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RESEARCH ARTICLE

Evaluation of the Difference of L-selectin, Tumor Necrosis Factor-α and Sialic Acid Concentration in Dairy Cows with Subclinical Ketosis and without Subclinical Ketosis

Z. G. Zhang[1,§], J. D. Xue§[2,§], R. F. Gao[3,§], J.Y. Liu[1,§], J. G. Wang[3], C. Y. Yao[2], Y. Liu[2], X. W. Li[3], X. B. Li[3], G. W. Liu[3] and Z. Wang[3,]*

[1]College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030,China;
[2]College of Animal Science and Technology, Inner Mongolia University for Nationalities, Tongliao 028000, China;
[3]College of Animal Science and Veterinary Medicine, Jilin University, Changchun 130062, China
*Corresponding author: wangzhe500518@sohu.com

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ABSTRACT

Ketosis is a major disease related with negative energy balance and immune suppression in dairy cows. The objective of this study was to examine the differences in β-hydroxybutyrate (BHBA), L-selectin, glucose, tumor necrosis factor-α (TNF-α), non-esterified fatty acids (NEFA), and sialic acid (SA) concentrations in serum in healthy dairy cows and those with subclinical ketosis during the early lactation period. The blood from 20 healthy cows and 20 subclinically affected cows were sampled. All the cows were within the first 2 months of lactation. Serum concentrations of the various aforementioned factors were measured using a number of different methods. The results demonstrated that in cows affected by subclinical ketosis, NEFA concentrations were significantly higher, and glucose and L-selectin concentrations were significantly lower than healthy cows. There was no significant difference in serum SA and TNF-α of dairy cows with subclinical ketosis compared to the control cows. The decrease in concentration of serum L-selectin may be related to immune suppression.

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INTRODUCTION

Transition from the dry to lactation period is a high-risk time for dairy cows (Goldhawk *et al*., 2009). Ketosis is a common metabolic disorder frequently observed in dairy cows during the early lactation period. It is a metabolic condition characterized by increased concentrations of ketone bodies in blood, urine, and milk. Subclinical ketosis is characterized by elevated concentrations of circulating ketone bodies and lacked visible clinical symptom. Subclinical ketosis can decrease economic income through decreased milk production (Ospina *et al*., 2010), impaired reproductive performance (Walsh *et al*., 2007), increased risk of displaced abomasum, mastitis (Moyes *et al*., 2009) and higher risk of clinical ketosis (Iwersen *et al*., 2009).

A key advance in the last decade has been the recognition of the importance of immune function in ketosis, and a better understanding of peripartum immunosuppression. Tumor necrosis factor–α (TNF-α) is a cytokine principally secreted by macrophages in response to much stress (Jain *et al*., 2002). It is associated with such pathological processes as inflammation, septic shock and metabolism disorder. TNF-α can promote the release of the prostaglandin, interleukins, and chemotatic factor (Kern *et al*., 2001). The effects of TNF-α include promoting apoptosis, suppressing the activity of lipoprotein lipase, and activating neutrophils (Halse *et al*., 2001). It is suggested that increased TNF-α concentration and decreased insulin sensitivity are related *in vivo* (Kern *et al*., 2001; Jain *et al*., 2002). Elevated circulating concentrations of TNF-α can indirectly increase monocyte-endothelial cell adhesion by promotion of proinflammatory cytokines and adhesion molecules (Jain *et al*., 2002). As pro-inflammatory cytokines, TNF-α can rugulate the metabolism of liver and many endocrine glands. Patterns of protein and glucose metabolism characteristic of the immune response can be altered by

[§]These authors contributed equally to this study.

cytokines and changes in blood hormone concentrations of the body (Dinarello and Grunfeld, 1992).

Sialic acid (SA) is an important immunology index. High serum SA concentration can increase the risk of type 2 diabetes (Nayak and Bhaktha, 2005), *Clostridium botulinum* nosotoxicosis (Yoneyama *et al*., 2008) and lameness (Seyrek *et al*., 2008). To date, no data has been published about the possible association between serum SA concentration and subclinical ketosis.

L-selectin belongs to the selectin family of proteins, and is a cell adhesion molecule which exists on leukocytes. L-selectin becomes free after shedding from cell surface (Redwine *et al*., 2003). It is involved in stretching, signal transduction and activation of the cell, and is the molecular basis of many physiological and pathological processes such as inflammation, immune response and wound healing (Nicholson, 2003).

