

Fatty Liver in High Producing Dairy Cows Kept in Evaporative Cooling System in a Commercial Dairy Herd in Thailand

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ABSTRACT

The occurrence of fatty liver was investigated in a commercial dairy farm under evaporative cooling system in Thailand by determination of triacylglycerol concentrations in the liver. Nineteen Holstein-Friesian cows were used. Liver samples were collected at -2, 1, 2, 3, and 4 wk from parturition. At -2 wk, mean liver triacylglycerol concentration was 24.3 ± 1.0 mg/g of liver. After parturition, mean liver triacylglycerol concentration from 6 cows was lower than 50 mg/g of liver (mild fatty liver group), the mean concentration from 8 cows was between 50 and 100 mg/g of liver (moderate fatty liver group), and the mean concentration from 5 cows was greater than 100 mg/g of liver (severe fatty liver group). The results showed that 68.4% of dairy cows in this investigation suffered from periparturient fatty liver. Milk production in severe fatty liver group tended to be higher than the milk production in mild or moderate fatty liver cows. This result indicated that cows with higher milk yield tended to enter deeper negative energy balance postpartum and as a consequence had more intensive lipolysis, leading to accumulation of a greater amount of triacylglycerols in the liver. In conclusion, an evidence of fatty liver was found in high producing dairy cows, kept under cooled condition in Thailand. Before any preventive measures could be made, further research is required to study the effect of fatty liver or negative energy balance on periparturient problems, i.e. infertility, metabolic disorders, in dairy cows raised in Thai conditions.

Key words: dairy cow, fatty liver, triacylglycerol

INTRODUCTION

Dairy cows usually go into a period of energy shortage or negative energy balance (NEB) during periparturient period because feed intake of the cows during that period cannot always provide sufficient energy to meet the increase of requirements as initiated by increasing milk production postpartum (Harrison *et al.*, 1990). Thus, the energy supply must be derived partly from lipolysis and proteolysis. Lipolysis in adipose

tissue raises the concentration of non-esterified fatty acids (NEFA) in the blood (Rukkwamsuk *et al.*, 1998). The mobilized NEFA are absorbed by the liver, are metabolized or re-esterified to triacylglycerols (TAG) (Bruss, 1993). Most of the TAG are secreted from the liver in very low density lipoproteins. Evidence exist that more intensive lipolysis as occurring in cows with severe NEB, plasma NEFA concentrations increase more substantially, resulting in an increase of TAG synthesis that exceeds the liver's capacity for

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excreting TAG in very low density lipoproteins, resulting in fatty liver or hepatic lipidosis (Van den Top *et al.*, 1995; Rukkwamsuk *et al.*, 1998; Rukkwamsuk *et al.*, 1999c). It is well documented that NEB or fatty liver has adverse effects on health, production, and reproduction in dairy cows, partly due to some consequences of intensive lipolysis causing fatty liver (Hill *et al.*, 1985; Gerloff *et al.*, 1986; West, 1990; Veenhuizen *et al.*, 1991; Kandefer-Szerszen *et al.*, 1992; Staufenbiel *et al.*, 1993; Rehage *et al.*, 1996; Rukkwamsuk *et al.*, 1999b; Rukkwamsuk *et al.*, 1999d;). In cows with severe fatty liver, apart from delayed bromsulphthalein clearance ability of the liver (West, 1990; Rukkwamsuk and Wensing 2002), hepatic gluconeogenesis is impaired (Rukkwamsuk *et al.*, 1999b), a phenomenon that may delay an inhibition of lipolysis.

Heat stress is a major contributing factor in infertility of lactating dairy cows in hot climate (Wolfenson *et al.*, 2000). In Thailand, some commercial dairy farms have adopted a cooling system for the cows in order to alleviate any adverse effects of severe heat stress. It is documented that cows raised in a barn with evaporative cooling system have lower rectal temperature and respiration rates than those raised in a barn without cooling system (Armstrong *et al.*, 1985; Armstrong *et al.*, 1993). The evaporative cooling system is suggested to improve cow comfort, and eventually could result in an improvement of production in high producing dairy cows.

The biochemical changes of liver in periparturient dairy cows that are raised in a farm with an evaporative cooling system are worth to be intensively studied. Therefore, the objective of this study was to determine liver triacylglycerol concentrations as an indicator of fatty liver or NEB during periparturient period in randomly selected cows from a commercial dairy farm.

