Prevalence and indicators of post partum fatty infiltration of the liver in nine commercial dairy herds in The Netherlands

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Abstract

A field study was performed with 218 dairy cows in nine dairy herds in order to investigate the prevalence of post partum fatty infiltration of the liver and its relationship to subsequent body condition scores, blood variables and milk production. The mean concentration of triacylglycerols in the liver was 61.2 mg/g wet liver tissue. The prevalence of fatty liver (more than 50 mg triacylglycerol in 1 g wet liver tissue) was 54.1%. Serum non-esterified fatty acids, urea and blood glucose concentrations appeared to be significant indicators of hepatic lipidosis between 6 and 17 days post partum ($R^2 = 0.33$). High milk production and large losses of body condition score in early lactation were significant indicators of hepatic lipidosis from a retrospective point of view ($R^2 = 0.22$). Single body condition scores were not significantly related to the concentration of triacylglycerol in the liver. It was concluded that, according to the classification used, fatty liver seems to be fairly common in early lactating dairy cows. Although correlation coefficients were too low to enable a precise and accurate description of liver triacylglycerol content, we found some biologically explicable variables in commercial herds that were significantly related to the liver triacylglycerol content. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Cattle; Pathology; Fatty liver; Prevalence; Body condition score

1. Introduction

Fatty liver syndrome or hepatic lipidosis is characterised by infiltration of triacylglycerol (TAG) in the liver. It may be related to metabolic disorders that are often associated with a variety of clinical abnormalities including reproductive and production disorders, and reduced immunity (Morrow et al., 1979; Reid et al., 1979; Reid, 1980; Reid and Roberts, 1982; Higgins and Anderson, 1983; Gerlof
and Herdt, 1984; Wentink et al., 1997). High concentrations of TAG in the liver are most often found in early lactation, with the exception of heifers (Reid et al., 1979, 1983a,b; Reid, 1980; Gerloff et al., 1986; Van den Top et al., 1996). Cases of high concentrations of TAG in the liver are rarely found in pregnant cows (Gerlof and Herdt, 1984).

The fatty infiltration of the liver is due to a negative energy balance, arising from a relatively low peri-parturient dry matter intake accompanied by high energy demands for milk production (Bertics and Grummer, 1999). In most cows, the fatty infiltration seems to be transient and not pathological (Veenhuizen et al., 1991; Van den Top et al., 1996). The reason why the negative energy balance that affects almost every dairy cow results in some cases in fatty infiltration of the liver and is almost absent in other cases, is still not fully understood.

The amount of TAG in the liver is usually quantified by taking a liver biopsy. Gaal et al. (1983) showed that two commonly used techniques are highly correlated. Testing of blood hepatic enzyme activities is poorly correlated with hepatic TAG with the exception of elevated aspartate aminotransferase (Reid et al., 1983b) and low glycerolphosphate acyltransferase activities (Van den Top et al., 1996). Hepatic lipidosis is frequently associated with increased serum non-esterified fatty acid (NEFA) concentrations and low blood glucose concentrations (Higgins and Anderson, 1983; Reid et al., 1983b; Veenhuizen et al., 1991; Wentink et al., 1997).

Although the negative impact of the TAG accumulation in the liver on health and reproduction of dairy cows is commonly discussed, data on its prevalence under commercial field conditions is limited. Also, more knowledge on the relationship of high liver TAG with variables that can be measured under field conditions, such as body condition scores (BCS) and blood values, would be of great relevance in order to determine the consequences of fatty liver on health and reproduction in commercial herds.

This paper describes a longitudinal field study. The goal was to determine the prevalence of fatty liver in cows from nine Dutch dairy herds and to investigate its relationship with commercial available variables, like blood chemistry concentrations, milk production and post partum BCS.

