ABSTRACT

Despite high numbers of cattle, milk production in many tropical countries such as Ethiopia is very low. Animals are managed traditionally, meaning they mostly depend on seasonal availability of natural pasture, grass, and crop residues with no supplementary feeds. Due to the lack of pasture management, there is overgrazing and soil erosion, and the land still must deal with extremely dry periods. All this has a negative effect on dairy cow productivity. Identification of the specific nutritional deficits would enable targeted interventions to improve milk yield performance, but nutrient and energy intakes are difficult to assess in ranging conditions. The aim of this research was, therefore, to evaluate the nutritional status of ranging dairy cows through blood metabolites, milk yield, and body condition in relation to environmental factors such as agro-ecology and season. The study was performed in a tropical region that is known to be exposed to the above-mentioned situation, the Arba Minch region in the southern Ethiopian Rift Valley. Blood samples were collected from 170 ranging dairy cows in 6 different districts, along a transect extending from the lowlands to the highlands, in both seasons (dry and rainy). Body condition score and milk yield of all cows were also determined for both seasons. Serum urea, creatinine, triglyceride, and nonesterified fatty acid concentrations were quantified spectrophotometrically. Dried serum spots were subject to quantitative electrospray tandem mass spectrometry to estimate changes in nutrient metabolism based on selected free AA and carnitine esters. Based on these measurements, nutritional status varied with season and geographical region. It can also be concluded that extensive metabolite analysis such as mass spectrometry can provide detailed insights, but the simpler spectrophotometric metabolite analysis can estimate the nutritional status of ranging animals.

Key words: nutrient metabolism, zebus, tropical rangeland, analytical techniques

INTRODUCTION

In many tropical areas, milk yield per dairy cow is still far below the estimated genetic potential (Ramírez-Rivera et al., 2019). Seasonal feed shortage and inefficient feed utilization are the major challenges affecting dairy cattle productivity (Manaye et al., 2009; Gelayenew et al., 2016; CSA, 2017). In many cases, dairy cattle are ranging in herds. In such a system, it is difficult to monitor and have control of the exact diet of the animals. Yet, the need to have information about their nutritional status is clear because imbalanced nutrition will not only lead to poor performance but also to more grazing pressure on the land due to the lower feed utilization efficiency. To identify the limiting nutrients in the ranging diet as a starting point for targeted interventions, strategies for nutritional status estimation are thus needed other than ration calculation, which is commonly done in stabled livestock. The voluntary intake of grazing ruminants depends on multiple factors linked to herbage and animal characteristics (Decruyenaere et al., 2009). If ration control is not feasible, blood metabolite analysis may be a way to identify and understand the nutritional limitations for ranging dairy cattle performance.

Blood glucose, BHB, and nonesterified fatty acids (NEFA) are the main metabolites used to assess the energy status of cattle (Ndlovu et al., 2007). Blood glucose has a moderate diagnostic value in the assessment of the nutritional status of cattle as it varies moderately in the blood (Reynolds et al., 2003).

During body fat mobilization, lipoprotein lipase hydrolyzes stored triglycerides into glycerol and NEFA, meaning that the latter is a reliable reflection of negative energy balance, and more specifically of body fat mobilization (Agenas et al., 2006; Han van der Kolk et al., 2017). Fatty acid oxidation turns these fatty acids into acetyl-CoA that can be used as an energy source in the citric acid cycle (CAC), but when glucogenic
sources are lacking, acetyl-CoA turns to less efficient ketone body formation, such as BHB (Drackley et al.,
2001; Turk et al., 2016). The event of elevated BHB concentrations often coincides with increased triglyceride concentrations because, at a certain point, the ketone pathway is saturated (Ospina et al., 2013).

High blood urea levels point to either high protein intake or excessive mobilization of muscle (Pulido et al., 2015). Serum urea concentration may thus also increase despite low protein feeding if energy intake does not meet requirements, hence reflecting the increased breakdown of endogenous proteins for energy production (Säkkinen, 2005). To differentiate the catabolism of mobilized body protein from the ingested protein use, serum creatinine can be taken as a measure of body muscle breakdown (Ndlovu et al., 2007). Creatinine is the breakdown product of creatine and phosphocreatine in the muscle (Otto et al., 2000; Miller et al., 2004).