MATERIALS AND METHODS

Farm, animals and diets

The study was conducted in a commercial dairy farm at Pakthongchai District, Nakhon Ratchasima Province, Thailand. The farm consisted of 532 lactating cows, 112 dry cows, and 550 replacement calves and heifers. The average milk production of the farm was 11,920 kg/d. The close-up and lactating cows were kept in a free-stall housing with an evaporative cooling system, which controls the inside temperature between 25-28°C. All cows in the evaporating barn were fed *ad libitum* with total mixed rations as shown in Table 1. During the dry period, the cows were offered 25 kg/d of the dry cow diet and 31 kg/d of the transition diet. After parturition, the cows were offered 39 kg/d of the lactating diet.

Twenty healthy, dry pregnant multiparous Holstein Friesian cows were randomly selected; mean age was 4.9 yr (SD = 2.2), mean 305-d cumulative milk yield was 7,629 kg (SD = 1453), and mean body condition score (BCS) was 3.6 (SD = 0.3) at the start of the experiment.

Sampling and assay procedures

Liver biopsy was obtained using the percutaneous biopsy method as described by Van den Top (1995). The liver samples were collected about 2 wk (12 ± 7 d, mean \pm SD) before the anticipated calving date and at 1 (7 ± 2 d), 2 (14 ± 2 d), 3 (21 ± 2 d), and 4 (28 ± 2 d) wk after parturition. During collection, the liver samples were placed on filter paper to remove any connective tissue and blood clots, and were placed in a tube with physiological saline. All samples were transported to the laboratory in an ice box. Thereafter in the laboratory, these samples were dried on the filter paper, and any remaining connective tissue or blood clots were removed. The samples were weighed in separated tubes for each TAG determination, and were kept at -20°C until analysis. Triplicate analyses were performed

Table 1 Composition of total mixed rations (TMR) as fed basis.

Ingredient	Dry period	Transition period	Lactating period
	kg as fed		
PCL-3 ¹	0.8
PCL-P ²	...	2.0	...
PCL-F ³	3.8
Wet brewer grain	8.0	7.0	10.0
Corn silage	8.0	12.5	12.5
Peanut hay	3.5	6.0	6.0
Rice straw	2.0
Cassava chips	...	1.0	1.3
Whole cotton seed	...	0.6	2.0
Ground corn	1.0
Molasses	1.0	0.5	0.5
Premixes	0.5	0.5	1.0

¹ Consisting of 33.0% soybean meal, 27% canola meal, 22.5% wheat bran, 11% dried brewer grain, 4.1% limestone, and 2.4% salts.

² Consisting of 54.5% soybean meal, 15.0% canola meal, 13.0% dried brewer grain, 11.0% corn gluten meal, 4.7% salts, and 1.8% biophos.

³ Consisting of 44.0% soybean meal, 27.0% corn gluten meal, 14.0% canola meal, 5.5% dried brewer grain, 1.9% salts, and 1.6% limestone.

in all liver samples.

Liver TAG concentrations were assayed by spectrophotometry with the use of a commercial kit (Triglyceride GPO-PAP; CLASS-1 Laboratories Co., Ltd., Bangkok, Thailand).

Statistical analyses

All cows calved normally. One cow was clinically abnormal after parturition, and this cow had an enormous increase of TAG in her liver. Therefore, data of milk production and liver TAG concentration of this cow were excluded from the analyses. Data were statistically analyzed using an SPSS computer program (SPSS Advance Statistic™, 1994). Data were tested for normal distribution using the Shapiro-Wilk W test (Petrie and Watson, 1999), and the homogeneity of variances was verified using the Levene's test (Petrie and Watson, 1999). Comparison of data

between sampling days was performed using the paired Student *t* test. The two-sided level of statistical significance was preset at $P \leq 0.05$.

RESULTS AND DISCUSSION

Liver triacylglycerols

Mean concentrations of TAG in the liver of 19 cows are presented in Figure 1. At 2 wk before parturition, mean liver TAG concentration was 24.3 ± 1.0 (mean \pm SEM) mg/g of liver wet weight, which was in the same range as reported previously (Van den Top *et al.*, 1995). At 1, 2, 3, and 4 wk after parturition, mean TAG concentrations were 61.4 ± 6.3 , 61.1 ± 6.4 , 57.3 ± 6.3 , and 57.8 ± 7.8 mg/g wet weight of liver, respectively. These values were significantly higher than the mean concentration before parturition and indicated that these cows had fatty liver as described by Gaal *et al.* (1983) In

