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Clinical-biochemical Observations in Periparturient Dairy Cows with Experimentally Induced Fatty Liver

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ABSTRACT

In 4 experimental studies, occurrences of health problems during the first 4 weeks of lactation were monitored in 36 control cows and 38 experimental cows that were overfed during the dry period to induce overconditioning at calving and deep negative energy balance and fatty liver postpartum. The experimental cows experienced postparturient problems, including milk fever (37%), acetonemia (8%), abomasal displacement (8%), mastitis (10%), and hoof problems (8%). Acetonemia and abomasal displacement were not observed in the control cows. Milk fever, mastitis, and hoof problems were observed in 3%, 6% and 3% of the control cows, respectively. The experimental cows gained more body weights during the dry period and lost more weights during the first 4 or 5 weeks of lactation than did the control cows. Between 1 and 2 weeks after parturition, the experimental cows had higher concentrations of blood nonesterified fatty acids, blood 3-hydroxybutyrate, and liver triacylglycerols, and had lower concentrations of liver glycogen than did the control cows. Blood glucose and insulin concentrations did not differ between the two groups. When data of the 4 studies were pooled, blood nonesterified fatty acid concentrations were positively correlated with blood 3-hydroxybutyrate and liver triacylglycerol concentrations, and were negatively correlated with liver glycogen concentrations. These results indicated that cows responded to a marked increase in energy requirements in early lactation by increased glycogenolysis in the liver and lipolysis in adipose tissue. In conclusion, overfeeding of cows during the dry period resulted in overcondition at calving. Overconditioning predisposed a cow to develop severe fatty liver postpartum and to experience more postparturient problems. The concentration of blood nonesterified fatty acids postpartum could be used as a routine indicator for the development of fatty liver in dairy cows postpartum.

Key words: dairy cow, fatty liver, negative energy balance, triacylglycerols

INTRODUCTION

The combination of a deep negative energy balance (NEB) and fatty liver is a common feature in high producing dairy cows after parturition (Herdt, 1988). There is evidence that fatty liver has deleterious effects on health, production, and reproduction of dairy cows postpartum (Morrow, 1976; Reid and Roberts, 1983; Gerloff *et al.*, 1986; Markusfeld *et al.*, 1988; Van den Top, 1995; Wentink *et al.*, 1997). Therefore, the combination of a deep NEB and fatty liver has an economical impact on dairy farming.

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Overconditioned cows are more likely to enter a more severe NEB than cows in normal condition (Garnsworthy and Topps, 1982; Harrison *et al*., 1990). As a physiological consequence, overconditioned cows increase lipolysis in adipose tissue more substantially, resulting in a greater increase in concentrations of blood nonesterified fatty acids (NEFA). Evidence exists that an increased concentration of circulating NEFA is related to increased accumulation of triacylglycerols (TAG) in the liver (Herdt *et al*., 1988; Rukkwamsuk *et al*., 1998). Epidemiological data provide evidence that overconditioned cows at calving undergo postparturient diseases more frequently than their herd mates (Gerloff *et al*., 1986; Treacher *et al*., 1986; Gearhear *et al*., 1990; Andrews *et al*., 1991). Negative energy balance is also reported to be related to health, production and reproduction problems in dairy cows (Collard *et al*., 2000).

The aim of this investigation was to check whether or not the mentioned health problems (Gerloff *et al*., 1986; Treacher *et al*., 1986; Gearhart *et al*., 1990; Andrews *et al*., 1991) also occur in cows that were induced overcondition at calving followed by a deeper NEB and fatty liver postpartum. Data from 4 experiments conducted between 1995 and 1998, including 36 control cows and 38 cows experimentally induced with fatty liver, were used. Selected blood and liver parameters were compared in those cows to characterize the degree of fatty liver. Correlation between blood and liver parameters were also evaluated.

