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THESIS

HEAT TOLERANCE INDICATORS FOR BEEF CATTLE IN THE TROPICS WITH SPECIAL REFERENCE TO BLOOD BIOCHEMICAL PROPERTIES

SUVIT BOONPRONG

A Thesis Submitted in Partial Fulfillment of
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Four experiments reported in this thesis were aimed to assess the heat tolerance indicators for beef cattle in the tropics with special reference to blood biochemical properties.

In Experiment I, the productivity of Simmental-Brahman crossbred (Kabinburi, K) cattle was compared to that of Thai Brahman (TB), which were kept under three different environmental conditions (Lamphayaklang Livestock Research and Breeding Center, LP; Nongkwang Livestock Research and Breeding Center, NK; Prachinburi Livestock Breeding Station, PC). The results revealed that bodyweight at birth, 200, 400 and 600 days of age of K cattle were significantly higher ($P<0.05$) than those of TB cattle. K heifers gave birth to their first calf at a younger age and had a shorter calving interval than TB cows. TB cattle kept at LP had significantly higher ($P<0.05$) bodyweight at 400 and 600 days than the animals kept at NK. TB cattle kept at LP were younger ($P<0.05$) at first calving and had a shorter calving interval than the animals kept at NK. K cattle kept at NK were heavier at birth and at 200, 400 and 600 days of age than the animals kept at PC. K cows kept at NK were significantly younger at first calving ($P<0.01$), but there was no difference in calving interval between the two groups kept at NK or PC.

In Experiment II, the relationship between the haemoglobin (Hb) type, reproduction and body weight was estimated in two cattle breeds, Thai indigenous and Simmental × Brahman crossbred cows. The results showed that five haemoglobin types were found in indigenous cattle: HbAA (35.59%), HbAB (28.81%), HbAC (20.34%), HbBB (11.6%) and HbBC (3.39%); three types were found in Simmental × Brahman crossbred cattle: HbAA (50.98%), HbAB (45.10%) and HbBB (3.92%). Thai indigenous cows with HbAB type were heavier at birth, but, by contrast, calves carrying HbBB type were the lightest animals at birth and were the youngest group at first calving. Simmental × Brahman crossbred cows with HbAA type were significantly heavier ($P<0.05$) than the animals with HbAB type at birth as well as on 200, 400 and 600 days of age whereas the animals with HbAB type gave birth to their first calf at a significantly younger ($P<0.05$) age than those with HbAA type.

In Experiment III, plasma biochemical profiles were studied to investigate the effect of breed and sex in Thai indigenous and Simmental × Brahman crossbred male and cyclic female cattle. The results showed that there were significant differences ($P<0.05$) in the levels of plasma glucose and GGT in both breeds. The levels of urea, creatinine, albumin, total protein, AST, ALT and ALP in Thai indigenous were significantly higher ($P<0.01$) than in crossbred cattle. Plasma urea concentration in male crossbred cattle was significantly lower ($P<0.05$) than in the other groups. Female crossbred cattle had significantly lower ($P<0.05$) plasma creatinine levels than the other animals. There were significant differences ($P<0.05$) in the levels of AST, ALT, ALP and GGT between male and female. Female crossbred cattle had the lowest ($P<0.05$) AST and GGT levels, whereas the lowest ($P<0.05$) ALT and ALP concentration was determined in male individuals of these breeds.

In Experiment IV, Twenty two mature (2^{nd} to 4^{th} lactation) healthy lactating cows including 11 Holstein Friesian (HF) and 11 Schwarz Bunt cows (SBT) were used. The animals were divided into groups according to their reproductive status. Group 1 was consisted of 9 pregnant animals (5 HF and 4 SBT) and group 2 was consisted of 13 cyclic animals (6 HF and 7 SBT), respectively. Lymphocytes were harvested from blood and incubated at 37.2°C; 5% CO_2 and at 41.0°C; 5% CO_2 for 72 h in presence or absence of phytohemagglutinin (PHA-M, Sigma; 100 µg/ml). Growth hormone was measured by a highly sensitive radioimmunoassay in duplicate. Nitric oxide was estimated using a colorimetric assay based on the Griess reaction. A significantly high secretion of GH was noticed from PHA-M – stimulated PBLs in cyclic SBT and HF, and pregnant SBT, but GH production was not different between un-stimulated PBLs and stimulated PBLs in pregnant HF. When the temperature of culture increased from 37.2°C to 41.0°C in un-stimulated PBLs of cyclic SBT, GH was decreased significantly ($P<0.05$). Moreover, GH levels from PBLs at 41.0°C in un-stimulated PBLs of the pregnant HF was significantly ($P<0.05$) higher than pregnant SBT. The present data indicate that PHA-M – stimulated PBLs produced significantly higher NO than un-stimulated PBLs. However, when the temperature of culture increased from 37.2°C to 41.0°C, NO production from un-stimulated PBLs was significantly increased. In contrast NO level from stimulated PBLs was significantly decreased. NO production was not significantly different between breeds and reproductive stages. In conclusion the present data indicate that the sensitivity of the lymphocytes differs in HF and SBT cattle breeds.

Heat tolerance indicators such as haemoglobin phenotypes or blood biochemical parameters could be enlisted together with other factors for selection of high performing animals in the tropics.

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Suvit Boonprong
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LIST OF ABBREVIATIONS

%	=	percent
°C	=	degree celcius
×g	=	standard acceleration of gravity
µg	=	microgramme
µl	=	microlitre
µM	=	micromole
µM/L	=	micromole per litre
2,3DPG	=	2,3-diphosphoglycerate
a.m.	=	ante meridiem
ACTH	=	adrenocorticotropic hormone
ALB	=	albumin
ALP	=	alkaline phosphatases
ALT	=	alanine aminotransferase
ANOVA	=	Analysis of Variance
AST	=	aspartate aminotransferase
AT	=	ambient temperature
BCS	=	body condition score
bpm	=	breaths per minute
BT	=	body temperature
BUN	=	blood urea nitrogen
BW	=	bodyweight
CK	=	creatine kinase
cm	=	centimetre
CRT	=	creatinine
d	=	time in day
DLD	=	Department of Livestock Development
DMI	=	dry matter intake
e.g.	=	exempli gratia
EDTA	=	ethylenediaminetetraacetic acid

LIST OF ABBREVIATIONS (Continued)

EPO	=	erythropoietin
FAL	=	Federal Agricultural Research Centre
FI	=	feed intake
FLI	=	Federal Research Institute for Animal Health
g	=	gramme
GGT	=	gamma glutamyl transferase
GH	=	growth hormone
GHRH	=	growth hormone-releasing hormone
GHRP-6	=	growth hormone releasing peptide-6
GHS-R	=	growth hormone secretagogue receptor
GLM	=	General Linear Model
GLU	=	glucose
h	=	time in hour
Hb	=	Haemoglobin
HbA	=	haemoglobin phenotype A
HbAB	=	haemoglobin phenotypes AB
HbAC	=	haemoglobin phenotypes AC
HbB	=	haemoglobin phenotype B
HbBB	=	haemoglobin phenotypes BB
HbBC	=	haemoglobin phenotypes BC
HbC	=	haemoglobin phenotype C
HbS	=	haemoglobin phenotype S
HBSS	=	Hanks' balanced salt solution
HF	=	Holstein Friesian cows
HSP	=	heat shock proteins
IGF-I	=	insulin-like growth factor-I
IU	=	International Unit
K	=	Kabinburi cattle
kg	=	kilogramme

LIST OF ABBREVIATIONS (Continued)

l/d	=	litre per day
LP	=	Lamphayaklang Livestock Research and Breeding Center
LSD	=	Least Significant Differences
LSM	=	Livestock Safety Monitor
Max	=	Maximum
Min	=	Minimum
min	=	time in minute
ml	=	millilitre
mm	=	millimetre
mmol/l	=	millimole per litre
mo	=	time in month
ng/ml	=	nanogramme per millilitre
NK	=	Nongkwang Livestock Research and Breeding Center
NO	=	nitric oxide
NRC	=	National Research Council
P<0.001	=	Probability at 99.9% level
P<0.01	=	Probability at 99% level
P<0.05	=	Probability at 95% level
PBLs	=	Peripheral Bovine Lymphocytes
PC	=	Prachinburi Livestock Breeding Station
PCV	=	packed cell volume
PHA-M	=	phytohemagglutinin M
pO_2	=	pressure of oxygen
PRL	=	prolactin
PTH	=	parathyroid hormone
RBC	=	red blood cell
RH	=	relative humidity
RIA	=	Radio Immuno Assay
RR	=	respiration rate

LIST OF ABBREVIATIONS (Continued)

RT	=	rectal temperature
s	=	time in second
SAS	=	Statistical Analysis System
SB	=	Simmental × Brahman crossbred cattle
SBT	=	Schwarz Bunt or German Black and White cows
SE	=	Standard Error
SRIF	=	somatostatin
TB	=	Thai Brahman cattle
THI	=	temperature humidity index
TI	=	Thai indigenous cattle
TNZ	=	thermoneutral zones
TP	=	total protein
U/l	=	International System of Units per litre
UR	=	urea
vs.	=	versus
yr	=	time in year

HEAT TOLERANCE INDICATORS FOR BEEF CATTLE IN THE TROPICS WITH SPECIAL REFERENCE TO BLOOD BIOCHEMICAL PROPERTIES

INTRODUCTION

Environments of high temperature and humidity are detrimental to the productivity of non-adapted farm animals (Fuquay, 1981; Morrison, 1983; Yousef, 1985). Thermoneutral zones (TNZ) for the animals are primarily dependent on the species, the physiological status of the animal, the relative humidity, velocity of ambient air and the degree of solar radiation (NRC, 1984). *Bos taurus* and *Bos indicus* cattle have TNZ between 2–21°C and 10-27°C, respectively (Bligh and Johnson, 1973). Considerable work has been performed to identify the physiological effects of heat stress and the mechanisms by which animal productivity is reduced. In growing cattle, heat stress may reduce dry matter intake (Lippke, 1975; West, 1994), the rate of weight gain (Ray, 1989; Mitlohner *et al.*, 2001; Mader, 2003), the fertility in males (Meyerhoeffer *et al.*, 1985) and females (Biggers *et al.*, 1987; Wolfenson *et al.*, 1997; Wilson *et al.*, 1998a). Quantification of these effects is complicated because of acclimation of animals (Robinson *et al.*, 1986) and breed differences in susceptibility to heat stress (Hammond *et al.*, 1998; Gaughan *et al.*, 1999).

In Thailand, a tropical country, beef industry was always under the influence of high ambient temperature, relative humidity, sunlight and ecto-parasites. All these factors would affect production and reproduction performance, especially during the long hot summer, the cow would have low production performance such as weight at birth, weaning weight, yearling weight, and weight at 600 days of age, low fertility and general weak conditions.

Thai indigenous cattle (*Bos indicus*) play an important role within agriculture in Thailand as a part of a crop-livestock integrated system. In the past, this breed has been important for the quality of life in the rural communities in terms of food,

transport and draught power. It even served as a kind of bank saving. Thai indigenous cattle is small [280-300 and 200-250 kg bodyweight (BW) for adult male and female, respectively], and have a low growth rate with a large variety of coat colours. It has been selected over generations for survival under stressful environment (heat stress, endo- and ectoparasites; Intaratham, 2002). Results of this selection are relatively high fertility as indicated by age at first calving (mean = 2.5 yr), calving interval (mean = 1 yr) and the length of productive age (mean = 13 yr) and high ability to use low quality roughage (Intaratham, 2002).

Moreover, imported American Brahman cattle have been used in breeding programmes by the Thai Department of Livestock Development (DLD) since 1954 in an attempt to improve performance of the native cattle for small-scale farmers. Over the last 50 years, an intensive selection for growth rate, fertility, heat tolerance and adaptation has been undertaken. This selection has led to the development of the Thai Brahman, an animal that shows good performance under tropical conditions (Animal Husbandry Division, DLD, 1995).

In order to improve cattle performance, a new cattle breed, the Kabinburi, has been established. The Kabinburi is a crossbred of 50% Simmental × 50% Brahman (*B. taurus* × *B. indicus*). The Kabinburi cattle was developed, in the Cattle Breed Established Project in central Thailand, from two breeds that are distinctly different, utilizes the strengths of both, and maximizes the hybrid vigor because of the extreme genetic differences. Combining the strengths of the Brahman breed, including longevity, heat tolerance, insects and diseases resistance, durability, grazing ability and calving ease with the superior Simmental traits of fertility, milking ability, rapid growth, and early sexual maturity led to Kabinburi cattle (Animal Husbandry Division, DLD, 1991).

OBJECTIVES

This study was conducted with the following objectives:

1. Investigation of performance of beef cattle under different environmental conditions in central Thailand.

2. To examine the relationship between haemoglobin phenotypes and productivity performance of beef cattle in central Thailand as indicated for selection of high productive animals.

3. Comparison of some blood biochemical profiles in beef cattle under tropical conditions in Thailand as indicator of animals' health, heat tolerance and reference values for beef cattle raised in Thailand and other Asian countries having similar climatic and nutritional conditions.

4. To investigate the effect of temperature and pregnancy on growth hormone (GH) and nitric oxide (NO) secretion from peripheral bovine lymphocytes (PBLs) as foundation for future exploration of the exact mechanism(s) by which PBLs sense and respond to differences in their environmental temperature.

LITERATURE REVIEW

1. Livestock heat stress

In a thermal environment in which the animal's heat production exceeds heat loss, an increasing amount of heat is stored in the animal's body, resulting in increased body temperature. When the body temperature is significantly elevated, a myriad of homeothermic events are initiated. These events include increases in both respiratory and cuteneous evaporative heat loss. However, when high temperatures and radiation lessen the ability of the animal to radiate heat from the body, feed intake, metabolism, body weight and milk yields decrease to help alleviate the heat imbalance (Johnson, 1987). Even though tissue substrates are mobilized, energy metabolism, growth and lactation decline.

Climate affects animal production in four ways:

(a) The impact of changes in livestock feed-grain availability and price;
(b) Impacts on livestock pastures and forage crop production and quality;
(c) Changes in the distribution of livestock diseases and pests; and
(d) The direct effects of weather and extreme events on animal health, growth and reproduction (Smitt *et al.*, 1996).

The impact of changes in livestock feed-grain availability and price has considered in several studies (Adams *et al.*, 1990, Bowes and Crosson, 1993; Easterling *et al.*, 1993; Rosenweig and Parry, 1994).

One important form of energy that flows through cattle is heat, and heat is major constraint on animal productivity, especially in tropical and arid areas (Silanikove, 2000). This is a concern because of the homeothermic condition of cattle. They need to maintain a constant body temperature, howeverthere is a diurnal fluctuation (Finch, 1984; Robertshaw, 1985), typically in the form of a monophasic rhythm with a maximum in the late evening and a minimum in late morning (Hahn,

1999). Environmental stressors may alter this monophasic temperature rhythm (Nienaber *et al.*, 1999). Hot environments have shown to cause phase shifts, increase amplitude and increase means of this diurnal rhythm (Hahn, 1999). An indication of acclimatization to heat stress displaces the normal rhythm (Nienaber *et al.*, 1999). Heat stress can lower animal production, especially those with high production such as *Bos tuarus*. Smith (1984) mentioned relationship between heat stress and productive cows by separating the heat resources from physical environment into 2 categories. There are direct and indirect results. The direct results are ambient temperature, radiation, and humidity and wind velocity. All of those have affected on working of central system. The indirect results are feed intake response, metabolic rate and endocrinological changes. All mentioned factors would affect production performance.

2. Heat stress impact on animals

Uncertainty is the most problematic aspect of climate change, thus limiting the usefulness of climate change impact assessments (St-Pierre *et al.*, 2003). At best, a range of impacts bound by a high and low extreme with a defined probability distribution can be produced (Jones, 2000). Quantitative simulation studies estimating impacts of future climate change directly on livestock are few. However, weather and extreme events have well documented effects on several aspects of animal production.

There is a range of thermal conditions within which animals are able to maintain a relatively stable body temperature by means of behavioural and physiological means (Johnson, 1987; Bucklin *et al.*, 1991; Blackshaw and Blackshaw, 1994). Heat stress results from the animal's inability to dissipate sufficient heat to maintain homeothermy. High ambient temperature, relative humidity and radiant energy compromise the ability of animals to dissipate heat. As a result, there is an increase in body temperature, which in turn initiates compensatory and adaptive mechanisms to reestablish homeothermy and homeostasis. These readjustments, generally referred to as adaptations, may be favorable or unfavorable to economic interests of humans, but are essential for survival of the animal (Stott, 1981).

The rate or velocity of exchange depends on the ability of the environment to accept heat and water vapour. High ambient humidity reduces the capacity of these exchanges because the air is already saturated with moisture. Resistance to these exchanges prohibits heat loss, resulting in an increase body temperature and a reduced appetite. Finch (1986) also stated that animals which increase their capacity for thermoregulation often do so through a diminution in energy metabolism, which probably is negatively related with their growth potential in less harsh environments.

Upon exposure to a hot environment, cattle respond initially with an acceleration of certain physiological processes to increase the rate of heat loss (McDowell, 1972). The two processes that are increased first are respiration rate and evapotranspiration rate; are mechanisms of achieving heat dissipation (Campbell and Lasley, 1985). Panting and sweating are paths of thermoregulatory water loss for heat dissipation (Silanikove, 2000) and are complementary in the sense that animals with less sweating capability normally have a higher capacity for panting (Yousef, 1985). In cattle with relatively large sweating response, skin temperature is kept cooler by sweat evaporation. The critical skin temperature that triggers panting seems to be one or two degrees higher than that for the onset of sweating. However, if the sweating response is poor, the panting reflex threshold is reached at lower temperatures (Curtis, 1981).

The cattle increase the rates of respiration, pulmonary ventilation, and respiratory vaporization with increasing environmental temperature (Worstell and Brody, 1953). Nonetheless, when the environmental temperature reaches in average 26.7°C for *Bos taurus*, and, 35.0°C for *Bos indicus*, these mechanisms become incapable of dissipating all of the excess heat (Brody, 1956). At higher ambient temperatures, the temperature gradient between an animal's body and the surroundings is reduced with a consequent reduction in convection and radiation cooling and heat loss is swung towards evaporative cooling. When the ambient temperature approaches the body surface temperature, convection and radiation cooling approaches zero (Worstell and Brody, 1953).

In addition to the environmental heat load, cattle also must cope with the heat production of the rumen (Brody, 1956), part of the heat increment of feeding. The heat increment of feeding is heat produced by an animal during fermentation in the gastroinate stinal tract and during the processing and use of nutrients by the body. Heat is released during the metabolism of the nutrients absorbed from the gut. The nutrients undergo chemical transformations related to productive and reproductive activities that are not very efficient and as a result, they are accompanied by heat increment. Estimations of the components of the heat increment of feeding should be carefully interpreted as they are influenced by numerous factors (Curtis, 1981). Furthermore, nutritional imbalance and deficiencies may aggravate the impact of heat stress (West, 1999).

Another factor that contributes to heat load into the core temperature of a bovine body is the level of production. Higher levels of production supply an extra thermal input and are reflected in the stress indices. Similar increases in heat load arise with pregnant cows and water-deprived cattle (Silanikove, 2000). Vulnerability to environmental forces is also influenced by life stage, conditioning and nutritional and health status (Worstell and Brody, 1953; Hahn, 1999).

Thus, an increase in air temperature, such as that expected in different scenarios of climate change, would affect directly animal performance by affecting animal heat balance. There are four modes of energy transfer: radiation, convection, evaporation, conduction, which is governed by physical laws. Several physical parameters control heat transfer by each mode. Air temperature affects energy exchanges through convection and evaporation (Hahn, 1976). When temperature increases, evaporation becomes the most important way of heat loss, since it does not depend on a temperature gradient (Ingram and Mount, 1975). Under those circumstances the combination of temperature and humidity acquire more relevance, since humidity enhances temperature effects. Therefore, it is universally accepted to evaluate the environment, from the heat stress stand-point, through the temperature humidity index (THI). Dairy cattle show signs of heat stress when THI is higher than 72 (Armstrong, 1994). In addition, the THI values exceeded the critical value of 75

for beef cattle (St-Pierre *et al.*, 2003). The comfort limit depends on level of production. Animals presenting higher level of production are more sensitive to heat stress (Johnson, 1987).

A heat wave is defined as a period of abnormally uncomfortable hot and usually humid weather of at least one-day duration, but conventionally lasting several days to several weeks (AMS, 1989). An operational definition (Hahn *et al.*, 2001) is 3 to 5 consecutive days with maximum temperatures above a selected threshold. During these heat waves, animal heat exchange is affected. They fail to dissipate the extra heat load accumulated during days when there are several hours with THI well above the comfort limit, and little opportunity to recover. Therefore, thermoregulation and feeding behaviour are affected (Hahn, 1999; Nienaber *et al.*, 2001). In a retrospective analysis of heat wave events, Hahn *et al.* (2001) summarize that the results support an environmental profile for single heat waves that create highly likely lethal conditions for *Bos taurus* cattle in feedlots. When THI at or above a base of 84 exceed 15 per day for 3 or more successive days with limited or no nighttime recovery opportunity, some death losses can be expected if relief measures are not provided (Hahn and Mader, 1997; Hahn *et al.*, 2001). It was concluded that heat waves produce an impact in performance of beef cattle or dairy cows in a grazing system.

In an effort to pull together the expected response of cattle to stressful environmental conditions, a Livestock Safety Monitor (LSM) was developed (Eigenberg *et al.*, 2003). The LSM collects continuous weather data from an onsite commercial private weather station including the measures of temperature, humidity, wind speed and solar radiation and outputs expected respiration rate (RR). The unit of measurement for RR is breaths per minute (bpm). The predicted RR is then compared to THI values and outputs an estimate of the severity of the weather conditions based on RR. The ranges are as follows: Normal < 85 bpm; Alert 85 to 110 bpm; Danger 111 to 133 bpm; and Emergency > 133 bpm (Eigenberg *et al.*, 2003). In a similar but independent effort to provide a descriptive and predictive weather index, Gaughan *et al.* (2002) developed the Heat Load Index, using both animal responses and weather measures to express the expected level of heat stress.

To estimate effects on beef growth, a model was developed by Frank *et al.* (2001) for yearling feeder cattle, excluding replacement heifers that are exposed to average daily temperatures greater than 15°C. The model applies to animals from 350 to 550 kg, and is composed of a series of interrelated calculations from NRC (1996) based on body weight and air temperature. An equation developed by the National Research Council (NRC, 1996) predicts feed intake as a function of weight, net energy content of the diet, and adjustment factors for the body condition of the animal, the animal's breed, usage of feed additives, the presence of mud, and temperature. Dietary net energy varies with feed composition. The temperature adjustment factor is a function of average daily temperature (NRC, 1996), and does not account for diurnal temperature changes, such as nighttime cooling. To account for night cooling, the temperature adjustment factor is modified to further decrease daily voluntary dry matter intake (DMI) by 0.15% per 1.0°C increase to a maximum 3.2% reduction at 40°C. While the model was developed to estimate the impact of global warming, it also demonstrates the responsiveness of cattle to thermal stress.

3. Performance responses

Under the challenge of several forces, homeotherms maintain their body temperature within a relatively narrow range (Das *et al.*, 1999; Frisch, 2000) and slight variations in internal temperature may disrupt normal processes and, as a result, their productive performance. The affected processes that have been studied the most are growth (McDowell, 1972; Turner, 1982; Finch, 1986; Vajrabukka, 1996; Hammond *et al.*, 1998), milk production (McDowell, 1972; Armstrong, 1993; Vajrabukka, 1996; Valtorta *et al.*, 1997; Ravagnolo *et al*., 2000) and reproduction (McDowell, 1972; Wolfenson *et al.*, 2000; Hansen *et al.*, 2001).

Growth under heat stress conditions appears to be directly related to the level of animal resistance to stressors which include high temperatures (Turner, 1982), low nutritional inputs, disease outbreak, and parasite infestation (Frisch, 1981). Cattle with better growth under harsh hot environments are those with lower respiration rates and rectal temperatures (Hammond *et al*., 1998). Animals might also reduce their

metabolism to compensate for the excessive external heat load. Cattle eat more frequent meals of smaller sizes in hot environments, perhaps trying to avoid peaks of heat load (Hahn, 1999).

Reproductive traits are influenced by many factors, both genetic and environmental. Environmental stressors can disrupt an animal from homeostasis, upsetting physiological functions such as reproduction. Reproduction is deleteriously influenced by hot weather due to reduction of estrus behaviour and conception rates (Turner, 1982) and also by lower embryonic survival (Hansen, 1997; Hansen *et al*., 2001). In addition, hot weather during pregnancy lowers calf birth weight (Collier *et al*., 1982) and may also disrupt male fertility by diminishing semen motility and increasing the incidence of sperm abnormalities (Meyerhoeffer *et al.*, 1985; Hansen and Arechiga, 1999). Timed insemination (Wolfenson *et al*., 2000, Cartmill *et al*., 2001), embryo transfer (Hansen *et al*., 2001) and in-vitro fertilization appear to be modern reproductive techniques that may help to overcome deleterious effects of heat stress on cattle fertility (Rutledge, 2001).

4. Coping capabilities

Coping with heat stress involves behavioural, physiological, and immunological functions, which are mobilized at different stressor levels to minimize adverse consequences. Thermoregulation and feeding behaviour are the principal responses of concern during heat waves. Respiration rate (RR) and body temperature (BT) are primary response measures related to thermoregulation, while feed intake (FI) is a primary measure of feeding behaviour (St-Pierre *et al.*, 2003; Nienaber and Hahn, 2007).