Briefly, the combined measurement of creatinine, urea, NEFA, and triglycerides should make a reasonable metabolite panel to estimate the nutritional status of ranging dairy cattle.

The main tool to estimate nutritional status to date is the BCS. Blood metabolites may, however, provide more background information on the underlying reasons for low performance, and BCS may not be as accurate and precise because of its subjective nature.

The above-mentioned metabolite panel can be easily measured through spectrophotometry. The question is to which extent more extensive metabolite profiling, for instance via MS, would be required to decipher the most pressing nutritional needs of ranging animals such as dairy cattle. In former studies in a wide array of animal species, the profile of free AA and carnitine ester in circulation allowed the identification of shifts in nutrient use for energy (cats: Verbrugghe et al., 2010; fish: Geda et al., 2017; frogs: Brenes-Soto et al., 2018).

We, therefore, screened the nutritional status of ranging dairy cattle in the southern Ethiopian Rift Valley in the Lake Abaya and Chamo catchment area, using body condition scoring at different seasons and agro-ecological conditions, combined with blood metabolite analysis through spectrophotometry as well as more detailed metabolite analysis through MS.

**MATERIALS AND METHODS**

**Study Area**

The study was conducted in 5 districts of Gamo zone (Chencha, Bonke, Boreda, Mirab Abaya, and Arba Minch Zuria) and the Dereshe special district, Southern Ethiopia. These 6 districts are found in the Chamo and Abaya Lakes catchments, which is a part of the southern Ethiopian Rift Valley. The mountainous catchment area of Lake Abaya and Lake Chamo is characterized by intensive land use, especially through grazing and crop production.

There are 3 agro-ecological regions in these catchments: the lowlands (Kolla) region, which is within 500 and 1,500 m above sea level; the midlands (Woyna Dega), within 1,501 and 2,300 m above sea level; and the highland (Dega) region at more than 2,300 m above sea level. It receives 600 to 1,600 mm rainfall per annum and the annual temperature ranges from 10°C to 34°C according to CSA (2017). The highlands are densely populated and have high grazing pressure because 80% of the cattle population is kept there (Daniel, 1988). In the highlands, better soil is used for cultivation and the steep slopes are used for grazing the animals (Alemayehu, 2004; Tolera et al., 2012).

About 65% of the feed for dairy cows is from natural meadows, and the rest comes from crop residues and agro-industrial by-products (Jahnke and Asemene, 1983; Birhan and Aduguna, 2014). The crop residues, like cereal straws, are more important in the dry season (Keftasa, 1988). To get an impression of what the ruminants are consuming, it is important to focus on the natural pasture. The 3 agro-ecological regions have different types of forage. The structure of the pastureland is mainly influenced by user pressure, topography, and the amount and distribution of rainfall (Tolera et al., 2012). As the altitude increases, the proportion of legumes forages also increases. *Trifolium* spp., and *Medicago* spp. grow above 2,200 m. In the lowlands, mainly browse and shrubs can be found (Alemayehu, 2004).

Because the quality of the natural meadow decreases, most palatable grasses have disappeared and have been replaced by indigestible and undesirable species such as *Aristida* and *Sporobolus* spp. and poisonous plant species.

**Animals and Data Collection Procedures**

All lactating dairy cows in each district of the study area were selected and grouped into 3 agro-ecological zones (lowlands, midlands, and highlands), which are reared under the same management system within the agro-ecological region. Every individual cow belonged to another smallholder farm to obtain a representation of the variety of management among the smallholders in a region. The blood sampling of the cows was done by a skilled person in accordance with the Guidelines for the Care and Use of Agricultural Animals in Research and Teaching (Vaughn, 2012). The cows were accustomed to close human contact, hence leading to a minimal level of distress. A total of 170 lactating local...
(zebu) breed dairy cows reared under extensive production were randomly selected for milk yield, blood sample collection, and body condition evaluation. Blood samples were collected twice from the same cows from the jugular vein at 2 seasons (dry and rainy) of the region; sampling started first in the dry season and all cows were at later lactation in the rainy season. The blood was transported to the laboratory in an icebox, where serum was collected and stored at −20°C until further analysis. A total of 170 blood samples were collected per season; 10 cows were sampled in each of the 3 agro-ecological zones in the 6 districts, with one district having only 2 agro-ecological zones (no highlands). Samples were collected preferably in the morning before the animals started ranging.

