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Fat Infiltration in the Liver of Finnish Ayrshire Cows during Early Lactation

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Gröhn, Y., K. Heinonen and L.-A. Lindberg: Fat infiltration in the liver of Finnish Ayrshire cows during early lactation. Acta vet. scand. 1987, 28, 143–149. – The incidence and severity of fat infiltration in the liver of Finnish Ayrshire cows at 1 and 8 weeks after calving were studied. All multiparous (n = 88) and some primiparous (n = 17) cows that calved in 20 commercial milk recorded herds were investigated. The cows lost weight and a condition score decreased significantly ($p < 0.05$) during early lactation. Blood samples were taken at 1, 4 and 8 weeks after calving. Ketone body concentrations were highest at 4 weeks after calving. Albumin and total protein concentrations and ornithine carbamyltransferase activity in the blood increased significantly ($p = < 0.05$) from 1 to 8 weeks after calving. The percentage of liver fat (v/v) was significantly ($p < 0.05$) greater at 1 (3.9 ± 0.5 %) than at 8 weeks (1.2 ± 0.3 %) after calving. Eighty-nine of 102 cows had less than or equal to 9 % of fat in the liver (mean 2.3 ± 0.2 %), and 13 cows had more than 9 % (mean 14.9 ± 1.2 %) at 1 week after calving. Only blood acetoacetate and plasma aspartate aminotransferase differed significantly between the groups. In the fatty liver group the incidence rate of treated cases of ketosis was 30.8 ± 13.3 % and of parturient paresis 23.1 ± 12.2 %. In the non-fatty liver group the rates were 10.1 ± 3.2 % and 7.8 ± 2.9 %, respectively. The differences were not significant.

fatty liver; bovine; parturition.

Introduction

Clinical and research reports on the "fat cow or fatty liver syndrome" in dairy cows have appeared with increasing frequency during the last 10 years (*Sommer* 1975, *Morrow et al.* 1979, *Reid & Roberts* 1983). An association between fat infiltration in the liver and increased susceptibility to periparturient diseases has stimulated research interest in this field. The syndrome occurs particularly in high yielding dairy herds where overfeeding in the dry period results in fat cows at calving. The significance of housing, husbandry and feeding of the dairy herd in prevention of fatty liver has been recognized (*Reid & Roberts* 1983).

In countries with small family farms like in Finland, it was uncertain that there has been a real increase in fatty livers in dairy cows. In earlier reports (*Gröhn et al.* 1983, *Gröhn* 1985) the extent of fat infiltration in the liver of spontaneously ketotic cows had been lower than that in healthy high yielding dairy cows reported e.g. from Compton (*Reid* 1980). Therefore it was decided to investigate 1) the incidence and severity of fat infiltration in the liver of Finnish Ayrshire cows at 1 and 8 weeks after calving, and 2) the possible association between a fatty liver and production, fertility measurements and the incidence of postparturient diseases. This paper describes the incidence and severity of fat infiltration in the liver, some blood characteristics, production parameters and postparturient diseases in Finnish Ayrshire cows in early lactation during the period of indoor feeding.

Material and methods

Animals and experimental design

Finnish Ayrshire cows, which calved between November 1, 1984 and March 31, 1985 in 20 commercial dairy herds in the vicinity of the Ambulatory Clinic of the Veterinary College, Hautjärvi, and which belonged to milk recorded herds, were selected for the study population. To get a convenient sampling procedure, the number of primiparous was limited to 17 (i.e. 1 heifer per farm).

The cows were milked in standings twice a day and individually fed a daily diet of hay silage (20–30 kg) and dry hay (2–5 kg). They were given 2–10 kg of grain based on the milk yield. The mean milk yield per year was 6060 kg of 4 % FCM (range 4000–8500 kg).

Liver samples were obtained by percutaneous needle biopsy from each animal at 1 week (range 4 to 10 days) and 8 weeks (range 53 to 59 days) after calving. Blood samples were taken at 1, 4 (range 25 to 31 days) and 8 weeks after calving. During the same visit that the blood samples were taken, a clinical examination was performed and a condition score (*Ministry of Agriculture, Fisheries and Food* 1978) was assessed for each animal. The cases of parturient paresis and ketosis treated by a veterinarian were obtained from the veterinary records of the Ambulatory Clinic.

