

Prevalence and risk factors associated with hypocalcemia in periparturient dairy cows in Thailand



Name: Lonneke Noorman
Student nr: 3259641
Supervisor in Thailand: Dr. T. Rukkamsuk
Supervisor in the Netherlands: Dr. ing J.T. Schonewille
Date: January - April 2011

Abstract

Milk fever is a clinical disorder in the periparturient period and is characterized by adaptations to the demands of lactation. The disease is life threatening itself and it is predisposing for several other disorders. It is an important disease, because of enormous economic consequences due to reduction in milk production, loss of animals and the treatment of the animals. In Thailand, milk fever is often observed among periparturient dairy cows, but data or reports concerning the status of blood calcium in periparturient dairy cows are limited. In this experiment the prevalence and the risk factors associated with hypocalcemia in periparturient dairy cows under Thai conditions are observed. The hypothesis that there may be some differences in importance in risk factors between western countries and Thailand is confirmed. Urine samples are collected to get information about the urine pH and therewith metabolic alkalosis. Also blood samples are taken to measure serum calcium before, during and after calving and the body condition score is observed. Questionnaires are used to collect data about feed and feeding management as well as pertinent data of cows and herds. Risk factors are discussed and low calcium intake and supplement of vitamin D seems to be the most important risk factors in Thailand. Also the intake of concentrate and roughage is important, probably because of association with low calcium intake. No correlation is found between urine pH and serum calcium value, which indicates DCAD (dietary cation-anion difference) is not important in Thailand.

Contents	
<u>Abstract</u>	<u>2</u>
<u>Contents</u>	<u>3</u>
<u>Introduction</u>	<u>4</u>
Economic consequences	4
Differences between Thailand and Western countries	4
Definition serum calcium levels	5
Clinical signs	5
Prevalence	6
Calcium homeostasis	6
Calcium-requirements	6
Risk factors	7
Oral calcium treatments	7
Vitamin D supplementation	8
Preventive feeding	8
Aim of the study and hypothesis	9
<u>Materials and methods</u>	<u>10</u>
Animals and Sampling, Sample analyses, BCS, Statistical analysis	10
<u>Results</u>	<u>11</u>
Prevalence	12
Change in serum calcium values and body condition score	12
Body condition score	13
Parity	15
Supplementation of mineral en vitamin	16
Vitamin supplement	17
Feed intake	18
Urine pH	19
Length of dry period	19
Milk yield	20
Comparison between hypocalcemia and negative energy balance (NEB)	22
<u>Discussion</u>	<u>23</u>
Prevalence	23
Change in serum calcium values and body condition score	23
Body condition score	23
Parity	24
Urine pH	24
Feed intake	25
Supplements	26
Supplementation of vitamin D	27
Length of dry period	27
Calcium intake before calving	28
Milk yield	28
Hypercalcemia	29
Related diseases	29
Comparison between hypocalcemia and negative energy balance (NEB)	30
Points of improvement	30
<u>Conclusion</u>	<u>32</u>
<u>Acknowledgements</u>	<u>33</u>
<u>References</u>	<u>34</u>
<u>Attachments</u>	<u>37</u>

Introduction

In Thailand, most dairy cows are kept in small-holder farms (of less than 40 dairy cows per farm), in which the farmers are confronting several production diseases (Chantalakhana et al. 1999). Clinical hypocalcemia or milk fever is often observed among these periparturient dairy cows. However, data or reports concerning the status of blood calcium in periparturient dairy cows in Thailand are limited.

The periparturient period is characterised by adaptations to the demands of lactation. Calcium requirement is increasing through the onset of lactation, causing a sudden loss of calcium into the milk. The foetal calf and the placenta need a lot of energy, protein and minerals during the end of gestation. Calcium disappears to the mammary gland faster than it can be replaced through mechanisms like intestinal calcium absorption and resorption of calcium from the bone (Kara et al. 2009). This may lead to subclinical or clinical hypocalcemia (Merck Veterinary Manual 2008, Thirunavukkarasu et al. 2010). Hypocalcemia is defined as a blood calcium concentration below 2 mmol/l (Merck Veterinary Manual 2008, Horst et al. 2003, Goff et al. 2008).

Cows with subclinical and clinical hypocalcemia are predisposed to other diseases, including dystocia, uterine prolapse, metritis, rumen disfunction, abomasal displacement, ketosis and mastitis. (Merck Veterinary Manual 2008, Horst et al. 2003, DeGaris et al. 2009, Rukkamsuk et al. 2010, Murray et al. 2010). Cows suffering from milk fever have longer intervals between parturition and first postpartum ovulation than cows with a subclinical or no hypocalcemia. Subclinical hypocalcemia reduces the size of the ovulatory follicles and their associated corpora lutea at the moment the normal calcium cycles is returned after calving (Murray et al. 2010). Further a relation between clinical hypocalcemia and retained placenta is observed. Cows with milk fever are more likely to have a retained placenta than cows with a normal calcium value (Kara et al. 2009, Murray et al. 2010).

Economic consequences

Milk fever is a serious problem in many countries, because of the enormous economic consequences of the disease. The most important economic loss is due to reduction in milk production, loss of animals and the treatment of the animals. In a research of Thirunavukkarasu et al. (2010), the costs are calculated due to milk fever in India. They found that farmers lost in average 13.55 American dollars (converted of Rs 618) for each cow for just the treatment. The costs were most for the medicine (49.2%), followed by veterinarians' fee and feed supplements. This trend was also seen in England in an earlier research of Kossaibati and Esslemont (1997). The economic loss due to reduced milk yield is measured as 7.59 dollar per affected cow in India in exotic cow. Kossaibati and Esslemont have reported the costs due to loss of milk yield as 64.79 dollar for a mild case, up to 161.96 dollar for a severe case in Holstein cows. There are not many cases of mortality in milk fever, but if there are, the economic loss is high. The value of the affected cows died in the research of Thirunavukkarasu et al. was 300.48 dollar each. This was an average loss of 2.30 dollar per affected cow. The total costs due to milk fever in this research were 23.42 dollar. Mike Hutjens (2003) reports milk fever costs of an average 334 American dollar per affected animal in dairy cattle in New York.

Differences between Thailand and Western countries

The environmental conditions of cows in Thailand are different compared to Western countries, like the Netherlands. In Thailand, there are a lot more problems with regard to contagious diseases, such as Foot and Mouth Disease and Paratuberculosis. They threat the cows suffering of these diseases, which causes a long period with suboptimal milk production. In Thailand farmers are more reluctant to cull cow, because of the economic conditions. Some

dairy herd health problems are related to low breeding efficiency (Markvichitr et al. 1995). In addition, there are a lot more stress factors during the gestation period in Thai condition according to a hot and humid environment and the floors are often slippery. A reduction of feed intake, a decrease in milk production and a reduction in fertility can be seen because of heat-stress (Avendano-Reyes et al. 2009). In Thailand, the dairy farms are typically small and in many cases the farmers own just a very limited amount of land. In 1999, more than ninety percent of dairy farms in Thailand were smallholder farms, with the total number of less than 40 dairy animals per farm (Chantalakhana et al. 1999). Nowadays, the number of farms is not increasing or is probably constant, but the number of animals per farm is increasing. The waste management and disposal systems in Thailand are weak. Dirty water and animal wastes cause degradation of the conditions of the environment in a long term. The water and soil conditions become worse (Chantalakhana et al. 1999).

The feeding practices, like mentioned above, are also different in Thailand. There is not much information about the feed of ruminants. Generally, specific feed evaluation system is not used in Thailand for feed formulation. Information about optimization of dairy ration given to the farmers is hardly present. The education of the farmers in Thailand is low. The grasses are cut and carried to the cows. Because of this grazing is not common (Ukawa et al. 2002). Green fodder is mainly fed in the rainy season.

Rice straw is used very much, just in the dry season or during the whole year because grass is not available. The feed is limited to mainly rice straw and purchased feed. This feeding system is less balanced than in the Netherlands. There is less concentrate food available, which means the cows eat less food that contains just all the necessary ingredients. All these different factors can lead to different risk factors with regard to hypocalcemia.

Definition serum calcium levels

Normal serum calcium levels are 2.1-2.5 mmol/L (8.5-10 mg/dL). Subclinical hypocalcemia is defined as serum calcium 1.4-2.0 mmol/L (5.5-8 mg/dL). When the serum calcium level declines to <1.4 mmol/L, the cow experiences a clinical hypocalcemia (Merck Veterinary Manual 2008, Horst et al. 2003, Goff 2008).

A high producing dairy cow loses 20 to 30 g of calcium a day with the milk or colostrum. The calcium is replaced by withdrawing calcium from bone or increasing the rate of absorption of calcium from the diet (Murray et al. 2008, Goff 2008, DeGaris et al. 2009).

Clinical signs

Milk fever has been recognised in the mid-nineteenth century. The clinical signs were first described by Victorian veterinary surgeons (Murray et al, 2008).

Clinical hypocalcemia usually occurs within 72 hours after parturition. It can be divided in three stages: stage 1, 2 and 3. Cows in stage 1 show signs of hypersensitivity and excitability. They are restless, they can be ataxic and they have tremors over the triceps and flanks. If cows in stage 1 are not treated, they will likely progress to stage 2. The cows in stage 2 are unable to stand. These cows are anorectic, have a dry muzzle, subnormal body temperature, cold extremities, tachycardia, decreased intensity of heart sounds and a weak peripheral pulse. Smooth muscle paralysis can lead to stasis of the gastro-intestinal tract, which can lead to a failure to defecate and loss of anal sphincter tone. The cows often tuck their heads into their flanks. Cows that progress to stage 3 lose consciousness and can go into a coma. The cows are unable to maintain sternal recumbency, have hypotonic muscles and do not response to stimuli. The peripheral pulse may be undetectable because the cardiac output declines. Without treatment, these cows will die within two or three days. (Merck Veterinary Manual, 2008)

Prevalence

Goff et al. (2008) and Sorenson et al (2002) found a prevalence of clinical hypocalcemia in the USA of 5-10% per lactation. In a research in India the prevalence of milk fever was 13.7% (Thirunavukkarasu et al. 2010).

Horst et al. (2003) did research on the prevalence of subclinical hypocalcemia in U.S. dairy operations. They found that subclinical hypocalcemia increased with advancing age. The prevalence of subclinical hypocalcemia in first lactation cows was 25.3% (n = 442), in second lactation cows 43.9% (n = 424) and in third or more lactation cows 57.8% (n = 580).

Goff (2008) and Sorenson et al. (2002) reported that the incidence of subclinical hypocalcemia in the USA is around 50% in third or more lactation cows. This is consistent with the research done by Horst et al. (2003).

Calcium homeostasis

The mobilisation of bone calcium is regulated by parathyroid hormone (PTH). PTH is secreted by the parathyroid glands as a response of decreasing calcium levels. PTH stimulates osteoclasts, which enhances bone resorption. PTH also increases the production of active vitamin D (1,25 dihydroxy-cholecalciferol) in the kidney, which enhances the absorption of calcium from the diet due to activation of the enzyme 1 α -hydroxylase. PTH also enhances tubular reabsorption of calcium. These endocrine controls are summarized in figure 1. (Murray et al. 2008, Goff 2008)

A normal periparturient cow will have an increased level of PTH and active vitamin D as a response to the decline of the serum calcium level. This leads to increased intestinal absorption, increased resorption from bone and increased renal tubular reabsorption of calcium (Goff 2008).

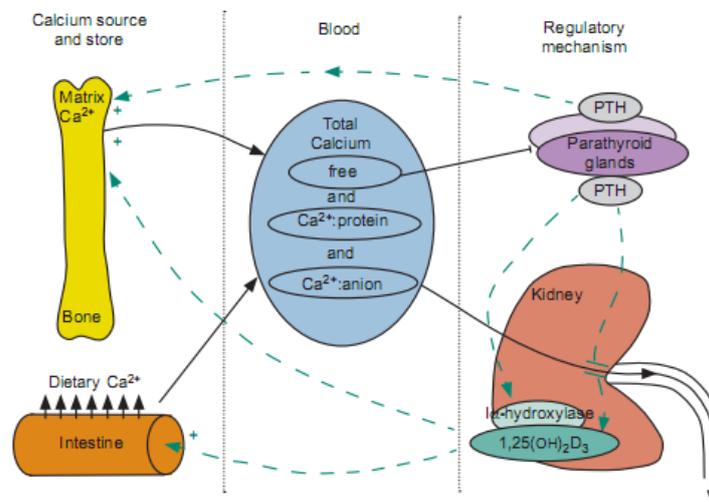


Figure 1: Calcium homeostasis (Murray et al. 2008)

Calcium-requirements

The calcium requirement is increasing through the onset of lactation, because of several factors. A lot of calcium is lost in the milk and also the foetal calf and the placenta need a lot of energy, protein and minerals at the end of gestation. Calcium is also lost in faecal and (in a very small part) urinary loss. (DeGaris et al. 2007)

The minimal calcium requirement is 31 gram in a cow of 600 kg. This is in a situation with a maximum absorption of calcium and a minimum loss of calcium.

Risk factors

High calcium intake (as described below) before the parturition is an important risk factor in the development of milk fever. High calcium leads to a low efficiency of calcium absorption and less rapid bone mobilisation in cows before calving and this leads to less available calcium at the parturition. After parturition low calcium intake is a risk factor, because of the high demands of calcium due to the onset of the lactation (Goff et al. 2008). In a research of Oetzel (1991) intermediate concentrations of calcium were associated with a higher risk at milk fever. Low calcium intake before calving had a beneficial effect at the prevalence at milk fever. Although large changes in calcium intake before calving had just a small affect in the incidence of milk fever (9%) (Oetzel 1991).

Hypocalcemia is more often seen in cows of 5 years or older. Older cows can mobilise less calcium from the bone, because they have fewer active osteoclasts (Murray et al. 2008, DeGaris et al. 2009). Clinical hypocalcemia does not usually affect cows before the third parturition (Horst et al. 2003).

Furthermore the body condition score has an effect at the development of milk fever (Rukkwamsuk et al. 1999, Ostergaard & Grohn 1999). Over-conditioned cows at the date of parturition are more risk full in the development of milk fever then cows with a lower body condition score. An explanation could be a low calcium intake in over-conditioned cows, because of reduced appetite in the critical period around parturition (Rukkwamsuk et al. 1999). An optimum body condition score for preventing milk fever should be between 3.25 and 3.75 (Thilsing-Hansen et al. 2002).

Metabolic alkalosis is also an important risk factor for hypocalcemia (Goff 2008). Metabolic alkalosis makes the cow less sensitive to PTH, which predisposes to (sub)clinical hypocalcemia. There is less mobilisation of calcium from the bones, the renal reabsorption is reduced and the kidneys will produce less active vitamin D. The cause of metabolic alkalosis is a diet that has more cation (K, Na, Ca, Mg) than anions (Cl, sulfate, phosphate), which lead to a positive diet cation-anion difference (DCAD). Generally with a high cations diet, the urine pH is above 8.2. An optimal urine pH for controlling milk fever in Holstein Friesian cows is described as a pH of 6.2-6.8. To reach this pH, it is essential to add anions to the ration. If the pH is lower than 5.5, the cow will suffer from a metabolic acidosis and will have a lower food intake (Goff et al. 2002).

Other important factors are magnesium and phosphor. Magnesium is necessary for the release of PTH and critical in the synthesis of vitamin D (1.25 dihydroxy-cholecalciferol) (DeGaris et al. 2009). Hypomagnesemia can interfere with PTH. Normally the binding of PTH to its receptor initiates activation of adenylate cyclase. This results in the production of a second messenger, cyclic AMP (Goff et al. 2008). Adenylate cyclase contains a magnesium-binding site, what makes a magnesium ion necessary (Goff et al. 2008). PO₄ concentrations are directly regulated by vitamin D (1.25 dihydroxy-cholecalciferol) and indirectly by the PTH/Calcium negative feedback loop (DeGaris et al. 2009).

Oral calcium treatments

Oral supplementation of calcium gives the possibility to force calcium across the intestinal tract due to passive diffusion between intestinal epithelial cells (Goff et al. 2008). This helps to maintain the normal calcium concentrations in the blood. For the best results one should give doses of calcium between 50 and 125 gram.

