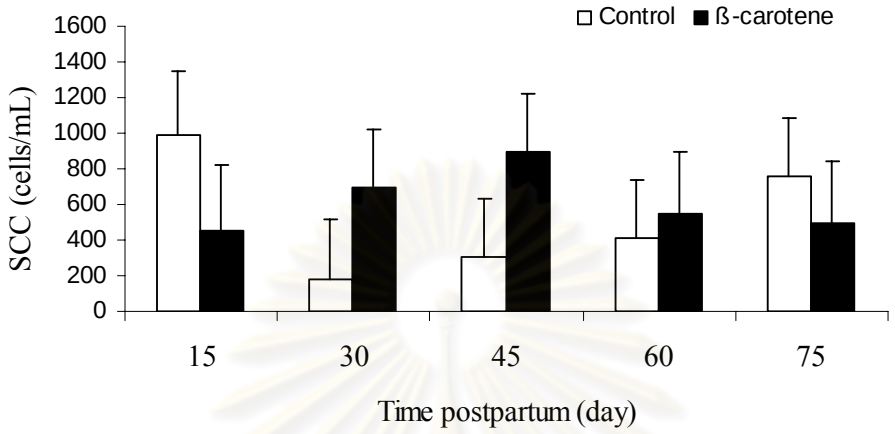


Figure 25 Somatic cell count (SCC) in dairy cows given either: a control diet (n=20) or a control diet plus 1g/d β-carotene (n=20) starting 8 wks before calving until calving. The samples were taken every 15 days post partum and lasted for 10 weeks. LSMean ± SEM.

4.3 The influence of a supplement of β-carotene given during the dry period to dairy cows on colostrum quality, and β-carotene status, metabolites and hormones in new born calves

4.3.1 Abstract

The objectives of the present study were to investigate whether a dietary supplement of β-carotene given to dairy cows during the dry period was able to: 1) increase their β-carotene status, 2) increase the amount of β-carotene in colostrum, 3) increase the concentrations of IgG in colostrum and 4) modify metabolic hormone, enzyme and metabolite status in their calves at birth. Forty Holstein cows were allocated to one of two dietary treatments: a control diet (C, n=20) or the same diet plus 1 g β-carotene/cow/d (BC, n=20) starting on the day of drying-off. The β-carotene supplement was given individually to the cows throughout the dry period.

From week 2 after the start of supplementation, blood concentrations of β-carotene were higher in BC compared to C cows ($P<0.0001$).

The β-carotene concentrations of colostrum were higher in BC than in C cows (3.10 ± 0.23 mg/L vs.1.44 ± 0.24 mg/L, $P<0.001$). Colostrum production was not different between groups (BC, 11.11 ± 1.21 kg vs. C, 10.05 ± 2.25 kg). The content of immunoglobulin G in colostrum was not affected by treatment (BC, 82.65 ± 8.79 mg/mL vs. C, 79.32 ± 9.02 mg/mL). Blood concentrations of β-carotene in calves at birth were unaffected by treatment (BC, 1.16 ± 0.21 mg/L vs. C, 1.27 ± 0.24 mg/L). A supplement of β-carotene given during the dry period to dairy cows did not affect metabolite and metabolic hormone concentrations and enzyme activities in newborn calves. The results of this study indicate that a dietary supplement of β-carotene given in late-gestation was able to increase β-carotene concentrations in dam blood and in colostrum but was unable to increase colostral IgG. In addition, hormone and metabolite status and enzyme activities in the neonatal calf were also unaffected.

4.3.2 Introduction

The natural precursor for vitamin A (retinol) in ruminants is β-carotene. Several studies have shown the importance of β-carotene, in its own right, on reproduction, immune function and health in the cow and calf (Michal et al., 1994, Kume and Toharmat, 2001). The majority of raw materials used to feed dairy cows are very poor sources of β-carotene (Nozière et al., 2006). In addition, plasma concentrations of β-carotene have been shown to decrease in dairy cows during the pre-partum period (Kawashima et al., 2009[a]). This may be due to the transfer of β-carotene from blood to colostrum or to the foetus. However, in many species liver stores of vitamin A are very low at birth. Therefore, the transfer of vitamin A and β-carotene to colostrum and its intake shortly after birth could be fundamental in providing adequate vitamin A and β-carotene to the neonate. The importance of immunoglobulin G (IgG) levels in colostrum for calf health is well recognized (Kume and Toharmat, 2001). However, little information on the possible effect of β-carotene supplementation to dairy cows on colostral IgG concentration is available. Various

studies have been conducted to study the effect of supplements of various antioxidants, including β-carotene, on cow health during the peripartum period (Chawla and Kaur, 2004, Spears and Weiss, 2008).

