Sodium and Protein Nutrition of Lactating Cows under Tropical Conditions

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Table of Contents

Chapter 1

General Introduction

GENERAL BACKGROUND OF THAI GEOGRAPHY, AGRICULTURE AND THE DAIRY INDUSTRY

Thailand, a country situated in the heart of southeast Asia, is located between 5° 37' to 20° 27' north latitude and 97° 22' to 105° 37' east longitude (TMD, 2007). The local climate is tropical and characterized by monsoon; from mid-May to September, there is a rainy, warm and cloudy southwest monsoon while from November to mid-March, the northeast monsoon can be characterized as dry and cool. The southern isthmus is hot and humid. The average of minimum and maximum temperature ranges between 24 °C and 33 °C, respectively (TMD, 2012). Rice is the country's most important crop. Other important agricultural products include fish and fishery products, cassava, rubber, grain and sugar (Falvey, 2000).

Historically, milk has not been an important food product within the Thai culture, and hence there were not many dairy cattle until the second half of the twentieth century (Kehren and Tisdell, 1998). Traditionally, meat from cattle and buffalo was obtained from retired working animals (Murphy and Tisdell, 1995) and surplus income was gained by farmers through the sale of animals for slaughter. In the 1950s, Indian settlers initiated dairying operations in Thailand, with Bangkok as the main market for their milk (Kehren and Tisdell, 1996). The commercial production of dairy cattle in Thailand commenced after the establishment of the Thai Danish Farm and Training Centre at Muek-Lek, which was a joint venture between the Thai and Danish governments in the early 1960s (Pichet, 1991). Another joint venture, the Thai-German Dairy Training and Processing Plant, was established in Chiang Mai in 1968, and this joint venture was taken over by the Thai Department of Livestock Development in 1977. Governmental promotion of milk consumption, in particular for children, resulted in a slow but steady increase in demand throughout the 1970s (ADC, 1993). Despite ongoing governmental actions to promote the dairy industry through various national economic and social initiatives, the dairy industry in Thailand does not yield sufficient milk to support current national demand. The national demand for milk keeps increasing due to an increase of both the population’s income and the tourist industry to date. As such, Thailand imports increasing amounts of dairy products from other countries, and milk powder as a substitute for mother's milk, represents a significant proportion of the total import of dairy products.

DAIRY INDUSTRY IN THAILAND

To date, there are approximately 512,000 dairy cows in Thailand. The cows are not equally distributed across the country. The majority of the total dairy population (61%) is found in the central part of Thailand and 24% in the northeastern part (Isan) of the country. Virtually all of the remaining cows of the Thai dairy population are found in the northern part of Thailand because only 1% of the population lives in the southern part of Thailand (DLD, 2014). Almost all dairy cows are Holstein Friesian crossbreds (87.5% HF) (Koonawootrittriron, 2014). Practically 95-99% of the dairy cow in Thailand are kept by small scale or small-holder farmers under mixed crop-livestock farming system (Chantalakhana and Skunmun, 2002; Wanapat, 1995). Typically, most dairy farmers in Thailand (59.9%) are small holders managing on average of 30 dairy cows per farm (DLD, 2014). There are a small number of farms (23.8%) with a herd size of 11 to 20 dairy cows and only 16.3% of the dairy farmers manage less than 11 dairy cows (DLD, 2014). Thai farmers keep their dairy cows in two types of housing systems, the tie-stall barn or a loose-stall barn system. The tie-stall barns are mainly used in the central and northern part of Thailand whereas loose-stall barns are typically used in the northeastern part of Thailand (i.e. Isan). Thai dairy cows produce 3733 kg per lactation, and daily milk yield averages 12.2 kg/cow (Pattamanont and Ruengpaibul, 2015). The mean composition of milk is 3.71% fat, 3.03% protein, 4.70% lactose, 8.43% non-fat solids and 12.13% total solids (Kamphusiri, 2013).

GENERAL NUTRITION OF THAI DAIRY COWS

In general, Thai dairy farmers own a small amount of land averaging < 1 ha (Kummanee *et al.*, 2012; Sompakdi and Phonprapai, 2014; Suriya, 2015) which hampers the possibility to graze cows on pastures. Therefore, the feeding of fresh grass is usually based on a cut and carry system; i.e. the grasses are manually collected from publically available land and fed to the cows on the same day. Grasses such as Ruzzi grass (*Bracharia ruziziensis*), Para grass (*Bracharia mutica*), Napier grass (*Pennisetum purpureum*), Guinea grass (*Panicum maximum)* and Pangola (*Digitaria eriantha*) are among the species most commonly used. In practice, the feeding of fresh grasses is restricted to the rainy season and given the labor intensive method of collection, quantitatively less important than roughage sources originating from agricultural activities such as cropping of rice, corn, cassava and sugar cane. In quantitative terms, the roughage supply typically depends on the availability of agricultural waste or by-products. Next to the fore mentioned roughages, concentrates also are widely used but regional differences exist on the use of compound feeds. For instance, in the north-

east of Thailand, farmers prefer on farm mixing of single, non-pelleted, feedstuffs while in the north and central part of Thailand, the use of pelleted compound feeds is more common. Feedstuffs used as concentrates are mainly agro-industrial and industrial by-products such as cassava chips, cassava pulp, soybean hull, pineapple waste, tomato waste, potato waste, molasses and distiller's grain waste. In principle, the farmers feed concentrates twice daily during milking while roughage is offered *ad libitum*. Computerized optimization of dairy rations is hardly practiced in Thailand and the amount of concentrate offered to the cows is calculated on the basis of the cow's milk yield. A commonly applied rule is the supply of 1 kg of concentrate for each 2 kg of milk produced (Wanapat *et al.*, 2000).

CONSTRAINTS OF DAIRY PRODUCTION IN THAILAND

Milk production is determined by both genetic and environmental factors and it was estimated by Wachirapakorn (2003) that 70% of the variation in milk production is explained by environmental factors, including nutrition. As already mentioned before, almost all dairy cows in Thailand are Holstein Friesian crossbreds. In view of such genetic merit of the Thai dairy cows, it is generally accepted that current milk yields are below the potential of these cows. Consequently, the main reason for the low milk production of dairy cows is related to the prevailing environmental conditions. The tropical climate is an important constraint for milk production because of two main reasons. First, it is well known that the digestibility of forages is negatively affected by tropical conditions. Tropical forages mature more rapidly than temperate forages (Leng, 1995). Unfavorable growth conditions can result in low contents of protein and minerals in the plant and high amounts of so called structural carbohydrates, i.e. neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). Especially the high ADF and ADL contents of tropical feedstuffs (Table 1) cause a poor digestion.

Next to the negative impact of tropical conditions on the nutritional quality of the forages, it is well known that during heat stress, feed intake and thus production performance, is reduced (McDowell, 1972; Rhoads *et al.*, 2009). Various strategies such as nutrition management schemes, physical protection and genetic development can be implemented to alleviate heat stress under hot and humid conditions. Although the relevance of this topic cannot be disputed, it is beyond the scope of the current thesis.

Table 1. Nutrient composition of roughages commonly used in Thailand

Item	DM	Ash	CP	EE	NDF	ADF	ADL	NFC	Na
	(% as fed)	(% of DM)							
Grasses and leaves									
Brachiaria mutica	20.7	13.7	7.5	2.9	63.4	38.7	3.6	12.5	0.02
Brachiaria ruziziensis	21.6	8.2	9.0	2.4	64.3	35.2	8.1	16.1	<0.01
Digitaria eriantha	18.9	12.1	8.2	2.4	63.1	38.5	3.5	14.2	0.03
Panicum maximum	19.5	5.5	12.5	2.4	70.1	43.5	7.8	9.5	0.04
Pennisetum purpureum	18.1	5.4	12.0	2.7	63.2	39.1	6.1	16.7	<0.01
Dried cassava leaves	90.2	9.0	25.6	5.9	30.7	27.0	26.4	28.8	-
Dried *Leucaena leucocephala*	90.7	6.9	22.4	3.2	31.2	21.7	10.8	36.3	-
Centrosema pascuorum cv. Calvacade	22.6	8.7	10.3	2.4	59.8	41.2	8.0	18.8	0.05
Crop residues									
Rice straw	93.5	14.1	3.0	1.9	74.7	53.2	4.5	6.3	0.01
Sweet corn stem	26.1	6.2	6.7	2.3	63.6	38.8	5.4	21.2	<0.01
Sweet corn cop	25.2	7.0	5.2	1.5	39.4	34.6	5.0	46.9	<0.01
Sweet corn husk	26.0	4.0	5.9	1.3	70.0	36.0	4.2	18.8	<0.01
Baby corn husk	14.0	2.2	12.6	1.8	50.5	24.3	3.5	32.9	<0.01
Baby corn stem	18.3	6.0	8.9	2.5	58.6	29.6	4.6	24.0	0.02
Pineapple waste	13.0	4.2	5.8	3.9	59.4	27.9	2.2	26.7	0.01
Sugar cane (top)	91.3	6.5	4.0	1.1	71.3	42.5	3.4	17.1	-

DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; NFC, non-fibre carbohydrate (NFC = 100 – ash – CP – EE – NDF); Na, sodium. Adapted from Kanjanapruthipong (2006).

Due to the low digestibility of the roughages, the use of concentrates is necessary to ensure dairy productivity (Wanapat *et al.*, 2000). The use of energy rich concentrates counteracts, at least partly, the low digestible energy content of roughages but it was already mentioned that the commonly used roughages also have low protein contents. Consequently, the concentrates to be used should also have a high protein content to ensure adequate protein supply of the cows. However, the majority of Thai farmers are small holders and they typically have a small margin between daily income and expenses in terms of liquid assets. Therefore, these farmers are reluctant to purchase protein rich concentrates because protein is relatively expensive (Kehren and Tisdell, 1998).

Since Thai dairy cows reduce their voluntary intake during heat stress, it is logical that mineral intake is less than optimal relative to potential productivity. Sodium is the major element in saliva and is utilized to buffer acid from ruminal fermentation. Sodium requirements and production responses are affected by temperature, humidity, sweating rate, stage of lactation, gestation, growth, and level of production (Suttle, 2010). Sweating aids in heat dissipation, and Na and K are secreted in sweat which sits on the surface of the skin. Sweat from the skin surface has a cooling effect due to evaporative cooling. The conversion of liquid water into vapor uses the thermal energy in the air, resulting in a lowering of the temperature of the skin. As a result, dilated blood vessels in the skin can lose heat more effectively to the surrounding air which subsequently reduces the body temperature. In unacclimatised cattle, the dribbling of saliva and sweating can result in daily losses of Na (Aitken, 1976; Collins and Weiner, 1968). In heat stressed dairy cows, supplementation of Na resulted in a significant increase in milk production (Schneider *et al.*, 1984, 1986). Forages contain relatively small amounts of Na (Table 1) due to soil, plant and husbandry factors (Suttle, 2010). The distribution of pasture Na concentrations worldwide is skewed towards low values, with 50% of samples containing < 0.15% of Na; moreover ever lower values are more common in tropical than in temperate pasture: tropical legume contain < 0.04% of Na (Minson, 1990).

AIM OF THE THESIS

The overall objective of the research described in this thesis was to provide a basis for improvement in milk production by dairy cows on small farm holders in Thailand. First a cross sectional study was conducted on farms varying in type and the amount of roughage supply to dairy cows on its effect to milk production (Chapter 2). This research led to indications of a low Na and protein supply on these farms and subsequent studies were conducted to investigate these nutritional aspects. In Chapter 3, the Na requirement of lactating dairy cows housed under tropical conditions was addressed while in Chapter 4 the physiology response of NaCl depletion and repletion was determined in lactating dairy cow under tropical conditions. The effect of concentrates with two levels of protein (17 and 21%) and the influence on rumen metabolism and milk production in mid lactating cows is reported (Chapter 5). Finally, the results of the studies are summarized and discussed in Chapter 6.

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Chapter 2

An Initial Investigation into the Relationship between Ration Composition and Milk Production of Dairy Cows in Thailand: A Cross Sectional Study

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ABSTRACT

The current study was conducted as a preliminary investigation into the relationship between ration composition and milk production in dairy cows in Thailand. A total of 45 multiparous cows (4-7 per farm) were monitored at 70 (n=33) and at 130 days (n=30) after calving. Body weight, milk yield and composition and feed intake as well as feed composition of each cow were determined. Cows fed with rations rich in crude protein (CP) and non-fibre carbohydrates (NFC) tended to have higher milk yields. Milk yield was inversely related with the forage to concentrate ratio and the dietary levels of neutral detergent fibre. Cows consumed 5 and 8% greater CP than requirement during early- and mid-lactation, respectively. Nevertheless, cows that ingested more CP responded with more milk yield, both during early- and mid-lactation. Intake of net energy for lactation (NE_L) in early lactation was 5% lower than recommended while NE_L intake during mid lactation was 14% greater than required. During early- but not mid-lactation, milk production responded positively to greater intakes of NE_L. The current observations are considered important with regard to dietary interventions to improve milk production in Thailand.

INTRODUCTION

The level of dairy milk production in Thailand is low, averaging 12 and 13 kg/d (DLD, 2011). The low level of milk production is most likely not caused by a poor genetic potential for milk production, because the dairy cows are generally crossbreds between Holstein-Friesian (> 87.5%) × indigenous breed. It is estimated that the milk yield of Thai dairy cows is only 50% of their potential to produce milk (Tumwasorn *et al.*, 1997).

Clearly, the normal Thai climatological conditions (average maximum temperature 33 °C, minimum temperature 27 °C and relative humidity 72-74%) do not facilitate a high dry matter intake (DMI) and thus not a high milk production (Rhoads *et al.*, 2009). In addition, the feeding value of roughages to dairy cows in Thailand is low because of low digestibility and this can be considered a major constraint in relation to DMI (Leng, 1995). The various sources of roughage that are typically used in Thailand include crop residues (rice straw, corn stem, corn sheath, corn silk, pineapple peal, etc.) and grasses (*Mauritius sp.*, *Panicum maximum* TD58, etc.). In general, the crop residues are low both in digestible energy and protein content (Balch, 1976). Furthermore, the quality of the grass is low due to a high content of lignin and low protein content (Leng, 1995). It is well known that at high environmental temperatures, the provision of poor quality roughage further lowers feed intake (Leng, 1995), leading to low levels of milk production. Therefore, roughages are supplemented with concentrates to support milk production. Under practical Thai conditions, the amount of concentrate fed to the cows is determined by the amount of milk produced; approximately 1 kg of concentrate is provided for each 2 kg of milk produced.

Besides the low energy and protein contents, vegetable feed compounds contain small amounts of sodium (Na). The amount of Na present in feed composed from these compounds may not be sufficient under tropical conditions. Furthermore, the current NRC (2001) requirements of Na for lactation cattle (0.22% in the DM) may not be valid under the conditions where the animal experiences heat stress (Sanchez *et al.*, 1994). It was suggested by Schneider *et al.* (1986), that feeding supplemental Na may increase milk production under heat stress conditions. To achieve a more optimal performance of dairy cows in Thailand, knowledge on the composition of the feedstuffs and the rations is essential as these data can improve the formulation of appropriate rations for dairy cows. Currently, the available data regarding the relationship between ration and milk production on Thai dairy farms are limited. A cross sectional survey was, therefore, conducted on farms in Thailand which varied in type

and amount of roughage supplied to dairy cows with both feed and milk samples collected and analysed. It was anticipated that this study would provide a first insight into the potential to improve milk production on Thai farms with the use of local feedstuffs.

MATERIALS AND METHODS

Animals and feeding

In the current study healthy, multiparous crossbred Holstein-Friesian cows housed at six different dairy farms in Thailand were used. The average size of the herd was 49 ± 17 cows. Four to seven multiparous cows per farm between the 2nd to 4th parity were selected with 33 cows being, on average, 70 ± 10 days in milk (DIM) and 30 cows 130 ± 17 DIM. Eighteen cows were followed during both early- and mid-lactation. Each farm was visited once or twice to monitor milk performance, feed intake and nutrient composition of the rations.

Table 1. Climatological indices and characteristics of the Thai farms included in the study

Farm	No cows	Concentrate type	Roughage type	T (°C)	RH (%)	THI
A	46	Commercial	Urea treated rice straw, Ruzi hay and *Panicum maximum* TD58	25	67	71
B	41	Commercial	Pineapple peal and *Panicum maximum* TD58	27	79	78
C	44	Farm-made	Rice straw	31	68	83
D	32	Commercial	Baby corn stem, baby corn husk and rice straw	29	79	81
E	49	Commercial	Baby corn husk and *Centrosema pascuorum cv. Cavalcade*	30	73	82
F	83	TMR	Total mixed ration	26	73	76

T, average temperature; RH, relative humidity; THI, temperature humidity index.

The participating farms were similar in farm management in terms of milking and feeding but used various feedstuffs (Table 1). During the study period, the farmers did not change the type of roughage used while the amount of concentrate was adjusted to milk yield and lactation period. The cows were housed in tie stall barn throughout the lactation period and had free access to water. The concentrates were given twice daily in two similar portions during milking times (~05:30 and ~15:00 h). The roughage was offered after the concentrate was consumed except for the 4 cows on farm F that received a total mixed ration (TMR).

Feeds offered and feed refusals were recorded per individual cow throughout 24 h and used to calculate feed intake. Each farm used a milking machine and milk yields of individual cows were recorded at each milking.

Sampling and laboratory analyses

Concentrate and roughage samples were collected on the same day as feed intake was determined. Feedstuffs were dried to constant weight in a forced air oven at 60 ºC and then allowed to equilibrate to the air. The dried feed samples were ground to pass a 1 mm sieve and stored at 5 ºC until chemical analysis. Dry matter (DM), ash, crude protein (CP) and ether extract (EE) were analysed according to the AOAC (1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed following the method of Van Soest *et al.* (1991). Total digestible nutrients (TDN) of all roughages and that of the farm made concentrates on farms C and F were calculated from the ingredients in the ration based on the Thai nutrient composition table (Kanjanapruthipong, 2006). The feed company provided the TDN values of the commercial concentrates. The non-fibre carbohydrates (NFC) content of concentrates was calculated as 100% minus the percentages of ash, CP, EE, and NDF in dry matter. Sodium was measured by flame emission spectroscopy (AA-6800, Shimadzu, Kyoto, Japan).

Milk samples were collected at the two consecutive milking times for determination of milk composition. Milk samples were placed in bottles containing 0.024% (w/v) of sodium azide and stored at 5 °C for ≤ 3 days until analysis of fat, protein, lactose and non-fat solids by the infrared method (Bentley, 2000, Agriyork Ltd. UK).

Body weight of the cows was determined by heart girth measurement (chest circumference) using a weight band (Heinrichs *et al.*, 1992). Body condition score was determined on a scale of 1 to 5 with 1 (emaciated) to 5 (obese) based on Edmonson *et al.* (1989). Body weights were determined 2 h after feeding in the morning on the day that feed intake and milk yield were recorded.