Liver morphology and biochemical studies

Liver samples were immediately cut into small (1 mm^3) pieces and processed as described previously (*Gröhn & Lindberg* 1982). Stereological analysis of liver morphology was also performed as described earlier (*Gröhn et al.* 1983). Plasma and perchloric acid supernatants of whole blood were stored at – 20°C until analysed. Ketone body concentrations were measured by the method of *Työppönen & Kauppinen* (1980) and glucose concentration by the glucose oxidase method (*Jacobsen* 1960). Plasma enzyme activity was determined by the following methods: Ornithine carbamyltransferase (OCT) by the method of *Ohshita et al.* (1976), iditol (sorbitol) dehydrogenase (ID) by that of *Gerlach & Hiby* (1974), aspartate aminotransferase (AST) by the standard method of the *Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology* (1974). Albumin was determined by the bromcresol green method (*Doumas et al.* 1971) and total protein by the biuret method (*Welchselbaum* 1946).

Statistics

To satisfy the distributional condition of normality, logarithms of liver-specific enzymes were used (*Dixon & Massey* 1969). The BMDP Biomedical Computer Program (*Dixon* 1981) was applied in the following way: one-way analysis of variance for 3 groups, Student's unpaired t-test for 2 groups and Chi-square statistics for frequency comparisons. The Bonferroni test was used as a post test in comparison of the groups in the analysis of variance.

Results

There were 105 Finnish Ayrshire cows in the original study population. Three cows were slaughtered before the first sampling (due to teat injury, prolapsus uteri and dystocia respectively). The distribution of the 102 cows by the number of parity was 1–2 = 46.1 %, 3–4 = 33.3 %, 5–6 = 12.8 %, and > 6 = 7.8 %. A comparison of some descriptive variables, blood characteristics and the percentage of fat in the liver for the 102 cows at 1, 4 and 8 weeks after calving is shown in Table 1. The cows lost weight and the condition score decreased significantly after calving. There were statistically significant differences among the groups in blood acetoacetate, beta-hydroxybutyrate and glucose concentrations and liver fat infiltration. Plasma activities of the liver-specific enzymes, OCT and ID, and albumin and total protein concentrations differed, as well.

The cows were grouped also by the percentage of fat in the liver at 1 week (4–10 days) after calving. The mean percentage of fat in the liver parenchyma of 102 cows was 3.9 ± 0.5 % (range from 0 % to 21.4 %). Eighty-nine out of 102 cows had less than or equal to 9 % of fat in the liver (mean 2.3 ± 0.2 %). Thirteen cows had more than 9 % (mean 14.9 ± 1.2 %). A comparison of several variables for the two groups is given in Table 2. Only blood acetoacetate and plasma AST differed significantly between the groups. Overall 10.4 % of the 102 cows were treated by a veterinarian for parturient paresis and 13.5 % for ketosis during the study period.

The third system under which the cows were grouped was by blood beta-hydroxybutyrate concentration at 4 weeks after calving (Table 3, *Gröhn et al.* 1983). Groups were designated control (< 1.0 mmol/l), mildly ketotic

Table 1. Comparison of some descriptive variables, blood characteristics, and the percentage of fat in the liver for 102 Finnish Ayrshire cows at 1 (4–10 days), 4 (25–31 days) and 8 (53–59 days) weeks after calving by one-way analysis of variance.