4.1 Thermoregulation

Respiration rate (RR) is easily observable in all animals by counting flank movements, but manual methods become tedious and labor intensive for long-term monitoring with frequent measurements. A respiration rate monitor was developed for

cattle described in Eigenberg *et al.* (2000). For cattle, an elastic cord was used to transfer flank movements to a pressure transducer. Digital outputs from the transducers are electronically recorded over a one-minute period every 15 minutes. Initial observations in the environmental chambers indicated that RR of feeder cattle increased by four bpm per °C above 21°C (Hahn *et al.*, 1997). Subsequent results, also for cattle in growth chambers, suggested a somewhat lower rate of increase: 3.0 bpm per °C from 18 to 34°C (Brown-Brandl *et al.*, 2002). The early study included data from 21°C and higher, while the more recent study included data for 18 to 34°C (±7°C). A study on effects of shade on feedlot cattle RR (Eigenberg *et al.*, 2000b) found that the RR increase for increasing temperature >25°C was 2.2 times greater for unshaded animals compared to animals having shade available. Changes in RR generally led changes in BT by two hr (Eigenberg *et al.*, 2002a). This demonstrates the effectiveness of thermoregulation attempts by cattle since increases in BT are delayed by increased RR. Compared to BT and feed intake, variation in measurements of RR among animals was lower, making it a good indicator of thermal stress (Brown-Brandl *et al.*, 2002).

4.2 Feeding behaviour

To investigate feeding behaviour of cattle as affected by heat stress, feed intake was measured from midnight to midnight, with feed delivery and weigh backs recorded at 07:30 h. Animals had ad-libitum access to both feed and water. Weighing feeders were recorded at 30 sec intervals (Nienaber *et al.*, 2001). Reports on the dynamics of eating showed that cattle require 3-4 days after the onset of heat stress to adjust (Hahn and Mader, 1997; Hahn, 1999; Nienaber *et al.*, 2001). The method of feed intake adjustment is likely a decrease in meal size and increase in number of daily meals as environmental temperature increases. Upon relief of heat stress, meal size increases dramatically and number of meals decreases (Nienaber *et al.*, 2001).

The thermoregulatory process also affects behaviour of cattle. Domestic animals are mainly diurnal in their habits, being active during daylight hours and inactive at night. In tropical and semi-arid regions, however grazing cattle tend to

reduce activity and seek shade in the daylight hours during hot weather. Instead, they graze in the late afternoon, at dawn and during the night (Gaalaas, 1945; Seath and Miller, 1946a; Silanikove, 2000). Seath and Miller (1946a) also reported that cows grazing during hot weather reduced their daytime grazing and tripled their grazing time at night. Tropically adapted cattle (Brahman, Senepol and Tuli) grazed more at midday while non-adapted *B. taurus* cattle (Angus) were more active at night during hot summer weather (Hammond *et al*., 1998). Other behavioural responses include avoidance of the font of heat, e.g., seeking shade, looking for water, and wallowing in mud (Robertshaw, 1985). This range of behavioural responses affects the heat exchange between the animal and its environment by reducing heat gain from radiation and by increasing heat loss by convection and conduction (Hafez, 1968).

Stress from hot weather also causes a reduction in daily feed consumption which primarily occurs through a reduction in meal size, and sometimes meal frequency (Nienaber *et al.*, 1999). One of the reasons why these changes in nutritional activities may occur is the exchange of blood flow that occurs between the thermoregulatory and the non – thermoregulatory tissues. Under the pressure of heat stress, blood flow tends to be shunted toward superficial tissues and respiratory muscles, diverting blood away from the gut (Christopherson, 1985, Beede and Collier, 1986). The increase of blood flow to the surface occurs very readily through vasodilatation of the arterioles near the skin (McDowell, 1972). Other studies have reported diminution of rumen motility and rumination when cattle are subjected to higher environmental temperatures, with opposite effects occurring under cold stress (Christopherson, 1985; West, 1999). Of course, the diminution of the blood flow to the gut and reduced rumen motility and rumination may be a cause-effect relationship.

Although management strategies can be implemented to buffer the animal against adverse environmental conditions, the primary factors limiting the precision of predicting performance are our ability to predict DMI (Hicks *et al.*, 1990). Additionally, a key component of performance is our ability to predict NEm requirements of cattle, particularly when they are exposed to adverse climatic conditions.

The effects of ambient temperature (AT) on DMI, as described in the NRC (1996), are based on incremental change in AT with adjustments ranging from a 16% increase for AT between -15°C and -5°C to -35% for AT > 35°C and no night cooling taking place. Although large variation exists among cattle relative to the effect of AT on DMI, the general relationships can be determined. However, the influence of no nighttime cooling on DMI is not completely accounted for in this equation. Frank *et al.* (2001) derived an algorithm that assumes the average effects of AT on DMI at AT > 24°C were in between those observed with and without night cooling.

At an average AT of 40°C, this equation would predict DMI to be approximately 50% of normal in feedlot cattle, which is a very likely scenario; however, it may not be the case for all cattle in general (Mader *et al.*, 2006).

To better account for effects of no night time cooling mentioned in NRC (1996), the negative effects of relative humidity (RH) on the evaporative cooling process need to be considered. The ability of cattle to lose body heat (cool down) at night is dependent not only on AT, but also on atmospheric moisture levels, or more specifically, RH, at night. Generally, RH is lower during daytime hours, but reach maxima when nighttime temperatures are typically the lowest, between 04.00 and 08.00 (Davis, 2001). Cattle feeding areas in the Southern Plains (AZ, NM, Western TX) often have high AT during the day, but can cool more and quicker at night due to the low RH. Whereas cattle fed in the Western Corn Belt can be subjected to more heat stress as a result of high RH even though actual average temperatures may be less than those found in the southern Plains. The temperature-humidity index (THI) was developed to adjust effects of AT for RH. Under hot conditions, assuming thermoneutral conditions range between 15 and 25°C (NRC, 1996), a separate equation can be used to describe effects of THI on DMI. Using the THI equation, more effectively accounts for the nighttime cooling effects on DMI. In addition, an increase in NEm requirements is found in cattle exposed to hot conditions. The NEm increase is largely dependent on the level and intensity of panting (NRC, 1981; 1996). However, NEm requirements under hot conditions are also dependent on body

condition. Cattle with greater body condition begin displaying signs of heat stress sooner than those with worse body condition. By combining data reported in NRC (1981) and Davis (2001), an adjustment for body condition score can be incorporated into a NEm requirement, based on THI. In this analysis, it is assumed that the BCS of cattle in previously reported studies (NRC, 1981) averaged five (scale of 1 to 9).

However, Kreikemeier and Mader (2002) reported over 20% greater DMI in winter vs. summer feedlot feeding studies. As indicated previously, large variation in DMI can exist in feedlot cattle. Seasonal patterns are likely dependent on normal vs. abnormal environmental conditions, as well as variations in these conditions. Short-term, sharp declines in DMI may be observed more often in the winter than in the summer due to the effects of winter storms that often accompany changing ambient temperatures (NRC, 1987). Lower DMI in the winter could be attributed to decreases in effective pen or bunk space due to pen conditions and/or negative social interactions among cattle. In addition, energy-dense diets provided to feedlot cattle and associated acidic end-products of fermentation are also factors limiting DMI (NRC, 1987). Increases in DMI brought on by cold stress, for instance, may be limited unless diet soluble starch content is reduced.

4.3 Water use

Water availability can interact with environmental conditions to affect production. For cattle, an adequate supply of clean, fresh water is vital for survival, especially during hot weather conditions. Expected water consumption is 75 l/d for a finishing beef animal for a hot weather condition (MWPS, 1987).

5. Signs of animal distress

Heat stress indicators may even measure the extent of a physiological displacement from the normal equilibrium or ground state, appropriately called homeostasis (Curtis, 1981; Yousef, 1982, 1985). Physiological measurements (heart rate, body temperature, respiration rate, etc.) can quantify the extent of the

physiological displacement from the normal state; the greater the physiological response, the nearer the animal is to collapse (Yousef, 1985). From an economic standpoint, weight gains (beef cattle) probably are the best indicators of the effects of heat stress during the summer. Production measurements are not the only factors to consider during the summer, but are very important and may be related to others (e.g., physiological indices) (Cartwright, 1955).

Extensive collection of environmental indices and milk production in Georgia allowed Ravagnolo *et al.* (2000) to find a specific environmental threshold where excessive heat load is evident by lowered milk output. Temperature-humidity index (THI) values above 72 seem to be the threshold where the effects of heat stress begin to be evident in reduced milk production (Ravagnolo *et al.*, 2000). Besides performance prediction, climatic records can also be used to quantify expected death or morbidity losses (Nienaber *et al.*, 1999).

5.1 Body temperature or rectal temperature (RT)

The most obvious index of thermal strain is the response in body temperature or rectal temperature (RT) (Brody, 1948). The temperature of a homeothermic animal's body is relatively uniform and constant, but various parts of the body do have different temperatures. Variations of temperature from site to site in the body are caused by a disparity in the insulation of each part (Curtis, 1981). Core or body temperature (RT) may be measured at several locations. The most frequently used site is the rectum which is perfectly adequate for steady – state conditions (Robertshaw, 1985), and RT is a very useful index of heat tolerance under field conditions (Hammond *et al.*, 1998). Deviation from the normal rectal temperature implies that the animal is under stress and that its homeothermic mechanisms are overtaxed (Brody, 1948).

Body temperature of the homeothermic animals is maintained within a relatively narrow range (Das *et al.*, 1999; Frisch, 2000) and slight fluctuations may upset the normal production processes. The efficiency of the body machine weakens

rapidly even with slight increases in core temperature (Brody, 1948). A rise in body temperature of only 4.4°C above normal is often quickly fatal. This concept is even clearer when we acknowledge that 38.5°C is normal temperature (Campbell and Lasley, 1985) and that most mammals die at core temperature of 42°C - 45°C (Brody, 1948; Silanikove, 2000).

The temperature of the central nervous system, in particular the brain, appears to be the most closely regulated deep body temperature as the function of the brain is very susceptible to temperature change. Tympanic temperature has been used as an indication of core temperature because the origin of the blood supply to the tympanic membrane and to the brain is the same (Robertshaw, 1985). In addition, Hahn (1999) has reported a high degree of association between tympanic temperatures and the feeding activities of cattle in thermoneutral environments. The use of measurement of tympanic temperatures with the infrared thermometer, as is done in humans could be of value considering the similarity between tympanic temperature and core body temperatures (Robertshaw, 1985). Tympanic temperatures are also known to be associated with feeding activities in cattle (Hahn, 1999). An appropriate procedure to take tympanic measurement with the infrared thermometer might improve body temperature records in cattle.

Blood temperature at the aorta is probably the best single indicator of average body temperature because it corresponds to a mixture of the blood from all over the body (Curtis, 1981) and responds much more rapidly to sudden changes of body temperature (Robertshaw, 1985). Rectal temperatures estimate average body temperature less accurately, especially when the temperature is changing. In addition, RT changes more slowly than doe's body temperature (Curtis, 1981). However, RT is an indicator of thermal balance and may be effective in quantifying the harshness of the thermal environment (Silanikove, 2000). The body temperature of homeotherms tends to be higher in the late afternoon and early evening than during the morning. In cows, body temperatures also vary with the stage of the estrous cycle (Curtis, 1981).

The rise in rectal temperature reflects or coincides with the cessation of increase in evaporative cooling and with rising environmental temperature. A decrease in the gradient between rectal and skin temperature causes a reduction of heat loss and rectal temperature increases (Worstell and Brody, 1953).

When ambient temperature is higher and cows could not dissipate heat effectively, heat stress would occur and resulted in higher rectal temperature. So it was popular to use rectal temperature represented the temperature on cows (Rosenberger, 1979). If the average rectal temperature of mature cows which were between 38.3°C to 39.1°C and they varied due to environment and heredity (Hafez, 1968).

Braton *et al.* (1966) found that the environment temperature between 25°C to 41°C have a highly positive correlationship (0.77) with body temperature for Holstein-Friesian, Jersey and American Brahman crossbred and American crossbred had the positive correlation between body temperature and ambient temperature. The experiment involved the environmental temperature and body temperature indicated that the higher environmental temperature could result in higher body temperature.

McDowell (1958) reported that body temperature of *B. taurus* would be increased when ambient temperature increased over 21°C Rhynes and Ewing (1973) found that Hereford cattle that were raised in the environmental temperature 21°C and 50% relative humidity for 7 weeks would have higher rectal temperature significantly (1.6°C) when compared with control group.

McDowell (1972) experimented with Angus, Shorthorn and Hereford in climatic chamber until the animal became heat stress. It found that every breed of cattle and in every experiment the cattle had increased rectal temperature when stayed in a room that had a high environment temperature (temperature 32°C and 60% relative humidity) when compare with those raised under comfortable temperature (18°C and 60% relative humidity). Elvinger *et al.* (1992) reported an increase in rectal

temperature of lactating cows ($P<0.01$) compare with cattle that become heat stress from the environmental temperature. Legates *et al.* (1991) found that when stress cattle under the environmental temperature of 40°C and the animal would have rectal temperature increased. Furthermore, relative humidity might have and effects on body temperature.

5.2 Respiration rate (RR)

Respiration is a form of convective heat transfer. The inhaled air closely corresponds to the body temperature by the time it reaches the trachea due to the heat and moisture exchange (Yousef, 1985). The greater the volume of air inhaled, warmed, and moisturized, the greater the resultant heat lost (McDowell, 1972). RR is a useful indicator of an animal's thermal load (Gaughan *et al.*, 2000); moreover, in panting animals that include cattle, it is an excellent index and the first visible sign of thermal stress (Brody, 1948; McDowell, 1972). The transference of heat through respiration is an imp ortant pathway of heat transfer for most mammals over a wide range of environmental conditions (Eigenberg *et al.*, 2000).

The changing threshold temperature demonstrates the dramatic difference in performance between acute and chronic exposures of heat stress. As stated in the earlier section on thermoregulation, RR is an excellent indicator of stress. For cattle, RR near 60 bpm is normal, indicating little or no stress load. However, RR >120 bpm reflect an increased stress load (Hahn *et al.*, 1997; Gaughan *et al.*, 2000). The onset of open mouth panting, with excessive drooling, indicates that an animal is failing to cope with heat stress and may need special attention, or close observation. Young and Hall (1993) listed other observable behaviours that are symptoms of impending heat stress. Listed in an increasing order of severity these include: alignment of body with solar radiation; shade seeking; refusal to lie down; reduced feed intake; crowding at the water; body splashing; agitation and restlessness; reduced rumination; and grouping to seek shade from other animals. Amelioration of heat stress assessment of the penalties to performance and well-being of livestock is essential to making

rational decisions for the selection, design, and management of their environments (Hahn, 1995).

Homeothermia under higher heat loads is easier to maintain when the respiration rate increases or panting begins, although they are not as effective as sweating for evaporative cooling (Robertshaw, 1985; Hahn *et al.*, 1997; Silanikove, 2000). A high respiration rate may be a useful means of increasing heat loss for short periods of time, but if the high rates continue for several hours, may cause serious problems for the animal. Continued panting may affect the efficiency of rumination and feeding and may lead to a reduction in the CO_2 combining capacity of the blood plasma because of hyperventilation. Hyperventilation normally is the origin of respiratory alkalosis that occurs when the CO_2 in the alveoli fails to reach the normal rate of diffusion (McDowell, 1972).

Measuring respiration rate appears to be the most accessible and easiest approach for evaluating the degree of heat stress in farm animals (low: 40 – 60 breaths per min, medium high: 60 – 80, high: 80 – 120, and severe stress: above 150 breaths per minute in cattle). All it requires is direct observation of the animal and a watch (Silanikove, 2000). As RR reaches 160 breaths per minute or higher, emergency actions should be activated to reduce heat loads (e.g., providing shade and/or wetting the animals) (Hahn *et al.*, 1997). Another type of breathing often observed in cattle under heat stress is open-mouth breathing, where drooling is present. This water loss mechanism of dealing with heat stress is very inefficient and is a cause of important mineral loss. “Open-mouth” breathers often show the poorest tolerance of heat, and normally, breeders in the tropics use this sign as a culling factor in selection for adaptation. Usually, a low respiratory rate under hot weather identifies animals with lesser discomfort. This fact is evident when comparing respiration rates of *B. taurus* vs. *B. indicus* under hot summer weather conditions where *B. indicus* (Zebu) cattle maintain lower respiration rates (McDowell, 1972; Gaughan *et al.*, 1999).

Respiration rate can be a valuable physiological parameter in conjunction with additional information such as ambient temperature, humidity, radiation heat loads, and air velocity (Eigenberg *et al.*, 2000; Silanikove, 2000). Highlighting the importance of RR, Brody (1948) commented that RR in *B. taurus* cattle is probably the most sensitive simple index of thermal stress, especially in conjunction with rectal temperature. Moreover, RR reacts faster than RT (Seath and Miller, 1946b) and may be useful when a rapid assessment of the heat load condition of cattle is needed.

Physiological responses of cattle have been studied intensively and RR has been shown to behave predictably, increasing with rising ambient temperature. However, the RR response is non linear. As ambient temperature increases beyond a threshold, RR increases more rapidly; this threshold occurs near heat stress conditions for cattle (Hahn *et al.*, 1997). A slight reduction in the respiration frequency during the days with highest environmental strains could be related to a shift in RR dynamics from rapid panting to a deep phase, open mouth panting which is slower (Gaughan *et al.*, 2000).

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The major stressor affecting respiration rate is ambient temperature, which has much more influence on RR than does humidity (Seath and Miller, 1946b). Some researchers have described specific relationships between ambient temperature and respiration rate indicating that increasing AT resulted in an approximate doubling of RR in adult lactating dairy cows for each 10°C rise in AT over the range from 4.4°C to 37.8°C (Regan and Richardson, 1938). Gaalaas (1945) suggested a quadratic relationship between RR and AT for mature lactating cows, as did Spain and Spiers (1996) for young dairy calves. The response of respiration rate to AT in lactating cows over a series of AT increasing from 10°C to 41°C in constant temperature environments was reported to be sigmoidal by Kibler and Brody (1953), and Worstell and Brody (1953). The sigmoidal function was a consequence of repeated changes in respiratory activity, from normal breathing at ground state, cool temperatures, to fast shallow breathing at moderately higher heat loads to a slower deep-phase of open mouth panting in hot conditions. The RR to AT relationship may help to define the threshold temperatures from which action to minimize the effect of higher heat loads will be needed (Hahn *et al.*, 1997).

Many researchers have attempted to identify the stress thresholds where homeothermal mechanisms are triggered and when heat balance is disrupted. Responses to high environmental temperatures that are reported in the literature include: intensification of the respiration rate and anorexia between 26.7°C and 29.9°C (Regan and Richardson, 1938), to increase respiration rate at 21.3°C of AT and to decline in feed intake at 25.0°C (Hahn, 1999), to decrease milk production at 24.0°C or 72 THI (Ravagnolo *et al.*, 2000; Ravagnolo and Misztal, 2000), and to die at body temperature of 42.7°C (Brody, 1948).

The development of biological response functions and their dynamic analyses will help to better understand the cause of the problem and recognize animals with problems. Moreover, they may lead to the establishment of standards for proactive environmental management of cattle during hot weather (Hahn, 1999).

Since heat stress occurs when the animal reaches the limit of heat loss without expending additional body energy, management of the stress depends on minimizing external or internal heat loads and maximizing the ability of the body to dissipate heat. Shade is a cost effective means of minimizing external heat; however, care must be taken to provide a suitable shade structure. Use of housing structures causes other limitations such as reduced air flow and increased moisture content of air, and increased radiant heat from other animals, especially important for cattle structures.

Management practices used to manage heat stress of beef cattle rarely include active cooling systems such as evaporative coolers or air conditioning. However, there are numerous practices used in dairy systems to reduce milk production losses. In addition to evaporative cooling in shelters (Wiersma and Scott, 1973), other techniques have been used such as: tunnel ventilation (Bray *et al.*, 2003; Gooch and Stowell, 2003); low speed/high air volume fans (Kammel *et al.*, 2003); and various spray techniques (Hillman *et al.*, 2001; Brouk *et al.*, 2003a, 2003b; Calegari *et al.*, 2003). Some of the dairy applications might be feasible for use in shade structures or outdoor pens for beef animals.

Additional planning might include the availability of shade (3.7 m^2/hd; MWPS, 1987), and/or a sprinkler system that delivers droplets of water as opposed to a mist which may exacerbate stress by increasing humidity and decreased evaporative heat transfer. Water droplets wet the hide, which draws heat from the body to evaporate moisture. Sprinklers should also be on an intermittent operation to allow time for evaporation and to reduce mud. A further benefit of sprinklers is reduction of dust in the feedyard (dust can lead to respiratory problems).

Feed intake has a large impact on heat production of all species and therefore control of eating has the potential for management of heat stress. However, timing of feed restriction is critical as outlined (Nienaber *et al.*, 2001) and successful restriction programs require close observation and planning (Gaughan *et al.*, 2001; Mader *et al.*, 2001), as well as accurate forecasts. Producers are reluctant to impose

potential performance losses resulting from feed restriction. Collier (2002) discussed the potential for utilizing genetic potentials for optimizing the stress tolerance of that species. Numerous references to systemic changes associated with acclimation to thermal stress of lab species were cited, but one citation included work on cattle (Manulu *et al.*, 1991). With the development of the full genomic map for the beef animal, more target tissue research is expected in order to enhance the acclimation of beef to heat.

5.3 Genetics of adaptation

There are two options to decrease the effects of heat stress on cattle: one is to modify the environment, which is expensive and not permanent and the other is to selectively bred cattle that are most adapted and productive (Finch, 1984). Genetic adaptation is defined by Yousef (1985) as a genetically fixed condition of a species or subspecies, or its evolution, which favors survival in a particular environment. The success of this endeavor has been a goal of farmers and scientists alike for a long time. Selection for resistance to thermal stress involves the use of physiological and production measurements; use of such quantitative measures may allow us to better understand the inheritance of the fitness (Brody, 1948). Examples of quantitative traits that may be related to adaptation to heat stress include rectal temperature, respiration rate, feed intake, weight gain, reproduction (McDowell, 1972), and other traits.

Turner (1982) researched the genetics of cattle in the tropics and found heritabilities of rectal temperature between 0.25 and 0.33 and genetic correlations of rectal temperature to female fertility and growth of -0.76, and -0.86, respectively. Burrow (2001) found a lower heritability for rectal temperature (0.17) with a repeatability of 27. Finch (1986) confirmed the existence of genetic control over body temperature and mentioned that selection within breeds for thermoregulatory characteristics would be a sound strategy for increasing productivity in the tropics. This idea is obvious when we consider that within a cattle population individuals have different levels of response or degree of adaptability to the environmental forces, such

differences are likely related to the genetic diversity within the population that could be exploited through selection.

It is possible to select for specific traits related to heat stress but the potential advantages of such an approach are diminished by the reduced selection pressure for traits of economic importance such as milk production. Perhaps it would be better to select for production in the hot climate itself (Hansen, 1997). The same belief was shared by Brody (1948) and Cartwright (1955), and was used by Frisch (1981) in Australia in the selection of beef cattle. Cartwright (1955) found a heritability of 0.19 for summer weight gain and considered it a useful selection tool for cattle that would resist hot weather. However, estimation of genetic parameters for growth and resistance to heat and other stresses in tropical cattle is not easy as it is complicated by various environmental interactions. Studying the genetics of tropical cattle in Australia, Burrows (2001) stated that in the tropics, growth is controlled not only of genes for direct growth, but also by genes for resistance to environmental stressors, and even which males and females may have different genes controlling growth and traits affecting insect resistance.

Frisch (1981) selected Hereford × Shorthorn cattle mainly for growth rate under conditions of moderate to high environmental stress beginning in 1966 as compared to an unselected line. Ten years later, the lines (selected vs. control) were compared and the selected line was shown to be more heat tolerant, to have lower nutritional requirements and greater resistance to infections and endo-parasites and hence, better growth under the presence of these stressors. However, the selected line did not express improved growth rate at low levels of stress. Finally, Frisch (1981) concluded that selection under stress appears not to increase growth rate *per se*, rather, the improvement of growth rate was achieved through increases in resistance to environmental strains that affected growth rate. This concept seems to be applicable for dairy cattle as well (Hansen, 1997; Pinheiro *et al*., 1998). In recent studies under tropical conditions in Brazil, Pinheiro *et al*. (1998) found genetic correlations of 0.56 between hair length and milk production in Holstein-Freisian cattle. Upon consideration at these results, they concluded that selection for milk

production under their environmental conditions results shortened hair length which was also expected to improve performance in subsequent generations.

Antagonisms exist between some components of adaptation and production potential. Al-Katanani *et al.* (1999) showed that when the milk yield increases the reproductive problems during the summer are magnified. It may be impossible to create an animal which has both high production potential coupled with a high level of adaptation (Frisch and Vercoe; 1979). This concern was shared by Ravagnolo *et al.* (2000) and Ravagnolo and Misztal (2000) who believe that Holstein cattle which have been selected intensively for improved milk production in temperate areas are now more susceptible to heat. They intend to validate the idea using extensive production and atmospheric data from producers and state weather stations, respectively. This could be one of the reasons why the difference between adapted and non-adapted animals disappears when the environmental conditions are not stressful enough and, in fact, the growth or production advantage could reverse in favor of non-adapted animals under such conditions (Frisch, 1981).