Cows were ranging out in the field during the day but housed overnight in a sheltered area. The animals stayed with their owner during the study. Milk yields of every cow in both seasons were reported by the owners and measured by locally available can or container of which the volume was checked by the researchers. The body condition of the animals was scored from 1 to 9 scale by 2 independent evaluators for each individual cow based on the criteria set by Nicholson and Butterworth (1986), and in case of a deviation in score between 2 evaluators, they made a decision based on consensus (Table 1).

Throughout the whole study, all animals consumed the feed obtained by grazing and what they received from the owner. The main feed consisted of hay or straw as roughage and brewer’s by-products, or grain residues as concentrate and kitchen waste. It was assumed that animals from the same district ingested similar diets. It must however be considered that the availability of feed resources varied in amount and composition. The free-grazing cattle drank water from the river.

**Laboratory Analysis**

A spectrophotometer (UV-3100 PC, VWR) was used to measure the following metabolites: urea, creatinine, triglycerides, and NEFA. Every morning and afternoon, a calibration curve was made to compensate for environmental temperature differences. Kits from Journilabs (Addis Ababa, Ethiopia) were used for urea (Watt and Chrisp, 1954), creatinine, and triglycerides according to the procedure of the product. A kit from Fujifilm (Lexington, MA) was used for NEFA [http://www.wakodiagnostics.com/r_nefa.html](http://www.wakodiagnostics.com/r_nefa.html).

Creatinine forms a colored complex if it is combined with picric acid in an alkaline solution. The concentration of creatinine is directly proportional with the amount of the complex formed. The absorbance of the complex formed. The absorbance of the samples needs to be measured at 500 nm. To make the calibration curve, the following reagents were used: R1, sodium hydroxide; R2, picric acid, and R3, standard (He et al., 2015). The triglycerides were determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone, and 4-chlorophenol under the catalytic influence of peroxidase. The absorbance of the samples was measured at 520 nm (Guglielmo et al., 2005). Urea is hydrolyzed by water and the enzyme urease into ammonia and carbon dioxide. The ammonia produced further reacts with ketoglutarate and NAD in the presence of glutamate dehydrogenase to reproduce glutamate and NAD⁺. The absorbance of the samples was measured at 340 nm. Nonesterified fatty acids are treated with acyl-CoA synthetase in the presence of ATP and CoA. Thiol esters of CoA then form as acyl-CoA along with by-products AMP and pyrophosphate. In the second portion of the procedure, the acyl-CoA is oxidized by added acyl-CoA oxidase to produce hydrogen peroxide. In the presence of added peroxidase, this allows for the oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline with 4-aminopyridine to form a purple color. The absorbance of the samples was measured at 550 nm (Johnson and Peters, 1993).

Acylcarnitine and AA profiles were conducted using dried bloodspots to obtain information about biomarkers as a relatively cheap and automated method (Dermauw et al., 2013). Thirty-five-microliter drops of serum were collected on Whatman Protein Saver 903 cards at the Arba Minch University College of Agriculture Animal Nutrition Laboratory, allowed to dry for 2 h, and stored at −20°C until transport to the laboratory for dried bloodspot analysis. Most of the analytes remain stable at room temperature for 1 wk or more, and in laboratory freezers, the analytes remain stable for long periods (McDade et al., 2007). The method of Zytkovicz et al. (2001) was used. Extraction of the dried bloodspots was done by shaking the bloodspot paper in 100 µL of methanol in a microtiter plate.

