



RESEARCH ARTICLE

Bioactivity Evaluation of Certain Hepatic Enzymes in Blood Plasma and Milk of Holstein Cows

Ping Liu^{1,2}, Bao Xiang He^{1,2,*}, Xian Ling Yang^{§2}, Xiao Lu Hou^{§2}, Jian Bin Han², Yin Hua Han², Pei Nie², Hui Fang Deng² and Xiang Hong Du²

¹Institute of Animal Reproduction, Guangxi University, Nanning, Guangxi 530005, People's Republic of China;

²Clinical Veterinary Laboratory, College of Animal Science and Technology, Guangxi University, Nanning, Guangxi 530005, People's Republic of China

*Corresponding Author: hbx5817@163.com

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ABSTRACT

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyltransferase (GGT), and alkaline phosphatase (ALP) are important catabolic enzymes, which play an important role in liver function of animals. The activities of these enzymes were monitored in milk and blood plasma and association between them was determined through regression analysis. Samples of milk and blood were collected from 35 housed multiparous Holstein cows 3-9 weeks postpartum. The results showed that there were significantly positive correlations for these four enzymes between the enzyme activities in milk and blood plasma (ALT, $r_s=0.852$, $P<0.001$; AST, $r_s=0.341$, $P<0.001$; GGT, $r_s=0.628$, $P<0.001$; ALP, $r_s=0.707$, $P<0.001$). Compared to the blood plasma, the activities of ALT ($P<0.05$) and AST ($P<0.001$) in milk appeared significantly lower, while the activities of GGT and ALP were significantly higher ($P<0.001$). With the regression models, it seems that detection of ALT, AST, GGT and ALP activities in milk may be an alternative way to monitor the liver function of cows. Therefore, this study is an initial step for studying the liver function through detection of these enzymes in milk.

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INTRODUCTION

The hepatic enzymes such as alanine aminotransferases (ALT EC 2.6.1.2), aspartate aminotransferase (AST EC 2.6.1.1), γ -glutamyltransferase (GGT EC 2.3.2.2) and alkaline phosphatase (ALP EC 3.1.3.1) are sensitive enzymes that primarily reflect hepatocellular necrosis, and cholestasis, respectively, so these are of special usefulness in the diagnosis of serious hepatic diseases such as hepatitis (Ray Kim *et al.*, 2008; Romeo *et al.*, 2010). In dog, cat, rabbit, and rat, ALT is a specific cytosol liver enzyme, the increase of which in the blood plasma reflects changes of the liver function as reported by Kramer *et al.* (1997). AST activity has been extensively studied in both animals and humans. For instance, it was found that the variation of AST had some relations with the function of liver, skeletal muscle and heart of cows (Sattler and Fürll, 2004; Kaneko *et al.*, 2008). In addition, AST is proposed to be one of the

common parameters for the detection and diagnosis of liver failure (Sattler and Fürll, 2004). The high concentrations of GGT in blood are detected in the liver, kidney, pancreas, spleen, intestine, heart, and the brain of mammalian tissue (Tennant, 1997). In the liver of cow, horse, sheep, and goat, the GGT activity is relatively high whereas it is considerably lower in dog and cat (Milinković-Tur *et al.*, 2005). Blood plasma ALT, AST and GGT activities were reported to be useful for postpartum Holstein cows (Stojević *et al.*, 2005; Šamanc *et al.*, 2011). ALP, which is a membrane-bound glycoprotein mainly found in animal tissues such as liver, bone, with a lesser amounts in kidney, intestine and placenta (Ali *et al.*, 2005; Webber *et al.*, 2010), is used as a biochemical marker to diagnose osteoporosis and hepatobiliary disorders as well as fatty liver disease (Webber *et al.*, 2010; Hanley *et al.*, 2005). While little information is available concerning about the activity changes of ALT, AST, GGT and ALP in milk.

In view of rare research on measuring liver function with enzyme activity in milk, the ALT, AST, GGT and

[§]These authors contributed equally to this paper.

ALP activities in blood and milk during postpartum lactation in Holstein cows were detected in the present study. In order to assess the associations between them, the correlation/regression model was also implied. Considering no systematic data for these important parameters reflecting the metabolic status of dairy cows was available, so the detection of activities of catabolic enzymes appears to be necessary, and the results obtained in this study may have a contribution to a better understanding of biochemical processes between activities of enzymes in milk and liver function of postpartum Holstein cows, as well as to estimating physiological status and diagnosing pathological status of liver function.