2. Materials and methods

2.1. Study population

The field study was performed in nine dairy herds during the housing seasons of 1995–1996 and 1996–1997. Of the nine herds, ranging in size from 50 to 120 cows, six herds were included in both seasons and three herds were included in only one season. The herds were selected as a convenience sample from those herds participating in a veterinary herd health programme with monthly farm visits by veterinary practitioners. All cows were housed in freestalls with cubicles and were fed twice daily a ration in which grass silage was the main component (>60% of dry matter). The dairy herds, as well as the veterinary practice, were located in the dairy-orientated northern part of The Netherlands. The prevalence of fatty liver was not evaluated in the herds before. There was, however, no history of abnormal disease incidence or confirmed fatty liver cases in any of the selected herds.

With the exception of heifers, all cows (no selection was executed) which calved during the housing season were included in the study. A total of 181 healthy cows were included in the study in 1995–1996. Due to haemolysis of blood samples, only another 37 cows were included in 1996–1997. Only 13 cows participated in both years. The number of cows from each herd during the two housing seasons is presented in Table 1. The average 4% fat-corrected milk production of all herds in 1996 is given in Table 1, in order to provide an approximate indication of the production level of the participating herds. These data were, however, not included in any of the analyses.

2.2. Sampling procedures

A blood and liver sample from each cow was taken between 6 and 17 days post partum, at which time serum NEFA concentrations and liver TAG contents are reported to be fairly stable (Studer et al., 1993; Van den Top et al., 1996). Although some diurnal variation in serum NEFA concentrations has been reported (Bines et al., 1983), we did not sample at a fixed time of the day. However, all participating
Table 1
Number of cows studied and recorded milk production per herd

| Herd | Number of cows studied 1995–1996 | Number of cows studied 1996–1997 | Milk yield
<table>
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<tr>
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<tbody>
<tr>
<td>A</td>
<td>43</td>
<td>9</td>
<td>9284</td>
</tr>
<tr>
<td>B</td>
<td>19</td>
<td>4</td>
<td>9632</td>
</tr>
<tr>
<td>C</td>
<td>23</td>
<td>6</td>
<td>8853</td>
</tr>
<tr>
<td>D</td>
<td>33</td>
<td>3</td>
<td>9735</td>
</tr>
<tr>
<td>E</td>
<td>17</td>
<td>0</td>
<td>9484</td>
</tr>
<tr>
<td>F</td>
<td>16</td>
<td>0</td>
<td>9092</td>
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<tr>
<td>G</td>
<td>19</td>
<td>2</td>
<td>8619</td>
</tr>
<tr>
<td>H</td>
<td>11</td>
<td>6</td>
<td>9233</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>7</td>
<td>10 304</td>
</tr>
</tbody>
</table>

*Average 4% fat-corrected milk yield per cow on each herd in 1996 (kg).

farmers ensured the permanent presence of feed in the bunk during the day, resulting in a considerable amount of daily feed refusals. This reduced the risk of a circumspect large feed intake that is most likely the cause of the diurnal variation in serum NEFA concentration. Also, we were especially interested in the use of some practically obtained variables to estimate the liver TAG content rather than elucidating its aetiology. This urged us to take samples irrelevant of the time of the day.

At the same time as the sampling for blood and liver tissue, a single BCS of the cows was recorded (BCS 1) on a scale from 1 (emaciated) to 5 (very fat) in steps of 0.25 (Edmonson et al., 1989). The recording of the BCS was repeated between 38 and 50 days post partum (BCS 2). A regular daily milk recording by the Dutch dairy board (CR Delta/NRS) was done on every herd, with an interval of 4 or 6 weeks (depending on the herd). We used only the second recording post partum in our models. This uncorrected recording, which was thus done between 4 and 12 weeks post partum, was judged to be a suitable and practically available variable for determining individual milk production. There are of course more accurate methods to describe the milk production of dairy cows in early lactation. Nevertheless, we decided to use the data which is present on the farm without any adjustments, in order to reflect field circumstances as much as possible.

