Agricultural Journal 8 (1): 51-55, 2013
ISSN: 1816-9155

Dynamic Monitoring of ALT and Correlation Analysis in Blood Plasma and Milk of Holstein Cows

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Abstract: Determination of Alanine aminotransferase (ALT) activity plays an important role in the diagnosis of liver function injury. Cows manifest hypernomic fat mobilization in early lactation which causes liver function injury and lead to variation of ALT activity. The study aim was to establish an advanced diagnostic method for determining the activity of ALT and analyze their dynamics characteristics and correlation in milk and blood plasma collected from postpartum dairy cows in early lactation. In this study, 35 healthy Holstein cows were involved. About 187 blood plasma and milk samples were collected from cows 4-10 weeks post-parturition. Samples were collected once weeks in the morning before the cows were feed. In the collected samples, the concentration of ALT was detected by spectrophotometer techniques. The results showed that: ALT activity was significantly lower in milk than that in blood plasma ($p<0.05$); ALT activity in both blood plasma and milk significantly increased and reached the peak at the same time at week 8 postpartum. According to the results of the study, there was significant positive correlation ($r = 0.84$, $p<0.001$) in the concentration of ALT between blood plasma and milk. Therefore, the outcome results suggest that ALT activity determination in milk can replace that in blood plasma in case of detection in the earlier lactation of cows days postpartum. This method may contribute to an extensive survey and monitoring systems.

Key words: ALT, blood, milk, dynamic monitoring, correlation analysis

INTRODUCTION

Alanine aminotransferase (ALT or GPT, EC 2.6.1.2) is one of the important enzymes for protein catabolism. Its main function is to catalysis alanine and alpha ketoglutarate and transfer them into generates pyruvate and glutamate. It plays an important role in the process of glucose and amino acid of intermediate metabolism (Gtiotti *et al*., 1991). The majority of ALT is found in liver cells which exist in all kinds of tissue cells. For instance, it was found that the variation of ALT have some relations with the function of the liver, heart, kidney, brain and skeletal muscle and other tissues and organs (Adolph and Lorenz, 1982; Kalhan *et al*., 2001; Weibrecht *et al*., 2010). Normally, only a little ALT release into the blood, lead to increase significantly the plasma ALT activity while the cytosol of ALT leak into the blood at the condition of a liver cell injury such as all kinds of hepatitis, drug toxicity liver cell necrosis and other diseases (Anderson *et al*., 2000; Puoti *et al*., 2005; Lai *et al*., 2007). Therefore, it is one of the important indexes of clinical diagnosis of liver function (Kaplwitz, 2000). Because of Holstein cows are world famous dairy cows with a high production thus in the early lactation of the cows, high body fat mobilization were formed which can easily lead to liver function injury (Stojevic *et al*., 2005). But, the researches of the dynamic ALT activity changes in Holstein cows in the postpartum period are few or less in milk.

Mostly, detections of ALT activity are reported in blood serum or blood plasma but detection from milk sample has not been reported. Besides, the process of sampling clinical blood is troublesome as well as disturbing cows caused by excessive stress. The aim of this experiment was to let people understand that postpartum ALT activity, dynamic variation and the correlation between blood plasma and milk could provide the basis for liver function injury diagnosis and monitoring methods of postpartum Holstein dairy cows in earlier lactation.

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MATERIALS AND METHODS

Experimental animal: All animals used in this experiment were approved by the Committee of Animal Welfare, Guangxi University, China.

A total of 35 Holstein cows (3-5 years old) which were randomly chosen from dairy farms of Guangxi, China. Body core of postparous experimental cows was approximate and the average weight of them was 510 kg with year milk yield of approximately 5700 kg.

Samples collection and preparation: About 187 samples (milk and blood) were collected from healthy Holstein cows at week 4-10 postpartum. Milk and blood collection from every cow was done simultaneously. Blood samples were taken from cows by jugular venepuncture into heparinized tubes and the blood plasma was separated by centrifugation timely at room temperature (1,500×g, 10 min). Milk samples were pre-centrifuged at 12,000×g for 30 min at 4°C by hypothermal ultracentrifuge and the pellucid milk serum was transferred into the new sterile tubes. Blood plasma and milk serum were stored at -20°C ready for biochemical measurements.