Zebu cattle are undoubtedly more heat tolerant than non-adapted *B. taurus*; part of this advantage is because *B. indicus* breeds have lower metabolic rates and energy levels which are closely related with reduced productivity levels (Worstell and Brody, 1953; McDowell *et al.*, 1955; Gaughan *et al.*, 1999). However, in many tropical regions around the world, the most common system to improve production of beef cattle is crossbreeding. Crossbred cattle (*B. taurus* × Zebu breeds or *B. taurus* × Sanga breeds) responded to heat stress similarly to the adapted parental breed (Seath and Miller, 1946b; Hammond *et al.*, 1996, 1997, 1998; Gaughan *et al.*, 1999) but with levels of production that are intermediate between both progenitors (Cartwright, 1955). The difference is rather constant through the stages of life (Seath and Miller, 1946b). Crossbreeding is a useful tool, particularly since heat tolerance is dominant (Hammond *et al.*, 1998).

Indigenous cattle in tropical areas have shorter hair compared to those that originated in temperate regions (McDowell, 1972), such as *B. taurus* breeds. Also, all

indigenous breeds of livestock in tropical areas have pigmented skin that avoids sunburn caused by solar radiation. There is abundant proof that longer hair cattle in the tropical areas can result in lower milk production (Lucena and Olson, 2000; Pinheiro *et al.*, 1998), lower weight gains, and higher levels of tick infestation (Bonsma, 1981; Frisch, 1981). The *B. taurus* breeds of Criollo cattle of Latin America, found in tropical areas probably have the shortest hair coat of any cattle. McDowell (1972) believed that only 12.5% to 25.0% of Zebu or Criollo genes are needed to insure a short coat.

5.4 Mitigation

Since climate change could result in an increase of heat stress, all methods to help animals cope with or, at least, alleviate the impacts of heat stress could be useful to mitigate the impacts of global change on animal responses and performance. Three basic management schemes for reducing the effect of thermal stress have been suggested (Beede and Collier, 1986):

(a) physical modification of the environment;
(b) genetic development of less sensitive breeds; and
(c) improved nutritional management schemes.

6. Effects of environmental conditions on blood parameters of cattle

Under chronic heat stress conditions, the value of haematocrits and haemoglobin would be lower. This was the result of heat balance mechanism activity via sweating which in turns made the body required more water intake. The animal will become one resulting anaemic more water diluting the plasma and more broken down of haemoglobin, hence lower haematocrits value (Hafez, 1968). Raghavan and Mullick (1962) found that when environmental temperature and relative humidity increased there was a negative correlationship between haematocrits and haemoglobin values, Furthermore, chronic heat stress conditions might affect amount of white and red blood cells of cattle. Ei-Masry and Marai (1991) found that haematocrits value of

Holstein-Friesian in summer was decreasing when compared with those of in winter. Moreover, an animal with better heat tolerance would fewer changes in haematocrits value than they are with worse heat tolerance. Nevertheless, Rusoff *et al.* (1954) found that in summer with high temperature of 27°C, there were no changes in haematocrit, haemoglobin, white blood cells values. Manresa *et al.* (1964) reported that in Philippines during hottest month of the year, the haemoglobin would have lowest and would reach value in coldest month of the year. There was also a diurnal variation of haematocrits. It would be high during morning, evening and cold night, and would be lowered during afternoon or hot part of the day. Nevertheless, Patterson *et al*. (1960) did not find diurnal variation of haemoglobin. Hence, Johnson (1987) suggested in that haemoglobin could be used as an index to indicate heat tolerating ability of cattle such that cattle with high haemoglobin value would tolerate heat better than those with lower haemoglobin value. Therefore, higher values of haematocrit and haemoglobin of less heat stressed cows might be due to the fact that the animals had more nutrient supplies for manufacturing red blood cells.

7. Haemoglobin synthesis

Haemoglobins are a group of proteins with the chief function to transport oxygen from the lungs to the tissues and carbon dioxide in the reverse direction. They are composed of polypeptide chains called globin and iron protoporphyrin heme groups (Jain, 1996). A specific sequence of amino acids constitutes each of four polypeptide chains. Each normal haemoglobin molecule contains one pair of alpha and one pair of beta chains (Huisman and Schroeder, 1971; Harvey, 1997). Polymorphism of haemoglobin beta (Hb) chains has been found in many different groups of vertebrates (Bangham, 1957; Bangham and Blumberg, 1958).

7.1 Control of haemoglobin synthesis

The syntheses of heme and globin chains are coordinated such that minimal amounts of free heme or globin monomers exist in the cytoplasm (Bunn, 1987). Heme plays a central role because it governs the initial step of translation in

globin chain synthesis (Traugh, 1989). Increased amounts of free heme increase globin synthesis, and the lack of free heme inhibits globin synthesis. In addition, α and β chain systhesis is coordinated; surplus α chains inhibit their own synthesis but stimulate β chain systhesis, and surplus β chains their own synthesis (Jandl, 1987).

7.2 Haemoglobin phenotypes in animals

Haemoglobin phenotypes are different in animal and human embryo than in fetuses or in adults. Embryonal Hbs are composed of either one or two pairs of peptide chains not found in adult Hbs (Kitchen and Brett, 1974). In ruminants and and humans, embryonal Hbs are replaced by fetal Hbs composed of two α and two γ chains. Most fetal Hb is replaced by adult Hb types in ruminants during the first month(s) after birth (Aufderheide *et al.*, 1980; Huisman *et al.*, 1969; Kitchen and Brett, 1974; Lee *et al.*, 1971). This switch from production of fetal Hb to the production of adult Hb appears to result from an inherent programming of hematopoietic stem cells (Wood *et al.*, 1985). In cat, dogs, horse, and pigs, embryonal Hbs are been replaced by adults Hb types during the fetal period. Hb types present in fetuses are identical to those found in adults (Bunn and Kitchen, 1973; Kitchen and Brett, 1974).

Considerable heterogeneity of Hb types occurs in adult animals. With the possible exception of pigs, two or more types are been reported to occur in domestic animal species (Braend, 1988). Most polymorphism of animal of animal Hbs is determined genetically and usually caused by multiple amino acid interchanges (Kitchen, 1974). Nongenetic alterations in Hb structure can also contribute to apparent Hb heterogeneity. Examples include the N-acetylation of β chains in cat HbB (Taketa *et al.*, 1972) and glycosylation of Hb, a function of intracellular glucose concentration and RBC lifespan (Higgins *et al.*, 1982; Rendell *et al.*, 1985). Increased glycosylation of Hb has been reported in diabetic dogs (Wood and Smith, 1980; Mahaffey and Cornelius, 1982).

Owing largely to its predominance in the erythrocyte and its ease of detection (no staining necessary), haemogloin phynotype was the first cattle protein polymorphism to be recognized (Cabannes and Serain, 1955; Bangham, 1957; Bangham and Blumberg, 1958). In both of these reports, alleles Hb^A and Hb^B were detected. In almost all breeds, Hb^A is the predominant allele and is virtually fixed in northern European and British cattle. In southern European, Channel Island, African and *B. indicus* cattle, Hb^B is found in moderate frequencies, approaching equality in the Jersey breed. The other *Hb* alleles have been reported; almost all of them are rare.

These alleles are: Hb^C (Crockett *et al.*, 1963; Carr, 1964), Hb^{DZAM} (Carr, 1965), Hb^D (Efremov and Braend, 1965), Hb^G (Braend, 1971), Hb^E (Khanna *et al.*, 1972), Hb^I (Osterhoff, 1975; Schwellnuss and Guérin, 1977), Hb^H (Han and Suzuki, 1976) and Hb^{XBAL} (Namikawa *et al.*, 1983). An allele, $Hb^{Khillary}$, reported by Naik *et al.* (1965) may be the same as Hb^{DZAM}. In a 1988 paper, Braend reported that by using isoelectric focusing he was able in Norwegian Red cattle to separate the Hb^A allele into Hb^{A4} and Hb^{A6} subtypes. The frequency of Hb^{A4} was 0.94. Whether or not there is a similar division in other breeds is not known. The locus *Hb* has been found to be linked to *EAA* (Larsen, 1966) and to the locus encoding parathyroid hormone, *PTH* (Fries *et al.*, 1988). The loci *Hb* and *PTH* have been mapped to BTA 15q13-q23 (Fries *et al.*, 1988).

7.3 Haemoglobin oxygen affinity

7.3.1 Oxygen dissociation curve

The initial binding of a molecule of O_2 to a monomer of tetrameric, deoxygenated Hb facilitates further binding of O_2 to the Hb molecule. Since the O_2 binding of one heme group influences the affinity of other heme group for O_2 this characteristic has been called the *heme-heme interaction*. The changing oxygen affinity of Hb with oxygenation results in a sigmoid oxygen dissociation curve (Figure 1) when the percent saturation of Hb with oxygen is plotted against the partial pressure of oxygen (pO_2).

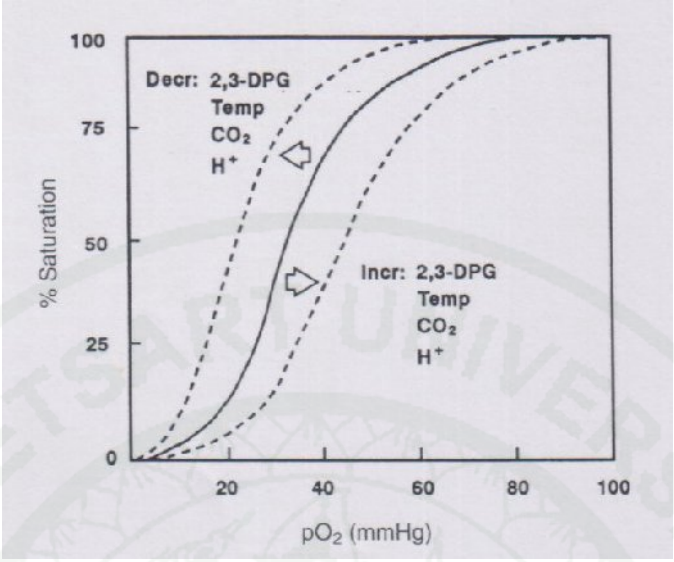

Figure 1 The haemoglobin-oxygen dissociation curve and factors influencing the position of the curve.

Source: Harvey (1997)

The pO_2 at which Hb is 50% saturated is the P_{50}. The steepness of the middle portion of the curve is of great physiologic significance because it covers the range of oxygen tensions present in tissues. Consequently, relatively small decreases in oxygen tension result in substantial oxygen release from Hb (Surgenor, 1975).

7.3.2 Effects of H^+, CO_2, and temperature

The oxygen affinity of Hb is influenced by H^+ in a manner termed the Bohr effect. In capillaries of metabolizing tissues, CO_2 enters RBCHs where it is rapidly converted to H^+CO_3 by carbonic anhydrase (carbonate dehydratase).This carbonic acid spontaneously ionizes to H^+ and HCO_3^-. The increased H^+ concentration decreases the oxygen affinity of Hb and facilitates oxygen delivery to the tissues. DeoxyHb is a weaker acid than OxyHb; therefore, DeoxyHb binds the excess H^+ and limits the decrease in pH. The increased HCO_3^- diffuses out of the cell down a

concentration gradient and $C1^{-}$ moves in (chloride shift) to maintain electrical neutrality. These processes are reversed at the lungs (Harvey, 1997).

The addition of H^{+} to a suspension of RBCs results in an increase in P_{50} and a shift of the oxygen dissociation curve to the right (Figure 1). The magnitude of the Bohr effect is defined numerically $\Delta \log P_{50} / \Delta$ pH. The relationship between the magnitude of the Bohr effect and average body size of various animal species is an inverse one (Riggs, 1960). The direct binding of CO_2 to Hb in carbamino groups also lowers oxygen affinity, but this effect is considered to be minor. Increased temperature decreases the oxygen affinity of Hb, a response that appears physiologically appropriate considering that increased heat production accompanies increased oxygen consumption in tissues (Surgenor, 1975).

Nevertheless, information about P_{50} for bovine RBCs is rather scanty. The P_{50} for greyhound RBCs in whole blood is lower than that for mongrel dogs, yet the groups have similar 2,3DPG concentrations (Sullivan *et al*., 1994). The cause of this difference remains to be determined but it is suggested that the higher PCV found in greyhound dogs may represent a compensatory response it a higher oxygen affinity of Hb in this species.

7.3.3 Effect of 2,3DPG

In RBCs from most mammalian species, 2,3DPG decreases the oxygen affinity of Hb, resulting in an increase in P_{50} (Bunn *et al.,* 1974). 2,3DPG reacts with Hb in a ratio of one molecule per Hb tetramer. Negatively charged groups of 2,3DPG bind to specific positively charged groups in the N-terminal region of Hb beta chains. There is a marked prepared to OxyHb due to differences in the conformation of the molecules. The interaction of 2,3DPG with Hb is represented as follows:

$$HbDPG + O_2 \rightleftharpoons HbO_2 + DPG$$

When 2,3DPG is increased, the reaction is displaced to the left, and when pO_2 is increased, the reaction is displaced to the right. ATP has a similar effect on Hb oxygen affinity, but is generally much less important than 2,3DPG, because it usually occurs in lower concentration and is complexed with Mg^{2+} (Bunn, 1971).

When the oxygen affinity of Hb is studied in hemolysates dialyzed to remove 2,3DPG and ATP, the "stripped" Hbs from species with low 2,3DPG RBCs have considerably lower oxygen affinities than stripped Hb from species with high 2,3DPG RBCs (Bunn,1971; Bunn *et al*.,1974). Furthermore, the oxygen affinity of stripped Hb from these low 2,3DPG RBCs is minimally affected by added 2,3DPG, because alterations are present in the β-chain-binding area that prevent or diminish 2,3DPG binding (Bunn,1981). However, the Hb oxygen affinity in cattle RBCs (a low 2,3DGP species) is modulated by chloride ions and be a lesser extent phosphate ions (Fronticelli, 1990; Gustin *et al.,* 1994). Because stripped Hbs from species with high 2,3DPG RBCs have high oxygen affinities, 2,3DPG is needed within RBCs of these species to maintain Hb oxygen affinity within a physiologically useful range (Benesch *et al*., 1975).

When blood from many mammalian species is studied, an inverse correlation is recognized between the P_{50} of whole blood and the log of body weight (Scott *et al.,* 1977). The relationship between metabolic rate (oxygen consumption per gram of tissue) and body weight (Kleiber, 1961) is an inverse one. Consequently, the higher P_{50} in smaller animals should be beneficial in meeting tissue oxygen requirement associated with their higher metabolic rate. Oxygen affinities of stripped Hb from various mammals dose not correlate with body weight (Nakashima *et al.,* 1985). The maintenance of 2,3DGP is energetically expensive because the ATP-generating PGK reaction is bypassed. 2,3DPG apparently allows for an evolutionary adaption of blood Hb oxygen affinity to metabolic rate.

Potentially, animals with high 2,3DPG RBCs can alter their Hb oxygen affinity to meet metabolic needs. The significance of (and in some cases the

appropriateness of) alterations in 2,3DPG in disease states is not always clear. RBC 2,3DPG increases in some anemic animals (Agar *et.al.*, 1977; King *et al.*, 1992; Studzinski *et al*., 1978). In the case of anemias, the resultant increasing in P_{50} would seem to be beneficial, but in the case of severe hypoxic hypoxemia the change might be detrimental, because Hb could not be fully saturated. Various studies in dogs indicate that cardiac output and microcirculation adjustments are much more important than changes in Hb oxygen affinity in adapting to hypoxia (Liard and Kunert, 1993; Schumacker *et al*., 1985; Zachara *et al*., 1981). However, a reduction in Hb oxygen affinity secondary to increased 2,3DPG can be beneficial, because it is far less energy demanding than is an increase in cardiacoutput (Liaed and Kunert, 1993; Mairbaurl, 1994; Teisseire *et al*., 1985). RBC 2,3DPG increases in hibernating mammals, but effect of the decrease in body temperature, associated with hibernation, on Hb oxygen affinity in vivo would more than offset of increased 2,3DPG (Bunn,1981). Because pH has substantial effect on Hb oxygen affinity, changes in RBC 2,3DPG concentration, in response to acidosis and alkalosis, produce effect on Hb oxygen affinity that counteract alterations induced by the respective in pH (Bellingham *et al*., 1971).

7.3.4 Haemoglobin phenotypes in resistance to parasite diseases

Interest in haemoglobin phenotypes in relation to resistance to parasite diseases arose when it was demonstrated that human carrier of the sickle cell trait who carrier an abnormal form (haemoglobin phenotypes S; HbS) were less susceptible to infection by *Plasmodium falciparum* (Vandepitte and Delaisse, 1957).

Although specific haemoglobinopathies have not been recognized in animals, claims have been made that certain haemoglobin types are correlated with the resistance of some bovine breeds to haemoprotozoan diseases. A study by Bangham and Biumberg (1958) showed a *prima facie* correlation between the absence of bovine HbB and relatively high tolerance to trypanosomiasis in African cattle. Francis and Little (1964) reported that the frequency of the HbB gene was substantially higher in *B. indicus* and *B. indicus* hybrid cattle than in *B. taurus*. It has

been establiished that *B. indicus* cattle and their crossbreds are more resistant to tick infestation and babesiosis than *B. taurus* cattle. Earlier Chandler (1952) presented evidence that two *B. taurus* breeds, N'Dama and Muturu, which have exclusively HbA, exhibit a greater tolerance to trypanosomal infections than do White Fulani cattle (*B. indicus*) which have a high incidence of HbB.

Naik *et al.* (1965) showed that the frequency of HbB was much higher in Indian breeds of cattle. Results of their work and other surveys on the frequency of haemoglobin phenotypes shows that the occurrence of HbB indicates an Asiatic rather than an African ancestry (Osterhoff, 1975).

One exception to this, in Jersey cattle which have a very high incidence of HbB, was reported previously by Bangham (1957) who obtained similar frequencies of haemoglobin types in Jersey and also the Guernsey and South Devon breeds. The fact that possession of a high frequency of HbB indicates an Asiatic ancestry has led to speculation about the genealogy of the Jersey (Boston, 1954; Bangham, 1957). Certainly, the high frequency of HbB exhibited by Jersey cattle is exceptional in *B. taurus* (Bachman *et al.*, 1978).

Bachman *et al.* (1978) studied on the frequency of haemoglobin phenotypes in several major breeds of cattle in northern Australia, all *B. taurus* cattle examined only the three common bovine type (AA, AB, BB) were found. F_2 Africander crossbreds showed only HbAA patterns. The frequency of haemoglobin B was significantly higher in *B. indicus* type cattle than in *B. taurus* breeds. In the pure Banteng cattle (*B. banteng*) with three phenotypes (HbBB, HbBC, HbCC) were present. The eleven buffaloes (*Bubalis bubalis*) typed each showed two haemoglobins (A1 and A2) in proportions of 71 to 29 respectively.

A unique occurrence in sheep and goats is the synthesis of HbC type in response to anemia (Huisman and Kitchen, 1968). This Hb switching from synthesis of HbA in sheep, and HbA and HbB in goats to HbC is mediated by EPO (a

34-kDa glycoprotein hormone that exhibits a high degree of sequence homology among mammals, Winslow *et al.*, 1989; Wen *et al.*, 1993; Adamu *et al.*, 2008)

8. Blood biochemicals profilres

In the last decades, the animal production was mainly focused on the maximal production of egg, milk, meat, etc. In recent years, the consumer has expressed his concern towards animal welfare and food safety. The intensive animal production is forced to produce high quality products with special attention to animal health and food safety. Exogenous factors such as management, diseases and stress have a major influence on growth, product quality and animal welfare.

Estimation of blood biochemicals such as enzymes, metabolites and proteins are helpful complementary diagnostic tools and support the common veterinary diagnostic techniques for a differentiated diagnostic of health and welfare status of the animal and competition of more specific treatment regiment (for review see Bogin, 1994; Jain, 1996; Kaneko *et al.*, 1997; Stockham and Scott, 2002). On the other hand, blood parameters can be influenced by several factors including breed, age, nutritional status, weather, season, physical exercise, diseases and stress (Kaneko *et al.*, 1997). The enzymes, metabolites and serum proteins, which are helpful complementary diagnostic tools as following:

8.1 Aspartate aminotransferase (AST)

Aspartate aminotransferase (AST) is present in many tissues and is useful in evaluating muscle and liver damage in small and large animals (Canfield *et al.*, 1985). This enzyme formerly was called serum glutamic oxaloacetic transaminase (SGOT). AST is not liver specific in any domestic animal species and the reference range in horses is rather broad (Kaneko *et al.*, 1997). Skeletal muscle is the second largest source of AST in animals. It is an absolute prerequisite to eliminate extrahepatic tissue damage as a possible source of serum AST when evaluating the enzyme in relation to the liver (Canfield *et al.*, 1985; Bogin, 1994).

In combinations with the physical examination and history, the evaluation of other serum enzymes should aid in differentiating the source of increased AST levels. AST is present in both the cytoplasm and mitochondria of hepatocytes (and many other cells) and will elevate in states of altered membrane permeability (Kaneko *et al*., 1997). In such cases, levels are expected to be less than in states of frank necrosis, when both cytoplasmic and mitochondrial enzymes are released (Canfield *et al.*, 1985; Bogin, 1994; Kaneko *et al*., 1997).

8.2 Alanine aminotransferase (ALT)

Alanine aminotransferase (ALT) is an enzyme that is normally present in liver and heart cell. ALT is also called serum glutamic pyruvic transaminase (SGPT) (Kaneko *et al*., 1997). This enzyme is present in high concentrations in the cytoplasm of hepatocytes. Plasma concentrations increase with hepatocellular, damage/necrosis, hepatocyte proliferation, or hepatocellular degeneration. ALT is a cytoplasmic enzyme, and is considered liver specific in dogs, primates and some other small animal species. There is little hepatic ALT activity in large domestic animals (Canfield *et al.*, 1985; Bogin, 1994).

Elevation of plasma levels of both AST and ALT can occur with states of altered hepatocellular membrane permeability. Because ALT is located only in the cytoplasm, plasma levels tend to be relatively higher than AST, because of membrane leakage from the hepatocyte (Stockham and Scott, 2002). Many causes of altered membrane permeability are potentially reversible but some may progress to hepatocellular necrosis that is essentially an irreversible change. Causes of increased cell membrane permeability include anoxia/circulatory hypoxia, metabolic disorders, exposure to toxins and toxaemia, hepatocyte proliferation and inflammation (Canfield *et al.*, 1985; Bogin, 1994; Radostits *et al.*, 1994).

The magnitude of both AST and ALT elevations in serum relates in general to the number of hepatocytes affected. However, the level cannot be used to predict either the type of lesion or whether cell damage is reversible *(leakage)* or

irreversible (frank necrosis) (Canfield *et al.*, 1985). In fact, focal necrosis may yield a lower concentration of both AST and ALT than would severe, transient hypoxia in which all cells may be affected resulting in a potentially reversible alteration in membrane permeability and diffuse enzyme leakage. Equally, increases in ALT and AST may be relatively mild in cases of severe cirrhosis/fibrosis of the liver since there is no ongoing hepatocellular damage (Bogin, 1994; Radostits *et al.*, 1994).

Another factor is been considered when interpretating AST and ALT levels are the rate of clearance from plasma. Both enzymes are molecularly too large to permit glomerular filtration and are primarily stereochemically denatured (Kaneko *et al*., 1997). The half-life of these enzymes is approximately 2-4 days and some prognostic information may be gleaned with this knowledge. Thus, if an elevated serum level falls by 50% after 2-4 days, the prognosis is generally more favourable than if the enzymes remain persistently elevated or are only slightly decreased after this time (Bogin, 1994; Radostits *et al.*, 1994; Kaneko *et al*., 1997).

8.3 Alkaline phosphatase (ALP)

The alkaline phosphatases (ALP) are a group of enzymes that catalyse the hydrolysis of a phosphate group from an organic molecule at an alkaline pH. ALP in plasma has been shown to consist of isoenzymes originating from the liver and bone which have a long half-life, and these isoenzymes are mainly utilized as indices of function or bone metabolism in cattle (Smith, 1996). Plasma ALP activity increases in cases of hepatitis, biliary disorders, pathologically in osseous alterations, fractures, vitamin D deficiency or during growth due to active bone metabolism (Smith, 1996; Miyazawa and Tomoda, 1991; Sato *et al.*, 2005).

ALP isoenzymes originating from bone and liver have different features of inhibition by heating or lectin affinity, and these isoenzymes have been measured individually using those features for clinical examination in cattle (Miyazawa *et al.*, 1991).

8.4 Gamma glutamyl transferase (GGT)

Gamma glutamyl transferase (GGT) was shown to be a sensitive marker of cholestasis. It may be used in conjunction with other tests, to determine the presence and origin of cholestasis (Canfield *et al.*, 1985). GGT was found to be a valuable tool in the diagnosis of hepatobiliary disorders. Most cells have some GGT activity, especially kidney, liver and pancreas, but most of the serum GGT derives from the liver. It is present in cell cytoplasm and bound to membranes. It is a carboxypeptidase, which cleaves glutamyl groups and transfers them to peptides and other appropriate receptors (Canfield *et al.*, 1985).

The physiological function of GGT is unknown, but it could be associated with glutathione metabolism. Elevation of serum GGT appears to be quite specific for intrahepatic or extrahepatic cholestasis. In liver damage, GGT may be used as an indication of chronic change, due to its slower release and metabolism, compared with transaminases (Canfield *et al.*, 1985). As such, it is often associated with cirrhosis. GGT is particularly useful for identifying chronic hepatic disease in horses. It is induced by corticosteroids in dogs and cannot be used to discriminate between steroid induced elevations of ALP and cholestasis (Canfield *et al.*, 1985; Kaneko *et al.*, 1997).