Statistical analyses

Overall effect of farm on the parameters (milk yield, milk composition, DM intake of feed and feed composition variable) was tested for significance by the use of one way ANOVA in the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The statistical model was:

$$Y = \mu + Farm_i + \varepsilon_{ij}$$

where Y = parameter to be tested, μ = mean, $Farm_i$ = effect of farm i, and ε_{ij} = error term. For each parameter, differences among means of farms were tested for significance by ANOVA using the Tukey multiple range test. The normality and independent assumptions of ε_{ij} were confirmed through residuals analysis and no indication of model inadequacy was found.

Pearson correlations between milk yield (kg/d) and CP intake (kg/d), NDF intake (kg/d), NFC intake (kg/d), EE intake (kg/d) and Na intake (g/d) were calculated using the CORR procedure of SAS (SAS Inst. Inc., Cary, NC, USA). Throughout, the level of statistical significance was pre-set at $P < 0.05$.

RESULTS

Feed composition, forage to concentrate ratio and nutrient intake

At 70 days in milk

The total CP content of the rations ranged from 10.5 to 26.3% of DM (Table 2). The ration provided to the cows on farm F contained the highest dietary CP content and these cows had the highest total CP intake (Table 3). Although the lowest dietary CP content was observed on farm A, CP intake was similar to the cows on farm C due to the greater DM intake. The cows on farms C and E received a ration with a similar dietary CP content and their protein intakes were similar to that of cows on farms A and D. The highest mean EE intake was observed on farm B while the lowest EE intake was observed at farms A, D and F, which is in line with the dietary EE contents on these farms. The percentage of NFC was the lowest on farm A while cows on farms D and E had the highest % of NFC in their ration. Taking differences in DM intake into account, the cows at farms D, E and F ingested the highest amounts of NFC while NFC intake was lowest at farms A and B. The NDF contents of the ration were similar on farms B, D and E. Rations containing the lowest and highest NDF contents were fed on farms F and A, respectively. However, NDF intake was only significantly different on farm A. The forage to concentrate ratio of the ration was similar on farms B, C, D and F and highest on

farm A. The TDN intake was significantly different between farms C and F. The dietary Na content ranged from 0.11 to 0.39% of DM with the lowest dietary Na content was observed on farm A, which was reflected in the low Na intake of the cows on this farm. However, Na intake by the cows on farm A was similar to those on farm D.

Table 2. Feed composition and forage to concentrate ratio (F/C) provided to cows at 70 and 130 days in milk at six Thai farms

Farm	Feed composition (% DM)[1]								F/C
	CP	EE	NFC	CF	NDF	ADF	TDN	Na	
70 days in milk									
A	10.5^{e}	2.9^{c}	16.7^{c}	29.5^{a}	56.3^{a}	37.4^{a}	0.56^{a}	0.11^{a}	2.28^{a}
B	18.7^{b}	7.2^{a}	26.4^{b}	15.5^{c}	37.2^{c}	19.2^{cd}	0.71^{d}	0.39^{d}	0.54^{c}
C	13.5^{d}	3.8^{b}	28.7^{b}	18.9^{b}	45.0^{b}	26.1^{b}	0.63^{b}	0.34^{d}	0.51^{c}
D	16.2^{c}	2.5^{cd}	33.8^{a}	16.0^{bc}	37.4^{c}	21.3^{c}	0.69^{d}	0.18^{b}	0.80^{bc}
E	13.0^{d}	4.5^{b}	34.6^{a}	18.0^{bc}	40.5^{bc}	20.7^{cd}	0.70^{d}	0.27^{c}	1.43^{b}
F	26.3^{a}	2.1^{d}	27.5^{b}	16.3^{bc}	30.7^{d}	18.0^{d}	0.66^{c}	0.24^{c}	0.47^{c}
SEM	0.82	0.33	1.06	0.87	1.41	1.15	0.01	0.02	0.13
130 days in milk									
A	11.8^{c}	3.1^{cd}	19.1^{b}	27.9^{a}	53.5^{a}	35.3^{a}	0.58^{a}	0.12^{a}	1.85^{a}
B	17.4^{a}	6.8^{a}	26.2^{ab}	14.6^{c}	38.2^{cd}	19.5^{d}	0.73^{c}	0.36^{b}	0.74^{c}
C	13.4^{bc}	3.8^{bc}	26.7^{a}	19.2^{bc}	45.3^{b}	26.4^{b}	0.63^{ab}	0.34^{bc}	0.53^{c}
D	15.3^{ab}	2.4^{d}	30.8^{a}	22.2^{ab}	41.1^{bc}	24.2^{bc}	0.61^{a}	0.16^{a}	1.02^{bc}
E	13.0^{bc}	4.4^{b}	32.2^{a}	18.2^{bc}	41.1^{bc}	20.8^{cd}	0.62^{ab}	0.27^{c}	1.54^{ab}
F	15.3^{ab}	5.9^{a}	33.0^{a}	16.5^{bc}	33.6^{d}	22.1^{bcd}	0.67^{b}	0.33^{bc}	0.48^{c}
SEM	0.41	0.29	1.07	0.94	1.20	1.05	0.01	0.02	0.11

[1]CP, crude protein; EE, ether extract; NFC, non-fibre carbohydrate; CF, crude fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre; SEM, standard error of mean; TDN, total digestible nutrients; Na, sodium; F/C, forage to concentrate ratio.

[abcd] Values within a column that do not have a common superscript differ significantly ($P < 0.05$) for cows at 70 days or 130 days in milk.

Table 3. Nutrient intake (kg/d) provided to cows at 70 and 130 days in milk at six Thai farms

Farm	DMI[1]	CP	EE	NFC	NDF	TDN	Na (g/d)
70 days in milk							
A	13.6^{ab}	1.44^{a}	0.40^{a}	2.29^{a}	7.63^{b}	7.59^{ab}	14.7^{a}
B	11.1^{a}	2.09^{b}	0.81^{c}	2.96^{ab}	4.11^{a}	7.90^{ab}	44.2^{b}
C	11.4^{a}	1.54^{a}	0.44^{ab}	3.29^{bc}	5.12^{a}	7.22^{a}	39.3^{b}
D	12.0^{ab}	1.93^{ab}	0.30^{a}	4.04^{cd}	4.56^{a}	8.25^{ab}	21.2^{a}
E	12.9^{ab}	1.68^{ab}	0.57^{b}	4.45^{d}	5.21^{a}	9.09^{ab}	35.2^{b}
F	14.5^{b}	3.81^{c}	0.30^{a}	3.99^{cd}	4.46^{a}	9.63^{b}	38.0^{b}
SEM	0.33	0.13	0.04	0.15	0.23	0.23	2.11
130 days in milk							
A	15.5^{bc}	1.83^{ab}	0.49^{a}	2.96^{a}	8.32^{b}	9.07^{a}	18.4^{a}
B	12.7^{ab}	2.27^{bc}	0.89^{b}	3.41^{ab}	4.68^{a}	9.22^{ab}	47.4^{cd}
C	11.3^{a}	1.51^{a}	0.43^{a}	2.99^{a}	5.13^{a}	7.13^{a}	38.4^{bcd}
D	13.8^{ab}	2.08^{abc}	0.32^{a}	4.21^{ab}	5.66^{a}	8.37^{a}	22.0^{ab}
E	13.5^{ab}	1.74^{ab}	0.59^{a}	4.33^{bc}	5.53^{a}	8.32^{a}	35.7^{abc}
F	17.2^{c}	2.64^{c}	1.02^{b}	5.66^{c}	5.78^{a}	11.47^{b}	56.6^{d}
SEM	0.44	0.09	0.05	0.20	0.24	0.31	2.85

[1]DMI, dry matter intake; CP, crude protein; EE, ether extract; NFC, non-fibre carbohydrate; NDF, neutral detergent fibre; SEM, standard error of mean; TDN, total digestible nutrients; Na, sodium.

[abcd]Values within a column that do not have a common superscript differ significantly ($P < 0.05$) for cows at 70 days or 130 days in milk.

At 130 days in milk

Cows on farm A were fed rations with similar dietary CP contents (Table 2) as the rations used on farms C and E. The CP content of the ration was highest on farm B. However, total CP intake (Table 3) by the cows was highest on farm F due to significantly higher DM intakes. Cows on farm C had similar CP intakes as the cows on farms A, D and E. The cows on farms B and F had the highest EE intakes, which was in line with the significantly higher EE contents of the ration on these farms. The NFC contents of the ration offered to the cows on farms C, D, E and F were similar and significantly higher than the dietary NFC content of the ration fed on farm A. However, the highest intake of NFC was observed at farm F. The intake of NFC by the cows on farms A, B, C and D were similar. Both, the dietary NDF contents and the NDF intakes of the cows that were 130 days in milk followed the same trend as described for the early lactating cows and are in line with the calculated forage to concentrate ratios of the ration. The highest dietary TDN values were calculated for the ration

fed on farm F while the ration with the lowest TDN value was fed on farm A and D. With respect to the dietary Na content, these were similar at both at 70 and 130 days in milk.

Table 4. Mean milk yield (MY), 4% fat corrected milk (FCM) and milk composition of cows at 70 and 130 days in milk for six Thai farms

Farm	No cows	MY (kg/d)	FCM (kg/d)	Milk composition (%)			
				Fat	Protein	Lactose	Non-fat solids
70 days in milk							
A	5	9.1^{a}	9.28^{a}	4.20^{ab}	2.75	4.84	8.43
B	7	17.3^{b}	18.7^{bc}	4.59^{ab}	2.97	4.87	8.64
C	5	16.9^{b}	17.8^{bc}	4.33^{ab}	3.04	4.74	8.45
D	7	17.2^{b}	16.0^{ab}	4.16^{ab}	2.99	4.71	8.80
E	5	18.7^{b}	17.9^{bc}	3.73^{b}	2.76	5.03	8.66
F	4	23.0^{b}	25.0^{c}	4.61^{a}	2.98	4.96	8.76
SEM[1]		0.88	1.17	0.16	0.06	0.04	0.08
130 days in milk							
A	5	8.2^{b}	7.8^{b}	3.76	2.87	4.65	8.33
B	5	17.2^{a}	15.6^{a}	3.81	3.23	4.83	8.88
C	5	17.8^{a}	17.8^{a}	4.02	2.85	4.85	8.54
D	5	13.7^{ab}	13.0^{ab}	4.02	3.20	4.82	8.84
E	6	17.7^{a}	18.0^{a}	4.11	3.02	4.93	8.67
F	4	13.6^{ab}	11.7^{ab}	3.06	2.74	4.87	8.45
SEM		0.99	0.91	0.18	0.06	0.05	0.07

[1]SEM, standard error of mean.

[abc]Values within a column that do not have a common superscript differ significantly ($P < 0.05$) for cows at 70 days or 130 days in milk.

Milk yield and milk composition

Milk yield was significantly lower at farm A as compared to farms B, C, D, E and F (Table 4) for 70 days in milk. In terms of fat corrected milk (FCM), milk yield was significantly higher at farm F. Milk fat content was significantly higher on farm F compared to farm E. The contents in milk protein, lactose and non-fat solids were not significantly different among the farms.

At 130 days in milk, the lowest milk yield was observed at farm A which was significantly different from the milk yields recorded at farms B, C and E. Trends of FCM

were similar to that of milk yield. The concentrations of fat, lactose, protein and non-fat solids in milk were not significantly different between the farms.

Body weight and body condition score

There were no significant differences in both body weight (BW) and body condition scores (BCS) of cows between farms. Moreover, BW and BCS were not significantly different between 70 and 130 days after calving. Mean BW at 70 and 130 days after calving were 470 kg (SD 70) and 480 kg (SD 70), respectively. Mean BCS were 2.90 (SD 0.35) and 2.80 (SD 0.35) at 70 and 130 days after calving, respectively.

Table 5. Correlations between nutrient intake (kg/d) and milk yield (MY, kg/d) for cows 70 and 130 days in milk at six Thai farms

Component	70 days in milk		130 days in milk	
	n	MY	*n*	MY
CP[a]	33	**0.65**	30	0.22
EE	33	0.17	30	0.31
NFC	33	**0.67**	30	0.24
NDF	33	**-0.43**	30	**-0.42**
TDN	33	**0.59**	30	0.09
Na (g/d)	33	**0.62**	30	**0.55**

[a]CP, crude protein; EE, ether extract; NFC, non-fibre carbohydrate; NDF, neutral detergent fibre; TDN, total digestible nutrients; Na, sodium.

Values in bold indicate significant ($P < 0.05$) correlations.

Correlations between milk yield and ration

The correlations between milk and nutrient intake are presented in Table 5. Milk yield was positively correlated with dietary CP, NFC, TDN and Na intake and negatively correlated with NDF intake ($P < 0.05$) at 70 DIM, while milk yield was positively correlated with Na intake and negatively correlated with NDF intake ($P < 0.05$) only in the mid lactating cows.

DISCUSSION

Among the farms investigated during early lactation (70 DIM), A had the lowest milk production, whereas the highest milk production was found for F. Although DMI was not different between these two farms, the intake of nutrients such as protein, NFC, TDN and Na was low on farm A, probably because of the high forage to concentrate ratio in the ration as indicated by the high intake of NDF of the cows on farm A. It is generally accepted that milk yield is positively correlated with concentrate intake and negatively with forage intake (McEvoy *et al.*, 2008; Tessmann *et al.*, 1991; Friggens *et al.*, 1998; Murphy *et al.*, 2000). Indeed, the dietary forage to concentrate ratio was numerically lowest on farm F. Concentrates have a high CP and TDN content whereas the forage in tropical countries are high in NDF and low in both CP and TDN. Therefore, cows which are fed high amounts of concentrate will receive more protein and TDN which affects milk production. In contrast to the data obtained at 70 days after calving, milk production of the cows at 130 days was similar on farms A and F. However, the significantly lower protein intake ($P < 0.02$) of the cows on farm F at 130 versus 70 days after calving might explain this apparent discrepancy as both energy and protein intake are related to milk production. These results are supported by previous reports showing that milk production increases in response to increased protein intake over a range of energy intakes (Frank and Swensson, 2002; Macleod *et al.*, 1984; Tessmann *et al.*, 1991).

Efficiency of milk production

Across herds and the two stages of lactation, the average efficiency of the conversion of feed into milk (kg FCM/kg DMI) was calculated to be 1.22. This value is lower than that can be calculated on the basis of data reported by Blake *et al.* (1986); i.e. 1.40. In the latter study, Holstein cows with mean BW of 537 kg were used and data were obtained at 75 and 143 DIM (Blake *et al.*, 1986). Therefore, it can be speculated the difference in feed efficiency (i.e. 1.22 *versus* 1.40 kg FCM/kg DMI) is caused by the nutrient composition of the rations. This notion is corroborated by the current observation that, at least at 70 DIM, the feed efficiency on farm F was greater than that on farm A (i.e. 0.50 and 1.72, respectively) and that the nutrient density (g/kg DM) of the ration on farm F versus farm A also was greater (Table 2).

During mid lactation, cows on farm C and E had a higher feed efficiency than cows on farms A and F (Figure 1). The percentage of NFC on farm E was high which is associated with a high concentrate intake, but the cows on farm E had a high F/C in combination with a

high %NFC. However, the higher F/C and lower protein intake on farm E did not cause the low milk production. This might indicate that the cows on farm E were fed with high quality roughage such as corncobs and legume (*Centrosema pascuorum cv. Cavalcade*). The relatively high %NDF on farm E might be associated with a higher quality of forage which leads to a better utilization of NDF which may have stimulated the fermentation activity, resulting in an increased DMI, leading to higher milk production (Grant, 1997).

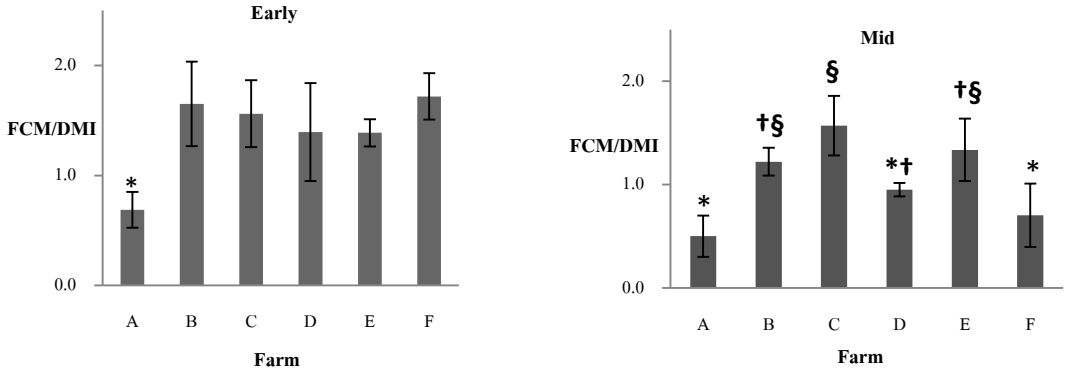

Figure 1. Average and standard deviation of feed efficiency of dairy cows in early and mid lactation on six farms (A-F) in Thailand. ***,†,§** different symbols indicate significant differences ($P < 0.05$) between bars. FCM, 4% fat corrected milk; DMI, dry matter intake.

Composition of milk

Over a wide range of nutrient intakes (Table 3), the contents of protein, lactose and non-fat solids in milk were found to be similar between farms. This observation is in line with the well accepted idea that the contents of protein, lactose and ash in milk are not very sensitive to changes in ration composition (Linn, 1988). In contrast, the fat content of milk showed more variation between farms and at 70 compared to 130 DIM and found to be different between farms E and F. A major dietary characteristic that influences the fat content of milk is F/C, at least in rations with low fat contents (7-8% DM) (Palmquist and Conrad, 1978), and a high F/C is generally associated with a high fat content of milk (Linn, 1988). However, F/C of the rations fed at farm F was lower than that of farm E while the cows had farm F had higher milk fat contents. Clearly, F/C ratio does not explain the difference in milk fat content between the two farms. Next to the dietary F/C, the amount and degradation rate of NFC is of interest with respect to the milk fat content and both a high NFC intake and rapid degradation rate of NFC are associated with lower contents of milk fat (Batajoo and Shaver, 1994). Thus, it may be speculated that the lower milk fat contents found on farm E are related, at least partially, to a difference in NFC intake. Indeed, NFC intake by the cows on farm E was 11.5%

greater than on farm F. It thus appears that the concentrates fed to the cows on farm E had a high content of NFC because F/C of the ration on farm E was higher than at farm F; i.e. 1.43 and 0.47, respectively. Unfortunately, the ingredient composition of the commercial concentrate fed at farm E is not known but perhaps the NFC had a rapid degradation rate. Finally, the cows at farm F produced 23% more milk than the cows at farm E and it can therefore not be excluded that the cows at farm F versus E, experienced a greater nadir negative energy balance (NEB). This condition also can potentially attribute to the difference in milk fat content between farm E and F because fatty acids mobilized during NEB are partially excreted with milk (Stoop *et al.*, 2009).