MATERIALS AND METHODS

Data collections

We used data from 74 cows from 4 experiments conducted between 1995 and 1998 at the Faculty of Veterinary Medicine of Utrecht University, Utrecht, and at the Institute of Animal Science and Health, Lelystad, The Netherlands. The 4 experiments included 3 control and 5 experimental cows, 16 control and 15 experimental cows, 6 control and 7 experimental cows, and 11 control and 11 experimental cows, respectively. The experimental cows were subjected to fatty liver-inducing regimen (Van den Top, 1995), whereas control cows were fed at restricted energy intake during the dry period. This regimen aimed at inducing overconditioning or excess body weight (BW) at calving. Details of cows, diets and feeding, sampling procedures, and sample analyses were presented elsewhere (Murondoti, 1998; Rukkwamsuk *et al*., 1998; Rukkwamsuk *et al*., 1999a,b). In all experiments, the daily clinical observations and procedures for clinical disease survey were monitored by veterinarians or well-trained animal care-takers.

Statistical analyses

Data of blood and liver parameters were tested for normal distribution using the Kolmogorov-Smirnov test (Petrie and Watson, 1999). Comparison of data between the two groups was performed using the Student's *t* test, or when data were not normally distributed, using the Mann-Whitney U test (Petrie and Watson, 1999). When data had normal distribution, homogeneity of variances was verified using the Levene's test (Petrie and Watson, 1999). Correlation between blood and liver parameters was performed using the Pearson Correlation test (Petrie and Watson, 1999). The relationship between blood NEFA, and other blood or liver parameters was performed using simple linear regression analysis (Petrie and Watson, 1999).

RESULTS AND DISCUSSION

In all experiments, experimental cows gained more BW during the dry period (Table 1). This finding was due to the fact that overfeeding during the dry period provided more energy substrates which led to deposition of body fat in

adipose tissue of dairy cows. This result also confirmed the hypothesis that overfeeding of dairy cows during the dry period leads to overconditioning or excess BW at calving. Furthermore, experimental cows lost more BW during the first 4 or 5 weeks of lactation than did control cows (Table 1). This result indicated that cows with excess BW at calving entered a deeper negative energy balance postpartum, thereby having a more intensive lipolysis than cows with normal BW. The mechanism underlying this finding was most likely related to the fact that cows with excess BW have lower feed intake after parturition than cows with normal BW (Garnsworthy and Topps, 1982).

Blood glucose, insulin, NEFA, 3-hydroxybutyrate (3-HB), liver glycogen and TAG concentrations measured between 1 and 2 weeks after parturition are presented in Table 2. Blood glucose and insulin concentrations did not differ between experimental and control cows, although it was expected as a consequence of more severe negative energy balance that experimental cows would have had lower values than control cows. One possible reason to explain these results was that we measured these parameters only once a week. Therefore, a part of the changes in the concentrations of these parameters could not be overseen. It was likely to be one of reasons that why we did not find any significant relationship between blood concentrations of glucose, insulin, and NEFA in our studies (Table 3).

Blood concentrations of 3-HB were higher in the experimental cows than in the control cows of Experiment II and III and tended to be higher in the experimental cows than in the control cows of Experiment I and IV. When data were pooled, overall experimental cows had higher concentrations of blood 3-HB than did overall control cows. Concentrations of blood 3-HB were positively correlated with liver TAG concentrations (Table 3) and with blood NEFA concentrations (Table 3). This finding was consistent with other observations that the extent of the fatty infiltration was positively correlated with the concentrations of ketone bodies in the blood (Gröhn *et al*., 1983).

In all experiments, blood NEFA and liver TAG concentrations were higher in experimental cows than in control cows (Table 2). In addition, overall experimental cows had lower concentrations of liver glycogen than did overall control cows. These findings strongly confirm that cows with excess BW at calving, as a consequence of deeper NEB postpartum, have increased lipolysis in adipose tissue and increased hepatic

Table 1 Body weight (BW) gain during the dry period (8 to 10 weeks) and BW loss (during the first 4 or 5 weeks of lactation) of control cows and experimental cows that were induced with fatty liver in the 4 experiments conducted between 1995 and 1998. Data were compared between control and Experimental cows.