**Table 1.** Body condition of the animals scored on a 1 to 9 scale based on the criteria set by Nicholson and Butterworth (1986)

<table>
<thead>
<tr>
<th>Score</th>
<th>Condition</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Emaciated</td>
<td>Shoulder, rib, and back visible</td>
</tr>
<tr>
<td>2</td>
<td>Very thin</td>
<td>Some muscle, no fat deposits</td>
</tr>
<tr>
<td>3</td>
<td>Thin</td>
<td>Some fat deposits, ribs visible</td>
</tr>
<tr>
<td>4</td>
<td>Borderline</td>
<td>Fore ribs not noticeable</td>
</tr>
<tr>
<td>5</td>
<td>Moderate</td>
<td>12th and 13th ribs not visible</td>
</tr>
<tr>
<td>6</td>
<td>Good</td>
<td>Ribs covered, sponginess</td>
</tr>
<tr>
<td>7</td>
<td>Very good</td>
<td>Abundant fat on the tail head</td>
</tr>
<tr>
<td>8</td>
<td>Fat</td>
<td>Fat cover thick and spongy</td>
</tr>
<tr>
<td>9</td>
<td>Obese</td>
<td>Extremely fat throughout</td>
</tr>
</tbody>
</table>
dried, whereafter 70 µL of butanol-HCl was added, then covered with Teflon and put in a 65°C oven for 15 min, and dried again. Deuterium-labeled standards were used at the Laboratory for Metabolic Diseases at Ghent University Hospital. The butanol derivates were reconstituted with 100 µL of acetonitrile:water (80:20 vol:vol). An aliquot of 20 µL was injected for tandem MS. This technique has been widely applied to identify metabolic disorders biochemically detectable in human beings (adults and newborns) in both serum and whole blood samples (Rinaldo et al., 2008), and recently has been also employed in studies with animals (Verbrugghe et al., 2009; Geda et al., 2017).

**Data Analysis**

Statistical analysis was performed using SPSS 21 (IBM Corp., Armonk, NY). Repeated measures variance analysis was used to evaluate the effects of season, agro-ecological zone, and their interactions on blood metabolites, BCS, and milk yields of dairy cows. Post-hoc differences between agro-ecological zones were evaluated using independent t-tests. Pearson correlation analysis and principal component analysis were performed to establish the relationship between pairs of variables and groups of variables. Significance was accepted at the 5% probability level.

**RESULTS**

Milk yield was lower ($P < 0.001$) in the dry season compared with the rainy season, with an interaction that showed the largest impact of the dry season in the highlands (Table 2). Overall, milk yield was highest in the midlands, followed by the highlands and lowlands ($P < 0.001$). Irrespective of the effect of the agro-ecological zone, the BCS was lower in the dry season compared with the rainy season ($P < 0.001$).

Serum creatinine, triglyceride, and NEFA concentrations were higher ($P < 0.001$) in the dry season than in the rainy season, whereas urea was lower (Table 3). However, there was no significant effect of the agro-ecological region on the concentrations of serum creatinine, triglyceride, urea, and NEFA. Yet, an interaction between season and the agro-ecological zone was found on serum urea concentration, with only a slight decrease in the lowlands toward the dry season, versus a major drop ($P = 0.003$) in serum urea in both midlands and highlands.

All measured free AA except citrulline showed distinctly lower ($P < 0.05$) concentrations in the dry season compared with the rainy season (only numerical for phenylalanine; Table 4). Only phenylalanine and tryptophan concentrations differed between the agro-ecological zones, being higher ($P < 0.01$) in the highlands.

---

### Table 2. Effects of season and agro-ecological zone on milk yield and BCS of ranging dairy cows reared in the southern Ethiopian Rift Valley (n = 20)

<table>
<thead>
<tr>
<th>Item</th>
<th>Rainy season</th>
<th>Dry season</th>
<th>$P$-value$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowlands</td>
<td>Midlands</td>
<td>Highlands</td>
</tr>
<tr>
<td>Milk yield (L/d)</td>
<td>1.89</td>
<td>2.99</td>
<td>2.89</td>
</tr>
<tr>
<td>BCS</td>
<td>5.05</td>
<td>5.68</td>
<td>5.61</td>
</tr>
</tbody>
</table>

$^1$S = season; AZ = agro-ecological zone; $S \times AZ$ = season and agro-ecological zone interaction.