8.5 Creatine kinase (CK)

Creatine kinase (CK) is useful in diagnosing skeletal muscle or cardiac muscle degeneration (Boyd, 1982). The clinical diagnosis of neuromuscular disease can be aided by serum enzyme determinations. Creatinine phosphate is the major form of high energy phosphate required by muscle for contraction. Increases in CK can be caused by skeletal muscle damage and excessive exercise, muscle anoxia, from prolonged recumbency, myositis, nutritional myopathy, and myocardial infarction (Canfield *et al.*, 1985). Frequently CK will increase after intramuscular injections due to local areas of muscle necrosis. CK in CSF may be useful in diagnosing disease of the central nervous system. The half-life of CK is very short and levels decrease

rapidly (Yazar *et al*., 2001). This is in contrast to the pattern which serum AST follows. AST is also useful in the diagnosis of muscle damage and can act as a prognostic indicator (Canfield *et al.*, 1985). Elevated CK values indicate that muscle damage is active or has recently occurred. If the CK continues to remain elevated, the muscle damage is continuing. If elevated AST levels are associated with decreasing or normal CK levels, the muscle damage is no longer active (Canfield *et al.*, 1985; Yazar *et al*., 2001).

8.6 Glucose

Plasma glucose (GLU) is an important source of energy for many cells. GLU in plasma normally maintains by the breakdown of dietary carbohydrates and a rather complex system of endogenous production (Kaneko *et al*., 1997). Endogenous glucose production results from glycogenolysis (glycogen broken down to glucose in the liver) and effects from gluconeogenesis. The maintenance of normal plasma glucose requires delicate balance of glucose availability with glucose utilization (Rook and Line, 1961; NRC, 1984).

Glucose is not the only energy source, which fuels the energy requirements of the body tissues. Fatty acids, proteins and other substances also provide energy. However, glucose is an obligate fuel for the central nervous system (NRC, 1984). Consequently, maintenance of a normal blood glucose concentration is essential for the survival of brain tissue. Glucose transport from the circulation into the brain can become rate limiting if the blood glucose falls into the hypoglycaemic range (NRC, 1984; Kaneko *et al*., 1997).

Many hormones are involved with glucose regulation (glucagon, epinephrine, cortisol, insulin). Insulin, secreted from the B cells of the pancreas, is the most noteworthy and dominant glucoregulatory factor. Insulin primarily stimulates glucose utilisation by a variety of insulin-sensitive tissues including muscle, fat and liver. Small changes in insulin result in substantial changes in blood glucose values (Bogin, 1994). An increase in insulin will generally lower plasma glucose levels.

Glucagon, epinephrine and cortisol are all glucose-raising hormones. Glucagon acts on the liver by stimulating both glycogenolysis and gluconeogenesis (Canfield *et al*., 1985; Guyton and Hall, 2006). Epinephrine both limits glucose utilisation and stimulates its production. Cortisol antagonises the effects of insulin and limits both the stimulation of glucose utilisation and the suppression of glucose production by insulin. It is the alterations in these glucoregulatory hormones, which cause hypoglycaemia and hyperglycaemia (Guidry *et al.*, 1976; Kumar and Pachauri, 2000).

Bulent *et al.* (2006) reported exponent decrease in serum glucose concentration as the parturition approached in dairy cattle and the significant decrease in blood glucose level during late pregnancy signifies rapid utilization of glucose towards the fag end of the pregnancy. Cows generally go ketoic during third trimester (Mandali *et al*., 2002) and insufficient feed intake during the winter months may also result in the lower glucose levels in pregnant cows. Several workers have reported blood glucose level in cattle averaging 2.40±0.03 mmol/l (Prudhvi Reddy *et al.*, 2003), 2.72±0.22 and 2.33±0.13 mmol/l (Nath *et al.*, 2004), respectively. Low temperature leads to the release of the enzyme phosphorylase in the biosystem due to the activation of adrenaline hormone (Guyton and Hall, 2006). Herdt (2000) claims that a change in glucose concentration is associated with possibly reflecting hormonal changes at calving that promote gluconeogenesis and glycogenolysis (Mir *et al.*, 2008)

8.7 Blood urea nitrogen

Blood urea nitrogen is a nutritional indicator related to protein intake and is formed in the liver. Moreover, is mainly excreted by the kidneys (Kaneko *et al.*, 1997; Coppo, 2004). Consequently, urea is useful in evaluating kidney function in conjunction with creatinine, which originates from the muscle and is filtered by the kidney (Jain, 1996). The majority of the blood urea nitrogen is synthesized in the liver from ammonia. Once formed, urea diffuses freely throughout all body fluids. The kidney is the most important route of urea excretion and as a result, urea has long been used as a barometer of renal function. Urea appears in the glomerular filtrate in

the same concentration as is found in the blood. This filtration process does not require energy (Kaneko *et al.*, 1997). Decreased glomerular filtration increases urea. Some urea is passively resorbed from the tubules back into the blood. The amount resorbed is inversely related to the rate of urine flow through the tubules the lower the urine flow rate the greater the tubular urea resorption resulting in an increased urea (Jain, 1996; Stockham and Scott, 2002).

8.8 Creatinine

Most creatinine originates from the non-enzymatic conversion of creatine in muscle. This spontaneous degradation of creatine to creatinine occurs at a rather constant and uniform daily rate (Kaneko *et al.*, 1997). Creatinine is freely filtered by the glomerulus and clearance of creatinine from the plasma to the urine can be used to provide an approximation of the glomerular filtration rate. A small amount of creatinine is secreted by proximal tubules in the kidney but, in contrast to urea, none is resorbed by the tubules (Bogin, 1994; Jain, 1996).

Causes of creatinine increases may generally be placed in the same three categories described for urea (Jain, 1996). However, creatinine values are not significantly affected by catabolic factors and diet. Diuresis and other factors affecting urine flow rate have less effect on creatinine than urea because creatinine is not resorbed by the renal tubules (Jain, 1996; Kaneko *et al.*, 1997).

8.9 Total serum proteins, albumin, globulins and the acute phase proteins

Serum proteins represent a heterogeneous group with albumin constituting the major portion (Bogin, 1994). Albumin serves as a regulator of osmotic equilibrium. Globulins are also important serum proteins and they are primarily associated with antibodies. Acute phase proteins are associated with the acute inflammatory response and are useful markers for acute and chronic active inflammation (Jain, 1996; Stockham and Scott, 2002).

The liver (Kaneko *et al.*, 1997) produces almost all proteins in the serum. Immunoglobulins are the notable exception and they are produced by lymphoid tissue. Serum proteins are relatively short-lived with most having half-lives of about 10 days. The breakdown of these proteins occurs mostly in the liver with some catabolic activity in the intestine and kidney. Cattle serum normally contains 30.3-35.5 g/l of albumin, which constitutes 40-60% of the total protein concentration (Kaneko *et al.*, 1997). Fluid accumulations in body cavities and tissue usually result when albumin levels drop below 10 g/l. However, fluid may accumulate with higher albumin concentrations if hypertension and loss of vessel integrity, etc. are present (Kaneko *et al.*, 1997). Plasma and serum proteins, act as anions in acid-base balance, take part in coagulation reactions, and serve as carriers for many compounds (Bogin, 1994). In addition to albumin, plasma contains globulins, fibrinogen (removed from serum by the clotting process), glycoproteins, lipoproteins, acute phase proteins and transport proteins (Jain, 1996; Stockham and Scott, 2002).

The globulin component is subdivided into important subfractions identified by electrophoresis as alpha, beta and gamma globulins. The alpha and beta fractions are important carriers of lipids, lipid soluble hormones and vitamins. Gamma globulins are primarily associated with antibodies. Conditions causing inflammation usually cause a measurable increase in serum levels of gamma globulins and often alpha-2 globulins (Bogin, 1994; Kaneko *et al.*, 1997).

Measurement of albumin, along with a separation of globulin into its fractions, can be accomplished with serum protein electrophoresis. When placed in an electric field, these proteins migrate at different rates yielding a familiar electrophoretic pattern. Values obtained from measuring serum proteins can provide an accurate reflection of an animal's health status (Kaneko *et al.*, 1997; Mir *et al.*, 2008).

Temperature stress, either febrile or hypothermia, is associated with nitrogen loss, increased adrenal activity, and increased protein turnover (Kaneko *et al.*, 1997). These stresses cause a decrease in total serum protein, decrease in albumin,

and often an increase in α_2-globulin. Similar findings are observed in crushing injuries, bone fractures, and extensive surgery. Tissue repair calls on protein reserves and the increased protein turnover results in decreased albumin and increased α_2-globulin (Ritzmann and Daniels, 1982; Kaneko *et al.*, 1997). In the inflammatory process, fluids and proteins move into the tissue fluids, inducing edema and contributing to a decrease in albumin. A rapid movement of interstitial fluid (without protein) into the plasma compartment to induce an acute hypoproteinemia follows haemorrhage or massive exudation with large external losses of plasma. Conversely, dehydration leads to haemoconcentration through reduction in fluid volume and consequent hyperproteinemia. During splendent contraction in the horse, a large mass of erythrocytes moves into the circulation with little or no change in the serum protein (Kaneko *et al.*, 1997).

9. Growth hormone and stress

The term 'somatotrophic axis' is generally used to refer to the integrated neural and endocrine mechanisms that control growth hormone (GH) production/secretion and the subsequent physiological responses to the secreted GH. Specialized cells of the anterior pituitary gland called somatotrophs produce and release GH. A variety of hormonal inputs can affect the somatotroph (Bertherat *et al*., 1995). Growth hormone-releasing hormone (GHRH) and somatostatin are important hypothalamic factors that exert stimulatory and inhibitory effects on GH secretion, respectively. One of the effects of GH is the stimulation of the liver to produce and release insulin-like growth factor-I (IGF-I). The growth and development of a variety of peripheral tissues are dependent on IGF-I. GH also exerts direct effects on numerous peripheral tissues (Holly and Wass, 1989).

Stress-induced reductions of GH and IGF-I secretion have been reported in rats (Armario *et al*., 1987; Straus, 1994; Peisen *et al*., 1995). However, data from other vertebrate species indicate that the somatotrophic axis responds to stress by concurrently increasing GH and decreasing IGF-I secretion (Vance *et al*., 1992; Kakizawa *et al*., 1995; Bruggeman *et al*., 1997; Carroll *et al*., 1998; McCusker, 1998).

These endocrine responses act to divert energy from growth to survival. The increase in circulating GH antagonizes the effects of insulin by direct GH receptor-mediated actions on peripheral target tissues, thus reserving blood glucose. A reduction in IGF-I is thought to minimize growth during times of distress, further preserving energy for purposes of survival. While evidence is limited, it is interesting to note that positive emotional experiences may be associated with decreased GH secretion (Berk *et al*., 1989).

10. Growth hormone secretion from peripheral lymphocytes

Lymphocytes orchestrate adaptive immune responses via antigen recognition and the secretion of cytokines and growth factors (Sprent and Trough, 1994). The expression of hormones and their receptors on lymphocytes are believed to play an important role in the maintenance of homeostasis during normal physiological processes as well as pathological states (Besedovsky and Rey, 1996; Dixit and Parvizi, 2001).

Lymphocytes express receptors for GH (Bresson *et al.*, 1999) and produce GH (Weigent and Blalock, 1991; Poppi *et al.*, 2002), which is apparently similar to its pituitary counterpart (Hattori *et al.*, 1990). GH increases the migration of fresh and activated lymphocytes and augments T cell adhesion via β1 and β2 integrins (Taub *et al.*, 1994). GH has been shown to play an important role in the development and regulation of the immune system (Suzuki *et al.*, 1990; Weigent *et al.*, 1991; Murphy and Lonngo, 2000; Koo *et al.*, 2001). It increases natural killer cell activity (Crist *et al.*, 1987), erythropoiesis (Golde *et al.*, 1977), lymphopoiesis (Astaldi *et al.*, 1973), granulopoiesis (Merchav *et al.*, 1988), the production of superoxide anions from neutrophils and macrophages (Edwards *et al.*, 1988). Moreover, lymphocytes express GHRH and somatostatin along with their specific receptors; however, contradictory data exist on the modulation of GH secretion from lymphocytes by these neuropeptides (Stephanou *et al.*, 1991; Roh *et al.*, 1998).

Stimulatory mechanisms involving with GH release seems to be similar between the pituitary gland and circulating lymphocytes; however, the inhibitory control mechanisms GH appear to differ (Poppi *et al.*, 2002).

11. Nitric oxide (NO)

Assignment of nitric oxide (NO), an inorganic free radical gas, as a biological messenger molecule was initially confroned with skepticism and disbelief (Dixit and Parvizi, 2001). However, over the last decade, diverse lines of evidence have converged to show that nitric oxide is a modulator of physiological and pathological processes in mammals (Moncada *et al.*, 1991; Nathan, 1992; Bredt and Snyder, 1994). Due to the diversity of its physiological functions and general ubiquity, NO has become very attractive to scientists in different areas of physiology (Dixit and Parvizi, 2001). It has emerged as an important mediator of a wide range of critical processes including neurotransmission, endocrine signal transduction, mediation of reproductive function (Dixit and Parvizi, 2000), vasodilation, and immune defense (Nathan, 1992). NO is implicated in the neuroendocrine control of pituitary GH secretion (Kato, 1992). Furthermore, some of leptin effects has been reported to be mediated via nitricoxididergic mechanism (Yu *et al*., 1997).

In biological systems, it is synthesized from L-arginine via an oxygen- and NADPH-dependent reaction that yields NO and L-citrulline (Bush *et al.*, 1992). Nitric oxide is a colourless gas at room temperature and pressure. Its maximum solubility in the water is similar to that of pure oxygen. Nitric oxide is a non-polar molecule which freely diffuses through membranes.

The role of nitric oxide in regulation of hypothalamic-pituitary-gonadal (HPG) axis is brought about by a complex network which involves hypothalamic, intrahyphophyseal signals. Growing evidence suggest that nitric oxide may act as a novel transmitter in hypothalamus (Rettori *et al.*, 1993; Moretto *et al.*, 1993; Bhat *et al.*, 1995), pituitary (Chatterjee *et al*., 1997) and gonads (Faletti *et al*., 1999; Dunnam *et al*., 1999).

MATERIALS AND METHODS

Experiment 1

Productivity of Thai Brahman and Simmental-Brahman crossbred (Kabinburi) cattle in central Thailand

1. Meteorological data

Maximum and minimum ambient temperatures, maximum and minimum relative humidity, and annual rainfall during January 1999 to October 2002 were collected from Photharam District weather station next to the Nongkwang Livestock Research and Breeding Center (NK; 13°39′N; 99°52′E; 40 m above sea level), Bua Chum District weather station next to the Lamphayaklang Livestock Research and Breeding Center (LP; 15°00′N; 100°33′E; 140 m above sea level) and Kabinburi District weather station next to the Prachinburi Livestock Breeding Station (PC; 13°59′N; 101°42′E; 74 m above sea level). The meteorological data were collected at 3-h intervals, and were averaged to obtain a daily value for each location. All locations are under a tropical monsoon climate with three seasons a year. The rainy season is from July to October, the winter runs from November to February and the summer is from March to June (Thai Meteorological Department, 2004). The Temperature – Humidity Index (THI) was also calculated (NOAA, 1976; Mader and Davis, 2004) as follows:

$$THI = (0.8 \times AT) + [(RH/100) \times (AT - 14.4)] + 46.4$$

Where: AT = Ambient temperature (°C)

RH = Relative humidity (%)

2. Animal data

Historical records for the period January 1999 to October 2002 were used. These data consisted of 1,316 records (597 from NK and 719 from LP) for Thai Brahman (TB) cows, and 756 records (351 from NK and 405 from PC) for Kabinburi (K) cows. The K cows data of F4 of *inter se* mating from original 15 Simmental sires and 500 Brahman dams were used in this survey. It is assumed that the Kabinburi to be a dual purpose (meat and milk) cattle (Animal Husbandry Division, DLD, 1995). Following data from each animal were analyzed: weight at birth, weight at weaning (180 – 220 days of age), yearling weight (380 – 420 days of age) and weight at 20 months of age (580 – 620 days of age), age at first calving and calving interval. The bodyweight of the individual animals at weaning, yearling and 20 months were adjusted at 200, 400 and 600 days of age, respectively by interpolation the values obtained from multiple weightings. Calculation was as follows:

$$WW = [(B - C)*200/D] + C$$

$$YW = [(E - B)/(F - D)]*200 + WW$$

$$MW = [(G - E)/(H - F)]*200 + YW$$

Where:

WW = weaning weight at 200 days of age
YW = yearling weight at 400 days of age
MW = 20 months weight
B = actual weaning weight (kg)
C = birth weight (kg)
D = actual weaning age (180 – 220 days of age)
E = actual yearling weight (kg)
F = actual yearling age (380 – 420 days of age)
G = actual weight at 20 months (kg)
H = actual age at 20 months (580 – 620 days of age)

At NK station, the 597 TB cows that were daughters of 14 sires and 258 dams, and the 351 K cows were offspring of 13 sires and 182 dams. At LP station, the 719 TB cattle were daughters of 17 sires and 315 dams. The 405 K cows kept at PC station were offspring of 13 sires and 206 dams.

3. Animal husbandry

The management was similar in all locations (Animal Husbandry Division, DLD, 1991). All animals were kept unrestrained in an open animal house and had free access to mixed pasture containing Para grass (*Brachiaria mutica*), Ruzi grass (*Brachiaria ruziziensis*), Guinea grass (*Panicum maximun*), Centro (*Centrosema pubescens*) and Hamata (*Stylosanthes hamata*). At night, they were also fed soilage crop or silage or hay and 1-2 kg of 14-16% (of dry matter) crude protein concentrate according to the recommendation of National Research Council (NRC, 1984). The animals had free access to mineral supplements and clean water. However, NK, LP and PC centers had no details of nutrient composition and digestibility of the diets fed during the period of this study.

Weaning occurred at age of 180-220 days in all locations. The replacement heifers were mated at age of 18-20 months or body weights of ≥ 280 kg. In all three locations, the cows were mated with the bulls of the respective breed by natural mating. The mating ratio of bull to dams was 1:25. The outcome of two breeding seasons of 120 days each (5 May to 1 September and 3 November to 2 March) were utilized. Both breeds were equally represented in these two breeding seasons.

Veterinarians monitored the health of animals regularly. The animals were vaccinated against foot and mouth disease (Type O, A and Asia 1; DLD, Bangkok, Thailand) twice a year in May and November. Vaccination against Brucellosis (Strain 19; DLD, Bangkok, Thailand) was between the ages of 4 and 8 months. They were examined for Brucellosis and Tuberculosis once a year in May, and were free from these two diseases. All animals were treated for endoparasites such as lungworms,

stomach roundworms, intestinal worms, tape worms and liver fluke using Valbazen® (11.36% albendazole; Pfizer, Thailand) twice a year in May and November.

4. Statistical analysis

Quantitative data were analyzed using GLM procedures of SAS (SAS, 1998). Meteorological data were analyzed for the effects of locations with the model including location, season and location × season. Differences were determined using Least Significant Differences (LSD) procedure. The bodyweight and reproduction data the included 4 treatment groups comprised of T1: TB cattle at NK, T2: K at NK, T3: TB at LP and T4: K cattle at PC. The effects of sires, dams, age of dams, year and season of birth were also included in the model for unbiased adjustment. The statistical model was:

$$Y_{ijklmno} = \mu + T_i + S_j + D_k + A_l + YSe_{mn} + \varepsilon_{ijklmno}$$

where

$Y_{ijklmno}$ = the record of the o^{th} cow of the i^{th} treatment, the j^{th} sire, the k^{th} dam, the l^{th} age group of dams and born in the m^{th} year × the n^{th} season interactions,

μ = the overall mean,

T_i = the i^{th} treatment (i = T1, T2, T3, T4),

S_j = the j^{th} sire (j = 1, 2, 3, …, 57),

D_k = the k^{th} dam (k = 1, 2, 3, …, 961),

A_l = the l^{th} age group of dams (l = 2, 3, 4, …, 12),

YSe_{mn} = born in the m^{th} year × the n^{th} season interactions (m = 1999, 2000, 2001, 2002 and n = summer, rainy, winter), and

$\varepsilon_{ijklmno}$ = the vector of residuals, which was assumed; $\varepsilon_{ijklmno} \sim NID\ (0, \sigma_e^2)$.

Orthogonal contrasts were used to determine treatment differences for T1 vs. T2, T1 vs. T3 and T2 vs. T4 treatment groups. Values are presented as means±SE.

Experiment 2

Relationship between haemoglobin types and productivity of Thai indigenous and Simmental × Brahman crossbred cattle

1. Animals

One hundred and ten mature (3 to 5–year–old) including 59 Thai indigenous cattle (*Bos indicus*) and 51 Simmental × Brahman (50% Simmental × 50% Brahman; *B. taurus* × *B. indicus*) crossbred cattle from different breeders were utilized. The Thai indigenous cattle were daughters of 35 sires and 59 dams. Crossbreds were offspring of 30 sires and 51 cows.

2. Animal husbandry and management

These animals were kept as a nucleus herd in Nongkwang Livestock Breeding and Research Center. All cattle used in the present study were mated with the same bull of the respective breed. The outcome of two breeding seasons of 120 days each (5 May to 1 September and 3 November to 2 March) were utilized. Both breeds were equally represented in these two breeding seasons.

Animals were maintained as a herd in Nongkwang Livestock Research and Breeding Center and were provided as one group with mixed pasture containing Para grass (*Brachiaria mutica*), Ruzi grass (*Brachiaria ruziziensis*), Guinea grass (*Panicum maximun*), Centro (*Centrosema pubescens*) and Hamata (*Stylosanthes hamata*). At night, all animals were housed and fed soilage crop or silage or hay and 1-2 kg of 14% meal concentrate modified from the National Research Council (NRC, 1984). Mineral supplements and clean water were also freely accessed. The animals were maintained under similar nutritional and environmental conditions at Nongkwang Livestock Research and Breeding Center, Department of Livestock Development (DLD) in Photharam District, Ratchaburi Province, Thailand (28.00±0.26°C and 70.46±1.30% RH; 13°39′ N; 99°52′ E). They were vaccinated

against foot and mouth disease and were free from brucellosis and tuberculosis. Treatment for control of endoparasites was performed twice a year. The body weight of the animals was recorded at birth, 200-, 400- and at 600-day of age. The age at first calving was also determined.

3. Sample collections

Blood samples (1 ml each) were collected via the jugular vein puncture from each cow during November 2003 to February 2004. The blood samples were withdrawn into a heparinized syringe (with 10% Na-Heparin 10 μl/ml blood) between 08:00 and 10:00 a.m. One part whole blood was added to 4 parts haemolysate reagent, mixed well and allowed to stand for 5 min. The sample was stored at –20°C until analysis, which was conducted within 7 days from withdrawal.

4. Haemoglobin phenotypes measurements

Haemoglobin phenotypes (Hb types) were estimated by electrophoresis at room temperature (25°C) via a cellulose acetate membrane as described by supplier (Huisman and Schroeder, 1971). Separations were performed using both Tris-EDTA and a boric acid buffer (Supre-Heme® Buffer; Cat. No 5802) at pH 8.2-8.6. Samples (haemolysate; 5 μl) were applied to the membrane and electrophoresis carried out for 25 min at 350 V. After separation, the membrane was stained with 0.5% ponceau S in 3.5% sulfosalicylic acid and 3.5% trichloroacetic acid for 6 min. To remove background staining, the membrane was then washed three times in 5% acetic acid for 2 min, rinsed in 95% ethanol and cleared in clearing solution (30 parts glacial acetic acid, 70 parts absolute methanol and 30 parts Clear Aid). The membrane was scanned using a densitometer (Model GS-670, Bio-Rad Laboratories USA). Quantification of the bands was performed using an electronic integrator attached to the densitometer.

5. Statistical analysis

Animals from each breed were allocated to different groups according to their Hb types. Differences in growth performance and age at first calving and their relationship to Hb types were statistically analyzed using the least squared analysis method (Harvey, 1975). The model included Hb type treatment groups in Thai indigenous or in Simmental × Brahman crossbred cows. The effects of sires, dams, age of dams, year and season of birth were also included in the model for unbiased adjustment. Comparison of means were tested for differences among groups by the least significant differences (LSD) using SAS program package (SAS, 1998).

Experiment 3

Blood biochemical profiles of Thai indigenous and Simmental × Brahman crossbred cattle in the Central Thailand

1. Animals and managements

One hundred and twelve mature (3 to 5 – year – old) cattle including 61 (45 non-pregnant cows and 16 bulls) Thai indigenous (*B. indicus*) and 51 (33 non-pregnant cows and 18 bulls) Simmental × Brahman crossbred cattle [50% Simmental × 50% Brahman (*B. taurus* × *B. indicus*)] from different breeders were utilized. Animals were kept as a beef cattle nucleus herd in the Nongkwang Livestock Research and Breeding Center, Department of Livestock Development, Ratchaburi Province and provided with mixed pasture containing Para grass (*Brachiaria mutica*), Ruzi grass (*Brachiaria ruziziensis*), Guinea grass (*Panicum maximun*), Centro (*Centrosema pubescens*) and Hamata (*Stylosanthes hamata*). At night all animals were housed indoors and fed soilage crop or silage or hay and 1-2 kg of a 14% (of dry matter) crude protein concentrate according to the recommendation of National Research Council (NRC, 1984). They had free access to mineral supplements and clean water. Ratchaburi Province is located in a tropic area of central Thailand at 13°39′ N, 99°52′ E and 40 m above sea level with an average temperature of 28.1°C (20–40°C) average relative humidity (RH) of 75% and an average of 1,266 mm annual rainfall (Thai Meteorological Department, 2004).

