Occurrence of subclinical metabolic disorders in dairy cows from western Santa Catarina state, Brazil

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The management of dairy herds during the transition period has been studied in several studies due to the severe adjustments to which cows are submitted in the metabolism of carbohydrates, lipids and minerals at early lactation. This is a period when occurs most of the metabolic disorders in dairy cows, especially in their subclinical form. Hitherto a lack of information exists on the occurrence of subclinical metabolic disorders in Brazil. The aim of this study was to determine the occurrence of subclinical metabolic disorders, including ketosis, liver lipidosis, hypocalcemia and hypomagnesemia, as well as phosphorous, copper and zinc deficiency in dairy cattle from the western region of Santa Catarina state, southern Brazil, during the first 30 days of lactation. Blood samples from 15 dairy herds managed in intensive production (free-stall) and semi-confined systems were collected. Milk yield, reproduction and health data of the herd were recorded in a questionnaire, based on the records of the farm and on the observations during samples collection. Blood samples were collected for the measurement of beta-hydroxybutyrate (BHB) and lactate using portable monitors at the farm and for obtaining serum to perform the following biochemical determinations: total calcium, phosphorus, magnesium, albumin, aspartate transaminase (AST) and creatine kinase (CK) by UV-visible spectrophotometry, and copper and zinc by atomic absorption spectrophotometry. In this study, the cutoff points considered were as follows: serum BHB concentrations >1.2mmol/L for subclinical ketosis, AST >140U/L and CK <94U/L for subclinical liver lipidosis, serum lactate concentrations >2.2mmol/L for lactacidemia, serum total calcium concentrations <7.5mg/dL for subclinical hypocalcemia, serum magnesium concentration <1.7mg/dL for hypomagnesemia, serum phosphorus concentration <2.5mg/dL for phosphorus deficiency, serum copper concentrations <32.8µg/dL for copper deficiency, and serum zinc concentrations <60µg/dL for zinc deficiency. The results showed an occurrence of 9% for subclinical ketosis, 11% for subclinical liver lipidosis, 44.5% for lactacidemia, 11% for subclinical hypocalcemia, 7.4% for subclinical hypomagnesemia, 10.7% for copper deficiency and 8.7% for zinc deficiency. According to the survey results, the occurrence of subclinical ketosis, lipidosis and hypocalcemia in western Santa Catarina differ from data found in the literature.

INDEX TERMS: Metabolic disorders, dairy cows, Brazil, cattle, epidemiology, ketosis, mineral deficiencies, clinics.

RESUMO.- Ocorrência de transtornos metabólicos subclínicos em vacas leiteiras da região Oeste do estado de Santa Catarina. O manejo dos rebanhos leiteiros durante o período de transição tem sido objeto de estudo de diversas pesquisas devido às severas adaptações sofridas pelas vacas no metabolismo dos carboidratos, lipídeos e minerais no início da lactação. Trata-se de um período em que ocorre...
a majority of the transtornos metabólicos in vacas leiteiras, especially in its form subclínica. At this time, there is
a lack of information about the occurrence of transtornos metabólicos subclínicos in Brazil. The objective of this study
was to determine the occurrence of some subclinical metabolic disorders in dairy cattle during the early lactation period
in the western zone of Santa Catarina state, southern Brazil.

**MATERIALS AND METHODS**

**Ethics statement.** All procedures with the cows of this study were approved by the Ethics Committee on Animal Use
of the Federal University of Rio Grande do Sul and authorized by the herd owners.

**Animals.** A total of 299 Holstein cows were evaluated during early lactation (from calving to 30 days of lactation)
in 15 herds located in the following seven municipalities of the western region of Santa Catarina state (southern Brazil):
Chapeco (27°05'47"S, 52°37'06"W, altitude 674m), Nova Erechim (26°54'49"S, 52°54'21"W, altitude 462m), Ouro Verde
(26°41'40"S, 52°18'43"W, altitude 758m), Quilombo (26°43'34"S, 52°43'14"W, altitude 425m), Xanxere (28°52'37"S, 52°24'15"W,
altitude 800m), Xavantina (27°04'07"S, 52°20'31"W, altitude 545m) and Xaxim (26°57'42"S, 52°32'05"W, altitude 770m).
According to the Köppen classification, those locations are in a transition area between the types of climate Cfb (mesothermal,
humid) and Cfa (mesothermal, humid without dry season, cool summers, with occurrence of severe and frequent frosts in
winter) and Csb (mesothermal, humid with dry season, hot summers, with occurrence of severe and frequent frosts in
summer) (Puchalski 2004).