this study, postpartum TAG concentrations were similar to the results of a field study in 9 commercial dairy herds in The Netherlands (Jorritsma *et al.*, 2001) in which the mean concentration of TAG in the liver of 218 cows sampled between 6 and 17 d after parturition was 61.2 ± 2.6 mg/g wet weight of liver. The prevalence of fatty liver (more than 50 mg of TAG in 1 g wet weight of liver tissue) is reported to be 54.1%. In this study, the occurrence of fatty liver was 68.4% (13/19). It was obvious that most cows increased their liver TAG concentrations immediately after parturition and the concentration remained at higher values throughout 4 wk after parturition (Van den Top, 1995).

According to liver TAG concentrations postpartum, cows were arbitrarily divided into three groups: mild fatty liver group (liver TAG concentrations lower than 50 mg/g wet weight of liver), moderate fatty liver group (liver TAG concentrations between 50 and 100 mg/g), and severe fatty liver group (liver TAG concentrations greater than 100 mg/g) (Gaal *et al.*, 1983). With this criterion, 6, 8, and 5 cows were in mild, moderate, and severe fatty liver groups, respectively. At 2 wk before parturition, mean liver TAG concentrations between the 3 groups of cows were $20.5 (\pm 3.1)$, $25.3 (\pm 5.0)$ and $23.6 (\pm 4.2)$ mg/g wet weight of liver, respectively. At 2 wk after parturition, mean liver TAG concentrations were $32.5 (\pm 9.3)$, $62.0 (\pm 13.8)$ and $101.1 (\pm 25.3)$ mg/g wet weight of liver, respectively. It is documented that the cows with severe fatty liver might enter deeper NEB, which could induce a more intensive lipolysis (Rukkwamsuk *et al.*, 1999a). In addition, severe fatty liver cows obviously have a prolonged fatty liver as the mean TAG concentrations in the liver remained high at least 4 wk after parturition. This result may relate to impaired gluconeogenic capacity of liver with high TAG levels (Rukkwamsuk *et al.*, 1999b), resulting in a prolonged period with decreased

blood glucose and insulin concentrations and as a consequence to longer lasting lipolysis.

Milk production

Mean milk yield during the first 30 d of lactation of the cows is demonstrated in Figure 2. The milk yield inclined very sharply during the first few days of lactation. All 19 cows produced an average of 31 ± 4 kg/d during first 4 wk of lactation. Mean milk yield of cows with mild, moderate, and severe fatty liver were 29 ± 6 (mean \pm SD), 31 ± 5 , and 34 ± 7 kg/d, respectively (Figure 3). The mean milk production of cows with severe fatty liver tended to be higher ($P = 0.1$) than the mean milk production of cows with mild fatty liver. This result indicated that a higher milk yield might be associated with NEB and, thus, was a predisposing factor to the fatty liver development.

CONCLUSIONS

It is well documented that fatty liver postpartum is associated with increased vulnerability for production diseases decreased immunocompetence (Wentink *et al.*, 1997), and reproductive disorders (Rukkwamsuk *et al.*, 1999e;) are found to be associated with fatty liver. This result demonstrated that Holstein Friesian cows raised in Thailand under cooled condition could develop fatty liver after parturition. It could be expected that this condition is connected with some health and reproduction problems as it is usually reported in Thailand. As far as the dairy cows under natural Thai conditions are concerned, no exact data either about the frequency that fatty liver occurs or about the seriousness of fatty liver postpartum are available. Taking into account that fatty liver in periparturient dairy cows can cause great economical losses, more research is necessary.

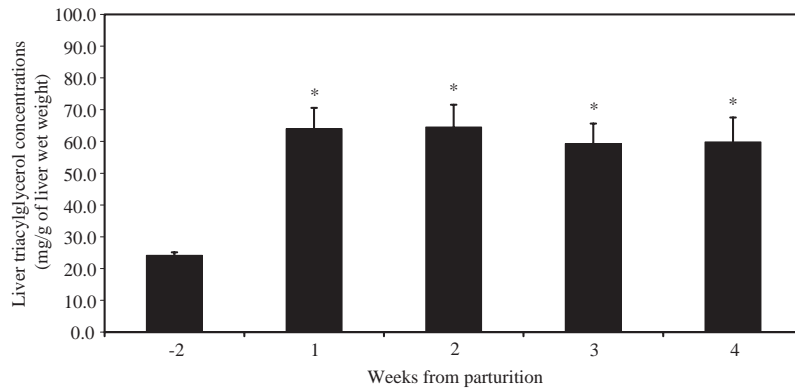


Figure 1 Liver triacylglycerol (TAG) concentrations at -2, 1, 2, 3, and 4 wk from parturition of 19 cows. Data represent mean \pm SEM. Asterisks indicate that mean concentrations of liver TAG at 1, 2, 3, and 4 wk after parturition significantly differed from the mean at -2 wk ($P \leq 0.05$).

