ABSTRACT

Metabolic and oxidative stress have been characterized as risk factors during the transition period from pregnancy to lactation. Although mutual relations between both types of stress have been suggested, they rarely have been studied concomitantly. For this, a total of 99 individual transition dairy cows (117 cases, 18 cows sampled during 2 consecutive lactations) were included in this experiment. Blood samples were taken at −7, 3, 6, 9, and 21 d relative to calving and concentrations of metabolic parameters (glucose, β-hydroxybutyric acid (BHBA), nonesterified fatty acids, insulin, insulin-like growth factor 1, and fructosamine) were determined. In the blood samples of d 21, biochemical profiles related to liver function and parameters related to oxidative status were determined. First, cases were allocated to 2 different BHBA groups (ketotic vs. nonketotic, N:n = 20:33) consisting of animals with an average postpartum BHBA concentration and at least 2 out of 4 postpartum sampling points exceeding 1.2 mmol/L or remaining below 0.8 mmol/L, respectively. Second, oxidative parameters [proportion of oxidized glutathione to total glutathione in red blood cells (%), activity of glutathione peroxidase, and of superoxide dismutase, concentrations of malondialdehyde and oxygen radical absorbance capacity] were used to perform a fuzzy C-means clustering. From this, 2 groups were obtained [i.e., lower antioxidant ability (LAA80%, n = 31) and higher antioxidant ability (HAA80%, n = 19)], with 80% referring to the cutoff value for cluster membership. Increased concentrations of malondialdehyde, decreased superoxide dismutase activity, and impaired oxygen radical absorbance capacity were observed in the ketotic group compared with the nonketotic group, and inversely, the LAA80% group showed increased concentrations of BHBA. In addition, the concentration of aspartate transaminase was higher in the LAA80% group compared with the HAA80% group. Both the ketotic and LAA80% groups showed lower dry matter intake. However, a lower milk yield was observed in the LAA80% group but not in the ketotic group. Only 1 out of 19 (5.3%) and 3 out of 31 (9.7%) cases from the HAA80% and LAA80% clusters belong to the ketotic and nonketotic group, respectively. These findings suggested that dairy cows vary in oxidative status at the beginning of the lactation, and fuzzy C-means clustering allows to classify observations with distinctive oxidative status. Dairy cows with higher antioxidant capacity in early lactation rarely develop ketosis.

Key words: transition period, β-hydroxybutyric acid, oxidative status

INTRODUCTION

The transition period around calving is a crucial period for highly productive dairy cows, characterized by tremendous physiological, metabolic, and nutritional changes. Similar to most mammals, cows will experience a period of endogenous reserve mobilization which will be aggravated due to nutrient prioritization toward lactogenesis and peaking of milk production in early lactation (Drackley et al., 2006; Lucy et al., 2009). However, it is well-documented that elevated levels of nonesterified fatty acids (NEFA) from fat mobilization and the partial oxidation of NEFA to BHBA will negatively affect the health status of cows, for instance, loss of appetite (Grummer, 1993; Ceciliani et al., 2018). A concentration of 1.2 mmol/L BHBA in the blood is a widely accepted threshold for the diagnosis of subclinical ketosis (Benedet et al., 2019).

In addition, enhanced metabolic activities in the transition period may result in the accumulation of reactive oxygen species (ROS; Folnožić et al., 2015;
ed concentrations of malondialdehyde (MDA) and enzyme activities are reported to increase during oxidative stress, resulting in damage to lipids, proteins, and DNA (Cross et al., 1987). Under normal physiological status, ROS are quickly neutralized by the antioxidant system. Various antioxidant enzymes and nonenzymatic antioxidants contribute to this system. Superoxide dismutase (SOD) forms the first barrier by converting superoxide to hydrogen peroxide (H₂O₂). Glutathione, a non-enzymatic antioxidant, exists in 2 states: a reduced glutathione (GSH) and an oxidized (GSSG) form. As a substrate for enzymes such as glutathione peroxidase (GPx), GSH plays a key role in maintaining the intracellular redox status by elimination of peroxidic radicals and peroxides (Luberda, 2005). Superoxide dismutase and GPx activities are reported to increase immediately after parturition, accompanied by elevated concentrations of malondialdehyde (MDA). As the latter is a marker for lipid peroxidation, this suggests an imbalance in the antioxidant system (Colakoglu et al., 2017; Bühler et al., 2018). Furthermore, there is increasing evidence that severe metabolic stress, especially relatively high peripheral levels of BHBA, is associated with an oxidative imbalance. Sun et al. (2021) reported that early-lactating cows (median 8 DIM) with serum BHBA exceeding 1.2 mmol/L show compromised GPx and SOD activities in mammary gland tissue compared with the control group (<0.6 mmol BHBA/L). Similar findings were documented by Song et al. (2021), where clinically ketotic cows (>3 mmol BHBA/L in serum) show greater oxidant indices (e.g., H₂O₂ and MDA) and lower antioxidant abilities including GPx and SOD activities in the mammary gland compared with healthy cows (<0.6 mmol BHBA/L in serum). These associations between ketosis and the oxidative status have been investigated on simultaneously taken samples (or sampled within consecutive days). However, particularly blood BHBA is expected to show quite some variability during the first 10 DIM (Dann et al., 2006), with elevated levels which may be transient (Horst et al., 2021). Therefore, more sampling points during the 3 wk after calving are essential to precisely identify ketotic cows. In addition to, and unlike the ketotic status which can be assessed by single parameters, little is known about how to classify cows according to antioxidant ability. Because the antioxidant system is a complex network of enzymatic and nonenzymatic components, no threshold value has been established to determine impaired oxidative status. Hence, a multivariate clustering approach could be used to identify impaired oxidative status animals as it has been applied to cluster cows with distinctive metabolic status in previous studies (De Koster et al., 2019; Xu et al., 2019).

Accordingly, the objective of this study was applying a multivariate approach to cluster cows with distinctive antioxidant ability in early lactation and to relate these clusters with the animal’s productive performance, intake as well as ketotic and overall metabolic status, during the transition period. Inversely, the oxidative capacity 3 wk after calving was assessed for animals either or not experiencing subclinical ketosis during the first 3 wk after calving.

**MATERIALS AND METHODS**

**Animals, Diets, and Management**

The animal experimental procedures were performed according to protocols approved by the Ethics Committee of Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Belgium (EC 2018/329). A total of 99 individual Holstein Friesian multiparous dairy cows were included in this experiment, housing at the research farm of ILVO (Melle, Belgium) from October 2018 to October 2020. Some cows were sampled twice, resulting in 117 observations overall. Indeed, as the experiment was running over a 2-yr period, 18 animals sampled in the first year of the trial were resampled in their subsequent lactation also.