### Table 3. Effects of season and agro-ecological zone on spectrophotometrically determined serum metabolite concentrations in dairy cows in the southern Ethiopian Rift Valley

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Rainy season</th>
<th>Dry season</th>
<th>$P$-value$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowlands</td>
<td>Midlands</td>
<td>Highlands</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.41</td>
<td>0.37</td>
<td>0.34</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.28</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>1.27</td>
<td>1.40</td>
<td>1.47</td>
</tr>
<tr>
<td>NEFA$^2$ (mmol/L)</td>
<td>1.21</td>
<td>1.19</td>
<td>1.10</td>
</tr>
</tbody>
</table>

$^2$NEFA = nonesterified fatty acids.
A more diverse picture appeared in the carnitine ester profile (Table 5). Free carnitine remained stable over seasons and agro-ecological zones, whereas acetyl carnitine ($C_2$) and propionyl carnitine ($C_3$) were lower ($P < 0.01$) in the dry season than in the rainy season, independent of the agro-ecological zone. The butyryl carnitine + isobutyryl carnitine ($C_4$) concentration did not differ between seasons but decreased ($P = 0.006$) with increasing altitude. Other markers for AA degradation such as isovaleryl carnitine + 2-methylbutyryl carnitine ($C_5$; isoleucine catabolism), methylmalonyl carnitine + succinyl carnitine ($C_{4DC}$; valine and methionine catabolism), glutaryl carnitine ($C_{5DC}$; lysine and tryptophan catabolism), and 3-methylglutaryl carnitine ($C_{6DC}$; leucine catabolism) remained constant over seasons and agro-ecological zones. Also, the marker for ketogenesis, 3-hydroxybutyryl carnitine ($3OHC_4$), was not affected by these factors. Malonyl carnitine, a marker for lipogenesis, also did not change over seasons, yet showed a tendency for a higher concentration in the cattle from the highlands.

**DISCUSSION**

The milk yield and body condition of the dairy cows reared under extensive, ranging systems were far below genetic potential (Cassandro, 2015). Similarly, the extensive, ranging dairy cows had lower BCS compared

---

**Table 4. Effects of season and agro-ecological zone on serum concentrations of free AA in ranging dairy cows in the southern Ethiopian Rift Valley**

<table>
<thead>
<tr>
<th>AA</th>
<th>Rainy season</th>
<th>Dry season</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowlands</td>
<td>Midlands</td>
<td>Highlands</td>
<td>Lowlands</td>
<td>Midlands</td>
</tr>
<tr>
<td>Glycine (µmol/L)</td>
<td>313.57</td>
<td>318.32</td>
<td>303.98</td>
<td>234.27</td>
<td>247.13</td>
</tr>
<tr>
<td>Alanine (µmol/L)</td>
<td>158.00</td>
<td>318.32</td>
<td>152.25</td>
<td>105.95</td>
<td>106.08</td>
</tr>
<tr>
<td>Valine (µmol/L)</td>
<td>168.65</td>
<td>177.13</td>
<td>161.31</td>
<td>156.18</td>
<td>149.19</td>
</tr>
<tr>
<td>Leucine (µmol/L)</td>
<td>139.68</td>
<td>146.44</td>
<td>141.31</td>
<td>120.95</td>
<td>118.75</td>
</tr>
<tr>
<td>Ornithine (µmol/L)</td>
<td>42.23</td>
<td>41.65</td>
<td>40.08</td>
<td>34.49</td>
<td>27.45</td>
</tr>
<tr>
<td>Methionine (µmol/L)</td>
<td>17.17</td>
<td>17.29</td>
<td>16.84</td>
<td>13.55</td>
<td>13.15</td>
</tr>
<tr>
<td>Phenylalanine (µmol/L)</td>
<td>24.94</td>
<td>25.03</td>
<td>26.57</td>
<td>20.74</td>
<td>20.78</td>
</tr>
<tr>
<td>Citrulline (µmol/L)</td>
<td>23.77</td>
<td>27.11</td>
<td>25.27</td>
<td>23.98</td>
<td>24.98</td>
</tr>
<tr>
<td>Tryptophan (µmol/L)</td>
<td>45.18</td>
<td>45.49</td>
<td>48.43</td>
<td>35.03</td>
<td>39.06</td>
</tr>
</tbody>
</table>

1S = season; AZ = agro-ecological zone; S × AZ = season and agro-ecological interaction.

