Evaluation of classification modes potentially suitable to identify metabolic stress in healthy dairy cows during the peripartal period

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ABSTRACT: The transition of pregnancy to lactation, with the concomitant negative energy balance during early lactation, requires substantial adaptive performance of the cow. Apart from clinical disease problems, the identification of cows with suboptimal adaptation is relevant to be able to adequately treat these animals or modify the ration. Effective approaches are necessary to provide maximal information at the earliest time possible. We therefore aimed to identify a measurement that, when applied at a defined point in time relative to calving, was relevant as much as possible to other information on metabolic and health status during early lactation. Blood samples were collected weekly from 4 wk antepartum to 12 wk postpartum from 38 high-yielding Holstein-Friesian cows. Nonesterified fatty acids, beta-hydroxybutyrate, IGF-I, and leptin were measured in serum, and BCS was recorded. Health status was characterized using the concentrations of haptoglobin, the number of leukocytes and neutrophils, as well as the activity of glutamate dehydrogenase (GLDH) in blood to evaluate liver status. Using the factors related to fat mobilization, the animals were classified according to their values recorded at one defined point in time or time interval as being above or below certain thresholds. For each criterion, the groups classified were compared with regard to the time-course yielded from all recordings. From 7 criteria of classification, the most closely related to the variables of fat mobilization was obtained when using NEFA and IGF-I (thresholds of 0.5 mM and 39 ng/mL in wk 1 postpartum, respectively). Both items were then combined into the criterion NEFA + IGF-I. Applying these criteria, the relations to indices of health and liver status were detectable on the basis of NEFA- and NEFA + IGF-I-classes, which yielded differences in both GLDH and leukocyte numbers. Animals with NEFA > 0.5 mM showed increased GLDH activity but decreased leukocyte numbers. The time and effort required for measuring the IGF-I-concentration in addition to NEFA is not justified for evaluating the metabolic status. Nonesterified fatty acid values ≥0.5 mM during the first week of lactation were considered as the most suitable criterion for identifying limited adaptive performance.

Key words: adaptation, cattle, fat mobilization, insulin-like growth factor-I, nonesterified fatty acid, transition period

INTRODUCTION

Dairy cows need to mobilize adipose tissue to compensate for the relative deficit of glucose occurring during the peripartum period due to the onset of lactation and the concomitant negative energy balance. The rapid adaptation of key metabolic pathways is central to successfully undergo the transition from pregnancy to lactation (Drackley et al., 2001). Failure of adequate adaptation may result in imbalances, leading to an increased risk of digestive, metabolic, or infectious disorders (e.g.,

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displaced abomasum, ketosis, or mastitis; Ingvartsen, 2006), or a combination of disorders.

Identification of animals having problems with adaptation is desirable, and several blood tests have been proposed for this (Jorritsma et al., 2003). However, most of the investigations so far have focused on finding risk factors related to various diseases. Differences between well-adapted animals and those with compromised adaptation might only be small, and fixing a limit to identify one or the other is difficult. Nevertheless, the detection of cows with an impaired capability of adaptation is important to treat individual cows adequately during the peripartum period.

The purpose of this study was to evaluate the information yielded when classifying dairy cows according to different criteria of fat mobilization in early lactation, using blood samples of apparently healthy, high-yielding dairy cows collected during the peripartum period. Threshold values from defined times in relation to parturition were applied, following mainly references from the literature, to retrospectively classify those cows showing values below or above these thresholds. The 2 groups were then compared with regard to the entire time-course of the other variables to test which classification would yield the most useful information. Thereby, an approach involving maximal information with minimal effort at the earliest time possible for identifying cows with suboptimal adaptive performance, albeit not clinically diseased, should emerge.

**MATERIALS AND METHODS**

**Animals and Diets**

All procedures involving animals met the standards of the German Animal Welfare Act and were approved by the local veterinarian authority; the experimental protocol was accredited by the commissary for animal ethics of the University of Bonn.