From the 15 herds studied, three were managed by confined (free-stall) system and 12 by semi-confined system. Of the 299 cows
that made up the study, 182 (60.9%) were raised in free-stall system and 117 (39.1%) in semi-confined system. All cows in
free-stall system and 105 cows (89.7%) in semi-confined system were supplemented with anionic salt (ammonium chloride,
calcium chloride, ammonium sulfate, calcium sulfate and magnesium sulfate) during the prepartum period for preventing
hipocalcemia. Only one herd of 12 lactating cows did not use anionic salt. Data concerning days in milk, number of lactation,
milk production and body condition score were obtained from all animals (Table 1).
The diet of the animals was composed of corn silage, pastures (ryegrass, oats and barley) plus hay. The concentrate contained corn, soybean meal and mineral supplement. The body score was assessed by subjective method, through observation on the scale of 1 to 5 (Edmonson et al. 1989), where score 1 is very lean and score 5 very fat. Production data (individual cow and herd mean), date of calving, days in lactation, body condition score, and number of lactations of the animals of all herds were recorded in a questionnaire filled with the help of veterinarian responsible for the herd. Also, in the questionnaire, clinical cases of postpartum diseases observed by the veterinarians were recorded (ketosis, ruminal acidosis, hypocalcemia, placental retention, abdomenum displacement, metritis and mastitis).

Sample collection and analysis. Blood samples were collected in cows up to 30 days of lactation through coccygeal venipuncture after local anespsis, using vacuum tubes without anticoagulant and with heparin (Vacuette, Brazil). The distribution of sampled animals according to the days in lactation (DEL) was as follows: 63 cows from 0 to 7 DEL, 78 from 8 to 15 DEL, 97 from 16 to 23 DEL and 61 from 24 to 30 DEL. All samples were collected during feeding period after milking. Immediately after collection, an aliquot of anti-coagulated blood was used for measurement of beta-hydroxybutyrate (BHB) through portable monitor and specific strip (Ketovet, TaiDoc, Korea) according to the manufacturer’s instructions. Another aliquot of this sample was used for measurement of lactate using a portable monitor and specific strip (Accutrend Plus, Roche, USA) according to the manufacturer’s instructions. During transportation, blood samples were put under refrigeration and forwarded to the laboratory where they were centrifuged (2,500rpm for 10 minutes) to obtain serum. Serum samples were placed in eppendorf tubes and stored at -20°C until biochemical determinations.

Biochemical measurements of total calcium, phosphorus, magnesium, aspartate transaminase (AST) and creatine kinase (CK) were carried out using semi-automatic equipment (Bio2000, Bioplus, Brazil) by UV-visible spectrophotometry using commercial kits (Labtest, Brazil). Copper and zinc were determined by atomic absorption spectrophotometry in automatic device (Aanaliser 200, PerkinElmer, USA). Cases of subclinical metabolic disorders and mineral deficiencies were considered, using the following cutoffs values: serum BHB concentrations >1.2mmol/L for subclinical ketosis (McArt et al. 2012), AST >140U/L and CK <94U/L for subclinical hepatic lipidosis (Kaneko et al. 2008), serum total calcium concentrations <7.5mg/dL for subclinical hypocalcemia (Goff et al. 1996), serum lactate concentrations >2.2mmol/L for lactacidemia (Kaneko et al. 2008), serum phosphorus concentration <2.5mg/dL for phosphorus deficiency (McDowell 2003), serum magnesium concentration <1.7mg/dL for magnesium deficiency (Kaneko et al. 2008), serum copper concentrations <32.8µg/dL for copper deficiency (Puls 1988), and serum zinc concentrations <60µg/dL for zinc deficiency (Puls 1988).

Statistics. Data were entered in Excel sheet (Microsoft, USA), for calculation of subclinical occurrence rates of ketosis, hepatic lipidosis, lactacidemia, hypocalcemia, hypomagnesemia and copper and zinc deficiency using the following formula: Occurrence rate of disorder = [number of new cases/number of cows under risk] x100. Cows under risk were considered those in the first 30 days of lactation.