Correlation between milk and nutrient intake

The correlation between TDN and NFC intake and milk yield was 0.59 and 0.67, respectively, in early lactation while this correlation was not evident in mid lactation. The current observation is corroborated by Coulon and Remond (1991), who reported that milk yield response to energy supply was greater for cows in early than mid lactation. In practice, TDN is used as a measure of energy to formulate rations of Thai dairy cows. However, energy requirements are expressed as MJ NE_L by the NRC (2001). Therefore, current TDN values were converted into NE_L using the equation: $NE_L = 0.0245 \times TDN - 0.12$ (NRC, 2001). It appeared that NE_L intake during early lactation was 5% lower than required while NE_L intake was 14% greater than required during mid lactation. Thus, it can be suggested that the intake of extra energy does not increase milk production in mid lactation. Therefore, in terms of milk production, it is not advantageous to provide more energy than NRC requirements to cows in mid lactation.

The positive correlation ($r = 0.65$) between milk yield and CP intake was clearly high during early lactation. Both during early and mid-lactation, cows consumed greater amounts of CP than recommended by the NRC (2001). The extra intake of CP was approximately 5 and 8%, respectively. It may be suggested that early lactating cows in Thailand fed locally available roughages require somewhat more CP than indicated by the NRC. Interestingly, significant positive correlations were calculated between Na intake and milk yield. This observations is somewhat difficult to explain because Na intake was highly correlated with concentrate intake ($r = 0.66$). In other words, the increase milk yield may be related to a higher intake of Na but this is confounded with the amount of concentrate intake. On the other hand, the temperature humidity index (THI) ranged from 71 to 83 (Table 1) indicating that the cows experienced mild to moderate heat stress (Armstrong, 1994) during the current study.

Sanchez *et al.* (1994) have suggested that the Na requirement is increased during heat stress due to the higher Na loss associated with sweating. Moreover, the current observation is line with results reported by Schneider *et al.* (1984, 1986) who suggested that the feeding of Na above the current NRC recommendations (i.e. 1.9-2.2 g/kg DM) may stimulate milk production in heat stressed cows.

In conclusion, protein, non-fibre carbohydrates and Na intake may have limited milk production by dairy cows on the Thai farms participating in the current study.

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Chapter 3

An Attempt to Define the Sodium Requirement of Lactating Dairy Cows in a Tropical Environment

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ABSTRACT

Lactating dairy cattle in the tropics may require more Na due to the hot and humid climatic conditions. It is unknown whether the current recommendations of Na for lactating cows can be quantitatively used in the tropical countries. This study attempted to define the sodium requirement of lactating dairy cows under tropical conditions by measuring Na in saliva, milk and faeces. The concentrations of Na and K in milk, faeces and serum were not affected by dietary treatments. The amount of Na absorbed by cows fed the basal diet containing 0.4 g Na/kg DM was equal to the amount of Na lost in the milk, showing that these animals were fed an Na-deficient ration. This observation was corroborated by the salivary Na and K levels, with the cows on the low-Na diet having salivary Na concentrations below 120 mmol/L in combination with salivary K concentrations above 20 mmol/L ($P < 0.05$). Consumption of a daily ration formulated to contain the current Na requirement set by the NRC appears to provide too much Na for lactating cows under tropical conditions. A tentative value of 1.2 g Na/kg DM is proposed as the Na requirement for dairy cows under tropical conditions.

INTRODUCTION

Sodium (Na) is a primary extracellular cation in animals and its main functions include maintenance of body fluid balance, osmotic pressure, and acid-base balance (McKenown, 1986). Furthermore, Na is a major component of minerals present in saliva and it buffers acids produced during ruminal fermentation (Blair-West *et al.*, 1970). In cows, Na deficiency has been associated with loss of appetite, decreased milk yield and pica (NRC, 2001). In ruminants the salivary Na concentrations drops to levels below 120 mmol/L with a concomitant increase in salivary K concentration during Na deficiency (Schonewille and Beynen, 2005a). Therefore an assessment of salivary Na and K concentration is highly instrumental in diagnosing Na deficiency. Clearly, a drop in the salivary Na concentration in combination with an increased level of salivary K results in a decreased Na/K ratio. Indeed, it was suggested by Schonewille and Beynen (2005a) that a salivary Na/K ratio lower than 6 is indicative of Na deficiency. The current Na requirements for dairy cows recommended by various authorities range from 0.5 to 2.2 g/kg DM (Table 1). However, it is unclear whether these recommendations can be quantitatively extrapolated to tropical conditions, because all requirements were set under temperate conditions. Consequently, the Na requirements listed in Table 1 do not specifically take into account Na loss through sweating during heat stress. Indeed, it was suggested by Sanchez *et al*. (1994) that the Na requirement is increased during heat stress owing to the higher Na loss associated with sweating. Furthermore, Schneider *et al.* (1986, 1984) reported that dry matter intake (DMI) was increased during heat stress when the dietary Na content was increased from 1.8 to 5.5 g/kg diet. This increase in DMI can be explained by the possibility that animals fed the low-Na ration were in negative Na balance.

In tropical countries such as Thailand the level of Na in feedstuffs used to formulate dairy rations is much lower than recommend (Minson, 1990) by the various authorities (Table 1) and therefore dairy rations are routinely supplemented with Na. The scientific basis for the amounts of Na addition to the ration, however, is scant. Generally, dairy cattle in tropical countries have lower production and consume poorer-quality feeds while exposed, at certain times to high temperature and relative humidity. In addition, excessive Na intake relative to the animals' requirement should be avoided in tropical countries in the light of responsible water management and water scarcity, as water consumption increases to excessive Na intake to maintain normal physiological Na concentrations in the extracellular fluid. The aim of this study was to attempt to define the Na requirement of lactating dairy cows under tropical conditions by measuring Na level in saliva, milk and faeces.

Table 1. Summary of sodium (Na) requirements set by different authorities

Reference	Na requirement (g/kg DM)
Underwood and Suttle, 1999	0.5 - 1.0
ARC, 1980	0.8 - 1.2
CVB, 2005	0.7[a] - 1.4[b]
DLG, 2001	1.0 - 1.5[b]
INRA, 1989	1.0 - 1.7
NRC, 2001	1.9 - 2.2

[a]Dry cows.

[b]Lactating cow, 40 kg of milk.

MATERIALS AND METHODS

Animals and experimental design

Fifteen crossbred multiparous Holstein Friesian (HF × indigenous) cows with a mean body weight of 485 kg (standard deviation (SD) 74 kg) and 93 days in milk (SD 39 days) were used. The cows were housed individually in a naturally ventilated tied stall for a period of 28 days, whereafter, during the following 9 days, they were housed in individual metabolic cages to facilitate the collection of faeces, saliva and blood. The experiment was conducted during the summer season from March to April with the cows exposed to local climatic conditions. Daily minimum and maximum temperatures directly outside the stall were measured throughout the study. Cows were fed individually and milked twice daily at 06:00 and 17:00. Prior to the start of the experiment, animals were randomly assigned to one of three dietary treatments, i.e. low, medium or high Na.

Experimental rations

All cows were fed a total mixed ration (TMR). The basal TMR was formulated to be deficient in Na (Table 2) and contained 0.4 g Na/kg DM. The medium- and high-Na rations were formulated by supplementing the basal ration with two levels of salt (NaCl). The Na contents of the medium- and high-Na rations were 1.6 and 4.0 g/kg DM, respectively. The ingredient and nutrient composition of the three dietary treatments are given in Table 2. The TMR was offered *ad libitum* twice daily and leftovers were removed twice daily before feeding. Water was available for all cows at all times.

Table 2. Ingredient and nutrient compositions of the low-, medium- and high-sodium (Na) experimental rations

Component	Low Na	Medium Na	High Na
Ingredient composition (g/kg as fed)			
Constant components[a]	630.0	630.0	630.0
Cassava chips	370.0	367.0	361.0
Salt (NaCl)	-	3.0	9.0
Analysed nutrient composition			
Dry matter, g/kg as fed	797.2	768.6	791.7
Crude protein, g/kg	173.3	171.2	166.1
Ether extract, g/kg	47.7	45.1	46.0
Crude ash g/kg	90.5	86.4	91.7
Neutral detergent fibre, g/kg	376.2	385.3	352.6
Acid detergent fibre, g/kg	231.8	240.8	212.9
Sodium, g/kg	0.4	1.6[b]	4.0[b]
Potassium, g/kg	10.2	10.7	10.2

[a]The constant components were 112 g soybean meal, 50 g dried tomato pomace, 100 g cottonseed meal, 51 g dried brewer's grain, 17 g tallow, 62 g molasses, 18 g urea, 1 g dicalcium phosphate, 3 g magnesium oxide, 6 g oystershell, 2 g sulphur, 205 g milled rice straw, 3 g mineral premix per kg diet. The mineral premix consisted of 440,000 IU vitamin A, 60,000 IU vitamin D_3, 30,000 IU vitamin E, 11.6 g Fe, 0.03 g Mn, 5.6 g Cu, 11.60 g Zn, 0.07 g I, 0.06 g Se, 10.0 g Mg and 15.0 g P per kg.

[b]Calculated on the basis of the analysed Na content of the low-Na ration and the amount of Na supplementation.

Sample collection and chemical analyses

Feed intake of each cow was recorded daily during the collection period. Samples of the TMR were taken weekly, dried at 60 °C, ground and pooled per treatment before analyses of dry matter, crude protein and crude fibre according to AOAC procedures (AOAC, 1990). Intrinsic Na and K of the TMR were measured using wet ashing and atomic absorption spectrophotometry.

Faeces were quantitatively collected and weighed each day during the 5 day collection period. The faeces collected each day were thoroughly mixed and samples of 10% of the total amount of faeces were taken and stored at -18 °C in plastic bags. At the end of the experiment the faeces samples were pooled for each cow and mixed thoroughly. The pooled samples were

dried at 60 °C, ground and stored until analyses for Na and K. Data from feed and faeces were used to derive the amount of Na and K being absorbed.

Milk production was recorded during the collection period and samples of approximately 30 mL per milking time (06:00 and 17:00) were collected for 5 days. Milk samples in proportion to yield on the sampling day were preserved by the addition of 0.2 g/L 2-Bromo-2-nitro-1, 3-propanediol and stored at 5 °C. Five-day milk samples from each cow were mixed, deproteinised with 100 g/L trichloroacetic acid, vortexed and centrifuged at 800 × *g* for 10 min, whereafter the supernatant was stored at -18 ° C until mineral analyses.

Blood samples (10 mL) were collected from the jugular vein of each cow at the last day of the collection period at 10:00. Serum samples were obtained by centrifuging of the blood samples at 800 × *g* for 5 min. All serum samples were stored at -20 °C until mineral analyses.

Saliva samples were collected on day 4 of the faeces collection period before the morning feeding to prevent Na contamination by the feed. Saliva sampling was done by placing sponges in the mouths of the cows at the third premolar in the maxilla for a period of 3 min. Subsequently the liquid in the sponges was collected into a plastic bag, transferred into a tube and stored at -18° C. Each sponge was washed thoroughly with demineralised water and dried at 40° C on a glass plate before being used to collect saliva.

All samples of faeces, milk, serum and saliva were analysed for Na and K using a flame emission atomic absorption spectrophotometer (AA-6800, Shimadzu, Kyoto, Japan) in accordance with the manufacturer's specification.

Calculations and statistical analysis

The data were statistically analysed by subjecting them to analysis of variance with treatment as factor. Multiple comparisons between groups were made by Duncan's test. All the statistical analyses were performed using SPSS for Windows 11.5 (SPSS Inc., Chicago, IL, USA) with the level of significance pre-set at 5%.

RESULTS AND DISCUSSION

The mean neutral detergent fibre content of experimental rations was found to be 371 g/kg (SD 16.9, $n = 3$), which is lower than recommended by NRC (2001). However, the animals showed normal rumination, were apparently healthy and remained in good condition during the experiment. DMI was not affected by dietary treatment ($P > 0.38$). The mean intake for all cows was 16.5 kg/d DM (standard error (SE) 0.33). The observed level of DMI was

somewhat lower than anticipated, i.e. 16.5 *vs* 18 kg/d. Although there was no significant differences in DMI among treatments, DMI tended to increase with Na supplementation (low, 15.9 kg/d; medium, 16.3 kg/d; high, 17.1 kg/d). In a study by Sanchez *et al.* (1997) dietary supplementation with NaCl did not alter DMI. In some earlier studies, DMI was increased when cows received a ration with an increased amount of both Na and K (Beede, 1991; Silanikove *et al.*, 1998). We only applied our treatment for a period of 37 days, which was not sufficiently long to induce severe Na deprivation in the low-Na fed animals. Aines and Smith (1957) reported that mild to severe symptoms of Na deficiency were shown after 16 months of feeding a low-Na diet, because the rumen could act as a buffer and contain up to 50% of the available body Na (Bell, 1995).

As expected, Na intake was significantly affected by treatment (Table 3). Faeces Na excretion was significantly higher in the medium- and high-Na groups than in the low-Na group. Apparent Na absorption expressed as a percentage of intake was significantly higher with the high-Na diet. Apparent Na absorption ranged between 80 and 96% of intake, which is in agreement with Kemp (1964) and Martz *et al*., (1988) who reported a similar range in apparent Na absorption in dairy cows. The relatively low level of apparent Na absorption in cows fed the low-Na ration can be explained by the inevitable faecal Na loss. Based on specific assumptions for estimating the endogenous faecal Na loss, a value of 1.6 g/d was calculated by Schonewille and Beynen (2005a). This value is similar to the faecal Na loss of 1.4 g/d in the low-Na fed cows. The cows fed the low-Na diet also had a very low Na concentration in the saliva (Table 4). Apparent K absorption was not significantly affected by dietary Na concentration and was 93% of intake (Table 3). This value is in agreement with the results of Hemken (1983) and Greene *et al*. (1983a,b) who reported an apparent faecal digestibility of 95% or higher for most feedstuffs.

Table 3. Sodium and potassium intake, faecal excretion, apparent absorption and milk content of dairy cows fed low-, medium- and high-sodium (Na) rations

Parameter	Treatment			Pooled SEM	*P* value
	Low Na	Medium Na	High Na		
Sodium					
Intake, g/d	6.9[a]	26.3[b]	67.8[c]	6.8	<0.001
Faeces, g/d	1.4[a]	3.0[b]	2.6[ab]	0.3	0.041
Absorption, g/d	5.5[a]	23.3[b]	65.2[c]	6.7	<0.001
Absorption, % of intake	80.2[a]	88.7[ab]	96.1[b]	2.4	0.013
Milk, g/d	5.5	6.5	6.9	0.4	0.446
Potassium					
Intake, g/d	162.7	173.8	173.7	3.4	0.328
Faeces, g/d	12.1	11.6	12.1	0.8	0.972
Absorption, g/d	150.5	162.2	161.6	2.9	0.758
Absorption, % of intake	92.6	93.3	93.2	0.4	0.198
Milk, g/d	23.6	25.4	29.3	1.7	0.422

Means with different letters in a row are significantly different ($P < 0.05$). SEM, standard error of mean.

Concentrations of salivary Na and K are given in Table 4. When dietary supply of Na is sufficient, the salivary Na concentrations is higher than 120 mmol/L (Morris *et al.*, 1980). In the present study the cows fed the low-Na ration had a salivary Na concentration of 95.6 mmol/L, indicating that these animals were fed below their Na requirement. The salivary K concentration was approximately 12 mmol/L after feeding the medium- and high-Na rations. This level is similar to the value set by Silanikove *et al.* (1998) as a tentative criterion for a sufficient supply of dietary Na. In Na-deficient ruminants, there is a replacement of Na^+ with K^+ in the saliva, causing a reduction in the Na/K ratio (Murphy and Connell, 1970; Underwood and Suttle, 1999). The use of the salivary Na/K ratio as a diagnostic tool to detect Na deficiency has been extensively reviewed by Schonewille and Beynen (2005a) and Suttle (2010), and it was concluded that an Na/K ratio lower than 6 (Schonewille and Beynen, 2005a) is associated with Na deficiency in ruminants. In the current study the salivary Na/K ratio was 4.8 when the cows were fed the low-Na ration. Such a low Na/K ratio corroborates our conclusion that Na deficiency occurred when the cows were fed the low-Na ration.

Table 4. Concentration of sodium (Na) and potassium (K) in saliva and serum of dairy cows fed low-, medium- and high-Na rations

Parameter	Treatment			Pooled SEM	*P* value
	Low Na	Medium Na	High Na		
Saliva					
Na, mmol/L	95.6[a]	121.0[ab]	132.0[b]	6.2	0.033
K, mmol/L	21.4[a]	11.8[b]	11.7[b]	1.7	0.020
Na/K ratio	4.82[a]	10.27[b]	12.46[b]	1.2	0.009
Serum					
Na, mmol/L	139	141	142	1.1	0.641
K, mmol/L	4.0	4.0	4.1	0.1	0.999

Means with different letters in a row are significantly different ($P < 0.05$). SEM, standard error of mean.

The apparent K absorption was greater than 150 g/d while 23.6 - 29.3 g/d of K was excreted in the milk. Inevitable urinary loss of K is estimated to be 38 mg/kg body weight (Schonewille and Beynen, 2005b). Thus in cows with a body weight of 485 kg the inevitable urinary K losses can be expected to be around 18.4 g/d. Based on this calculation, approximately 100 g of K was available for excretion in sweat, at least in adult, non-pregnant cows. Quantitative data on K loss through sweating are at least to our knowledge, not available. The clinical signs of K deficiency are not well documented, but reduced appetite, poor growth, muscular weakness, stiffness paralysis and intracellular acidosis have been reported (Underwood and Suttle, 1999). In the current study the animals appeared healthy, indicating that K deficiency did not occur during the trial.

The increase in Na intake did not induce any changes in serum concentrations of Na and K. These results are consistent with previous studies that reported an increased intake of Na via supplementation of the diet with NaCl and $NaHCO_3$ without any effect on plasma Na concentration (Eicher *et al.*, 2003; O'Connor *et al.*, 1988; Schneider *et al.*, 1984; Tucker and Hogue, 1990). Also, homeostatic regulation, which is controlled by several hormones, can maintain serum Na levels during Na deficiency (NRC, 2005). Aldosterone responds to a decline in serum Na concentration or to systemic low blood pressure. As a result, there is an increase in renal conservation of Na which increases in renal K excretion (NRC, 2005). It was reported by Schneider *et al.* (1986) that plasma Na concentration increased when $NaHCO_3$ was used to increase dietary Na from 1.8 to 8.8 g/kg DM. However, no increase in level of Na was seen when NaCl was utilised as Na source. It should be noted that the present study only

used the different diets for a period of 37 days, so there is still sufficient Na present in the body to maintain plasma Na levels within the normal physiological range even if the cow is fed a low dietary Na concentration.