	BW gain (kg)			BW loss (kg)		
Experiment[1]	Control	Experimental	SED[2]	Control	Experimental	SED
I	54.7	82.4	12.6[a]	-32.0	-88.0	20.0[b]
II	18.0	80.0	15.0[d]	8.3	-36.7	9.2[d]
III	72.8	124.0	19.2[b]	-16.3	-60.8	5.3[d]
IV	21.5	113.5	22.5[c]	-16.5	-46.3	10.4[c]

[1] Experiment I included 3 control and 5 experimental cows; Experiment II included 16 control and 15 experimental cows; Experiment III included 6 control and 7 experimental cows; Experiment IV included 11 control and 11 experimental cows.

[2] Standard error of difference.

[a]$P < 0.1$, [b]$P < 0.05$, [c]$P < 0.01$, [d]$P < 0.001$.

glycogenolysis more substantially. We also observed the negative correlation between blood NEFA concentrations and liver glycogen concentrations, although the correlation was not high (Table 3). Our results provide consistent evidence that increased concentrations of blood NEFA predominantly contribute to the accumulation of TAG in the liver. This hypothesis

Table 2 Blood glucose, insulin, nonesterified fatty acid (NEFA), 3-hydroxybutyrate (3-HB), and liver glycogen and triacylglycerol (TAG) concentrations (mean ± SEM), measured between 1 and 2 weeks after parturition, of control cows and experimental cows that were induced with fatty liver in the 4 experiments conducted between 1995 and 1998. Data were compared between control and treatment cows.

	Experiment[1]				
Parameters, unit	I	II	III	IV	Overall
Glucose, mmol/L					
Control	2.77 ± 0.15	3.08 ± 0.08	2.88 ± 0.14	3.02 ± 0.13	3.00 ± 0.06
Experimental	2.74 ± 0.37	3.09 ± 0.11	2.61 ± 0.22	3.06 ± 0.10	2.95 ± 0.08
Insulin, μU/ml					
Control	8.2 ± 2.2	6.0 ± 0.7	8.5 ± 0.8	8.3 ± 1.6	7.3 ± 0.6
Experimental	7.7 ± 1.0	6.1 ± 0.7	8.7 ± 1.0	7.6 ± 1.1	7.2 ± 0.5
NEFA, mmol/L					
Control	0.32 ± 0.04	0.48 ± 0.08	0.64 ± 0.08	0.51 ± 0.06	0.50 ± 0.04
Experimental	1.20 ± 0.30[a]	1.03 ± 0.14[c]	1.15 ± 0.11[c]	0.81 ± 0.14[a]	1.01 ± 0.08[d]
3-HB, mmol/L					
Control	0.67 ± 0.09	0.48 ± 0.08	1.00 ± 0.17	0.70 ± 0.14	0.65 ± 0.07
Experimental	1.47 ± 0.53	0.74 ± 0.08[b]	2.06 ± 0.16[d]	0.90 ± 0.06	1.12 ± 0.11[d]
TAG, mg/g wet wt of liver					
Control	49.4 ± 10.8	30.8 ± 3.6	50.0 ± 7.3	22.0 ± 2.3	32.5 ± 2.7
Experimental	117.2 ± 29.5[a]	86.0 ± 7.6[d]	124.7 ± 23.5[b]	70.7 ± 8.4[d]	93.0 ± 7.6[d]
Glycogen, mmol of glucose/g wet wt of liver					
Control	0.15 ± 0.05	0.12 ± 0.01	0.08 ± 0.01	0.15 ± 0.01	0.13 ± 0.01
Experimental	0.10 ± 0.02	0.09 ± 0.01[a]	0.06 ± 0.01	0.11 ± 0.01[c]	0.09 ± 0.01[d]

[1] number of animals as in Table 1.

[a]$P < 0.1$, [b]$P < 0.05$, [c]$P < 0.01$, [d]$P < 0.001$.