Quantitative variables were described by mean, standard deviation, minimum and maximum values, and compared between groups by Student t test for independent samples. Categorical variables were determined applying a test of proportions (Chi square). In both tests a range of 95% confidence was considered (P<0.05).

RESULTS

The dairy herds analyzed in the western region of Santa Catarina state, had a total occurrence of 9% for subclinical ketosis, 11.4% for subclinical hepatic lipidosis, 44.5% for lactacidemia, 11% for subclinical hypocalcemia and 7.4% for subclinical hypomagnesemia. The occurrence of mineral deficiencies was 10.7% for copper and 8.7% for zinc. There were no cases of phosphorus deficiency. The production system (confined and semi-confined) did not show difference in the occurrence of the various disorders (Table 2). When analyzing the occurrence of subclinical metabolic disorders in relation to milk production (Table 3), a higher occurrence of subclinical hepatic lipidosis were seen in cows with milk yield below 15 L/cow/day (P<0.05).

Table 1. Production traits (mean ± standard deviation) of the cows studied

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Free-stall confinement</th>
<th>Semi-confinement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cows</td>
<td>182</td>
<td>117</td>
</tr>
<tr>
<td>Days in lactation</td>
<td>16.7 ± 8.5</td>
<td>14.6 ± 8.6</td>
</tr>
<tr>
<td>Number of lactations</td>
<td>2.2 ± 1.4</td>
<td>2.9 ± 1.7</td>
</tr>
<tr>
<td>Milk yield (L/cow/day)</td>
<td>24.5 ± 9.7</td>
<td>23.0 ± 7.3</td>
</tr>
<tr>
<td>Body condition score (1-5)</td>
<td>2.7 ± 0.5</td>
<td>3.0 ± 0.5</td>
</tr>
</tbody>
</table>

Table 2. Occurrence (%) of subclinical metabolic disorders in cows from western Santa Catarina state, Brazil, according to the production system

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Free-stall confinement</th>
<th>Semi-confinement</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>182</td>
<td>117</td>
</tr>
<tr>
<td>Ketosis</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Liver lipidosis</td>
<td>14.8</td>
<td>6.0</td>
</tr>
<tr>
<td>Lactacidemia</td>
<td>26.8</td>
<td>17.7</td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>6.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Hypomagnesemia</td>
<td>5.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Phosphorus deficiency</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Copper deficiency</td>
<td>6.7</td>
<td>4.0</td>
</tr>
<tr>
<td>Zinc deficiency</td>
<td>4.7</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Table 3. Occurrence (%) of subclinical metabolic disorders in cows from western Santa Catarina state, Brazil, according to the milk yield level

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Milk yield (L/cow/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 15</td>
</tr>
<tr>
<td>N</td>
<td>59</td>
</tr>
<tr>
<td>Ketosis</td>
<td>8.5</td>
</tr>
<tr>
<td>Liver lipidosis</td>
<td>23.7</td>
</tr>
<tr>
<td>Lactacidemia</td>
<td>4.75</td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>13.6</td>
</tr>
<tr>
<td>Hypomagnesemia</td>
<td>8.5</td>
</tr>
<tr>
<td>Phosphorus deficiency</td>
<td>0</td>
</tr>
<tr>
<td>Copper deficiency</td>
<td>11.9</td>
</tr>
<tr>
<td>Zinc deficiency</td>
<td>16.9</td>
</tr>
</tbody>
</table>

a,b Different letters in the same line indicate statistical difference among groups (p<0.05).
There was no significant difference (P>0.05) in the occurrence of subclinical hypocalcemia comparing the cows that consumed (N=287, occurrence 17.7%) to the cows that did not consume (N=12, occurrence 16.7%) anionic salt during prepartum period. The multiparous (N=197) and the primiparous (N=102) cows did not show any difference in the occurrence rates of subclinical metabolic disorders. The cows with a body condition score between 3.25 and 3.75 had a higher occurrence (P<0.05) of subclinical ketosis (20%) than cows with less score (7.3%).

**DISCUSSION**

The metabolic profile test has been used as a practical tool in dairy herds to improve feeding management, detect subclinical metabolic disorders and prevent production diseases (Kida 2002). We used the measurement of specific biochemical analytes in blood to detect the most important subclinical metabolic disorders in dairy herds from southern Brazil.