Milk Na concentration and milk yield ($P < 0.292$) were not affected by the dietary Na content. The mean milk production for all cows was 15.0 kg/d (SE 0.68) which was lower than anticipated. The discrepancy between the predicted and the observed milk yield was at least partially explained by the observed level of DMI. The dietary NaCl content had no effect on the level of milk production in the current study, which agrees with earlier studies (Coppock *et al.*, 1982; Granzin and Gaughan, 2002). The Na excretion via the milk was numerically lower after feeding the low-Na ration because of lower milk yield (low, 13.7 kg/d; medium, 14.9 kg/d; high, 16.4 kg/d). The apparent faecal Na absorption in cows fed the low-Na diet was equal to the amount of Na lost in milk. In addition to the losses in milk and faeces, there are some inevitable Na losses in urine (Michell, 1995) as well as losses due to sweating caused by the warm humid conditions (Underwood and Suttle, 1999). Mean minimum and maximum daily temperatures were 24.6 and 34.8 °C respectively and mean minimum and maximum daily relative humidity were 50.0 and 88.1% respectively. Because the thermal neutral zone for cows has a temperature humidity index lower than 72, it is likely that the animals in the present study experienced heat stress during some parts of the day (Hahn and Mader, 1997). Therefore it is speculated that cows fed the low-Na diet were in negative Na balance owing to the inevitable Na loss with both urine and sweat. Because cows maintain Na balance by Na excretion with urine, it is anticipated that the excess of Na absorbed by cows fed the medium- and high-Na diets was excreted with urine. The outcome of the current study clearly showed that an Na content of 1.6 g/kg DM was sufficient to meet the Na requirement of dairy cows under tropical conditions. Clearly, the minimum Na requirement could not be derived from this study, but the current estimate of the Na requirement for dairy cows set by the NRC (2001) is too high. On the basis of the data provided in Table 1, a mean Na requirement of 1.2 g/kg DM can be calculated. This value is in line with the conclusion of Suttle (2010) who suggested that 1.2 g Na/kg DM is sufficient for cows with a DMI and milk yield similar to those observed in the current study. Therefore, we propose a tentative value of 1.2 g/kg DM as the Na requirement for dairy cows under tropical conditions.

CONCLUSIONS

A large increase in Na intake did not induce any changes in serum, faecal and milk Na concentrations but did affect the Na concentration in the saliva. The salivary Na/K ratio was a good indicator of Na intake by dairy cattle. Consumption of a diet containing Na at the level recommended by the NRC appears to provide too much Na for lactating cows under tropical conditions.

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Chapter 4

Response of Saliva Na/K Ratio to Changing Na Supply of Lactating Cows under Tropical Conditions

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ABSTRACT

Factorial determination of the sodium (Na) requirement of heat-stressed lactating cows is hindered by accurate estimates of the Na losses through sweat. Direct studies, therefore, may be needed requiring information on the time course of healthy animals to become Na depleted and the subsequent rate of repletion. The rate of Na depletion and subsequent rate of Na repletion with two levels of dietary Na to lactating dairy cows housed under tropical conditions were investigated using the salivary Na/K. The 12 lactating cows (salivary Na/K ratio 14.6) rapidly developed clinical signs of Na deficiency, including pica, polyuria and polydipsia, reduced body weight and reduced milk yield when fed a low-Na ration (0.33 g/kg dry matter (DM)) for 3 weeks. Deficiency symptoms were associated with a rapid decrease in salivary Na/K ratio to < 4.3 from 7 to 21 days. Subsequent repletion of the cows with NaCl to a ration concentration of 1.1 or 1.6 g Na/kg DM for 5 weeks did not restore salivary Na/K ratio to values of > 6. A daily Na intake of heat-stressed lactating cows to a ration intake of 1.6 g Na/kg DM was insufficient to restore Na deficiency. One week was sufficient to deplete heat-stressed lactating cows of Na, allowing for rapid dose-response studies utilizing the salivary Na/K ratio as a parameter for Na status of cows under tropical conditions.

INTRODUCTION

Sodium (Na) is essential for dairy cows to maintain water balance: in the event of Na deficiency polyuria and polydipsia occur (Whitlock *et al.*, 1975). Furthermore, Na deficiency is associated with clinical signs such as pica, loss of appetite and a decreased milk yield in dairy cows (Suttle, 2010a). Sodium is also a major component of salts in saliva to buffer acid generated during ruminal fermentation (Blair-West *et al.*, 1963). It is generally accepted that in Na-deficient ruminants the salivary Na concentration decreases to a value below 120 mmol/L with a concomitant increase in salivary potassium (K) concentration. As such, the use of the salivary Na/K is a sensitive diagnostic tool to detect Na deficiency in ruminants (Schonewille and Beynen, 2005). Previously, Thiangtum *et al.* (2011) showed the value of salivary Na/K in detecting Na deficiency in dairy cows housed under tropical conditions.

The current recommendations regarding an adequate Na supply for dairy cows ranges from 0.7 to 2.2 g/kg dry matter (DM) (ARC, 1980; CBV, 2005; DLG, 2001; INRA, 1989; NRC, 2001). However, these recommendations were derived from studies with animals housed under temperate conditions and thus do not take into account Na loss through sweating (Sanchez *et al.*, 1994) during heat stress. Indeed, it was suggested by Sanchez *et al*. (1994) that the Na requirement is increased during heat stress due to the higher Na loss associated with sweating. Furthermore, Schneider *et al*. (1986) reported an increased DM intake when the Na content of rations of lactating cows during heat stress was raised from 1.8 to 5.5 g/kg DM. The issue of Na requirement of dairy cows in tropical countries was addressed by Thiangtum *et al*. (2011), who arbitrarily suggested that dairy cows require a dietary Na content of 1.2 g/kg DM under tropical conditions. However, generalization of this tentative value of 1.2 g Na/kg DM is currently not warranted owing to a dearth of studies addressing the issue of Na requirements of dairy cattle in the Tropics.

The assessment of Na requirement of heat-stressed dairy cows is hindered by the fact that Na losses through sweat are difficult to quantify, and therefore direct studies of the Na requirements under tropical conditions are needed. Important information for such studies is the time course of healthy animals to become deplete of Na and the subsequent rate of repletion. In the current study, the rate of Na depletion and subsequent rate of Na repletion using two levels of dietary Na were investigated using salivary Na/K in heat-stressed lactating dairy cows. Lactating cows were initially fed a Na low ration (0.33 g/kg DM) for 3 weeks before being provided supplemental NaCl to a dietary concentration of 1.2 g (LNa) or 1.7 g Na/kg DM (HNa). The aim of the current study was to determine both the rate of depletion

and repletion and it was hypothesized that high *versus* low Na repletion would restore the salivary Na/K ratio more rapidly.

MATERIALS AND METHODS

Animals and management

Twelve cross-bred (Holstein Friesian × indigenous) multiparous cows with a mean body weight (BW) of 476 kg (SD = 43) and 61 (SD = 37) days in milk were used. The cows were housed individually in a tie stall for a period of 8 weeks. The experiment was conducted during the summer season from the end of February to April. Ambient temperature and relative humidity inside the stall were recorded every 2 hours from 10:00 Friday to 10:00 Saturday during the experiment (Maxim's iButton devices, San Jose, CA, USA).Temperature and relative humidity records were also obtained from the Nakhon Pathom meteorological station, located 500 m from the dairy barn of Kasetsart University (Kamphaengsaen, Nakhon Pathom, Thailand). Rectal temperature of each cow was measured weekly on Friday between 09:00 and 10:00. Cows were fed individually four times daily at 02:00, 07:00, 15:00 and 21:00 and the animals had free access to water. 24 h water intake was recorded between 09:00 and 10:00. The cows were milked twice daily and milk production was recorded at 14:30 Friday and 05:30 Saturday. BW was measured on days 0, 20 and 56 of the experiment.

Experimental design and rations

The experiment consisted of a depletion and a repletion period, with all cows offered the Na-deficient, total mixed ration (TMR, Table 1) *ad libitum* each day throughout the experiment. Orts for each individual cow were collected daily. After the 3-week depletion period, cows were blocked by milk yield and, within each block, cows were randomly allocated to either LNa or HNa. Dry matter intake observed during weeks 2 and 3 was used to determine the amount of supplemental NaCl to achieve 1.2 and 1.7 g Na/kg DM, resulting in a supplementation of 23.5 and 38.7 g/day of NaCl, respectively. During the 5-week repletion period, supplemental NaCl was offered daily to each cow in two equal portions fed at 10:00 and 15:00. Throughout the repletion period, all cows consumed all the supplemental salt offered.

Table 1. Ingredient and nutrient composition of the basal ration

Component	Content
Ingredient composition, (g/kg as fed)	
Rice straw	200.0
Cassava chips	420.0
Cotton seed	100.0
Soybean meal	120.0
Brewer's grain	50.0
Coconut oil	10.0
Molasses	64.0
Urea	18.0
Calcium phosphate	7.0
MgO	3.0
Sulphur	3.0
Premix[a]	5.0
Analysed nutrient composition	
Dry matter (DM, g/kg as fed)	903.0
Crude protein (g/kg DM)	144.3
Ether extract (g/kg DM)	62.5
Neutral detergent fibre (g/kg DM)	275.4
Acid detergent fibre (g/kg DM)	207.2
Sodium (g/kg DM)	0.33
Potassium (g/kg DM)	10.5

[a]The mineral premix consists of 440,000 IU vitamin A, 60,000 IU vitamin D3, 30,000 IU vitamin E, 11.6 g Fe, 0.03 g Mn, 5.6 g Cu, 11.60 g Zn, 0.07 g I, 0.06 g Se, 10.0 g Mg and 15.0 g P per kg.

Sample collection and chemical analyses

Samples of TMR (~2 kg) were collected weekly, dried at 60 °C, ground and pooled before analysis of dry matter, crude protein and ether extract according to AOAC procedures (1990). Neutral detergent fibre and acid detergent fibre were analysed according the method described by Van Soest *et al.* (1991). During the depletion period and approximately 30 min prior to the supplementation of salt during the repletion period, saliva samples were collected each Friday by placing sponges in the mouths of the cows at the third premolar in the maxilla for a period of 3 min. Subsequently, the liquid in the sponges was collected into a plastic bag, transferred

to a tube and stored at -20 °C until mineral analysis. Blood samples (~10 mL) were collected from the jugular vein of each cow on each Friday at 10:00. Serum samples were obtained by centrifuging blood samples at 800 × *g* for 5 min. All serum samples were stored at -20 °C until mineral analysis by means of a blood gas analyzer (Nova Biomedical Corporation, Waltham, MA, USA). A milk sample (~30 mL) of the Friday pm and Saturday am milking of each cow was collected in a tube containing 0.2 g/L of 2-bromo-2-nitro-1, 3-propanediol. Preserved milk samples were pooled in proportion to the milk yield of each milking, and stored at 4 °C. The following Monday, fat was removed manually and milk was deproteinated by adding 10% trichloroacetic acid, vortexed and centrifuged at 800 × *g* for 10 min. The collected supernatant was stored at -18 °C until mineral analysis.

During the repletion period, urine was quantitatively collected from each cow for 24 h every Friday. Urine was collected manually either through manual stimulation of the vulva or collection of urine that was spontaneously voided. After 24 h, total urine volume was recorded, thoroughly mixed and a sample (~100 mL) stored at -20 °C until analysis.

All samples of milk, saliva, urine and feed were analysed for Na and K using a flame emission atomic absorption spectrophotometer (AA-6800, Shimadzu, Kyoto, Japan) in accordance with the manufacturer's specifications.

Calculations and statistical analysis

The temperature humidity index (THI) was calculated according to the following formula (NRC, 1971):

$$THI = (1.8 \times T_{db} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26)]$$

where: T_{db} = dry bulb temperature (°C) and RH = relative humidity (%). Data from the depletion period were subjected to repeated-measures analysis of variance (ANOVA). The data from the repletion period were subjected to repeated-measures ANOVA with block and sodium treatment as factors, with the corresponding data of week 2 of the depletion period as a covariate. All statistical analyses were performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). Time effects were tested by mean of orthogonal polynomials. The level of statistical significance was pre-set at a probability level below 0.05 with a trend considered of $0.10 < P \leq 0.05$.

RESULTS

During the depletion period, the daily THI calculated from the temperature and humidity inside the barn ranged from 79.5 to 83.1 (Figure 1) with a mean value of 81.6 (SD 1.5). The temperature and humidity recorded in the barn agreed closely with the values recorded by the meteorological station located nearby (Figure 1). Likewise, a similar range in THI was measured during the repletion period and a mean daily THI value of 82.6 (SD 0.8) was found. Despite the high THI, cows were able to maintain their body temperature within the physiological range during both the depletion and repletion period and, for the two periods combined, the group mean values ranged between 37.9 and 38.9 °C.

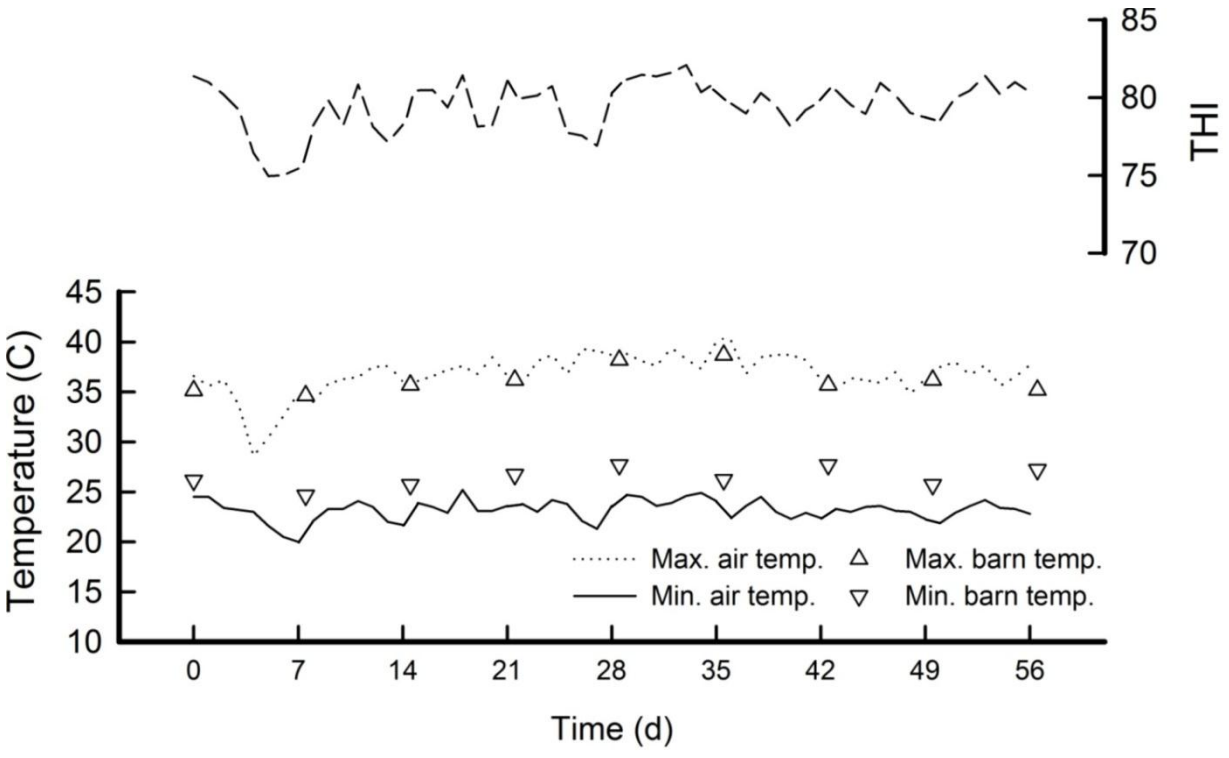

Figure 1. Atmospheric and barn temperatures (maximum and minimum) and temperature humidity index (THI) during the 56 day experiment.

Table 2. Dry matter (DM) intake, milk yield, water intake, urine production and concentrations of sodium and potassium in saliva and serum in the course of the depletion period

Item	Days of Na depletion				SEM	Significance of time effect
	0	7	14	21		
DM intake (kg/d)	12.5	12.3	11.8	10.8	0.7	NS
Milk yield (L/d)	16.9	13.7	12.9	14.3	0.6	L,Q
Water intake (L/d)	71	89	94	123	7.9	L
Urine production (L/d)	23	26	39	58	6.1	L,Q
Saliva						
Na (mmol/L)	128.7	97.7	82.1	98.1	4.2	L,Q
K (mmol/L)	10.2	28.8	26.9	26.7	1.8	L,Q
Na/K	14.6	3.8	3.4	4.3	0.6	L,Q
Serum						
Na (mmol/L)	138	136	138	138	0.3	Q
K (mmol/L)	4.5	2.7	3.1	3.4	0.1	L,Q

SEM, standard error of mean; NS, not significant; L, linear; Q, quadratic.

Depletion period

BW of the cows decreased ($P < 0.001$) from 476 (SD 43.2, $n = 12$) to 426 kg (SD 47.0, $n = 12$) during the 3-week depletion period. Likewise, DM intake decreased from 12.5 to 10.8 kg/d (Table 2) during the depletion period but the difference in DM intake over time was not statistically significant. In contrast, milk yield was 15.4% lower ($P < 0.001$) at the end of the depletion period compared to initial yield. Both water intake and urine production significantly increased during the depletion period, and values were found to be 1.7 and 2.5 times greater respectively, at the end of the depletion period compared to the initial values. In addition, pica and skin licking were observed starting at 7 days. Salivary Na concentration significantly decreased, with a concomitant increase in salivary K concentrations (Table 2), and values of 98.1 and 26.7 mmol/L, respectively, were observed at the end of the depletion period. Furthermore, the salivary Na/K ratio was found to decrease significantly during the depletion period, with a value at 7 days already significantly lower than the value found at the start of the depletion period. Serum Na and K concentrations responded in a quadratic fashion over time and the lowest values were found 7 days after the start of the depletion period, i.e. 136 and 2.7 mmol/L, respectively (Table 2). However, in contrast to serum Na concentrations, serum K values at the end of the depletion period were found to be 24.4% lower than the value at day 0.

Repletion period

BW of the cows remained unchanged (P = 0.121) and was similar between Na intake (P = 0.893) during the repletion period. Across time and Na intake, mean BW was found to be 433 kg (SD 8.6). Dry matter intake was neither influenced by the amount of supplemented salt nor by time × Na intake. However, for DM intake a quadratic time trend was observed during the repletion period (Table 3). Milk yield did not respond to the increase in DM intake and remained constant throughout the repletion period. Furthermore, milk yield was similar for the two levels of Na intakes and, across time and for the two Na intakes combined, it was found to be 14.6 L/day (SD 2.8). The intake of water was neither affected by Na intake nor by time or by time × Na intake. Average water intake of the two groups over the 5 week-repletion period was 120 L/d. Likewise, urine production was not affected by the amount of supplemented salt, and urine production over time (Table 3) was not significant; however, a time × Na intake trend (P = 0.063) was observed.

