Blood clinicopathological differences between type I and II ketosis in dairy cows

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ABSTRACT
The purpose was to determine the difference of blood clinicopathological changes between type I and type II ketosis in dairy cow. Fifty-eight cows, from dairy cattle farm in Heilongjiang of China, were included. An ELISA test was used to evaluate the blood indicators. The plasma concentrations of beta-hydroxybutyric acid (BHBA) and insulin sensitivity decreased, and the plasma concentration of glucose (Glc), non-esterified fatty acid (NEFA) and bilirubin content increased in type II ketosis group compared with the type I ketosis group. These results showed that there was a difference in etiology between type II ketosis and type I ketosis. Type II ketosis was not only associated with energy metabolism and insulin resistance, but also with oxidative stress and liver function. It laid the foundation for further investigate the mechanism and prevention of type II ketosis in the future.

Key words: Dairy cow, Energy metabolism, Insulin resistance, Liver function, Oxidative stress, Type II ketosis.

INTRODUCTION
During the transition dairy cow undergo a period of negative energy balance for the demands of milk synthesis, which cannot be met by feed intake (Wang et al., 2010). Ketosis, one of the most prevalent metabolic diseases in dairy cows during transition period, was a condition that the concentration of ketone in blood was exceeding than 3,000μmol/L (Oetzel, 2007) during the transition (Shire et al., 2013; Zhang et al., 2013). Cows with ketosis were characteristic with dry matter intake (DMI) and milk production decreased, and reduced reproductive performance. In addition, it was known that ketosis was at an increased risk of additional postpartum diseases such as displaced abomasum (DA), fatty liver and metritis, which may increase their risk of removal from the herd during early lactation (de Roos et al., 2007; Itle et al., 2015).

From an aetiological viewpoint, there were two quite different types of metabolic disorders in which ketosis can occur, type I ketosis and type II ketosis, respectively (Holtenius and Holtenius 1996). Type I, generally occurs 3-6 weeks after calving in cows whose milk secretion is so extensive that the demand for glucose exceeds the capacity for glucose production, was characteristic with a decrease in appetite and milk production, and weight loss. In addition, there was a decreased on glucose, but the concentrations of beta-hydroxybutyric acid (BHBA) and non-esterified fatty acid (NEFA) were increased (Berge and Vertenten 2014). However, type II, generally occurs earlier in lactation, leads to the concentrations of BHBA, glucose and NEFA were increased (Oikawa and Oetzel 2006).

It was reported that the prevalence of ketosis was 5.0% in America; 14.7% in India; 43.1% in Japan, and 15-30% in China. And there was 151-312$ per dairy cow losing on the therapy (Ke-he 2008). Therefore, we made an investigation on the dairy farm, and found that there were higher incidence on type II ketosis than type I ketosis. And the mechanism of type II ketosis was unclear, thus, an experiment was conduct to provide a review of differences in dairy cows with type I and II ketosis on the energy metabolic, liver function, stress oxidation and insulin resistance.

MATERIALS AND METHODS
Ethics statement: The study was approved by the farm owner and all experimental animals were conducted according to the International Guiding Principles for Biomedical Research. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Heilongjiang Bayi Agricultural University.

Animals and Groups: Fifty-eight Holstein cows were selected from an intensive 2000 dairy cattle farm in Heilongjiang, China. All cows were 3 to 5 year old. All cows had a body condition score (BCS) between 2.75 and 3.50. BCS was recorded by the same person immediately after parturition. BCS was expressed on a 5-point scale with 0.25 increments as described by Edmonson et al. (1989). Cows were divided in 3 classes based on their BCS: (1) under-conditioned, <3; (2) normal condition, 3 to 3.5; and (3) over-conditioned, >3.5 (Meganck, et al. 2015). All the cows were fed a total mixed ration (TMR) at 4:00 am, 10:00pm and...
16:00 pm daily during transition period, which consisted of concentrated feed, silage corn, brewer’s grain, cooked soybeans, Chinese hay, melon pulp, surface melon shell, and fat. The nutritional analysis was 55.6% dry matter (DM), 17% crude protein, 1.75mcal/DM net energy for lactation (NEL), 5.60% fat, 39.10% neutral detergent fiber (NDF), 20.60% acid detergent fiber (ADF), 180g Ca, and 116g P.