The occurrence of subclinical ketosis in western Santa Catarina was lesser (9%) than the values reported by other studies in the southern region of Brazil, as Gonçalves (2015) who mentioned an prevalence of 35% of this disorder or Garcia et al. (2011) who found 24% in cows from the state of Rio Grande do Sul. Cucunubo et al. (2013) found 14.8% of subclinical ketosis in dairy cows from southern Chile and Garro et al. (2014) in Argentina found similar values of subclinical ketosis to our study (10.3%). Those differences in the incidence rates of subclinical ketosis may be ascribed to the cutoff point of BHB considered for declaring a cow with the disorder, and also to the day of lactation when the cow was sampled. The current consensus is to consider a cutoff point of BHB at least of 1.2mmol/L for subclinical ketosis (McArt et al. 2012).

Herds with high prevalence of subclinical ketosis in early lactation tend to have an increased incidence of displaced abomasum and clinical ketosis (Oetzel 2004). Duffield et al. (2009) stated that hyperketonemia is also a risk factor for metritis. Though it was not an objective of our work to establish risk factors due to the low number of animals, proportionally more cows with subclinical ketosis suffered metritis (11%) compared to those that did not have subclinical ketosis (8%). Asl et al. (2011) stated that the prevalence of subclinical ketosis is greatest in high-yielding dairy cows and in cows with two or more lactations. In our study, although no significant differences were observed in subclinical ketosis occurrence among cows with different production levels (Table 3) the occurrence of this disorder was almost double in cows yielding more than 30L/day that in cows producing 15-30L/day (14.9 vs. 6.9%). Primiparous and multiparous cows, however, had a similar occurrence of subclinical ketosis (7.8 and 9.6%, respectively). In our study, it was likely that the body condition score is a parameter more related to occurrence of subclinical ketosis once the higher the score at early lactation the more occurrence of the disorder.

Bobe et al. (2004) reviewed data of European dairy herds between 1980 and 2001, and found that the incidence of moderate hepatic lipidosis is 20 to 65%, while the incidence of severe lipidosis is 5 to 24%. In our study, the occurrence of subclinical hepatic lipidosis based on AST levels above 140 U/L concomitant with CK levels below 94 U/L, was 15.7%. Both enzymes are present in muscle tissue but the increase in AST without the increment in CK indicates liver lesion, most probably from esteaostis in early lactation cows (Liu et al. 2012). Liver lipidosis may be related to disorders after calving, as hypocalcemia, ketosis, displaced abomasum or any situation that cure with anorexia, as retained placenta or dystocia (Allen & Piantoni 2013). In our study 9% of the cows with subclinical liver lipidosis suffered displaced abomasum, comparing to only 1% of the healthy cows.

Lactacidemia may occur as a consequence of ruminal acidosis, a very common disorder derived from the use of high grain diets, which is broadly used in high-yielding dairy cows (Tajik & Nazifi 2011). Other causes of lactacidemia include hypovolemic shock, septic shock, and any other cause of decreased tissue perfusion leading to ischemia and necrosis (Russell & Roussel 2007), which can be disregarded in the present work. The only accepted tool of diagnosis of ruminal acidosis is the measurement of ruminal pH, not always a method easy to perform at the field (Enemark et al. 2002). As in this study we did not measured ruminal pH, we intended to estimate a condition of lactacidemia, based on the measurement of blood lactate. Rodrigues (2009) correlated ruminal pH and ruminal concentrations of lactate in cattle, observing a high negative correlation between these two variables (R²=0.9127). The same author correlated ruminal lactate and blood lactate, noting a significant positive correlation between the two variables (R²=0.718). Therefore, it has been suggested that the measurement of blood lactate has good correlation with ruminal lactate. The occurrence of lactacidemia (above 2,2mmol/L) in our study was 44.5%. Field studies have shown that in the US, more than 19% of cows in early lactation and 26% of cows in mid-lactation have subclinical rumen acidosis (Garret et al. 1997) while Kleen et al. (2013) stated that, in German herds, the prevalence of subclinical acidosis is up to 50% regardless of the milk production level. In the present study, we do not have reliable data for suggesting the occurrence of ruminal acidosis. We suggest that an approach for diagnosing metabolic acidosis can comprise the measurement of blood and ruminal lactate.