**Table 5. Effects of season and agro-ecological zone on serum concentrations of selected carnitine esters in ranging dairy cows in the southern Ethiopian Rift Valley**

<table>
<thead>
<tr>
<th>Carnitine ester (µmol/L)</th>
<th>Rainy season</th>
<th>Dry season</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lowlands</td>
<td>Midlands</td>
<td>Highlands</td>
<td>Lowlands</td>
<td>Midlands</td>
</tr>
<tr>
<td>C0</td>
<td>11.36</td>
<td>11.282</td>
<td>11.172</td>
<td>11.411</td>
<td>11.492</td>
</tr>
<tr>
<td>C2</td>
<td>1.212</td>
<td>1.363</td>
<td>1.304</td>
<td>1.045</td>
<td>0.933</td>
</tr>
<tr>
<td>C3</td>
<td>0.123</td>
<td>0.102</td>
<td>0.119</td>
<td>0.106</td>
<td>0.085</td>
</tr>
<tr>
<td>C4</td>
<td>0.340</td>
<td>0.319</td>
<td>0.285</td>
<td>0.346</td>
<td>0.313</td>
</tr>
<tr>
<td>C5</td>
<td>0.130</td>
<td>0.124</td>
<td>0.128</td>
<td>0.146</td>
<td>0.141</td>
</tr>
<tr>
<td>3OHC4</td>
<td>0.038</td>
<td>0.040</td>
<td>0.041</td>
<td>0.039</td>
<td>0.035</td>
</tr>
<tr>
<td>C3DC</td>
<td>0.016</td>
<td>0.019</td>
<td>0.036</td>
<td>0.018</td>
<td>0.018</td>
</tr>
<tr>
<td>C4DC</td>
<td>0.062</td>
<td>0.063</td>
<td>0.057</td>
<td>0.058</td>
<td>0.055</td>
</tr>
<tr>
<td>C5DC</td>
<td>0.025</td>
<td>0.027</td>
<td>0.030</td>
<td>0.022</td>
<td>0.026</td>
</tr>
<tr>
<td>C6DC</td>
<td>0.023</td>
<td>0.023</td>
<td>0.023</td>
<td>0.022</td>
<td>0.021</td>
</tr>
</tbody>
</table>

1S = season; AZ = agro-ecological zone; S × AZ = season and agro-ecological interaction; C0 = free carnitine; C2 = acetyl carnitine; C3 = propionyl carnitine; C4 = butyrylcarnitine + isobutyryl carnitine; C5 = isovaleryl carnitine + 2-methylbutyryl carnitine (isoleucine catabolism); C4DC = methylmalonyl carnitine + succinyl carnitine (valine and methionine catabolism); C5DC = glutaryl carnitine (lysine and tryptophan catabolism); C6DC = 3-methylglutaryl carnitine; 3OHC4 = 3-hydroxybutyryl carnitine; DC = decarboxyl esters.
with animals reared under a confinement system (Kolver and Muller, 1998). The national average milk yield per cow per day is 1.54 L for local zebu cows in Ethiopia (CSA, 2008). It has long been known that season of the year has a major effect on dairy animal performance including growth, reproduction, and lactation (Collier et al., 2006). According to Javed et al. (2004), seasonal variation in animal performance in the tropics is indeed expected to be primarily a manifestation of variation in feed quality and quantity. Lanyasunya et al. (2006) found higher performance of dairy cattle in the rainy season compared with the dry season.