To date there has been little research into the possible effects of a supplement of β-carotene during late pregnancy on calf health at birth. In new born calves there are great morphological and functional changes (Blum and Hammon, 2000) and there are several hormones and metabolites involved in these processes including insulin, cortisol, insulin-like growth factor-1 (IGF-1), glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), urea, albumin and protein. The changes in these parameters reflect the health status of newborn calves. Determination of enzyme activity is a useful tool to monitor health status. In cattle, changes in the enzyme activities of: γ-glutamyl transferase (γ-GT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP) and creatinine kinase (CK) are associated with liver, heart and skeletal function (Zanker et al., 2001, Hussein and Abd Ellah, 2008).

The objectives of the present study were to investigate whether a dietary supplement of β-carotene given during the dry period to dairy cows was able to 1) increase their β-carotene status, 2) increase the amount of β-carotene in colostrum, 3) increase the concentration of IgG in colostrum and 4) modify metabolic hormone, (cortisol, insulin, IGF-1), enzyme activity (ALP, ASAT, γ-GT, CK), and metabolite, (glucose, NEFA, urea, BHB, creatinine, albumin, and protein) status in their calves at birth.

4.3.3 Materials and methods

4.3.3.1 Animals and management

This study was conducted using the same animals and management as described in experiment 4.1.

4.3.3.2 Sampling and data collection

Blood samples were obtained at -8, -6, -4, -2 weeks before and on the day of calving by caudal venipuncture before the morning feed. Cows were moved to the calving pen

one month before the expected calving date. Ease of calving was noted on a 0 to 2 scale (where 0 = no assistance to 2 = requiring heavy traction, Jacobsen et al., 2000). The cows were filmed during calving by four cameras. The time required for the calves to move from the lying position to a sterno-abdominal position was noted and used as an indicator of calf vitality at birth. Calf rectal temperature was measured at birth. Immediately after the calf had changed position a jugular blood sample was taken and its live weight measured. The total amount of colostrum produced by each cow was recorded and a sample of 20 mL of colostrum was collected. The concentrations of β-carotene in blood and colostrum were measured as described previously (Schweigert et al, 2007). Analysis was performed after one-step denaturation and extraction into organic solvent by the iEx™ assay system using a carotene photometer (iCheck™; BioAnalyt GmbH, Germany). The blood samples obtained from the calves were centrifuged at 4 ºC, 2000 g for 10 min. Plasma and colostrum samples were stored at -20 ºC until required for assay.

4.3.3.3 Blood metabolites assays

Plasma samples were analysed by photometric methods for glucose (Glucose-RTU®, BioMérieux, Lyon, France), NEFA (NEFA C®, Wako Chemicals, Neuss, Germany), urea (Urea-kit S®, BioMérieux, Lyon, France), BHB (3-hydroxybutyrate dehydrogenase, Roche Diagnostics, Meylan, France), protein (Protein-kit®, BioMérieux SA, Lyon, France), albumin (Albumin-kit®, BioMérieux SA, Lyon, France), and creatinine (Creatinine cinetique, BioMérieux® SA). Inter-assay coefficients of variation were 4.3 % at 3.47 mmol/l, 12.65 % at 0.31 mmol/l, 8.3 % at 2.99 mmol/l, 6.5 % at 0.82 mmol/l, 1.3 % at 64.8 g/l and 1.3 % at 36.3 g/l and 2.2 % at 358.1 g/l for glucose, NEFA, urea, BHB, protein, albumin and creatinine respectively.

4.3.3.4 Cortisol

Plasma cortisol was measured on an Elecsys 2010 immunoanalyser system (Roche Diagnostics, Meylan, France) using the Roche Cobas cortisol assay according

to the manufacturer's instructions. The inter-assay coefficient of variation was 0.33 % at 389 nmol/l.

4.3.3.5 Immunoglobulin G (IgG)

The quantity of IgG in colostrum was determined using Bovine IgG ELISA Quantitation kit (Bethyl Laboratories, InC, USA). The assay was conducted according to the manufacturer's instructions using standard dilutions from 7.8 to 500 ng/ml of Bovine IgG. The intra-assay coefficient of variation was 12.1 %.

