ABSTRACT

As left-displaced abomasum (LDA) often occurs in cows with high contents of fat in the liver (fatty liver), a postpartum fatty liver-inducing regimen was applied to 16 cows. The main interest of the study was whether there were productive or metabolic changes in cows prior to LDA. Therefore, feed intake and milk production were monitored and blood samples were collected from the cows. The LDA occurred in 4 out of 16 dairy cows that were included in the feeding regimen. Compared to cows not developing LDA, LDA-cows had a significantly lower feed intake, 6.5 kg/d less, and milk production, 8 kg/d less, prior to clinical diagnosis of LDA. In the 10-d period preceding clinical diagnosis of LDA, blood concentrations of calcium, glucose, and insulin were significantly lower, whereas blood concentrations of nonesterified fatty acids and beta-hydroxybutyrate, as well as aspartate aminotransferase activities were significantly elevated compared to cows not developing LDA. These preclinical changes may play an important role in the pathogenesis of LDA. It is not certain, however, whether there is a causal association between these parameters and LDA.

(Key words: left-displaced abomasum, dairy cow, fatty liver)

Abbreviation key: LDA = left-displaced abomasum, NEB = negative energy balance, TAG = triacylglycerol.

INTRODUCTION

Left-displaced abomasum (LDA) is a disorder that occurs mainly in high producing postpartum dairy cows (Geishauser, 1995). The economic consequences of LDA are becoming more important as the incidence rate has been increasing to 5% of postpartum dairy cows (Geishauser et al., 2000). Normally, the abomasum contains fluid and is positioned in the ventral part of the abdo-
Table 1. Composition of the total mixed ration (TMR, DM = 455 g/kg, containing 6.42 MJ NE\textsubscript{L}/kg DM) fed throughout the experiment.

<table>
<thead>
<tr>
<th>Component</th>
<th>TMR composition (%)</th>
<th>DM basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize silage\textsuperscript{1}</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Rape seed meal\textsuperscript{2}</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Soybean meal\textsuperscript{3}</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Sugar beet pulp\textsuperscript{4}</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Minerals</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1}Characteristics of maize silage per kg DM: 213 g crude fiber, 73 g crude protein, 179 g crude ash, 335 g starch, 422 g NDF, 228 g ADF, 25 g ADL.
\textsuperscript{2}DM = 877 g/kg.
\textsuperscript{3}DM = 870 g/kg.
\textsuperscript{4}Extracted, DM = 905 g/kg.

Cows that showed sudden loss of appetite or a decreased milk production during the experimental period were clinically examined. Based on a tympanic, resonant, high-toned ping at the left abdominal wall, LDA was clinically diagnosed, and was confirmed by right flank laparotomy. After correcting the position of the abomasum the omentum major was fixated in the wound during closure of the abdomen (Trent, 1990).

Sampling Procedure and Laboratory Analyses

As we expected to detect the largest variation in blood parameters during the first three weeks, and less variation later in lactation, blood was collected from the jugular vein daily at 0700 and 1000 h during the first 22 d postpartum. Subsequently, blood was collected on d 24, 26, 28, and 31 of lactation. Additionally, blood was collected into appropriate tubes at 1200, 2200, and 2400 h on days 1 to 7, 9, 11, 12, 15, and 21 after calving. The values of multiple samples that were collected on one day were averaged. Percutaneous liver biopsy samples were taken in the 11th intercostal space during the second week of lactation (Van der Top et al., 1995). Immediately after sampling, connective tissue and blood clots were disposed of and the samples were weighed and stored at −20°C. After thawing the samples, the amount TAG was determined, expressed as mg/g wet weight of liver tissue, using a commercial test kit (kit number 337-A; Sigma Chemical Co., St. Louis, MO). Cows were categorized as moderate fatty liver cows (TAG between 50 and 100 mg/g liver tissue), severe fatty liver cows (TAG between 100 and 200 mg/g liver tissue), and extreme fatty liver cows (TAG higher than 200 mg/g liver tissue) (Gaal et al., 1983).

In all blood samples, glucose and beta-hydroxybutyrate (BHBA) concentrations were determined. In the samples of the first 21 d postpartum NEFA and insulin concentrations were determined. On d 3, 7, 9, 13, 15, and 17 postpartum gamma-glutamyl transpeptidase
(GGT) activity, aspartate amino transferase (ASAT) activity, cortisol concentrations, and calcium concentrations were determined. These parameters were analyzed using standard test kits on a Beckman CX automatic analyzer.

Additionally, cortisol concentrations were determined on d 19 and 21 after calving. Cortisol and insulin concentrations were determined by radioimmunoassay (Coat-a-Count, Diagnostic Products Corp., Los Angeles).