During the repletion period, interactions between time and Na intake did not occur with respect to the intakes of Na and K and their respective excretions with milk and urine (Table 4). Sodium intake was significantly different between the two levels of salt supplementation. Furthermore, a quadratic trend of Na intake over time was found and this observation is in line with the observed time trend of DM intake during the repletion period. Likewise, for K intake also a quadratic time trend was found but K intakes were similar between the two levels of Na intake (Table 4). Na excretion with urine was not significantly different between the two levels of salt supplementation or between the different time points (Table 4). For all time points combined, mean urinary Na excretion was found to be 0.3 and 1.7 g/d for the LNa and HNa group, respectively. K excretion with urine showed a linear trend (P = 0.055) over time, while no effect was found for Na intake or the interaction between time and Na intake. The Na and K concentrations of milk (data not shown) increased linearly (P < 0.024) in time, i.e. from 0.33 to 0.35 g/L and from 1.47 to 1.65 g/L, respectively. These increases were independent (P > 0.559) from the amount of supplemental salt and were not affected (P > 0.281) by time × Na intake. Sodium and K excretion with milk was not influenced by Na intake, time or their interaction (Table 4). For the two Na intakes combined, the mean Na and K excretion with milk over the repletion period was found to be 5.1 and 23.1 g/d, respectively.

There was no effect observed for salivary Na concentrations due to the supplementation of Na (Table 5). Furthermore, salivary Na concentrations were not affected

by time × Na intake but a linear trend (P = 0.050) in time was observed. Neither the salivary K concentrations nor the salivary Na/K ratio was statistically different between the dietary Na intake (Table 5) and no time or interaction was found. The serum concentrations of Na and K were not influenced by the amount of supplemental salt, time or by time × Na intake (Table 5).

Table 3. Dry matter (DM) intake, milk yield, water intake and urine production in the course of the repletion period

Item	Na intake	Days of Na repletion						SEM	Significance		
		0	7	14	21	28	35		Time	Na intake	Time × Na intake
DM intake (kg/d)	Low	11.4	11.4	12.3	11.9	13.6	13.3	0.88	Q[a]	NS	NS
	High	10.2	10.6	13.0	12.6	13.1	13.7				
Milk yield (L/d)	Low	14.2	14.5	14.8	15.2	13.4	14.2	0.56	NS	NS	NS
	High	14.4	15.2	15.4	14.9	15.2	14.1				
Water intake (L/d)	Low	97	104	106	117	102	105	16.1	NS	NS	NS
	High	148	122	151	143	121	125				
Urine production (L/d)	Low	40	37	38	49	35	37	14.0	NS	NS	L[a]
	High	75	57	73	68	56	50				

SEM, standard error of mean; NS, not significant; L, linear; Q, quadratic.

[a]Trend ($0.10 < P \leq 0.05$).

Table 4. Intake and excretions by means of milk and urine of sodium and potassium during the repletion period

Item	Na intake	Days of Na repletion						SEM	Significance		
		0	7	14	21	28	35		Time	Na intake	Time × Na intake
Sodium (g/d)											
Intake	Low	13.0	13.0	13.3	13.2	13.8	13.7	0.29	Q[a]	<0.001	NS
	High	18.6	18.8	19.5	19.4	19.6	19.8				
Milk	Low	4.7	4.5	4.9	5.7	5.0	4.9	0.51	NS	NS	NS
	High	4.9	5.0	5.6	5.2	5.6	5.0				
Urine	Low	0.3	0.3	0.2	0.2	0.2	0.7	0.38	NS	NS	NS
	High	1.0	2.4	1.7	1.1	1.2	2.9				
Potassium (g/d)											
Intake	Low	119	119	128	124	142	139	9.25	Q[a]	NS	NS
	High	107	111	136	132	136	143				
Milk	Low	21.1	21.9	24.3	26.4	21.6	23.5	0.73	NS	NS	NS
	High	20.9	22.5	23.4	24.8	23.9	23.2				
Urine	Low	65.8	73.5	56.2	67.8	69.0	85.5	6.69	L[a]	NS	NS
	High	68.7	72.4	67.0	63.0	96.0	83.7				

SEM, standard error of mean; NS, not significant; L, linear; Q, quadratic.

[a]Trend ($0.10 < P \leq 0.05$).

Table 5. The concentrations of sodium and potassium in saliva and serum during the repletion period

Item	Na intake	Days of Na repletion						SEM	Significance		
		0	7	14	21	28	35		Time	Na intake	Time × Na intake
Saliva											
Na (mmol/L)	Low	88	104	88	85	91	100	3.10	L[a]	NS	NS
	High	108	108	104	95	100	103				
K (mmol/L)	Low	31.8	44.2	35.1	39.2	36.7	31.2	2.20	NS	NS	NS
	High	21.5	26.1	27.6	26.3	28.6	25.5				
Na/K	Low	3.4	2.4	2.6	2.3	2.9	3.4	0.29	NS	NS	NS
	High	5.3	4.3	3.8	3.8	3.6	4.7				
Serum											
Na (mmol/L)	Low	138	138	137	139	138	140	0.37	NS	NS	NS
	High	138	139	138	139	139	140				
K (mmol/L)	Low	3.5	3.4	3.5	3.5	3.4	3.4	0.09	NS	NS	NS
	High	3.4	4.0	3.8	3.6	3.5	3.7				

SEM, standard error of mean; NS, not significant; L, linear.

[a]Trend ($0.10 < P \leq 0.05$).

DISCUSSION

During the depletion period, cows developed clinical signs of Na deficiency such as polyuria, polydipsia, pica and a decrease in milk yield, 1 to 2 weeks after the start of feeding of the ration containing 0.33 g Na/kg DM. The occurrence of polyuria and polydipsia during Na deficiency is caused by the inability of the cow to maintain the osmotic pressure of the extracellular fluid (ECF). Briefly, Na is the main cation of the ECF and, together with its associated anions, it accounts for more than 90% of the osmotic pressure of the ECF (Houpt, 1993). A deficit of Na in the ECF hampers the release of antidiuretic hormone (ADH), thereby causing a rapid renal excretion of excess water to counteract the initial decrease of the Na concentration of the ECF (Houpt, 1993). However, at this stage the volume of the ECF is lowered, which triggers activation of angiotensin II, leading to an increased water intake to restore the volume of ECF (Blair-West *et al.*, 1988). Clearly, the latter action leads to lower Na concentrations of the ECF, thereby triggering the aforementioned physiological actions. The clinical observations of Na deficiency were associated with decreased salivary Na and increased salivary K concentrations, leading to a salivary Na/K ratio of < 4.3, which is indicative of Na deficiency (McSweeney *et al.*, 1988). The current observations on salivary Na and K concentrations are in line with data reported by Thiangtum *et al.* (2011) who reported that the salivary Na/ K ratio decreased to 4.8 when a ration was fed containing 0.4 g of Na/kg DM for 32 days. In the latter study, however, no clinical signs such as pica were observed. The observed changes in salivary electrolyte concentrations are most likely explained by the action of aldosterone, because it has been shown by Riad *et al.* (1986) and McSweeney *et al.* (1988) that during Na deficiency this hormone responds to a decline in serum Na concentration and inhibits the salivary secretion of Na and concomitantly stimulates salivary K secretion.

The low intakes of Na during the depletion period caused transient changes in the plasma concentrations of Na and K. It is unknown whether this observation can be generalized because, to the authors' knowledge, time-related changes of plasma Na and K concentrations during a diet-induced Na deficiency have not been previously reported in dairy cattle. These changes are difficult to explain but the data indicate that initial responses of plasma Na and K concentrations to Na deficiency are alleviated over time. It can be speculated that the initial decrease in plasma Na concentration is counteracted by an aldosterone-mediated action on the renal conservation of Na (Alan and Lingtak-Neander, 2009). Furthermore, a severe decrease in plasma Na concentration can be prevented for

several months because the rumen can act as a buffer containing up to 50% of the available body Na (Bell, 1995). Moreover, high levels of circulating aldosterone are known to shift K from the extracellular to the intracellular pool (Rastegar, 1990) and enhance K secretion into the tubular lumen, thereby increasing the urinary excretion of K (Palmer and Frindt, 2000). Plasma K concentrations initially may have over-responded to the action of aldosterone, causing plasma K concentrations to decrease to critically low levels (~2.5 mmol/L), as previously reported (Palmer and Frindt, 2000). Since aldosterone is the key regulator of extracellular K concentration (Suttle, 2010b), such low plasma K concentrations may have alleviated the initial aldosterone responses on the plasma K concentration.

The data clearly show that supplemental Na did not restore the salivary Na/K to values > 6 (Schonewille and Beynen, 2005). This was unexpected as the highest level of supplementation was calculated to exceed (1.7 g/kg DM) the dietary requirement (1.2 g/kg DM) (Thiangtum *et al.*, 2011) of lactating dairy cows in a tropical environment. The actual Na consumed was found to be 1.6 g/kg DM for the HNa group. It thus appears that the amount of Na that was withdrawn from the mobilizable Na pool during the 3 weeks of Na depletion was not replenished by the supplemental Na during the 5-week repletion period. The average amount of Na not accounted for in milk and urine for the LNa and HNa groups during the repletion period was 8.1 and 12.4 g/d. The losses due to sweat, as well as unabsorbed dietary and endogenous fecal Na, were not measured in the present study. Using the dietary Na absorption data of Thiangtum *et al.* (2011), it can be estimated that the apparent Na absorption of the LNa and HNa cows should have been approximately 85% and 87%, respectively. Using these values, 2.0 and 2.4 g/d Na would have been excreted via the feces of the cows in the LNa and HNa groups, leaving 6.1 and 9.9 g/d unaccounted for, respectively. These amounts of Na would have been used by the cow to replenish the mobilizable Na pool of the rumen and sweat production. During the 3 weeks of Na depletion, a total amount of 44 g Na was minimally withdrawn from the animal's mobilizable Na pool.

Throughout the 56-day experiment, the cows experienced, on average, 4 h of mild (THI 72-79), 19 h of moderate (THI 79-89) and 1 h of severe (THI > 89) heat stress (Armstrong, 1994; Akyuz *et al.*, 2010) each 24 h. Jenkinson and Mabon (1973) reported that Na losses with sweat ranged from 7.1 to 10 mg/m^2/h when THI ranged from 79 to 81. Gebremedhin *et al.* (2011) reported sweating rates in Holstein cows of up to 660 g/m^2/h at a THI of 79.6. When a sweating rate of 600 g/m^2/h is used and an estimated surface area of 4.5 m^2 (BW 433 kg) (Brody, 1945), the Na concentration of sweat under the current conditions were maximally 6.6 mmol Na/kg, assuming the aforementioned apparent Na absorption and

no replenishment of the mobilizable Na pool of the rumen. In the case of replenishment of the rumen pool, the Na concentration of sweat was 5.8 mmol Na/kg. The latter two values are much higher than the value of 2 mmol Na/kg sweat reported by Johnson (1970). Clearly, there is a considerable discrepancy between the Na losses with sweat calculated here and the data provided by Jenkinson and Mabon (1973) which cannot easily be explained. The issue on Na losses with sweat in cattle remains and requires further research to obtain accurate values. The lack of unequivocal estimates on the Na losses with sweat, in combination with the fact that the Na-supplemented cows did not restore their salivary Na and K concentrations, hinders potential refinement of the previous recommendation regarding the Na requirement of lactating cows under tropical conditions (Thiangtum *et al.*, 2011).

Across dietary Na intake and time, cows ingested 128.0 g K/d. Using the average dietary K absorption data (93.0%) of Thiangtum *et al.* (2011), it can be estimated that the apparent K excretion with feces was 9.0 g/d. The associated K excretions with milk and urine were found to be 23.1 and 72.4 g/d. Consequently, 23.5 g/d of K was available for excretion with sweat. Using the data on K excretion with sweat reported by Jenkinson and Mabon (1973) and the aforementioned values of surface area and time of exposure to heat stress, K excretion with sweat is estimated to be ~2 g/d. Therefore, it can be excluded that the animals suffered from K deficiency in the current experiment, corroborated by the lack of clinical signs of K deficiency such as muscular weakness, stiffness and paralysis (Suttle, 2010b).

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Chapter 5

Effect of Dietary Protein Levels on Rumen Metabolism and Milk Yield in Mid-Lactating Cows under Hot and Humid Conditions

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ABSTRACT

An experiment was conducted to investigate the effects of 2 levels of dietary Crude Protein (CP) in concentrates with similar proportions of Rumen Undegradable Protein (RUP) on rumen metabolism, milk yield and composition in mid lactating cows in Thailand. Eight 87.5% Holstein × 12.5% indigenous multiparous cows were used in a crossover design with two successive 25 day periods. Diets contained 30% paragrass and 70% concentrate on a Dry Matter (DM) basis. Concentrate feeds were formulated to provide low dietary CP [17.3%; LCP] or high dietary CP [19.0%; HCP]. The proportion of Rumen Degradable Protein (RDP) and RUP was 61% and 39% in both diets. Diets were isocaloric in terms of net energy for lactation. Milk yield, milk lactose yield, Dry Matter Intake (DMI), and apparent digestibility of DM, CP and Neutral Detergent Fibre (NDF) were greater in cows fed HCP than in those fed LCP. Concentration of blood urea nitrogen was elevated in cows fed HCP diets. Rumen NH_3-N concentration and pH tended to increase in cows fed HCP diet. Rumen microorganism counts and volatile fatty acids levels in the rumen did not differ between treatments. The increasing CP content in mid-lactating cow was beneficial to increase DMI, apparent digestibility of DM, CP and NDF and therefore milk yield.

INTRODUCTION

The majority of forage in the tropics is low in digestibility and nutrient contents and thus limits feed intake and milk output in ruminants (Camero *et al*., 2001; Leng, 1990). Consequently, lactating Thai dairy cows mostly ingest poor quality roughage. Therefore, it is common practice in Thailand to reduce the relative amount of roughage, while increasing the proportion of concentrate in order to increase nutrient supply to the animals (Kanjanapruthipong *et al*., 2001). The protein level of the concentrates is important to ensure an adequate supply of dietary protein in supporting milk production in the tropics because of low Crude Protein (CP) content in typical tropical roughage (Korhonen *et al*., 2002).

Providing sufficient protein to dairy cows depends on the balance between the availability of nitrogen for microbial growth in the rumen and nitrogen for productive functions (Ferguson *et al*., 1988). At the same intake of dietary protein, a shortage of Rumen Degradable Protein (RDP) may reduce microbial growth in the rumen. Consequently, rumen digestibility (Chalupa, 1984), Dry Matter Intake (DMI) (Weigel *et al*., 1997) and microbial protein synthesis and thus protein flow from the rumen (Argyle and Baldwin, 1989) may be reduced. Nowadays there is a tendency towards an increase of dietary protein supply above NRC requirements (NRC, 2001) to optimize the production of dairy cows under hot and humid climates. However, experimental data that corroborate this tendency are scarce. Therefore, the aim of the present study was to investigate the effect of 2 levels of dietary CP with equal proportions of rumen RDP and RUP on digestion, rumen metabolism, blood urea nitrogen, milk yield and milk composition in mid-lactating cows.

MATERIALS AND METHODS

Animals and feeding

Eight Holstein × indigenous (87.5 × 12.5) multiparous dairy cows with 113 ± 12 Days in Milk (DIM) and weighing 469 ± 46 kg were used. The parity of the cows ranged from 2-5 and the mean was 3 (± 1.3).

The trial had a 25 × 25 days cross over design with a 16 days wash out period (Cochran and Cox, 1957). Data were collected from day 17-25 of each experimental period. The animals were randomly assigned to the two treatments, i.e. a low protein (17%, LCP) and a high protein (19%, HCP) concentrate. The proportions of RDP and RUP were similar for both concentrates; i.e. 61 and 39%, respectively. The energy density was the same for both

experimental concentrates (1.7 Mcal NE_L/kg DM). Each day, the cows were first fed with concentrates at a level of 2.1% of Body Weight (BW) in DM and then fed paragrass (*Brachiaria mutica*) at a level of 0.9% of BW in DM. Each feed was offered 3 times per day at 08:00, 14:00, and 21:00 h. Paragrass was harvested at a 45-50 day interval. Individual feed intakes and refusals were recorded daily.

Table 1. Ingredient composition of the experimental concentrates

Ingredients (%)	LCP[a]	HCP
Full fat soybean	7.1	11.5
Cassava chip	30.9	23.4
Palm kernel cake	11.5	12.0
Soybean meal	1.0	6.0
Kapok seed meal	13.4	12.0
Whole cotton seed	10.0	10.0
Coconut meal	16.0	15.0
Molasses, sugarcane	5.5	5.5
Urea	0.6	0.6
Mono dicalcium phosphate	1.0	1.0
Sodium bicarbonate	1.5	1.5
Sulphur	0.3	0.3
Magnesium oxide	0.2	0.2
Trace mineral and vitamin mix[b]	0.5	0.5
NaCl	0.5	0.5

[a]LCP, concentrate containing a low CP content; HCP, concentrate containing a high CP content.

[b]Consists of (per kg) 2 million IU of vitamin A; 0.40 million IU of vitamin D3; 3,000 IU of vitamin E; 0.46 g of vitamin K; 10.0 g of Fe; 4.0 g of Cu; 7.4 g of Mn; 0.20 g of Co; 15.0 g of Zn; 0.20 g of I and 0.08 g of Se.

Concentrate ingredients and the chemical composition of the feedstuffs are given in Tables 1 and 2, respectively. Cows were housed in tied-stalls with individual feed bins. Animals had free access to water. Cows were milked by a milking machine twice daily at 06:00 and 15:30 h. The experiment was conducted in the months of January and February with mean minimum and maximum temperatures at 21.3±2.4 and 33.1±2.5 ºC, respectively. Mean maximum and minimum relative humidity was 96.1±1.1 and 48.6±10.0%, respectively. The average maximum and minimum Temperature Humidity Index (THI) was

90.9±4.2 and 66.9±3.3, respectively. The climatic conditions during the study are shown in Figure 1.

Table 2. Chemical composition of the experimental concentrates and grass

Chemical analysis (% DM)	LCP[a]	HCP	Grass
Crude protein	17.30	19.05	10.67
Rumen undegradable protein[b]	6.90	7.33	1.28
NDF	52.75	51.38	75.94
ADF	19.06	17.75	39.20
Ether extract	8.79	9.45	1.09
Ash	12.16	12.41	13.05
AIA	0.78	0.60	7.16
NE_L (Mcal/kg DM)	1.68 [c]	1.71[c]	1.30[d]
TDN	73.47	74.67	57.84

[a]LCP, concentrate containing a low CP content; HCP, concentrate containing a high CP content.