Blood samples were taken from the coccygeal blood vessels into evacuated tubes (Vacuette) with either NaFl for glucose determination or with Li-heparin/paraoxon tubes for NEFA determination. A tube without additive was used for serum urea analysis. Percutaneous biopsy of the liver was performed with a Urocut biopsy needle, without using the core of this instrument. Prior to the biopsy, clipping and desinfection was applied to the biopsy site. Subsequently, a stab incision was made at the level of the greater trochanter in the 11th intercostal space on the right side of the cow. The liver tissue, obtained by moving the biopsy needle several times in the direction of the contralateral elbow, was kept in physiologic saline under refrigeration until analysed (there was no effect of this short term storage on the measured TAG amount in liver tissue; unpublished data). The unprocessed blood samples were, together with the liver tissue, refrigerated before and during transport to the laboratory, once every week.

Blood was analysed for glucose (mmol/l), serum urea (mmol/l) and NEFA (mmol/l). The concentration of TAG was measured (mg/g) in the liver biopsy and classified as low (0–50 mg/g), moderate (51–100 mg/g) and severe (>100 mg/g) TAG accumulation in the liver (Gaal et al., 1983). All analyses were performed at the laboratory (Utrecht) by methods described elsewhere (Van den Top et al., 1996).

2.3. Applied statistical methods

A paired t-test was used to compare mean BCS 1 to mean BCS 2. The difference between the two
measurements (\(\Delta BCS\)) was computed for each individual cow and was included in one of the models described below.

Four general linear models were developed with the liver TAG as the dependent variable. Herd was included as a fixed-effect classification variable in all models. All other variables were treated as fixed-effect continuous variables. Main effects of each variable were evaluated before considering quadratic effects and two-way interactions. Only one variable was removed or entered manually at each step depending on their significance in the model.

The first model, annotated as the estimation model, contained variables measured between 6 and 17 days post partum in order to estimate with those variables the amount of TAG in the liver at that moment. Blood glucose, serum NEFA and serum urea concentrations were included in this model.

The second model, annotated as the retrospective model, contained variables available several weeks after the liver TAG measurement: the regular milk recording between 4 and 12 weeks post partum and the change in BCS during the first period of lactation. Interactions between these variables were also evaluated. This model may be used in retrospect to detect herds with a high prevalence of cows with elevated concentrations of TAG in the liver.

Two other models (third and fourth model) contained only BCS 1 and BCS 2, respectively, as independent variables. Additionally, the correlation (Pearson's) between BCS 1 and \(\Delta BCS\) was calculated.

Residuals were output and examined for normality. All analysis were done with the Statistix software (Statistix 4.1, Analytical Software) and statistical significance was defined at \(P < 0.05\).

3. Results

The milk production of the participating cows at week 4 to 12 post partum varied between 24.1 and 60.4 kg per day. The mean milk production of these cows in that period was 41.0 (S.D. = 6.71) kg per day.

The prevalence of moderate plus severe fatty liver (Gaal et al., 1983) in this cohort of 218 cows was 54.1%. The class with low fatty liver contained 100 cases (45.9%), the moderate class 87 cases (39.9%) and the severe class 31 cases (14.2%). The concentration of TAG in the liver varied between 12.7 and 208.6 mg/g wet weight with a mean of 61.2 mg/g (S.E. mean 2.6). A frequency histogram is presented in Fig. 1.

The mean blood glucose concentration was 2.93 mmol/l (S.D. = 0.44), mean serum NEFA was 0.48 mequiv./l (S.D. = 0.23) and mean serum urea was 5.28 mmol/l (S.D. = 1.13).

The mean BCS between 6 and 17 days post partum (BCS 1) was 3.0 (S.D. = 0.7) and between 38 and 50 days post partum (BCS 2) was 2.3 (S.D. = 0.5). These two mean BCSs were different from each other \((P < 0.0001)\) according to the \(t\)-test. The mean difference between BCS 1 and BCS 2 was 0.7 (S.D. = 0.5). The correlation between BCS 1 and \(\Delta BCS\) was 0.6 \((P < 0.0001)\).