From 3 wk before calving, cows received the partial mixed ration of the lactating cows supplemented with a dry cow mineral premix and on average 1 kg of balanced concentrate per cow per day. The partial mixed ration was calculated to achieve the requirements for lactating cows as the experiment was running over a 2-yr period, 18 animals sampled in their subsequent lactation also. The supply of the balanced diet was adjusted following nutrition values of the silages used during the whole trial period. Concentrate intake on d 3 after calving started at 1.7 kg of balanced concentrates, 0.2 kg formaldehyde-treated soybean meal (Covasoy Braz., FeedValid, Poederoijen, NL), 0.3 kg soybean meal. Covasoy was increased over a period of 7 d to 1 kg, whereas the balanced concentrate was increased to 6 kg over a period of 20 d. Detailed information about diet chemical composition during early lactation is shown in Table 1 and the buildup of supplemented part for lactating cows is given in Table 2. Roughage was provided twice.
per day and intake of individual cows was monitored automatically using Roughage Intake Control feeding bins (Insentec, Hokofarm Group) and concentrate intake was monitored through the concentrate dispensers (De Laval). Lactating cows were milked twice daily approximately at 0530 and 1630 h, and milk yield (kg/d) was recorded electronically for each cow. Methods for milk analysis (fat, protein, lactose) were described by Heirbaut et al. (2023). All cows had free access to the Roughage Intake Control bins and drinking water. Clinical diseases were diagnosed and recorded by a veterinarian or experienced farm staff.

**Blood Collection and Processing**

Whole blood samples were obtained from the jugular vein using lithium heparin tubes (10 mL; BD Diagnostics) at 21 d after parturition and then centrifuged at 1,500 × g for 10 min instantly at room temperature. After centrifugation, the upper layer was transferred into Eppendorf tubes for determining the activities of GPx, SOD, and the concentrations of MDA, as well as oxygen radical absorbance capacity (ORAC). The intermediate layer was discarded, then exactly 300 µL of the red blood cells from the lower layer was transferred into Eppendorf tubes and 10 µL of 1 mmol/L bathophenanthrolinedisulfonic acid disodium salt hydrate solution (stored at 4°C for a maximum of 1 wk) was added for determining the proportion of oxidized glutathione to total glutathione in red blood cells (GSSG %). All the Eppendorf tubes were immediately snap frozen in liquid nitrogen and stored at −80°C until analysis.

Serum and plasma were harvested in the morning, approximately between 0915 and 0945 h, at −7, 3, 6, and 9 d relative to calving from coccygeal vessels, and at 21 d from jugular vein, by using serum blood tubes (10 mL) and NaF tubes (4 mL; BD Diagnostics), respectively. In brief, serum tubes were kept at room temperature for at least 30 min before centrifuging (1,500 × g for 15 min) and NaF tubes were kept in an ice box until centrifuged at 1,000 × g for 10 min at room temperature. After centrifugation, the upper layer of serum and NaF tubes was divided and transferred into labeled Eppendorf tubes. For serum analysis, the samples for determining the concentrations of BHBA, NEFA, insulin, and fructosamine were stored at −20°C, whereas samples for IGF-1 were stored at −80°C until analysis. In addition, extra serum samples were taken for biochemical indices and acute phase protein assay at 21 d and stored at −20°C. Meanwhile, plasma samples for assessing the concentrations of glucose were stored at −20°C until analysis.

**Table 1.** Chemical composition (mean ± SD) of the diet calculated based on the observed individual intake of the partial mixed ration (PMR) and concentrates during the 2 wk before calving as well as the diets offered between d 3 and 21 in lactation

<table>
<thead>
<tr>
<th>% of DM (unless noted otherwise)</th>
<th>d −14 to 0</th>
<th>d 3 to 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (% of fresh material)</td>
<td>39.8 ± 3.83</td>
<td>42.0 ± 3.49</td>
</tr>
<tr>
<td>VEM (VEM/kg DM)</td>
<td>944 ± 65.1</td>
<td>1,009 ± 21.5</td>
</tr>
<tr>
<td>CP</td>
<td>14.1 ± 2.15</td>
<td>16.7 ± 1.24</td>
</tr>
<tr>
<td>FOM</td>
<td>49.3 ± 3.73</td>
<td>51.8 ± 1.46</td>
</tr>
<tr>
<td>Starch</td>
<td>18.1 ± 2.73</td>
<td>18.0 ± 1.32</td>
</tr>
<tr>
<td>NDF</td>
<td>35.6 ± 8.66</td>
<td>31.6 ± 7.15</td>
</tr>
<tr>
<td>ADL</td>
<td>21.0 ± 5.30</td>
<td>18.5 ± 4.23</td>
</tr>
<tr>
<td>NEL (MJ/kg of DM)³</td>
<td>6.51 ± 0.449</td>
<td>6.96 ± 0.149</td>
</tr>
</tbody>
</table>

³VEM = feed unit lactation (Van Es, 1975).
²FOM = fermented OM (Tammenga et al., 1994).
⁴Calculated based on the Belgian-Dutch net energy evaluation system (i.e., 1.000 VEM = 6.9 MJ NE₃; Van Es, 1975).

**Table 2.** Buildup of the supplemental part of the diet, individually supplied to the cows during milking and via the concentrate dispenser

<table>
<thead>
<tr>
<th>Type of dietary supplement</th>
<th>Supplement at 3 d, amount¹ (kg/d)</th>
<th>Increment duration (d)</th>
<th>Supplement at 20 d, amount (kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balanced compound feed A²</td>
<td>0.2</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Balanced compound feed B³</td>
<td>1.5</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Covasoy⁴</td>
<td>0.2</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>0.3</td>
<td>20</td>
<td>0.3</td>
</tr>
</tbody>
</table>

¹Supplement start was given from d 3 after calving and limited to the same amount per animal.
²Contains: beet pulp (10%), soybean meal (27%), wheat (8.5%), maize (43%), molasses (7%), salt (0.6%), feed phosphate (1%), microminerals (1%), lignin-sulfonate (1%), chalk (0.4%), magnesium oxide (0.5%). Chemical composition (mean ± SD % of DM, unless noted otherwise): 89.6 ± 0.34% DM, 1,165 ± 9.8 feed unit lactation (VEM; Van Es, 1975)/kg DM, 20.2 ± 0.25% CP, 11.6 ± 0.29% NDF, 5.98 ± 0.28% ADF, 37.4 ± 0.68% starch, 57.5 ± 0.51% fermented OM (FOM; Tammenga et al., 1994).
³Contains beet pulp (37%), soybean meal (21%), wheat (18.5%), maize (12%), molasses (5%), salt (1.2%), soya oil (1%), feed phosphate (1%), microminerals (1%), lignin-sulfonate (1%), chalk (0.8%), magnesium oxide (0.5%). Chemical composition (mean ± SD % of DM, unless noted otherwise): 90.3 ± 0.59% DM, 1,114 ± 16.5 VEM/kg DM, 17.6 ± 0.31% CP, 19.6 ± 0.29% NDF, 10.8 ± 0.36% ADF, 22.5 ± 0.57% starch, 62.9 ± 2.13% FOM.
⁴Covasoy = formaldehyde-treated soybean meal to bypass rumen degradation.
Assessment of Oxidative Status

The GPx activity assay follows the method described by Hernández et al. (2004) by measuring the consumption of NADPH at 340 nm, with adjusted volumes of reagents to fit the 96-well plate. One unit of GPx activity was expressed as 1 µmol NADP+ from NADPH per minute at 25°C.