	Weeks post-partum		
Variable	1	4	8
Weight, kg	573 ± 6^a	–	538 ± 6^b
Condition score	3.20 ± 0.10^a	2.80 ± 0.10^b	2.60 ± 0.10^c
Acetoacetate, mmol/l	0.14 ± 0.01^a	0.29 ± 0.03^b	0.21 ± 0.02^c
Beta-hydroxy butyrate, mmol/l	0.83 ± 0.04^a	1.52 ± 0.09^b	1.47 ± 0.12^b
Glucose, mmol/l	2.09 ± 0.04^a	2.38 ± 0.06^b	2.89 ± 0.06^c
Fat in liver, %	3.90 ± 0.50^a	–	1.20 ± 0.30^b
Log AST^d	1.95 ± 0.02^a	1.89 ± 0.01^b	1.90 ± 0.01^b
log IU liter^{-1}	(98.50 ± 7.80)	(79.80 ± 2.10)	(82.30 ± 2.00)
Log OCT^e	0.71 ± 0.01^a	0.80 ± 0.02^b	0.78 ± 0.02^b
log IU liter^{-1}	(5.50 ±0.20)	(6.70 ± 0.30)	(6.60 ± 0.30)
Log ID^f	1.00 ± 0.02^a	1.01 ± 0.01^a	1.05 ± 0.02^a
logIU liter^{-1}	(10.70 ± 0.50)	(10.80 ± 0.40)	(12.20 ± 0.70)
Albumin, g liter^{-1}	36.90 ± 0.30^a	36.80 ± 0.30^a	38.30 ± 0.30^b
Total protein, g liter^{-1}	74.50 ± 0.70^a	75.60 ± 1.00^a	78.30 ± 0.60^b

The values are means and standard errors. $^{a, b, c}$Means within variable groups sharing common superscripts do not differ significantly ($p > 0.05$). In parentheses are actual means in IU liter^{-1}. ^{d}Aspartate aminotransferase, ^{e}Ornithine carbamyltransferase, ^{f}Iditol (sorbitol) dehydrogenase.

Table 2. Comparison of some descriptive variables, blood characteristics and the incidence rate of ketosis and parturient paresis for 102 Finnish Ayrshire cows grouped by the percentage of fat in the liver at one week (4–10 days) after calving.

Variable	Fat in liver, %	
	$\leq$ 9.0 (n = 89)	> 9.0 (n = 13)
Fat in liver, %	2.30 ± 0.20^{a}	14.90 ± 1.20^{b}
Weight, kg	570.00 ± 6^{a}	591.20 ± 29^{a}
Condition score	3.20 ± 0.10^{a}	3.30 ± 0.30^{a}
Parity	3.10 ± 0.20^{a}	3.90 ± 0.70^{a}
Milk yield, kg yr^{-1} 4 % FCM	6339 ± 131^{a}	6019 ± 467^{a}
Maximum milk yield kg day^{-1}	26.70 ± 0.50^{a}	$25.90 \pm 1{,}50^{a}$
Acetoacetate, mmol/l	0.13 ± 0.01^{a}	0.21 ± 0.04^{b}
Beta-hydroxybutyrate, mmol/l	0.81 ± 0.05^{a}	0.98 ± 0.1^{a}
Glucose, mmol/l	2.08 ± 0.04^{a}	2.09 ± 0.12^{a}
Log AST[c] log IU $liter^{-1}$	1.94 ± 0.02^{a} (96.30 ± 9,10)	2.04 ± 0.03^{b} (113.80 ± 8.40)
Log OCT[d] log IU $liter^{-1}$	0.71 ± 0.01^{a} (5.40 ± 0.20)	0.71 ± 0.04^{a} (5.60 ± 0.70)
Log ID[e] log IU $liter^{-1}$	1.01 ± 0.02^{a} (10.80 ± 0.50)	0.98 ± 0.04^{a} (10.20 ± 1.00)
Albumin, g $liter^{-1}$	37.10 ± 0.40^{a}	35.50 ± 1.00^{a}
Total protein, g $liter^{-1}$	74.10 ± 0.60^{a}	77.10 ± 3.10^{a}
Incidence rate of paresis puerperalis, %	7.80 ± 2.90^{a}	23.10 ± 12.20^{a}
Incidence rate of ketosis, %	10.10 ± 3.20^{a}	30.80 ± 13.30^{a}

The values are means and standard errors. [a, b]Means within variable groups sharing common superscripts do not differ significantly ($p > 0.05$). In parentheses are actual means in IU $liter^{-1}$. [c]Aspartate aminotransferase, [d]Ornithine carbamyltransferase, [e]Iditol (sorbitol) dehydrogenase.

(1.0–3.0 mmol/l) and severely ketotic (> 3.0 mmol/l). Blood ketone body and glucose concentrations differed among the groups. Severely ketotic cows had clinical paresis puerperalis and ketosis more frequently than mildly ketotic and control cows. Ketotic cows were slightly older and produced more milk, but the differences were not statistically significant (Table 3).