[b]Calculated RUP and TDN based on nutrient compositions of Thai feed table (Angthong *et al*., 2004; Kanjanapruthipong, 2006; Moran, 2005).

[c]NE_L (Mcal/kgDM) = 0.0245*TDN(%)-0.12 (NRC, 1989).

[d]NE_L (Mcal/kgDM) = 2.3977 –(0.0280*ADF%) (Ishler *et al.*, 1996).

Sampling and chemical analyses

Cows were weighed on the first and again on the final day of both periods before morning feeding. Milk yields were recorded from days 16-24 in each experimental period. Milk samples (approximately 30 mL per milking) were collected during days 22, 23 and 24 at each of the two milkings. Milk samples were placed in bottles containing 0.02% of 2-bromo-2-nitro-1,3-propanediol and stored at 5 °C until determination of milk composition (fat, protein, lactose and non fat solids) by infrared spectroscopy (Bentley 2000, Agriyork Ltd.,UK). Concentrates and grass were sampled once a week, to determine DM content. The weekly samples were dried at 60 °C and were pooled based on CP content per period. The pooled samples were analysed for crude protein, ether extract (EE) and ash according to the Association of Official Analytical Chemists (AOAC, 1990) procedure and Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) following the method by Van Soest *et al*. (1991).

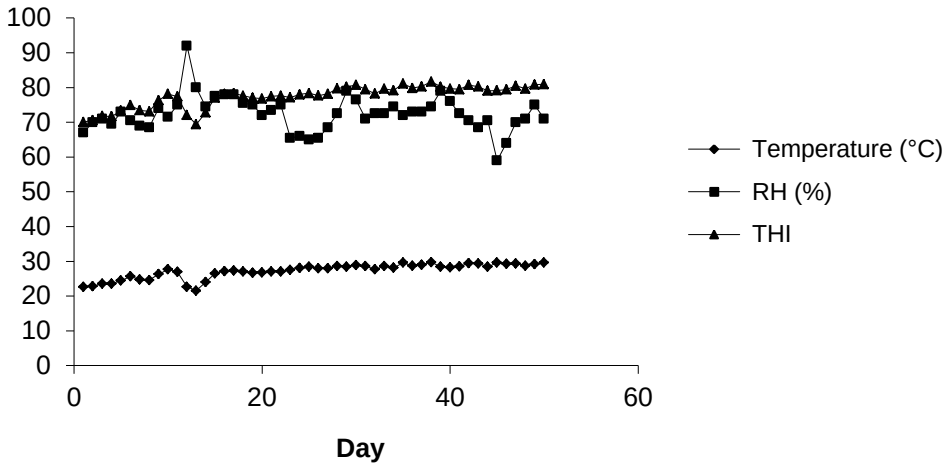

Figure 1. The average temperature (°C), relative humidity (RH, %) and temperature humidity index (THI) during the experimental periods.

Blood samples, 10 mL were collected from the jugular vein of each cow at days 23 and day 24 of each period at 4 h after morning feeding. Then, blood samples were centrifuged for 5 min at 500 *g*. All serum samples were stored at -20 °C until analysis for Blood Urea Nitrogen (BUN) concentration by spectrophotometry (Urease-Berthelot).

Faecal samples from each cow were collected after the morning feeding on the last day of each period. They were dried in a 60 °C forced-air oven and ground in a Wiley mill to pass through a 1-mm screen. Samples were subsequently analysed for NDF, CP and Acid Insoluble Ash (AIA). The AIA was used as an internal marker to determine apparent total-tract DM digestibility (Van Keulen *et al*., 1977).

Rumen fluids were collected by means of a nasogastric tube on day 25 of each period. Whole ruminal contents (100-200 mL) were taken 4 h after the morning feeding. Rumen fluid pH was measured immediately by using pH meter (EC 20, HACH, USA). The rest of rumen fluid was strained through four layers of cheesecloth. Two sub-samples were taken from the strained fluid: one 10 mL sample was preserved by the addition of 1 mL of 50% (vol./vol.) H_2SO_4 for later analysis of ammonia (NH_3-N) and Volatile Fatty Acids (VFAs) and one 9 mL sample was preserved by the addition of 1 mL of 10% formalin solution for later analysis of total direct count of bacteria, protozoa and zoospores (Galyean, 1989). All ruminal samples were stored at -20 °C. Just before analysis, samples were thawed, centrifuged (15000 × *g* for 15 min), and analysed for NH_3-N concentration (Bremner and Keeney, 1965) and for VFAs by gas chromatography following the method modified from Erwin *et al*. (1961).

Statistical analysis

Milk yield, milk composition, DM intake, digestibility, BUN, ruminal pH, NH_3-N, VFAs and microbial counts were subjected to ANOVA (SAS, 2001). Experimental period (fixed) and dietary treatment were factors (SAS, 2001). Effects were considered significant at $P \leq 0.05$.

Table 3. Intake of DM and selected nutrients and digestibilities of DM, CP and NDF in cows fed either the low (LCP) or the high CP (HCP) concentrates

Contents	Diets		SEM[a]	*P* value
	LCP	HCP		
Intake				
Total DMI (kg/d)	11.71	12.24	0.37	0.01
Concentrate DMI (kg/d)	8.41	8.77	0.35	0.10
Paragrass DMI (kg/d)	3.30	3.48	0.13	0.04
CP intake (kg/d)	1.81	2.04	0.09	<0.01
CP intake (%)	15.43	16.63	0.18	<0.01
RUP intake (kg/d)	0.63	0.69	0.03	0.01
RDP intake (kg/d)	1.18	1.35	0.05	<0.01
NE_L intake (Mcal/d)	18.41	19.42	0.61	<0.01
EE intake (kg/d)	0.78	0.81	0.03	0.09
NDF intake (kg/d)	6.94	7.16	0.20	0.03
Digestibility (%)				
Dry matter	54.88	63.06	2.70	<0.01
CP	63.80	72.22	1.84	<0.01
NDF	61.76	69.02	2.47	<0.01

[a]Standard error of the mean.

RESULTS

Body weight, nutrient intake and digestibility

Body weight was not affected by dietary treatments. Mean BW at the end of the experiment was 468 kg (SE = 13.8; n = 8), which is similar to pre-experimental values ($P > 0.1$). Nutrient intake and digestibilities of DM, CP and NDF are shown in Table 3. Total DM intake was 0.53 kg/d higher in cows fed HCP diet than in those fed the LCP diet which was mainly caused by the difference in concentrate intake. However, in contrast with the difference in

grass intake between the two experimental diets, the difference in concentrate intake appeared to be non-significant. The intakes of CP, RUP, RDP, NDF and NE_L were significantly increased when the cows were fed the HCP diet. Digestibilities of DM, CP and NDF were influenced by dietary treatments. Cows that were fed the LCP diet had significantly lower digestibilities of DM, CP and NDF.

Table 4. Lactational performance of cows fed either the low (LCP) or the high CP (HCP) concentrates

Contents	Diets		SEM[a]	*P* value
	LCP	HCP		
Milk production				
Milk yield (kg/d)	12.28	13.31	0.65	<0.01
3.5% FCM yield (kg/d)	12.90	13.65	0.79	0.24
Fat yield (kg/d)	0.47	0.49	0.03	0.50
Protein yield (kg/d)	0.36	0.38	0.02	0.21
Lactose yield (kg/d)	0.57	0.62	0.04	0.01
Milk composition				
Milk fat (%)	3.81	3.66	0.19	0.37
Milk protein (%)	2.94	2.85	0.06	0.22
Milk lactose (%)	4.57	4.62	0.07	0.18
Solid not fat (%)	8.17	8.13	0.13	0.53

[a]Standard error of the mean.

Milk production

Effects of dietary treatments on milk production are shown in Table 4. Cows fed the LCP diet had a significantly lower milk yield and lactose yield than those fed the HCP diet. The amount of 3.5% fat corrected milk and the contents of fat, protein, lactose, and non-fat solids in milk were similar on both CP treatments.

Table 5. Selected indices of rumen fermentation, blood urea nitrogen (BUN) and rumen microorganisms of cows fed either the low (LCP) or the high CP (HCP) concentrates

Contents	Diets		SEM[a]	*P* value
	LCP	HCP		
Ruminal metabolism				
pH	6.22	6.29	0.04	0.21
NH_3-N (mg%)	24.31	28.28	1.46	0.11
Acetate (%)	65.77	65.75	1.01	0.98
Propionate (%)	23.37	22.73	1.16	0.68
Butyrate (%)	10.23	10.85	0.43	0.24
Valerate (%)	0.63	0.66	0.06	0.67
Total VFA (mmol/L)	138.47	141.81	5.02	0.82
A/P[b]	3.04	2.95	0.20	0.74
BUN (mg/dL)	12.23	14.73	0.70	0.01
Rumen microorganisms				
Bacteria (10^{11} cell/mL)	2.20	2.16	0.17	0.88
Protozoa (10^5 cell/mL)	5.81	3.78	0.64	0.09
Zoospore (10^5 cell/mL)	1.69	1.48	0.13	0.42

[a]Standard error of the mean.

[b]A/P, Acetate/Propionate ratio.

Rumen metabolism and blood urea nitrogen

Effects of dietary treatments on selected indices on rumen metabolism and BUN are presented in Table 5. Rumen NH_3-N concentrations and rumen pH were similar between experimental treatments. However, rumen NH_3-N concentrations and pH had a tendency to increase when the cows were fed the HCP diet. Mean BUN concentrations were significantly higher in cows fed the HCP diet. The profile of individual VFAs in the rumen and the acetate to propionate ratio did not show clear differences between the two experimental treatments. Furthermore, bacteria, protozoa and zoospore counts in the rumen content of the cows were similar for both dietary treatments.

DISCUSSION

Dry matter intake and digestibility

In this experiment, the mean Temperature Humidity Index (THI) was 77, which is above the upper point of 72 for optimal dairy cow productivity (Ravagnolo *et al*., 2000). The THI in the current study indicated that the cows experienced mild heat stress (THI 72 to 78) (Armstrong, 1994). Feed intake in cows with heat stress may be reduced by 8% or more, thereby negatively affecting milk production (Kabuga, 1990). In the current study, feed intake was lower than the NRC (2001) estimation for these cows; i.e. 14 and 11% lower for the LCP and HCP treatment respectively. However, NRC (2001) estimations of feed intake are based on temperate climatological conditions, it may be suggested that the climatological condition during the current experiment was at least partly, responsible for the relative low level of feed intake. The lower DMI was likely to reflect the poor quality of roughage in tropical regions (Leng, 1990). During heat stress, cows reduce feed intake in particular with high fibre diets (Kanjanapruthipong *et al.*, 2010). In contrast, the present study showed that roughage intake was greater in cows fed HCP diet than those fed LCP. This high DMI for cows fed HCP diet can be explained in part by differences in apparent DM, CP and NDF digestibility between diets. This is in agreement with other studies (Blauwiekel and Kincaid, 1986; Jones-Endsley *et al*., 1997).

In the current study, DM, CP and NDF digestibility were increased by 15, 13 and 12%, respectively when the HCP diet was fed. This observation is corroborated by Tyrrell *et al.* (1981), Weigel *et al.* (1997) and Kendall *et al*. (2009) who observed an increased nutrient digestibility when the dietary CP content was raised from 11 to about 18% (DM basis). Concentration of rumen microbial yield did not differ between cows on HCP and LCP. This result on digestibility was in agreement with earlier reports in sheep that the increasing levels of concentrate in the diet resulted in increased total tract apparent digestibility of DM, organic matter and CP and similar microbial population (Ramos *et al*., 2009).

Milk production and milk composition

An increase in dietary CP intake may affect milk yield by increasing the availability of NH_3, peptides and amino acids for microbial growth in the rumen (Bequette *et al*., 1998). Providing adequate protein to dairy cows increases milk production under tropical conditions (Promma *et al*., 2002). Supplementing diets with CP increased DMI and as a consequence

also milk yield (Reynal and Broderick, 2003). Similar results were observed in the present study, cows on the diet containing HCP produced more milk than those fed the LCP diet. The responses of milk yield agree with higher intakes of NE_L (+2.12 Mcal) and CP (+220 g) intake than recommended by the NRC (2001). Promma *et al*., (2002) suggested that for dairy cows in tropical ambient conditions the energy and CP intake should be 20% higher than the NRC recommendation for dairy cows in moderate climatic condition. Similarly, Kalscheur *et al*. (2006) showed that cows fed a high RDP supplement (11% RDP, 17% CP) produced more milk yield than cows on a low RDP supplement diet (8.2% RDP, 14% CP). In contrast, supplemental CP at a higher level did not affect milk composition in the current study. This observation is in line with the results reported by Reynal and Broderick (2003) and Mulligan *et al*. (2004).

Rumen metabolism and blood urea nitrogen

When CP intake exceeds the requirements for microbial growth or when there is an insufficient supply of fermentable carbohydrates for microbial growth, there is a potential for nitrogen (N) loss from N surplus in the form of ammonia (Agle *et al*., 2010; Kim *et al*., 2000). In this study, rumen NH_3-N concentration (P = 0.1) tended to increase in cows fed HCP diet. However, these rumen NH_3-N concentrations (24.31-28.28 mg/dL) were in line with results of earlier studies indicating that the optimum rumen NH_3-N should be between 10-30 mg/dL for rumen fermentation on low-quality roughage (Chanjula 2004; Khampa and Wanapat, 2006). As a consequence of increased rumen NH_3-N, NH_3-N will be converted to urea. In general there is a positive correlation between rumen NH_3-N and BUN concentration (Odensten *et al*. 2005; Ropstad *et al*. 1989). The current study also showed that BUN in cows fed HCP diet was higher than those fed LCP diet. However, BUN concentration in cows fed HCP diet was still in the normal range. The optimal BUN concentration ranges from 12-17 mg/dL according to Baker *et al*. (1995).

It can be concluded that DMI and therefore milk production in mid-lactation cows under hot and humid conditions could be increased by increasing CP content higher than the CP level recommended by NRC (2001).

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Chapter 6

General Discussion

INTRODUCTION

The relative low milk production of Thai dairy cows is, as outlined in the general introduction of the current thesis, related to the prevailing environmental conditions. The combination of a high air temperature and high humidity leads to heat stress in lactating cows (Davison *et al.*, 1996; Gaughan, *et al.*, 1998), thereby, negatively influencing dry matter intake (DMI) and subsequently milk production, reproduction and cow health (Beede *et al.*, 1986; McDowell, 1972; McGuire *et al.*, 1989; Rhoads *et al.*, 2009; Sanchez, *et al.*, 1994; West, 1994). Heat stress can be attenuated by reducing the heat load of the animal through measures such as provision of shade to reduce incoming radiation, an increase of air velocity and/or lowering of environmental temperature by evaporative cooling. Alternatively, the development of specific dairy breeds can be considered opportune to combat the negative impact of heat stress on dairy production (Beede *et al.*, 1986). Thus measures related to the housing, either or not in combination with the use of cows genetically equipped to cope better with heat stress, are probably needed to ultimately increase dairy production in Thailand towards levels comparable with those commonly observed under temperate conditions. Although the relevance of these topics cannot be disputed, they are, however, beyond the scope of the current thesis.

Needless to say that nutrient supply also has a major impact on milk production of cows kept under hot and humid conditions. The overall objective of the research described in this thesis was, therefore, to provide a nutritional basis for improvement of milk production of Thai dairy cows. Therefore, the results of the experimental studies reported in Chapters 3, 4 and 5 are mainly discussed from a practical point of view.

GENERAL CONSIDERATIONS RELATED TO THE CROSS SECTIONAL STUDY

The outcome of the cross sectional study reported in the second chapter of this thesis suggests that both the sodium (Na) and protein supply of dairy cows in Thailand are important constraints for milk production. However, in this study the sample size was small (45 cows housed in 6 different farms) and thus the representativeness of the results can be considered a concern and may, therefore, limit extrapolation of the outcomes. On the other hand, the mean (n = 63) milk yield (MY), milk fat and protein contents of the cows sampled during the cross sectional study were calculated to be 15.9 kg milk/cow/day, 4.06% fat and 2.96% protein and these values are not too different from the nation's mean values, i.e. 12.2 kg milk/cow/day (Pattamanont and Ruengpaibul, 2015), 3.70% fat and 3.03% protein (Kamphusiri, 2013), respectively. It must be kept in mind that MY was only measured at 70 and 130 days in milk

during the cross sectional study. Thus, MY was not monitored during the second half/end of the lactation, thereby, explaining the somewhat greater mean MY as observed during the cross sectional study.

In Thailand, the types of feedstuffs fed to the dairy cows, especially forages, depend on geographical location of the farm. In the northeastern part of Thailand (Isan) for instance the principle source of roughage is rice straw while in Chiang Mai province (Northern part of Thailand) by-products of the corn industry such as corn stem and corn cobs (without grain) are the main source of roughage. Moreover, commercial pelleted compound feeds are hardly used in Isan while its use is common in the central part of Thailand. It can, therefore, be argued that data should be collected from each region in Thailand in proportion to the number of cows in each region in order to achieve a representative estimate of the overall Thai feeding conditions. Unfortunately, the geographical distribution of the farms sampled during the cross sectional study does not fully meet this criterion of representativeness. Consequently, the feedstuffs used on the farms that participated in the cross sectional study do not fully reflect the average Thai farm. On the other hand, in terms of nutrient contents, almost all roughages share the same characteristics; i.e. high ADF (≥ 243 g/kg DM), low Na (≤ 0.5 g/kg DM) and with the exception of cassava leaves and *Leucaena leucocephala*, low crude protein (CP) contents (≤ 126 g/kg DM) (Table 1, General introduction). Thus, in terms of nutrient composition of the used roughages, the six selected farms can be considered as a fair representation of the average Thai farm.

SODIUM SUPPLY AND REQUIREMENTS OF THAI DAIRY COWS

The outcome of the cross sectional study indicated that the sodium (Na) supply of dairy cows in Thailand is an important constraint of milk production because an observed positive correlation between Na intake and MY. This positive correlation could not be easily explained due to the confounding effect of concentrate intake. Thus, the cause and effect relationship of Na intake and MY was not clear but there are indications that heat stressed *versus* non heat stressed cows require greater amounts of Na. Sanchez *et al*. (1994) suggested that heat stressed cows require greater amounts of Na due to greater Na losses with sweat. Thus, the current recommendations, which were set from studies conducted with cows maintained under temperate conditions (i.e. 0.5 to 2.2 g/kg DM, Chapter 3) may not apply to cows living under hot and humid environmental conditions as present in Thailand. Unfortunately, there is a dearth of studies addressing the issue of Na requirements of heat

stressed cows and it was, therefore, considered relevant to gain more insight into this, for milk production under tropical environmental conditions, important topic.