As supplementation calcium chloride has been used. However this can induce side-effects which are caused by an irritating effect on the mucous lining of the gastro-intestinal tract (Thilsing-Hansen et al. 2002). It could also induce an uncompensated metabolic acidosis. Calcium propionate is a better solution and has no acidogenic effects. Best is to give the dose at calving and 24 hours later again. Careful treatment is necessary, because toxic doses can be

reached. About 250 gram soluble calcium is enough to kill some cows. (Goff et al. 2008, Thilising-Hansen et al. 2002)

Vitamin D supplementation

Already in 1943, Campbell and Turner noticed vitamin D could be used to buffer the demands for calcium and increased activity of the parathyroid glands and in 1977 B.F. Sansom described the use of vitamin D metabolites for preventing milk fever. The fact that supplementation of vitamin D will reduce the chance of getting hypocalcemia is proven many times (Goff et al. 2008, Murray et al. 2010, DeGaris et al. 2009, Thilising-Hansen et al. 2002). To prevent milk fever, the best way of supplementation vitamin D can be disputed (Murray et al. 2008). There is some discussion about the use of vitamin D supplementation 10-14 days before calving. In some researches this is described as a way to prevent milk fever. The vitamin D increases the intestinal Ca absorption. However this supplementation is difficult, because of problems with dose and timing. The dose to prevent milk fever is very close to the toxic dose, which can cause several clinical symptoms (Goff et al. 2002, Thilising-Hansen et al. 2002, Goff et al. 2008); lower doses sometimes induce milk fever because the vitamin suppresses the PTH secretion. Treatment with 1.25-dihydroxyvitamin D is more effective and much safer. The problem with suppression of 1.25-dihydroxyvitamin D production can be minimized by giving the hormone over a period of more days after calving (Goff et al. 2008).

Preventive feeding

There are several factors important at the food management in the dry period to prevent milk fever. To start with calcium itself, it is necessary to restrict the calcium intake before calving. Actually the norm of calcium intake was set at 40-60 gram calcium a day, but the best evidence for the utility of low calcium was proved with diets in which calcium was less than 25 gram a day (Goff et al. 2003). Due to the low calcium, the parathyroid gland is stimulated before calving. That results in bone calcium mobilization and production of 1,25-dihydroxyvitamin D. At this way the cow is more prepared to the calcium demands of lactation. Mobilisation of calcium from the bone is less rapid in cows who are feed with food that contains high calcium prior to calving (DeGaris et al. 2009). It can be quite difficult to give a low calcium diet to especially a non-grazing cow. Two methods are developed to solve this problem. One method is supplementation of zeolite. This binds calcium and this cause fecal excretion of calcium. However this method is not used in common, because of very large amounts of zeolite is necessary (0.25-1 kg/day for 2 weeks before calving). Another disadvantage is that it interacts with phosphor. That is why zeolite is often chemically modified. At this way it is possible to increase the affinity en specificity to calcium, so fewer amounts can be given. It also contains vegetable oils that interact and bind with calcium and form an insoluble soap, to prevent absorption of calcium from the diet. After calving the calcium intake should be changed to a high intake (Goff et al. 2008).

Another important factor is the diet cation-anion difference (DCAD). The incidence of hypocalcemia can be declined by reducing the amount of dietary cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+} and added anionic salts) and/or increasing the amount of dietary anions (Cl^- , SO_4^{2-} , P^{3-}) (Sorenson et al. 2002, Goff 2008, DeGaris et al. 2009). NaCl and KCl do not have an effect on the DCAD, because Na^+ , K^+ and Cl^- are absorbed with an efficiency of almost 100%. Therefore it is important to use strong cations that are absorbed in a lesser extent than the strong anions (CaCl_2 , CaSO_4 , MgCl_2 , MgSO_4 , NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$, HCl and H_2SO_4) (DeGaris et al. 2007). Roughage contains relatively many cations. Straw and hay contains mainly a lot of potassium (2.16 and 2.31% DM) (Robinson et al. 2005). A linear relationship is seen, but with a linear regression of 0.061 (so almost negligible). Between a DCAD of -30 and 60 mEq/100 g of DM incidence of milk fever is seen, with a big spreading of incidence between

these two values (Lean et al. 2006). Cations probably reduce the sensitivity of renal tissue to PTH. The consequence is less formation of active vitamin D which leads to less absorption of calcium from the intestine (Murray et al. 2008). Horst et al. (2003) found that cows fed a ration with a negative DCAD had a lower prevalence of subclinical hypocalcemia. Horst et al. (2003) also found lower serum non-esterified fatty acids levels in cows with a serum calcium level higher than 8 mg/dL. This indicates that the cows with a normal calcium level were in better energy status than the cows with hypocalcemia. This means that they have a less negative energy balance and there is less fat mobilized. In a research of Lean et al. (1991), sulfur is found as the most important factor that influences the risk at milk fever. Sulfur acts as a strong anion and that is why (as described above) it has a reducing effect at the risk of milk fever.

Other factors in the preventive feeding are phosphor and magnesium. Phosphor increases milk fever risk because there have a negative impact on calcium homeostasis (DeGaris et al. 2009). A cow with a phosphor intake of more than 80 g/day (8%) has an increased risk to develop milk fever, because the phosphor will block the renal production of vitamin D (1.25 dihydroxy-cholecalciferol). For most cows it is the best the diet contains about 4 g/kg (Goff et al. 2008). A diet that contains high levels of magnesium could induce a lower renal calcium excretion than a diet low in magnesium. So, a cow feed with a low magnesium diet will lose more calcium due to renal excretion what leads to poor calcium mobilisation (DeGaris et al. 2009). To guarantee the cow has enough magnesium, the diet should contain 3.5-4.0 gram magnesium/kg (Goff et al. 2008). However this phenomenon is not very important in dairy cows.

A high value of potassium in the diet also increases the risk at milk fever (DeGaris et al. 2009). The general requirement of potassium of the dry cow is about 10 g/kg (Goff et al. 2008). Potassium is one of the cations which are important for the DCAD.

Aim of the study and hypothesis

The aim of the current study is to trace out the most relevant risk factors for milk fever under the Thai conditions. This is important because of the high economic consequences of milk fever as mentioned above. It is anticipated that the outcome of this study provides a scientific basis for further optimization of rations for Thai dairy cows. Imaginable the risk factors are most similar to other countries, but there may be another distribution in importance because of different conditions of the cows.

Material and methods

Animals and Sampling

In this research 40 dairy cows (crossbreeds of Holstein Friesian) in Ratchaburi and Kanchanaburi in Thailand are used. These are random cows (selected at calving date), with different ages and different farms. The data are collected in January till March 2011.

Questionnaires are used to collect information on feed and feeding management as well as pertinent data of cows and herds. Body condition scores from all cows are recorded at -4, -1 and 4 weeks from parturition.

Blood samples are collected from all cows at -4, -1, 0, and 4 weeks from parturition to determine serum concentrations of calcium. Urine samples are collected in week -1 to determine pH.

Sample analyses

Blood samples are collected of the coccygeal vein. After collecting blood samples, serum is separated through centrifuging. Serum calcium concentrations are measured using atomic absorption spectrophotometry. Midstream urine samples are collected through massaging of the perineum. The pH-values of the urine are measured directly at the farm with a pH-indicator, which is flushed with distilled water between measurements.

Body condition score

The body condition score is determined using palpation and visual examination. The score uses a five-point scale (one is lean, five means fat), with increments of 0.25 according to the system of Elanco Animal Health Bulletin AI 8478. The numbers and the quarter-point divisions could be determined using the bend of the tuber coxae and tuber ischiadica. Using the fat covering true looking at the spinous processes and the span between the tuber coxae and backbone gives the information which numbers the body condition should be scored.

Statistical analysis

Prevalence data on milk fever (blood calcium concentration below 5.5 mg/dL) are reported as descriptive statistics. Risk factors associated with hypocalcemia are analyzed using linear correlation coefficient, chi-square test and paired t-test.

Results

Sample	L. No.	Ca wk -6 - -2	Ca wk -3 - 0	Ca wk 0	Ca wk 3 - 5	BCS wk -6 - -2	BCS wk -3 - 0	BCS wk 3 - 5	pH wk -2 - 1
1	1	8.9	8.6* ₋₂	7.6	9.2	2.75	2.75* ₋₂	2.25	8.11
5 (1)	5	8.7	10.0	8.4	10.1* ₊₃	3.75	4.25	3.75* ₊₃	7.11
6 (1)	4	8.4	10.5* ₋₃	11.5	x	2.50* ₋₆	2.75* ₋₃	x	7.91
7 (1)	3	6.7	x	11.4	7.4	2.50* ₋₃	2.50* ₀	2.50* ₊₅	7.27* ₀
8 (2)	5	7.6	10.9* ₋₃	8.5	x	2.50* ₋₆	2.50* ₋₃		x
9 (2)	h	10.4	9.1	10.1	x	4.00* ₋₃	4.00	3.25* ₊₅	8.17
10	2	12.2	9.6* ₀	9.1	10.5	3.25* ₋₂	3.50* ₀	2.50* ₊₃	7.81* ₀
11 (3)	h	10.0	x	9.1	11.5	3.50* ₋₂	4.00* ₀	3.00	7.53* ₀
12 (3)	1	10.3	10.8* ₀	8.5	9.4	3.25* ₋₅	3.50* ₀	2.50	7.40* ₀
13 (4)	3	9	9.6	8.2	x	3.00	3.00	2.75	8.02
14 (4)	h	11	10.3	8.3	x	3.00	2.25	2.50	7.89
18	1	12.6	8.6* ₋₂	8	x	2.75* ₋₆	2.75* ₋₂	2.50* ₊₃	6.89* ₋₂
20	1	9.7	10.1* ₋₂	13	10.3* ₊₃	2.75* ₋₆	2.75* ₋₂	2.75* ₊₃	8.09* ₋₂
22	1	8.2	x	7.7	7.5	2.75	3.00* ₀	2.75	7.62* ₀
25 (5)	1	9.9	8.7* ₋₂	7.6	x	2.75	2.75* ₋₂	2.25	7.45* ₋₂
26 (5)	1	8.6	11.5	8	10.9* ₊₃	2.75	2.75	2.50* ₊₃	7.97
27 (5)	3	7.8* ₋₁	x	7.4* ₊₁	7.5	2.75* ₋₁	3.00	2.50	7.21
28 (5)	h	9.2	x	8.3	9.6	2.50* ₋₃	2.25* ₀	2.25	7.41* ₀
29	5	8.2	9* ₋₂	4.7	8.3	2.75	3.50* ₋₂	3.00	7.90
30	6	7.6	12.3	7.6	8.3	3.75	4.00	2.75	7.71
31	2	8.4	8* ₋₂	4.8	12	2.75* ₋₃	2.75* ₋₂	2.50* ₊₅	7.41
34	5	7.9	10.2	5.5	7	4.00* ₋₃	3.75	2.75* ₊₅	7.68
36	2	8.7	7.9* ₋₂	8.3	10.1	3.50	4.00* ₋₂	2.75	6.96
42 (6)	h	x	7.9	7.7	9* ₊₃	x	3.50	3.00* ₊₃	8.00
43 (6)	h	11.3	x	10.4	x	4.00* ₋₃	3.50* ₀	x	7.50* ₀
46 (5)	4	7.8	x	7.7	x	4.00* ₋₃	3.25* ₀	x	5.61* ₀
47	3	9.6	9.1* ₋₂	10.5	x	2.75* ₋₅	3.00* ₋₂	x	7.37* ₋₂
48 (7)	1	8.9	9	10.8	x	3.00	4.00	x	7.35* ₀
49 (7)	h	9.1	x	8.9	x	2.75* ₋₃	2.75* ₀	3.00* ₊₃	8.07* ₀
51	h	15.3	x	9.5	x	3.50* ₋₃	2.75* ₀	x	7.46* ₀
54 (8)	h	10.1	8.8	6.7	x	3.00	3.00	x	7.31
55	6	9.4	7.8	8.7* ₊₁	x	3.00* ₋₃	3.00* ₋₂	2.75* ₊₃	5.16* ₊₁
56	h	9.3	9.3	7.4	x	3.00* ₋₃	3.00	3.00* ₊₃	5.73
57	7	9.3	x	7.1	x	2.75* ₋₃	2.75* ₀	x	7.62* ₀
58	2	8.4	9.5	7.4	x	2.75* ₋₃	2.75	x	7.13
59	h	9.2	x	8	x	3.25* ₋₃	3.00* ₀	x	7.80* ₀
60 (8)	3	8.2	9.6	9.1	x	3.00	2.75	x	7.71
66	h	11.7	x	7.6	x	3.25* ₋₂	3.25* ₀	x	x
74	2	6.6	x	5.4	x	2.75* ₋₂	2.50* ₀	x	7.47* ₀
76	4	8.2	x	7.9	x	2.50* ₋₂	2.25* ₀	x	7.27* ₀

Table 1: All results

* h means heifer; this is equal to lactation no. 0

*₂ If the sample no. is cursive and red, it means the cow had a serum calcium value < 5.5 mg/dL at week 0. If the sample no. is blue, it means the cow had a serum calcium value between 5.5 and 8.5 mg/dL at week 0

*₃ Some of the numbers are missing, because of some animals are excluded of the research due to different reasons (cow is sold/culled, missing sample)

*₄ Not all data are collected at exact the same time. In the schedule is through *-*,_o or +* noticed at which moment the sample is actually taken. The samples who are not marked with * are taken at -4, -1, 0 or +4

*₅ If samples are of the same farm, this is mentioned through the number after the sample. Cows with the same number after the number of the sample are of the same farm

Prevalence

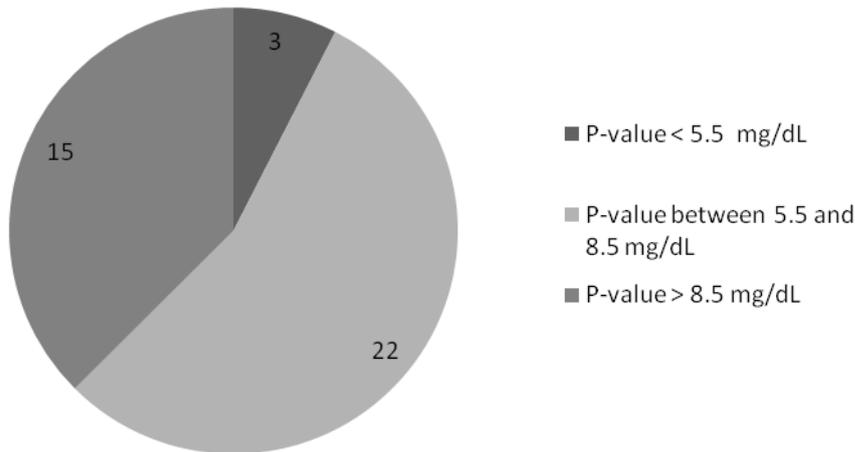


Figure 3: Prevalence of sub- and clinical hypocalcemia in this study

In this study the prevalence of a plasma calcium value below 5.5 mg/dL is 7.5%. The incidence of a p-value between 8.5 and 5.5 mg/dL is 55%. In third or more lactation cows the prevalence of a p-value between 8.5 and 5.5 mg/dL is 53.33%.

Change in serum calcium values and body condition score (BCS)

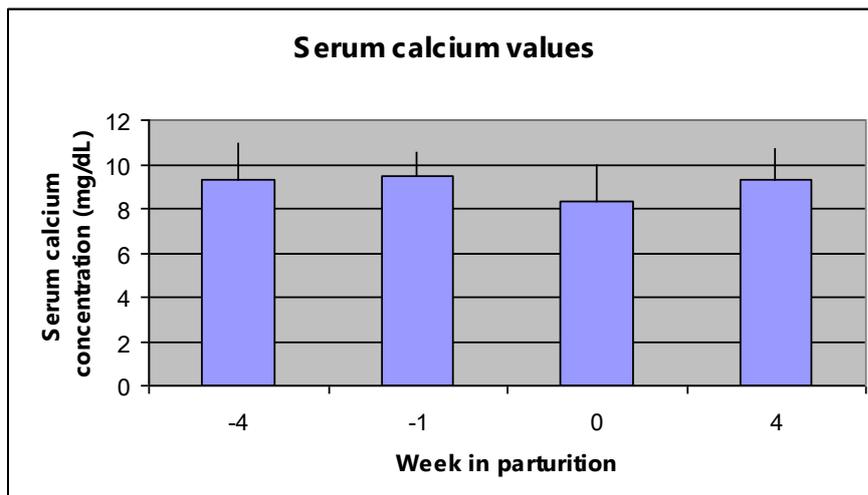


Figure 4: average serum calcium values in week -4, -1, 0 and 4

$P < 0.05$ between -1 -0 and 0 -4

* Error bars show the standard deviation

*₂ In average the plasma calcium is above 8 mg/dL, which means in average there is no hypocalcemia

A significant change ($p = 0.005$ at $-1 - 0$ and $p = 0.017$ at $0 - 4$) can be seen in the serum calcium value of week 0. The serum calcium value is clearly the lowest around the parturition.