The animals had a body condition score (BCS; Long *et al.*, 1979; Herd and Sprott, 1986) between 4 and 7 (see table 8 and 9) at the time of blood sampling as assessed on a scale from 1 (severe emaciation) to 9 (obese). The health of animals was monitored by veterinarians regularly. The animals were vaccinated against foot and mouth disease (Type O, A and Asia 1; DLD, Bangkok, Thailand) and were free from Brucellosis and Tuberculosis. They were treated for control of endoparasites twice a year. All cattle were accustomed to humans and procedure of blood sampling.

None of the cows, neither the crossbreds nor the Thai indigenous ones were milked or suckled during the experimental period.

2. Sample collections and measurements

Blood samples (15 ml each) were collected via the puncture of a jugular vein from each animal during November 2003 to February 2004 between 08:00 to 10:00 AM. During this period, the average ambient temperature and relative humidity was 24.2°C (night: 15.8±0.36°C; day: 33.28±0.29°C) and 72% respectively. The blood samples (10 ml each) were withdrawn in heparinized syringes containing 10% Na-Heparin (10 μl/1 ml blood). Samples were immediately centrifuged (1000 × *g*) for 20 min at 4°C. Plasma was separated and stored at –20°C until analyzed. Moreover, 5 ml blood from each animal was collected in a glass tube without heparinized then allowed the blood to clot for 30 min to 1 h at room temperature. Serum was then separated and stored at –20°C until analyzed. All analyses were conducted within 7 days.

Plasma concentrations of the enzymes aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and creatine kinase (CK) as well as glucose (GLU), urea (UR) and creatinine (CRT), were determined by standard enzymatic methods. Total serum protein (TP) and serum albumin (ALB) levels were determined by Biured method (Scoffone and Fontana, 1975) and Bromocressol-green method (Drupt *et al.*, 1974), respectively. All analyses were performed with a Hitachi Model 917 multichannel biochemical analyzer (Roche Diagnostics, IN, USA) for measurements of: GLU (in the range 0.3-55.5 mmol/l), UR (0.8-66.6 mmol/l), CRT (88-2210 μmol/l), ALB (2-100 g/l), TP (2-150 g/l), AST (4-800 U/l), ALT (4-400 U/l), ALP (3-1000 U/l), GGT (1-1,200 U/l) and CK (3-1500 U/l).

3. Statistical analysis

The 2 × 2 factorial in completely randomized design was used for statistical verification, with breed (Thai indigenous and Simmental × Brahman crossbred cattle) and sex (female and male) as effective factors. Each combination consisted of 45, 16, 33 and 18 replications, respectively. Least significant differences test (LSD; Harvey, 1975) was used for differences between groups. All statistical analyses were performed using SAS Program Package [Statistical Analysis System (SAS), 1998]. Values are presented as means±SE.

Experiment 4

Effect of temperature on growth hormone and nitric oxide secretion from peripheral bovine lymphocytes

1. Animals

Twenty-two mature (5.52±0.22 yr old, 2nd to 4th lactation) healthy lactating cows including 11 Holstein Friesian [HF; 821.00±9.36 kg (±SE) bodyweight (BW)] and 11 Schwarz Bunt or German Black and White cows (SBT; 672.50±19.81 kg BW) were used in this study during January – April, 2006. All dairy cows were provided indoors with a total mixed ration balanced to meet the requirements for their reproductive status (Institute for Animal Nutrition, Federal Agricultural Research Center, FAL, Braunschweig, Germany) and water was freely available at all times. Cows were milked at 05:30 and 14:30 h daily. Pregnancy was confirmed by transrectal sonography on day 45, after insemination. Animals were used in accordance with procedures approved by Animal ethic Committee, Germany.

The animals were divided into groups according to their reproductive status. Group 1 consisted of 9 pregnant animals (5 HF and 4 SBT) and Group 2 consisted of 13 cyclic animals (6 HF and 7 SBT).

2. Preparation of lymphocyte

Blood samples (350 ml each) were collected in EDTA-coated tubes by jugular venipuncture. Blood was centrifuged at 1,285 ×*g* for 20 min at 20°C; the buffy coat was harvested. This blood was subsequently layered on lymphocyte separating medium, Lymphodex (inno-TRAIN Diagnostik, D-61476 Kronberg/Taunus, Germany), and centrifuged at 800 ×*g* for 30 min at 20°C to enrich the mononuclear cells (Hattori *et al.*, 1990; Dixit and Parvizi, 2001). Upon enrichment, mononuclear cells (in following will be addressed to as lymphocytes or PBLs) were removed, resuspended, and washed twice with Hanks' balanced salt solution (HBSS).

Contaminating erythrocytes were lysed by hypotonic shock in double distilled water, followed by an immediate three times – wash with HBSS. Throughout the experimental period, each animal was subjected to blood sampling twice. 3 ml blood from each animal was colleted in a separate tube and was centrifuged at 1,710 ×*g* for 20 min at 4°C; the plasma was separated and stored at -20°C until analysis for GH.

3. Cell culture

Peripheral bovine lymphocytes (1.0×10^6 cells/ml) were resuspended in a combination of 1:1 RPMI 1640 (Sigma, Munich, Germany) and HBSS solutions with 1% antibiotic and antimycotic mixture containing 10,000 IU penicillin, 10 mg streptomycin, and 25 µg/ml amphotericin B (Sigma). The cells were cultured in medium at 37.2°C; 5% CO_2 and at 41.0°C; 5% CO_2 for 30 min. The cell viability as assessed by the trypan blue exclusion test was always greater than 95%. Subsequently, the cells were seeded in four-well culture plates (Nunc Brand Product, Roskilde, Denmark) in medium with 100 µl of fetal calf serum (Sigma, Munich, Germany) and then incubated in presence or absence of phytohemagglutinin from *Phaseolus vulgaricus* (PHA-M, Sigma; 100 µg/well). These cultures were incubated at 37.2°C; 5% CO_2 or at 41.0°C; 5% CO_2 for 72 h.

For each treatment, a total of 264 cell cultures were conducted (22 animals; three plates per each treatment; four wells per each plate). After the end of the incubation period (72 h), cell viability as assessed by the trypan blue exclusion test was more than 60%. One ml culture supernatant from one of the culture plates from each treatment was stored at -20°C for nitric oxide (NO) measurement.

4. Gel chromatography

The cell cultures from the three plates (11 wells) of one treatment were pooled and centrifuged at 800 × *g* at 20°C for 10 min. The 11 ml supernatant from the cultures were eluted for GH by chromatography using 1.5 × 30 cm Sephadex G-50

fine column. The column was equilibrated with 0.1 M PBS containing 5% BSA, and 26 aliquots of 1.5 ml each were collected and stored at -20 °C pending analysis. These samples were subsequently lyophilized using β1 lyophilizer (Christ, Osterode, Germany) and reconstituted in 300 µl of assay buffer at pH 7.4 containing 0.01 M PBS, 0.025 M EDTA, 0.25% BSA and 0.01% thimerosal (Dixit *et al*., 2003).

5. Growth hormone assay

A homologous double antibody was used for measurements of growth hormone in duplicate. This RIA (Bauer and Parvizi 1996) was adapted for GH measurements in cell culture medium (Poppi *et al.*, 2002; Dixit *et al.*, 2003). The first antibody, a specific, highly purified porcine GH (Biogenesis, Dorset, UK), with a potency of 1 × United States Department of Agriculture B-1 standard was used for iodination and as standard. A goat anti-rabbit antiserum (goat anti-rabbit, Biogenesis) was diluted 1:45 and utilized as second antibody. The antiserum shows no cross-reactions with other adenohypophysial hormones. All reagents were diluted in assay buffer (0.01 M PBS, 0.025 M EDTA, 0.01% thimerosal, 1% egg albumin, pH 7.4). GH was also measured in 100 µl aliquots of culture medium (not incubated with cells). This background level (0.7±0.1 ng/ml) was deducted from the level measured in each sample. Half maximum displacement (ED 50) was achieved at 6 ng/ml. The intra- and inter-assay coefficients of variation were 4.15% and 5.63%, respectively. GH was measured in all 26 aliquots from 11 ml of one treatment. For statistical evaluations, GH levels measured in fractions 9 to 12 were pooled. To evaluate the nature of GH secreted by lymphocytes, 100 ng of highly purified (PGH, Biogenesis) I^{125}-labeled porcine pituitary GH diluted in 1 ml of culture medium was eluted on a column similar to the one used for elution of media from cell culture. The profiles of the elution resembled those of media from cell cultures (Poppi *et al.*, 2002).

6. Nitric oxide (NO) measurement

In aqueous solutions that contain no heme proteins, NO is oxidized to nitrite only (Ignarro *et al.*, 1993), which can serve as an indirect marker for the presence of NO (Hirvonen *et al.*, 1997). All reagents were freshly prepared before each assay. Assay was performed as described previously (Dixit and Parvizi, 2001); briefly, total nitrite in sample was assayed using equal amounts of sample and Griess reagent [1% sulfanilamide and 0.1% *N*-(1-naphthylene) ethylenediamine in 5% concentrated phosphoric acid]. Amounts of nitrite were estimated from a standard curve of sodium nitrite, and the absorbance was measured at 540 nm spectrophotometrically with an assay sensitivity of 1 μM (Dixit *et al.*, 2003).

7. Statistical analysis

The results are expressed as the mean ± SE. The differences between means and the effects of treatments were determined by ANOVA followed by Tukey's test (SAS, 1998), which protects the significance of all pair combinations (Tukey, 1991).

RESULTS

Experiment 1 – Results

Meteorology

Maximum, minimum and average ambient temperatures (Table 1) were recorded at PC station followed by NK and LP ($P<0.05$; Table 1). Maximum, minimum and average RH and average THI were measured at PC station (Table 1). It was found that maximum RH and minimum THI were not significantly different among the three locations. Annual rainfall was significantly higher at LP than NK and PC stations ($P<0.05$; Table 1).

Table 1 Means±SE values of the meteorological data of the three locations during the period 1999–2002.

Parameter	PC	NK	LP
Average temperature (°C)	28.93±0.34[a]	28.00±0.26[b]	27.96±0.18[b]
Max temperature (°C)	34.10±0.42[a]	32.88±0.41[b]	32.74±0.27[b]
Min temperature (°C)	23.67±0.42	23.20±0.28	23.20±0.11
Average RH (%)	72.58±0.46[a]	70.46±1.30[ab]	69.58±1.19[b]
Max RH (%)	92.25±1.32	91.96±0.56	90.50±1.63
Min RH (%)	53.21±1.67[a]	48.54±2.49[b]	46.83±2.22[b]
Average THI	80.09±0.34[a]	78.38±0.54[b]	78.20±0.45[b]
Max THI	91.85±1.35[a]	89.44±1.67[b]	89.19±1.78[b]
Min THI	70.27±1.26	69.23±2.52	69.08±1.90
Rainfall/yr (mm)	1,317.53±38.76[c]	1,395.95±31.65[b]	1,481.23±26.34[a]

Means±SE within the same row with different superscripts were significantly different ($P<0.05$).

The seasonal meteorological variations of the three locations PC, NK, and LP are shown in Table 2.

Table 2 Means±SE values of the seasonal meteorological data of the three locations during the period 1999–2002.

Parameter	Summer			Rainy			Winter		
	PC	NK	LP	PC	NK	LP	PC	NK	LP
Average temp (°C)	30.5±0.4^{a}	29.6±0.5^{a}	29.8±0.2^{a}	28.9±0.3^{ab}	28.4±0.5^{b}	27.6±0.3^{b}	26.8±0.4^{c}	26.3+0.2^{c}	26.0±0.3^{c}
Max temp (°C)	35.4±0.5^{a}	35.2±0.2^{a}	34.7±0.4^{ab}	33.7±0.6^{b}	32.7±0.5^{c}	32.7±0.3^{c}	32.7±0.5^{c}	30.3±0.2^{d}	32.1±0.3^{c}
Min temp (°C)	24.2±0.5^{a}	24.3±0.4^{a}	24.8±0.3^{a}	22.8±0.5^{b}	24.1±0.4^{a}	22.5±0.3^{b}	20.8±0.6^{c}	21.5±0.4^{c}	21.5±0.4^{c}
Average RH (%)	74.7±0.6^{a}	70.7±1.1^{c}	70.5±1.2^{c}	75.0±1.0^{a}	73.2±0.8^{b}	73.9±1.1^{b}	67.4±0.7^{d}	68.2±0.6^{d}	65.2±0.8^{e}
Max RH (%)	92.0±1.2^{b}	91.1±0.7^{b}	90.4±1.3^{b}	94.2±1.3^{a}	91.7±1.4^{b}	92.4±1.2^{b}	88.4±0.7^{c}	88.8±0.9^{c}	88.8±1.1^{c}
Min RH (%)	52.5±1.6^{c}	50.4±1.8^{c}	40.4±1.5^{d}	56.7±1.6^{a}	54.3±1.8^{b}	56.4±2.2^{a}	43.2±1.7^{d}	42.4±2.2^{d}	41.7±2.2^{d}
Average THI	82.8±0.6^{a}	80.9±0.7^{b}	81.1±0.8^{b}	80.5±0.9^{b}	79.3±0.4^{bc}	78.2±0.5^{c}	76.2±1.2^{d}	75.5±0.6^{d}	74.8±0.6^{d}
Max THI	94.0±1.5^{a}	93.5±1.8^{a}	92.6±1.7^{a}	91.5±1.6^{ab}	89.4±1.8^{b}	89.5±1.3^{b}	88.7±1.5^{b}	84.8±1.6^{c}	87.8±1.9^{b}
Min THI	70.9±1.4^{a}	70.8±2.3^{a}	70.5±1.9^{a}	69.4±1.1^{a}	70.9±2.0^{a}	68.9±1.7^{a}	65.9±1.8^{b}	66.6±2.1^{b}	66.6±1.8^{b}
Rainfall (mm)	179.6±30.3^{d}	194.1±37.1^{d}	394.1±26.2^{c}	938.7±23.1^{ab}	990.3±24.2^{a}	905.9±20.2^{b}	199.2±36.4^{d}	211.6±30.6^{d}	181.2±42.3^{d}

Means±SE within the same row with different superscripts were significantly different ($P<0.05$).

Productivity of Thai Brahman and Kabinburi cows

Kabinburi cows had a significantly (P<0.01; Table 3) higher bodyweight than Thai Brahman cattle at birth as well as at 200, 400 and 600 days of age. Furthermore, K heifer gave birth to their first calf at a younger age and had a shorter calving interval than Thai Brahman cows (P<0.01).

Table 3 Means±SE values of productivity of Thai Brahman and Kabinburi cattle at NK center.

Parameter	Thai Brahman (n = 597)	Kabinburi (n = 351)
Birth weight (kg)	28.51±0.32[b]	32.16±0.55[a]
Weaning weight (kg)	142.82±2.28[b]	188.24±3.47[a]
Yearling weight (kg)	228.22±4.42[b]	296.97±7.28[a]
Weight at 20 months (kg)	348.75±13.26[b]	421.58±17.86[a]
Age at first calving (yr)	3.45±0.05[a]	2.73±0.07[b]
Calving interval (d)	502.52±5.37[a]	425.67±18.58[b]

Means±SE within the same row with different superscripts were significantly different (P<0.01).

Productivity of Thai Brahman cows at different locations

Thai Brahman (TB) cows kept at LP had significantly (P<0.05) higher bodyweight at 200 and 400 days of age than the animals of the same breed kept at NK. In contrast, bodyweight at birth and 600 days of age were not different. Thai Brahman cows kept at LP were younger at age of first calving (Table 4) and had a shorter calving interval than animals kept at NK (P<0.05).

Table 4 Means±SE values of productivity of TB cattle at different locations.

Parameter	LP (n = 719)	NK (n = 597)
Birth weight (kg)	28.43±0.23	28.51±0.32
Weaning weight (kg)	169.06±1.12[a]	142.82±2.28[b]
Yearling weight (kg)	251.12±3.14[a]	228.22±4.42[b]
Weight at 20 months (kg)	370.63±9.36	348.75±13.26
Age at first calving (yr)	3.09±0.06[b]	3.45±0.05[a]
Calving interval (d)	470.67±6.34[b]	502.52±5.37[a]

Means±SE within the same row with different superscripts were significantly different (P<0.05).

Productivity of Kabinburi cows at different locations

At birth, 200, 400 and 600 days of age Kabinburi (K) cattle kept at NK were significantly (P<0.01) heavier than those kept at PC (Table 5). In addition, K cows kept at NK were younger at first calving (P<0.01), but the calving interval was not significantly different between Kabinburi cows housed at different locations.

Table 5 Means±SE values of productivity of K cattle at different locations.

Parameter	NK (n = 351)	PC (n = 405)
Birth weight (kg)	32.16±0.55[a]	30.56±0.47[b]
Weaning weight (kg)	188.24±3.47[a]	169.07±2.97[b]
Yearling weight (kg)	296.97±7.28[a]	244.73±4.07[b]
Weight at 20 months (kg)	421.58±17.86[a]	349.12±8.38[b]
Age at first calving (yr)	2.73±0.07[b]	3.01±0.08[a]
Calving interval (d)	425.67±18.58	448.37±12.63

Means±SE within the same row with different superscripts were significantly different (P<0.01).

Experiment 2 – Results

The results revealed five Hb types in Thai indigenous cows as follows: HbAA (35.59%), HbAB (28.81%), HbAC (20.34%), HbBB (11.6%) and HbBC (3.39%). In the Simmental × Brahman (SB) crossbred cattle, three Hb types, 50.98% HbAA, 45.10% HbAB and 3.92% HbBB, were present. The observed distributions of genotypes and frequencies of alleles within these breeds are shown in Table 6.

Table 6 Distribution of haemoglobin genotypes and frequencies of alleles in Thai indigenous (TI) and Simmental × Brahman (SB) crossbred cattle.

Breed	No.	Genotypes						Gene frequency		
		AA	AB	AC	BB	BC	CC	Hb^A	Hb^B	Hb^C
TI	59	21	17	12	7	2	-	0.60	0.28	0.12
	(%)	(35.59)	(28.81)	(20.34)	(11.86)	(3.39)	-			
SB	51	26	23	-	2	-	-	0.74	0.26	-
	(%)	(50.98)	(45.10)	-	(3.92)	-	-			

Table 7 shows the performances of Thai indigenous cows with different Hb types. Animals with HbAB type were heavier at birth, however, the best growth performance was recorded in HbAC animals. In contrast, HbBB type animals were the lightest ($P<0.05$) animals at birth and they were the youngest group ($P<0.05$) at first calving. This group of Thai indigenous cows was more than 4 months younger at first calving than the heifers with HbAA type.

Table 7 Effects of haemoglobin phenotypes on production performance of Thai indigenous cattle.

Parameter	HbAA	HbAB	HbAC	HbBB
BW at birth (kg)	16.32±0.32^{ab}	16.36±0.23^{a}	15.56±0.31^{b}	15.08±0.55^{b}
BW at 200 days (kg)	85.39±1.99^{b}	76.82±1.45^{c}	94.94±1.92^{a}	72.42±3.39^{c}
BW at 400 days (kg)	117.51±2.57^{b}	108.82±1.87^{c}	125.19±2.48^{a}	100.92±4.37^{c}
BW at 600 days (kg)	140.92±1.75^{a}	133.25±1.28^{b}	143.69±1.69^{a}	129.92±2.98^{b}
Age at first calving (mo)	28.71±0.48^{a}	27.21±0.48^{b}	27.75±0.64$^{a\ b}$	24.50±0.74^{c}

Means±SE within the same row with different superscripts were significantly different ($P<0.05$).

The results from Simmental × Brahman crossbred cows with HbAA and HbAB types were statistically verified. As indicated in Figure 2, animals with HbAA type [32.10±0.90 kg bodyweight (BW)] were significantly (P<0.05) heavier than the animals with HbAB (30.10±0.90 kg BW) at birth as well as on days 200 (208.00±2.70 kg BW vs. 197.20±2.90 kg BW), 400 (269.90±6.50 kg BW vs. 249.90±6.90 kg BW) and 600 (361.50±11.80 kg BW vs. 329.60±12.50 kg BW) of age. Whereas, the animals with HbAB type gave birth to their first calf at a significantly (P<0.05) younger age than those with HbAA type (Figure 3).

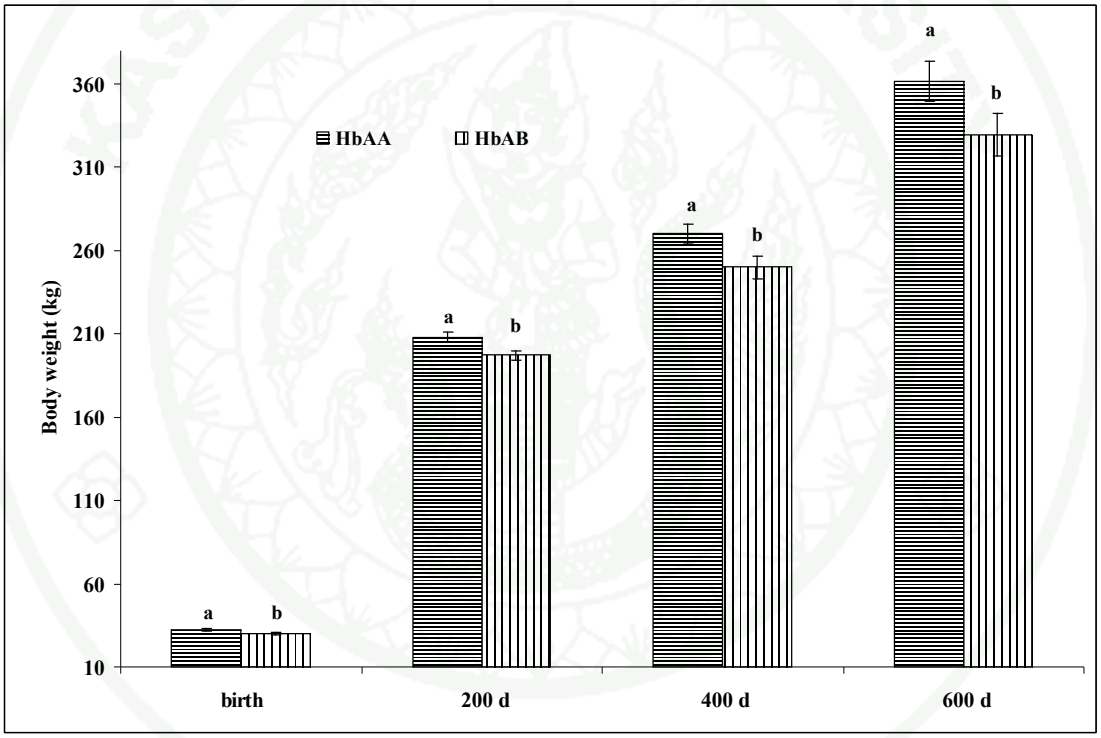

Figure 2 Effects of haemoglobin phenotypes on growth performance of Simmental × Brahman crossbred cattle. Values are expressed as means±SE,
a = P<0.05 vs. b at birth, 200, 400 and 600 days of age; GLM followed by LSD test.

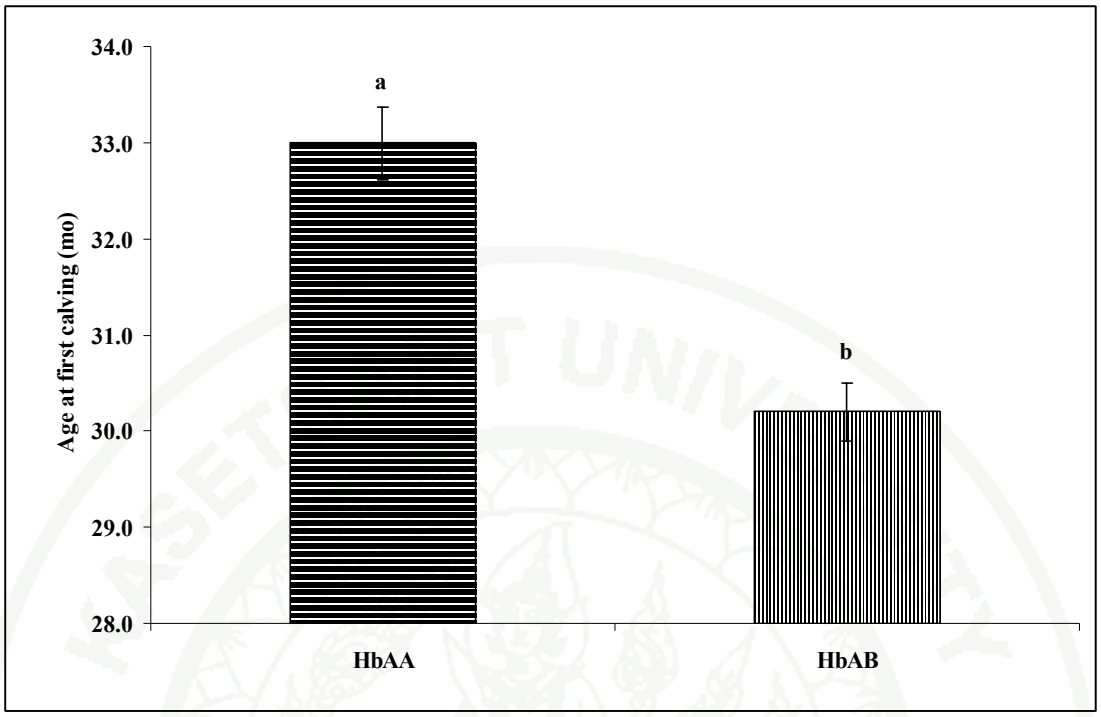

Figure 3 Effects of haemoglobin phenotypes on age at first calving of Simmental × Brahman crossbred cattle. Values are expressed as means±SE, a = P<0.05 vs. b; GLM followed by LSD test.