Discussion

Both overfeeding in the dry period (e.g. *Morrow et al.* 1979) and negative energy balance in early lactation (e.g. *Roberts et al.* 1981) are important factors contributing to fatty liver. Generally, in countries with small family farms like Finland, the cows are individually fed and poor individual feeding management may be more uncommon than in countries with large farm units. On the other hand, a negative energy balance is very likely for cows producing an average 6060 kg 4 % FCM and kept indoors for 8 months per year (*Gröhn et al.* 1984). The period of negative energy balance during early lactation was indicated by blood ketone body and glucose concentrations. The cows lost weight and the condition

Tabel 3. Comparison of some descriptive variables, blood characteristics, and the incidence rate of ketosis and parturient paresis for control (n = 40), mildly ketotic (n = 46) and severely ketotic (n = 10) Finnish Ayrshire cows at four weeks (25–31 days) after calving.

Variable	Control	Mildly ketotic	Severely ketotic
Parity	2.90 ± 0.30^{a}	3.30 ± 0.30^{a}	3.60 ± 0.60^{a}
Milk yield kg yr^{-1}, 4 % FCM	6061 ± 197^{a}	5377 ± 199^{a}	6532 ± 432^{a}
Maximum milk yield kg day^{-1}	25.70 ± 0.80^{a}	26.80 ± 0.70^{a}	27.10 ± 1.30^{a}
Condition score	2.90 ± 0.10^{a}	2.80 ± 0.10^{a}	2.90 ± 0.10^{a}
Acetoacetate, mmol/l	0.10 ± 0.01^{a}	0.34 ± 0.03^{a}	0.85 ± 0.07^{b}
Beta-hydroxybutyrate, mmol/l	0.79 ± 0.03^{a}	1.72 ± 0.09^{b}	3.51 ± 0.09^{c}
Glucose, mmol/l	2.57 ± 0.09^{a}	$2.33 \pm 0.09^{a,b}$	1.84 ± 0.16^{b}
Log AST[d] log IU $liter^{-1}$	1.87 ± 0.02^{a} (76.60 ± 3.70)	1.91 ± 0.01^{a} (82.40 ± 2.40)	1.83 ± 0.10^{a} (78.80 ± 9.50)
Log OCT[e] log IU $liter^{-1}$	0.77 ± 0.02^{a} (6.30 ± 0.40)	0.82 ± 0.02^{a} (7.00 ± 0.30)	0.86 ± 0.05^{a} (7.60 ± 0.80)
Log ID[f] log IU $liter^{-1}$	0.99 ± 0.02^{a} (10.30 ± 0.60)	1.02 ± 0.02^{a} (11.00 ± 0.50)	1.05 ± 1.05^{a} (11.90 ± 1.30)
Albumin, g $liter^{-1}$	36.40 ± 0.50^{a}	36.90 ± 0.50^{a}	37.50 ± 0.90^{a}
Total protein, g $liter^{-1}$	75.60 ± 1.00^{a}	75.50 ± 2.00^{a}	75.50 ± 1.80^{a}
Incidence rate of paresis puerperalis, %	2.50 ± 2.50^{a}	$13.00 \pm 5.00^{a,b}$	$30.00 \pm 15.30^{b,c}$
Incidence rate of ketosis, %	15.00 ± 5.70^{a}	6.50 ± 3.70^{a}	40.00 ± 16.30^{b}

96 cows grouped by blood beta-hydroxybutyrate concentration with cut off points of 1.0 and 3.0 mmol/l. The values are meand and standard errors. [a, b, c]Means within variable groups sharing common superscripts do not differ significantly ($p > 0.05$). In parentheses are actual means in IU $liter^{-1}$. [d]Aspartate aminotransferase, [e]Ornithine carbamyltransferase, [f]Iditol (sorbitol) dehydrogenase.

score decreased, as well. The percentage of cows treated for parturient paresis and ketosis was higher than in an earlier report in Finland (*Gröhn et al.* 1986), which may indicate a more active veterinary service and better record keeping. The percentage of liver fat at one week after calving was much lower than that reported in the review by *Reid & Roberts* (1983). Using the classification by *Reid & Roberts* (1983), only 2 out of 102 cows in our study had a fatty liver (more than 20 % fat in the liver) 1 week after calving. Therefore, it was decided to use our own, milder fatty liver grouping. Thirteen cows (12.7 % of the cows) had more than 9 % fat in the liver (mean 14.9 ± 1.2 %). Only blood acetoacetate and plasma AST activity distinguished these cows from the other cows. A fatty liver does not seem to be a problem in this study.