The SOD assay kit 19160–1KT-F (Sigma Aldrich) was used to determine the SOD activity by an indirect spectrophotometric method. Superoxide radicals are generated by the xanthine oxidase reaction which converts water-soluble tetrazolium salt to water-soluble tetrazolium salt formazan dye. Superoxide dismutase can inhibit formazan dye formation by converting superoxide radicals to hydrogen peroxide, and 1 unit of SOD activity is defined as inhibiting the rate of formazan dye formation under the assay conditions. The analysis was conducted following the manufacturer’s protocol.

The concentration of MDA was assessed based on the modified method of Grotto et al. (2007). Briefly, 300 µL of plasma or standard solution was transferred to a centrifuge tube and incubated with NaOH (200 µL, 1.5 mol/L) at 60°C for 30 min. After this, H₃PO₄ (500 µL, 6%) and thiobarbituric acid (TBA: 500 µL, 0.8%) were added and the mixture was heated at 90°C for 45 min. Malondialdehyde combines with 2 equivalents of TBA forming the MDA-TBA₂ complex which absorbs maximally at 532 nm. In this experiment, 1,1,3,3-tetramethoxypropane was used as the standard. The optical density at 532 nm values obtained from different concentrations of standard were used to make a linear equation and calculate the concentrations of each sample.

The ORAC assay was performed according to the method of Nimalaratne et al. (2011). For this purpose, an F-bottom black, 96-well microplate (VWR) was used with Trolox (0.02 mol/L) as the stock solution to build the standard curve. In short, 25 µL of sample or Trolox standard and 150 µL of fluorescein sodium salt was added to each well in the plate and incubated for 10 min at 37°C. After incubation, 25 µL of 2,2’-azobis(2-methylpropionamide) dihydrochloride (153 mmol/L) was automatically added to the mixture through the equipped dispenser. After that, the microplate fluorometer (Thermo Fisher Scientific) recorded fluorescence for each well at 1 min intervals for 90 min at 485 nm (excitation)/525 nm (emission). The net area under the curve was calculated for each sample or standard as the area under the curve minus the average area under the curve from all blank assays with the same plate. Regression equations obtained from the net area under the curve of the standard series were used to calculate for samples assay. Final ORAC values were expressed as micromoles of Trolox equivalents.

The GSSG (%) analysis was performed according to the method of Degroote et al. (2012) by HPLC [Agilent 1200 series, with autosampler, quaternary pump, column oven, and DAD detector (Agilent Technologies)]. To efficiently separate GSH and GSSG, the HPLC was equipped with an aminopropyl column (250/4.6 Nucleosil 120–7 NH₂, Macherey-Nagel) protected by the same NH₂ guard column (CC8/4). Solvent A was a water-methanol (1:4, vol/vol) and solvent B was 0.5 mol/L sodium acetate in 64% methanol. A multistep gradient was carried out to separate glutathione with a flow rate of 1.5 mL/min at 25°C, which started at 10% solvent B for 5 min, followed by a linear increase to 100% solvent B in 15 min, and then linearly decreased for 5 min to 10% solvent B, followed by a 3 min isocratic period for equilibration. Reduced glutathione and GSSG were identified by retention times of authentic standards (L-glutathione reduced and L-glutathione oxidized). Regression equations were obtained from the peak area of external standard series to calculate the concentration of GSH and GSSG.

Blood Metabolic Status Parameters and Biochemical Profiles

Glucose, BHBA, NEFA, total protein, albumin, α-globulin, β-globulin, γ-globulin, urea, and aspartate aminotransferase were analyzed using a Gallery Discrete Analyzer (ThermoFisher Scientific), Randox kits (Randox Laboratories Ltd.), and MINICAP PROTEIN(E) Analyzer (ThermoFisher Scientific), Randox kits (Randox Laboratories Ltd.), and haptoglobin were analyzed using a Gallery Discrete Analyzer (ThermoFisher Scientific). Serum amyloid a and haptoglobin were analyzed using the serum amyloid a assay-multispecies ELISA kit and haptoglobin assay kit (BioRepair), respectively. Serum IGF-1 was analyzed by a radioimmunoassay method (RIA) using the Non-Extraction IGF-1 IRMA DSL-2800 (LifeSpan Biosciences) by Poznań University of Life Sciences. The concentration of this hormone was determined with isotope I125 by using the automatic gamma radiation reader (Wizard2 2-Detector Gamma Counter, Perkin Elmer). In this method, the peptide being determined is sandwiched between 2 antibodies. The tubes were coated with the first antibody. The second one [Anti-IGF-1 (J-125) Reagent] was radiolabeled for detection. The analytic peptide (IGF-1) present in blood serum, standards, and controls were bound by both antibodies to form a sandwich complex. Unbound compounds were removed by decanting. The Mercodia Bovine Insulin ELISA kit (Bio-connect Diagnostics) was used for insulin analysis. Fructosamine was determined using the adapted method of Johnson et al. (1983) based on...
the ability of the ketoamine group of glycated proteins to reduce tetrazolium salt to formazan (measured at 530 nm).

**Calculation and Statistical Analysis**

Due to missing values, 7 cases were excluded from the study, resulting in 93 unique cows and 110 cases in total.

The range (maximum minus minimum) of metabolic parameters including glucose, BHBA, NEFA, insulin, IGF-1, and fructosamine were calculated within each animal observation based on data from 3, 6, 9, and 21 d after calving. Then, the means ± standard error of the mean were calculated for each group.

Data analysis was performed by R (version 4.0.3) and RStudio (version 1.4.1717–3). Visualization of data analysis was achieved by R package ggplot2 v.3.3.5 (Wickham, 2016) and GraphPad Prism 7 (GraphPad Software).