4.3.3.6 Insulin and insulin-like growth factor–1

Insulin and IGF-1 were analysed by radioimmunoassays respectively based on porcine insulin (Insulin-CT®, CIS Bio International, Gif-sur-Yvette, France) and human recombinant IGF-1 (IGF-1-RIACT®, CIS Bio International, Gif-sur-Yvette, France). IGF-BPs were removed following the manufacture's instructions. Intra-assay coefficients of variation were 4.8 % at 153.8 pmol/l and 3.8 % at 57.45 ng/ml for insulin and IGF-1 respectively.

4.3.3.7 Enzyme assays

Enzyme activities in the plasma samples were determined using the commercial kits (BioMérieux, Lyon, France): Enzyline® Standardised PAL for alkaline phosphatase (ALP), Enzyline® Standardised ASAT/GOT for aspartate aminotransferase (ASAT), Enzyline® γ-GT S for γ-glutamyltransferase, Enzyline® optimized CK NAC for creatinine kinase. These enzymes activities were determined kinetically at 37 ºC (COBAS MIRA).

4.3.4 Statistical analysis

Statistical analysis was performed either by ANOVA using a general linear model or with the MIXED procedure of the SAS Software for repeated measures,

including a random female effect. Results are presented as LSmeans ± standard error of the mean (SEM). Significant differences are reported at $P<0.05$.

4.3.5 Results

Measurements made in cows and calves on the day of calving are shown in Table 11. Treatment had no effect on the quantity of colostrum produced at first milking after calving. The concentration of colostral β-carotene was increased ($P<0.001$) in the BC compared to the C group. Colostral IgG concentrations were not different between groups. Blood concentrations of β-carotene in calves at birth were unaffected by treatment (BC, 1.16 ± 0.21 mg/L vs. C, 1.27 ± 0.24 mg/L). Calf live weight, body temperature, ease of calving and vitality were unaffected by treatment. Plasma concentrations of metabolites (glucose, NEFA, BHB, urea, creatinine, albumin and protein), hormones (cortisol, IGF-1 and insulin) and enzyme activities (alkaline phosphatase, aspartate aminotransferase, γ-glutamyl transferase and creatinine kinase) were not influenced by treatment (Table 11).

The birth weight of male calves was higher than female calves ($P=0.02$). There was an effect of calf gender on the concentrations of plasma glucose ($P=0.03$) at birth, males had higher concentrations than females (Table 12).

4.3.6 Discussion

The results of the present study showed that plasma concentrations of β-carotene decreased during the dry period. Similar results have been previously published, with (Kawashima et al., 2009[b]) or without (Calderón et al., 2007 and Kawashima et al., 2009[a]) a supplement of β-carotene. This finding may be explained, in part, by the transfer of β-carotene from the blood to colostrum since colostrogenesis starts several weeks before calving (Barrington et al., 2000).

Table 11 Differences in colostrum characteristics, calf measurements and plasma metabolite, enzyme and hormone concentrations measured on the day of calving in calves from Holstein cows which had received either a control diet or a control diet plus 1g/d β-carotene starting -8 wk before calving until calving (LSMeans ± SEM).