Ketosis during the transition period, which is characterized by increased concentrations of circulating β-hydroxybutyrate (BHBA) and decreased concentration of glucose, contributes to the suppression of immune system function (Moyes *et al*., 2009). Immune cell functions such as neutrophil chemotaxis (Suriyasathaporn *et al*., 2000), respiratory burst (Hoeben *et al*., 1997), and lymphocyte proliferation (Franklin *et al*., 1991) were impaired when culture media were added with various concentrations of BHBA *in vitro* (Moyes *et al*., 2009). Ketosis can cause suppressed immune function and energy metabolic disorder, so it may provide novel interactions between infectious and metabolic disorders of cows during the transition period. Reduced immunoresponsiveness during ketosis in ewes and cows has been previously reported (Franklin *et al*., 1991; Lacetera *et al*., 2002).

To further determine the interactions between the immune system and metabolism of dairy cows affected with subclinical ketosis, difference of serum TNF-α, SA and L-selectin concentrations were assessed in postpartum dairy cows affected with subclinical ketosis.

MATERIALS AND METHODS

Twenty healthy and 20 affected by subclinical ketosis Holstein dairy cows, within the first two months of lactation were used in this study. Approximate milk production was 23-28 kg/d per cow. The cows were fed the same mixed ration feed in compliance with the nutrition requirements of dairy cows at the early postpartum period. All the cows had no disease with visible clinical symptom. The experimental procedure was approved by the Academic Committee of Jilin University, China.

Cows having serum BHBA concentrations greater than 1,200 mM were diagnosed with subclinical ketosis (Duffield, 2000; Geishauser *et al*., 2000; Zhang *et al*., 2012a). Blood samples were collected in the morning before feeding. The blood was obtained by jugular venipuncture using evacuated tubes without anticoagulant. The blood was centrifuged at 4,000 × g for 15 min, serum was separated and stored at -70°C. Samples exhibiting visual hemolysis were removed from the analysis.

BHBA and glucose level were measured with corresponding test kit (Randox Clinical Diagnostic Company, UK) and these tests were finished in a Hitachi 7170 auto-analyzer (Hitachi, Japan). Non-esterified fatty acids (NEFA) and SA were detected with corresponding test kit (Nanjing Jian Cheng Institute of Bioengineering, China) (Nayak and Bhaktha, 2005). These detections were finished in a T60 UV-VIS spectrophotometer (Beijing Purkinje, China). TNF-α and L-selectin were measured using correponding ELISA kit (Adlitteram Diagnostic Laboratories, San Diego, CA, USA). TNF-α and L-selectin tests were performed in a Power Wave XS Universal Microplate Spectrophotometer (BIO-TEK Instruments. Inc., USA).

The SPSS18.0 software (SPSS Inc., Chicago, USA) was used for the statistical analysis of the results. The data were expressed as means ± standard deviation. Gaussian distribution was tested by Kolmogorov-Smirnov test. Student's *t*-test was used for comparison of means, and $P<0.05$ was referred to as statistical significance.

RESULTS

The results showed that serum glucose concentration in cows suffering subclinical ketosis was significantly lower ($P<0.01$) than control cows. NEFA and BHBA concentrations in cows with subclinical ketosis were significantly higher ($P<0.01$) than control cows (Table 1). There were no visible differences in the TNF-α and SA concentrations between two groups of cows ($P>0.05$). The serum concentration of L-selectin in cows with subclinical ketosis was significantly decreased ($P<0.01$) compared to the healthy cows (Table 1).

Table 1: Serum BHBA, glucose concentration, NEFA, TNF-α, L-selectin and SA concentration (means±SD) in healthy cows and those affected by subclinical ketosis (means±SD)

Components	Healthy cows (n=20)	Effected cows (n=20)	P
BHBA (mM)	0.32±0.33	2.21±0.11	<0.01
Glucose (mM)	4.15±0.35	3.37±0.17	<0.01
NEFA (mM)	3.29±0.11	7.80±0.42	<0.01
TNF-α (pg/mL)	39.94±1.98	38.88±1.73	<0.28
L-selectin (ng/mL)	66.37±0.76	45.36±1.12	<0.02
SA (mM)	2.25±0.24	2.10±0.64	<0.17