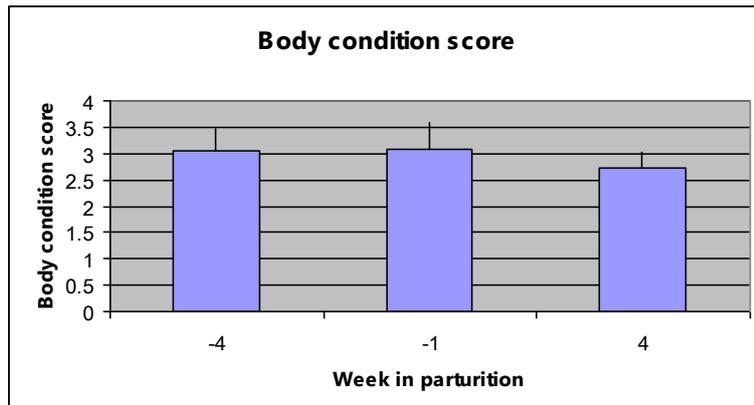


Figure 5: average BCS in week -4, -1 and 4

$P < 0.01$ between -1 and 4

* Error bars show the standard deviation

A significant drop ($p < 0.01$) in BCS can be seen between week -1 and week 4. No significant change exists between week -4 and -1.

Body condition score

BCS -4	No. of cows	Average calcium wk 0
2.25	0	-
2.50	5	9.52 ($\sigma = 1.78$)
2.75	15	7.83 ($\sigma = 2.16$)
3.00	7	8.46 ($\sigma = 1.31$)
3.25	4	8.30 ($\sigma = 0.07$)
3.50	4	8.65 ($\sigma = 2.13$)
3.75	2	8.00 ($\sigma = 0.75$)
4.00	4	8.43 ($\sigma = 1.30$)
4.25	0	-

Table 2a: BCS -4 against serum calcium value at parturition ($p > 0.05$)

BCS -1	No. of cows	Average calcium wk 0
2.25	3	8.17 ($\sigma = 0.23$)
2.50	3	8.43 ($\sigma = 3.00$)
2.75	13	8.61 ($\sigma = 2.05$)
3.00	8	8.08 ($\sigma = 1.15$)
3.25	2	7.65 ($\sigma = 0.07$)
3.50	5	8.08 ($\sigma = 2.13$)
3.75	1	5.50
4.00	5	9.18 ($\sigma = 1.30$)
4.25	1	8.40

Table 2b: BCS -1 against serum calcium value at parturition ($p > 0.05$)

In table 2a the BCS of one month before calving is placed against the average calcium at parturition. The linear correlation coefficient is 0.075; there is no significant correlation. In

table 2b the same comparison is shown, but with BCS of one week before calving instead of one month before calving. In this case the linear correlation coefficient is 0.058, so also at this comparison no significance is present.

BCS -1	No. of cows	Average calcium wk 0
< 3.25	27	8.38 ($\sigma = 1.75$)
3.25 – 3.75	8	7.65 ($\sigma = 1.84$)
> 3.75	6	9.05 ($\sigma = 1.20$)

Table 3: BCS -1 against serum calcium, divided in groups ($p > 0.05$)

Thilising-Hansen et al. (2002) mentioned a BCS between 3.25 and 3.75 as optimal BCS to prevent milk fever. Because of this statement and to create bigger groups, also a comparison is made between optimal BCS and no optimal BCS (table 3). Also in this comparison no significant difference is measured ($p > 0.05$; paired t-test). Also if one group is made of < 3.25 and > 3.75, no significance is detected.

In figure 6 the average BCS is plotted for cows with a serum calcium value > 8.5, serum calcium between 8.5 and 5.5 and cows with a serum calcium value < 5.5 mg/dL. In this study a calcium value between 5.5 mg/dL and 8.5 mg/dL is considered as a subclinical hypocalcemia. Because not all samples are complete, the n is different between different weeks (see below figure). One could see the BCS of the cows suffering from milk fever is lower than the BCS of the cows with a subclinical or without a hypocalcemia. However there is no significant difference between the different groups ($p > 0.05$; paired t-test).

For a complete overview the BCS (week before calving) is plotted against average calcium in figure 12a. The linear correlation coefficient is 0.058. This value is very close to 0, so the positive correlation that exists is almost negligible and not significant.

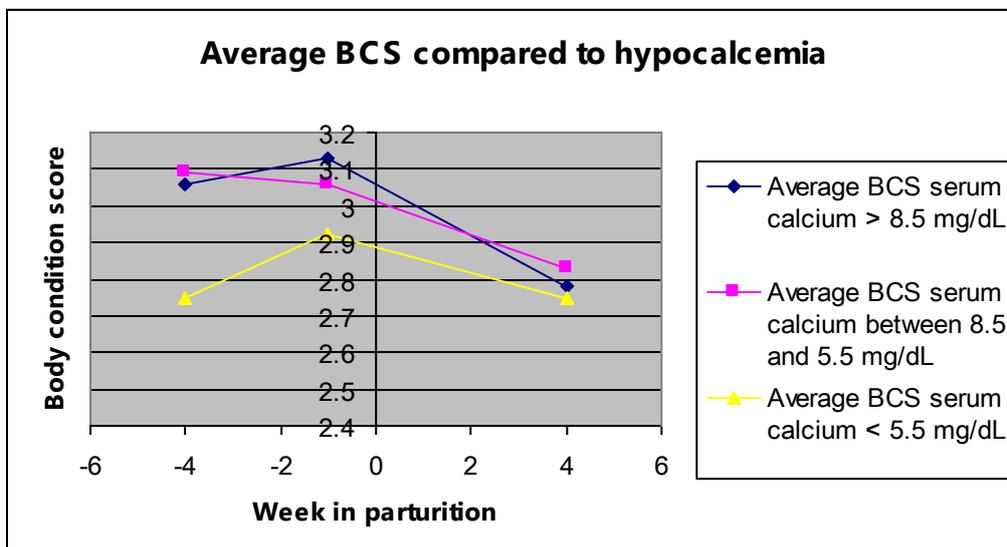


Figure 6: BCS compared to serum calcium

Average BCS no hypocalcemia: n wk -4 = 16; n wk -1 = 16, n wk 4 = 8

Average BCS subclinical hypocalcemia: n wk -4 = 22; n wk -1 = 22, n wk 4 = 15

Average BCS clinical hypocalcemia: n wk -4 = 3; n wk -1 = 3, n wk 4 = 2

Parity

No. of lactation	No. of cows	Average calcium wk 0
0	13	8.57 ($\sigma = 1.11$)
1	7	8.90 ($\sigma = 1.96$)
2	5	7.00 ($\sigma = 1.85$)
3	5	9.32 ($\sigma = 1.64$)
4	3	9.03 ($\sigma = 2.14$)
5	4	6.78 ($\sigma = 1.96$)
6	2	8.15 ($\sigma = 0.78$)
7	1	7.10

Table 4: Lactation number against serum calcium at week 0 ($p > 0.05$)

To compare the lactation number with the serum calcium value, in table 4 the average serum calcium value on calving date against the number of lactation is shown. A linear correlation coefficient of -0.205 is seen, which means there is a weak (and not significant) negative correlation between number of lactation and average calcium at week 0.

In figure 7a the number of cases of serum calcium < 5.5 mg/dL and serum calcium between 5.5 and 8.5 mg/dL are shown in a bar chart. Two of the three serum calcium values < 5.5 mg/dL of this study belong to lactation number two. The other value < 5.5 mg/dL belongs to lactation number five. Almost every lactation number shows a higher number of cows with subclinical hypocalcemia compared to cows with no hypocalcemia (except lactation number three and six). A chi-square test notify a p-value of 0.247 ($p > 0.05$), which means there is no significant difference between different lactation numbers in this study. Because of low number of samples of cow with lactation number > 2 and this is the described risk factor in other studies, in figure 7b these samples are taken as one group. Also in this comparison, no significance is found ($p = 0.15$; $p > 0.05$; chi-square). The mean calcium value of lactation number 0 is 8.57 mg/dL and the mean calcium value of lactation number 1 is 8.90 mg/dL, which means the mean calcium value of both groups is > 8.5 mg/dL.

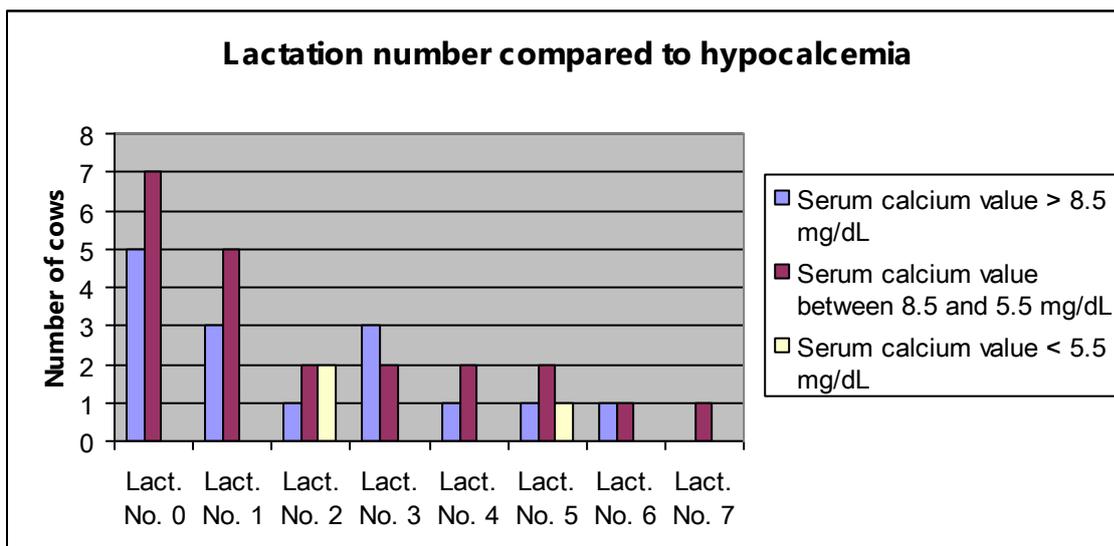


Figure 7a: Lactation number as a risk factor for hypocalcemia ($p > 0.05$)

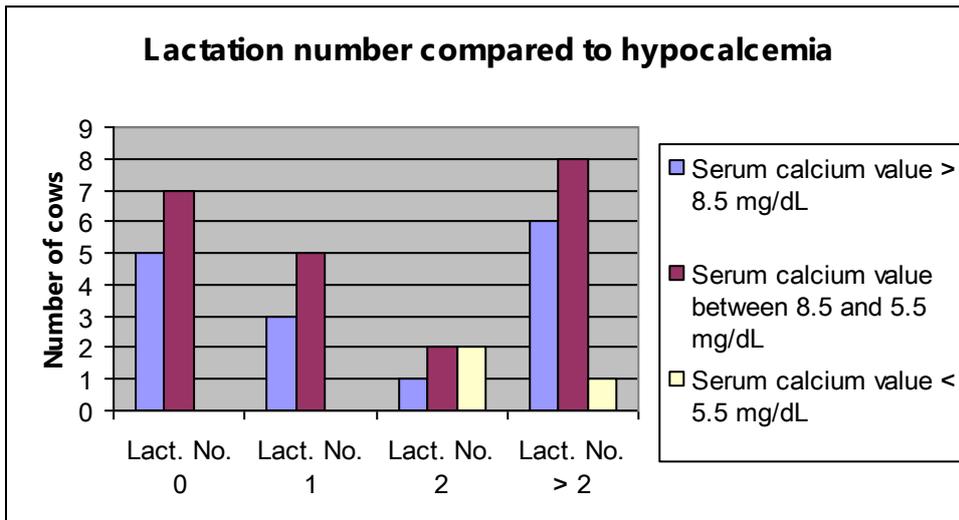


Figure 7b: Lactation number as a risk factor for hypocalcemia, divided in groups ($p > 0.05$)

Supplementation of mineral en vitamin

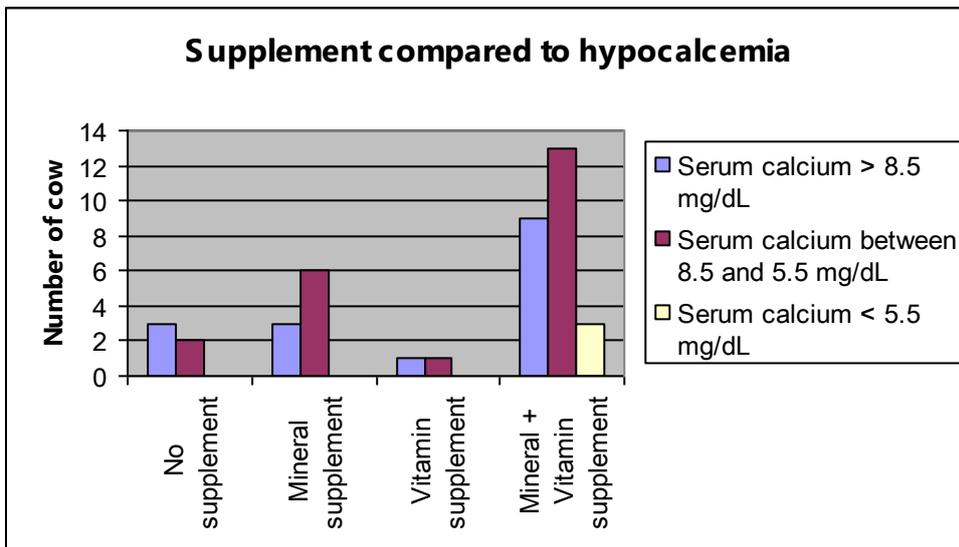


Figure 8: Supplements to prevent hypocalcemia ($P > 0.05$)

In figure 8 the cows are divided into four categories of vitamin and mineral supplementation. As one could see in the bar chart all three milk fever cases occurred in the category ‘mineral + vitamin supplement’. Calculated with a chi square test, a p-value of 0.789 ($p > 0.05$) is found, so there is no significant difference between these four categories in this research.

To compare the calcium course, in figure 9 the average calcium values of these four categories are plotted against the time. There is just a significant difference between the serum calcium value of one month before calving between ‘no supplement’ and ‘mineral+vitamin supplement’ ($p = 0.032$; $p < 0.05$; paired t-test). Furthermore no significant difference between different groups is seen. A big problem is that both amount and ingredient composition of the supplements is not provided and the groups size is not the same (see discussion). A lot more cows received mineral+vitamin, so this lead to an higher chance to detect a cow with hypocalcemia.

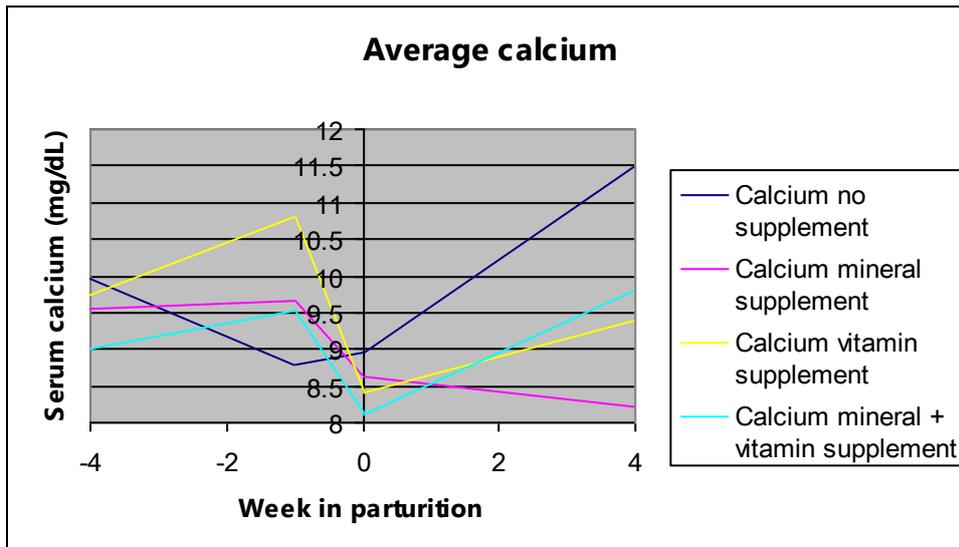


Figure 9: Influence of supplements on average serum calcium values
 Calcium no supplement: n wk -4 = 5; n wk -1 = 2, n wk 0 = 5, n wk 4 = 1
 Calcium mineral supplement: n wk -4 = 8; n wk -1 = 5, n wk 0 = 9, n wk 4 = 3
 Calcium vitamin supplement: n wk -4 = 2; n wk -1 = 1, n wk 0 = 2, n wk 4 = 1
 Calcium mineral + vitamin supplement: n wk -4 = 22; n wk -1 = 15, n wk 0 = 25, n wk 4 = 9

Vitamin supplement

Vitamin D supplementation is described as a specific risk factor of milk fever. Because of this, in figure 10 the supplementation of just vitamin D is been shown. The cows are divided in two categories: cows who did not receive vitamin D and cows that were supplemented with vitamin D. In this research the vitamin D that is used is vitamin AD₃E. All other cows are placed in the first category, which means the category 'no vitamin D' includes different cows that could be supplemented with other vitamin, mineral or they did not get any supplementation before calving. In this figure one could see the prevalence of a serum calcium value between 8.5 and 5.5 mg/dL in the category 'no vitamin D' is much higher than in 'ADE'. There is a significant difference between these two categories (chi square test) and the corresponding p-value is 0.024 ($p < 0.05$).