Holstein-Friesian cows (n = 38), with an average age of 3.9 ± 1.6 yr, were studied 4 wk antepartum (AP) until 12 wk postpartum (PP). They were housed in free-stall barns in 2 units (organic, n = 21; and conventional, n = 17) of the agricultural research center from the North Rhine Westphalian chamber for agriculture matters (Riswick, Kleve, Germany). We intended to integrate cows of comparable nutritional status and performance regardless of how their feed was produced (i.e., grown according to conventional or organic farming practices). Based on the metabolite and hormone profiles recorded in blood samples, as described below, no differences were detectable between the 2 groups.

All cows were fed a total mixed ration based on grass and corn silage for ad libitum intake; the amounts offered and refused were recorded daily to maintain approximately 10% (5 to 15%) orTs. On average, the nutrient supply was similar in both groups and is shown in Table 1. During the dry period, cows were fed a ration that satisfied the demand for maintenance and the additional energetic needs of the conceptus and the mammary gland (i.e., 13 MJ of NE₂/d from wk 6 to 4 AP and 18 MJ of NE₂/d from wk 3 AP until calving, respectively) according to the recommendations of the German Society of Nutrition Physiology (GfE, 2001). Body condition was scored every 2 wk during the entire experiment, as described by Edmonson et al. (1989), using 0.25 increments on a 1 to 5 scale; milk yield and milk composition were recorded every second week by the regular milk recording organization (Landeskontrollverband Nordrhein Westfalen e.V., Bischofstrasse 85, 47749 Krefeld, Germany). During the first 12 wk of lactation, daily milk yield was 39.2 ± 1.14 kg of energy-corrected milk.

**Blood Collection and Laboratory Analyses**

Blood samples were collected weekly via jugular venipuncture from 4 wk AP to 12 wk PP, approximately 4 h after the morning feeding. For total blood leukocyte counts, EDTA-treated blood was used; for differential leukocyte counts, blood smears were prepared and analyzed with the ADVIA 120-hematology-system (VLK, Laboratory for Veterinary Medicine, Cologne, Germany). For all other measurements, serum was prepared by centrifugation (20 min, 3,000 × g, 4°C) of clotted blood and stored at −20°C until analysis.

![Table 1](image-url)
The concentrations of haptoglobin (Hp) and leptin were measured using double antibody, enzyme immunoassays, as described by Hiss et al. (2004) and Sauerwein et al. (2004), respectively. For progesterone (P4), the enzyme immunoassay protocol of Sauerwein et al. (2006), which is based on the technique described by Prakash et al. (1987), was used. The minimal detectable dose in the assays for Hp, leptin, and P4 was 0.07 μg/mL, 0.3 ng/mL, and 0.1 ng/mL, respectively. The intra- and interassay CV were 2.9 and 5.8% for Hp, 3.6 and 7.8% for leptin, and 9.3 and 17.2% for P4. The concentrations of IGF-I were measured by RIA, as described by Daxenberger et al. (1998). The sensitivity of the RIA was 1 ng/mL, and the intra- and interassay CV were 5.1 and 13.4%, respectively.

Serum concentrations of NEFA were measured using an enzymatic test kit (kit Nr. 1 383 175, Roche, Mannheim, Germany), in which the volumes were reduced for use on microtiter plates. Beta-hydroxybutyrate (BHB) and glutamate dehydrogenase (GLDH) were measured at VLK using kits from Randox, Antrim, Ireland (kit Nr. RB 1008) and from Roche (kit Nr. 197734), respectively.