DISCUSSION

Subclinical ketosis is a major disease associated with negative energy balance. Increased energy requirements due to lactation and/or fetal development, and decreased energy intake because of depressed appetite in dairy cows during perinatal period. This transition results in decreased blood glucose level and body fat mobilization (Zhang *et al*., 2012b). Therefore, serum NEFA and BHBA concentrations are subsequently increased resulting in subclinical ketosis (Goldhawk *et al*., 2009; Ospina *et al*., 2010). BHBA detection in blood has been used as an index to distinguish between cows with and without subclinical ketosis (Zhang *et al*., 2011). Blood NEFA concentrations are valuable in evaluating the periparturient energy level of dairy cows (Duffield *et al*., 2009; Roche *et al*., 2009). Elevations in NEFA and BHBA reflect the degree of negative energy balance in dairy cows with subclinical ketosis.

In recent years, much attention has been given to the relationship between inflammation and metabolic diseases of dairy cows. Inflammation is proposed as a missing relation in the pathology of metabolic diseases of transition cows (Bertoni *et al*., 2008; Katok and Yanar, 2012). During the transition period, the metabolic effects of acute systemic inflammation such as breakdown of liver glycogen, adipose mobilization, and liver triglyceride storage can take place in cows (Bertoni *et al*., 2008). Higher ketones can impaire immunocompetence of cows during early lactation (Suriyasathaporn *et al*., 2000). Cytokines in blood can affect the metabolism of liver and endocrine glands. The immune response of host can be affected by the energy and protein metabolism, which are regulated by the levels of cytokines and circulating hormone. In addition, cytokines can promote the breakdown of fat through direct promotion of lipolysis, decreased insulin sensitivity and feed intake (Kushibiki *et al*., 2003). These conditions are related with ketosis in dairy cows (Ingvartsen, 2006).

TNF-α can decrease liver glucose production and promotes triglyceride storage when mobilized NEFA enter the liver (García-Ruiz *et al*., 2006). Cytokines on liver metabolism may take an important effect in promoting metabolic disorders in cows with infectious disorders or high body condition score (Bertoni *et al*., 2008). Blood TNF-α concentrations in high body condition score were elevated. Hyperketonemia can promote the secretion of TNF-α in a monocyte cell culture model and in type 1 diabetic patients *in vivo* (Jain *et al*., 2002). There were no significant differences between TNF-α production in cows with subclinical ketosis and healthy cows.

Serum SA, an acetylated derivative of neuroaminic acid, has been refered as a marker of inflammatory diseases and the acute phase response (Erdogan *et al*., 2008). SA is abundantly present in all biological membranes, and is released from cell membranes when cells are damaged. SA concentrations in dairy cows were not increased in this study, revealing that liberation of SA from cell membranes into circulation did not increase in dairy cows with subclinical ketosis.

Lymphocytes play a central role in the immune response. L-selectin is mainly expressed by lymphocytes, neutrophilic leukocyte and monocyte, and plays a biological role by mediating leukocyte adhesion to endothelial cells. Sato *et al*. (1995) reported that increased serum concentrations of acetoacetate and BHBA in ketosis-affected cows suppressed lymphocyte blastogenesis *in vitro*. The mitogenic response of the lymphocytes from cows with ketosis was significantly suppressed, and the suppression persisted for 2 weeks. This suggested that suppressed functions of lymphocytes were related with the increased susceptibility of cows to infections during ketosis. Reduced immunoresponsiveness during ketosis of ewes is likely to be associated with an increase in plasma concentration of NEFA, not with an increase in plasma concentration of BHBA (Lacetera *et al*., 2002). Therefore, decreased blood level of free L-selectin in dairy cows with subclinical ketosis may be related to increase in serum concentrations of ketones and/or NEFA. High concentrations of BHBA and NEFA may decrease the production of L-selectin by lymphocytes, and then decrease the recruitment of lymphocytes and rolling velocity of leukocytes. This may decrease the releasing of free L-selectin from surface of leukocytes.

Conclusion: The decrease in serum concentration of L-selectin may be related to immune suppression in dairy cows with subclinical ketosis. Further studies involving *in vitro* lymphocytes culture need to be conducted in order to elucidate the role of ketone bodies and NEFA on the secretion of L-selectin and the production of free L-selectin.

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