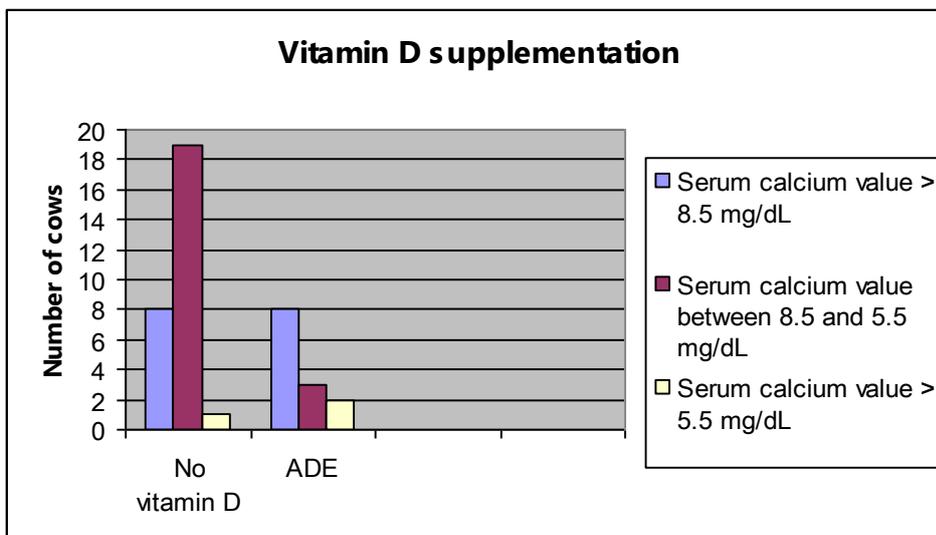


Figure 10: Vitamin D supplementation to prevent hypocalcemia ($p < 0.05$)

Feed intake

In this research the reported intakes of concentrate and roughage (in kg/day) differs a lot in different cows. The concentrate intake varies between 0.5 and 27 kg/day and the intake of roughage between 8 and 70 kg/day. However, these highest values for feed intake are very high and are not possible unless the dry matter is rather low. Because of this variation, a comparison is made between average calcium values at parturition and intake of roughage and concentrate (figure 12). Also a comparison is made between number of cows with a serum calcium value < 5.5 mg/dL and a serum calcium value between 5.5 and 8.5 mg/dL and the intake of concentrate and roughage. In table 5 the comparison between concentrate intakes are shown. As one could see all three serum calcium values < 5.5 mg/dL occurred at an concentrate intake of less than 5 kg/day. However a p-value of 0.099 ($p > 0.05$) is calculated and no significant difference is noticed (chi square test). In figure 13d the average plasma calcium is plotted against intake concentrate. A little increase in serum calcium is seen at more concentrate intake each day. The linear correlation coefficient between intake of concentrate and blood calcium value at calving is 0.636 ($p < 0.05$). This means there is a positive correlation between these two values, but not very strong.

In table 6 the intake of roughage (kg/day) is shown against the different serum calcium values. At a higher intake of roughage, a higher incidence of a serum calcium value < 8.5 mg/dL can be seen. However, this is not clearly. The p-value (calculated by a chi-square test) is 0.021 ($p < 0.05$), so there is a significant difference in the different categories of roughage intake.

In figure 12e a decrease in blood calcium value at a higher roughage intake can be seen, but also this is not a clear line. The linear correlation coefficient between intake of roughage and blood calcium values is -0.786 ($p < 0.05$), which means there is quite a strong negative correlation between these two values.

Intake concentrate (kg/ day)	No. of cows	Prevalence of cows with serum calcium value between 8.5 and 5.5 mg/dL (= no. of cows)	Prevalence of cows with serum calcium value < 5.5 mg/dL (= no. of cows)
< 1	1	0 (0)	1 (1)
1-1.99	3	1 (3)	0 (0)
2-2.99	3	0.67 (2)	0 (0)
3-3.99	7	0.29 (2)	0 (0)
4-4.99	10	0.4 (4)	0.2 (2)
5-5.99	1	1 (1)	0 (0)
6-6.99	3	0.33 (1)	0 (0)
7-7.99	0	0 (0)	0 (0)
8-8.99	9	0.44 (4)	0 (0)
10-11	2	0 (0)	0 (0)
27-28	2	0.5 (1)	0 (0)

Table 5: Concentrate intake against serum calcium values at week 0 ($p > 0.05$)

Intake roughage (kg/ day)	No. of cows	Prevalence of cows with serum calcium value between 8.5 and 5.5 mg/dL (= no. of cows)	Prevalence of cows with serum calcium value < 5.5 mg/dL (= no. of cows)
---------------------------	-------------	---	---

< 10	4	0.25 (1)	0 (0)
10-19.99	18	0.72 (13)	0 (0)
20-29.99	7	0.43 (3)	0.14 (1)
30-39.99	8	0.25 (2)	0.125 (1)
40-49.99	1	1 (1)	0 (0)
50-59.99	1	0 (0)	1 (1)
60-69.99	0	0 (0)	0 (0)
70-79.99	2	1 (2)	0 (0)

Table 6: Roughage intake against serum calcium values at week 0 ($p < 0.05$)

Urine pH

In figure 12c the pH of the urine (collected between a week pre-parturition and parturition) is plotted against the average serum calcium value at parturition. No clear relation is perceptible in this figure. The linear correlation coefficient between pH and blood calcium value at calving is 0.134, so no significant correlation is present ($p = 0.422$; $p > 0.05$).

Length of dry period

Dry period (days start dry period till day of calving)	No. of cows	Cows with serum calcium value between 8.5 and 5.5 mg/dL (= no of cows)	Cows with serum calcium value < 5.5 mg/dL (= no. of cows)
40-49.99	1	0 (0)	0 (0)
50-59.99	1	0 (0)	0 (0)
60-69.99	5	0.6 (3)	0 (0)
70-79.99	8	0.63 (5)	0.13 (1)
80-89.99	4	0 (0)	0.25 (1)
90-99.99	5	0.8 (4)	0 (0)
120-129.99	1	0 (0)	1 (1)
130-139.99	2	0 (0)	0 (0)
310-320	1	0 (0)	0 (0)

Table 7: Length of dry period in relation to prevalence of hypocalcemia at week 0 ($p > 0.05$)

In table 7 the dry period (in days from start of dry period till day of calving) is shown against the serum calcium values below 5.5 and between 5.5 and 8.5 mg/dL at week 0. No significant difference is seen ($p = 0.124$; $p > 0.05$; chi-square test).

The cow with a dry period of 318 (310-320) days, was suffering of infertility problems. That is why the dry period is that long.

Milk yield

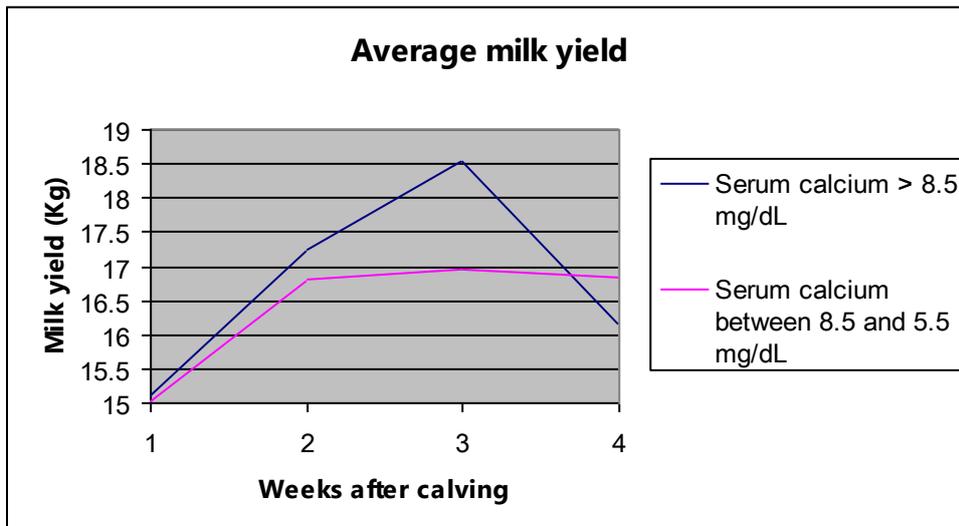


Figure 11: Length of dry period relation to hypocalcemia and BCS

No hypocalcemia: week 1 $n = 9$; week 2 $n = 8$; week 3 $n = 7$; week 4 $n = 4$

Subclinical hypocalcemia: week 1 $n = 15$; week 2 $n = 14$; week 3 $n = 14$; week 4 $n = 14$

* Because of the availability of the milk yield of just one cow with a serum calcium < 5.5 mg/dL (that is not complete), this category is excluded of this figure
($P > 0.05$)

In figure 11 the average milk yield is presented, divided into two groups: serum calcium > 8.5 mg/dL and serum calcium between 8.5 and 5.5 mg/dL. Because of lacking data of cows with a serum calcium value < 5.5 mg/dL, this group is excluded from this comparison. The figure shows a difference between the two groups in milk yield, but not significant ($p > 0.05$; paired t-test). The first three weeks the average milk yield of the cows with a serum calcium > 8.5 mg/dL is higher compared to the cows with a serum calcium value between 5.5 and 8.5 mg/dL. The last (fourth) week this is contrary.

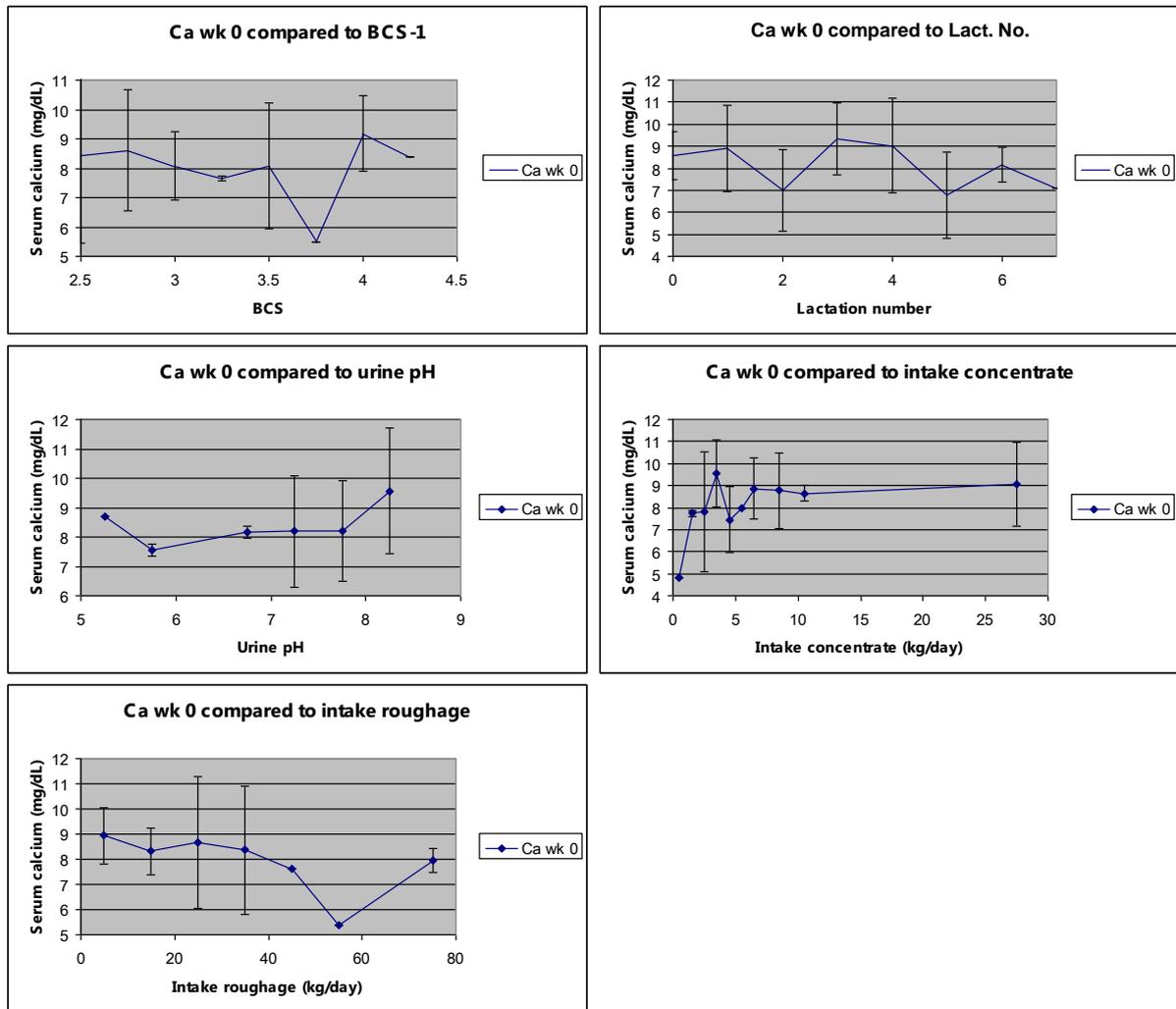


Figure 12: Mean serum calcium concentrations by (A) BCS -1 ($r=0.058$), (B) Lactation no. ($r=-0.205$), (C) Urine pH ($r=0.134$), (D) Concentrate intake ($r=0.636$), (E) Roughage intake ($r=-0.786$)

- A. BCS 2.25 $n = 3$; BCS 2.5 $n = 3$; BCS 2.75 $n = 13$; BCS 3.0 $n = 8$; BCS 3.25 $n = 2$; BCS 3.5 $n = 5$; BCS 3.75 $n = 1$; BCS 4.0 $n = 5$; BCS 4.25 $n = 1$
- B. Lact. No. 0 $n = 13$; Lact. No. 1 $n = 9$; Lact. No. 2 $n = 5$; Lact. No. 3 $n = 5$; Lact. No. 4 $n = 3$; Lact. No. 5 $n = 4$; Lact. No. 6 $n = 2$
- C. pH 5-5.5 $n = 1$; pH 5.51-6 $n = 2$; pH 6.01- 6.5 $n = 0$; pH 6.51-7 $n = 2$; pH 7.01-7.5 $n = 14$; pH 7.51-8 $n = 15$; pH 8.01-8.5 $n = 5$
- D. Intake <1 $n = 1$; intake 1-1.99 $n = 3$; intake 2-2.99 $n = 3$; intake 3-3.99 $n = 7$; intake 4-4.99 $n = 10$; intake 5-5.99 $n = 1$; intake 6-6.99 $n = 3$; intake 7-7.99 $n = 0$; intake 8-8.99 $n = 9$; intake 10-11 $n = 2$; intake 27-28 $n = 9.05$
- E. Intake <10 $n = 4$; intake 10-19.99 $n = 18$; intake 20-29.99 $n = 7$; intake 30-39.99 $n = 8$; intake 40-49.99 $n = 1$; intake 50-59.99 $n = 1$; intake 70-79.99 $n = 2$

* Error bars show the standard deviation

Comparison between hypocalcemia and negative energy balance (NEB)

Because of the same cows are used for a research about negative energy balance, also the NEFA and glucose values are available. As well hypocalcemia as NEB are important metabolic diseases around parturition. To compare negative energy balance and hypocalcemia, the NEFA and glucose-values are compared to the serum calcium values. A significant correlation is seen between several values. The significant linear correlation coefficients that are calculated are summarized in table 8.

	Linear correlation coefficient	Significance
Calcium wk 0 and NEFA wk 0	-0.369	0.025
Calcium wk -4 and glucose wk -4	0.493	0.003
Calcium wk -4 and glucose wk 0	0.440	0.008

Table 8: significant linear correlation coefficients between serum calcium and glucose and NEFA

Because Reinhardt et al. (2010) found a significant difference between NEFA in serum calcium values > 8.5 mg/dL and < 8.5 mg/dL, this calculation is repeated in this study. In figure 13 the two serum calcium values of week 0 are compared with the average NEFA concentration of week 0 (mmol/liter). A significant difference is found (p = 0.019; p < 0.05; paired t-test). No significant correlation (r = -0.225; p = 0.181) between the changes in NEFA (NEFA wk 0 – wk -4) and serum calcium week 0 is found, but a significant difference between change of NEFA and the different serum calcium values is found (p < 0.01; figure 14).