	Dietary Treatment		*P*-value
	Control group n = 15	β-carotene group n = 19	
Colostrum			
Quantity (kg)	10.1 ± 2.3	11.1 ± 1.2	0.54
β-carotene (mg/L)	1.44 ± 0.24	3.10 ± 0.23	< 0.001
IgG (mg/mL)	79.3 ± 9.0	82.7 ± 8.8	0.79
Calf			
Live weight (kg)	43.5 ± 1.4	45.4 ± 1.3	0.34
Temperature (ºC)	39.1 ± 0.2	39.1 ± 0.2	0.99
Blood β-carotene (mg/L)	1.27 ± 0.24	1.16 ± 0.21	0.75
Calving ease (scale 0-2)	0.9 ± 0.2	0.4 ± 0.3	0.19
Vitality (min)	4.0 ± 2.9	8.9 ± 2.1	0.18
Calf plasma			
Glucose (mmol/L)	5.10 ± 0.51	4.88 ± 0.44	0.75
NEFA (mmol/L)	0.64 ± 0.07	0.64 ± 0.06	0.96
BHB (mmol/L)	0.42 ± 0.06	0.32 ± 0.05	0.23
Urea (mmol/L)	2.86 ± 0.41	3.07 ± 0.37	0.78
Cortisol (nmol/L)	398.0 ± 37.68	451.7 ± 33.83	0.31
IGF-1 (ng/mL)	188.8 ± 14.06	177.9 ± 12.62	0.56
Insulin (pmol/L)	19.6 ± 2.45	17.1 ± 2.2	0.45
ALP (μkat/L)	2.9 ± 0.24	2.4 ± 0.21	0.32
ASAT (μkat/L)	0.45 ± 0.07	0.41 ± 0.06	0.61
γGT (μkat/L)	123 ± 0.03	125 ± 0.03	0.42
CK (μkat/L)	4.41 ± 1.75	3.29 ± 1.57	0.62
Creatinine (μmol/L)	240.53 ± 17.02	261.49 ± 15.28	0.36
Albumin (g/L)	32.53 ± 0.53	33.52 ± 0.47	0.17
Protein (g/L)	44.26 ± 0.59	44.23 ± 0.53	0.97

ALP = alkaline phosphatase, ASAT = aspartate aminotransferase, γGT = γ-glutamyl transferase, CK = creatinine kinase, NEFA = non-esterified fatty acids, BHB = beta-hydroxybutyrate, IGF-1 = insulin-like growth factor-1.

Table 12 The effect of sex on some blood parameters measured in newborn calves (LSMeans ± SEM).

	Male (n =18)	Female (n = 16)	P value
Live weight (kg)	46.7 ± 1.33	42.1 ± 1.38	0.02
Glucose (mmol/L)	5.7 ± 0.47	4.2 ± 0.48	0.03
NEFA (mmol/L)	0.66 ± 0.65	0.62 ± 0.68	0.76
BHB (mmol/L)	0.35 ± 0.57	0.39 ± 0.59	0.65
Urea (mmol/L)	2.96 ± 0.38	2.98 ± 0.40	0.97
Cortisol (nmol/L)	451.8 ± 35.20	397.7 ± 36.40	0.2
IGF-1 (ng/mL)	182.4 ± 13.13	184.3 ± 13.58	0.92
Insulin (pmol/L)	18.5 ± 2.29	18.2 ± 2.37	0.93

NEFA = non-esterified fatty acids, BHB = beta-hyroxybutyrate, IGF-1 = insulin-like growth factor-1.

However, Wise et al. (1947) reported that circulating β-carotene concentrations decreased in cows which had prematurely calved and had undergone a mammectomy. Therefore, there would, in this situation, be no synthesis of colostrum. Very little information concerning the physiology of β-carotene transport into mammary secretions is available. Some cleavage of β-carotene into vitamin A by the mammary gland may occur (Schweigert and Eisele, 1990). In addition, it is known that dry matter intake decreases before calving and therefore the amount of β-carotene eaten could be lower than the amounts consumed earlier in gestation. In the present experiment this was not the case since the cows ate all the diet offered and the supply of β-carotene in the two diets was practically the same.

Another possibility to explain the decrease in β-carotene in the weeks before calving is that β-carotene is transported to the foetus during the period of exponential foetal growth. A supplement of 1 g/cow/d of β-carotene increased cow blood and colostral β-carotene concentrations but did not modify calf blood β-carotene at birth. Similar results have been obtained in other species such as the horse (Gay et al.,

2004). The epitheliochorial placenta does not allow easy transfer of fat-soluble vitamins from the dam to the foetus. This finding was in agreement with Kume and Toharmat (2001) who reported that placental transfer of β-carotene to the calf is very low and they concluded from their study that low plasma β-carotene 6 days after birth, which resulted from the ingestion of low colostral β-carotene levels was associated with an increased risk of producing low dry matter faeces or having diarrhoea. Although we did not measure placental transfer of β-carotene it could be inferred from our data that it was not possible to increase transfer by giving a β-carotene supplement to the dam.