Despite clear differences in milk yield per cow and BCS between agro-ecological regions, no major differences in metabolite profiles (urea, creatinine, triglyceride, and NEFA) were found, suggesting that the nutritional status of ranging dairy cattle does not differ that much between the agro-ecological regions. This implies that cattle seem to maintain their condition and convert any additional nutrient and energy excess into milk. This is an important insight because it means that improved feeding will increase milk production rather than being converted to a higher body condition of the cows. Yet, the season has clear effects: the dry season seems to be such a severe challenge that the animals can no longer maintain their minimum body condition and must decrease milk production. Before going into detail on the individual metabolites, this already points to the added value of measuring blood metabolites: body condition only reflects the reserves of energy in the body, but this does not necessarily indicate whether the animal experiences limitations in metabolizing nutrients for milk production. Indeed, if an animal has a lower body condition, but will convert any additional feed efficiently into milk (or vice versa), the BCS is neither enough nor accurate to estimate nutritional status.

In the dry season, all spectrophotometric analyses indicate point to difficulties in maintaining energy and nutrient balance: the distinctly higher NEFA concentrations in the dry season reflect a stronger negative energy balance, urging for body fat mobilization. Unfortunately, the cows have difficulties in using the mobilized fatty acids efficiently in the CAC. A study on sows by Hulten et al. (2002) indicated that during lactation animals can mobilize considerable amounts of body fat to maintain milk production and also justified techniques that measure circulating NEFA and triglyceride concentrations at specific points in time to provide knowledge of the current energy status of the animal. Ospina et al. (2013) also reported that NEFA is a good source of energy for several tissues in the body and can be used to synthesize milk fat. However, elevated levels of NEFA can result in excessive accumulation of triglycerides in the liver, resulting in hepatic lipidosis. More recent research has demonstrated that elevated NEFA concentrations can also adversely affect immune function (Contreras et al., 2010; Ster, et al., 2012). High serum NEFA concentration in the dry season was an indicator of negative energy balance, confirming the higher losses of BCS and the lower milk yield (Berton and Trevisi, 2013).

To use fatty acids in the CAC, oxaloacetate is needed to react with acetyl-CoA to form citric acid (Walsh et al., 2008). In ruminants on a fibrous diet, the 2 possible sources of oxaloacetate are propionic acid from rapid fermentation, and AA provided by dietary or body protein. In the dry season, urea was lower than in the rainy season, indicating that less protein was available to support the CAC in the dry season, despite a higher body muscle breakdown, reflected in the increased creatinine levels. The CP content of grazing land is indeed markedly lower in the dry season compared with the rainy season in the Ethiopian highlands (Gizachew and Smit, 2012), and according to Safari et al. (2011), serum urea concentration was lowest in the late dry season. In a study on sows, Cools et al. (2014) concluded that more balanced entry metabolites in the CAC are needed to better support the maternal peripartum metabolism period. Merrall (1976) was able to relate changes in urea concentrations of herds in New Zealand to changes in the protein content of pasture; the higher concentrations of urea in summer in this country probably also results from an increased dietary intake of available nitrogen during the grazing season. Monitoring of blood urea levels can be used for measuring protein status in cattle from different feeding regimens and seasons (Hammond, 2006). High blood urea levels could indicate a high protein intake or the excessive mobilization of muscle (Chimomyo et al., 2002). In ruminants, a decrease in blood urea concentration is related to low dietary intake of protein due to the recycling of urea from the blood back to the rumen when dietary protein intake is low (Sakkinen, 2005). Protein deposition, which affects AA requirements, and ruminal synthesis of microbial protein, which affects AA supply, are both energy-dependent processes (Tedeschi et al., 2015), implying that these metabolite concentrations may not only reflect better protein provision but also increased energy supply in the rainy season. The interaction term for urea pointed to a clear difference in seasonal response for the lowlands: unlike the other agro-ecological zones, urea did not decrease in the dry season. This may suggest that lowland cattle maintain a similar rate of protein use, but combined with their low milk yield, this higher urea concentra-
tion may rather reflect a lack of fermentable OM in their diet, leading to a relative excess of nitrogen in the rumen that is not converted to milk protein.

The above already demonstrates that the fairly accessible spectrophotometric analysis of routine measurements can provide useful insights that clearly go beyond body condition scoring. The higher concentrations of most free AA in the mass spectrometric analysis in the rainy season agree with the increased access of dairy cows to good quality feed in the rainy season. The fact that citrulline did not change with the season, whereas ornithine decreased in the dry season, again confirms the image of lower availability of protein as an energy source because the conversion of ornithine to citrulline occurs when the urea cycle needs to detoxify the ammonium molecules from AA deamination into urea (Fontana and Lavriková, 2018).