When the results of this study are compared with other studies on fatty liver, disease definition, diagnostic criteria, the source and method of collecting data and the method for the estimation of fat infiltration have to be considered. We used Toluidine blue stained Epon sections from liver tissues immediately fixed in glutaraldehyde and osmium tetroxide. The morphology in these sections is superior to that achieved with paraffin embedding, and fat droplets are easily recognized and distinguished from other components. This method has also been used in recent work on fatty liver (*Reid*

& Collins 1980). Our objective was not to sample a risk population, but to investigate a representative sample of Finnish Ayrshire cows in milk recorded herds. A random sample would have been an ideal choice. However, we decided to use the purposive sampling procedure in the vicinity of the Ambulatory Clinic. This more practical approach may limit the possibility of generalizing the results to other milk recorded herds in Finland. On the other hand, the sample, which included all dairy cows in 20 commercial dairy herds in a typical dairy cow area during the study period, should be representative.

Another consideration is the sample size. If 1 of 3 dairy cows had a fatty liver 1 week after calving (*Reid & Roberts* 1983), the sample size of 102 cows should have been large enough to indicate that. However, if the incidence rate of fatty liver is much lower, as in this study, a sample size should be larger to obtain an exact incidence rate. In our earlier report (*Gröhn et al.* 1983), the extent of fat infiltration of the liver even in severely ketotic cows was less than 20 % (16.9 ± 2.6 %).

Although there were not statistically significant differences in the incidence rate of parturient paresis and ketosis between the cows with < 9 % and the cows with > 9 % fat in the liver, the incidence rates suggest that such differences might be associated with fatty liver. One has to consider that the occurrence of ketosis and parturient paresis was investigated using the binomial scale, and the threshold characteristics are statistically difficult to handle.

Based on previous (*Gröhn et al.* 1983, *Gröhn* 1985) and current results the severity of fat infiltration in the liver of Finnish Ayrshire cows does not seem to be as problematic as in other countries. It is obvious that fat infiltration, per se, in the liver does not explain all postparturient disturbances. Other metabolic and management events are also involved.

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Sammendrag

Fettinfiltration i levern hos finska Ayrshire kor under tidig laktation.

Förekomsten och graden av fettinfiltrering i levern hos finska Ayrshire kor en samt åtta veckor efter kalvningen studerades. Alla multipara (n = 88) och några primipara (n = 17) kor som kalvade i 20 besättningar hörande till mjölkkontrollregistret undersöktes. Korna gick ner i vikt och konditionspoängen minskade signifikativt ($p < 0.05$) under tidig laktation. Blodprov togs en, fyra och åtta veckor efter kalvningen. Koncentrationen av ketonkroppar var störst fyra veckor efter kalvningen. Albumin- och totalproteinkoncentrationerna samt ornitin carbamyltransferasaktiviteten i blodet ökade signifikativt ($p < 0.05$) från en till åtta veckor efter kalvningen. Fettprocenten i levern (v/v) var signifikativt högre ($p < 0.05$) en vecka (3.9 ± 0.05 %) än åtta veckor (1.2 ± 0.3 %) efter kalvningen. Åttionio av 102 kor hade mindre än eller lika med 9 % fett i levern (medeltal 2.3 ± 0.2 %) och tretton kor hade mera än 9 % (medeltal 14.9 ± 1.2 %) en vecka efter kalvningen. Bara värdena för acetoacetat i blodet och aspartat aminotransferas i plasma var signifikativt olika i grupperna. I gruppen med fettlever (> 9 %) var frekvensen behandlade fall av ketos 30.8 ± 13.3 % och kalvningsförlamning 23.1 ± 12.2 %, respektive 10.1 ± 3.2 % och 7.8 ± 2.9 % i gruppen med lägre fettinfiltrering. Skillnaderna var inte signifikanta.

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