**Comparison of Oxidative Status Between Ketotic and Nonketotic Cows.** Cows with an average concentration of BHBA >1.2 or <0.8 mmol/L after calving and for at least 2 of all 4 sampling points a BHBA concentration fulfilling the criterion were considered as the ketotic group or the nonketotic group, respectively. The upper BHBA concentration of 1.2 mmol/L often has been used as threshold for hyperketonemia or subclinical ketosis (Duffield et al., 2009). As there is no strict cutoff for nonketotic cows in the literature, the concentration of BHBA <0.8 mmol/L was selected by retrospective assignment: observations were organized from low to high BHBA concentrations and a concentration of 0.8 mmol/L BHBA as cutoff value resulted in: (a) 2 groups with clearly distinct blood BHBA concentration (i.e., on average 1.2 vs. 0.8 mmol/L), (b) the inclusion of about half of the observations in the 2 groups (53 out of 110 observations), (c) a more or less balanced sample size for the ketotic versus nonketotic group (N:n = 20:33), and (d) ensuring that animals with the same average BHBA were kept in a single group. An independent t-test was performed to compare the oxidative status between groups. The Wilcoxon rank sum test was used for non-normal distribution data. The assumption of homogeneity of variance was checked by Levene’s test of equality of variances. The significance level was set at 0.05.

**Clustering Based on Oxidative Status and Selection of Subclusters Differing in Antioxidant Ability.** We used data of parameters related to oxidative status (GPx, SOD, GSSG (%), MDA, and ORAC) to sort cows into different clusters. The fuzzy C-means (FC) clustering algorithm and checking of cluster stability was achieved by R package geocmeans (Gelb and Apparicio, 2021). Before running the FC clustering, data were standardized (z-scores) and the number of clusters was determined by k-means clustering using R package NbClust where all 30 indices were included (Charrad et al., 2014). The fuzziness degree (m) of the FC clustering was set from 1.2 to 2.0 at 0.1 intervals and the silhouette index as well as explained inertia were used to determine the best m value for the FC clustering. To ensure the clustering stability, boost_group_validation function in package geocmeans was used. The Jaccard index was calculated at each replication and values above 0.75 were considered as acceptable clustering results (Supplemental Figure S1, https://doi.org/10.6084/m9.figshare.21277557.v1, Zhang et al., 2022). A membership matrix was outputted, and cows with a likelihood of belonging to a particular cluster equal or higher than 80% were selected for comparison of cows of different oxidative status. As such, the 2 clusters were referred to as LAA<sub>80%</sub> and HAA<sub>80%</sub>, with 80% referring to the retention of observations with 80% membership to a cluster and LAA and HAA referring to low and high antioxidative ability, respectively.

**Comparison of Oxidative Status Between LAA<sub>80%</sub> and HAA<sub>80%</sub>.** An independent t-test was performed to compare the oxidative status between groups. The same approach was used as described for comparison of ketotic and nonketotic cows.

**Comparison of Blood Biochemical Profiles and Production Performance Between LAA<sub>80%</sub> and HAA<sub>80%</sub>.** Statistical comparisons of metabolic status (glucose, BHBA, NEFA, IGF-1, insulin, and fructosamine) and production performance (DMI and milk yield) were analyzed using linear mixed models by R package lmer4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017). Multicollinearity of linear mixed models was checked using VIF function by R package car (Fox and Weisberg, 2019). The values of variance inflation factor were lower than 10 in all models. The model included 80%-membership groups (G), days relative to calving (D), parity (P, 3 parity levels were set as 2, 3, or 3<sup>+</sup>), and health status (H, 2 levels were set as presence or absence of clinical symptoms) and their interactions including G × P, G × D, D × P, and G × D × P as fixed effects and individual cow as random effect. The fixed effects were always included in the model, whereas interactions were removed from the model if P > 0.1. Nevertheless, all tested interactions were kept in the tabular overviews of the results to clearly indicate the original model. In case the interaction was removed from the final model, a hyphen (−) is mentioned in the tables. Post hoc pairwise comparisons were performed using R package multcomp by Tukey test (Hothorn et al., 2008) to assess the differences.
among least-squares means when significant effects were indicated for interactions or parity. Then, the average and range of metabolic status parameters and other blood biochemical parameters at 21 d were analyzed by linear mixed effects models which were built in the same way as mentioned before while excluding the fixed effect of day. The assumption of residuals’ normal distribution of the model was assessed by quantile-quantile plots. Log-transformed data were used when the above assumptions were rejected. The significance level was set at 0.05.

RESULTS

The cows had an average BW of 709 ± 67.2 kg and produced on average 48.2 ± 7.19 kg of fat- and protein-corrected milk and 48.7 ± 8.25 kg 3.5% FCM.

Oxidative Status of Cows with Distinct BHBA Levels in Early Lactation

By retrospective assignment, cows were allocated to 2 BHBA groups (ketotic vs. nonketotic) based on threshold BHBA concentrations (>1.2 mmol/L and <0.8 mmol/L, respectively). Of the 110 observations, 20 were grouped in the ketotic group (Supplemental Table S1, https://doi.org/10.6084/m9.figshare.21277557.v1, Zhang et al., 2022), whereas 33 had an average BHBA below 0.8. In addition to the selection criterion of BHBA <0.8 mmol/L and frequencies fulfilling the defined BHBA thresholds, all cows in the nonketotic group also were characterized by BHBA concentrations which did not exceed the threshold of 1.2 mmol/L on any of the 4 postcalving sampling days. The oxidative status of cows with distinct BHBA levels is shown in Figure 1. No difference was found in GPx activity and GSSG (%) in red blood cells. Compared with the nonketotic group, increased concentrations of MDA, decreased SOD activity, and impaired ORAC were observed in the ketotic group.

Production Performance and Health Status of Cows with Distinct BHBA Levels in Early Lactation

Dry matter intake and milk yield of dairy cows in both BHBA groups are given in Figure 2. In addition, more detailed information is given in the supplementary files (Supplemental Figure S2, https://doi.org/10.6084/m9.figshare.21277557.v1, Zhang et al., 2022) for production performance in both BHBA groups where interaction effects with parity is shown. The differences in DMI between the ketotic and nonketotic group particularly depended on the cows’ parity: a higher DMI in the nonketotic compared with the ketotic group particularly was observed for cows with parity greater than 3 (p_{group × parity} < 0.05). Despite the trend for a higher DMI, a higher milk yield was noticed only on d 2 and 4 in the ketotic group compared with the nonketotic group (p_{group × day} < 0.05). Nevertheless, third parity animals of the nonketotic group achieved a higher milk yield at d 1, 9, and 16 to 20 compared with the same parity group in the ketotic group (p_{group × day × parity} < 0.05). Irrespective of the ketotic status, both DMI and milk yield increased during the first 3 wk (p_{time} < 0.001).

Both in the ketotic as well as the nonketotic group 6 cases developed one or more clinical diseases which required intervention by a veterinarian (Supplemental Table S1). Proportionally, this represented 33% of the cases in the ketotic group versus 18% of the nonketotic cases. Interventions were related to hypocalcemia (n = 3), mastitis (n = 3), displaced abomasum (n = 7), clinical ketosis (n = 2), uterine infection (n = 4), and other undefined health problems during the transition period (n = 3).