Although carotenoids are known to have an immunostimulatory action (Chew, 1993), this may not be functional in the newborn calf since its immune system is immature. Therefore, the role of vitamin A may be more important than β-carotene in protecting the newborn calf through its ability to maintain epithelium integrity, creating a barrier to bacterial and viral infection. Under our experimental conditions, there was no effect of a dietary supplement of β-carotene given to dairy cows during the dry period on colostral IgG content. It has been reported in an *in vitro* study, that β-carotene enhances mitogen-induced lymphocyte proliferation (Bendich and Shapiro, 1986). Likewise, some researchers have reported that β-carotene enhanced bovine blood and mammary gland phagocytic cell kill ability (Chew, 1993). The mechanisms by which β-carotene or carotenoids regulate immunity are not fully understood. Concerning the colostral IgG content in our study regardless of the effect of a supplement, we found that the average colostral IgG concentrations were higher than in previous studies; 51.7 g/L (Gulliksen et al., 2008), 76 g/L (Maunsell et al., 1999) and 65.8 g/L (Quigley et al., 1994). It is generally accepted that a minimum concentration of 50 g/L of IgG in colostrum is required to ensure adequate protection of the calf (Gulliksen et al., 2008).

The only ‘natural’ source of vitamin A in ruminants is through the cleavage of β-carotene into vitamin A during; absorption by enterocytes and to a much lesser extent metabolism in the liver (Debier and Larondelle, 2005). Even if placental transport of β-carotene occurred, the calf would not be able to synthesize vitamin A from it since β-carotene needs to be absorbed to be cleaved. Therefore, in order to

ensure sufficient vitamin A (β-carotene) supply, colostrum must contain adequate amounts of β-carotene and its absorption by enterocytes is primordial in calves. However, more research is necessary on this topic because conflicting information exists in the literature as to the ability of pre-ruminant calves to convert oral β-carotene to vitamin A. Hoppe et al. (1996) showed that calves were able to convert β-carotene to vitamin A and they estimated that conversion was as efficient as that seen in adult ruminants, while Nonnecke et al. (1999) were unable to show that supplemental oral β-carotene given to calves increased circulating vitamin A concentrations. In addition, Poor et al. (1992) showed that there existed, in their study, animals for which vitamin A concentrations did not respond to the β-carotene supplement. This has also been observed in humans (Dimitrov et al., 1988). However, the latter two experiments were only conducted with single oral doses of β-carotene.

In the present study, there was no positive effect of dietary β-carotene supplement given to the dairy cows during the dry period on calf birth weight, rectal temperature, ease of calving and vitality. The enzyme activities and metabolites measured to assess liver and kidney function at birth were not affected by the supplement, which is logical in view of the fact that we were unable to increase placental β-carotene transport to the foetus by giving a dietary β-carotene supplement to the dam during the last two months of gestation. Colostrum is the main source of β-carotene in calves due to apparent poor placental transport.

Satisfactory supply of β-carotene (vitamin A) to the newborn calf requires the ingestion of adequate amounts of colostrum which is rich in β-carotene. Additionally, colostrum appears to be the most important source of hormones, nutrients and other factors that influence the morphological and functional changes observed in the new born calf (Blum and Hammon, 2000, Blum, 2006). However, the effects of β-carotene supplementation on such parameters in colostrum were not measured in this study.

We observed a gender effect on birth weight. Male calves were heavier than female calves. It has long been recognized that the gender of a calf influences birth weigh (Holland and Odd, 1992). The weight difference between genders at birth is related to androgenic hormone production. Androgen production in the male foetus exceeds that of the female foetus resulting in higher weights at birth (Holland and Odde, 1992).

Findings from this study showed that a dietary supplement of β-carotene given to dry dairy cows increased β-carotene concentrations in both their blood and colostrum. However, the β-carotene status of the newborn calf was not improved suggesting that placental transfer of β-carotene was low. Colostrum appears to be the main source of β-carotene for new-born calves. This suggests that satisfactory supply of β-carotene (vitamin A) to the newborn calf requires the ingestion of adequate amounts of colostrum which is rich in β-carotene. Supplementation of β-carotene during the dry period did not improve colostral IgG content, hormone and metabolite concentrations and enzyme activities in the new-born calf.