Classification of the Animals According to Thresholds Set for BCS, Blood Metabolites, and Hormones Related to the Mobilization of Body Fat

The animals were retrospectively grouped, according to the results from the BCS scoring and the blood concentrations of metabolites and hormones related to fat mobilization, as being below or above the threshold values that were defined as explained below. Considering the metabolic and physiological backgrounds, being below the threshold was classified as beneficial in the case of NEFA and BHB, and the groups were termed using the suffix -ok (e.g., NEFA-ok). Animals with values above the threshold were accordingly judged as being potentially at risk for health impairments and the groups were termed using the suffix -ltd for limited adaptive capability (e.g., BHB-ltd). For IGF-I, leptin, and BCS, the approach was the opposite; i.e., values below the threshold were judged as negative and the suffixes were used accordingly. For the BHB threshold, the values recorded during wk 2 PP were used because the peripartum increase is well detectable at this time (Doepel et al., 2002). A concentration of 1,200 mM, described previously as appropriate to identify fresh cows with subclinical ketosis (Enjalbert et al., 2001) was used; i.e., cows with BHB concentrations ≥1,200 mM were classified as BHB-ltd and those with concentrations <1,200 mM as BHB-ok animals.

No defined threshold for plasma NEFA exists for postpartum cows (Nafikov et al., 2006). For prepartum cows, a NEFA threshold of 0.5 mM was defined; cows exceeding this threshold would have a greater likelihood for developing postpartum disorders (Oetzel, 2004; LeBlanc et al., 2005). We herein used the NEFA concentrations measured in wk 1 PP, because the peripartum increase was then obvious, occurring approximately 1 wk earlier than for BHB (Doepel et al., 2002). To establish a threshold, we used a Receiver-Operating-Characteristic curve [sensitivity vs. (1 – specificity); Perkins and Schisterman, 2006], in which the aforementioned BHB threshold provided the basis for the classification. Youden’s index (Youden, 1950), a function of sensitivity and specificity (sensitivity + specificity – 1), was used to define the cut-off value (0.5 mM NEFA). At the maximal Youden Index value, the differentiating ability of NEFA concentrations is optimized when equal weight is given to sensitivity and specificity. Sensitivity and specificity were 79 and 75% in this approach, respectively. The area under the curve was 0.786.

For IGF-I, 2 modes of setting a threshold were tested. First, the median of IGF-I concentrations (39.0 ng/mL) during the first wk PP was used to define the groups IGF-I-ltd (<39.0 ng/mL) vs. IGF-I-ok (≥39.0 ng/mL). Second, the magnitude of the decrease in the concentrations from wk 1 AP to wk 1 PP was considered; the ratio of the PP/AP values was calculated, expressed as a percentage, and the median of these percentages was used to form the classes IGF-I-%-ltd (≥76.9) and IGF-I-%-ok (<67.9). For all calculations using IGF-I, 1 cow had to be excluded due to abnormally high values that could not be adequately measured by diluting the sample.

To establish threshold values for leptin, an analogous approach as that taken for IGF-I was used. That is, we used the median recorded during wk 1 PP to classify the groups, leptin-ok (≥4.1 ng/mL) and leptin-ltd (<4.1 ng/mL). In addition, we considered the percentage decrease from wk 1 AP to wk 1 PP and used the median (71.4%) as threshold for the respective leptin-% groups.

For BCS, again 2 thresholds were tested: 1 used the categorization of the animals according to their BCS during the last 2 wk AP using a threshold of 3.5, according to Busato et al. (2002) and Lopez-Gatius et al. (2003; i.e., classifying BCS-ltd, ≥3.5; and BCS-ok, <3.5). In addition, BCS losses (Δ-BCS) of more than 0.5 from wk -2 to -1 AP until wk 3 to 4 after calving were applied for a second threshold that yielded the groups Δ-BCS-ltd, ≥0.5; and Δ-BCS-ok, <0.5.

Statistical Analyses

All statistical analyses were performed with the SPSS program, version 12.0 (SPSS GmbH Software, Munich, Germany). For the groups classified at a defined point in time or time interval according to the thresholds described above, the various blood concentrations as dependent variable were compared between each of the -ok and the -ltd groups for the entire time-course recorded via the GLM. Given that the classification was done at a certain time and we considered the entire time-course for the variables, we also included analyses to compare the curves of the variables that were used as criteria of classification (e.g., the concen-
trations of NEFA during the study were compared also between the NEFA-ok and the NEFA-ltd groups. Fixed effects tested were the respective classification and also parturition, the latter being considered via nested periods (before and after parturition) within sampling weeks. Sampling week was considered as a repeated effect. Three covariance structures (first order antedependence, first order autoregressive, and heterogeneous first order autoregressive) were initially tested, and the one with the lowest values of the Akaike’s information criterion was then applied.