FC Clustering Based on Oxidative Status

We further performed FC clustering based on oxidative status-related parameters and obtained 2 clusters (N:n = 31:19) by a cutoff membership value of 80% as a selection criterion. Parameters reflecting the oxidative status of observations with 80% membership to either one of the clusters are shown in Figure 3. No difference was found in the concentration of SOD between the 2 clusters. However, a trend of increased GPx activity was observed in cluster2 compared with cluster1 (0.05 < P < 0.1). Meanwhile, ORAC and GSSG (%) were higher whereas the concentration of MDA was lower in cluster2 compared with cluster1 (P < 0.05). The oxidative status of both clusters was then allocated based on these differences (i.e., cluster1 grouped cows with LAA_{80%} whereas cows of cluster2 had HAA_{80%}).

The distribution of cows that were sampled twice is shown in the supplementary files (Supplemental Table S1). In the LAA_{80%} and HAA_{80%}, there were 8 and 2 cases that developed one or more clinical diseases which required intervention by a veterinarian, respectively including hypocalcemia (n = 4), mastitis (n = 1), displaced abomasum (n = 4), clinical ketosis (n = 2), uterine infection (n = 3), and other undefined health problems during the transition period (n = 3). Furthermore, both groups of cows had a median parity of 3 (range 2–4 and 2–6, respectively; Supplemental Table S1).
Production Performance of Cows in HAA$_{80\%}$ Versus LAA$_{80\%}$

Dry matter intake and milk yield of dairy cows in different oxidative status groups are given in Figure 4. In addition, more detailed information was given in the supplementary files (Supplemental Figure S3, https://doi.org/10.6084/m9.figshare.21277557.v1, Zhang et al., 2022) for production performance in different oxidative status groups that showed interaction effects between group and parity. In the HAA$_{80\%}$ group, an increased DMI was observed when parity was greater than 2 compared with the LAA$_{80\%}$ group ($p_{\text{group} \times \text{parity}} < 0.05$). Dairy cows of the HAA$_{80\%}$ group showed an increased milk yield compared with the LAA$_{80\%}$ group except for d 1, 2, and 20 after calving ($p_{\text{group} \times \text{day}} < 0.05$). The increased milk yield was most pronounced for the third parity HAA$_{80\%}$ cows throughout the entire first 3 wk compared with the LAA$_{80\%}$ group, driving the 3 ways interaction ($p_{\text{group} \times \text{day} \times \text{parity}} < 0.05$). Irrespective of the oxidative status, both DMI and milk yield were increased during the first 3 wk ($p_{\text{time}} < 0.001$).

Metabolic Status and Biochemical Profiles of Cows in HAA$_{80\%}$ Versus LAA$_{80\%}$

Metabolic status-related parameters of dairy cows in different oxidative status groups are given in Figure 5. In addition, more detailed information was given in the supplementary files (Supplemental Figure S4, https://doi.org/10.6084/m9.figshare.21277557.v1, Zhang et al., 2022) for parameters that showed interaction effects between group and parity (BHBA and IGF-1). The concentrations of BHBA were significantly higher in the LAA$_{80\%}$ group compared with the HAA$_{80\%}$ group on 6, 9, and 21 d after calving ($P < 0.05$), tending to an interaction effect ($0.05 < p_{\text{group} \times \text{day}} < 0.1$). In addition, cows of the third parity showed an increased BHBA concentration compared with those of the second parity, but only on d 21 after calving ($p_{\text{day} \times \text{parity}} < 0.05$).

![Figure 1](image1.png)

**Figure 1.** Comparison of oxidative parameters at 21 d after calving between groups with different β-hydroxybutyric acid (BHBA) levels postpartum ($^*P < 0.05$, $^{**}P < 0.01$). The 2 groups, ketotic (n = 20) versus nonketotic (n = 33), were defined by the average concentration of BHBA >1.2 mmol/L or <0.8 mmol/L at 3, 6, 9, and 21 d relative to calving, respectively. Furthermore, on at least half of the observation days, individual observations were above or below the threshold, respectively. The minimum and maximum values of lower and upper whiskers were calculated as the first quartile (Q1) − 1.5 × interquartile range (IQR) and the third quartile (Q3) + 1.5 × IQR, respectively. The boxes represent the central 50% of the data with a central line marking the median value. The dots represent observations outside the central 50% of the data. SOD, superoxide dismutase; GPx, glutathione peroxidase; ORAC, oxygen radical absorbance capacity; MDA, malondialdehyde; GSSG (%), proportion of oxidized glutathione to total glutathione in red blood cells.
Irrespective of the oxidative status, all metabolic status–related parameters measured in this study changed significantly over time: glucose, fructosamine, IGF-1, and insulin decreased whereas BHBA and NEFA increased after parturition \( (p_{time} < 0.001) \). The within-animal observation range of the concentrations of metabolic parameters postpartum in different oxidative status groups is given in Table 3. The insulin concentra-

![Figure 2](image-url)

**Figure 2.** The production performance of dairy cows in different β-hydroxybutyric acid (BHBA) levels after calving (mean ± SEM). The 2 groups, ketotic \( (n = 20) \) versus nonketotic \( (n = 33) \), were defined by the average concentration of BHBA >1.2 mmol/L or <0.8 mmol/L at 3, 6, 9, and 21 d relative to calving, respectively. Furthermore, on at least half of the observation days, individual observations were above or below the threshold, respectively. Parity = 2, 3, or >3. Fixed effects of the linear mixed model were used to assess differences between ketotic groups \( (G) \), days relative to calving \( (D) \), parity \( (P) \), health status (i.e., presence or absence of clinical symptoms; \( H) \), and their interactions including \( G \times P, G \times D, D \times P, \) and \( G \times D \times P \). In case of a \( G \times D \) interaction, * symbols indicate the days on which significant differences were observed.
tion postpartum showed a greater range in the LAA_{80\%} group compared with the HAA_{80\%} group (p_{group} < 0.05).

Only weak correlations were observed between ORAC and blood BHBA concentrations at d 6, 9, and 21 as well as MDA and BHBA concentrations at d 6, respectively (correlation coefficients <0.3; Table 4), while other oxidative parameters did not correlate with blood BHBA concentrations. To further study the relation between blood BHBA and the oxidative status based on multiple oxidative parameters, the mutual overlaps of ketotic and LAA_{80\%} as well as nonketotic and HAA_{80\%} groups were assessed. For this, a scatter plot of all individual observations based on antioxidant ability and BHBA levels postpartum has been introduced (Figure 6). This figure shows that 10 out of 31 (32.3\%) of the LAA_{80\%} and 9 out of 19 (47.4\%) of the HAA_{80\%} observations belong to the ketotic and the nonketotic group, respectively. In contrast, only 1 out of 19 (5.3\%) and 3 out of 31 (9.7\%) cases from the HAA_{80\%} and LAA_{80\%} groups belong to ketotic and nonketotic groups.