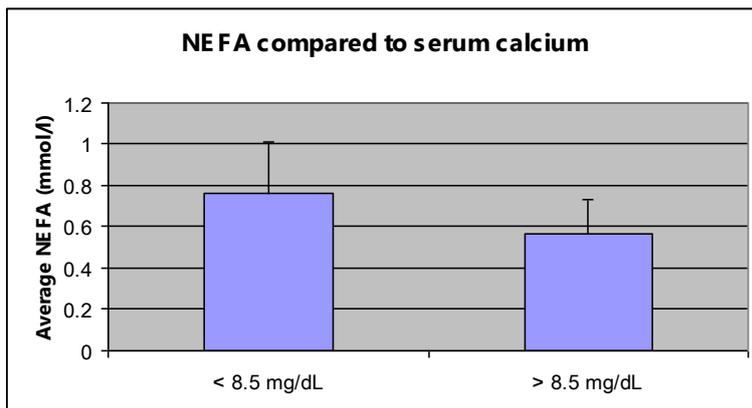


Figure 13: NEFA wk 0 compared to serum calcium wk 0 < 8.5 and > 8.5 mg/dL
 < 8.5 = 0.7639; > 8.5 = 0.5631; p < 0.05

* Error bars show the standard deviation

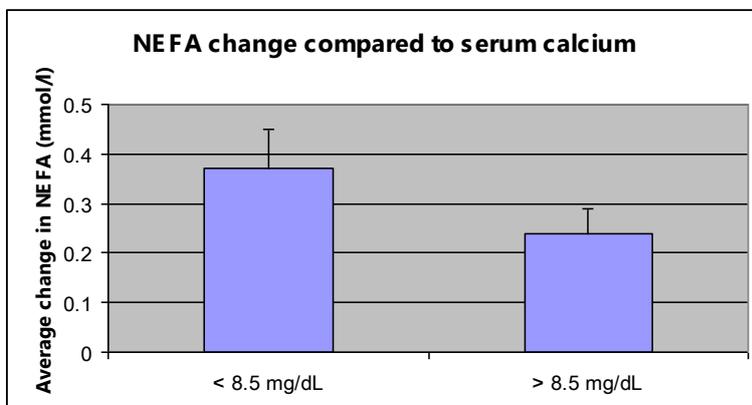


Figure 14: NEFA change (wk 0 – wk -4) compared to serum calcium wk 0 < 8.5 and > 8.5 mg/dL
 < 8.5 = 0.36; > 8.5 = 0.27; p < 0.01

* Error bars show the standard deviation

Discussion

Prevalence

In this research a serum calcium value < 8.5 mg/dL is classified as (sub)clinical hypocalcemia. None of the cows did actually show symptoms of milk fever.

The prevalence of a serum calcium value below 5.5 mg/dL in this study is 7.5%. The incidence of a serum calcium value between 8.5 and 5.5 mg/dL is 55%. This is comparable with other researches. Goff et al. (2008) and Sorenson et al. (2002) found a prevalence of clinical hypocalcemia in the USA of 5-10% and Thirunavakkarasu et al. (2010) found a prevalence of 13.7% in India. Compared to the study in India, the incidence of milk fever in Thailand is quite low. However this is based on a single research of a single place in both of the countries, so to say more about this, more researches are necessary.

Goff et al. (2008) and Sorenson et al. (2002) showed a prevalence of subclinical hypocalcemia of around 50% in third or more lactation cows and Horst et al. (2003) found a prevalence of 57.8% in third or more lactation cows. In this research eight cows showed subclinical hypocalcemia in this category, that leads to an incidence of 53.33% in third or more lactation cows. This incidence is not irregular of the incidences found by Horst et al., Goff et al. and Sorenson et al.

The prevalence of subclinical hypocalcemia of 55% in all lactation cows is quite high. Horst et al. (2003) reported a prevalence of subclinical hypocalcemia in first lactating cows of 25.3%. In second lactation cows they found a prevalence of 43.9%. In this research in first lactating cows a prevalence of 62.5% is found, which is very high (see below).

As mentioned above, none of the cows showed symptoms of milk fever, even the cows with a serum calcium < 5.5 did not show any symptoms. Probably if the serum calcium value decline below 5.5 mg/dL, cows adapt their metabolism and prevent the symptoms of milk fever.

Change in serum calcium values and body condition score

A significant change is seen in the serum calcium value of week 0. This is in agreement with the literature. Because of a calcium disappearance in the milk, the calcium requirement increases. The disappearance of calcium in the milk is faster than blood calcium can be replaced through mechanisms like intestinal calcium absorption and resorption of calcium from the bone (Kara et al. 2009). Because of this, a drop in calcium is seen in the week of parturition. After parturition this mechanisms take care for an increase in calcium again and this causes the significant increase in serum calcium after calving. Furthermore the calcium concentration in colostrum is higher compared to milk (Murray et al 2010, Ferneborg 2010).

A significant drop in BCS is seen between week -1 and week 4. This could be explained through the fact the cows produce a lot of milk after parturition and this requires a lot of energy. The levels of feed intake could not reach the requirements for supporting milk production in early lactation (Beever et al. 2006).

Body condition score

Another statement which has a high agreement is the fact that cows with a high body condition score (>3.75) at calving have an increased risk in developing milk fever (Thilising-Hansen et al. 2002). This is not being seen in this research. Cows that had a serum calcium value < 5.5 mg/dL had a average body condition score of 2.92 one week before calving and cows who did not suffer from (sub)clinical hypocalcemia had a average body condition score of 3.13 (one week before calving). This means that in this study cows with clinical hypocalcemia actually had a lower body condition score compared to cows with no

hypocalcemia, but not significant. Probably the lower body condition score can be explained due to less feed intake and so on less calcium intake in critical period (around calving).

There is some evidence that over-conditioned cows with a higher risk at milk fever are related to the milk production before. Higher producing cows have an increased risk at developing hypocalcemia (DeGaris et al. 2007). To research these statements, milk yields of the year before are necessary and those are not available in this study.

No relation exists in this study for body condition score and serum calcium. The body condition in this research was quite low, so maybe there is another optimal range in Thailand. But more likely is that there is not enough variation in body condition score to make a good comparison. Just six cows in this study showed a BCS (in week -1) above 3.75. There is not enough variation in body condition score to find a significant relationship between plasma calcium and body condition score.

Parity

In 1987 Schmidt already described milk fever as a disease that attacks cows seldom before third or fourth parturition (Murray et al. 2008). Other researches support the statement that older cows have a higher risk in developing milk fever (DeGaris et al. 2009, Reinhardt et al. 2002). This statement is described in many researches and in different countries, for example in Australia, England and the USA. In this research, the statement is not supported. Of the three cows that showed a serum calcium value below 5.5 mg/dL, two had lactation number 2. This is not in agreement with the researches of DeGaris et al. (2009) and Reinhardt et al. (2002). No significant difference is seen between serum calcium at calving date and lactation number. Also a chi square test of the number of cases of sub- and clinical hypocalcemia notified a p-value > 0.05 , so no significance.

The first explanation of a high incidence of a p-value between 8.5 and 5.5 mg/dL at heifers and first lactating cows could be that the values are most close to 8.5 mg/dL, but because of this grouping the cows are placed in the category between 5.5 and 8.5 mg/dL. To research this hypothesis, the p-values of heifers and first lactation cows are studied again. Two of the seven cows with lactation number 0 that showed a p-value between 8.5 and 5.5 mg/dL, showed a p-value between 8 and 8.5 mg/dL, which means they showed a p-value very close to 8.5 mg/dL. Most of the other heifers within this range (<8.5 , >5.5 mg/dL) showed a p-value around 7.5 mg/dL. In first lactating cows two cows with a p-value between 8.5 and 5.5 mg/dL showed a p-value of 8 mg/dL. The other cows of this category showed a p-value between 7.5 and 8 mg/dL. This means that most of the cows with lactation number 0 or 1 showed a p-value between 7.5 and 8.5 mg/dL, so in the upper range of this category. This could be one explanation of the high prevalence of subclinical hypocalcemia. However the difference between the cut-off values and the p-value is still 1 mg/dL.

Maybe the cows with lactation number two that showed a serum calcium value < 5.5 mg/dL had a higher milk production before. If they had a higher production they will lose more calcium in the milk. Just five cows include the group of lactation number two, so the two cows with the serum calcium value below 5.5 mg/dL have quite a big influence on the mean serum calcium value.

Another reason could be these two cows were suffering from a subclinical disease that is not detected. Perhaps this disease could have led to a reduced food intake, which could have led to less calcium intake in the critical period after calving.

As mentioned before, the cows with lactation number one also showed a relatively high prevalence of subclinical hypocalcemia compared to researches in other countries. Maybe the quality of the food plays a role in this phenomenon or the environmental stress factors, described in the introduction.

Urine pH

In a research of Goff et al. (2008) metabolic alkalosis is described as a predisposing factor for milk fever. Many countries described reducing DCAD (diet cation-anion difference) as an important factor to prevent milk fever. Goff et al. (2008; USA), DeGaris et al. (2009; Australia), Sorenson et al. (2002; Denmark), Wu et al. (2008; China) are all examples of researches who agree with the fact DCAD should be low before calving. In this research the urine pH is used to collect information about the cation-anion balance. With a high cations diet, the urine pH is generally above 8.2 (Goff et al. 2002). For reducing the pH to 6.2-6.8 (which is an optimal pH for controlling milk fever in Holstein Friesian cows) it is essential to add anions to the ration. If the pH decreases to 5-5.5, a metabolic acidosis is induced and the cow will suffer from a decline in dry matter intake (Goff et al. 2002). In 2008, Wu et al. proved a significant relationship between urinary pH and DCAD.

The cause of metabolic alkalosis is a diet that has more cation (Na^+ , K^+ , Ca^{2+} , Mg^{2+}) than anions (Cl^- , SO_4^{2-} , P^{3-}). Roughage contains relatively many cations. Straw and hay contains mainly a lot of potassium (2.16 and 2.31% DM) (Robinson et al. 2005). Concentrate contains usually less potassium; however Soya bean is a source which contains also a lot of potassium (Kolver 2000).

In this research no significant difference is seen between pH of the urine and average serum calcium at parturition. Also no correlation is found between pH and serum calcium value. The optimal range of the urine pH is described as 6.2-6.8, but in this research no cow had a pH between these values.

It is advised to use low DCAD in the transitional period to reduce milk fever. This leads to a decrease in pH and so on a reducing risk at milk fever. However in Thailand this method is not used. In the transitional period cows are not feed with a special diet which leads to a low DCAD. This does not mean the diet never is low in DCAD, but it does mean they do not pay attention to this strategy to prevent milk fever. In Thailand they feed the cow before the transitional period much roughage. In the transitional period they usually change the feed intake and they start to feed more concentrate. Generally, the cow has a low calcium intake before calving due to this feed management and is the low calcium intake the critical factor for reducing milk fever. A lot more researches describe feeding low calcium pre-calving to reduce the risk at milk fever (DeGaris et al. 2009). There has been suggested a period of 14 days before calving is necessary to prevent milk fever (Thilising-Hansen et al. 2002). In this study almost every cow has a transitional period of 14 days or longer. Because of low calcium intake, the parathyroid gland is stimulated before calving and there is a rapid bone calcium mobilization and production of 1.25-dihydroxyvitamin D (DeGaris et al. 2009). In Western countries it can be quite difficult to give a low calcium diet (Goff et al. 2008). That is why the DCAD is at these places a lot more important compared to the low calcium intake in the transitional period. In Thailand the low calcium intake is much more important compared to DCAD, because low calcium intake is possible to realise much easier here. Because of the absence of the method of DCAD, the lacking correlation between urine pH and serum calcium value can be explained.

Not all urine samples are taken at the right time (one week before calving) and to make sure this did not interfere with the results, a new correlation test between urine pH and blood calcium (at calving) is done, just with the samples that have a urine pH measurement at the right time. This also leads to no significant correlation between urine pH and serum calcium value.

Feed intake

A significant correlation is seen between intake of concentrate and serum calcium value at week 0. Also a significant correlation is present between intake of roughage and serum

calcium value at week 0. The linear correlation coefficient between intake of concentrate and blood calcium value at calving is 0.636 ($p < 0.05$), so there is a positive correlation between these two values, but not very strong. The linear correlation coefficient between intake of roughage and blood calcium values is -0.786 ($p < 0.05$), which means there is quite a strong negative correlation between these two values.

Also the incidence of hypocalcemia is compared between different intakes of concentrate and roughage. Remarkable is that all three cases of milk fever occurred at an concentrate intake of less than 5 kg/day. No significant relationship is seen ($p = 0.099$) between the different intakes of concentrate. However the p-value is close to 0.05 and our sample size is quite small. Perhaps more samples would have lead to a significant difference. In the comparison of intake of roughage against incidence of hypocalcemia, a significant difference is measured between different groups. This means intake of roughage does have an effect at the serum calcium value of week 0.

It could be the correlation between intake of roughage and concentrate can be explained through the amount of calcium in the food. As described above, the feed system in Thailand usually change the amount of concentrate in the transitional period to a higher intake. This leads to a lower calcium intake. A positive correlation is seen between concentrate and blood calcium value, so if the intake of concentrate increases, the blood calcium value also increases. This is in agreement with the fact that the increase of intake of concentrate in the transition period reduces the risk at milk fever; probably because of a low calcium intake. A negative correlation is seen between intake of roughage and serum calcium value and also these findings agree with the method to change the intake of many roughage to more concentrate in the transition period. To be sure of these findings, the calcium value in the food should be measured. These correlations are measured in the transitional period and around parturition it is important to feed high calcium. If they feed also low concentrate around parturition, this will increase the risk on milk fever.

Another explanation could be the difference in amount of anions between concentrate and roughage. As mentioned in the introduction, generally concentrate contains more anions compared to roughage. A change in the intake of concentrate and roughage could possibly lead to a change in pH and because of this a change in risk at developing milk fever. However, there is mentioned above that no correlation is seen between pH and serum calcium value. To check this thought, the intake of concentrate and roughage is compared to the urine pH; no significant correlation is calculated. Also these measurements support the suggestion DCAD is not important in Thailand and low calcium intake is the determined factor in risk of developing milk fever.

Supplements

In this study the supplement of mineral and vitamin is compared to each other. No significant difference between the four groups (no supplement; mineral supplement; vitamin supplement; supplement of both vitamin and mineral) is found. Actually this comparison could not be made; all kinds of mineral are mixed in one group and also in dose no separation is made. The same one could say about the vitamin supplementation. So, that no significance is found is not unexpected. Also the significant difference between 'no supplement' and 'mineral+vitamin' is probably not related to hypocalcemia, because most supplements are given just before calving. At week -4 the supplements are not given yet.

Supplementation of vitamin D

Thirteen cows were supplemented with vitamin D before calving. Of the thirteen cows that got vitamin D supplemented before calving, five cows showed a serum calcium value < 8.5 mg/dL. Twenty-eight cows did not get vitamin D before calving and twenty of them showed a

serum calcium value < 8.5 mg/dL. The difference in prevalence between these two groups is significant. In agreement with several articles, cows with a vitamin D supplementation before calving had less chance to develop milk fever. Already in 1943, Campbell and Turner said vitamin D could be used to buffer the demands for calcium and increased activity of the parathyroid glands and in 1977 B.F. Sansom described the use of vitamin D metabolites for preventing milk fever. The fact that supplementation of vitamin D will reduce the chance of getting hypocalcemia is proven many times and so is in this research (Goff et al. 2007, Murray et al. 2010, DeGaris et al. 2009, Thilsing-Hansen et al. 2002). In this research most cows are treated with AD₃E. This contains vitamin D₃, which is in the active form vitamin 1.25-dihydroxyvitamin.

Though, in Thailand there is a lot of solar radiation, that could lead to the production of vitamin D. This leads to the thought that cows in Thailand should be able to produce enough vitamin D under the influence of the sun, without any supplements. UV light cleaves 7-dehydrocholesterol in the skin and at this way it cause the production of pre-vitamin D₃. At body temperature this pre-vitamin D₃ isomerizes spontaneously into vitamin D₃ (Hymoller et al. 2010). Still, a significant difference between cows who were supplemented with vitamin D and cows that did not get any supplementation of vitamin D is seen. An explanation could be that the cows in Thailand have not so much possibility to graze. Most of the cows are standing in sheds all day, which means there is a roof above them and they are not exposed to the UV light of the sun.