CHAPTER V

GENERAL DISCUSSION AND CONCLUSIONS

In Thailand, heat stress is one of the main problems that affect reproductive performance in dairy cows (Aiumlamai, 2007, Suadsong et al., 2008). The mechanisms by which heat stress affect reproduction are not fully understood (De Rensis and Scramuzzi, 2003). Attempts have been made and several studies have been conducted to gain more knowledge on, and eliminate or, at least, alleviate the effect of heat stress on reproductive performance in dairy cows (Rodtian et al., 1996, Virakul et al., 2001, Pongpiachan et al., 2003[a,b], Kornmatitsuk et al., 2008,). However, the additional knowledge related to heat stress and reproduction in dairy cows still requires further studies. There are no retrospective studies which consider the data across the country to determine the effect of heat stress on reproductive performance in dairy cows in Thailand. Although there have been reports that THI remains high throughout the year, the experiments mentioned above were mostly designed to study and compare reproductive performance at particular time periods. Additionally, heat stress causes a great change in metabolism especially in the peripartum period. It has been reported in stressed cows that high production of oxygen-derived free radicals occurrs (Chawla and Kaur, 2004). This change occurs before the expected time for breeding and may subsequently affect reproduction (Padilla et al., 2006). Thus, the studies in this thesis were designed, to investigate the effects of heat stress by classifying heat stress by region and month of calving (MOC) on reproductive performance using registered data, to investigate the effect of heat stress on reproductive performance in first lactation cows throughout the year, and to investigate the effect of a dietary supplement of β-carotene on reproductive performance in dairy cows.

5.1 Effect of region and MOC on DO in dairy cow: a retrospective study

The results from this study showed that there were regional variations in climatic conditions among regions in this study; Central, Eastern, Northeastern, and

Northern regions. In the Northern region, mean THI was lower than the other regions. Also, the length of DO in dairy cows in the North was lower than that in the other regions. This finding indicates that high THI affects reproductive performance in dairy cows. The highest frequency of calving was found in September and October and the lowest frequency of calving was observed from December to February. Calving during these periods corresponds to insemination success in December and January and from April to June respectively. Considering together with the THI, minimum THI values were observed in December and January while, December and January were the coolest months of the year. In addition, Cows that calved in February had the longest length of DO compared to cows that calved in the other months. The time for rebreeding in cows calving in February is about in April and May when the success rate of insemination was low and probably due to high THI as mentioned above. This finding is in agreement with previous reports which showed a marked decrease in conception rates during the hot season (De Rensis and Scaramuzzi, 2003; García-Ispierto et al., 2007). To achieve the economic reproductive cycle in dairy cattle in Thailand, the findings from this study lead to the suggestions that dairy cows especially replacement heifers, should be inseminated from November to February to calve from August to November. Additionally, utilization of proper cooling systems, which may be modified to be suitable for different regions, to alleviate the effect of heat stress should be recommended. These strategies may useful to reduce days-open and improve dairy farming profitability in Thailand.

It has been reported that heat stress affects milk production (Igono et al. 1992). The variations among MOC may not only affect reproductive performance thhrough mean DO but also via milk yield. Cows calving at an improper time of the year for example in hot months may suffer frome heat stress or insufficient or low quality feed supply leading to decreas milk yield. The study to test this hypothesis is needed. Additionally, the study on the effects of heat stress on follicular development and postpartum uterine involution would be of interest. Further studies to improve conception rate in hot months for example, by the utilization of hormonal intervention together with cooling system may benefit to the farmers.

5.2 Effect of heat stress on ovarian function, plasma metabolites and embryonic loss in first lactation cows

In this study the degree of heat stress were classified by the THI value as described by Armstrong (1994). The proportion of cows with normal ovarian activity that calved in the mild stress months was higher than that which calved in the severe stress months. However, other parameters; interval from calving to first ovulation, interval from calving to first AI, DO, first service conception rate were not different between the MS and SS groups. Also, body weight, BCS, blood concentrations of NEFA, IGF-1 and cortisol were unaffected by heat stress. The lack of a detrimental effect of heat stress on reproduction in this study may be due to the fact that a cooling system was employed in the farm where the study was conducted especially during the hot season of the year when the handling of cooling cows was more intensive. It has been reported that cooling the cows improved their reproductive performance (Ryan and Boland, 1992). Additionally, the nutritional status of the experimental animals in this farm was good. Body weight, BCS and blood concentrations of NEFA, insulin, IGF-1 and cortisol in this study fall within the acceptable ranges. The cooling system together with good nutritional management may improve the effect of heat stress in this study. The effects of heat stress on the frequency of embryonic mortality were also not observed in this study. The explanation for this finding is the same as mentioned above or the lack of difference in frequency of EM may be due to the fact that the conditions induced by MS were detrimental to embryonic development and were negative enough to induce a rise in the frequency of EM.