The blood biochemical indices of liver function of dairy cows in different oxidative status groups are given in Table 5. The percentage of α-globulin and β-globulin as well as the concentrations of γ-globulin were greater whereas the percentage of γ-globulin and the aspartate aminotransferase concentration were lower in the HAA_{80\%} group compared with the LAA_{80\%} group (p_{group} < 0.05). Moreover, the concentrations of albumin decreased with parity in the LAA_{80\%} group whereas parities in the HAA_{80\%} group did not (tend to) differ (p_{group × parity} < 0.05).

**DISCUSSION**

The imbalance of metabolic and oxidative status in dairy cows has been addressed as risk factors during the transition period, especially the first weeks of lactation (Sordillo and Raphael, 2013; Yehia et al., 2020). However, the relationship between these 2 stressors is not yet completely understood. In our study, this relationship has been assessed from 2 angles. First, by
Figure 4. Production performance of dairy cows in different oxidative status at 21 d relative to calving (mean ± SEM). The 2 groups, LAA₈₀% (lower antioxidant ability, n = 31) versus HAA₈₀% (higher antioxidant ability, n = 19), were obtained by fuzzy C-means clustering and a cutoff membership value of 80% was applied as a selection criterion. Parity = 2, 3, or >3. Fixed effects of the linear mixed model were used to assess differences between 80%-membership groups (G), days relative to calving (D), parity (P), health status (i.e., presence or absence of clinical symptoms; H), and their interactions including G × P, G × D, D × P, and G × D × P. In case of a G × D interaction, * symbols indicate the days on which significant differences were observed.
Figure 5. Blood parameters related to metabolic status of dairy cows differing in oxidative status at 21 d relative to calving (mean ± SEM). The 2 groups, LAA<sub>80%</sub> (lower antioxidant ability, n = 31) versus HAA<sub>80%</sub> (higher antioxidant ability, n = 19), were obtained by fuzzy C-means clustering and a cutoff membership value of 80% was applied as a selection criterion. Parity = 2, 3, or >3. Fixed effects of the linear mixed model were used to assess differences between 80%-membership groups (G), days relative to calving (D), parity (P), health status (i.e., presence or absence of clinical symptoms; H), and their interactions including G × P, G × D, D × P, and G × D × P. In case of a G × D interaction, * symbols indicate the days on which significant differences were observed. BHBA, β-hydroxybutyric acid; NEFA, nonesterified fatty acids.
retrospective selection of a subdata set based on blood BHBA concentrations (approximately 50% of observations were included), we found that nonketotic cows showed a higher antioxidant ability compared with ketotic cows which were characterized by increased concentrations of MDA, diminished ORAC, and decreased SOD activity. Second and inversely, it was investigated whether the impaired antioxidant ability of animals was consistently reflected in increased BHBA levels and in other parameters related to metabolic status.

However, assessing the oxidative status is more challenging than the ketotic status as for none of the oxidative parameters specific golden standard thresholds exist and unreliable findings may be obtained by evaluation of single parameters. For instance, significantly increased activities of SOD and GPx—normally representing a better oxidative status—were observed whereas the total antioxidant ability decreased after parturition (Wullepit et al., 2012; Bühler et al., 2018). Such conflicting results may relate to the homeostatic control of the antioxidant system and endogenous antioxidant increases when oxidative damage occurs (Đidara et al., 2015). Therefore, a combination of parameters that can represent both the production of ROS and antioxidant ability are required to comprehensively determine the oxidative status. In previous studies, a combination of enzymatic antioxidants (SOD and GPx) and nonenzymatic antioxidants [GSSG (%)] as well as oxidative damage biomarkers (e.g., MDA) were widely used to evaluate the antioxidant ability of dairy cows (Liu et al., 2013; Song et al., 2016; Chalmeh et al., 2021).

In this study, FC clustering based on SOD and GPx activity, ORAC, MDA concentration, and GSSG (%) has been used to group cows with distinctive oxidative status at 21 d in lactation. Compared with k-means clustering that assigns data points to a particular cluster, FC algorithm is a soft clustering approach that calculates the membership value of data point belonging to each cluster. Research reporting the choice of cutoff membership value is limited. Dembélé and Kastner (2003) applied FC algorithm with certain threshold levels of membership values (the median of the highest membership values) to select genes that are tightly associated with a cluster. In our study, when using a cutoff membership value of 80%, approximately 50% of the individual observations of the original data set were included and a distinctive oxidative status between LAA80% and HAA80% was noticed, with the lat-

Table 3. The range of blood parameters related to metabolic status postpartum (d 3–21) of dairy cows of different oxidative status (LAA80% vs. HAA80%) at 21 d after calving (mean ± SEM)1

<table>
<thead>
<tr>
<th>Item</th>
<th>LAA80%</th>
<th>HAA80%</th>
<th>Group Parity3</th>
<th>Health</th>
<th>Group × parity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.817 ± 0.1253</td>
<td>0.611 ± 0.0633</td>
<td>0.101</td>
<td>0.773</td>
<td>0.146</td>
</tr>
<tr>
<td>BHBA (mmol/L)</td>
<td>0.809 ± 0.1351</td>
<td>0.464 ± 0.1513</td>
<td>0.524</td>
<td>0.905</td>
<td>0.005</td>
</tr>
<tr>
<td>NEFA (mmol/L)</td>
<td>0.606 ± 0.0732</td>
<td>0.540 ± 0.0901</td>
<td>0.915</td>
<td>0.637</td>
<td>0.189</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>0.373 ± 0.0875</td>
<td>0.274 ± 0.0278</td>
<td>0.009</td>
<td>0.554</td>
<td>0.010</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>39.2 ± 6.02</td>
<td>43.6 ± 6.76</td>
<td>0.646</td>
<td>0.504</td>
<td>0.828</td>
</tr>
<tr>
<td>Fructosamine (µmol/L)</td>
<td>52.3 ± 7.14</td>
<td>51.9 ± 8.82</td>
<td>0.971</td>
<td>0.249</td>
<td>0.567</td>
</tr>
</tbody>
</table>

1The 2 groups, LAA80% (lower antioxidant ability, n = 31) versus HAA80% (higher antioxidant ability, n = 19), were obtained by fuzzy C-means clustering and a cutoff membership value of 80% was applied as a selection criterion.  
2The range (maximum − minimum) of parameters related to metabolic status was calculated for each animal from the 4 postpartum sampling points (3, 6, 9, and 21 d relative to calving). Then, the means ± SEM were calculated for each group. BHBA = β-hydroxybutyric acid; NEFA = nonesterified fatty acids.  
3Parity = 2, 3, or >3.