A surprising finding is that some of the detailed acylcarnitine markers for AA catabolism (e.g., C4DC, C5, C5DC, and C6DC) remained stable, whereas urea, creatinine, and the AA clearly pointed to a seasonal effect on protein metabolism. Still, the carnitine ester analysis provided additional insights. For instance, the seasonal changes in C2 and C3 mirror the available CAC energy substrates and thus nutrient utilization. Although not specifically, their higher concentrations in the rainy season are likely derived from increased acetate and propionate production from ruminal fermentation. This link between fermentative propionate production and peripheral propionyl carnitine concentration has clearly been demonstrated in an experiment with cats (Verbrugghe et al., 2010). The spectrophotometric analysis already showed that the dry season not only leads to a lack of energy, but also to less efficient use of that energy due to the lack of a glucogenic precursor for oxaloacetate. This is confirmed by the mass spectrometric analysis since C3 as well as C2 are substantially higher in the rainy season. Propionyl carnitine tells how much propionate (from ruminal fermentation) is entering the metabolism. This propionate is crucial to support the CAC, as it enters the CAC through succinyl CoA, and then forms oxaloacetate (van Knegsel et al., 2007). In the Krebs cycle, oxaloacetate is often a limiting compound. More acetyl-CoA can then be used in the CAC, increasing the energetic efficiency. The unchanged concentrations of 3OHC4, as a measure of the ketone body synthesis, suggests that the ketone pathway is not more activated in association with milk production, as is often observed in high-producing dairy cows in intensive husbandry (Duffield, 2000; Bertoni and Trevisi, 2013).

As with the spectrophotometric analyses, the seasonal effects far outweighed the influence of the agro-ecological zone on the metabolite concentrations. However, in contrast to the spectrophotometric analyses, several effects were found in the free AA and acylcarnitine profiles. Although C4 was not affected by season, its concentration increased from lowlands to highlands. Butyrate can originate from diets rich in water-soluble carbohydrates, but concentrations in tropical grasses typically do not exceed 4% (e.g., Rigueira et al., 2013), especially not in the dry season. Therefore, it is unlikely that the diets contained much substrate for butyrate production. Butyryl carnitine can originate from butyrate production in the rumen but can also be formed from valine catabolism, although the latter did not change with the agro-ecological zone. The reason for the underlying cause of this effect is yet unclear but may warrant further investigation into the differences between agro-ecological zones, including the associated altitude aspects. Similarly, the higher concentrations of (only) phenylalanine and tryptophan might not be attributed to factors at hand yet may point to interesting agro-ecological differences.

In this study, the mass spectrometric analyses do bring additional insights, but the chosen spectrophotometric panel is already capable of identifying the crucial attention points for nutritional interventions, such as the increased use of body protein for energy. Sustainable intervention strategies for such situations may include the use of multinutrient blocks with local protein sources (e.g., Moringa stenopetala) especially during the dry season. Certainly, the serum metabolite profiling provides much more information than just body condition scoring. The information from this study can therefore be used to deploy targeted nutritional interventions that consider differences in agro-ecological zones, and especially how this interacts with the challenge of the dry season.

ACKNOWLEDGMENTS

This work was realized as part of the Flemish Interuniversity Council-University Development Cooperation (VLIR-UOS) institutional university cooperation (IUC) program, which is carried out with financial support from the Arba Minch University (AMU-IUC) bilateral research funding agreements. The authors thank the Donna Vanhausteghem, Floortje van de Meulengraaf, and Annet Ligtenberg (Ghent University, Merelbeke, Belgium), for their support in UV spectrometer analysis as well as the personnel from the Ghent University hospital, and we also thank farmers and management of Arba Minch University agricultural college and stuff. The authors declare that they have no conflict of interest. We confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. We confirm that
we have followed EU standards for the protection of animals used for scientific purposes.

REFERENCES


Worku et al.: SEASONAL AND AGRO-ECOLOGICAL EFFECTS ON NUTRITIONAL STATUS

4348