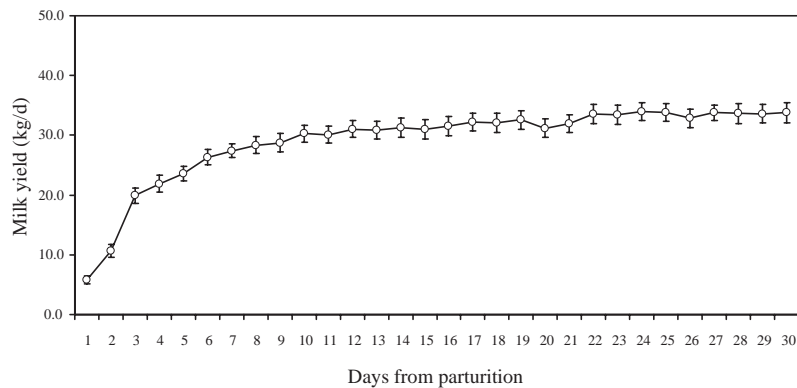


Figure 2 Milk production during the first 30 days after parturition of 19 cows .

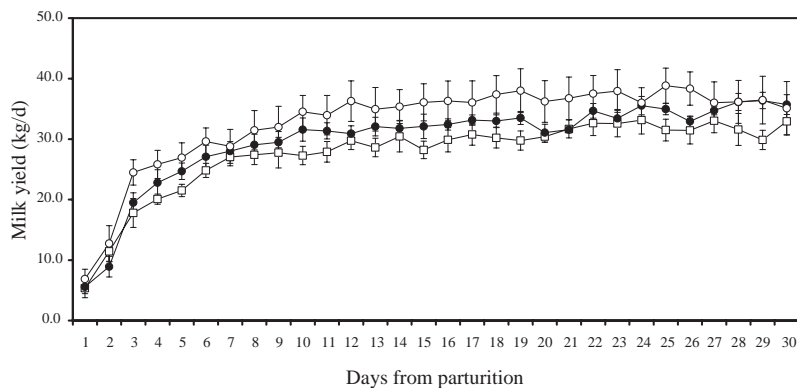


Figure 3 Milk production during the first 30 days after parturition of cows with mild (□; $n = 6$), moderate (●; $n = 8$), and severe (○; $n = 5$) fatty liver.