In general, Na status of animals can be determined using Na balance studies as the amount of Na absorbed in excess of requirement is rapidly excreted in urine. During Na deficiency (negative Na balance), there is an aldosterone induced decrease of Na urinary excretion with a concomitant decrease of the salivary Na concentration which is accompanied by a simultaneous increase of the salivary K concentration. Therefore, the salivary Na/K ratio is a more practical indicator of Na status in ruminants. A salivary Na/K ratio lower than 6 is indicative for Na deficiency (Schonewille and Beynen, 2005).

The impact of dietary sodium on milk yield

In the current studies into the Na metabolism of dairy cows (Chapters 3 and 4), both dry matter intake and MY were affected by the Na content of the ration (Table 1). For the two studies combined (Thiangtum *et al.*, 2011, 2017), it was found that an increase of 1 g Na/kg DM resulted in an increase of 0.91 kg of milk and 0.27 kg dry matter. This result is corroborated by Schneider *et al.* (1984, 1986, 1988) and Silanikove *et al.* (1998) who also reported increased MY and dry matter intake in response to a greater dietary Na content (Table 1). The latter four studies were all conducted under heat stress conditions and across those four studies, MY and dry matter intake were increased by 0.47 and 0.26 kg, respectively when the dietary Na content was increased by 1 g/kg DM. Moreover, Sanchez *et al*. (1994) modeled milk production and nutrition data from 15 studies that were conducted during summertime in southern USA and they found maximum MYs when the ration contained ~7 g Na/kg DM. The greater milk yields can be explained by the greater intake of dry matter but it is not easy to explain why the intake of dry matter increases in response to a greater Na content in the ration. Perhaps, the greater Na intakes caused greater water intakes, thereby, increasing the influx of water into the rumen and subsequently accelerated the flow of liquid digesta out of the rumen. Such conditions may increase the efficiency of fibre digestion (Rogers and Davis, 1982; Rogers *et al.*, 1982) and, therefore, increase dry matter intake.

Table 1. Dry matter intake (DMI) and milk yield (MY) in response to the dietary Na content

Reference	Dietary Na	DMI	MY	Response[a]	
	g/kg DM	kg/d	kg/d	DMI	MY
Schneider *et al.*, 1984	6.6	18.1	17.9	0.43	0.20
	9.6	19.4	18.5		
Schneider *et al.*, 1986	1.8	18.2	19.1	0.03	0.24
	5.5	18.3	20.0		
Schneider *et al.*, 1988	1.9	21.0	17.4	0.06	0.18
	6.8	21.3	18.3		
Silanikove *et al.*, 1998	2.2	15.4	26.2	0.53	1.27
	3.7	16.2	28.1		
Thiangtum *et al.*, 2011	1.6	16.3	14.9	0.33	0.62
	4.0	17.1	16.4		
Thiangtum *et al.*, 2017	1.2	12.5	14.4	0.20	1.20
	1.7	12.6	15.0		

[a]Within studies the response in DMI to the dietary Na content is calculated as ΔDMI (kg/day) / Δdietary Na (g/kg DM). The response in MY is calculated similarly.

Sodium requirement of heat stressed cows

A first estimation of the Na requirement of lactating cows in a tropical environment based on literature data was provided in Chapter 3. The value of 1.2 g Na/kg DM was the mean of the various estimates on Na requirements as set by different authorities (ARC, 1980; CVB, 2005; DLG, 2001; INRA, 1989; NRC, 2001; Underwood and Suttle, 1999) instead of the result of experimental data. In case the factorial method is used (Table 2) to calculate the Na requirement of a cow with body weight (BW) of 485 kg producing 15 kg of milk (Thiangtum *et al*., 2011), the daily Na requirement is calculated to be 11.3 g and this value is 43% lower than the estimated Na requirement using the value of 1.2 g/kg DM in combination with a DM intake of 16.5 kg/day (Thiangtum *et al*., 2011). Thus, at first glance, the tentative value set in Chapter 3 appears to overestimate the Na requirement. However, the values used by the CVB (2005) do not take into account the extra Na losses with sweat in heat stress conditions and as such Na losses with increased sweat production are not taken into account in this estimate.

Table 2. Sodium (Na) requirement of a dairy cow with a body weight of 485 kg, producing 15 L of milk.

Parameter[a]	Na losses			
	Faeces	Urine	Dermal	Milk
Endogenous losses				
mg/kg body weight	5.1	1.0	0.6	
g/day	2.5	0.5	0.3	
Milk				
g/L				0.46
g/day				6.9
	Na requirements (g/day)			
Net requirement for maintenance (M)			3.2	
Net requirement for M and 15 L milk			10.1	
Gross requirement for M			3.6	
Gross requirement for M and 15 L milk			11.3	

[a]The gross Na requirement is calculated using an efficiency of true Na absorption of 90% (Schonewille and Beynen, 2005).

Quantitative data on the Na losses with sweat are provided by Jenkinson and Mabon (1973) and they reported that Na losses with sweat range from 7.1 to 10 mg/m^2/h when THI ranged from 79 to 81. Under the assumption that a cow with a body weight of 485 kg (estimated surface area 4.75 m^2 (Brody, 1945)) experience 4 h of mild (THI 72–79), 19h of moderate (THI 79–89) and 1h of severe (THI > 89) heat stress each 24h (Thiangtum *et al.*, 2017), it can be calculated that such a cow loose ~3 g Na/day via sweat. Addition of the latter value to the Na requirement already mentioned (i.e. 11.3 g/day) results in a maximum Na requirement of 14.6 g/day for a cow weighing 485 kg, producing 15 kg of milk and suffering from heat stress. Thus, in case the ration contains of 1.2 g Na/kg DM, the cows need to ingest minimally 12.2 kg DM/day to meet their Na requirement. This minimum level of DM intake is generally achieved under Thai feeding conditions (Chapter 2). It, therefore, seems that the tentative value on the Na requirement set by Thiangtum *et al.* (2011) is a reasonable estimate for the required dietary Na content under Thai feeding conditions. However, it appeared that there is a considerable discrepancy between the Na losses with sweat calculated on the basis of data obtained by Na depletion and subsequent Na repletion (Thiangtum *et al.*, 2017) and the data provided by Jenkinson and Mabon (1973), and without further research they cannot be explained. Thus, the lack of unequivocal estimates on the Na losses with sweat, hinders

potential refinement of the previous recommendation (i.e. 1.2 g Na/kg DM) regarding the Na requirement of lactating cows under tropical conditions.

Apart from the Na losses with sweat, the endogenous Na losses with faeces, and the Na content of milk are major determinants of the net Na requirement (Table 2). Data on endogenous fecal Na losses were not obtained during the current studies (Chapters 3 and 4) but in Chapter 3 it was reported that cows consuming either 6.9 or 26.3 g Na/day lost respectively, 1.4 or 3.0 g Na/day with their faeces. Under the assumption that the efficiency of true Na absorption is 90% of intake (Schonewille and Beynen, 2005) and body weight of the cows is 485 kg, endogenous fecal Na losses are calculated to be 1.5 and 0.8 mg/kg BW when cows ingest 6.9 or 26.3 g Na/day, respectively. These calculated values are much lower than those listed in Table 2 but the issue of endogenous fecal Na loss is complicated and hitherto there is much uncertainty on this topic.

The current value on the endogenous fecal Na loss is estimated from Na balance data and derived from regressing fecal Na output against Na intake (Schonewille and Beynen, 2005). Using this approach, the endogenous faecal Na losses are assumed to be represented by the faecal Na losses at zero Na intake; i.e. the intercept of the regression formula. It can, however, be disputed whether this approach can be conceptually defended because considerable exchange of Na can occur between the extracellular body pool and the contents of the gastro-intestinal tract (ARC, 1980; Bell, 1995). Moreover, it was suggested by Schonewille and Beynen (2005) that inevitable Na losses associated with mucosal cells and gastro-intestinal juices are, at least in quantitative terms, negligible. From this viewpoint, the German (DLG, 2001) approach to estimate the maintenance requirement of Na on the basis of the Na content of fecal water is from a physiological viewpoint more attractive and they (DLG, 2001) adopted a value of 0.35 g Na/kg faecal water as an estimate for the maintenance requirement of Na. However, the latter value most likely overestimates the inevitable loss of Na with faeces because the DLG (2001) reports also that the Na concentration in faecal water can drop at least to 0.12 g/kg faecal water. These values are more or less in line with the values reported by Boehncke *et al*. (1983) and Renkema *et al.* (1962) who reported that the Na concentration in fecal water can drop to value as low as 0.07 g Na/kg. Clearly, the issue on the inevitable faecal Na excretion is not settled yet and may also depend on the dietary intake of K. It has been shown, at least in sheep that an increase of the K intake results in a decrease of the faecal Na excretion (Greene *et al*., 1983; Rahnema and Fontenot, 1986; Suttle and Field, 1967). It thus appears that a drop in the Na concentration of faecal water is accompanied by a rise in the K concentration of faecal water, thereby, keeping the sum of Na

and K more or less constant (DLG, 2001; Van Weerden, 1959). Taken the aforementioned into account, it is clear that it seems without doubt that more research is needed to properly estimate the maintenance requirement of Na under various physiological conditions. It is obvious that the issue of endogenous faecal Na loss also affects the estimate of true Na absorption and the value listed in Table 2 may underestimate the efficiency of true Na absorption. Indeed, Delaquis and Block (1995a) reported values on apparent Na absorption ranging from 93.3 to 95.9% of intake and the latter value is almost the same as the value reported in Chapter 3 (96.1%).

In the studies on Na reported in Chapter 3 and 4, the Na contents in milk were found to range from 0.21 to 0.62 g/L with an overall mean Na content of 0.35 g/L. Thus, the overall mean Na content of milk as measured in the current studies was ~24% lower than the value listed in Table 2. The latter value, however, is the overall mean that was calculated using the values on the Na content of milk (i.e. ranging from 0.40 to 0.63 g Na/L) adopted by various authorities (ARC, 1980; CVB, 1996; DLG, 2001; NRC, 2001; INRA, 1989) and values from individual studies (Delaquis and Block, 1995a; Kemp, 1964; Shalit *et al*., 1991; Silanikove *et al*., 1997). Kemp (1964) observed a Na content of milk ranging from 0.31 to 0.49 g Na/L, with a mean value of 0.38 g Na /L and mean Na contents of milk reported by Delaquis and Block (1995b), Shalit *et al*. (1991) and Silanikove *et al*. (1997) were 0.29, 0.42 and 0.45 g/kg, respectively. It thus appears that the Na contents of milk as measured in the current study fall well within the range of values reported in the literature.

Taken all aforementioned considerations into account, it can be suggested that the endogenous faecal Na losses as presented in Table 2, are overestimated and the efficiency of true Na absorption is underestimated. Moreover, in the current study, Na contents in milk were lower than 0.46 g/L (Table 2). Thus, it seems that the previous calculation on the Na requirement of cows, i.e. 11.3 g/day, already overestimates the actual Na requirement of cows living under temperate conditions. This implies that the gap in Na requirements of heat stressed versus non heat stressed is even greater than previously assumed. This notion strengthens the previous conclusion that the Na losses associated with heat stress are much greater than indicated by Jenkinson and Mabon (1973).

In the experiments reported in Chapter 3 and 4, salivary Na concentrations in the Na depleted cows ranged from 132 to 82.1 mmol/L while salivary K concentrations ranged from 10.2 to 44.2 mmol/L. In Na-deficient cattle there is a replacement of Na^+ with K^+ in the saliva, causing a reduction in Na/K ratio (Murphy and Connell, 1970; Underwood and Suttle, 1999). Based on these experiments, the Na/K ratio ranged from 14.6 to 2.3. The salivary

Na/K ratio lower than 6 was used for the diagnostic tool of Na deficiency (Schonewille and Beynen, 2005). After dietary induced Na deficiency (Chapter 4), cows showed a Na/K ratio in saliva lower than 4 in the first week with associated with signs of pica. The present study thus shows that the Na/K ratio in the saliva of cows is an accurate and convenient measure to diagnose Na deficiency.

PROTEIN SUPPLY AND MILK PRODUCTION OF THAI DAIRY COWS

A positive correlation was found between protein intake and MY in early lactating dairy cows in Thailand (Chapter 2). This observation can be interpreted in that protein intake is relevant to improve dairy production in Thailand. Unfortunately, the correlation between protein intake and MY cannot be easily explained because the cause and effect relationship is not clear due to the confounding effect of energy intake.

The issue on protein supply was addressed in Chapter 5 as well and in this study, an increase of the dietary CP content from 15.4 to 16.6% (DM basis) was associated with an increase of 1.0 kg of milk. The observed increase in MY in response to the increase in the dietary CP content, is similar to the amount that can be predicted with regression formula derived from a meta-analysis conducted by Ipharraguerre and Clark (2005). The increase in MY (Chapter 5) was associated with an increase in DMI, i.e. 0.5 kg. Thus, in this study too, the effect of dietary CP content on MY was confounded by DM intake and, therefore, with energy intake. On the other hand, the observation of a stimulatory effect on DM intake in response to an increase of the dietary CP content, is in line with a substantial body of evidence as reviewed by Oldham *et al.* (1984). It thus appears that, at least under the condition of *ad libitum* supply of feed, it is not possible to demonstrate a cause and effect relationship between the dietary CP content and MY. In contrast to MY, the yield of milk protein (kg/day) was not significantly affected by the increase of the dietary CP content and thus protein intake. Consequently, the increase in dietary CP content was associated with a decrease in the efficiency of nitrogen utilization (i.e. N-milk/N intake × 100%). This result can be interpreted in that the protein ingested in surplus to the low CP ration (i.e. 1.8 to 2.0 kg/day, Chapter 5) was used as a source of energy rather than a source of protein to synthesize milk protein. This notion is corroborated by the observation that the blood urea nitrogen (BUN) concentration increased from 12.2 to 14.7 mg/dL when the cows were fed the high instead of the low CP ration. On the other hand, BUN is also related to the ammonia (NH_3) concentration in rumen (Odensten *et al.*, 2005; Ropstad *et al.*, 1989) and rumen NH_3 concentrations increased from 24.3 to 28.3 mg/dL when cows were fed low or high CP

rations, respectively (Chapter 5). Although NH_3 is important for growth of rumen microbes, it can be disputed whether an increase from 24.3 to 28.3 mg/dL in rumen NH_3 concentrations has the potency to effectively stimulate microbial growth (Dijkstra *et al.*, 1998). Under the assumption that N supply to rumen microbes is the only limiting factor for their growth, maximum growth rate of rumen microbes is achieved when rumen NH_3 concentration is ~51 mg/dL (Dijkstra *et al.*, 1998, value is converted for the sake of comparison). However, in case the rumen NH_3 concentration is 24.3 mg/dL, maximum growth rate is estimated to be only ~5% lower compared to a rumen NH_3 concentration of 51 mg/dL. It can be suggested that the increase in rumen degradable protein (Chapter 5) was too small to effectively enhance microbial growth and, therefore, the flow of microbial protein from the rumen to the small intestine.

Next to N, either in the form of NH_3, amino acids or small peptides (Hackmann and Firkins, 2015), rumen microbes also require energy (i.e. ATP) to grow. This ATP is synthesized during rumen fermentation when organic matter is converted into volatile fatty acids (i.e. predominantly acetic acid, propionic acid and butyric acid). Thus, the amount of fermentable organic matter (FOM) that is ingested by ruminants is an important determinant of microbial growth and, therefore, the amount of microbial protein available for, amongst others, milk production. From this viewpoint, the content of fermentable carbohydrates in Thai dairy rations is of interest. In tropical countries, including Thailand, forages generally have high fibre contents (i.e. NDF, ADF and ADL, general introduction) and are, therefore, difficult to ferment by the rumen microbiota. It may, therefore, be that the forages used in Thai dairy practice are a poor source of energy for rumen microbes and thus limit microbial protein synthesis. From this viewpoint, caution is warranted to supplement Thai dairy rations with rumen degradable protein (RDP) because the N that originates from this RDP may not be incorporated in microbial protein and subsequently in milk protein, thereby, leading to low efficiency of N utilization. Hitherto, the FOM contents of Thai dairy feedstuffs are unfortunately not quantified and it can be suggested that this lack of knowledge hinders optimization of Thai dairy rations in terms of protein supply to the cows and, therefore, milk production in Thailand.

MILK YIELD AND MILK FAT CONTENT IN THAI DAIRY COWS

The most important trait for Thai dairy producers is MY because it is directly associated with the income of the farmer. In addition, the content of milk fat is also of importance because it is considered in setting the price of milk (Thai Milk Board, 2016). The % of milk fat relative to other countries is quite low in Thailand (i.e. 3.70% fat, Kamphusiri, 2013) and the Thai Milk Board has currently set a standard for the minimum fat content of milk. The milk prize of milk that contains less than 3.4% of fat is reduced by the milk collecting center. Currently, an increasing number of farmers face the problem that their milk is not accepted by cooperatives due to the low fat content in milk. The issue of low milk fat is, therefore, of considerable interest in Thailand.

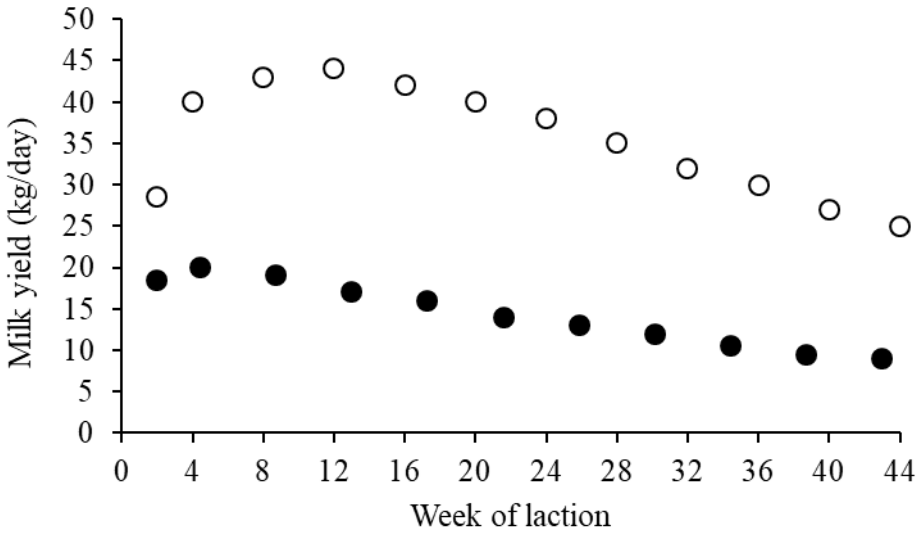

Figure 1. Milk yield in the course of time of cows producing either ~4,400 (●, Seangjun *et al.*, 2009) or ~10,800 kg of milk (○, Wu and Satter, 2000) during 305 days of lactation.