Another important notification is the average calcium values of both groups (supplementation with vitamin D against no supplementation of vitamin D). The average calcium value of the group with cows that were supplemented with vitamin D is 8.51 mg/dL. The average calcium value of the cows who were not supplemented with vitamin D is 8.21 mg/dL. This means there is a very small difference between these two groups and probably because of grouping, a small difference in calcium had lead to a significant difference.

The amount of supplemented vitamin D in this study is not discussed. For better results one should notice the dose of vitamin that is supplemented and the days the supplement is given. Supplemental vitamin D can only work when there is enough calcium in the feed. Probably the supplemental vitamin D is offered in Thailand because of vitamin D deficiency instead of reducing milk fever, which means the doses are not turned to prevent milk fever.

Length of dry period

Cows with a long dry period before calving have a long period without lactation, which gives a predisposition to becoming fat. This can lead to a higher risk of milk fever, because of a high BCS leads to a higher risk for milk fever (Thilsing-Hansen et al. 2002). In this research a weak positive relation (which is not significant) is found between BCS and the length of the dry period. As described earlier also no correlation is found between BCS and serum calcium.

A longer dry period can result itself in a more stable calcium homeostasis. In 1992 a post parturient calcium drop in blood of cows is compared with a mean dry period of 4 days and 8 weeks. They found a less profound drop in the cows with a mean dry period of 4 days. However side-effects are present like a reduction in milk production. In 1991 there is noticed a significant drop in milk production when shortening the dry period from 7 to 4 weeks (Thilsing-Hansen et al. 2002). In 1997 is observed that dairy cows that did not have a dry period produced 5.6 kg less milk per day compared to cows with a dry period of 60 days (Bachman et al. 2003). In this research no significant difference is seen in prevalence of sub- and clinical hypocalcemia compared with the length of the dry period. A very weak negative relation can be seen between blood calcium value and length of dry period, but this is almost negligible and not significant. However in this research there was not that much variance in length of dry period compared to the researches mentioned above.

Calcium intake before calving

There are many researches about the effect of high or low calcium intake before calving at the development of milk fever. Both a high and a low intake of calcium are described as a reduction in the risk of milk fever (Thilising-Hansen et al. 2002, DeGaris et al. 2009). An effect of diets with a low difference in cations en anions could lower de available bone calcium and it has been suggested feeding high calcium intake before calving could reduce this effect. Lean et al. (2006) described that short exposure to high calcium 12-18 days before calving greatly increase the risk of milk fever. Probably a low calcium (less than 25 gram a day) before calving and a high calcium post-calving should result in the best effect. A lot more researches describe feeding low calcium pre-calving to reduce the risk at milk fever (DeGaris et al. 2009). There has been suggested a period of 14 days before calving is necessary to prevent milk fever (Thilising-Hansen et al. 2002).

In this study the calcium intake should be measured through food analysis. Unfortunately, this could not be done within time, so in this study that results could not be used. Without food analysis it is almost impossible to compare the calcium intake before calving and that is why it is not done in this research. It is just possible to look at the mineral supplement, but this is most of the time given just before calving. Furthermore this is a way to increase the calcium intake and not to reduce the calcium intake during the dry period. However it is a very important factor in reducing the risk of milk fever, so it definitely should be done in a bigger research. In a research in Denmark, Sorenson et al. (2002) have collected the most relevant options to control and prevent milk fever due to experts. Also in this research 'low calcium in dry-cow ration' is mentioned first.

Generally, concentrate contains less calcium compared to roughage (Kolver et al. 2000). However this depends on the kind of roughage and concentrate. A positive correlation between intake of concentrate and serum calcium values at week 0 is seen, so maybe this could partly suggest less calcium intake has a favorable effect at reducing the risk at milk fever. As mentioned above data about feed analysis are necessary to say more about this and be sure.

Milk yield

Ostergaard et al. (2002) did a study about the influence of calcium status at calving at the milk yield. They found no relation between milk yield and the calcium status at calving. However they did not use the cows' own milk yield as reference, which is suggested for a next research. Moreover this has been done by Rajala-Schultz et al. They did not found a milk-reducing effect, but the results were saying that cows with milk fever actually were higher yielding cows. It is very difficult to research the potential milk reducing effects of milk fever, because cows who are suffering from milk fever seem to be higher yielding cows (Rajala-Schultz et al. 1999). In the research of Rajala-Schultz et al. the milk yield of 8 weeks after calving is used as reference, but it could be that the cows were still suffering from other diseases that reduced the milk yield.

In this research just the data about milk yield are available of the first four weeks after calving. Not all data are complete and no milk yield of other years is available. There is no significant difference between milk yield of cows with a serum calcium > 8.5 mg/dL and cows with serum calcium between 5.5 and 8.5 mg/dL. This is in agreement with the research of Ostergaard et al.; they did not use the cows' own reference as well. Also there are not many cows in this comparison and that could interfere with the results and possibly that is a reason why the lines are crossing at week 4. Not every week has the same number of cow that is used for the average milk yield and also that could be a reason in week 4 the contrary is seen of week 1, 2 and 3.

To compare better, like Ostergaard et al. said, it is possibly better to use the milk yield of the cows' own milk yield as reference. Maybe it is better to use the reference of the cows' milk yield of the year before the research instead of the milk yield of 8 weeks after calving, but this will be very hard to manage. This would just be possible with an average lactation number, because in young cows and cows with a high lactation number the milk production will be less.

Hypercalcemia

In this study many cows showed a serum calcium value above 10 mg/dL, which means they have a hypercalcemia due to the reference values of Horst et al (2003) and Goff et al (2008). At week 0, seven cows showed a serum calcium value > 10 mg/dL. These values vary between 10.1 and 13 mg/dL and the precise values are 10.1; 10.4; 10.5; 10.8; 11.4; 11.5 and 13 mg/dL. So, most of the values are rather close to 10 mg/dL, but there are also values that are not that close to the cut-off value.

A high serum calcium value is possibly due to supplementation of vitamin D. Vitamin D is already described as a method for reducing the risk at milk fever. In 1965, Capen et al. described the effect of supplementation of vitamin D at possible hypercalcemia. A supplementation of vitamin D for 3 days resulted in a serum calcium increase from 9.1 mg/dL to 10.6 mg/dL. The supplementation of vitamin D for 5 days resulted in an increase in serum calcium from 10.9 mg/dL to 12.2 mg/dL. After 7-10 days of vitamin D supplementation the serum calcium value increased to 13.5 mg/dL and after 14 days the value increased above 13.9 mg/dL.

There are many other reasons for a high serum calcium value, like malignancies, but that is very unlikely to be the case in these cows. These cows did not show any symptoms of disorders and probably these high calcium values do not have a real impact on the cows' health. Vitamin D supplementation could play a role, however no significant correlation is seen between average serum calcium values at week 0 and vitamin D supplementation. But in this research the dose of given vitamin D is not known and that is very important for this comparison. The comparison of number of cases of hypocalcemia between vitamin D supplementation and no supplementation did show a significant difference. The reference values calculated by Horst et al. (2003) and Goff et al. (2008) are averages and probably the higher serum calcium values in this study are not very important and irregular.

Related diseases

A relation between hypocalcemia and retained placenta is described. Cows with milk fever are more likely to have a retained placenta than cows with a normal calcium value (Kara et al. 2009, Cagdaz et al. 2008). In this research we have seen one cow with a retained placenta and this cow showed a serum calcium value < 5.5 mg/dL. This is in agreement with the research of Kara et al. and Cagdaz et al..

Milk fever also predisposes for mastitis. In this study there was one cow who suffered of mastitis, but this cow did not suffer from milk fever. However this cow had a calcium value of 7.6 at the calving date, so she had a subclinical hypocalcemia. This is in agreement with Horst et al. 2003, DeGaris et al. 2009 and Rukkamsuk et al. 2010.

Both relationships are not significant due to low number of cows.

Comparison between hypocalcemia and negative energy balance (NEB)

Reinhardt et al. (2010) found that cows with serum calcium > 2.0 mM had a lower serum NEFA concentration than cows with serum calcium < 2.0 mM. This phenomenon indicates that cows with calcium within the reference values were in better energy balance than the subclinical hypocalcemic cows. In this research the similar difference is seen. A significant

difference in NEFA concentration between serum calcium < 8.5 and > 8.5 mg/dL is calculated. This supports the statement of Reinhardt et al. that cows with calcium > 8.5 mg/dL are in better energy balance than the subclinical hypocalcemic cows. The positive linear correlation that is found between calcium week 0 and NEFA week 0 also supports this statement. An explanation for this phenomenon could be that the cow eats less in the critical period (around parturition) due to the negative energy balance. This leads to less calcium intake and because of that a higher risk at milk fever. Or on the contrary: because of hypocalcemia, the cow eats less in the critical period and this leads to negative energy balance. Between serum calcium value and the difference in NEFA's (wk 0- wk -4) also a significant difference is found.

A significant positive linear correlation is calculated between serum calcium of week -4 and glucose (as well as from week 4 as from week 0). In a cow suffering from NEB, lowered blood glucose will be seen (Rukkwamsuk et al. 2006). If the statement is true that serum calcium concentration has an association with NEB, it should be that a higher serum calcium value is attended with a better energy balance, and so on a higher glucose concentration in blood. In this study a positive linear correlation is found, which supports the statement above. A higher serum calcium concentration is associated with a higher glucose concentration in blood. However no significant relation is seen between the serum calcium value of week 0 and glucose. The serum calcium value of week 0, is the most important value for indicating milk fever. Milk fever usually occurs within 72 hours after calving (Merck Veterinary Manual, 2008).

Because of the significant difference in NEFA's and change in NEFA's between serum calcium < 8.5 and > 8.5 mg/dL and the significant correlation between NEFA and serum calcium at week 0, it is plausible to say there is a relationship between NEB and milk fever.

Points of improvement

This research is part of a bigger research and this result in fewer samples in this part of the study. This was because of time and available (pregnant) cows. To make sure more results are significant, more samples should be used. Because of fewer samples, also cows in an early lactation stadium are used and also heifers are included in this study. As described in the introduction, milk fever occurs merely in older cows and normally it does not occur in cows with a lactation number below 3. This is partly a reason why just three cows developed a serum calcium value below 5.5 mg/dL in this study and no cow showed symptoms of milk fever.

The samples are not taken all at the right time. Some samples of one week before calving are missing, because the calving dates changes. Actually one should collect more than one time the sample of one week before calving, so the right sample can be used.

In this study it is quite difficult to examine the risk factors like vitamin and mineral supplementation. A lot of questionnaires do not say which mineral or vitamin supplement is given and also the amount is seldom known. In a next research these amounts should be known of all cows to make a better comparison.

Actually the food should be analyzed to determine calcium, sodium, potassium, magnesium, chloride and sulfur. With all these data, it is possible to determine risk factors like amount of these minerals. Furthermore one also need quantitative information on feed intake. In this research the measurements could not be done within time, so I do not have enough data to make a conclusion about these specific minerals.

The vet who was collecting the sample also determined the body condition score, which means different vets have determined the body condition score in this research. To improve the results, it should be better to have one person who determines all the body condition scores.

Conclusion

Milk fever is not a problem with a high appearance in Thailand. The incidence of serum calcium below 5.5 mg/dL is 7.5% and these cows did not even show symptoms of milk fever.

There is some difference between risk factors in western countries compared to Thailand. Some risk factors seem to be not important and other risk factors have another distribution in importance. The most relevant risk factor in Thailand is feeding low calcium in the transitional period. DCAD, which is another important method used to reduce the risk at milk fever, is in Thailand less important and the farmers do not pay attention to this method. Because of this, metabolic alkalosis is not an important risk factor for milk fever. To realize a low calcium intake in Western countries is much more difficult and that is why the DCAD is there the common method to prevent milk fever. That explains the risk factors in their articles differ from Thailand. Because of the importance of low calcium intake more research should be done about the calcium intake before calving due to food analysis.

A significant correlation is seen between intake of roughage and concentrate compared to serum calcium value of week 0. This possibly might indicate the current feed system, in which roughage changes in more concentrate intake in the transitional period, is a good working system that can be improved. A lot of difference exists in feeding patterns observed in these cows.

Another important method to reduce the risk at milk fever is the supplementation of Vitamin D. The intake of vitamin D enhances the absorption of calcium from the diet and tubular reabsorption of calcium. This is in agreement with other studies and other countries.

Risk factors like BCS and duration of dry period appear to be not very important for reducing the risk at milk fever in Thailand, but more research is necessary to say more about these risk factors.

The best is to focus on the most important factor in Thailand as low calcium intake. To do more research about the best way of this method to prevent milk fever will improve the results and improve the blood calcium concentration. Although the incidence of milk fever is low, many cows showed subclinical hypocalcemia, which also could lead to other diseases.

Acknowledgements

I want to thank all persons who have made this research possible: our room to stay, collecting the samples, going to the university to work and discuss about our paper, collecting serum and measuring the samples. All veterinarians of Nong Pho I want to thank for collecting samples together with us and collecting all the data. I am also very thankful to Dr. T. Rukkwamsuk and Dr. Wandee for their help and measuring the samples. Also the laboratory staff I want to thank we could use the centrifuge to collect the serum every time we wanted. Furthermore I am very grateful to all farmers who give us the opportunity to get the samples and data. Last, but not least, I would like to thank Dr. ing J.T. Schonewille for his help in writing this report and writing the research proposal.

References

- Avendano-Reyes, L., Fuquay, J.W., Moore, R.B., Liu, Z., Clark, B.L., Vierhout, C. (2009) Relationship between accumulated heat stress during the dry period, body condition score, and reproduction parameters of Holstein cows in tropical conditions. *Trop Anim Health Prod* (2010) **42**: 265-273
- Bachman, K.C., Schrairer, M.L. (2003) Invited review: Bovine studies on optimal lengths of dry periods. *J. Dairy. Sci.* **86**: 3027-3037
- Beever, D.E. (2006) The impact of controlled nutrition during the dry period on dairy cow health, fertility and performance. *Animal reproduction science* **96**: 212-226
- Capen, C.C., Young, D.M. (1965) Fine structural alterations in thyroid parafollicular cells of cows in response to experimental hypercalcemia induced by vitamin D. *Capen and Young*, **57**, 2
- Chantalakhana, C., Korpraditsakul, R., Skunmun, P., Poondusit, T. (1999) Environmental conditions and resource management in smallholder dairy farms in Thailand. II Effects of dairy wastes on water and soil. Asian, Kasetsart University, Bangkok
- DeGaris, P. J., Lean, I. J. (2009) Milk fever in dairy cows: A review of pathophysiology and control principles. *The Veterinary Journal* **176**, 58-69
- Ferneborg S. (2010) Calcium homeostasis at calving in cows milked prepartum. SLU: Swedish University of Agricultural Science, Animal nutrition and management
- Goff, I., Horst, R.L. (2003) Milk Fever control in the United States. *Acta vet. Scand, Suppl.* **97**, 145-147
- Goff, J.P., Horst, L. (1997) Physiological changes at parturition and their relationship to metabolic disorders. *Journal of Dairy Science* **80**, 1260-1268
- Goff, J. P. (2008) The monitoring, prevention and treatment of milk fever and subclinical hypocalcemia in dairy cows. *The Veterinary Journal* **176**, 50-57
- Horst, R. L., Goff, J. P., McCluskey, B. J. (2003) Prevalence of subclinical hypocalcemia in US dairy operations. *Journal of Dairy Science* **86** (Suppl. 1), 247
- Hutjens, M. (2003) An alternative to metabolic disorders; Looking at hypocalcemia. *Dairy decision column*, February 18
- Hymoller, L and Jensen, S.K. (2010) Vitamin D₃ synthesis in the entire skin surface of dairy cows despite hair coverage. *Journal of dairy science* **93**, 2025-2029
- Kara, C., Orman, A., Udum, D., Melih Yavuz, H., Kovanlikaya, A. (2009) Effects of calcium propionate by different numbers of applications in first wee postpartum of dairy cows on hypocalcemia, milk production and reproductive disorders. *Ital. J. Anim. Sci.* **8**, 259-270
- Kolver, E. (2000) Nutrition Guidelines for the high producing dairy cow. *Proceedings of the Ruakura Dairy Farmers' Conference*, **52**

Markvichitr, K., Tumwasorn, S., Thanindrathran, B. (1995) Losses due to health and fertility problems in purebred Holstein Friesian cows under Thai farmer condition. *Thai J. Agric. Sci*

Murray, R. D., Horsfield, J. E., McCromick, W. D., Williams, H. J., Ward, D. (2008) Historical and current perspectives on the treatment, control and pathogenesis of milk fever in dairy cattle. *Veterinary Record* **163**, 561-565

Ostergaard, S., Grohn, Y.T. (1999) Effects of diseases on test day milk yield and body weight of dairy cows from Danish research herds. *J. Dairy. Sci.* **82**, 1188-1201

Oostergaard, S., Larsen, T. (2000) Short communication: Associations between blood calcium status at calving and milk yield in dairy cows. *Journal of dairy science* **83**, No. 11

Sorensen, J. T., Ostergaard, S., Houe, H., Hindhede, J. (2002) Expert opinions of strategies for milk fever control. *Preventive Veterinary Medicine* **55**, 69-78

Parturient paresis. *The Merck Veterinary Manual*, 2008.