To evaluate the relationship between NEFA and BHB as well as NEFA and IGF-I concentrations, nonparametric coefficients of correlation (Spearman) were calculated. Variation in the data is expressed as SEM. Significance was declared at $P < 0.05$. As a consequence of the actual calving dates, the intervals of BCS evaluation could not be met for all cows. Eventually missing values were calculated by using the mean of the BCS score before and after the interval without recordings.

**RESULTS AND DISCUSSION**

**Fat Mobilization**

The timely changes of the concentrations of NEFA, BHB, IGF-I, and leptin in serum as well as BCS data recorded throughout the study, are shown in Figures 1 and 2, respectively. For all variables recorded, effects of calving were observed ($P < 0.001$).

Mobilization of body fat associated with the negative energy balance PP could be recorded using the variables described above. Elevated NEFA and BHB concentrations from fat mobilization serving as energy source at a time in which intake of energy is limited (Pullen et al., 1989; Bell, 1995) have been reported previously (Doepel et al., 2002; Pushpakumara et al., 2003; Murondoti et al., 2004) and are related to a compromised metabolic status. The maximal concentrations of NEFA (1.25 mM) during the first week after parturition in our study were less compared with the reports quoted above, but the temporal changes of the concentrations
of NEFA are in agreement with the works of Murondoti et al. (2004) and Doepel et al. (2002), who reported maximal NEFA concentrations of 1.7 and 1.9 mM, respectively, in wk 1 PP.

The peripartum decrease of the concentrations of leptin we found is in line with the observations of Block et al. (2001) and Chilliard et al. (2005). For IGF-I, the peripartum pattern obtained in our study agrees with earlier works (Schams et al., 1991; Kim et al., 2004).

Cows were in proper body condition at the start of the study, and the observed loss of condition throughout the study can be judged as moderate (López-Gatius et al., 2003). The BCS at the time of calving observed herein corresponds well to the report by Kokkonen et al. (2005) and showed no noticeable deflection from this during the first weeks of lactation. A potential loss of information due to the every 2 wk scoring interval is considered as being of minor importance in view of the relatively slow changes of body condition. In general, the average mobilization of body fat, compared with other findings, was judged as moderate.

**Health Status**

Concentrations of Hp in serum, the number of leukocytes, the number of neutrophils, as well as the activity of the liver specific enzyme GLDH during the experiment are presented in Figure 3. For Hp, GLDH, and the number of leukocytes and neutrophils (PMNL), effects of calving (P < 0.001) were observed. Leukocyte number was also affected (P < 0.01) by calving.

Immediately after calving, Hp reached maximal values corresponding to a 4.5-fold increase of the prepartum concentrations. During the third week of lactation, baseline values were reestablished. Haptoglobin is one of the major acute phase proteins in cattle, and increasing concentrations are attributable to inflammatory reactions. Increasing concentrations of Hp at parturition agree with the observations from Uchida et al. (1993) and are probably related to the concomitant hormonal changes and to tissue lesions occurring during birth.

The sequential changes of the pre- and postpartum activities of GLDH recorded in our study were similar to those reported by Hoedemaker et al. (2004). Compared with healthy nonlactating and nonpregnant cows (West, 1997), these values were increased and might be attributed to liver cell damage caused by triacylglycerol accumulations as discussed elsewhere (Staufenbiel et al., 1993). Nevertheless, the increase of GLDH activity observed herein is only marginal compared with cows with clinical hepatic lipidosis or other liver disease (West, 1997).