### Statistical Analysis

A cow that developed LDA was matched with two other healthy cows that did not develop LDA. Matching was based on parity, the level of fatty infiltration of the liver and the day of lactation. The day of clinical diagnosis of LDA is defined as d 0. Days prior to LDA were set from d –1 to d –10. The variables were analyzed with Linear Mixed Effects method, which takes dependency of the data into account and therefore is suitable to statistically analyze repeated measurements (S-PLUS 2000, MathSoft Inc., Cambridge, United Kingdom). This method uses maximum likelihood techniques for estimating the fit of the model. The identity of the cow was used as random effect. The following formula for the fixed effects was used: 

\[ Y = \text{FL-severity} + \text{Day PP} \times \text{PRIOR} \times \text{LDA}, \]

where 

- \( Y \) = feed intake, milk production and values of blood analyses;
- \( \text{FL-severity} \) = category of fatty liver, ordinal: moderate, severe, and extreme;
- \( \text{Day PP} \) = number of days postpartum, as a polynomial of the third order;
- \( \text{PRIOR} \) = day prior to LDA, between –10 and –1, as polynomial of the third order;
- \( \text{LDA} \) = occurrence of LDA, binomial; and
- \( \text{PRIOR} \times \text{LDA} = \) interaction term to analyze whether the trend over time differed between the LDA cows and control cows. By putting day postpartum and severity of fatty infiltration of the liver in the beginning of the fixed effects formula, the term PRIOR and LDA, as well as the interaction of both are adjusted for the moment after calving and the severity of fatty liver. To determine whether the day postpartum, as well as the day prior to LDA should be linear, or a polynomial of the second, third or fourth order, a likelihood ratio test was performed in order to test whether the model did significantly improve compared to the less complex model. This resulted both for the day postpartum as well as day prior to LDA in a polynomial of the third order. Significance of the difference between LDA-cows and controls prior to DA, concerning feed intake, milk production, and values of blood analyses, were analyzed in separate models. For obtaining the point estimates of LDA, the formula had been run again without interaction term. The former formula was used to determine differences between LDA-cows and their matched controls, concerning the 10-d period prior to DA. In order to evaluate significant differences on one specific day, the analysis was performed with day prior to LDA as a factor. When only one value was present on that day, either in the LDA or the control group, significance could not be analyzed, since then there is no variation. Autocorrelation structures were tested and selected based on LR-test. The normality of the residuals and the random effects in the final model were visually checked with Q-Q plots.

### RESULTS

Out of 16 cows, four developed LDA on d 4, 11, 21, and 29 after calving. Of the DA-cows, three had a severe fat infiltration of the liver (119, 187 and 200 mg/g liver tissue), while in one DA-cow the fatty infiltration of the liver was extreme (307 mg/g liver tissue). Out of 16 cows, 14 from both experimental groups revealed liver fat contents higher than 50 mg/g liver tissue in the second week postpartum. Of these cows two had extreme fatty liver, severe fatty liver occurred in seven cows, and a moderate fatty infiltration of the liver oc-

<table>
<thead>
<tr>
<th>Variable</th>
<th>LDA-cows (n = 4)</th>
<th>Control cows (n = 8)</th>
<th>Reference value (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake, kg/d of DM</td>
<td>8.64 ± 0.73</td>
<td>15.27 ± 0.68</td>
<td>. . .</td>
</tr>
<tr>
<td>Milk production, kg</td>
<td>23.36 ± 6.05</td>
<td>31.50 ± 7.64</td>
<td>. . .</td>
</tr>
<tr>
<td>NEFA, mmol/l</td>
<td>1.23 ± 0.11</td>
<td>0.79 ± 0.05</td>
<td>0–0.8</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>2.46 ± 0.22</td>
<td>2.84 ± 0.05</td>
<td>2.5–4.0</td>
</tr>
<tr>
<td>Insulin, μIU/ml</td>
<td>1.12 ± 0.20</td>
<td>3.73 ± 0.21</td>
<td>0–5</td>
</tr>
<tr>
<td>BHBA, mmol/l</td>
<td>3.45 ± 0.39</td>
<td>0.99 ± 0.09</td>
<td>0–1.2</td>
</tr>
<tr>
<td>GGT, U/l</td>
<td>45.38 ± 8.02</td>
<td>26.13 ± 1.62</td>
<td>0–27</td>
</tr>
<tr>
<td>ASAT, U/l</td>
<td>130.00 ± 20.97</td>
<td>75.13 ± 5.87</td>
<td>10–70</td>
</tr>
<tr>
<td>Cortisol, nmol/l</td>
<td>2.27 ± 0.71</td>
<td>4.18 ± 1.31</td>
<td>15–19</td>
</tr>
<tr>
<td>Calcium, mmol/l</td>
<td>2.56 ± 0.10</td>
<td>2.74 ± 0.06</td>
<td>2.3–3.2</td>
</tr>
</tbody>
</table>
curred in five out of 14 cows. Maximum TAG contents in the liver was 307 mg/g tissue in the second week postpartum. The LDA-cows originated from the fatty liver induced group (n = 3) as well as the control diet group (n = 1). From the matched controls, nine came from the fatty liver induced group, and one from the group that had received the control diet.