Laboratory analyses: With the improvement of Wright's method, the ALT activities of plasma and milk were strictly measured by the usage of commercially available kits by semi automatic biochemical analyzer (MS-500E).

Statistical analysis: All analyzes were conducted by the usage of the statistical package SPSS version: 18.0. Mean (M)±Standard Error (±SEM) were used to calculate the average activity of ALT in the plasma and milk. Student's t-test was performed by testing the significant difference between enzyme concentration in plasma and milk. The average ALT activity in plasma and milk were analyzed by the analysis of variance and multiple comparisons analysis; the activity of ALT correlation analysis with the assistance of Pearson method statistical processing. In all cases, significance was declared when the probability fell <0.05.

RESULTS AND DISCUSSION

Determination on the activities of blood plasma and milk ALT: About 187 samples (milk and blood) were used to detect the average activity of ALT in the sampled 35 healthy Holstein cows at week 4-10 postpartum. The result showed that the mean activity of ALT in blood plasma (14.37±0.63 UL^{-1}) was significantly higher than that in the milk (13.93±0.61 UL^{-1}) (Table 1). The mean activity of ALT in the blood plasma was in the normal range (1~40 UL^{-1}).

Dynamic change of ALT activity in the blood plasma and milk: Dynamic change of ALT activity in the blood plasma and milk showed in Fig. 1. ALT activity in blood plasma and milk showed a certain similarity trend that was increased at the outset and then decreased. The peak of the activity in the blood and milk was appearing at week 8 postpartum. Simultaneously at week postpartum, the activity of ALT in the milk was markedly higher than that in the blood plasma ($p<0.05$). Nonetheless at the time of week 4, 5 and 8 postpartum, the activity of ALT in the blood was higher than that at the other weeks and it was obviously higher than that in the milk ($p<0.05$).

The difference of significance test about ALT at the different weeks: Table 1 shows the results of the difference of significance test. The activity of ALT in blood plasma and milk reached the peak at week 8. Concurrently, ALT activity at week 8 was dramatically higher than that at previous weeks ($p<0.001$). The activity

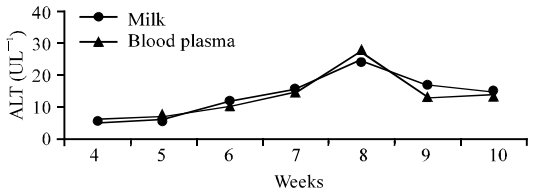

Fig. 1: Cows after delivery of 3-9 in peripheral blood and milk in the ALT activity

Table 1: ALT concentrations in blood and milk samples during the experiment (mean±SE)

	ALT of blood plasma (UL^{-1})		ALT of milk (UL^{-1})		Correlation	
Weeks	M±SE	No. of samples	M±SE	No. of samples	r	p-value
4	$6.58±0.40^{b}_{Cd}$	22	$5.42±0.28^{b}_{Dcd}$	24	0.35^{NS}	0.122
5	$6.73±0.48^{a}_{Cd}$	27	$5.59±0.26^{b}_{Dcd}$	27	0.62^{**}	0.001
6	$11.00± 0.44^{a}_{Bc}$	27	$11.48 ±0.44^{a}_{Cc}$	27	0.45^{**}	0.017
7	$15.84±1.23^{a}_{Bb}$	29	$15.09± 1.21^{a}_{BCbc}$	29	0.51^{**}	0.005
8	$27.37± 0.63^{a}_{Aa}$	33	$24.68 ±0.86^{b}_{Aa}$	33	0.66^{***}	0.000
9	$14.72±1.08^{b}_{Bb}$	32	$17.56± 1.58^{a}_{Bb}$	32	0.78^{***}	0.000
10	$13.82 ±1.27^{a}_{Bbc}$	15	$13.41 ±1.33^{a}_{BCbc}$	15	0.94^{***}	0.000
Total	$14.37±0.63^{a}$	187	$13.93±0.61^{b}$	187	0.84^{***}	0.000