Increasing numbers of PMNL toward the time of calving (Reisler et al., 2000; Burvenich et al., 2003) were also found in the cows studied herein, which were without other pathological findings. However, this increase was hardly reflected in leukocyte numbers. In general, the criteria used to evaluate health were judged as being within the physiological range of high-yielding dairy cows.

**Evaluation of the Classification According to Criteria Related to Fat Mobilization**

The criteria related to fat mobilization were used to classify the cows according to their values recorded at 1 defined point in time (NEFA, BHB, IGF-I, and BCS) or time interval (ΔBCS, IGF-I-%, and Leptin-%) as being below or above a certain threshold (-ok or -ltd groups). Between each of the 2 groups thereby defined, the temporal patterns of the various variables tested were compared. Table 2 summarizes the results of these comparisons. The presentation is limited to significant relationships. In addition, the effort required for analysis as well as the earliest possible time of analysis is considered.

**BCS AP and BCS Loss.** Differentiating the animals by their AP BCS values yielded effects on leptin (P = 0.007); as expected, cows with BCS scores ≥ 3.5 (BCS-ok) had relatively greater concentrations of leptin in serum than cows with lower scores. When animals were grouped according to the degree of BCS loss (Δ BCS), cows with a BCS decline of more than 0.5 points also had lower concentrations of leptin (P = 0.017) and of IGF-I (P = 0.001) during the study. However, when considering that the time necessary to detect BCS losses in early lactation is more than 2 wk PP, BCS evaluation is of limited use when requiring earlier information about adaptive status.

**BHB and NEFA.** Cows with BHB values greater than 1.2 M at the second week PP had elevated NEFA concentrations during the study (P = 0.01). Due to the high variability of the concentrations of BHB throughout the study, the BHB threshold used did not allow for the detection of differences in BHB serum concentrations between the BHB-ok and the BHB-ltd group throughout the time interval recorded.

Increased NEFA as well as decreased IGF-I concentrations were observed in NEFA-ltd cows, with major differences during the first 4 (NEFA: P < 0.04) and first 2 (IGF-I: P < 0.0058) wk PP compared with the NEFA-ok cows. Mean maximal concentrations of 0.86 ± 0.06 mM NEFA for NEFA-ltd cows were found in the first week after calving, whereas the concentrations in NEFA-ok cows were less than half of this (0.38 ± 0.04 mM). Again, due to the variability, no differences were found for BHB concentrations.

In contrast to NEFA, BHB concentrations peaked at the second week PP. This lag-time might be explained by increasing NEFA concentrations during the first week PP, which provide the substrate for BHB synthesis (Doepel et al., 2002). We therefore used the BHB concentration during the second week PP to evaluate adaptive capability. Nonesterified fatty acids and BHB were correlated (r = 0.4; P < 0.001) during the entire time, a finding that is corroborated elsewhere (Meikle et al., 2004) and which further supported us to establish
Figure 3. Changes of the concentrations of 4 variables related to animal health [haptoglobin, glutamate dehydrogenase (GLDH), and leukocyte and neutrophil (PMNL) numbers] in serum of dairy cows during late gestation and early lactation. Calving was a significant effect for all 4 variables ($P < 0.001$; for leukocyte numbers, calving was significant at $P < 0.01$).

the 0.5 mM NEFA threshold in week 1 PP. This threshold for NEFA is similar to the ones proposed to identify cows at risk for subclinical ketosis (0.5 mEq/L; Gooijer et al., 2004) or abomasal displacement (0.5 mEq/L; LeBlanc et al., 2005), but the data basis as well as the intended purpose of the studies need to be differentiated; the works quoted used prepartal NEFA values to retrospectively relate them to the incidence of disease. In contrast, we intended to evaluate adaptive performance after calving and therefore needed to define a separate NEFA threshold. Using BHB as reference in the Receiver-Operating-Characteristic curve approach yielded reasonable sensitivity and specificity, and the threshold thereby selected is comparable to the ones defined in the literature for prepartum cows.