Experiment 3 – Results

There were no significant differences in age and body condition score (BCS) between the two breeds (Table 8 and 9). BCS of the animals ranged between 4 and 7 with an average of 5.4±0.2 in the crossbred and 5.6±0.2 in the Thai indigenous cattle. The levels of plasma glucose (GLU) and gamma-glutamyl transferase (GGT) in the two breeds were significantly ($P<0.05$) different (Table 1). Furthermore, the plasma concentrations of urea (UR), creatinine (CRT), aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP), and the serum levels of albumin (ALB) and total serum protein (TP) in Thai indigenous were significantly ($P<0.01$) higher than in Simmental × Brahman crossbred (Table 8). Whereas, the creatine kinase (CK) levels did not significantly differ in crossbred and indigenous animals (Table 8).

Table 8 Comparisons of plasma levels of metabolites and proteins between Thai indigenous and Simmental × Brahman crossbred cattle.

Parameter	Thai indigenous (n = 61)		Simmental × Brahman crossbred (n = 51)		*P* > |t|	Reference range
	Mean±SE	Range*	Mean±SE	Range*		
Age (year)	4.2±0.1	2.8-5.3	4.1±0.1	2.8-5.0	0.3810	-
BCS	5.6±0.1	4.0-7.0	5.4±0.1	4.0-7.0	0.4590	-
GLU (mmol/l)	3.9±0.1	3.6-4.3	3.6±0.1	3.4-3.8	0.0481	2.5-4.2[1]
UR (mmol/l)	10.6±0.3	10.0-11.8	9.1±0.4	6.8-10.4	0.0036	7.1-10.7[1]
CRT (μmol/l)	141.4±2.6	132.6-150.3	114.9±4.4	92.8-147.6	<0.0001	88.4-177[1]
ALB (g/l)	32.0±0.4	28.0-34.0	25.7±0.5	23.0-27.0	<0.0001	27-39[2]
TP (g/l)	87.0±0.9	82.0-89.2	76.3±0.9	72.0-88.0	<0.0001	61-81[2]
AST (U/l)	81.2±1.9	77.2-90.0	68.6±2.4	63.4-77.0	<0.0001	78-132[1]
ALT (U/l)	32.6±0.8	30.5-33.7	20.4±0.7	16.6-22.8	<0.0001	11-40[1]
ALP (U/l)	120.2±6.1	96.2-176.9	166.5±13.4	83.2-214.8	0.0025	0-488[1]
GGT (U/l)	17.0±0.7	13.6-18.5	14.5±0.7	11.9-18.9	0.0172	6.1-17.4[1]
CK (U/l)	209.5±12.4	183.4-276.6	192.8±18.7	156.1-230.4	0.4587	0-200[3]

*Range is the mean ± 2 SD of the respective levels.

[1] Kaneko *et al.* (1997); [2] Jain (1996); [3]Radostits *et al.* (1994)

Statistical analysis revealed interactions between sex and breed ($P<0.05$). Plasma levels of GLU in the male Thai indigenous were significantly higher ($P<0.05$) than in other three groups (Table 9). Plasma urea concentration in the male Simmental × Brahman crossbred was lower than in the other group ($P<0.05$). The female Simmental × Brahman crossbred had significantly ($P<0.05$) lower CRT than other animals. Furthermore, serum levels of ALB in the male and TP in the female Simmental × Brahman crossbred were the lowest ($P<0.05$) among the groups.

Table 9 Plasma levels of metabolites, serum proteins and the activity of enzymes in the cattle.

Parameter	Thai indigenous		Simmental × Brahman crossbred	
	female	male	female	male
No. of animals	45	16	33	18
Age (yr)	4.2±0.1	4.1±0.2	4.0±0.1	4.1±0.1
BCS	5.5±0.1	5.6±0.2	5.4±0.1	5.4±0.2
GLU (mmol/l)	3.7 ± 0.1^{b}	4.1 ± 0.2^{a}	3.7 ± 0.1^{b}	3.5 ± 0.1^{b}
UR (mmol/l)	10.4 ± 0.4^{a}	11.2 ± 0.6^{a}	10.0 ± 0.4^{a}	7.4 ± 0.6^{b}
CRT (μmol/l)	141.4 ± 3.5^{a}	144.1 ± 6.2^{a}	97.2 ± 4.4^{b}	141.4 ± 6.2^{a}
ALB (g/l)	33.4 ± 0.5^{a}	29.1 ± 0.8^{a}	26.4 ± 0.6^{b}	24.5 ± 0.8^{c}
TP (g/l)	88.2 ± 1.0^{a}	83.8 ± 1.6^{b}	73.2 ± 1.1^{c}	81.3 ± 1.5^{b}
AST (U/l)	79.5 ± 2.3^{ab}	86.1 ± 3.9^{a}	66.1 ± 2.7^{c}	73.3 ± 3.7^{b}
ALT (U/l)	32.8 ± 0.9^{a}	32.0 ± 1.5^{a}	21.8 ± 1.0^{b}	18.0 ± 1.4^{c}
ALP (U/l)	105.6 ± 9.4^{bc}	161.1 ± 15.8^{b}	203.8 ± 11.0^{a}	98.1 ± 14.9^{c}
GGT (U/l)	17.7 ± 0.8^{a}	14.9 ± 1.3^{ab}	12.8 ± 0.9^{b}	17.7 ± 1.2^{a}
CK (U/l)	200.9±17.5	242.5±34.1	197.6±19.7	183.5±27.4

Means±SE in the same row with different superscripts were significantly different ($P<0.05$).

From Table 9 it can be seen that there were no significant differences in plasma CK levels between males and females. However, plasma levels of AST, ALT, ALP and GGT were significantly ($P<0.05$) different between males and females. The female crossbred cattle had the lowest ($P<0.05$) AST and GGT levels, whereas the lowest ($P<0.05$) ALT and ALP concentrations were determined in male individuals of these breeds (Table 9).

Experiment 4 – Results

GH levels in plasma

As indicated in Figure 4, the pregnant HF and SBT cows had significantly ($P<0.01$) lower plasma GH levels than cyclic SBT animals. GH secretion in cyclic SBT cows was 7.71±0.22 ng/0.5 ml, which decreased to 6.52±0.40 ng/0.5 ml in pregnant SBT cows. Likewise, the levels of plasma GH in cyclic HF cows were 7.27±0.31 ng/0.5 ml and decreased to 6.91±0.41 ng/0.5 ml in pregnant HF cows. However, GH levels of HF cows were not significantly different between cyclic and pregnant groups.

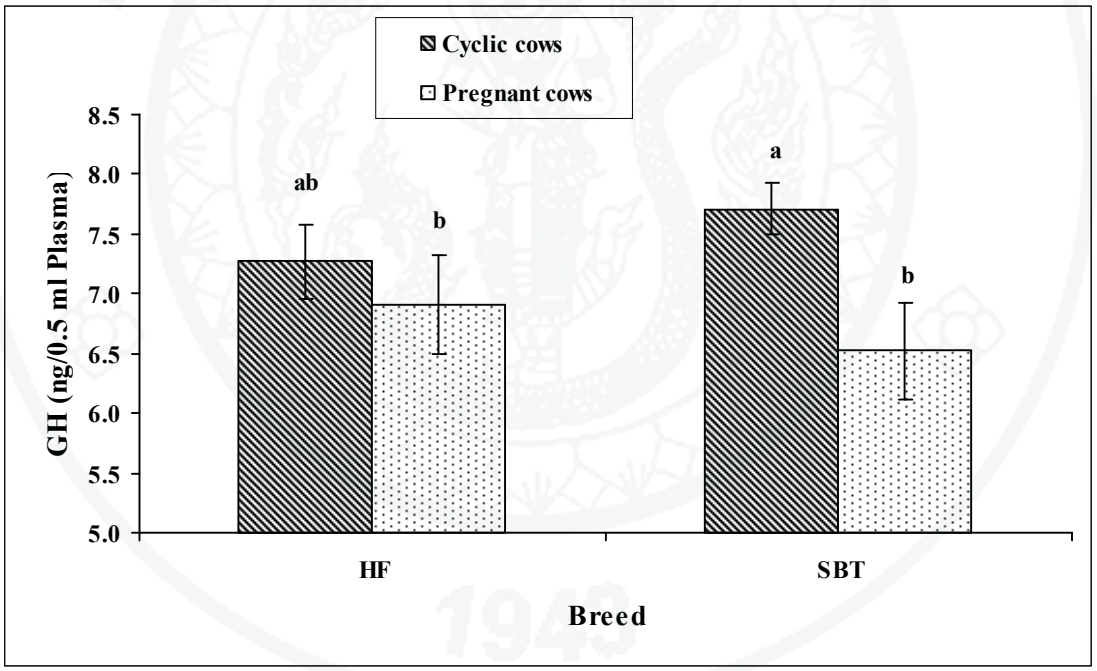

Figure 4 GH levels in plasma of HF and SBT cows expressed as mean±SE; a vs. b = $P<0.01$ (two – way ANOVA followed by Tukey's test).

GH secretion from PBLs of cyclic HF cows at different temperature

As indicated in Figure 5, un-stimulated PBLs (control) of cyclic HF cows in the culture secreted 31.47±1.04 ng/ml GH at the temperature of 37.2°C and 29.52±1.45 ng/ml at 41.0°C. This secretion increased significantly ($P<0.05$) when PBLs were incubated with PHA-M. In the presence of PHA-M the lymphocytic GH secretion increased to 35.70±1.15 ng/ml at 37.2°C and 35.82±2.60 ng/ml at 41.0°C.

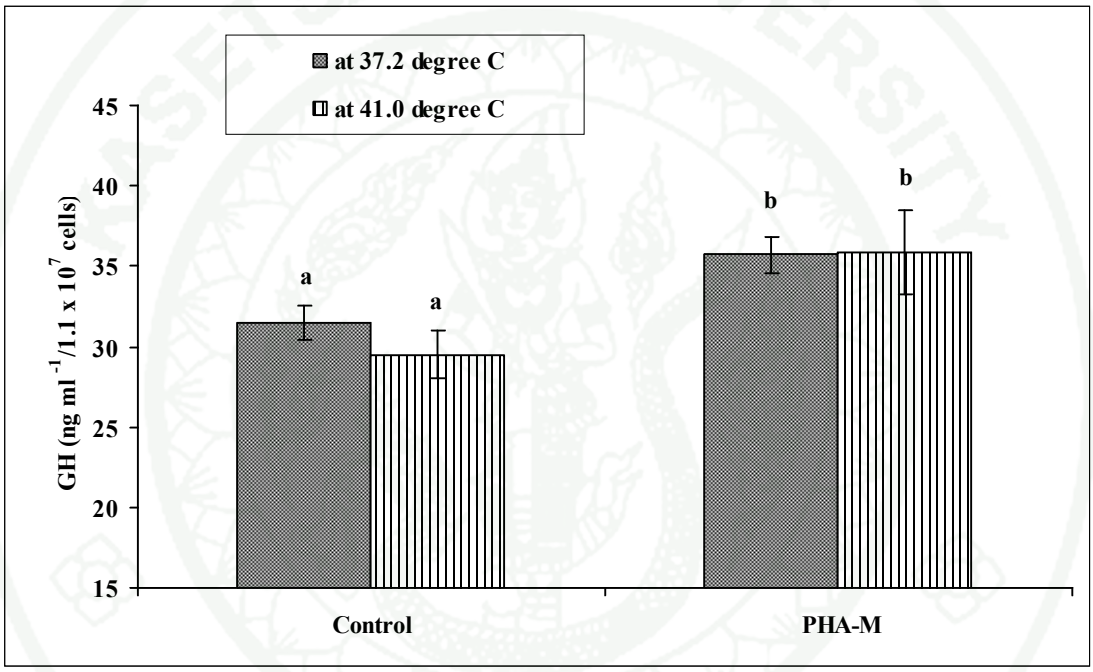

Figure 5 Secretion of GH by un-stimulated (control) and PHA-M – stimulated PBLs from cyclic HF cows; values are expressed as mean±SE; a vs. b = $P<0.05$ (two – way ANOVA followed by Tukey's test).

GH secretion from PBLs of cyclic SBT cows at different temperature

As shown in Figure 6 un-stimulated PBLs (control) cultures from cyclic SBT cows secret at temperature of 37.2°C 30.37±1.24 ng/ml GH The release significantly ($P<0.001$) increased (37.83±0.96 ng/ml) when the cells were incubated with PHA-M. At 41.0°C, un-timulated PBLs (control) secreted 29.52±1.45 ng/ml of GH. This secretion increased significantly ($P<0.05$) in PBLs incubated with PHA-M (37.18±2.45 ng/ml).

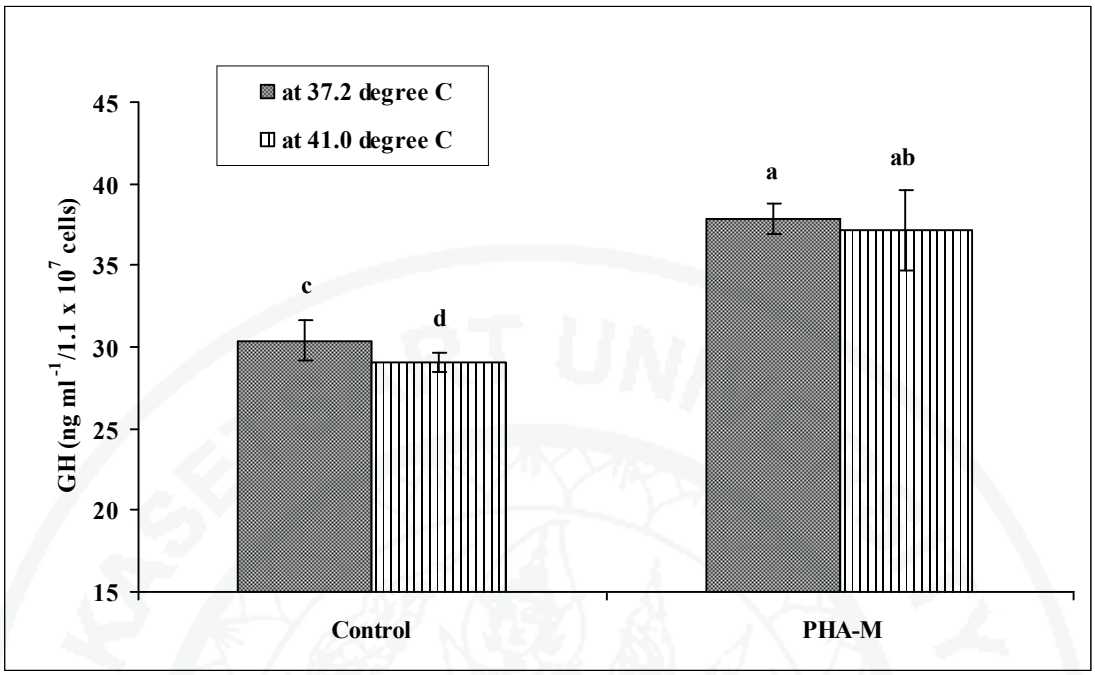

Figure 6 Secretion of GH by un-stimulated (control) and PHA-M – stimulated PBLs from the cyclic SBT cows; values are expressed as mean±SE; b vs. c and d = $P<0.05$; c vs. d = $P<0.05$; a vs. c and d = $P<0.001$ (two – way ANOVA followed by Tukey's test).

Comparison of GH production by PBLs of cyclic HF and SBT cows

At the temperature of 37.2°C, as well as at 41°C the GH secretion from un-stimulated PBLs of cyclic HF (31.47±1.04 ng/ml) and cyclic SBT (30.37±1.24 ng/ml) were not significantly different (Figure 5 and 6).

GH secretion from PBLs of pregnant HF cows at different temperature

The basal GH secretion from PBLs obtained from pregnant HF cows was at 37.2°C and at 41.0°C 33.68±4.31 and 32.72±1.55 ng/ml, respectively. When the PBLs were incubated with PHA-M, GH secretion was increased significantly to 37.72±3.40 ng/ml at 37.2°C and to 36.66±1.80 ng/ml at 41.0°C (Figure 7).

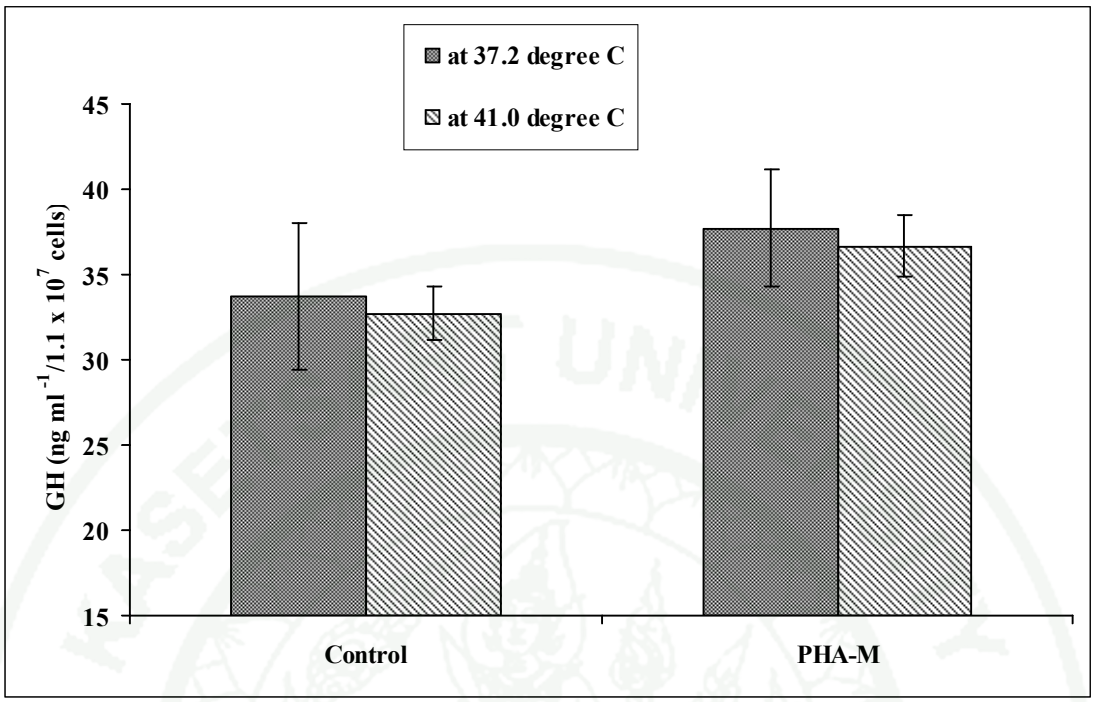

Figure 7 Secretion of GH by un-stimulated (control) and PHA-M – stimulated PBLs from pregnant HF cows in the culture at different temperature; values are expressed as mean±SE; No significant differences were observed, two – way ANOVA followed by Tukey's test.

GH secretion from PBLs of pregnant SBT cows at different temperature

Figure 8 shows that un-stimulated PBLs (basal secretion) of pregnant SBT cows in the culture at 37.2°C secret 32.20±2.42 ng GH/ml. When the lymphocytes were incubated with PHA-M, the GH secretion was significantly ($P<0.05$) increased to 39.10±1.90 ng/ml. At 41.0°C, un-stimulated PBLs secreted 29.02±1.24 ng/ml of GH. This secretion increased significantly ($P<0.05$) when PHA-M was added to the culture media (33.88±1.10 ng/ml). Although, there was no significant effect of temperature on GH secretion from un-stimulated PBLs, the effect of PHA-M was temperature dependent in pregnant SBT cows (Figure 8).

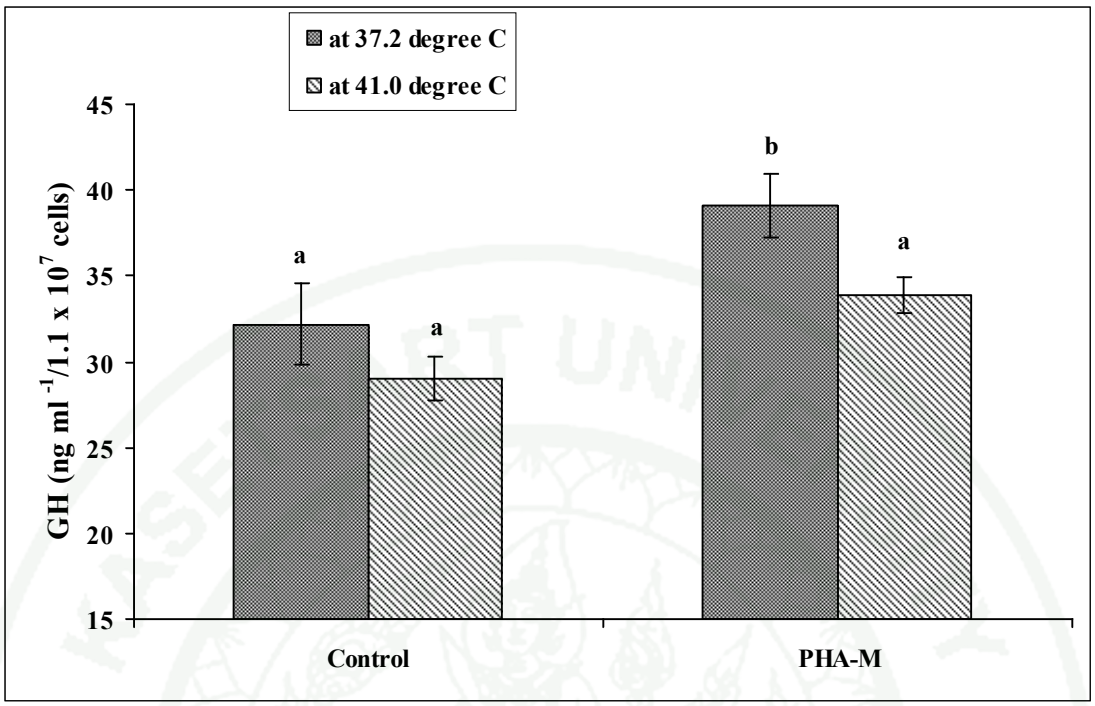

Figure 8 Secretion of GH by un-stimulated (control) and PHA-M – stimulated PBLs from pregnant SBT cows; values are expressed as mean±SE; a = P<0.05 vs. b; two – way ANOVA followed by Tukey's test.

Comparison of GH production by PBLs of pregnant HF and SBT cows

At 37.2°C, there was no significant difference in basal or PHA-M –stimulated GH release from PBLs harvested from pregnant HF and SBT cows (Figure 7 and 8).

At 41.0°C, it was found that basal GH levels of the pregnant HF (32.72±1.55 ng/ml) were significantly (P<0.05) higher than that of the pregnant SBT (29.02±1.24 ng/ml) cows. However, GH levels from PBLs incubated with PHA-M did not significantly differ between pregnant HF (36.66±1.80 ng/ml) and pregnant SBT (33.88±1.10 ng/ml) cows (Figure 9).

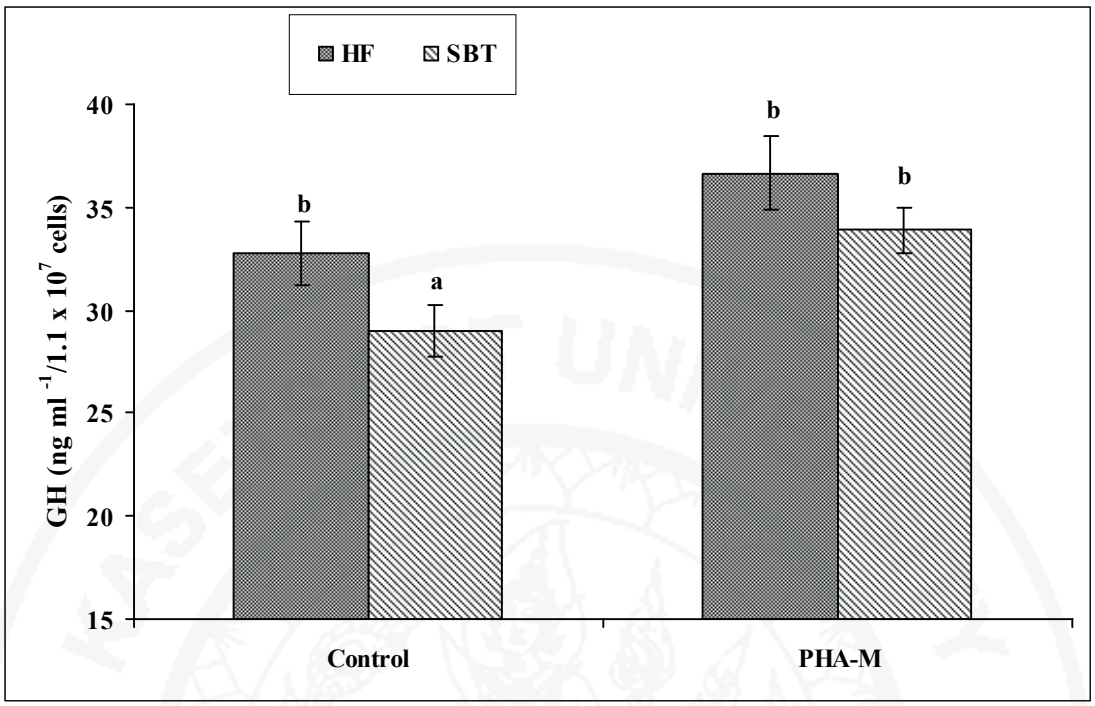

Figure 9 Comparison of GH levels from un-stimulated (control) and PHA-M – stimulated PBLs cultured at 41.0°C; values are expressed as mean±SE; a vs. b = P<0.05 (two – way ANOVA followed by Tukey's test).