Reinhardt, T.A., Lippolis, J.D., McCluskey, B.J., Goff, J.P., Horst, R.L. (2010) Prevalence of subclinical hypocalcemia in dairy herds. *The veterinary journal*

Robinson, T.F., Roeder, B.L., Schaalje, G.B., Hammer, J.D., Burton, S., Christensen, M. (2005) Nitrogen balance and blood metabolites of alpaca (*Lama pacos*) fed three forages of different protein content. *Elsevier: Small ruminant research* **58**, 123-133

Rukkwamsuk, T., Homwong, N., Bumkhuntod, W., Rohitakane, P., Sukcharoen, R. (2006). Negative energy balance in periparturient dairy cows raised in small-holder farms in Kamphaengsaen district, Nakhon Pathom province. *Nat. Sci.* **40**, 1000-1004

Rukkwamsuk, T. (2010). A field study on negative energy balance in periparturient dairy cows kept in small-holder farms: Effect on milk production and reproduction. *Afr. J. Agr. Res.* (In press).

Rukkwamsuk, T., Kruip, T.A.M., Wensing, T. (1999). Relationship between overfeeding and overconditioning in the dry period and the problems of high producing dairy cows during the postparturient period. *Vet. Quarterly* **21**, 71-77

Sansom, B.F. (1978) The use of vitamin D metabolites and analogues for the prevention of milk fever in dairy cattle. *Veterinary science communications* **1**, 323-335

Thilsing-Hansen, T., Jorgensen, R.J., Ostergaard, S. (2002). Milk fever control principles: a review. *Acta vet. Scand* **43**, 1-19

Thirunavukkarasu, M., Kathiravan, G., Kalaikannan, A., Jebarani, W. (2010) Quantifying economic losses due to milk fever in dairy farms. *Agricultural economics research review* **23**, 77-81

Ukawa, H., Ando, M. (2002) Feed management and significance of storage of roughage for dry season in dairy farming. *Jircas.affrc.go.jp*

Wu, W.X., Liu, J.X., Xu, G.Z., Ye, J.A. (2008) Calcium homeostasis, acid-base balance, and health status in periparturient Holstein cows fed diets with low cation-anion difference. *Livestock science* **117**, 7-14

Attachments: Questionnaires

Sample No.: 1

Farm: Narong Luangkravi

Subdistrict: Photaram

Province: Rachaburi

No. of lactating cows: 16

Cow No.: Chompu

Lactation No.: 1

AI date: 3 May 2010

Dry date: 2 December 2010

Calving date: 15 February 2011

Transitional feed date (before calving): When decrease of milk production on dry period, the owner will increase the concentrate feed.

Type of feed (~1 week before calving)

Concentrate:

- 16% protein ~ 4 kg/day

Roughage:

- Grass: 6 kg/day
- Straw: 6 kg/day
- Silicate (pine-apple): 2 kg/day

Remarks: /

Mineral supplement before calving: yes

Mineral block: no

Vitamin: yes, KT (from radio advertisement) + Catosal, 20 ml

Has cow got a milk fever before? No

Sample No.: 5

Farm: Theera

Subdistrict: Bangpae

Province: Ratchaburi

No. of lactating cows: 24

Cow No.: 4

Lactation No.: 5

AI date: 10 May 2010

Dry date: 23 Nov 2010

Calving date: 17 February 2011

Transitional feed date (before calving): 1 month

Type of feed (~1 week before calving)

Concentrate:

- NP 20: ~ 0.5*2 kg/day
- Cassava residue: ~ 5*2 kg/day

Roughage:

- Baby corn: ~ 5*2 kg/day
- Hay: ~ 4 kg/day

Remarks: /

Mineral supplement before calving: yes, Sotext (1 table spoon)

Mineral block: no

Vitamin: yes, AD₃E

Has cow got a milk fever before? No

Sample No.: 6

Farm: Vichai

Subdistrict: Tamakha

Province: Kanchanaburi

No. of lactating cows: 15

Cow No.: Num Kang

Lactation No.: 4

AI date: 18 May 2010

Dry date: 18 January 2011

Calving date: 02 March 2011

Transitional feed date (before calving): after dry off for 2 weeks

Type of feed (~1 week before calving)

Concentrate:

- NP (16% ep): ~ 3-4 kg/day

Roughage:

- Napier grass: ~ 30 kg/day

Remarks: pangola grass

Mineral supplement before calving: yes

Mineral block: no

Vitamin: yes, Catasal ~ 20 ml + Vit. ADE ~ 15 ml (both 15 days before calving)

Has cow got a milk fever before? Yes, 6 months ago (2 cows were died)

Sample No: 7

Farm: Vichai

Subdistrict: Tamakha

Province: Kanchanaburi

No. of lactating cows: 15

Cow No.: Khao phong

Lactation No.: 3

AI date: 9 May 2010

Dry date: 22 November 2010

Calving date: 6 February 2011

Transitional feed date (before calving): after dry off for 2 weeks

Type of feed (~1 week before calving)

Concentrate:

- NP (16% ep): ~ 3-4 kg/day

Roughage:

- Napier grass: ~ 30 kg/day

Remarks: pangola grass

Mineral supplement before calving: yes

Mineral block: no

Vitamin: yes, Catasal ~ 20 ml + Vit. ADE ~ 15 ml (both 15 days before calving)

Has cow got a milk fever before? Yes, 6months ago (2 cows were died)

Sample No: 8

Farm: Vichai

Subdistrict: Tamakha

Province: Kanchanaburi

No. of lactating cows: 15

Cow No.: Kra-tae

Lactation No.: 5

AI date: 15 May 2010

Dry date: 18 October 2010

Calving date: 02 March 2011

Transitional feed date (before calving): after dry off for 2 weeks

Type of feed (~1 week before calving)

Concentrate:

- NP (16% ep) ~ 3-4 kg/day

Roughage:

- Napier grass ~ 30 kg/day

Remarks: pangola grass

Mineral supplement before calving: Yes

Mineral block: no

Vitamin: yes, Catasal ~ 20 ml + Vit. ADE ~ 15 ml (both 15 days before calving)

Has cow got a milk fever before? Yes, 6months ago (2 cows were died)

Sample no.: 9

Farm: Wisoot

Sub district: Potaram

Province: Ratchaburi

No. of lactating cows: 11

Cow No.: Wandee

Lactation No.: heifer

AI date: 9 May 2010

Dry date: -

Calving date: 9 February 2011

Transitional feed date (before calving): 2 months before calving

Type of feed (~1 week before calving):

Concentrate:

- Powder: 3 kg/d
- Concentrate: 3 kg/d

Roughage:

- Rice straw: 10 kg/d

Remarks: cow eats concentrate about 2 kg/day and increase to 4 kg/day 2 months before calving.

Mineral supplement before calving: no

Mineral block: yes, SK Best Lick

Vitamin: yes, injectavit + catosol

Has cow got a milk fever before? No

Sample no.: 10

Farm: Wisoot

Sub district: Potaram

Province: Ratchaburi

No. of lactating cows: 11

Cow No.: Petch

Lactation No.: 2

AI date: 1 May 2010

Dry date: 1 December 2010

Calving date: 1 February 2011

Transitional feed date (before calving): 1 month before calving

Type of feed (~1 week before calving):

Concentrate:

- Concentrate: 3 kg/d
- Powder: 3 kg/d

Roughage:

- Rice straw: 10 kg/d

Remarks: cow eat concentrate about 2 kg/day when start dry period and increase to 6 kg/day when 1 month before calving.

Mineral supplement before calving: no

Mineral block: yes, SK Best Lick

Vitamin: yes, injectavit + catosol

Has cow got a milk fever before? No

Sample no.: 11

Farm: Piw-Ngam

Sub district: Potaram

Province: Ratchaburi

No. of lactating cows: -

Cow No.: Kaem

Lactation No.: heifer

AI date: 23 April 2010

Dry date: -

Calving date: 30 January 2011

Transitional feed date (before calving): 3 weeks before calving

Type of feed (~1 week before calving):

Concentrate:

- Concentrate: 4 kg/d
- Pine apple powder: 4 kg/d

Roughage:

- Rice straw: 8 kg/d

Remarks: eat concentrate since yearling to heifer about 1 kg/day and increase to 4 kg/day 3 weeks before calving.

Mineral supplement before calving: no

Mineral block: no

Vitamin: no

Has cow got a milk fever before? No

Sample no.: 12

Farm: Piw-Ngam

Sub district: Potaram

Province: Ratchaburi

No. of lactating cows: -

Cow No.: Phrae

Lactation No.: 1

AI date: 22 April 2010

Dry date: 27 October 2010

Calving date: 26 January 2011

Transitional feed date (before calving): 3 weeks before calving

Type of feed (~1 week before calving):

Concentrate:

- Concentrate: 4 kg/d
- Pine apple powder: 4 kg/d

Roughage:

- Rice straw: 8 kg/d

Remarks: eat concentrate about 1 kg/day since start dry period and increase to 3 kg/day when 3 weeks before calving.

Mineral supplement before calving: no

Mineral block: no

Vitamin: yes, AD₃E

Has cow got a milk fever before? No

Sample no.: 13

Farm: Sumran 1

Sub district: Potaram

Province: Ratchaburi

No. of lactating cows: 18

Cow No.: 70-472957 (1)

Lactation No.: 3

AI date: 14 May 2010

Dry date: 5 December 2010

Calving date: 20 February 2011

Transitional feed date (before calving): 5 January 2011

Type of feed (~1 week before calving): 1 month before calving

Concentrate:

- Concentrate pure pride: 2 kg/d
- Concentrate Nhong Pho: 2 kg/d

Roughage:

- Corn: 10 kg/d
- Rice straw: 3 kg/d

Remarks: cow eats concentrate about 1 kg/day since 1 month before calving.

Mineral supplement before calving: yes, about 50 g/day (no brand)

Mineral block: no

Vitamin: no

Has cow got a milk fever before? No

Sample no.: 14

Farm: Sumran 1

Sub district: Potaram

Province: Ratchaburi

No. of lactating cows: 18

Cow No.: Ree (1)

Lactation No.: heifer

AI date: 17 May 2010

Dry date: -

Calving date: 20 February 2011

Transitional feed date (before calving): -

Type of feed (~1 week before calving):

Concentrate:

- Concentrate pure pride: 2 kg/d
- Concentrate Nong Pho: 2 kg/d

Roughage:

- Corn: 10 kg/d
- Rice straw: 3 kg/d

Remarks: cow eats concentrate about 1 kg/day since yearling.

Mineral supplement before calving: yes, 50 g/day (no brand)

Mineral block: no

Vitamin: no

Has cow got a milk fever before? No

Sample no.: 18

Farm: Outumporn

Sub district: Potaram

Province: Ratchaburi

No. of lactating cows: 27

Cow No.: 15

Lactation No.: 1

AI date: 25 May 2010

Dry date: 20 December 2010

Calving date: 27 February 2011

Transitional feed date (before calving): 2 weeks before calving

Type of feed (~1 week before calving):

Concentrate:

- Concentrate 16% protein: 3 kg/d

Roughage:

- Grass: 10 kg/d
- Corn: 8 kg/d

Remarks: when dry period, cow eat concentrate about 2 kg/day and increase to 4 kg/day 2 weeks before calving.

Mineral supplement before calving: no

Mineral block: no

Vitamin: no

Has cow got a milk fever before? No

Sample no.: 20

Farm: Sumran 2

Sub district: Potaram

Province: Ratchaburi

No. of lactating cows: 10

Cow No.: Lukesow

Lactation No.: 1

AI date: 15 May 2010

Dry date: 11 December 2010

Calving date: 25 February 2011

Transitional feed date (before calving): 2 weeks before calving

Type of feed (~1 week before calving):

Concentrate:

- Concentrate: 8 kg/d

Roughage:

- Straw: 2.5 kg/d
- Grass: 10 kg/d
- Silate: 8 kg/d

Remarks: when dry period, cow eats concentrate about 2 kg/day and increase to 4 kg/day 2 weeks before calving.

Mineral supplement before calving: no

Mineral block: yes, KMZ

Vitamin: no

Has cow got a milk fever before? No

Sample no.: 22

Farm: Montree

Sub district: Potaram

Province: Ratchaburi

No. of lactating cows: 28

Cow No.: 10

Lactation No.: 1

AI date: 10 May 2010

Dry date: 15 November 2010

Calving date: 14 February 2011

Transitional feed date (before calving): -

Type of feed (~1 week before calving):

Concentrate:

- Concentrate: 1 kg/d

Roughage:

- Grass: 20 kg/d
- Rice straw: 3 kg/d
- Corn peel: 2 kg/d

Remarks: -

Mineral supplement before calving: yes, KT super selenium plus ½ tablespoon with ½ table spoon salt

Mineral block: no

Vitamin: no

Has cow got a milk fever before? No

Sample no.: 25

Farm: Wan-Pen

Sub district: Ban-Pong

Province: Ratchaburi

No. of lactating cows: 35

Cow No.: Num-Kang

Lactation No.: 1

AI date: 14 May 2010

Dry date: 30 November 2010

Calving date: 20 February 2011

Transitional feed date (before calving): 1 month before calving

Type of feed (~1 week before calving):

Concentrate:

- Concentrate: 8 kg/d

Roughage:

- Straw: 8 kg/d
- Looktao: 5 kg/d

Remarks: when dry period, cow eat concentrate about 2 kg/day and increase to 4 kg/day 1 month before calving.

Mineral supplement before calving: yes, about 1 table spoon BIO-MIX

Mineral block: no

Vitamin: yes, phosphonotonic 20% + Fercobsang 12

Has cow got a milk fever before? No

Sample no.: 26

Farm: Wan-Pen

Sub district: Ban-Pong

Province: Ratchaburi

No. of lactating cows: 35

Cow No.: Yod-num

Lactation No.: 1

AI date: 14 May 2010

Dry date: 30 November 2010

Calving date: 16 February 2011

Transitional feed date (before calving): 1 month before calving

Type of feed (~1 week before calving):

Concentrate:

- Concentrate: 8 kg/d

Roughage:

- Straw: 8 kg/d
- Looktao: 5 kg/d

Remarks: when dry period, cow eat concentrate 2 kg/day and increase to 4 kg/day 1 month before calving.

Mineral supplement before calving: yes, about 1 table spoon BIO-MIX

Mineral block: no

Vitamin: yes, Fercobsang + phosponontonic

Has cow got a milk fever before? No

Sample no.: 27

Farm: Wan-Pen

Sub district: Ban-Pong

Province: Ratchaburi

No. of lactating cows: 35

Cow No.: Ying

Lactation No.: 3

AI date: 5 April 2010

Dry date: 25 October 2010

Calving date: 27 January 2011

Transitional feed date (before calving): 1 month before calving

Type of feed (~1 week before calving):

Concentrate:

- Concentrate: 8 kg/d

Roughage:

- Straw: 8 kg/d
- Looktao (made of pineapple): 5 kg/d

Remarks: when dry period, cow eat concentrate about 2 kg/day and increase to 4 kg/day 1 month before calving.

Mineral supplement before calving: yes, about 1 table spoon BIO-MIX

Mineral block: no

Vitamin: yes, Fercobsang + phosphonotonic

Has cow got a milk fever before? No

Sample no.: 28

Farm: Wan-Pen

Sub district: Ban-Pong

Province: Ratchaburi

No. of lactating cows: 35

Cow No.: Flower

Lactation No.: heifer

AI date: 13 May 2010

Dry date: -

Calving date: 5 February 2011

Transitional feed date (before calving): 2 months before calving

Type of feed (~1 week before calving):

Concentrate:

- Concentrate: 8 kg/d

Roughage:

- Straw: 8 kg/d
- Looktao: 5 kg/d

Remarks: heifer eats concentrate about 2 kg/day since yearling and increase to 4 kg/day 2 months before calving.