**IGF-I and IGF-I-%.** Comparing the time-course of the IGF-I concentrations in the IGF-I-ok and the IGF-I-ltd cows, the classification from wk 1 PP was maintained [i.e., IGF-I-ok cows had greater ($P < 0.001$) IGF-I concentrations throughout the study than the IGF-I-ltd cows]. The difference between the 2 groups was particularly distinct in wk 1 and 2 PP. In addition, the curves of the leptin concentrations recorded in the IGF-I-ok class were elevated compared with the IGF-I-ltd class. Both IGF-I and leptin showed a distinct decrease ($P < 0.001$) in concentration around parturition. We speculated that not only the concentrations at early lactation might be related to the extent of metabolic stress, but also the relative change from pre- to postpartum values. We therefore formed a criterion considering the decrease (i.e., classified the animals according to the relative decrease from wk 1 AP to wk 1 PP). The effect on NEFA and IGF-I concentrations obtained by classifying with the IGF-I-% criterion was like the effects described above, with the exception of leptin, for which no differences were detected. Compared with the IGF-I-criterion, 2 more cows were classified as -ltd.

Declining IGF-I concentrations correlate with negative energy balance and indicate a decreased functionality of the liver (Spicer et al., 1990; Beam and Butler, 1998; Taylor et al., 2004). To our knowledge, there is no IGF-I-threshold for clinically or subclinically diseased animals published; the median used herein is a first attempt to classify animals by their IGF-I concentrations during the first week PP. There was no additional benefit using the criterion IGF-I-%, which required 2 samples for classifying, implying that the effort for analysis did not improve the information yielded about the adaptive status.

**Leptin and Leptin-%.** When comparing the Leptin-ok vs. the -ltd group, the variables affected were leptin and IGF-I ($P < 0.001$ and $P < 0.05$, respectively). The relation between leptin and IGF-I were thus confirmed, but in contrast to the classification according to IGF-I, NEFA concentrations were not different. The classification using leptin was thus considered as less informa-
Table 2. Criteria of classification and variables affected in considering the earliest possible time of analysis (t) and the effort required for analysis (e)

<table>
<thead>
<tr>
<th>Criterion of classification (time relative to parturition), and groups (threshold):</th>
<th>Time-courses of variables found affected</th>
<th>Variable</th>
<th>P-value</th>
<th>ok</th>
<th>ltd</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS (1 to 2 wk AP)†</td>
<td>+++ 0</td>
<td>Leptin</td>
<td>0.007</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>BCS-ltd (≥35): 6</td>
<td></td>
<td></td>
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<tr>
<td>BCS-ok (&lt;35): 25</td>
<td></td>
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<tr>
<td>∆ BCS (2 wk AP to wk 4 PP)‡</td>
<td>− 1</td>
<td>Leptin</td>
<td>0.017</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>∆BCS-ltd (≥35): 8</td>
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<td></td>
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<tr>
<td>∆BCS-ok (&lt;35): 22</td>
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<tr>
<td>β-hydroxybutyrate (wk 2 PP)</td>
<td>+ 1</td>
<td>NEFA</td>
<td>0.001</td>
<td>↓</td>
<td>↑</td>
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<tr>
<td>BHB-ltd (≥1200 mM): 14</td>
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<tr>
<td>BHB-ok (&lt;1200 mM): 24</td>
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<tr>
<td>NEFA (wk 1 PP)</td>
<td>++ 1</td>
<td>NEFA</td>
<td>&lt;0.001</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>BCS-ltd (≥20.5 mM): 17</td>
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<tr>
<td>IGF-I (wk 1 PP)</td>
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<td>IGF-I</td>
<td>&lt;0.001</td>
<td>↑</td>
<td>↓</td>
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<tr>
<td>IGF-I-ltd (&lt;39 ng/mL): 19</td>
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<tr>
<td>IGF-I-% (wk 1 AP to wk 1 PP)</td>
<td>++ 2</td>
<td>IGF-I</td>
<td>&lt;0.001</td>
<td>↑</td>
<td>↓</td>
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<tr>
<td>IGF-I-%-ltd (&lt;67.9%): 18</td>
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<tr>
<td>Leptin (wk 1 PP)</td>
<td>++ 1</td>
<td>Leptin</td>
<td>&lt;0.001</td>
<td>↑</td>
<td>↓</td>
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<tr>
<td>Leptin-ltd (&lt;4 ng/mL): 19</td>
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<td>Leptin-ok (≥4 ng/mL): 18</td>
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</table>

1The entire time-course of the blood concentrations of the different variables was compared between each of the -ok and the -ltd groups (fixed effect); only those variables for which significant differences between the groups were found are shown.