Table 3 Correlation coefficients of the correlation of concentrations of blood nonesterified fatty acids (NEFA), glucose, insulin, 3-hydroxybutyrate (3-HB), and liver triacylglycerols (TAG) and glycogen between 1 and 2 weeks after parturition of 74 cows in 4 experiments conducted between 1995 and 1998.

	NEFA	Glucose	Insulin	3-HB	TAG
Glucose	-0.06	...	...	...	...
Insulin	-0.10	0.21[a]	...	...	...
3-HB	0.33[c]	-0.60[d]	0.07	...	...
TAG	0.57[d]	-0.33[c]	-0.03	0.68[d]	...
Glycogen	-0.26[b]	0.24[b]	0.29[b]	-0.33[c]	-0.47[d]

[a]$P < 0.1$, [b]$P < 0.05$, [c]$P < 0.01$, [d]$P < 0.001$.

was also supported by the result that the blood concentration of NEFA was positively correlated with the liver concentration of TAG (Table 3).

It was clear that dairy cows that had developed deeper NEB and more severe fatty liver postpartum experienced more postpartum problems (Table 4). This result was in agreement with the finding in practice (Van Dijk *el al*., 1989). We observed a high occurrence of milk fever in cows with fatty liver as also had been reported previously (Andrews *et al*., 1991). Ketosis was observed only in cows with fatty liver which is consistent with other field observations (Gröhn *et al*., 1983; Lean *et al*., 1994). Displaced abomasum was also observed only in the experimental cows. It has been demonstrated that cows with displaced abomasum have livers which are mildly to severely infiltrated with fat (Muylle *et al*., 1990). However, so far no clear mechanism has accounted for the association between the development of displaced abomasum and fatty liver.

In this study, we did not observe a high occurrence of mastitis in experimental cows compared with control cows. Although it was not possible to conclude from our study that cows with fatty liver suffered more frequently from mastitis, most cases of mastitis that we observed in our experimental cows were relapsed. Evidence exist that cows with an increased liver TAG concentration have a delayed or impaired specific immunoreactivity against tetanus toxiod (Wentink *et al*., 1997). It was likely that cows with fatty liver had an impaired defense mechanism; however, further research is required to elucidate the role of fatty liver in the susceptibility of cows for infections. Our observation was also in agreement with another report that cows with overconditioning at calving were more prone to hoof problems after calving (Gerahart *et al*., 1990). Because our experimental cows also became overweight, hoof problems might be due to the increased mechanical stress on their

Table 4 The occurrence of diseases during the first 4 weeks after parturition of control and experimental cows that were induced with fatty liver in the 4 experiments conducted between 1995 and 1998.

Experiment	Diseased cases				
	Milk fever	Aceto-naemia	Displaced abomasum	Mastitis	Hoof problems
Experiment I					
Control (n = 3)	1	...	...	...	...
Experimental (n = 5)	1	...	2	...	...
Experiment II					
Control (n = 16)	...	...	...	1	...
Experimental (n = 15)	3	2	1	1	1
Experiment III					
Control (n = 6)	...	...	...	1	1
Experimental (n = 7)	4	1	...	3	2
Experiment IV					
Control (n = 11)	...	...	...	...	...
Experimental (n = 11)	6	...	...	...	...
Experiment (Overall)					
Control (n = 36)	1	...	...	2	1
Experimental (n = 38)	14	3	3	4	3

musculoskeleton system as also suggested by Gearhart *et al*. (1990).

CONCLUSIONS

It is clear that overconditioned dairy cows went into a deeper NEB, a more intensive mobilization of body fat and a higher accumulation of liver TAG after parturition, Although it is not advisable to overfeed dairy cows during the dry period, cows with overconditioning at calving and the related problems are still observed in practice. Therefore, it is of importance to closely monitor the condition of dairy cows, particularly during the entire lactation period, to prevent overconditioning or excess BW at calving. This would decrease the likelihood of a cow to develop a severe fatty liver postpartum, thereby decreasing consequent health problems related to the combination of a deeper NEB and fatty liver.

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