Table 4. Pearson correlation coefficients between β-hydroxybutyric acid (BHBA) and oxidative status–related parameters in blood sampled on 4 d postpartum (n = 110)

<table>
<thead>
<tr>
<th>Item1</th>
<th>BHBA d 3</th>
<th>BHBA d 6</th>
<th>BHBA d 9</th>
<th>BHBA d 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>−0.088</td>
<td>−0.037</td>
<td>−0.076</td>
<td>−0.109</td>
</tr>
<tr>
<td>GPx</td>
<td>−0.068</td>
<td>−0.030</td>
<td>−0.112</td>
<td>0.136</td>
</tr>
<tr>
<td>ORAC</td>
<td>−0.182</td>
<td>−0.282**</td>
<td>−0.236*</td>
<td>−0.216*</td>
</tr>
<tr>
<td>MDA</td>
<td>0.093</td>
<td>0.231*</td>
<td>−0.169</td>
<td>0.049</td>
</tr>
<tr>
<td>GSSG (%)</td>
<td>−0.103</td>
<td>−0.102</td>
<td>−0.038</td>
<td>0.039</td>
</tr>
</tbody>
</table>

1SOD = superoxide dismutase; GPx = glutathione peroxidase; ORAC = oxygen radical absorbance capacity; MDA = malondialdehyde; GSSG (%) = proportion of oxidized glutathione to total glutathione in red blood cells.  
*P < 0.05, **P < 0.01.
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Table 5. Blood biochemical indices of liver function of dairy cows of different oxidative status (LAA_{80%} vs. HAA_{80%}) at 21 d relative to calving (mean ± SEM)^1

<table>
<thead>
<tr>
<th>Item^2</th>
<th>LAA_{80%}</th>
<th>HAA_{80%}</th>
<th>Group</th>
<th>Parity^3</th>
<th>Health</th>
<th>Group × parity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total protein (g/L)</td>
<td>75.6 ± 0.95</td>
<td>82.6 ± 6.32</td>
<td>0.167</td>
<td>0.384</td>
<td>0.626</td>
</tr>
<tr>
<td></td>
<td>Albumin (%)</td>
<td>42.3 ± 0.75</td>
<td>42.3 ± 1.26</td>
<td>0.974</td>
<td>&lt;0.001</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>Albumin (g/L)</td>
<td>31.9 ± 0.55</td>
<td>35.0 ± 2.85</td>
<td>0.102</td>
<td>0.134</td>
<td>0.082</td>
</tr>
<tr>
<td></td>
<td>α-Globulin (%)</td>
<td>15.6 ± 0.28</td>
<td>16.4 ± 0.35</td>
<td>0.041</td>
<td>0.103</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>α-Globulin (g/L)</td>
<td>11.8 ± 0.22</td>
<td>13.5 ± 1.06</td>
<td>0.135</td>
<td>0.947</td>
<td>0.905</td>
</tr>
<tr>
<td></td>
<td>β-Globulin (%)</td>
<td>8.98 ± 0.226</td>
<td>9.53 ± 0.327</td>
<td>0.029</td>
<td>0.192</td>
<td>0.363</td>
</tr>
<tr>
<td></td>
<td>β-Globulin (g/L)</td>
<td>6.79 ± 0.179</td>
<td>7.86 ± 0.634</td>
<td>0.115</td>
<td>0.659</td>
<td>0.820</td>
</tr>
<tr>
<td></td>
<td>γ-Globulin (%)</td>
<td>33.2 ± 0.86</td>
<td>31.7 ± 1.24</td>
<td>0.005</td>
<td>0.002</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td>γ-Globulin (g/L)</td>
<td>25.2 ± 0.86</td>
<td>26.3 ± 2.22</td>
<td>&lt;0.001</td>
<td>0.171</td>
<td>0.981</td>
</tr>
<tr>
<td></td>
<td>A:G</td>
<td>0.741 ± 0.0227</td>
<td>0.751 ± 0.0387</td>
<td>0.833</td>
<td>0.002</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>Urea (mmol/L)</td>
<td>2.71 ± 0.113</td>
<td>2.86 ± 0.096</td>
<td>0.709</td>
<td>0.828</td>
<td>0.478</td>
</tr>
<tr>
<td></td>
<td>AST (IU/L)</td>
<td>95.2 ± 4.26</td>
<td>83.8 ± 6.07</td>
<td>0.003</td>
<td>0.203</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td>SAA (µg/mL)</td>
<td>82.3 ± 24.99</td>
<td>39.4 ± 12.38</td>
<td>0.178</td>
<td>0.506</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>HP (mg/mL)</td>
<td>0.228 ± 0.0507</td>
<td>0.171 ± 0.0249</td>
<td>0.278</td>
<td>0.375</td>
<td>0.026</td>
</tr>
</tbody>
</table>

^1The 2 groups, LAA_{80%} (lower antioxidant ability, n = 31) versus HAA_{80%} (higher antioxidant ability, n = 19), were obtained by fuzzy C-means clustering and a cutoff membership value of 80% was applied as a selection criterion.

^2A:G = ratio of albumin to globulin; AST = aspartate transaminase; SAA = serum amyloid A; HP = haptoglobin.

^3Parity = 2, 3, or >3.

Figure 6. Scatter plot of all individual observations based on antioxidant ability and β-hydroxybutyric acid (BHBA) levels postpartum. The 2 groups, LAA_{80%} (lower antioxidant ability, n = 31) versus HAA_{80%} (higher antioxidant ability, n = 19), were obtained by fuzzy C-means clustering and a cutoff membership value of 80% was applied as a selection criterion. The NA group (not assigned, n = 60) consists of observations that did not fulfill the fuzzy C-means clustering membership criterion for any of the 2 clusters. The horizontal dotted lines further indicate the average BHBA cut-offs, used as one of the criteria to separate the ketotic (BHBA >1.2 mmol/L) and nonketotic (BHBA <0.8 mmol/L) groups. Additionally, to be classified in these groups, the former conditions had to be fulfilled on at least 2 of the 4 postpartum observation days. All cases with an average BHBA concentration below 0.8 mmol/L fulfilled this additional criterion, whereas a part of the cases with average BHBA >1.2 mmol/L did not fulfill the second condition and hence are not classified into the ketotic group. These are indicated by open symbols.
ter characterized by a greater total antioxidant ability (ORAC), increased GSSG (%), and reduced MDA concentrations. Although higher proportions of glutathione in the oxidized stage (GSSG %) were considered as a negative sign of redox homeostasis in some previous studies (Dobbelaar et al., 2010; Zhao et al., 2015), the increased GSSG proportion potentially could be partially explained by an increased GPx activity (trend, \( P = 0.065 \)) which uses GSH as substrate in redox reactions to protect the cellular redox balance. Accordingly, Correa et al. (2022) reported decreased concentrations of GSH and GSH or GSSG values in association with increased activities of GPx as well as greater GSSG concentrations in the liver of Nellore cattle that received antioxidant supplements.