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LITERATURE CITED

- Armstrong, D.V., F. Wiersam, T.J. Fuhrmann, J.M. Tappan and S.M. Cramer. 1985. Effect of evaporating cooling under a corral shade on reproduction and milk production in a hot arid climate. **J. Dairy Sci.** 68 (Suppl. 1): 167 (Abstr.).
- Armstrong, D.V., S.K. DeNise, F.J. Delfino, E.J. Hayes, P.H. Grundy, S. Montgomery and A. Correa. 1993. Comparing three different dairy cattle cooling systems during high environmental temperatures. **J. Dairy Sci.** 76 (Suppl. 1): 24 (Abstr.).
- Bruss, M.L. 1993. Metabolic fatty liver of ruminants, pp. 421-422. In C. E. Cornelius (ed.). **Advances in Veterinary Science and Comparative Medicine**. Academic Press Inc., San Diego, U.S.A.
- Gaal, T., I.M. Reid, R.A. Collins, C.J. Roberts and B.V. Pike. 1983. Comparison of biochemical and histological methods of estimating fat content of liver of dairy cows. **Res. Vet. Sci.** 34: 245-248.
- Gerloff, B.J., T.H. Herdt and R.S. Emery. 1986. Relationship of hepatic lipidosis to health and performance in dairy cattle. **J. Am. Vet. Med. Assoc.** 188: 845-850.
- Harrison, R.O., S.P. Ford, J.W. Young, A.J. Conley and A.E. Freeman. 1990. Increased milk production versus reproductive and energy status of high producing dairy cows. **J. Dairy Sci.** 73: 2749-2758.
- Hill, A.W., I.M. Reid and R.A. Collins. 1985. Influence of liver fat on experimental *Escherichia coli* mastitis in periparturient cows. **Vet. Rec.** 117: 549-551.
- Jorritsma, R., H. Jorritsma, Y.H. Schukken, P.C. Bartlett, T. Wensing and G.H. Wentink. 2001. Prevalence and indicators of post partum fatty infiltration of the liver in nine commercial dairy herds in The Netherlands. **Livest. Prod. Sci.** 68: 53-60.
- Kandefer-Szerszen, M., J. Filar., A. Szuster-Ciesielska and W. Rzeski. 1992. Suppression of interferon response of bovine leukocytes during clinical and subclinical ketosis in lactating cows. **Dtsch. Tierarztl. Wochenschr.** 99: 440-443.
- Petrie A. and P. Watson. 1999. **Statistics for Veterinary and Animal Science**. Blackwell Science Ltd., Oxford, United Kingdom. 243 p.
- Rehage, J., M. Mertens, N. Stockhofe-Zurwieden, M. Kaske and H. Scholz. 1996. Post surgical convalescence of dairy cows with left abomasal displacement in relation to fatty liver. **Schweiz Arch Tierheilkd** 138: 361-368.
- Rukkamsuk, T., T. Wensing and M.J.H. Geelen. 1998. Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. **J. Dairy Sci.** 81: 2904-2911.
- Rukkamsuk, T., T.A.M. Kruip, G.A. Meijer and T. Wensing. 1999a. Hepatic fatty acid composition in periparturient dairy cows with fatty liver induced by intake of a high energy diet in the dry period. **J. Dairy Sci.** 82: 280-287.
- Rukkamsuk, T., T. Wensing and M.J.H. Geelen. 1999b. Effect of fatty liver on hepatic gluconeogenesis in periparturient dairy cows.

- J. Dairy Sci.** 82 : 500-505.
- Rukkwamsuk, T., T. Wensing and M.J.H. Geelen. 1999c. Effect of overfeeding during the dry period on the rate of esterification in adipose tissue of dairy cows during the periparturient period. **J. Dairy Sci.** 82: 1164-1169.
- Rukkwamsuk, T., T. Wensing and H.J. Breukink. 1999d. Clinical-biochemical observations in dairy cows experimentally induced with fatty liver, pp. 339-343 *In Proceedings 25th Ann. Conf. Thai Vet. Med. Assoc.*, Bangkok, Thailand.
- Rukkwamsuk, T., T. Wensing and T.A.M. Kruij, 1999e. Relationship between triacylglycerol concentration in the liver and first ovulation in postparturient dairy cows. **Theriogenology** 51: 1133-1142.
- Rukkwamsuk, T. and T. Wensing. 2002. Delayed hepatic bromsulphthalein clearance in postparturient dairy cows with increased triacylglycerol accumulation in the liver, pp. 353-359. *In Proceedings 40th Kasetsart Univ. Ann. Conf.*, Bangkok, Thailand.
- SPSS Advance Statistic™ Version 6.1. 1994. SPSS Inc., Chicago, Illinois, U.S.A.
- Staufenbiel, R., B. Staufenbiel, N. Rossow, H. Klukas and U. Johannsen. 1993. Diagnosis of fatty liver in dairy cows. **Dtsch. Tierarztl. Wochenschr.** 100: 225-230.
- Van den Top, A.M. 1995. **Diet and lipid metabolism in ruminants with particular reference to fatty liver development.** Ph.D. Thesis. Utrecht University, Utrecht, The Netherlands.
- Veenhuizen, J.J., J.K. Drackley, M.J. Richard, T.P. Sanderson, L.D. Miller and J.W. Young, 1991. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. **J. Dairy Sci.** 74: 4238-4253.
- Wentink, G.H., V.P.M.G. Rutten, T.S.G.A.M. van den Ingh, A. Hoek, K.E. Müller and T. Wensing. 1997. Impaired specific immunoreactivity in cows with hepatic lipidosis. **Vet. Immunol. Immunopathol.** 56: 77-83.
- West, H. J., 1990. Effect on liver function of acetonemia and the fat cow syndrome in cattle. **Res. Vet. Sci.** 48: 221-227.
- Wolfenson, D., Z. Roth and R. Meiden. 2000. Impaired reproduction in heat-stressed cattle: basic and applied aspects. **Anim. Reprod. Sci.** 60-61: 535-547.