2The effort required for analysis (e) and the earliest possible time of analysis (t) for the different parameters were considered and rated as follows: t, +++ = determination at wk 2-1 antepartum (AP); ++ = determination at wk 1 postpartum (PP); + = determination at wk 2 PP; and − = determination at wk 3 PP; e, 0 = no blood sample required; 1 = 1 blood sample required; and 2 = 2 blood samples required.

3↑ = greater values for the variable than the other group; and ↓ = lower values than the other group.

4Only those animals that could be scored during the last 2 wk before calving were included (n = 31).

In consequence of using combined criteria, one-third of the animals could not be classified. This indicates the limitations of using the criteria of classification in field studies, implying that using both, the criteria by themselves and also the combined criteria, will not be able to properly evaluate this one-third of animals. If metabolic stress would only be identifiable on the basis of combined classes, those animals with 1 criterion (NEFA or IGF-I) above the threshold are not at risk.

Classifying Cows According to the Criteria of Fat Mobilization: Affection of Variables Related to Health

Both the effort required for analysis and the earliest possible time of analysis should be considered when using different items to evaluate the adaptive status. An extensive analysis should not disqualify an item per se, if the information obtained exceeds the effort. Thus, we tested whether the classifications based on thresholds for the factors related to fat mobilization would be reflected by significant differences in the time-course of those variables related to animal health (Hp, P4, leukocyte and PMNL number, and GLDH activity). As summarized in Table 3, the classifications using ∆BCS,
NEFA, IGF-I, and NEFA + IGF-I yielded differences between the respective -ok and -ltd groups for leukocyte number. For GLDH activity, differences were detectable between the respective BHB, the NEFA, and the NEFA + IGF-I groups. Using BCS and IGF-I-% to classify the animals did not result in any differences between the -ok and the -ltd groups. Significant relationships with both variables indicative for animal health were limited to the NEFA and the NEFA + IGF-I classes. Independently of dividing the animals by NEFA alone or by the combined factors, the temporal changes of GLDH activity and leukocyte numbers were similar in the respective -ok and the -ltd groups (Figure 4). The NEFA-ok and the NEFA + IGF-I-ok animals had different GLDH activities ($P = 0.001$ and $P = 0.05$, respectively), with lower values at wk 3 to 5 PP.

Considering that the classification was done according to the values of wk 1 PP, the retarded increase of GLDH in -ltd cows might thus be a result from stressors beginning to affect liver function at earlier stages. Leukocyte counts were continuously greater in the NEFA-ok and NEFA + IGF-I-ok animals than in the corresponding -ltd cows. In general, the decreased leukocyte counts in -ltd cows might be interpreted as reflecting an immuno-compromised status, but additional data on whether the function of these cells is impaired would be helpful to judge the situation. The concentrations of Hp and the number of PMNL were not different between the 2 groups in either classification nor for the concentrations of P4, which were used as indicators of reproductive health. When using the first increase of P4 to characterize the onset of ovarian cyclicity, again no differences were detectable between the -ok and -ltd groups (data not shown). Classifying cows according to their leptin blood concentrations during wk 1 PP yielded no differences between the variables used to evaluate health status.