Tables 2 and 3 present outcomes of the estimation and retrospective models, respectively. Both analyses used the liver TAG content as the dependent variable. Serum NEFA and urea concentrations and blood glucose concentrations were the only significant variables in the estimation model. No quadratic effects and two-way interactions were significant. In the retrospective model, only the milk recorded between weeks 4 and 12 post partum and the difference between BCS 1 and BCS 2 were significantly related to the hepatic TAG. Quadratic variables and interactions between the variables were also not significant in this model. The estimation model explained approximately a third \((R^2 = 0.33)\).
Table 2
Regression coefficients, standard errors (S.E.s) and P values of the estimation model for the description of the liver triacylglycerol content (mg/g) between 6 and 17 days post partum (n = 218 cows) (R² of the model: 0.33)

<table>
<thead>
<tr>
<th>Regression coefficient</th>
<th>S.E.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>151.15</td>
<td>24.94</td>
</tr>
<tr>
<td>Herd ± ± ±</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>NEFA⁴</td>
<td>68.42</td>
<td>11.13</td>
</tr>
<tr>
<td>Glucose²</td>
<td>–31.56</td>
<td>5.92</td>
</tr>
<tr>
<td>Urea³</td>
<td>–4.50</td>
<td>2.37</td>
</tr>
</tbody>
</table>

⁴ Serum non-esterified fatty acids between days 6 and 17 post partum (mequiv./ml).
⁵ Blood glucose between days 6 and 17 post partum (mmol/l).
⁶ Serum urea between days 6 and 17 post partum (mmol/l).

and the retrospective model explained approximately a fifth (R² = 0.22) of the variation in TAG concentration. The relationships between the liver TAG and the most significant variable in the estimation and retrospective models are depicted in Figs. 2 and 3.

The BCS 1 and BCS 2 values that were included in the third and fourth models, respectively, were not significant (P = 0.15 and P = 0.21).

4. Discussion

The percentage of moderate plus severe liver TAG fatty liver of 54.1% found in this study is within the range of those reported in other studies (Reid, 1980; Reid et al., 1983a; Gerloff et al., 1986). It is not discussed in this paper whether the encountered severity of fatty liver is of clinical importance or not, as is suggested in many publications (Morrow et al., 1979; Reid et al., 1979; Reid, 1980; Reid and Roberts, 1982; Higgins and Anderson, 1983; Gerloff et al., 1986). The percentages obtained do not necessarily reflect the prevalences in the involved herds or in The Netherlands, as only a certain number of cows from nine dairy herds were used in this study. We expect, however, that these prevalences are fairly typical, because the percentages of this study are comparable with percentages reported in other studies and cows were selected by calving dates only. Thus, the potential impact of fatty liver may be is substantial, because approximately 14% of
the cows in this study appeared to have severe fatty liver.

The combination of high serum NEFA and low blood glucose concentrations is commonly seen and biologically explicable during the negative energy balance (NEB) of post partum dairy cows, because it reflects the shortage of energy and the subsequent mobilisation of body fat reserves. A relationship with these variables and with liver TAG content, which is found in the estimation model, is therefore not surprising. The significant effect of low serum urea concentrations in the same model may be explained by a saving in NH₃ metabolism due to NEB, although NEB could also evoke higher NH₃ and urea concentrations when proteins are metabolised for energy supply.

The two single BCS measurements were not significant in any of the models that described the liver TAG. The differences between the two BCSs, ΔBCS, was significant in the retrospective model and may therefore be of some use. Although cows with a high BCS have more fat to mobilise, which is illustrated with our data, a lot of them did not have a high concentration of TAG in the liver. It is theoretically possible that the cows with a high concentration of liver TAG had an even higher BCS at parturition and experienced already a severe loss in BCS before our first measurement. It is also possible that at least a part of the high TAG concentration depends on differences between cows in how they cope with the high energy demands and concomitant NEFA mobilisation in early lactation. As a consequence of this theory, cows with high as well as with low BCS between 6 and 17 days post partum can have high concentrations of TAG in the liver, which would be in agreement with our results. Because a measurement of the BCS immediately before or after parturition is lacking in this study, it is not possible to discern between these two possibilities.