According to the concentrations of Glc, NEFA, BHBA and the clinical symptoms the cows were classified into three groups by using the classify method of Holtenius, Control group, Type I ketosis group (I group) and type II ketosis group (II group), respectively. The classification standard is that if the concentration of Glc more than 3.75 mmol/L, BHBA concentration was less than 0.6 mmol/L, NEFA concentration was less than 0.5 mmol/L, describing it as control group; if the concentration of Glc less than 2.5mmol/L, BHBA concentration exceeds 1.2mmol/L, NEFA concentration greater than 0.5mmol/L, describing it as type I ketosis, otherwise, the concentration more than 2.8mmol/L, and other remaining indicators are equal, describing it was type II ketosis. There were 12 cows which were not occurred ketosis during 0-28 day, divided them into C group. On the 7 day after culling, there are 6 cows with type I ketosis, and 8 cows with type II ketosis. And there are 8 cows with type I ketosis and 12 cows with type II ketosis after 14 days. There were 10 cows with type I or type II ketosis on the day of 21 day. There are 5 cows with type II ketosis and 7 cows with type II ketosis on the 28 day.

Sample collection: Blood samples were collected from the vena caudalis mediana using sodium heparin at the time of day of parturition, and again at 0d, 7d, 14d, 21d and 28d after parturition and then immediately centrifuged at 1,400 \( \times \) g for 10 min at room temperature. The supernatants were aliquoted into Eppendorf tubes (1 mL plasma/tube) and stored at -80°C until analysis.

Parameters and method: Clinical blood biochemical data included glucose (Glc, Oxidase method, purchased from Biosino Bio-technology and Science INC), beta-hydroxybutyric acid (BHBA, Enzyme rate method, purchased from Beijing jiujing Biotechnology Company), and non-esterified fatty acids (NEFAs, colorimetric method, purchased from Sekuisi Medical Technology(China)) for assessing energy balance status, and aspartate aminotransferase (AST), alanine aminotranserase (ALT), total bilirubin (TBIL), were devised by kit method, purchased from Biosino Bio-technology and Science INC) for measuring liver function, and insulin (INS, Elisa, purchased from Nanjing jiancheng Technology Company) and Adiponectin (ADP, Elisa, RD Company in America) for evaluation the insulin resistance status, and then malondialdehyde (MDA) and nitric oxide (NO) (Nitric acid reduction method)and SOD (Hydroxylamine method) they were purchased from Nanjing jiancheng technology Company for determining oxidative stress status.

Statistics analysis: The data was used SPSS17.0 software for statistical analysis using the single factor variance; the results were expressed as “means ± standard deviation”. The revised quantitative insulin sensitivity check index, \( \text{RQUICKI} \), following formula: \( \text{RQUICKI}=1/\left[\log \text{ (glucose in mg/dL)} + \log \text{ (insulin in mU/mL)} + \log \text{ (NEFA in mmol/L)}\right] \).

RESULTS AND DISCUSSION

The cows were divided into three groups according the dates from Table 1, the Glc concentration of type II was significantly higher than type I ketosis. And there were also differences between BHBA and NEFA, and they were all significant higher than C group. In addition, BHBA and Glc got the peak at the 14d; NEFA got the peak at the 7d in the cows with type I ketosis. And BHBA, Glc and NEFA got the peak at the 7d in the cows with type II ketosis.

The figure 1 showed that the concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of type II ketosis were higher than type I ketosis on the 7-28 days, and they were all significant higher than C group. The total bilirubin (TBIL) concentration of type II ketosis was higher than type I ketosis on the 7 day at postpartum, and there was no significantly difference at the others day.

Table 1: The parameters of two type ketosis and healthy cows

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Time of postpartum (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>BHBA</td>
<td>C</td>
<td>0.511±0.07^a</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>0.671±0.07^a</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0.583±0.12^ab</td>
</tr>
<tr>
<td>Glc</td>
<td>C</td>
<td>4.706±0.8</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>4.28±0.76</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>4.642±0.43</td>
</tr>
<tr>
<td>NEFA</td>
<td>C</td>
<td>0.57±0.11^a</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>0.81±0.09^b</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>1.06±0.14^c</td>
</tr>
</tbody>
</table>

Note: the groups were divided compared with control dairy cows. Different capital letters in same row represent very significant difference (P <0.01), different lowercase letters represent significant difference (0.01<P <0.05), and the same letter or without letters mean no significant difference (P > 0.05).
The INS concentration of type II was significantly higher than C group, and there was no significantly difference between the type I and II ketosis at the 0-28day. The ADP concentration was decreased compared with type I ketosis except the 21day. The concentration of RQUICKI was significantly lower than the type I ketosis on the 0-8 day (Fig 2).