Average daily feed intake, milk production and blood values in the 10-d period before development of LDA, as well as the values of the matched control cows are given in Table 2. Not adjusted for the day after calving, the concentrations of NEFA, BHBA, GGT and ASAT are higher in cows developing LDA compared with the matched control cows. Table 3 shows significant differences between LDA- and control cows for the variables tested in the linear mixed effects model. Adjusted for the day postpartum and the severity of fatty liver, and leaving out the interaction term (Prior × LDA), we found that feed intake (−9.5 kg/d), milk production (−6.4 kg/d), calcium-, glucose- and insulin concentrations (respectively −0.26 mmol/l, −0.37 mmol/l, and −2.7 μIU/ml) were significantly lower in cows that developed LDA. Nonesterified fatty acids (0.36 mmol/l), BHBA (2.2 mmol/l) and ASAT (52.2 U/l) levels were elevated in those cows. These differences can also be detected when looking at Figures 1 to 6. These figures show, besides the difference contributed by LDA, also the trends over time: milk production, feed intake, BHBA, NEFA, insulin and ASAT in DA-cows and their control cows within the 10-d period before LDA diagnosis are presented. When comparing the parameters on individual days, BHBA concentrations were elevated, whereas the insulin concentrations were lowered in nearly the complete 10-d period in LDA-cows compared to their matched control cows (Figures 3 and 5). At d 4 prior to LDA detection, the DM intake was significantly lower in LDA-cows, which was, with respect to milk production, also the case at three days prior to LDA (Figure 1 and 2). NEFA was significantly elevated on one day before LDA occurred (Figure 4).

DISCUSSION

Retrospective analysis of results of a feeding trial revealed differences in parameters between animals that developed a clinically persistent LDA and animals...
that did not. Cows that would develop displacement of
the abomasum in general had lower feed intake, lower
milk production, decreased blood calcium levels, ele-
vated blood ketone body and NEFA concentrations, and
high activity of ASAT compared to the matched ani-
mals. These findings are in accordance with previous
reports concerning differences in the preclinical stage
of LDA (Detillieux et al., 1997; Geishauser et al., 1998;
Geishauser et al., 2000; Østergaard and Grøhn, 2000).
In the present study, two different phases in the 10-
d period can be identified based on the parameters that
were evaluated. First, the period between ten and five
days prior to LDA, and secondly the phase from four
days before LDA until the day of DA. The latter period
can be interpreted in two ways. First, the abomasum
can be sub-clinically dislocated several days before clin-
ical diagnosis and the preclinical changes in the param-
eters are due to alterations of the position of the aboma-
sum. The other interpretation is that changes in these
last 4 days prior to LDA are putative (co-)initiators for
DA, which is also mentioned in literature (Østergaard
and Grøhn, 2000).
Reduced feed intake prior to LDA in fatty liver cows
is the key element in the observed changes in param-
ters. A result of reduced feed intake is poor rumen fill.
The poorly filled rumen enables the abomasum to shift
to the left and finally the abomasum dislocates clinically
(Dirksen, 1962; Van Winden et al., 2002b). As a conse-

Figure 2. Feed intake (dry matter, DMI, kg/cow/day) in LDA-cows prior to LDA (○, – –) and their matched controls (●, —). The line represents the polynomial regression line of the third order. Day 0 is the moment of LDA-development. *: significant difference ($P < 0.10$) between LDA- and control cows at that day.

Figure 3. Beta-hydroxybutyrate (BHBA, mmol/l) concentration in blood of LDA-cows prior to LDA (○, – –) and their matched controls (●, —). The line represents the polynomial regression line of the third order. Day 0 is the moment of LDA-development. *: significant difference ($P < 0.10$) between LDA- and control cows at that day.

Figure 4. Nonesterified fatty acid concentration (NEFA, mmol/l) in blood of LDA-cows prior to LDA (○, – –) and their matched controls (●, —). The line represents the polynomial regression line of the third order. Day 0 is the moment of LDA-development. *: significant difference ($P < 0.10$) between LDA- and control cows at that day.

Figure 5. Insulin concentrations (μIU/ml) in blood of LDA-cows prior to LDA (○, – –) and their matched controls (●, —). The line represents the polynomial regression line of the third order. Day 0 is the moment of LDA-development. *: significant difference ($P < 0.10$) between LDA- and control cows at that day.
Aspartate aminotransferase (ASAT, U/l) activity in blood of LDA-cows prior to LDA (○, —) and their matched controls (●, —). The line represents the polynomial regression line of the third order. Day 0 is the moment of LDA-development.

Figure 6.