Production of NO by PBLs of cyclic HF cows at different temperatures

Nitrite levels were measured in culture supernatants as an indirect measure of nitric oxide production. Measurement of nitrite from lymphocyte-conditioned medium at 37.2°C in the cyclic HF cows (Figure 10) showed a significant increase (P<0.001) in presence of PHA-M when compared to that of un-stimulated PBLs. Un-stimulated PBLs had a nitrite production of 14.13±2.47 μM/L, which increased to 36.40±1.46 μM/L after treatment with PHA-M.

At 41.0°C (Figure 10), un-stimulated PBLs in the culture produced 24.24±1.11 μM/L of NO. This value was significantly (P<0.01) higher than the level obtained at 37.2°C. Levels obtained in response to PHA-M at 41.0°C (29.24±0.92 μM/L) were lower (P<0.01) than the levels measured after PHA-M treatment at 37.2°C.

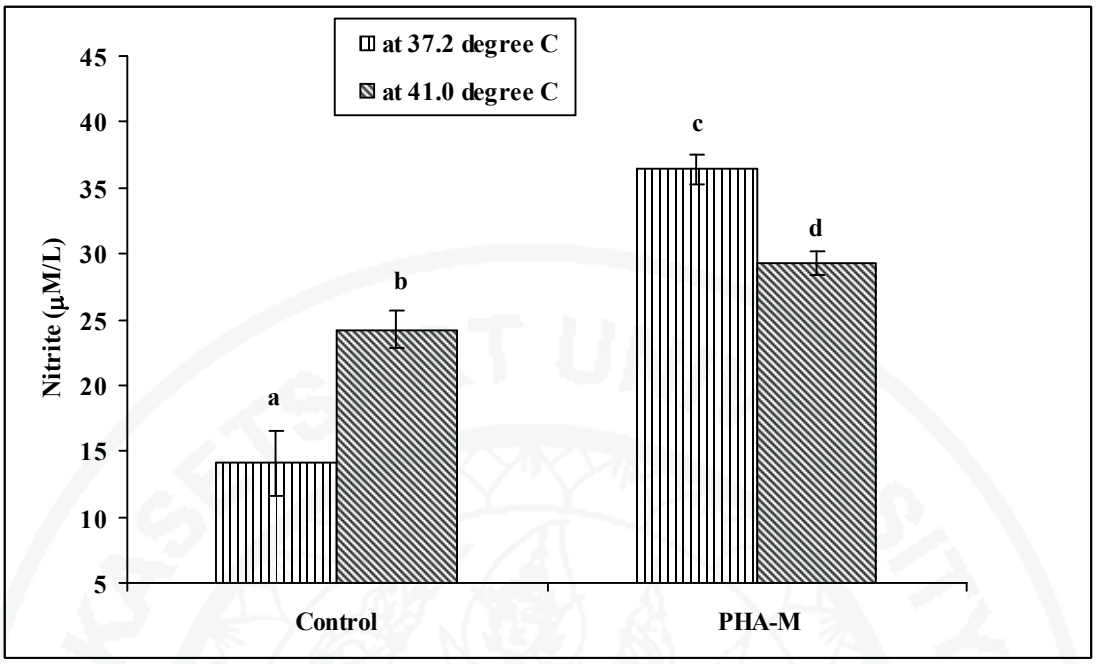

Figure 10 Production of NO from un-stimulated (control) and PHA-M-stimulated PBLs of cyclic HF cows at different temperatures; values are expressed as mean±SE; a = P<0.01 vs. b, a = P<0.001 vs. c, b = P<0.01 vs. d, c = P<0.01 vs. d (two – way ANOVA followed by Tukey's test).

Production of NO by PBLs of cyclic SBT cows at different temperatures

Figure 11 shows that in cyclic SBT cows, the secretion of NO (32.10±1.95 µM/L) by PBLs incubated with PHA-M was significantly (P<0.001) higher than that of un-stimulated PBLs (18.89±1.74) at 37.2°C. Interestingly, NO production by PBLs at 41.0°C was not significantly different between control (24.05±2.62 µM/L) and PHA-M treatment (27.09±1.16 µM/L).

The un-stimulated PBLs released at 41.0°C 24.24±1.11 µM/L of NO. This value was significantly (P<0.05) greater than the secreted amount of NO at 37.2°C (18.89±1.74 µM/L). In contrast, incubated PBLs with PHA-M at 41.0°C produced less NO (27.03±1.16 µM/L, P<0.01) when compared to the levels obtained after incubations with PHA-M at 37.2°C (32.10±1.95 µM/L) as in cyclic HF cows (Figure 11).

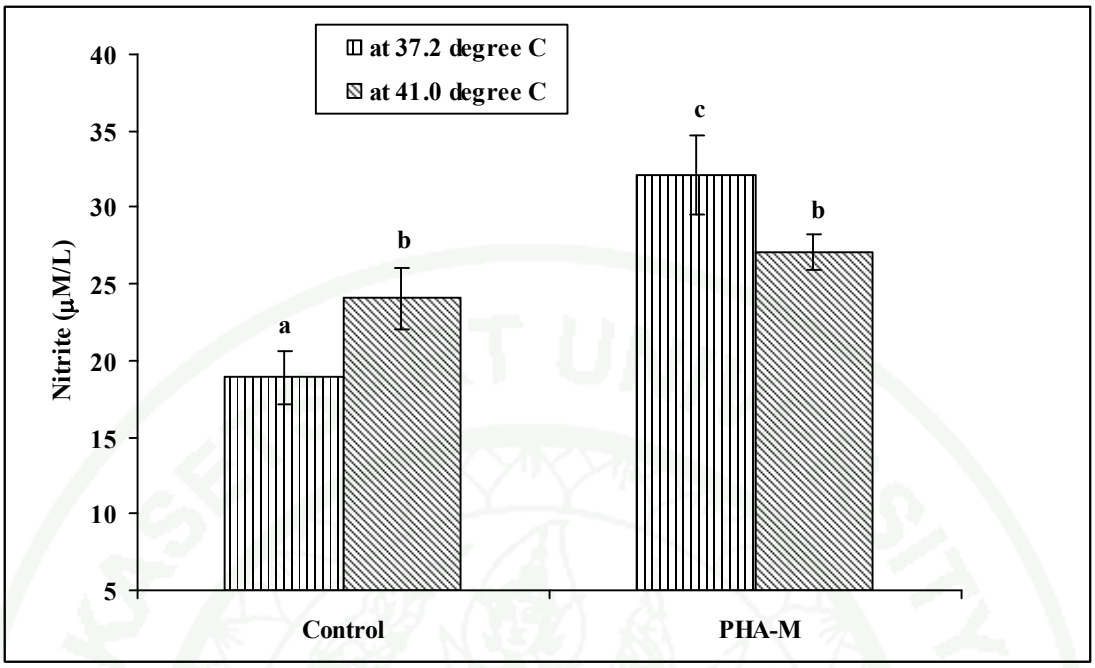

Figure 11 Production of NO from un-stimulated (control) and PHA-M – stimulated PBLs of the cyclic SBT cows in the culture at different temperature; values are expressed as mean±SE; a = P<0.05 vs. b, a = P<0.001 vs. c and b = P<0.05 vs. c within incubation with PHA-M (two – way ANOVA followed by Tukey's test).

Production of NO by PBLs of pregnant HF cows at different temperature

As indicated in Figure 12 NO production (33.08±2.36 µM/L) after, incubation with PHA-M at 37.2°C, (15.42±5.37µM/L) was significantly (P<0.001) higher than those of control incubations. At 41.0°C, basal NO release (25.66±4.07 µM/L) was not different from NO secretion in response to PHA-M (31.03±1.00 µM/L). Moreover, this study indicates (Figure 12) that NO levels of un-stimulated PBLs (basal levels) at 37.2°C were significantly lower than the basal levels at 41.0°C (P<0.05).

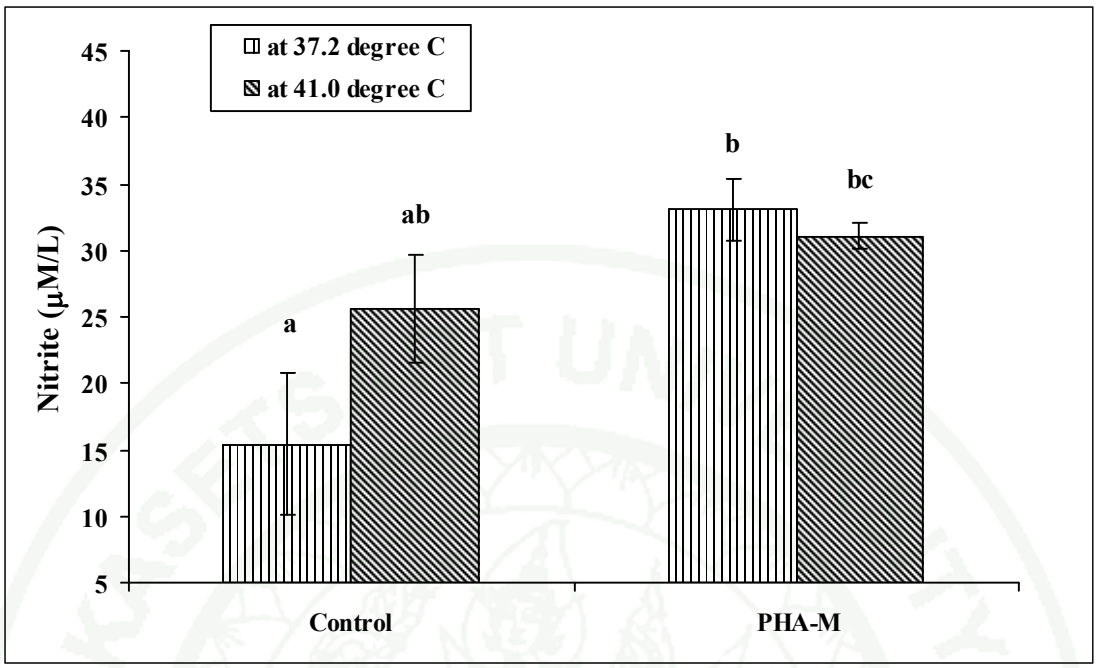

Figure 12 Production of NO from un-stimulated (control) and PHA-M – stimulated PBLs of the pregnant HF cows in the culture at different temperature; values are expressed as mean±SE; a = P<0.001 vs. b (control at 37.2°C vs. PHA-M at 37.2°C) and a = P<0.05 vs. c (control at 41.0°C vs. PHA-M at 41.0°C) (two – way ANOVA followed by Tukey's test).

Production of NO by PBLs of pregnant SBT at different temperature

From Figure 13 it can be seen that NO production by PBLs of pregnant SBT incubated with PHA-M in the culture at 37.2°C (17.90±4.01 µM/L) was significantly (P<0.05) higher than those of the control (33.18±1.44 µM/L). At 41.0°C, the NO secretion after PHA-M treatment (29.43±1.06 µM/L) was not different from control levels (24.30±4.28 µM/L).

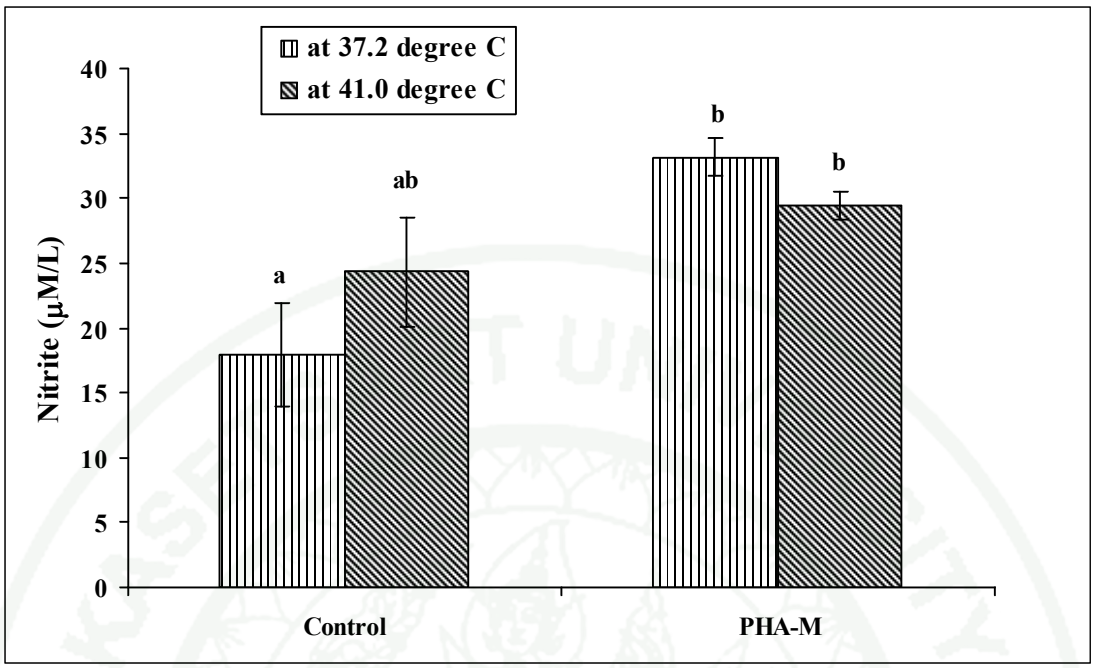

Figure 13 Production of NO from un-stimulated (control) and PHA-M – stimulated PBLs of pregnant SBT cows in the culture at the different temperature; values are expressed as mean±SE; a = P<0.05 vs. b (two – way ANOVA followed by Tukey's test).

Comparison of production of NO by PBLs of cyclic cows

At 37.2°C the basal NO production from PBLs of cyclic HF cows (14.13±2.41 µM/L) was not significantly different from that of cyclicSBT cows (18.89±1.74 µM/L). Likewise, at 41.0°C, the NO release did not differ between cyclic HF (24.24±1.11 µM/L) and SBT (24.05±2.62 µM/L) cows.

Further, NO production from PBLs treated with PHA-M at 37.2°C as well as 41°C was not significantly different between cyclic HF (36.40±1.46 µM/L) and SBT (32.10±1.95 µM/L) cows.

Comparison of production of NO from PBLs of pregnant cows

We could not observe any differences in lymphocytic NO release between pregnant HF and pregnant SBT cows. The effect of PHA-M and the action of temperature on in vitro NO release was similar in these two breeds.

DISCUSSION

Experiment 1 – Discussion

Prachinburi Livestock Breeding Station had the highest average and maximum ambient temperature followed by NK and LP. However, ambient temperature at all locations in summer and rainy seasons exceeded the upper critical temperature of 27°C given for *B. indicus* (Bligh and Johnson, 1973). The average THI values were also over the critical value of 75 given for beef cattle (St-Pierre *et al.*, 2003).

The seasonal meteorological variations indicate that presumably the animals were only during wintertime and nights not under heat stress. High environmental temperature stimulates the peripheral thermal receptors resulting in a suppression of nerve impulses to the centers controlling the appetite in the hypothalamus. This would cause a decrease in feed consumption (Habeeb *et al.*, 1992; Brosh *et al.*, 1998; Mader, 2003). Furthermore, Yousef (1985) reported that high ambient temperature could influence thyroid activity both directly and indirectly resulting in attenuation of appetite (Johnson, 1987).

Central Thailand gets almost all the annual rainfall during the monsoon season between July and October. The dry season stretches for 6-8 months and can be generally divided into two phases; cool and dry (November to February) and hot and dry (March to June). The first part of the dry season is characterized by food shortage with low quality of the food. The later part of the dry season is the most critical with limited quantity and quality of food (Crowder and Cheda, 1982). Rainfall is the most important factor controlling the composition and productivity of a pasture (Butterworth, 1985). Lamphayaklang had the highest annual rainfall followed by NK and PC. The low rainfall at NK and PC during the summer can result in poor nutritive values of the food (Crowder and Cheda, 1982). In USA, economic losses are incurred by the livestock industries when farm animals are raised in locations where effective temperature conditions venture outside their zone of thermal comfort. St-Pierre *et al.* (2003) reported that beef cows and finishing cattle kept in Kansas (heat stress = 925

h/year; max THI = 83.3), Oklahoma (heat stress = 1,504 h/year; max THI = 85.9) and Texas (heat stress = 1,991 h/year; max THI = 85.4) were, in fact, production losses. These authors (St.-Pierre *et al.*, 2003) report that reduction of dry matter intake is 23.0, 42.7 and 56.6 kg/head/year in Kansas, Oklahoma and Texas, respectively and growth loss is 6.9, 12.8 and 17.0 kg/head/year in Kansas, Oklahoma and Texas, respectively. The importance of heat stress to livestock industry is increasing with the time, because of the global warming and because animals with greater genetic potential for growth produce more body heat due to their greater metabolic activity (West, 1994).

Kabinburi cattle have, in present study, significantly higher bodyweight at birth as well as at 200, 400 and 600 days of age than Thai Brahman cattle. It is likely that Kabinburi cattle profit from as a heterosis effect, resulting in an increase in growth rate and fertility (Koger, 1980; Turner, 1980) and lower calving interval than Brahman cows. However, it is also possible that Kabinburi as a new synthetic breed possesses a better heat tolerance and adaptation capacity to the tropical climate than the Brahman breed. Likewise, comparative maternal productivity of beef breeds evaluated in Botswana showed that Simmental crossbred had a significantly higher bodyweight than Brahman cattle at birth as well as at weaning and 18 months (Trail *et al.*, 1977). These authors report that birth weight is 36.9 and 27.0 kg, and weaning weight is 214 and 182 kg in Simmental crossbred and Brahman cattle, respectively. The 18-month weight of Simmental crossbred and Brahman cattle at 325 and 305 kg, respectively. In Swaziland, Vilakati (1990) reported that Simmental crossbred (444 days) had a shorter calving interval than Brahman cows (472 days), but the age at first calving was not significantly different between both groups.

Thai Brahman cattle raised at LP station had higher growth rate and a better reproductive performance than those raised at NK. In addition, Kabinburi cattle kept at NK had higher bodyweight at birth, at 200, 400, and 600 days of age and were younger at first calving than the animals kept at PC. It could be due to the fact that the animals kept in locations with high temperature and low annual rainfall would only have a low quality food available. In general, beef calves are raised with their dams

until approximately 7 months. After weaning, more roughage and supplements will be provided. Post-weaning growth rate has been usually found negatively correlated with the age at the puberty (Bergfeld *et al*., 1994). Responsible for this seems to be the insufficient quality and quantity of the food which limits the growth rate and subsequently causes the late onset of puberty (Fox *et al.*, 1988). Heifers with a greater growth rate are younger and heavier at puberty than heifers fed a lesser amount of energy (Yelich *et al.*, 1995). They would be also more fertile at puberty (Murphy *et al.*, 1991; Bergfeld *et al.*, 1994). Furthermore, the nutrient intake before and after parturition, can influence the interval from calving to the first ovulation. The follicular growth after the calving is also influenced by the energy intake (Spitzer *et al.*, 1995; Bossis *et al.*, 2000).

In conclusion, the demand for high quality beef has been increasing due to the change in the socio-economic pattern of population in Thailand. The market system in the country demands animals that produce heavy carcasses with less fat trims. Animals with high fertility and efficiency in growth performance are preferred. This survey confirms that Kabinburi cattle is an ideal Thai beef cattle for high production under tropical climate.

Experiment 2 - Discussion

Several studies report on Hb types in cattle (Bangham and Blumberg, 1958; Osterhoff, 1975; Han and Suzuki, 1976; Bachmann *et al.*, 1978), however, very little is known about Hb types of Thai cattle breeds. A study on Korean cattle (Han and Suzuki, 1976) verified eight Hb types in this breed: HbAA type (80.10%), HbAB (16.40%), HbAC (1.50%), HbBB (1.10%), HbBC (0.20%), HbAH (0.50%), HbCH (0.10%) and HbHH (0.10%). Bachmann *et al.* (1978) studied the frequency of haemoglobin types in several major breeds of cattle in northern Australia. All *B. taurus* cattle examined carried the three common bovine types (AA, AB and BB). While, F_2 Africander cross-breeds represented the AA patterns only. The frequency of haemoglobin B was significantly higher in *B. indicus* type cattle than in *B. taurus* breeds. In the pure Banteng cattle (*B. banteng*) three genotypes (BB, CB and CC) were present, whereas in Brahman × Banteng cross a further two genotypes (AB and AC) were detected. In Thailand, Sangsomrit (2002) reported three haemoglobin types in beef cattle (50% Charolais × 25% Brahman × 25% Indigenous cattle) were HbAA, HbAB and HbBB. Our results support these finding and indicate that Thai indigenous and Simmental × Brahman crossbred cattle in Thailand have several gene diversities of Hb types.

The relationship between Hb types and productivity of beef cattle is not reported previously. Nevertheless, data obtained from other species such as ewes show that the variants of haemoglobin were significantly correlated to the reproduction (Dally *et al.*, 1980). It was found that HbBB ewes had the highest and HbAA ewes had lowest prolificacy in terms of lambs born per ewe exposed to mating, total number of lambs weaned and total kilograms of lamb weaned. Olson and Loggins (1979) also reported that among Florida native sheep, ewes with HbBB were more fertile than ewes with HbAA. Likewise, our data suggests a beneficial effect of the Hb^B allele on the age of first calving. In contrast to these results, King *et al.* (1958) reported that in the Scottish Blackface breed, HbAA ewes were more fertile than either HbBB or HbAB ewes. Meyer *et al.* (1967) reported that among Blackhead Mutton sheep in Germany, HbAA ewes were more fertile than HbBB ewes. Work

done on Australian Merinos by Mayo *et al.* (1970) indicated no difference in fertility that was attributable to Hb type.

Our results, in Thai indigenous cattle indicated that HbAC had significantly higher bodyweight at 200, 400 and 600 days of age than HbAA, HbAB and HbBB respectively, whereas HbAA of Simmental × Brahman crossbred cattle had significantly higher bodyweight than HbAB at all ages studied. It is speculated that the difference in Hb type could affect the oxygen transport and oxygen binding properties, resulting in changes in the metabolism and growth rates. Huisman *et al.* (1958) reported that HbAA had a higher oxygen affinity than HbBB. The oxygen affinity of Hb C in sheep and goats has been reported (Huisman and Kitchens, 1968) to be substantially less than that of Hb A, and suggested that the switch from A to C may be advantageous in anemia. Winslow *et al.* (1989), however, showed that purified Hb A and C had very similar oxygen affinities but in the presence of carbon dioxide (CO_2), the oxygen affinity of Hb C decreased below that of Hb A.

In conclusion, the data presented in this communication suggest that Hb types may be related to growth performance and age at first calving in Thai indigenous and Simmental × Brahman crossbred cattle. Furthermore, haemoglonbin phenotype could be enlisted together with other factors for selection of high performing animals in Thailand and in countries with similar climatic and nutritional conditions.

Experiment 3 – Discussion

Plasma levels of GLU in Thai indigenous cattle was slightly, but significantly (P<0.05) higher than in Simmental × Brahman crossbred. Nevertheless, the levels were found to be within the range of reported values for cattle (2.5 – 4.2 mmol/l; Kaneko *et al.*, 1997). This may indicate an adequate energy supply of animals (Rook and Line, 1961; Prasanpanich *et al.*, 2002; Singh *et al.*, 2002). It is, however, to consider that the role of glucose as a superior indicator for energy supply in the mature cattle is a matter of dispute, because it is regulated by an array of different factors (Mudron *et al.*, 2005).

Plasma urea is a nutritional indicator related to protein intake (Kaneko *et al.*, 1997; Coppo, 2004) and useful in evaluating kidney function in conjunction with creatinine which originates from the non-enzymatic conversion of creatinine in muscle and is filtered by the kidney (Jain, 1996; Stockham and Scott, 2002). In the present study, mean levels of plasma urea were significantly higher in Thai indigenous than in crossbred animals. Albeit, the values in all animals were within the range given for the cattle (Kaneko *et al.*, 1997). In the Paraguayan cattle in South Africa (Otto *et al.*, 1992) and Angoni cattle in Mozambique (Otto *et al.*, 2000) the mean concentration of plasma urea appeared to be lower than the range given for the cattle (5.4 mmol/mean level in Angoni cattle versus 7.1-10.7 range given for cattle). Furthermore, in Angoni cattle, the level was varying with the amount of the rainfall. Thus, it seems that not only the genetic background and nutrition but also the climate determines the plasma concentrations of urea in the cattle.