Production diseases occur when physiological mechanisms for the adaptation to negative energy balance fail (Herdt, 2000). It has already been demonstrated that the immune defense is compromised during the periparturient period, particularly around calving (Mallard et al., 1998; Piccinini et al., 2004). The classification according to NEFA values recorded in wk 1 PP yielded different runs of the curves for both GLDH activity and leukocyte numbers; in contrast, classifying the cows according to their absolute and relative IGF-I concentrations did not result in differentiable effects on GLDH activity. In addition, a dominant role of NEFA for liver function is supported by the observation that the combination of NEFA + IGF-I allowed for the detection of cows with increased activities of GLDH. Although the observed increases of GLDH are well below the values typical for severe clinical liver diseases, they do indicate disturbances of liver function and knowingly correlate with the degree of hepatic lipidosis (West, 1990; Staufenbiel et al., 1993). Unrestricted functionality of the liver during the transition period is meaningful for animal welfare (Rehage and Kaske, 2004), and the early detection of such disturbances, as proposed by using NEFA values during the first wk PP, might be useful to take metaphylactic measures for individual cows. Based on the difference in leukocyte numbers we detected in cows classified according to NEFA, to IGF-I, and to the combined criterion NEFA + IGF-I, immune function seems to be related to these 2 items. For IGF-I, decreasing concentrations during early lactation have been suggested to be involved in the reduced quality and functionality of blood cells (Vangroenweghe et al., 2005). For NEFA, there are some indications from in vitro experiments that they may exert direct inhibitory effects at concentrations comparable to the one we established herein on leukocyte function, particularly in lymphocytes (Lacetera et al., 2004). In contrast, at similar dosages, significant effects were not detectable for neutrophil function (Scalia et al., 2006). However, our data are limited to leukocyte and neutrophil numbers; cell function could not be addressed. Moreover, the correlation we found for NEFA and IGF-I with leukocyte numbers and GLDH activity neither necessarily re-

### Table 3. Criteria of classification for adaptive performance and their relation to parameters indicative for animal health

<table>
<thead>
<tr>
<th>Criterion of classification</th>
<th>Threshold</th>
<th>Hp</th>
<th>P4</th>
<th>Leuko</th>
<th>PMNL</th>
<th>GLDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS</td>
<td>3.5 BCS</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Δ-BCS</td>
<td>0.5 Δ-BCS</td>
<td>—</td>
<td>—</td>
<td>&lt;0.001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>BHB</td>
<td>1200 mM</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.037</td>
</tr>
<tr>
<td>NEFA</td>
<td>0.5 mM</td>
<td>—</td>
<td>—</td>
<td>&lt;0.001</td>
<td>—</td>
<td>0.001</td>
</tr>
<tr>
<td>IGF-I</td>
<td>39 ng/mL</td>
<td>—</td>
<td>—</td>
<td>0.002</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>IGF-I</td>
<td>67.9%</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>NEFA+IGF-I</td>
<td>—4</td>
<td>—</td>
<td>—</td>
<td>&lt;0.001</td>
<td>—</td>
<td>0.05</td>
</tr>
</tbody>
</table>

1. = not significant.
2. Hp = haptoglobin; P4 = progesterone; Leuko = leukocyte number; PMNL = number of polymorphonuclear neutrophilic leukocytes; and GLDH = activity of glutamate dehydrogenase.
3. BHB = β-hydroxybutyrate.
4. Thresholds as used for NEFA and IGF alone were used in combination.
flects a causal relationship nor gives it evidence for a mechanism linking these elements. Nevertheless, the correlation observed supports the applicability of NEFA for monitoring purposes.

Besides diagnosing of disease problems in dairy cows, blood testing may be used to identify cows that are apparently healthy but are potentially at risk for health disturbances. Hereby effective approaches are necessary to provide maximal information at the earliest time possible. Rather than evaluating or predicting clinical conditions, a test should be identified that, when applied at a defined point in time relative to calving, is composed of as much as possible other information on metabolic and health status during early lactation. Classifying cows according to their blood concentrations of NEFA during the first week after calving was most effective in picking up information about other variables during the peripartum period.

LITERATURE CITED


cows during the periparturient period. J. Dairy Sci. 84 (E. Suppl.)E100–E112.