Mineral supplement before calving: no

Mineral block: no

Vitamin: yes, Phosphonontonic + Fercobsang

Has cow got a milk fever before? No

Sample No.: 29

Farm: Suchada

Subdistrict: Tamaka

Province: Kanchanaburi

No. of lactating cows: 14

Cow No.: Jun

Lactation No.: 5

AI date: 01 May 2010

Dry date: 24 November 2010

Calving date: 12 February 2011

Transitional feed date (before calving): 3 weeks after dry off, 2 kg of 20% ep (NP)/day

Type of feed (~1 week before calving)

Concentrate:

- 20% ep (NP): ~ 4 kg/day

Roughage:

- Bawa grass: ~ 32 kg/day
- Rice straw ~ 5 kg/day

Remarks: -

Mineral supplement before calving: yes, mineral powder (super selenium)

Mineral block: yes

Vitamin: yes, catasal 14 days before calving (phosphorour + vit B12 supplement)

Has cow got a milk fever before? Yes, 6 years ago

Sample No.: 30

Farm: Khui

Subdistrict: Tamakha

Province: Kanchanaburi

No. of lactating cows: 34

Cow No.: Kularb

Lactation No.: 6

AI date: 7 May 2010

Dry date: 3 December 2010

Calving date: 13 February 2011

Transitional feed date (before calving): no concentrate in dry off period until 1 week before calving, then increase to 1 kg

Type of feed (~1 week before calving)

Concentrate:

- NP (20% ep): ~ 1 kg/day

Roughage:

- Corn stalk: ~ 10kg/day
- (Corn stalk ~ 20 kg/day)
- Silage (corn stalk, pine apple peel, cassava peel) ~ 15 kg/day

Remarks: /

Mineral supplement before calving: yes

Mineral block: yes

Vitamin: yes, catosal

Has cow got a milk fever before? Yes, 1 year ago

Sample No.: 31

Farm: Somnuke

Subdistrict: Tamakha

Province: Kanchanaburi

No. of lactating cows: 10

Cow No.: Dok-Oy

Lactation No.: 2

AI date: 3 May 2010

Dry date: 29 November 2010

Calving date: 9 February 2011

Transitional feed date (before calving): After dry off feed asith 16% ep (NP) ~ 10,5 kg/day. One month before calving, 16% ep (NP) ~ 4,5 kg. One week before calving 0,5 kg of 16% ep (NP).

Type of feed (~1 week before calving)

Concentrate:

- 16% ep: ~ 0,5 kg/day

Roughage:

- Corn stalk: ~ 25 kg/day or napier grass (if corn cannot harvest)
- Rice straw: ~ 3 kg/day

Remarks: -

Mineral supplement before calving: Yes

Mineral block: No

Vitamin: yes, AD₃E 2 weeks before calving + vit.B12

Has cow got a milk fever before? No

Follow up disease outbreak:

Disease: retent foetal membrane, Date: 10 February 2011

Sample No.: 34

Farm: Somkiat

Subdistrict: Tumkang

Province: Kanchanaburi

No. of lactating cows: 24

Cow No.: Praew

Lactation No.: 5

AI date: 8 May 2010

Dry date: 8 November 2010

Calving date: 7 February 2011

Transitional feed date (before calving): 15 days before calving give 20% ep (NP) ~ 2 kg/day + mineral supplement (selenium plus, 0.02%)

Type of feed (~1 week before calving)

Concentrate:

- 20% ep (NP): ~ 2 kg/day
- Soy bean cake (byproduct from tofu production): ~ 4 kg/day

Roughage:

- Corn stalk: ~ 35-40 kg/day
- Rice straw: ~ 1 kg/day

Remarks: /

Mineral supplement before calving: yes, mineral powder, 15 days before calving

Mineral block: no

Vitamin: no

Has cow got a milk fever before? No

Sample No.: 36

Farm: Sank-petch

Subdistrict: Tamakha

Province: Kanchanaburi

No. of lactating cows: 5

Cow No.: Lay

Lactation No.: 2

AI date: 6 May 2010

Dry date: 2 December 2010

Calving date: 14 February 2011

Transitional feed date (before calving): 1 month after dry off, 1 kg/day. Before calving 2,5 kg/day

Type of feed (~1 week before calving)

Concentrate:

- 20% ep (NP): ~ 2,5 kg/day

Roughage:

- Corn stalk: ~ 70 kg/day

Remarks: /

Mineral supplement before calving: yes

Mineral block: yes

Vitamin: yes, powder -> goto 1000 (source of vitamin B complex)

Has cow got a milk fever before? No

Sample no.: 42

Farm: Sukit

Sub district: Ban-Pong

Province: Ratchaburi

No. of lactating cows: 43

Cow No.: Kaew

Lactation No.: heifer

AI date: 13 May 2010

Dry date: -

Calving date: 11 February 2011

Transitional feed date (before calving): 10 days before calving

Type of feed (~1 week before calving):

Concentrate:

- Ethanol residual: 13 kg/d
- Pine apple peel: 13 kg/d
- Concentrate: 1 kg/d

Roughage:

- Grass: 4 kg/d
- Rice straw: 3 kg/d

Remarks: cow eats concentrate about 2 kg/d (14% protein) and 10 days before calving, cow eats concentrate 18% prot 2 kg/d.

Mineral supplement before calving: yes, Premix 60 g/d

Mineral block: no

Vitamin: yes, AD₃E

Has cow got a milk fever before? No

Sample no.: 43
Farm: Sukit
Sub district: Ban Pong
Province: Ratchaburi
No. of lactating cows: 43

Cow No.: Luke Soy
Lactation No.: heifer
AI date: 30 May 2010
Dry date: -
Calving date: 1 March 2011

Transitional feed date (before calving): 10 days before calving

Type of feed (~1 week before calving):

Concentrate:

- Ethanol residual: 13 kg/d
- Pine apple peel: 13 kg/d
- Concentrate: 1 kg/d

Roughage:

- Grass: 4 kg/d
- Rice straw: 3 kg/d

Remarks: cow eats concentrate about 2 kg/d (14% protein) and 10 days before calving, cow eats concentrate 18% protein 2 kg/d.

Mineral supplement before calving: yes, Premix 60 g/d

Mineral block:

Vitamin: yes, AD₃E

Has cow got a milk fever before? No

Sample no.: 46

Farm: Wan-Pen

Sub district: -

Province: Ratchaburi

No. of lactating cows: 25

Cow No.: Tua-Lek

Lactation No.: 4

AI date: 26 May 2010

Dry date: 20 November 2010

Calving date: 26 February 2011

Transitional feed date (before calving): 1 month before calving

Type of feed (~1 week before calving):

Concentrate:

- Concentrate: 8 kg/d

Roughage:

- Straw: 8 kg/d
- Looktao: 5 kg/d

Remarks: when dry period, cow eat concentrate about 2 kg/d and increase to 4 kg/d 1 month before calving.

Mineral supplement before calving: yes, BIO-MIX about 1 table spoon

Mineral block: no

Vitamin: yes, Fercobsang + phosphonontonic

Has cow got a milk fever before? No

Sample no.: 47

Farm: Sinsumrit

Sub district: Bangphae

Province: Ratchaburi

No. of lactating cows: 21

Cow No.: Yhee

Lactation No.: 3

AI date: 2 June 2010

Dry date: 1 November 2010

Calving date: 15 March

Transitional feed date (before calving): none

Type of feed (~1 week before calving):

Concentrate:

- Concentrate: 3 kg/d

Roughage:

- Chopped corn: 10 kg/d
- Straw: 4 kg/d
- Cassava residue: 4 kg/d

Remarks: concentrate 1 kg/d with straw until calve

Mineral supplement before calving: yes, sodium carbonate + dicalcium + salt

Mineral block: no

Vitamin: no

Has cow got a milk fever before? No

Sample no.: 48

Farm: Sirijun

Sub district: Meaung

Province: Nakhon Pathom

No. of lactating cows: 31

Cow No.: F78

Lactation No.: 1

AI date: 4 June 2010

Dry date: 20 April 2010

Calving date: 04 March 2011

Transitional feed date (before calving): yes

Type of feed (~1 week before calving):

Concentrate:

- Concentrate: 2 kg/d

Roughage:

- Straw: 10 kg/d
- Soybean byproduct: 2 kg/d
- Kak-mun: 4 kg/d

Remarks: -

Mineral supplement before calving: no

Mineral block: no

Vitamin: no

Has cow got a milk fever before? No

Sample no.: 49

Farm: Sirijun

Sub district: Meaung

Province: Nakhon Pathom

No. of lactating cows: 31

Cow No.: F78

Lactation No.: heifer

AI date: 4 June 2010

Dry date: -

Calving date: 22 February 2011

Transitional feed date (before calving): yes

Type of feed (~1 week before calving):

Concentrate:

- Cassava residual: 8 kg/d
- Concentrate: 2 kg/d

Roughage:

- Rice straw: 6 kg/d
- Corn chopped: 8 kg/d

Remarks: -

Mineral supplement before calving: no

Mineral block: no

Vitamin: no

Has cow got a milk fever before? No

Sample no.: 51

Farm: Rungsalit

Sub district: Tamakha

Province: Kanchanaburi

No. of lactating cows: 8

Cow No.: Mini

Lactation No.: heifer

AI date: 25 May 2010

Dry date: -

Calving date: 02 March 2011

Transitional feed date (before calving): 20 days before calving

Type of feed (~1 week before calving):

Concentrate:

- 16% ep (NP) ~ 4 kg/day

Roughage:

- Corn stalk ~ 30 kg/day

Remarks: /

Mineral supplement before calving: yes, mineral powder (GT1000)

Mineral block: yes

Vitamin: yes, vitamin AD₃E

Has cow got a milk fever before? No

Sample no.: 54
Farm: Manop
Sub district: Tamaka
Province: Kanchanaburi
No. of lactating cows: 7

Cow No.: Bai Fern
Lactation No.: heifer
AI date: 31 May 2010
Dry date: -
Calving date: 08 March 2011

Transitional feed date (before calving): 1 month before calving (give 1 kg 20% ep NP)

Type of feed (~1 week before calving):

Concentrate:

- 20% ep (NP) ~ 4 kg/day

Roughage:

- Corn stalk ~ 30 kg/day
- Or Napier (taniaan) ~ 30 kg/day

Remarks: /

Mineral supplement before calving: yes, mineral powder (GT1000)

Mineral block: yes

Vitamin: yes, vitamin AD₃E

Has cow got a milk fever before? No

Sample no.: 55
Farm: Utumporn
Sub district: Potaram
Province: Ratchaburi
No. of lactating cows: 27

Cow No.: Pen-Porn
Lactation No.: 6
AI date: 23 May 2010
Dry date: 20 December 2010
Calving date: 27 February 2011

Transitional feed date (before calving):

Type of feed (~1 week before calving):

Concentrate:

- Concentrate 16% protein: 3 kg/d

Roughage:

- Grass: 10 kg/d
- Corn: 8 kg/d

Remarks:

Mineral supplement before calving: yes

Mineral block: no

Vitamin: yes, inject with AD₃E

Has cow got a milk fever before? No

Sample no.: 56
Farm: Su-Chart Lord
Sub district: Potaram
Province: Ratchaburi
No. of lactating cows: 15

Cow No.: Dum-Sao
Lactation No.: heifer
AI date: 21 May 2010
Dry date: -
Calving date: 22 February 2011

Transitional feed date (before calving): yes

Type of feed (~1 week before calving):

Concentrate:

- Concentrate: 6 kg/d

Roughage:

- Chopped corn: 15 kg/d
- Corn plant: 8 kg/d

Remarks:

Mineral supplement before calving: yes

Mineral block: yes

Vitamin: no

Has cow got a milk fever before? No

Sample no.: 57

Farm: Samban

Sub district: Bang-pae

Province: Ratchaburi

No. of lactating cows: 12

Cow No.: Dao-Reaung

Lactation No.: 7

AI date: 1 June 2010

Dry date: 10 December 2010

Calving date: 28 February 2011

Transitional feed date (before calving): yes

Type of feed (~1 week before calving):

Concentrate:

- Concentrate 16% protein: 2 kg/d

Roughage:

- Grass: 10 kg/d
- Straw: 2.5 kg/d

Remarks: 15 day after dry date eat concentrate 1 kg/d with grass, straw, chopped corn, until calve.

Mineral supplement before calving: yes

Mineral block: no

Vitamin: yes, eat KT super selenium plus

Has cow got a milk fever before? No

Sample no.: 58

Farm: Boon-Kra

Sub district: Potaram

Province: Ratchaburi

No. of lactating cows: 11

Cow No.: Sang-Jun

Lactation No.: 2

AI date: 31 May 2010

Dry date: 27 December 2010

Calving date: 3 March 2011

Transitional feed date (before calving): yes

Type of feed (~1 week before calving):

Concentrate:

- Tablet: 2.5 kg/d
- Powder: 2 kg/d

Roughage:

- Grass: 8 kg/d
- Soy-milk residue: 2 kg/d

Remarks: 15 day before calve: eat soy-milk residue 4 kg/d, concentrate 3.2 kg/d, grass 20 kg/d, straw 5 kg/d.

Mineral supplement before calving: yes

Mineral block: no

Vitamin: yes, selenium 0.02% V Plus

Has cow got a milk fever before? No

Sample no.: 59

Farm: Virat

Sub district: Ban-Pong

Province: Ratchaburi

No. of lactating cows: 15

Cow No.: Thong-cum

Lactation No.: heifer

AI date: 7 June 2010

Dry date: -

Calving date: 06 March 2011

Transitional feed date (before calving): none

Type of feed (~1 week before calving):

Concentrate:

- Home-made concentrate: 5 kg/d

Roughage:

- Grass: 15 kg/d
- Straw: 2 kg/d

Remarks:

Mineral supplement before calving: no

Mineral block: no

Vitamin: no

Has cow got a milk fever before? No

Sample no.: 60

Farm: Manop

Sub district: Tamaka

Province: Kanchanaburi

No. of lactating cows: 5

Cow No.: Pao-Reving

Lactation No.: 3

AI date: 07 June 2010

Dry date: 16 January 2011

Calving date: 09 March 2011

Transitional feed date (before calving): 1 month before calving (give 1 kg 20% ep NP)

Type of feed (~1 week before calving):

Concentrate:

- 20% ep (NP) ~ 4 kg/day

Roughage:

- Corn stalk ~ 30 kg/day
- Or Napier ~ 30 kg/day

Remarks: /

Mineral supplement before calving: yes, mineral powder (GT1000)

Mineral block: yes

Vitamin: yes, vitamin AD₃E

Has cow got a milk fever before? No

Sample no.: 66

Farm: Surapohol

Sub district: Tamaka

Province: Kanchanaburi

No. of lactating cows: 13

Cow No.: Waw

Lactation No.: heifer

AI date: 6 June 2010

Dry date: -

Calving date: 02 March 2011

Transitional feed date (before calving): 20 days before calving

Type of feed (~1 week before calving):

Concentrate:

- 20% ep (NP) ~ 2 kg/day
- 16% ep (NP) ~ 2 kg/day

Roughage:

- Pangola grass ~ 50 kg/day
- Corn stalk silage ~ 20 kg/day

Remarks: /

Mineral supplement before calving: yes

Mineral block: yes

Vitamin: no

Has cow got a milk fever before? No

Sample no.: 74

Farm: Teerachai

Sub district: Tamuang

Province: Kanchanaburi

No. of lactating cows: 9

Cow No.: Fai

Lactation No.: 2

AI date: 16 June 2010

Dry date: 08 November 2010

Calving date: 09 March 2011

Transitional feed date (before calving): 1 month before calving

Type of feed (~1 week before calving):

Concentrate:

- NP (16% ep): ~ 4 kg/day (when calving give 20% ep NP)

Roughage:

- Napier grass: ~ 50 kg/day

Remarks: Pankota grass

Mineral supplement before calving: yes, mineral powder -> Goto 1000

Mineral block: yes

Vitamin: yes, AD₃E for 1 month

Has cow got a milk fever before? No

Sample no.: 76

Farm: Tamakrit

Sub district: Tamaka

Province: Kanchanaburi

No. of lactating cows: 13

Cow No.: Ma-dee

Lactation No.: 4

AI date: 10 June 2010

Dry date: 06 January 2011

Calving date: 09 March 2011

Transitional feed date (before calving): 20 days before calving

Type of feed (~1 week before calving):

Concentrate:

- NP (16% ep): ~ 1 kg/day
- DS_CP: ~ 0.5 kg/day

Roughage:

- Bawa grass: ~ 25 kg/day
- Rice straw: ~ 3 kg/day

Remarks: /

Mineral supplement before calving: yes

Mineral block: no

Vitamin: yes, Biocatalin (source of Amino acid + vit B complex)

Has cow got a milk fever before? No