No or at most, rather weak correlations have been observed between single anti- or pro-oxidant plasma parameters with blood BHBA concentrations (correlation coefficients <0.3). However, the antioxidant system is complex, and synergy may exist between various antioxidant enzymes as discussed above. Therefore, a multivariate oxidative classification rather than a classification based on one single indicator was applied. The scatterplot of Figure 6 reveals only 1 out of 19 (5.3%) and 3 out of 31 (9.7%) cases from the HAA80% and LAA80% groups belong to ketotic and nonketotic groups, respectively, suggesting that cows with a higher antioxidant ability in early lactation rarely develop metabolic issues (e.g., elevated BHBA levels). Nevertheless, still quite a lot of animals in the intermediate group (not assigned due to lower than 80% membership value based on antioxidant ability clustering) are included in the extreme ketotic or extreme nonketotic group and vice versa, suggesting that high BHBA and low oxidative capacity do not coincide consistently.

A ketone body, BHBA, is produced in the liver due to the restricted condensation with oxaloacetate of acetyl CoA (Krebs, 1966). Excessive amounts of the latter originate from β-oxidation of NEFA in case of a severe negative energy balance. In our study, average NEFA concentrations tended to be higher after calving in the LAA80% group compared with the HAA80% group. Enhanced metabolic activity (e.g., reflected in enhanced concentrations of NEFA and ketone bodies) is associated with enhanced production of ROS (Perdemera et al., 2010). Moreover, in vitro studies found that treatments inducing high levels of BHBA (1.2–2.4 mmol/L) were correlated with increased ROS levels and impaired antioxidant ability which further could induce hepatocytes’ apoptosis or injury (Song et al., 2016). In the current study, also some other parameters, such as the increased blood aspartate aminotransferase concentrations in the LAA80% group may indicate an impaired liver function (Seifi et al., 2007; Sun et al., 2015). Additionally, total blood proteins and their fractions are useful indicators of health status and liver function (Cozzi et al., 2011; Cattaneo et al., 2021). Alberghina et al. (2011) reported reference intervals of blood proteins in dairy cows. The concentrations of albumin (reference intervals: 31.86 ± 4.60 g/L), α-globulin [reference intervals: α1-globulin (5.77 ± 2.20 g/L) and α2-globulin (5.84 ± 1.90 g/L)] and β-globulin (reference intervals: 7.46 ± 1.94 g/L) were within the reported normal range, whereas γ-globulin (reference intervals: 16.73 ± 4.54; mainly antibodies) concentrations were higher and the albumin to globulin ratio (reference intervals: 0.88 ± 0.43) on average was lower compared with reference intervals. Nevertheless, the study of Alberghina et al. (2011) was conducted on the Modicana cows, a multipurpose breed from the south of Sicily with low milk yield. Bobbo et al. (2017) reported the higher concentration of total protein and globulin, and a lower albumin to globulin ratio of specialized breeds (especially Holstein Friesian) compared with dual-purpose breeds that produced less milk.

No differences could be observed between the 2 antioxidant ability groups on other metabolic parameters, except for a higher range of insulin concentrations postpartum in the LAA80% group, due to a pronounced peak concentration at d 6. Meanwhile, the glucose levels did not differ between the 2 groups. Although, the current concentration-based data do not allow any mechanistic conclusions. The observed variation in insulin without glucose response in the LAA80% group suggests further research toward the association between oxidative stress in early lactation and the development of insulin resistance could be of interest, as insulin plays a key role in the regulation of energy partition. Indeed, recently an increasing number of studies emphasize that oxidative stress at early lactation is associated with the development of insulin resistance (Abuelo et al., 2016; Youssef and El-Ashker, 2017). Nevertheless, to quantify insulin secretion and assess resistance a hyperinsulinemic euglycemic clamp test is required (De Koster et al., 2016; De Koster et al., 2017).

A suppressed appetite before parturition which gradually recovered postpartum already has been reported in previous studies (Hayirli et al., 2002; Huzzey et al., 2007). In line with previous studies, we also observed the lowest DMI in the first week after calving with a gradual increase after the onset of lactation. In addition, a lower DMI was observed in the LAA80% group compared with the HAA80% group. The cause of the anorexic phenomenon around calving is not yet elucidated, but an elevated inflammatory response and liver dysfunction around calving (Horst et al., 2021) were suggested as potential major factors. Nevertheless, the positive acute phase proteins (i.e., serum amyloid a and...
During the transition period, however, the LAA80% milk yield reduction compared with nonketotic cows (Rathbun et al., 2017), ketotic cows did not show a reduction in milk yield. In line with some previous studies, approaches of the current study showed different results. In line with some previous studies (Rathbun et al., 2017), ketotic cows did not show a decreased trend of milk yield compared with the nonketotic group. However, despite some overlap between the LAA80% and the ketotic group, the approaches of the current study showed different results regarding milk yield. In line with some previous studies (Rathbun et al., 2017), ketotic cows did not show a milk yield reduction compared with nonketotic cows during the transition period. However, the LAA80% group (with an average BHBA concentration of 1.19 mmol/L) achieved a lower milk yield compared with the HAA80% group. As suggested by Horst et al. (2021), ketosis should not always be considered as problematic, the increased circulating ketones should not be considered as causal toward negative outcomes but in some cows could be a necessary adjustment for optimum production. Furthermore, impairment of the oxidative status may be a process which has greater effect on production performance than ketosis. However, in our study, no difference could be observed between cases which concomitantly belonged to the LAA80% group as well as the ketotic group (35.2 ± 4.739 kg/d, n = 10) versus the other, exclusively ketotic cases (34.8 ± 5.824 kg/d, n = 10). Nevertheless, the limited number of cases in each category may have restricted the production evaluation.

CONCLUSIONS

This study started from 2 different entry points to obtain a subdata set of the transition cows. Ketotic cases showed reduced antioxidant ability [characterized by lower ORAC, GSSG proportion, and GPx (trend) and elevated MDA] and inversely, observations with impaired antioxidant ability, classified based on a multivariate clustering approach, showed increased concentrations of BHBA. Both ketotic cases as well as cases with a lower antioxidant ability showed lower DMI. However, for ketotic cases this was not associated with reduced milk yield, whereas lower milk production was observed in the group with impaired antioxidant ability. Cases with a higher antioxidant ability in early lactation rarely develop ketosis. Therefore, sufficient dietary antioxidants supply could be paramount to alleviate metabolic stress of the transition period by maintaining the balance of redox status. However, further investigation is still needed to elucidate underlying mechanisms of interacting factors.

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REFERENCES


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