According to a survey done by Reinhardt et al. (2011) subclinical hypocalcemia in US dairy herds has an incidence of 25-54%, in the first 48h after calving, considering as the cutoff point serum calcium concentration of 8.0mg/dL. In our study the occurrence of hypocalcemia was 11%. Goff et al. (1996) reinforce that the occurrence of subclinical hypocalcemia has been reported up to 10 days after calving. The early period of lactation studied and the cutoff point considered by those authors may explain the lower occurrence in our study, since we included cows up to 30 days in milk and use a cutoff point of 7.5mg/dL. Hypocalcemia may affect approximately 50% of the adult dairy cows not receiving anionic salts in prepartum period and 30% of the cows that consume anionic salts (Melendez et al. 2002). In our study, however, no significant difference was observed in the occurrence of subclinical hypocalcemia comparing cows that consumed with those that did not consume anionic salts before calving. Bouda et al. (2000) cited that subclinical hypocalcemia leads to decreased muscle tone, which can cause displacement of abomasum. Nevertheless, in our study only 1 cow out of 51 cows (less than 2%) that suffer subclinical hypocalcemia had displaced abomasum. Also, the milk production levels did not influence the occurrence of subclinical hypocalcemia,
although Correa et al. (2010) consider the high milk yield as a predisposing factor for clinical hypocalcemia, so that low yielding cows rarely have hypocalcaemia.

According to McDowell (2003), the incidence of clinical hypomagnesemia in dairy cattle is lower (<2%) but few studies indicate the incidence of subclinical condition. We found an occurrence of hypomagnesemia of 7.4%. Magnesium is involved in various enzymatic reactions of the intermediary metabolism and its deficiency can affect various physiological activities, mainly by diminishing the reproductive performance (Dugmore et al. 1987). McDowell (2003) also cites immunity and muscle contraction as physiological events affected by magnesium deficiency, which may be a risk factor in the occurrence of metritis. Nevertheless, this was not the case in our study, where 25 cases of clinical metritis occurred in cows with normomagnesemia and no one case in cows with hypomagnesemia. Deficiency in magnesium absorption may be caused by high levels of phosphorus, potassium and nitrogen in the diet (McDowell 2003). In our study, the mean phosphorus concentration in serum (10mg/dL) was above the reference value for cows (5.6-6.5mg/dL), which may reveal excessive intake of this mineral and one possible cause for the occurrence of subclinical hypomagnesemia. In other regions of Brazil, like the state of Minas Gerais, serum phosphorus range of 4.45 to 5.63mg/dL was found in lactating cows (Dayrell & Resz 1984).

The occurrence of subclinical copper deficiency in our study was 10.7%. In Santa Catarina, Tokarnia et al. (2000) reported copper deficiency in ruminants. Risk factors for copper deficiency include older animals, high mineral demand during pregnancy or lactation, poor availability of copper in the mineral supplement, soil characteristics and concentrations of other minerals such as molybdenum and sulfur (Overton et al 2004). It is suggest more studies regarding this deficiency and its relation with the occurrence of abomasal displacement. In our study the only one case of displaced abomasum belonged to a cow with copper deficiency.

The low occurrence of zinc deficiency in our study (8.7%) contrast with the finding of Moraes (1998) in the states of Ceara and Piauí (northeastern Brazil) when more than 50% of the animals showed low levels of this mineral, suggesting the occurrence of subclinical zinc deficiency, although without any evidence of clinical disorder. In Brazil, most research on zinc deficiency has been conducted with beef cattle. Sousa et al. (1982) in the state of Mato Grosso found low levels of hepatic zinc in cattle from six herds while Lisboa et al. (1996) did not find evidence of zinc deficiency analyzing the hepatic content of zinc in bovines in the state of São Paulo.

The occurrence of zinc and copper deficiencies, although relatively low, are worth of considering by veterinarians from the studied region.

**REFERENCES**


**CONCLUSIONS**

In the western region of Santa Catarina state, the occurrence of subclinical ketosis is lesser than in other Brazilian regions and more similar to that of Argentinean herds.

Subclinical hepatic lipidosis has lower occurrence than those reported by authors from Europe and it is suggested that this disorder may be a risk factor for displaced abomasum.

The occurrence of subclinical hypocalcemia is lower than those found in the literature, fact attributable to the lactation period and the cutoff values used in our work.

It is likely that in the studied herds exist excess intake of phosphorus in the diet.