To date, there has been no studies to calculate a the heat stress index specificially for dairy cows in Thailand. The studies conducted in Thailand, used the temperature and humidity indices which were formulated from studies conducted in foreign contries. This may not suitable for the conditions in Thailand where several factors are different for example milk yield and breed. The severity of the response to the particular level of heat load may different between high producing dairy cows, with generally higher metabolic heat increment, and low producing dairy cows, with generally lower metabolic heat increment. Heat stress is one of the main problems that

farmers in Thailand are facing to. Because of the climate change, global warming, the impact of this problem is likely to increase in dairy farming. How to reduce/alleviate the impact of heat stress to acheive the good reproducteve performance is the subject for the future research.

5.3 Effect of a dietary supplement of β-carotene on reproductive and productive performance in dairy cows and on their calves' health status

The relationship between β-carotene and reproduction, production and immunity in dairy cows has long been investigated. However, the studies so far have produced different findings and the mechanisms by which β-carotene could function on these parameters are not fully clear (Bindas et al., 1984[a,b], Rake et al., 1985, Akordor et al., 1986, Wang et al., 1986, Arikan and Rodway, 2000). The importance of β-carotene on the health status of neonatal calf has also been reported (Kume and Toharmat, 2000). However, little information on the possible effect of a β-carotene supplementation given to dairy cows on calf health status at birth is available. Therefore, in an effort to resolve conflicting evidence concerning the effects of β-carotene supplementation of dairy cows during the dry period on health and reproduction and to investigate the possible effects on calf health status at birth, the studies in this thesis were designed.

In agreement with the other studies, we found that supplementation with β-carotene during the dry period increased blood concentrations of β-carotene in dairy cows (Kawashima et al., 2009[b]) above the recommended level of 3 mg/L (Frye et al, 1991). The length of the interval from calving to first ovulation, ovarian activity, progesterone production, were not affected by β-carotene supplementation in this study.

Uterine involution as judged by the changes in uterine horn and cervical diameters was not different between groups. The absence of β-carotene effect is in agreement with Wang et al. (1998) but contrasts with Rakes et al. (1985).

Collagen is a fibrous protein whose molecule consists of three polypeptide chains which contain significant amounts of the amino acids; glycine, proline and hydroxyproline (Sarges et al., 1998). Collagen degradation releases glycine and

hydroxylproline in blood. Hydroxyproline is not found in feed stuff and is unique to collagen and can be used as an indicator of the speed and completeness of the uterine involution (Abribat et al., 1992). In this study we found that dietary supplement of β-carotene had a positive effect on an indictor of uterine involution by increasing blood concentrations of hydroxyproline.

We found in this study that the percentage of PMN in the uterus and cervix was lower in cows supplemented with β-carotene compared to controls. The difference between groups in blood concentrations of β-carotene was observed until the second week postpartum. This difference may explain the lower numbers of PMN in the uterus and cervix during the spontaneous recovery period.

Milk production, circulating hormones (insulin, IGF-1) and metabolites (glucose, NEFA, urea) and milk composition (milk protein, milk fat, milk urea and SCC) were unaffected by the supplementation of β-carotene during the dry period. The absence of the effect of supplementation of β-carotene on these parameters may be due to the fact that the supplementation was stopped at calving. The concentrations of blood β-carotene during the postpartum period were not large enough to influence on these parameter. While supplementation of β-carotene during the dry period modified dam blood concentrations of β-carotene, blood concentrations of β-carotene in calves at birth were unaffected. This was in agreement with previous reports and indicated that placental transfer of β-carotene is limited (Kume and Toharmat, 2001). Since the blood concentrations of β-carotene in calves at birth were not different between groups, β-carotene could not modify enzyme activities, blood concentrations of hormones, metabolites in the neonatal calves. However, supplementation of β-carotene of dairy cows during the dry period increased β-carotene concentrations in colostrum. The suggestion from this study is that satisfactory supply of β-carotene (vitamin A) to the newborn calf requires the ingestion of adequate amounts of colostrum which is rich in β-carotene.

In this study we found the positive effects of supplementation of β-carotene on the indicator of uterine involution (hydroxyproline) and on the clearance of PMN in postpartum cows. However, we used a small number of animals. Therefore, the

further study conducting in a large, controlled, multi-location study is needed in order to test robustness of out finding.