In the same line of different superscript lowercase letters indicate a significant difference ($p<0.05$); the same column among the two groups with different subscript lowercase letters mean significant difference ($p<0.05$); the capital letters indicate the significant difference ($p<0.001$); the same letters indicate no significant difference ($p>0.05$); ** indicates significant correlation ($p<0.05$), *** indicates an extremely significant correlation ($p<0.001$); NS indicated no significant correlation ($p>0.05$)

of ALT in the blood plasma and milk showed a obvious difference at week 4, 5, 8 and 9 ($p<0.05$) and a slightly difference at week 6, 7 and 10 ($p<0.05$); throughout a further statistical analysis, ALT activity in milk was obviously higher at week 9 ($p<0.05$) and lower at week 4, 5 and 8 ($p<0.05$) compared to the ALT activity recorded in blood plasma.

Correlation analysis of ALT activity between blood plasma and milk: In this study, significant positive correlation of ALT activity were found between blood plasma and milk ($r = 0.84$, $p<0.001$) and they had a trend to be a straight line in the scatter diagram; the blood sample and corresponding milk sample showed a normal distribution (Fig. 2). Box plots in the Fig. 3 demonstrated that measured value of ALT in the blood plasma and milk had no abnormality except two abnormal values observed in the blood plasma, even though they had a comparative quartile.

Aminotransferase become one of the widest and most valuable biochemical indicator in liver function tests and it is a primary criterion of hepatocytes' damage and ALT is used as a sensitive marker of the liver disease

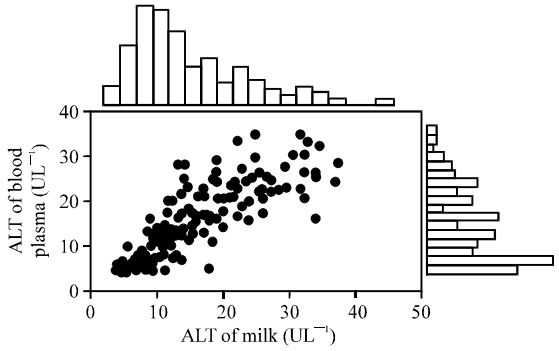

Fig. 2: Cows after delivery in plasma and milk ALT activity phase analysis chart

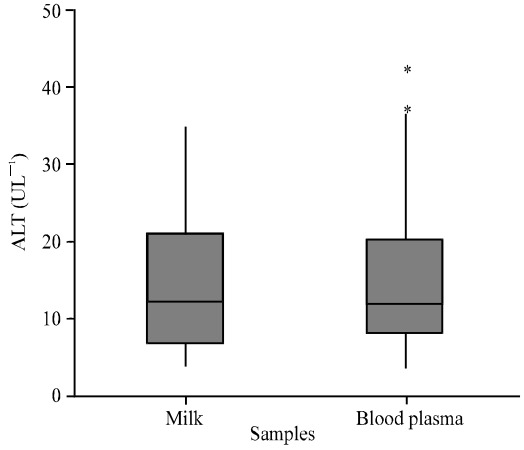

Fig. 3: Box diagram

such as the diagnosis of acute liver injury (Ray *et al*., 2008). In this study, the result showed that the mean activity of ALT in the blood plasma was 14.37±0.63 UL^{-1} and similar findings have been reported previously by Stojevic *et al*. (2005) (14.89±5.88 UL^{-1}); the result showed that the mean activity of ALT in the milk was 13.93±0.61 UL^{-1}. In the knowledge, this is the first reports about detecting the activity of ALT in milk.