From the figure 3, the trend of superoxide dismutase (SOD) concentration were first increased and then decreased,
and got the peak on the 14 day both two types ketosis. There was a significant difference in malondialdehyde (MDA) concentration at the 0-7 days, and the content of type II ketosis was higher than the type I ketosis. The other days there was no significantly different. There was no significantly difference on the nitric oxide (NO) between type I and II ketosis. The trend of concentration was firstly decreased and then increased, and both of them got the peak at the 21 day.

**Energy metabolic and type II ketosis:** During the perinatal period dairy cows undergo pregnancy, culling and lactation. As a result of nutrient demand for milk productions increasing faster than DMI, cows experienced a period of negative energy balance (NEB). To adapt to this negative energy balance, metabolic disorder occurred in this time (Bouwstra et al., 2008; Gonzalez et al., 2011; Santschi et al., 2011; McArt et al., 2012). BHBA and NEFA are the products of fat metabolism. BHBA content reflects the oxidation of fat metabolic, and NEFA content reflects the mobilization of fat. When the contents of BHBA and NEFA increased in the blood, it indicts that there are a lot of mobilization of fat (Li et al., 2012). The dairy cow induced ketosis when the concentration of NEFA product more than the demands of body. The concentrations of BHBA and NEFA are higher than the normal in the figure 1, it reflects that the body is in negative energy balance. Glc mainly offer energy, once the Glc concentration is lower than the demand for the milk product, NEFA will be mobilization (Bobe et al., 2004). From figure 1 there are a significant difference between type I and type II ketosis. The Glc concentration of type II ketosis is higher than the type I ketosis, and higher or equal with normal dairy cow.

**Liver function and type II ketosis:** The liver aminotransferases-ALT and AST are both found in the liver, serum and various organ tissues. ALT and AST are found predominantly in the liver and its serum levels become elevated whenever disease process affects liver cells (Vozarova et al., 2002; Kunutsor et al., 2013). From figure 2 there are higher ALT and AST concentrations in type II ketosis than type I ketosis, and they are all higher than normal level, it indicts that there are liver damage when happened ketosis whether type I or type II, and type II ketosis has bigger damage than type I ketosis. Total bilirubin (TBIL) is produced as an unconjugated from by hemoglobin after the end of life of red blood cells from the reticuloendothelial system such as in spleen. The bilirubin is increased after damage of liver due to infection (Giannini et al., 2005). The concentration of TBIL of type II ketosis is more than type I ketosis on the 0- 14 days of partum. And they are all increased compared with the normal level of dairy cow. It suggests that there is a damage in the liver.

**Insulin resistance and type II ketosis:** Insulin is secreted by the pancreas, it mainly plays a role of liver and peripheral tissue. When the liver damaged, the sensitivity of insulin changed (Bossaert et al., 2008). Adiponectin (ADP), an adipose-specific secretary protein, exhibits anti diabetic and antiatherogenic properties (Nishizawa et al., 2002). And ADP
plays an important role in the accumulated adipose tissue, it is thought to be partially responsible for the development of insulin resistance (IR) in obesity. IR is defined as a state in which normal concentration of insulin produce a less than normal biological response (Zachut et al., 2013). IR may be the main reason what cause the high glucose in dairy cow. States of IR may consist of decreased insulin sensitivity, decreased insulin responsiveness on a combination of both decreased insulin sensitivity and responsiveness (Oetzel et al., 2003). From figure 3, the concentration of MDA in type II ketosis is higher than type I on the 0-28d, it indicts that the cows with type II ketosis is in the oxidation. NO contents of type II ketosis is higher than type I ketosis on the 0-28d, it indicts that the oxidation of type II ketosis enhanced than type I ketosis. And then the concentrations of the MDA and NO increased, it indicts the capacity of oxidation enhanced. Overall, The Oxidation and antioxidation system imbalanced in dairy cow with the type II ketosis.

CONCLUSION
To sum up, study on the energy metabolic, liver function, insulin resistant and stress oxidation showed that type II ketosis with higher glucose and NEFA, lower BHBA, liver damaged, emergent insulin resistant and within oxidation statement compared with type I ketosis. These blood clinicopathological changes of type II Ketosis play an important role in the further study of pathogenesis of type II ketosis, and it also have significance means to make a new measure to prevent the ketosis.

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