It was already mentioned that Thai dairy cows produce, on average, 12.2 kg milk/day (Pattamanont and Ruengpaibul, 2015) containing 3.70% fat (Kamphusiri, 2013). Thus, the amount of milk produced in 305 days is, on average, 3,721 kg/cow. In the central part of Thailand, the most important dairy region in the country, the 305 d milk production is around 18% greater than the nations mean (i.e. ~4,400 kg) but still can be considered low. Compared to cows producing ~10,800 kg milk/305 days, peak MY is low and this is considered the primary cause of the low 305 d milk production in Thai dairy cows because MY persist well over time (Figure 1).

In light of the low level of MY in Thailand, a high milk fat content is expected because it is well known that MY and milk fat content are inversely related (Gaunt, 1980). However, as already mentioned, the fat content of milk is generally low in Thailand. The milk fat content of the cows sampled during the cross sectional study was found to be 4.06% (Chapter 2), while Kamphusiri (2013) and Yeamkong *et al.* (2010) reported 3.70% and 3.4%, respectively. In contrast, Wu and Satter (2000) for instance reported milk fat contents of 3.99% and 3.92% in combination with 39.1 and 32.6 kg milk, respectively in US dairy cows. It, therefore, seems plausible that the low fat% in milk of Thai dairy cows is not caused by a high level of MY. Instead, it can be speculated that the cows lack sufficient precursors of milk fat, at least relative to that of lactose (i.e. the principle determinant of MY (Linn, 1988)) to maintain an acceptable fat content in the milk.

The predominant fat in milk is triacylglycerol, which contains short-(C_4-C_{10}), intermediate-(C_{12}-C_{16}), or long-chain (C_{18}) fatty acids (Linn, 1988). The short-chain fatty acids are synthesized within the mammary gland from acetate and ß-hydroxybutyrate; long-chain fatty acids are almost exclusively derived from blood plasma fatty acids of dietary origin; and intermediate-chain acids arise from both sources. In broad terms, approximately 50-60 percent of the fatty acids in milk are synthesized in the mammary gland and the other 40-50 percent are derived directly from blood (Linn, 1988). Thus, both acetate and ß-hydroxybutyrate are important precursors of fatty acid synthesis in ruminant mammary cells (Palmquist, 2006) and both volatile fatty acids originate from organic matter that is fermented in the rumen, i.e. FOM. It is well known that at a high rate of fermentation, the profile of volatile fatty acids shifts from acetate to propionate (Moran, 2005). Thus in case of a given amount of FOM, MY is enhanced because of greater availability of propionate to generate lactose. At the same time, lower amounts of milk fat can be generated due to lower amounts of precursors to generate milk fat and thus % of milk fat will drop. In view of the latter, the amount of FOM that originates from roughages can be considered important to potentially increase the milk fat% because this type of FOM generally has a low rate of fermentation (Noziere *et al.*, 2011) and thus yields greater proportions of acetate. In view of the chemical composition of the major roughages used in Thailand (General introduction), it can be speculated that the contribution of absolute amounts of acetate that originates from those roughages is low because of their low FOM and thus only marginally contribute to the generation of milk fat. Fibrous feeds are characterized in general by less propionate shifted fermentation with more methane production as compared with concentrate. Improvement of roughage quality is crucial in the utilization of fibrous feeds for sustainable livestock

production. There are many methods to improve roughage quality such as breeding, physical methods, chemical treatment methods and biological methods. Each method has different advantages and disadvantages, such as: breeding methods are effective but progress is slow, thermal treatment by steam improves nutritive value of waste product. Chemical treatments i.e. oxidizing agents (peroxyacetic acids, acidified sodium chloride, ozone etc.) decompose fairly efficiently the lignin and hydrolyzed agents (NaOH, KOH, CaOH, ammonia, urea, etc.) are able to hydrolyze the chemical bonds formed between the indigestible lignin and the parietal polysaccharides (cellulose, hemicellulose) resulted in complete digestion or partial digestion (Chenost and Kayouli, 1997). Biological treatment i.e. enzymatic agents with cellulolytic and hemicellulolytic capability are added to fibrous feed in attempts to improve nutrient digestibility (Abdel-Aziz *et al.*, 2015) and fungal treatments such as white rot fungi can decrease lignin and increase digestion (Shrivastava *et al.*, 2011).

GENERAL CONCLUSIONS

- The Na supply of dairy cows in Thailand is an important constraint for milk production because of an observed positive correlation between Na intake and milk yield.
- Both dry matter intake and milk yield were affected by the Na content of the ration. An increase of 1 g Na/kg DM resulted in an increase of 0.91 kg of milk and 0.27 kg DM.
- The salivary Na/K ratio is a more practical indicator of Na status in ruminants. A salivary Na/K ratio lower than 6 appears to be a good indicator for Na deficiency.
- The Na requirements of heat stressed lactating dairy cows are much greater than non-heat stressed cows. The Na losses associated with heat stress are much greater than indicated by different authorities.
- Dry matter intake responds to an increase of the dietary crude protein content.
- The milk protein yield (kg/day) was not significantly affected by an increase of the dietary crude protein content and thus protein intake.

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Summary

Dairy cows in Thailand are Holstein Friesian crossbreds (87.5% HF) and it is generally accepted that current milk yields of these cows are below their potential. The main reason for the low milk production of these dairy cows in Thailand is related to the prevailing environmental conditions. The tropical climate is an important constraint for milk production because of two main reasons. First, it is well known that the digestibility of forages is negatively affected by tropical conditions. Unfavorable growth conditions can result in low contents of protein and minerals in the plant and high amounts of so called structural carbohydrates. Secondly, heat stress affects animal performance by affecting feed intake.

The objectives of this thesis were to provide a basis for improvement of milk production in small farm holders in Thailand through supplementation of protein and salt (NaCl). Four experimental objectives were identified for this research, and it was anticipated that the outcome of the research can be applied so as to improve dairy production in Thailand and potentially other countries where dairy cows are maintained under tropical conditions.

In **Chapter 2**, a preliminary investigation is described into the relationship between ration composition and milk production of dairy cows in Thailand. The positive correlation between milk yield and crude protein (CP) intake was high during early lactation (r = 0.65). Cows consumed 5 and 8% greater CP than requirement during early- and mid-lactation, respectively. Interestingly, significant positive correlations were found between Na intake and milk yield. This observations is somewhat difficult to explain as Na intake was highly correlated with concentrate intake (r = 0.66). In other words, the increased milk yield may been related to a higher intake of Na but this was confounded with the amount of concentrate intake. In conclusion, protein, non-fibre carbohydrates and Na intake may have limited milk production by dairy cows on the Thai farms participating in the current study.

The study in **Chapter 3** investigated the Na requirement of lactating dairy cows under tropical conditions by measuring Na in saliva, milk and faeces. The Na intake did not change the concentrations of Na and K in milk, faeces and serum, but did affect sodium concentration in the saliva. This observation was corroborated by the salivary Na and K concentrations with the cows on the low NaCl diet having a salivary Na concentrations < 120 mmol/L in combination with salivary K concentrations > 20 mmol/L ($P < 0.05$). The ratio of Na:K in saliva was a good indicator of Na intake by dairy cattle. Consumption of a daily ration formulated to contain the current Na requirement as set by the NRC appears to be too high for lactating cows under tropical conditions. A tentative value of 1.2 g Na/kg DM is proposed as the Na requirement for dairy cows under tropical conditions.

In **Chapter 4**, the assessment of the Na requirement of heat stressed lactating cows is hindered by accurate estimates of the Na losses through sweat. Direct studies, therefore, are needed on the time course of healthy animals to become Na depleted and the subsequent rate of repletion. The rate of Na depletion and subsequent rate of Na repletion with two levels of dietary Na to lactating dairy cows housed under tropical conditions was investigated using the salivary Na/K. The 12 lactating cows rapidly developed clinical signs of Na deficiency, including pica, polyuria and polydipsia, reduced body weight and reduced milk yield when fed a Na-low (0.33 g/kg DM) ration during 3 weeks. Deficiency symptoms were associated with a rapid decrease in salivary Na/K ratio to < 4.3 from 7-21 d. Subsequent repletion of the cows with NaCl to a ration concentration of 1.1 or 1.6 g Na/kg DM for 5 weeks did not restore salivary Na/K ratio to values of > 6. A daily Na intake of heat-stressed lactating cows to a ration intake of 1.6 g Na/kg DM was insufficient to restore Na deficiency. One week was sufficient to deplete heat-stressed lactating cows of Na allowing for rapid dose-response studies utilizing the salivary Na/K ratio as a parameter for Na status of cows under tropical conditions.

In **Chapter 5**, an experiment is reported where the effects of two levels of dietary CP in concentrates with similar proportions of rumen undegradable protein (RUP) on rumen metabolism, milk yield and composition in mid lactating cows was investigated. Concentrate feeds were formulated to provide low dietary CP [17.3%; LCP] or high dietary CP [19.0%; HCP]. The proportion of rumen degradable protein and RUP was 61% and 39% in both diets, respectively. Diets were isocaloric in terms of net energy for lactation. Milk yield, milk lactose yield, dry matter intake (DMI), and apparent digestibility of DM, CP and neutral detergent fibre (NDF) were greater in cows fed the HCP than in those fed the LCP. Concentration of blood urea nitrogen (N) was elevated in cows fed HCP diets. Rumen ammonia-N concentration and pH tended to increase in cows fed the HCP diet. Rumen microorganism counts and volatile fatty acids levels in the rumen did not differ between treatments. In conclusion, increasing CP content in mid-lactating cow was beneficial to increase DMI, apparent digestibility of DM, CP and NDF and therefore milk yield.

In **Chapter 6**, the results of the experimental studies reported in this thesis are discussed in light of Thai dairy production conditions.

บทสรุป

โคนมในประเทศไทยส่วนใหญ่เป็นโคนมพันธุ์โฮลสไตน์ฟรีเชียนที่มีสายเลือดของโคนมพันธุ์แท้อยู่ 87.5 % แต่ความสามารถในการผลิตน้ำนมต่ำกว่าความสามารถของพันธุกรรมที่ผลิตนมได้ สาเหตุหลักมาจากภูมิอากาศของประเทศเป็นแบบเขตร้อนชื้น สภาพอากาศดังกล่าวทำให้พืชอาหารหยาบมีคุณภาพต่ำส่งผลให้เกิดการย่อยยาก เนื่องจากมีองค์ประกอบของเยื่อใยสูง มีปริมาณโปรตีนและแร่ธาตุต่ำ นอกจากนี้ความเครียดเนื่องจากความร้อนยังส่งผลต่อประสิทธิภาพโคนมโดยทำให้การกินได้ของโคลดลง

วิทยานิพนธ์นี้มีวัตถุประสงค์เพื่อเป็นข้อมูลพื้นฐานในการปรับปรุงการผลิตน้ำนมในฟาร์มโคนมรายย่อยของประเทศไทย โดยการศึกษาเรื่องการเสริมโปรตีนและเกลือในอาหาร ซึ่งประกอบด้วย 4 เรื่องการศึกษา จากผลการศึกษานี้หวังว่าจะสามารถนำไปประยุกต์ใช้ในฟาร์มโคนมที่เลี้ยงในเขตร้อนชื้นเพื่อช่วยเพิ่มผลผลิตน้ำนม

บทที่ 2 เป็นการศึกษาเบื้องต้นเกี่ยวกับความสัมพันธ์ระหว่างองค์ประกอบอาหารและผลผลิตน้ำนมของโคนมในประเทศไทย พบว่าปริมาณน้ำนมในช่วงต้นของการให้นมมีความสัมพันธ์ในเชิงบวกกับปริมาณโปรตีนที่โคได้รับ (r=0.65) โดยแม่โคที่อยู่ในระยะต้นและระยะกลางของการให้นมที่ได้รับโปรตีนมากกว่าค่าความต้องการโปรตีนระดับมาตรฐานอยู่ 5 % และ 8 % ตามลำดับ และยังพบว่าปริมาณโซเดียมที่โคได้รับมีความสัมพันธ์ในเชิงบวกกับปริมาณน้ำนม (r=66) แต่ความสัมพันธ์นี้อาจไม่ใช่ความสัมพันธ์ที่แท้จริงเนื่องจากปัจจัยกวนจากปริมาณอาหารข้นซึ่งมีโซเดียมเป็นองค์ประกอบอยู่ค่อนข้างมาก โดยสรุปจากการศึกษาพบว่าปริมาณโปรตีน คาร์โบไฮเดรตที่ไม่ใช่เยื่อใย และโซเดียมที่โคได้รับ อาจส่งผลต่อการผลิตน้ำนมในประเทศไทย

บทที่ 3 เป็นการศึกษาความต้องการโซเดียมของโครีดนมในเขตร้อนชื้น โดยการวัดระดับโซเดียมในน้ำลาย น้ำนม และมูล พบว่าปริมาณการกินโซเดียม ไม่ส่งผลต่อความเข้มข้นของโซเดียมและโปแทสเซียมในน้ำนม มูล และซีรั่ม แต่ส่งผลต่อความเข้มข้นของโซเดียมในน้ำลาย พบว่าโคนมที่กินอาหารที่มีเกลือ (NaCl) ในระดับต่ำ จะมีความเข้มข้นของโซเดียมในน้ำลายน้อยกว่า 120 มิลลิโมลต่อลิตร และมีความเข้มข้นของโปแทสเซียมในน้ำลายมากกว่า 20 มิลลิโมลต่อลิตร (P<0.05) สัดส่วนของโซเดียมต่อโปแทสเซียมในน้ำลายเป็นตัวบ่งชี้ที่ดีของปริมาณโซเดียมที่โคได้รับ จากการศึกษาครั้งนี้พบว่าความต้องโซเดียมที่กำหนดโดย NRC สูงกว่าความต้องการของโครีดนมที่อยู่ในเขตร้อนชื้น ซึ่งมีความต้องการโซเดียม 1.2 กรัมต่อกิโลกรัมวัตถุแห้ง

บทที่ 4 เป็นการศึกษาความต้องการโซเดียมของโครีดนมที่อยู่ในภาวะความเครียดจากความร้อน โดยนำการสูญเสียโซเดียมทางเหงื่อมาร่วมคำนวณด้วย ทำการศึกษาในโครีดนม 12 ตัวให้อยู่ในภาวะขาดโซเดียม (0.33 กรัมต่อกิโลกรัมวัตถุแห้ง) เป็นระยะเวลา 3 สัปดาห์ พบว่าโคทั้งหมดแสดงอาการขาดโซเดียม โคกินน้ำและขับปัสสาวะมาก น้ำหนักตัวและปริมาณนมลดลง ร่วมกับการลดลงของสัดส่วนโซเดียมต่อโปแทสเซียมในน้ำลายน้อยกว่า 4.3 ในระหว่างวันทดลองที่ 7-21 และหลังจากนั้นทำการแบ่งโคเป็น 2 กลุ่ม เพื่อทดแทนโซเดียมในระดับต่ำ (1.1 กรัมต่อกิโลกรัมวัตถุแห้ง) และระดับสูง (1.6 กรัมต่อกิโลกรัมวัตถุแห้ง) เป็นระยะเวลา 5 สัปดาห์ ผลการศึกษาพบว่าการทดแทนโซเดียมทั้ง 2 กลุ่ม ไม่สามารถทำให้สัดส่วนโซเดียมต่อโปแทสเซียมในน้ำลาย กลับมามีสัดสัดที่มาก 6 โดยสรุปสัดส่วนโซเดียมต่อโปแทสเซียมในน้ำลายสามารถบ่งชี้ภาวการณ์ขาดโซเดียมได้ภายใน 7 วัน

บทที่ 5 เป็นการศึกษาเมตาบอลิซึมในกระเพาะรูเมน ปริมาณนม และองค์ประกอบน้ำนมของโครีดนมระยะกลาง โดยทดลองให้อาหารข้นที่มีโปรตีนต่างกัน 2 ระดับคือระดับต่ำ (17.3%) และระดับสูง (19.0%) ซึ่งมีสัดส่วนของโปรตีนไหลผ่านในอาหาร (RUP, 39%) และพลังงานสุทธิในการให้น้ำนมเท่ากัน ผลการทดลองพบว่าปริมาณน้ำนม ปริมาณแลคโตส ปริมาณการกินได้ที่คิดเป็นวัตถุแห้ง และการย่อยได้ปรากฏของวัตถุแห้ง โปรตีนและเยื่อใยที่ละลายในสารฟอกที่เป็นกลาง (NDF) ในโคที่ได้รับโปรตีนระดับสูงจะสูงกว่าในโคที่ได้รับโปรตีนระดับต่ำ ความเข้มข้นของยูเรียในกระแสเลือดเพิ่มขึ้นในโคที่ได้รับโปรตีนระดับสูง โดยที่ความเข้มข้นของแอมโมเนียไนโตรเจน และความเป็นด่างในกระเพาะรูเมนมีแนวโน้มมีค่าสูงขึ้นในโคที่ได้รับโปรตีนระดับสูง ปริมาณโปรตีนในอาหารไม่ส่งผลต่อจำนวนจุลชีพ และ กรดไขมันระเหยง่ายในกระเพาะรูเมน โดยสรุปการเพิ่มขึ้นของโปรตีนในอาหารโคนมระยะกลางส่งผลต่อการเพิ่มขึ้นของการกินได้ของวัตถุแห้ง การย่อยได้ปรากฏของวัตถุแห้ง โปรตีน NDF และปริมาณน้ำนม

บทที่ 6 ผลการศึกษาทั้งหมดที่อยู่ในวิทยานิพนธ์นี้ได้ถูกนำมาอภิปรายภายใต้สภาพการเลี้ยงโคนมในประเทศไทย

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Wandee

About the author

Curriculum vitae

List of publications

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Wandee Thiangtum was born on October 21, 1970 in Chanthaburi province, Thailand. She obtained the degree of Doctor of Veterinary Medicine (D.V.M.) in 1996 from Kasetsart University, Thailand. After her graduation, she worked for 8 months as a small animal practitioner at Kasetsart University Veterinary Teaching Hospital at the Kamphaeng Saen campus. Thereafter she became a staff member at the Large Animal and Wildlife Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University. In 2004, she received a M.Sc. degree in Veterinary Epidemiology and Economics from Utrecht University, the Netherlands. That same year, she started her Ph.D. studies through a sandwich program with Faculty of Veterinary Medicine, Utrecht University and made the switch in continuing her studies at the Animal Nutrition Group, Wageningen University & Research in 2010. She has been promoted to an Assistant Professor in 2013. During the Ph.D. program she started preliminary experiments and conducted her research work focusing on sodium and protein requirement of lactating cows under tropical conditions. This thesis provides information on the work she did during her Ph.D. and was defended in public on May 7, 2018, at Wageningen University & Research, the Netherlands.

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