To date, there has been no study on the effects of a dietary supplement of β-carotene on productive or reproductive performance in dairy cows in Thailand. Dairy cows in Thailand are exposed to heat stress almost throughout the year. It has been reported that in stressed cows the production of free radicals was drastic increased (Padilla et al., 2006). An initial study that aimed to determine the blood β-carotene status in dairy cows in different conditions would provide the oveall picture of β-carotene concentrations in dairy cow in Thailand. This informationwould be usefull to design furthure experiments on the relationship between β-carotene and reproductive or productive performance under Thai production condition.

5.4 Conclusions

THI profiles showed that dairy cows in Thailand are exposed to heat stress throughout the year. Even if the conception rate was not directly measured, the results from a retrospective study indicated that the conception rate in the period with high THI was low. The strategies to inseminate the cows to calve at the proper time should be considered by farmers and dairy cow producers. The preventive measures such as cooling systems may be useful to alleviate the impact of heat stress. However, since the climate condition is different between regions, the measures or method used need to be adapted so as to be suitable for the different farming conditions.

Supplementation of β-carotene during the dry period in dairy cows may improve uterine health, since higher blood concentrations of hydroxyproline and lower PMN were found in cervical and uterine smears compared to controls. In this thesis, the study on the effect of dietary supplement of β-carotene was conducted in France, where the temperature and humidity is very different from that in Thailand. Thus it is not possible to draw any conclusions between β-carotene and heat stress in dairy cows. The research to determine the beneficial effect of β-carotene on reproduction under climate condition in Thailand is warranted.

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APPENDIX

List of publications and conferences

Local

1. **Kaewlamun, W.,** Suwimonteerabutr, J. and Techakumphu, M. 2006. Infertility treatment using ear progesterone ear implant and fixed time AI in dairy cows. In: Proc. RGJ Seminar Series 47th: Reproductive biotechnology for improving animal breeding stratergies. Nan, Thailand. 29.
2. **วินัย แก้วละมุล** กุลภัทร์ โพธิกนิษฐ์ จันทร์เพ็ญ สุวิมลธีระบุตร นิกรสาง ห้วยไพร มงคล เตชะกำพุ. 2007. The Use of ovsynch protoccol to induce of ovulation in sub-fertile swamp buffalo cows. In: Proc 33rd Veterinary Medicine and Livestock Development Annual Conference. Bangkok, Thailand. 179.
3. **Kaewlamun, W.**, Suvimonteerabutr, J., Chaimee, T., Virakul, P., Techakumphu, M. 2008. Low pregnancy rate in dairy cattle after fixed time AI using norgestomet + PGF2α + eCG program during hot -humid months in Thailand. Thai J. Vet. Med. 38: 53-58.
4. **Kaewlamun, W.,** Okouyi, M., Humblot, P., Techakumphu, M., Tristant, D. and Ponter, A. 2010. The influence supplement of β -carotene given during the dry period to dairy cows on calf beta-carotene. In: Proc RGJ Seminar Series LXXI: Perspectives and Innovation in Veterinary Biosciences, 24th 2010, Fac. Vet. Sci. CU. 29.
5. **Kaewlamun, W.**, Shayarattanasinp, R., Tummaruk, P., Suadsong, S., Virakul, P., Ponter, A.A., Humblot, P., and Techakumphu, M. 2010. Effects of region and month of calving on days open in dairy cows in Thailand. Thai J. Vet. Med. (In preparation)
6. **Kaewlamun, W**., Okouyi, M., Humblot, P., Rémy, D., Techakumphu, M., Duvaux-Ponter C., and Ponter, A.A. 2010. Effects of a dietary supplement of β-carotene given during the dry period on milk production and circulating hormones and metabolites in dairy cows. Revue de Médecine Vétérinaire. (in preparation)

International

1. **KAEWLAMUN W**., OKOUYI M., HUMBLOT P., TECHAKUMPHU M., TRISTANT D., REMY D., PONTER A.A. Effect of a dietary supplement of β-carotene during the dry period on the metabolism of dairy cows and colostrums quality (Effet d'un supplément alimentaire de β-carotène pendant le tarissement sur le métabolisme des vaches laitières et la qualité du colostrum). 2009. In: Proc 16th3R (Rencontres, Recherches, Ruminants) Conference. Paris, France. 16:81
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VITAE

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