In terms of ALT dynamic variation, data from the study suggest that ALT activity in both blood plasma and milk from calved dairy cows may reach the peak at week 8. Thereby, they had a similar dynamic variation trend. Results in the study on the changes of enzyme activity of ALT in blood plasma are in accordance with that reported by Stojevic *et al*. (2005) and Liu *et al*. (2012). Nevertheless, activity change of ALT in milk has not yet been reported. Alteration of ALT activity could be caused by the appearance of lactation peak and energy metabolism and the damage of liver function. As for energy metabolism, metabolism of macro minerals which is the configurable part or ingredients of enzymes plays an obvious role in the regulation of physiological functions and other sorts of body's chemical reactions during the time of pregnancy and lactation. Although, minerals for growth, reproduction and lactation are required by all animals (Ahmed *et al*., 2000; Krajnicakova *et al*., 2003). During the period of the postpartum, negative energy balance was caused by the body's physical consumption which was the result of the mobilization of body fat and liver injury and the variation of liver cell permeability (Roche *et al*., 2000; Rukkwamsuk *et al*., 2000; Hayirli *et al*., 2003; NRC, 2001). Furthermore, ALT in the liver cell was released into blood and brought about the increase of activity in the blood (Anderson *et al*., 2000; Puoti *et al*., 2005; Lai *et al*., 2007). Besides, it has been demonstrated that liver function is frequently associated with the attack of postpartum abomasal displacement, ketosis, mastitis, parturient paresis and endometritis which often occur in postpartum cows (Sarwar *et al*., 2002; Sevinc and Aslan, 2003; Takaike *et al*., 2004; Amany and Dina, 2008; Sahinduran *et al*., 2010). It has been reported that the serum ALT activity has significantly increased in ketotic cows (Dann *et al*., 2005; Sahinduran *et al*., 2010) which is also significant in mastitic cows compared to healthy cows (Amany and Dina, 2008). When compared with cows with abomasal displacement and parturient paresis, ALT concentrations of healthy cows were significantly lower (Sevinc and Aslan, 2003; Aslan *et al*., 2003) and in endometritis, ALT showed an increase (Sarwar *et al*., 2002).

According to the results of this study, there was a significant positive correlation ($r = 0.84$, $p<0.001$) by the

way of correlation analysis between ALT activity in blood plasma and milk. With the same study, it has been demonstrated that urea level showed a strong link between milk and blood in buffalo ($R^2 = 0.769$; $p<0.01$) (Campanile *et al*., 1998); the present study of estrone and 17β-estradiol of bovine were significantly correlated between milk and plasma ($r = 0.77$; $r = 0.93$) by Pape-Zambito *et al*. (2008). There is an indication that the variation of enzyme in the milk has an intimate relation with liver function. In this study, the change of ALT observed in milk was accompanied with its change in the blood. There are some reasons accounted for this phenomenon:

- A majority of enzymes which were exist in the breast cells derived from some enzymes of blood (Fox and Kelly, 2006)
- As previously reported, some enzymes including ALT, AST, GGT, LDH which had intense secretions in milk owing to the presence of these enzymes from the blood (Walentin *et al*., 1988). It is easily coming to the consequence that ALT correlation between blood and milk is significant

In short, the present study draws attention to the fact that ALT activity in dairy milk instead of blood served as an indicator of surveying early lactation and diagnosing liver function. This gives a new method of choosing samples to be tested in the diagnostic of liver function in dairy herds at postpartum period. This study is intended to make contribution in detecting liver functions with milk enzyme instead of blood enzyme. Except, this method appear to be more economic and time saving and also can reduce animal stress during sample collection. To better realize the relationship between other enzymes in milk and blood and whether they have something in common with the liver function or not. More experiments need to be conducted to confirm a direct correlation between enzymes in blood and in milk.

CONCLUSION

In the present study, the activities of enzymes ALT were measured in blood plasma and milk from healthy Holstein cows. ALT activity in blood plasma and milk significantly increased and reached the peak at the same time, week 8 postpartum. According to the results of the study, there was significant positive correlation ($r = 0.84$, $p<0.001$) in the concentration of ALT in blood plasma and milk. Interestingly, it seems that the detection of these enzymes in milk may be an alternative way of analyzing the liver function in dairy cows at lactating period. More experiments need to be conducted to furthermore understand and confirm a direct correlation between ALT activity in blood plasma and milk and recognize the detection of these enzymes in milk as an alternative tour of analyzing the liver function in dairy cows at lactating period.

ACKNOWLEDGEMENTS

The researchers thank the staff of Jin-Guang dairy farm of Nanning for caring the experimental animals. This research was financially supported by Chinese National Natural Scientific Foundation (No. 30960294) and Postgraduate Innovation Program (No. 105931001005; GXU11T32527). Ping Liu and Xiao Lu Hou: These 1st authors contributed equally to this study; Pei Nie, Yin Hua Han and Yun Fei Huang: These 2nd authors contributed equally to this study; Hui Fang Deng and Zhen Xing Zou: These 3rd authors contributed equally to this study.

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