Although BCS in early lactation was not a significant variable in any of the models, we found a fairly large correlation between BCS 1 and ΔBCS, which is probably due to the fact that cows with a high BCS 1 have more possibilities to show a large ΔBCS during early lactation than cows with a low BCS 1. Thus, although a single BCS between day 6 and 17 post partum may give no information about a mobilisation process, there may be some effects of BCS 1 on liver TAG that are completely modulated by the ΔBCS, which in turn reflects how animals cope with NEB. Similar conclusions were drawn by other researchers (Reid, 1980).

Minimising NEB in order to avoid high TAG concentrations in the liver is theoretically possible by either maximising energy uptake or minimising the demands for energy. The demands for energy are necessary for the genetically-related high milk production; therefore, high energy intake should be achieved. In order to achieve a high energy intake, it is important to provide a palatable diet with a high energy concentration in early lactation. Restricting feed according to requirements in the dry period is another factor that has impact on the post partum feed intake. In comparison with an ad libitum feeding regime, restriction had no effect on total milk production and resulted in higher blood levels of glucose, lower serum levels of NEFA post partum and increased peri-parturient dry matter intake (Kunz et al., 1985; Van den Top et al., 1996). Post partum energy deficit in the restricted feeding group was also less marked.

Body condition score at parturition seems to be another factor that modulates feed intake in early lactation. Investigations (Garnsworthy and Topps, 1982; Garnsworthy and Jones, 1987) showed that cows with a higher BCS are more likely to have poor appetites post partum and have a larger gap between calving and the time of maximum dry matter intake. This observation is, however, not demonstrated in every study (Van den Top et al., 1996). Our data showed that BCS 1 was indeed related to ΔBCS (correlation coefficient = 0.6), but a relationship between BCS 1 and liver TAG was lacking. Because negative effects of a high BCS (relative to low) are more often reported, we would suggest a BCS of 3–3.5 at calving to be a reasonable goal.

A dry cow transition period (consisting of cows in their last weeks of gestation) is a fourth factor that affects NEB. It is currently believed that the development of the ruminal flora and the adaptation of the ruminal papillae that is necessary for a smooth transition from dry cow to early lactation is ameliorated when a dry cow transition group is created (Garnsworthy and Jones, 1987; Studer et al., 1993;
Goff and Horst, 1997; Dirksen et al., 1999). In The Netherlands for example, a dry cow transition period of 1 or 2 weeks is commonly advised.

Determining BCS at several times during lactation, in order to get a ΔBCS, may be useful function in monitoring the effect of the measures taken in the dry period on NEB in early lactation in order to avoid a possible TAG accumulation in the liver.

5. Conclusion

Milk production in early lactation, together with the ΔBCS, is a retrospective indicator and the combination of blood glucose, serum NEFA and serum urea is a good estimator of a high TAG concentration in the liver. This is probably useful as a first approach to suspected fatty liver herds in practice. Biopsy is, however, more accurate, but also more invasive in diagnosing high concentrations of TAG in the liver than combined measurements of blood glucose, serum urea and NEFA concentrations or the observation of milk production and BCS loss. Both to confirm a diagnosis in practice and in research, biopsy will therefore remain preferable. Because our data showed that ΔBCS was, in contrast to the BCS in early lactation, significantly associated with the liver TAG, suggests that the differences of TAG between cows may be explained by differences in how cows cope with NEB. Further research should be focussed on elucidating the differences between cows as to how they cope with NEB and on assessing the effects of post partum NEB and (subclinical) fatty liver on health and performance in dairy cows, as the prevalence of fatty liver is rather high.

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