Albumin levels in Simmental × Brahman crossbred were lower than in Thai indigenous cattle (P<0.01). Further, the males in the breed had the lowest levels (P<0.05) among the groups. The levels were slightly lower than normal range of 27-39 g/l (Jain, 1996) given for cattle. This biochemical parameter has been shown to be significantly correlated with the health in cattle and good nutritional condition (Kaneko *et al.*, 1997; Coppo, 2004). Albumin concentration would significantly decrease during malnutrition (Bogin, 1994; Singh *et al.*, 2002); alimentary restriction

alters quickly the albumin levels in serum (Jain, 1996). Crossbred animals used in this study showed no clinical signs or pathological symptoms. Furthermore, animals had access to fresh water *ad libitum* at all times. We find no correlation between the body score and the concentration of blood albumin. Thus, the slightly lower levels in males can be due to the breed and/or environmental conditions (Rowlands *et al.*, 1975; Otto *et al*., 1992).

Total protein level of serum is associated with evaluation of hydration status or possible hemorrhage and is a useful marker for acute and chronic active inflammation (Stockham and Scott, 2002). In this study, total protein in Thai indigenous was significantly ($P<0.01$) higher than in Simmental × Brahman crossbred. Furthermore, the range of total protein levels in Thai indigenous was slightly higher than the range of 61-81 g/l which is estimated for European breeds (Jain, 1996). Likewise, in the Paraguayan cattle in South Africa (Otto et al., 1992) and Angoni cattle in Mozambique (Otto *et al.*, 2000), the mean concentration of total protein appeared to be higher than the range given for the cattle (85.0 g/l in Paraguayan cattle and 83.8 g/l in Angoni cattle, respectively). Several investigators have mentioned that the serum protein profile of a particular individual is relatively constant over a considerable length of time (Rowlands *et al.*, 1973; Anderson *et al.*, 1987; Knowles *et al.*, 2000; Coppo, 2004). This constancy is apparently controlled genetically. In fact, Kaneko *et al.* (1997) suggested that the serum protein patterns were so characteristic that it can be used to differentiate species and in some cases the strain and the sex of an animal. Furthermore, heritability of serum protein in the cattle has been reported by Smithies and Hickman (1958).

The enzymes AST, ALT, ALP, GGT and CK are considered as indicators of the health of the animal. They reveal the damage to tissue or cells in the muscle, liver, skeleton and heart (Canfield *et al.*, 1985; Kaneko *et al.*, 1997; Stockham and Scott, 2002). Our results showed that AST, ALT, ALP and GGT levels in Thai indigenous were significantly higher than in Simmental × Brahman crossbred. The sex affects AST, ALT, ALP and GGT significantly ($P<0.05$), whereas the CK levels are similar in both sexes as reported previously (Radostits *et al.*, 1994).

In conclusion, the data presented in this communication can serve as reference values for Thai indigenous and Simmental × Brahman crossbred cattle grown in Thailand and the other Asian countries having similar climatic and nutritional conditions.

Experiment 4 – Discussion

A great deal of evidence has been presented demonstrating the involvement of hormones, cytokines, and neuromodulators in the bidirectional communication and integration of the immune and endocrine systems (Besedovsky and Rey, 1996). In this study it was found that PHA-M caused a significant ($P<0.05$) increment in basal GH release from cyclic HF and SBT cell cultures at the temperature of 37.2°C and at 41.0°C. In pregnant cows, GH levels from PHA-M – treated and control PBLs from HF did not significantly differ in 37.2°C and 41.0°C. However, PHA-M caused a significant ($P<0.05$) increment in basal GH release from pregnant SBT cell cultures at the temperature of 37.2°C and at 41.0°C. Similar results have been obtained in other species as mitogenic stimulation increased PRL and GH release from T and B lymphocytes in rats (Hattori *et al.*, 1993), human (Hattori *et al.*, 1994) and ACTH secretion from lymphocyte of cycling cows (Dixit and Parvizi, 2001). Poppi *et al.* (2002) noted that porcine and bovine lymphocytes respond to low concentrations of GHRH by increasing the GH production. This is contradictory to reports in humans where it was observed that GHRH was ineffective in modulating GH secretion by lymphocyte cell lines (Kao and Meyer, 1992) and lymphocytes in absence of leptin (Hattori *et al.*, 1990).

Because lymphocytes are exquisitely sensitive to depletion of the cellular energy supply (Sanna *et al.*, 2003), their effector functions are particularly influenced by endocrine signals that control cellular metabolism, resulting in cell activation and proliferation. Poppi *et al.* (2002) reported that stimulation of ghrelin receptor, by a synthetic analog, hexarelin, induces a substantial increase in GH production from porcine lymphocytes (Poppi *et al.*, 2002)

GH secretion from PBLs at different temperatures

The results of Experiment 4 indicated that un-stimulated PBLs in the cultures at temperature of 41.0°C has GH levels lower than at the treatments of 37.2°C in cyclic

HF (P>0.05), cyclic SBT (P<0.05), pregnant HF (P>0.05) and pregnant SBT (P>0.05) cows. Likewise, stimulated PBLs with PHA-M in the cultures at temperature of 41.0°C has GH levels lower than at the treatments of 37.2°C in cyclic SBT (P>0.05), pregnant HF (P>0.05) and pregnant SBT (P<0.05) cows. Therefore, the stress temperature (41.0°C) could lead to attenuation of GH release and most mammals die when deep body temperature reaches 42-45°C (Bianca, 1968; Silanikove, 2000), which exceeds normal body temperature by about only 3 - 6°C.

Dixit *et al.* (2003) have assumed that stimulatory mechanism involved in GH release is similar between the pituitary gland and PBLs. However, the inhibitory control mechanism regulating GH appear to differ (Poppi *et al.*, 2002)

Mitra *et al.* (1972) studied GH secretion in cattle at thermoneutral (18.0°C, 50%RH) and at high ambient temperature (35.0°C, 50%RH). They found that the mean values obtained at thermoneural ambient were 18.2 ng GH/ml plasma, and decreased to 13.5 ng/ml at high ambient temperature.

Therefore, a reduced secretion of GH seems to be necessary for survival of the homeotherm species in high ambient temperature. Since GH is related intimately to the physiological processes of growth and lactation which are known to be affected by heat stress (Mitra *et al.*, 1972; Hart *et al.*, 1978).

GH secretion from PBLs by different breeds

In present study there was no breed difference in lymphocytic GH secretion when the cells were incubated at 37°C. However, cells from pregnant SBT cows were more sensitive to a thermal stress than the cells from HF cows. The plasma GH levels were greater in cyclic SBT than in pregnant SBT cows. Whereas, in HF cows the plasma GH levels were similar in the cyclic and the pregnant animals. The reason for this breed difference is not known. But, HF cows are high–milk–yield breed while the SBT cow is rather a dual purpose (milk and meat) one. Hirose (2002) reported a

positive correlation between milk yield and GH during lactation period. Generally, GH is likely to be one of several metabolic hormones that act to direct flow of nutrients to the mammary gland for milk production (Baccari *et al.*, 1983).

Production of NO from PBLs at different temperatures

Nitric oxide (NO) is a free-radical gas generated by the enzyme NO synthase (Palmer and Moncada, 1989; Bush *et al.*, 1992) using arginine as a precursor. It is now known to be an important biological messenger in animals, and it is also produced from lymphocytes (Kirk *et al.*, 1990; Reiling *et al.*, 1996). It mediates critical processes such as neuromodulation, endocrine signal transduction, vasodilation, and immune defense. It also exerts immunosuppressive effects by contributing to TH-2 shift (Kolb and Kolb-Bachofen, 1998; Roozendaal *et al.*, 1999) via inactivation of the zinc finger transcription factors (Chang *et al.*, 1997; Berendji *et al.*, 1999). Moreover, NO is implicated in the neuroendocrine control of pituitary GH secretion (Kato, 1992).

NO is believed to play a critical role in regulating inflammation (Nathan, 1992), and it has recently been shown to be up-regulated in response to proinflammatory challenge and is inhibited after anti-inflammatory pathway downstream of liver × receptor (Joseph *et al.*, 2003).

NO production often occurs in environments of elevated temperature (Pritchard *et al.*, 2005). The data in this study reveal, that physiologically relevant elevations in temperature increase NO production. Therefore, fever or inflammation or high ambient temperature in which cells may experience a temperature shift, may play an important regulatory role in NO production (Pritchard *et al.*, 2005).

Additional levels of complexity in the thermal regulation of NO production were revealed in PBLs from cyclic cows. When PBLs of cyclic cows were incubated with PHA-M at 41.0°C, there was an inhibition of NO production. As a high level of NO is known to negatively regulate the continued production of NO (MacMicking *et*

al., 1997), it is likely that incubation with PHA-M of PBLs at 41.0°C over-stimulated the PBLs and thus, inhibited the further synthesis of NO.

It is important to note that the data presented here are both in contrast (un-stimulated PBLs) and similar (incubation with PHA-M) to previously published reports. However, there are potentially important differences between the current study and those performed previously. One important difference may be that the models used in the previous studies did not use cells of the immune system. These earlier studies demonstrated that exposure of rat and human hepatocytes (de Vera *et al.*, 1996a; 1996b), rat pulmonary artery smooth muscle cells (Wong *et al.*, 1995), and rat astrocytes (Feinstein *et al.*, 1996; Heneka *et al.*, 2000) to heat-shock conditions decreases cytokine-induced NO and iNOS production. It is intriguing to speculate that PBLs may have evolved to respond to temperature shifts as part of their selective response to the host, as they generate fever in the presence of bacterial or viral infections.

Moreover, another difference between the earlier studies and those presented here is that temperatures used in the previous studies are higher (i.e., between 41.0°C and 43.0°C) than those used here (Mackowiak and Boulant, 1996). It is reasonable to assume that there could be important differences in the response, depending on the temperature to which the cells were exposed and that there may be a narrow window for physiological response to temperature gradients. A more thorough examination of how temperature affects NO production in various cell types, including the macrophage, would illuminate just how much heat is required to obtain the enhanced NO that we have already observed and at what temperature, enhancement of NO ceases. However, Pritchard *et al.* (2005) who studied NO production in response to thermal stimulation in murine macrophages, found that altering the thermal microenvironment is an important mean by which the host can manipulate macrophage responses. Increases in temperature (e.g., during fever) may function to lower the activation threshold needed for NO production of effectors molecules in times of infection.

The present data show significant differences in NO production by control or PHA-M stimulated PBLs from cyclic and pregnant cows both in HF and SBT cows. This is similar to previous findings of Dixit and Parvizi (2001) which also demonstrated an elevation of ACTH and NO during pregnancy implying a new role for ACTH and NO from PBLs in recognition and, probably, maintenance of pregnancy.

In conclusion, these data indicate that PBLs may be highly sensitive to physiologically relevant thermal gradients and that condition such as fever or inflammation can lower the threshold of activation required for NO production. The hyperthermia may thus serve as a danger signal similar to a role recently described for Heat Shock Protein (HSP) (Colaco, 1998; Asea *et al.*, 2000; Basu *et al.*, 2000; Vabulas *et al.*, 2002; Campisi *et al.*, 2003). These data provide the foundation for future exploration of the exact mechanism(s) by which PBLs sense and respond to differences in their environmental temperature, and suggest the presence of cellular signaling pathways or gene regulatory mechanisms that are activated by thermal stimuli.

General Discussion

In recent years, the demand for high quality beef has been increasing due to the change in the socio-economic pattern of population in Thailand. The consumer has expressed his concern towards animal welfare and food safety. Likewise, the intensive animal production is forced to produce high quality products with special attention to animal health and food safety. Animals with high fertility and efficiency in growth performance are preferred. Exogenous factors such as management, diseases and stress have a major influence on growth, product quality and animal welfare. At present, no other studies have assessed the heat tolerance for beef cattle in Thailand with special reference to blood biochemical properties.

This thesis presents four experiments that:

(a) evaluated and compared the productive and reproductive performance of the Thai Brahman and Simmental-Brahman crossbred (Kabinburi) cattle under different environmental conditions in central Thailand (Experiment I);

(b) examined the relationship between haemoglobin types and productivity of Thai indigenous and Simmental × Brahman crossbred cattle. (Experiment II);

(c) examined the blood biochemical profiles of Thai indigenous and Simmental × Brahman crossbred cattle in the Central Thailand (Experiment III); and

(d) investigated the effect of temperature on growth hormone and nitric oxide secretion from peripheral bovine lymphocytes (Experiment IV).

The seasonal meteorological variations indicate that presumably the animals were not under heat stress only during wintertime and nights. It could be because the animals kept in locations with high temperature and low annual rainfall would only have a low quality food available. The reason for this seems to be insufficient quality and quantity of food which limits the growth rate and subsequently causes the late onset of puberty. Kabinburi cattle has significantly higher bodyweight at birth as well as at 200, 400 and 600 days of age than Thai Brahman cattle. It is likely that the Kabinburi cattle due to heterosis effect would have an increase in growth rate and

fertility and lower calving interval than Brahman cows. Nevertheless, it is also possible that Kabinburi as a new synthetic breed possesses a better heat tolerance and adaptation capacity to the tropical climate than Brahman breed (Experiment I).

The findings of the second study revealed that the Thai indigenous cattle with HbAC had significantly higher bodyweight at 200, 400 and 600 days of age than those with HbAA, HbAB and HbBB respectively, whereas the Simmental × Brahman crossbred cattle with HbAA had significantly higher bodyweight than those with HbAB at all ages studied. It is speculated that the difference in Hb type could affect the oxygen transport and oxygen binding properties, resulting in changes in the metabolism and growth rates. Huisman *et al.* (1958) reported that HbAA had a higher oxygen affinity than HbBB. The oxygen affinity of Hb C has been reported (Huisman and Kitchens, 1968) to be substantially less than that of Hb A, and suggested that the switch from A to C may be advantageous under existing stressful environmental conditions. Winslow *et al.* (1989), however, showed that purified Hb A and C had very similar oxygen affinities but in the presence of carbon dioxide (CO_2), the oxygen affinity of Hb C decreased below that of Hb A.

In the third study, the animals used showed no clinical signs or pathological symptoms, they were considered "healthy" and the data obtained can serve as reference values for future use of these animals in veterinary medicine and animal production. Majority of blood biochemical profiles levels such as plasma glucose, urea, creatinine, AST, ALT, ALP, GGT and CK in all animals were within the range given for the cattle (Kaneko *et al.*, 1997). However, total protein in Thai indigenous was significantly ($P<0.01$) higher than in Simmental × Brahman crossbred. Furthermore, the range of total protein levels in Thai indigenous was slightly higher than the range of 61-81 g/l which is estimated for European breeds (Jain, 1996).

Several investigators have mentioned that the plasma protein profile of a particular individual is relatively constant over a considerable length of time (Rowlands *et al.*, 1973; Anderson *et al.*, 1987; Knowles *et al.*, 2000; Coppo, 2004). This constancy is apparently controlled genetically. In fact, Kaneko *et al.*

(1997) suggested that the plasma protein characteristic patterns can be used to differentiate species and in some cases the strain and the sex of an animal.

The results of the fourth study indicated that the heat stress (41.0°C) could lead to attenuation of GH release from PBLs. Therefore, a reduced secretion of GH seems to be necessary for survival of the homeotherm in high ambient temperature. Because lymphocytes are exquisitely sensitive to depletion of the cellular energy supply (Sanna *et al.*, 2003), their effectors' functions are particularly influenced by endocrine signals that control cellular metabolism, resulting in cell activation and proliferation. Over stimulation of PBLs (e.g. PHA-M treatment at 41.0°C) caused a rebound effect resulting in an inhibition of NO release.

CONCLUSION AND RECOMMENDATION

Conclusion

Based on the available results it is concluded that:

1. Kabinburi cattle is an ideal Thai beef cattle for high production under tropical climate.

2. Haemoglobin phenotypes may be related to growth performance and age at first calving in Thai indigenous and Simmental × Brahman crossbred cattle. Furthermore, haemoglonbin phenotypes could be enlisted together with other factors for selection of high performing animals in Thailand and in countries with similar climatic and nutritional conditions.

3. Blood biochemical profiles of animal describe the normal reference values of a unique cattle breed, adapted to existing environmental, nutritional and pathogenic exposures. This communication can serve as reference values for Thai indigenous and Simmental × Brahman crossbred cattle grown in Thailand and the other Asian countries having similar climatic and nutritional conditions.

4. PBLs may be highly sensitive to physiologically relevant thermal gradients and that condition such as fever or inflammation can lower the threshold of activation required for NO production. These data provide the foundation for future exploration of the exact mechanism(s) by which PBLs sense and respond to differences in their environmental temperature, and suggest the presence of cellular signaling pathways or gene regulatory mechanisms that are activated by thermal stimuli.

Recommendation

Heat tolerance indicators such as haemoglobin phenotypes or blood biochemical parameters could be enlisted together with other factors for selection of high performing animals in Thailand and in other countries with similar climatic and nutritional conditions.

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APPENDIX

Appendix Figure 1 Characteristic of Thai Brahman bulls (800-1,000 kg bodyweight at maturity, ~5 yr) kept in Thailand.

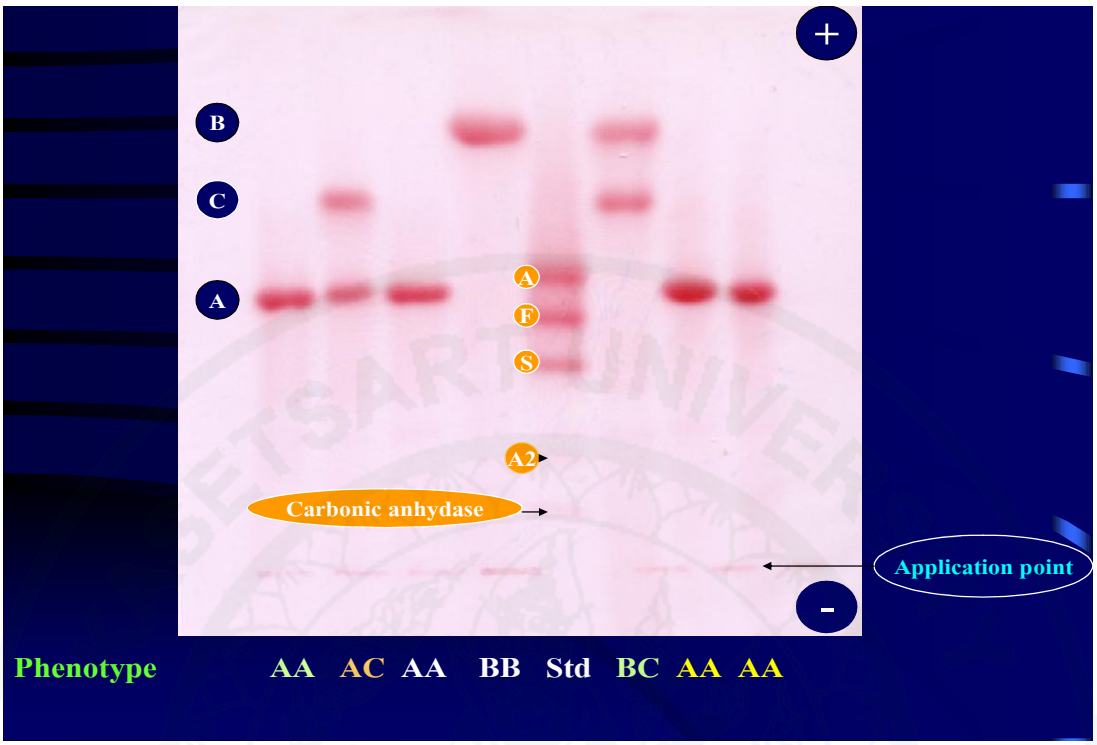
Appendix Figure 2 Characteristic of Thai Brahman cows (500-600 kg bodyweight at maturity, ~5 yr) used in Experiment I.

Appendix Figure 3 Characteristic of Kabinburi bull (800-900 kg bodyweight at maturity, ~5 yr), a new synthetic breed, kept in central Thailand.

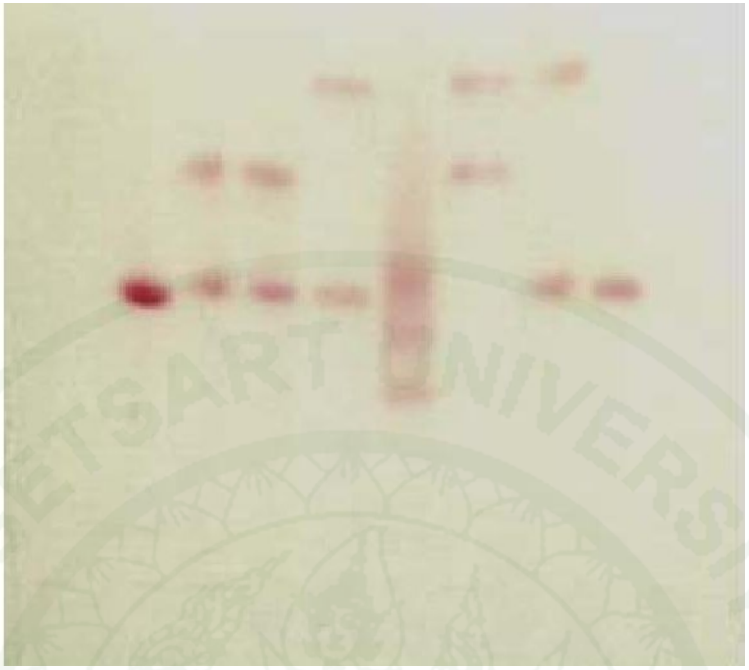
Appendix Figure 4 Characteristic of Kabinburi cows (500-650 kg bodyweight at maturity, ~5 yr) used in Experiment I, II and III.

Appendix Figure 5 Characteristic of Thai indigenous bull (350-450 kg bodyweight at maturity, ~5 yr), generally kept in Thailand.

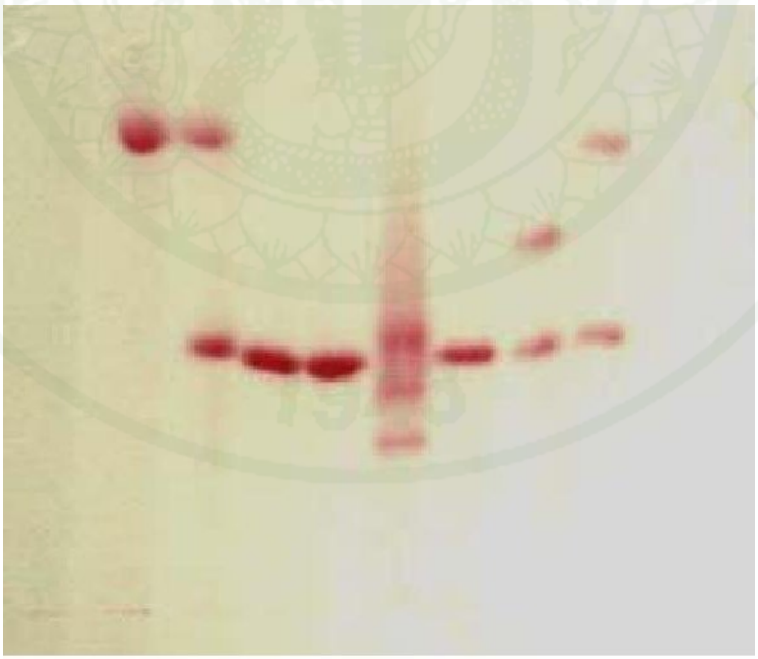
Appendix Figure 6 Characteristic of Thai indigenous cows (230-350 kg bodyweight at maturity, ~5 yr) used in Experiment II and III.



Appendix Figure 7 Haemoglobin phenotypes were separated using both Tris-EDTA and a boric acid buffer (Supre-Heme® Buffer; Cat. No 5802) at pH 8.2-8.6. Samples (haemolysate; 5 µl) were applied to the membrane at application point and electrophoresis carried out for 25 min at 350 volts. Each purified haemoglobin phenotype moved from cathode (-) to anode (+) in electric field, was different (experiment II). This figure show haemoglobin phenotype of selected seven animals compared to standard sample (Std). Haemoglobin phenotypes shown from the left to the right are as follows: HbAA, HbAC, HbAA, HbBB, Std, HbBC, HbAA and HbAA, respectively.



Appendix Figure 8 Haemoglobin phenotype are from the left to the right: HbAA, HbAC, HbAC, HbAA, Std, HbBC, HbAB and HbAA



Appendix Figure 9 Haemoglobin phenotypes shown from the left to the right are as follows: HbBB, HbAB, HbAA, HbAA, Std, HbAA, HbAC and HbAB

Publications

This thesis is based on the work contained in the following papers:

I. Boonprong, S., A. Choothesa, C. Sribhen, N. Parvizi and C. Vajrabukka. 2007. Relationship between haemoglobin types and productivity of Thai indigenous and Simmental × Brahman crossbred cattle. **Livest. Sci.** 111: 213-217.

II. Boonprong, S., C. Sribhen, A. Choothesa, N. Parvizi and C. Vajrabukka. 2007. Blood biochemical profiles of Thai indigenous and Simmental × Brahman crossbred cattle in the Central Thailand. **J. Vet. Med. A** 54: 62-65.

III. Boonprong, S., A. Choothesa, C. Sribhen, N. Parvizi and C. Vajrabukka. 2008. Productivity of Thai Brahman and Simmental-Brahman crossbred (Kabinburi) cattle in central Thailand. **Int. J. Biometeorol.** 52: 409-415.

IV. Boonprong, S., A. Choothesa, C. Sribhen, C. Vajrabukka and N. Parvizi. 2010. Effect of temperature on growth hormone and nitric oxide secretion from peripheral bovine lymphocytes. (in preparation)

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