MATERIALS AND METHODS

Experimental animals: The use of all animals in this experiment was approved by the Committee of Animal Welfare, Guangxi University, China. Milk and blood samples used in this study were collected from 35 Holstein cows (3-6 years old), which were randomly chosen from more than 1000 lactating dairy in Guangxi province, China. Samples were collected in the morning before feeding once a week or between 3 to 9 weeks after calving. Three weeks before collecting samples, the cows were fed with a careful-formulated diet and under disease control to ensure all the samples were collected from healthy cows. Before milk samples collected, California mastitis test (CMT) (Pohn *et al.*, 2009) was used on them to screen those with mastitis.

Sample collection: Blood and milk samples were taken simultaneously from each lactating cow. Blood samples (10 ml) were taken by jugular venipuncture under sterile conditions into a heparinized tube from each animal, and the blood plasma was separated by centrifugation at room temperature (1,800×g, 15 min). Milk samples were centrifuged at 12,000×g for 30 min at 4°C and the supernatant was transferred into the new sterile tubes. Blood plasma and milk were stored at -20°C until being used for biochemical measurements.

Laboratory analyses: Samples were analyzed at the Clinical Veterinary Laboratory, College of Animal Science and Technology, Guangxi University. They were measured by Semi-automatic biochemical Analyzer. The activities of ALT and AST in blood plasma and milk were measured by colorimetric Reitman-Frankel method (Yang *et al.*, 2009). The GGT and ALP in blood plasma and milk were measured as described by the International Federation of Clinical Chemistry (IFCC) (Ceriotti *et al.*, 2010).

Statistical analysis: The data was analyzed with the statistical package SPSS version 18.0. In order to detect statistically significant differences, the data was summarized with descriptive statistics: mean (M) ± standard error (± SE), and frequencies (n). Student's t-test was used to evaluate data of enzyme activity in blood plasma and milk. The correlations were analyzed by Spearman rank among continuous datasets by calculating the correlation coefficient (r_s). Regression models (simple) (Gelman and Hill, 2007) were performed on curvilinear relationship of the enzymes in milk and blood plasma.

RESULTS

Blood and milk samples, which were collected once a week during 3 to 9 weeks after calving from 35 housed multiparous Holstein cows, were used in the present study. Among them, 246 samples were measured for the activities of ALT, 245 samples for AST, 247 samples for GGT, and 248 samples for ALP. The results clearly showed that there was a significant difference for the enzyme activity in individual cow's blood plasma and milk (Table 1). The AST activity in milk was much lower than that in blood plasma ($P<0.001$). Compared to other three enzymes, the enzyme activity of ALT in blood plasma and milk were the lowest. Surprisingly, the enzyme activities of both GGT and ALP in milk were significantly higher than that in blood plasma, especially for GGT. Furthermore, the activity of GGT in milk was almost thirty fold as much as that in blood plasma ($P<0.001$), and the ALP activity in milk was nearly three fold as much as that in blood plasma ($P<0.001$) (Table 1).

To further analyze the correlations between enzyme activities in blood plasma and in milk, a series of statistical analysis were used to evaluate the enzyme activity. As indicated in Table 1, the significantly positive correlations were found for these four enzymes ALT ($r_s=0.852$, $P<0.001$), AST ($r_s=0.341$, $P<0.001$), GGT ($r_s=0.628$, $P<0.001$), and ALP ($r_s=0.707$, $P<0.001$) between the enzyme activities in blood plasma and milk, and high correlation of enzyme activities was obtained for ALT, GGT, and ALP. Furthermore, regression models were also used in the present study, which show a curve correlation between variables through calculation. On the basis of the analysis concerned, the regression models, in which the blood plasma is dependent variable and milk independent variable, described a powerful function equation. The resulting exponential equation showed that there were highly significant relationships occurred for these four enzymes in which the enzyme activities in blood plasma increased with the increasing enzyme activities in milk (ALT, $R^2=0.72$, $P<0.001$; AST, $R^2=0.11$, $P<0.001$; GGT, $R^2=0.43$, $P<0.001$; ALP, $R^2=0.52$, $P<0.001$) (Fig 1-4). Concentrations were estimated by using these formulas: ALT, $\hat{y}=1.78(x^{0.79})$; AST, $\hat{y}=57.48e^{0.009x}$; GGT, $\hat{y}=2.22(x^{0.34})$; ALP, $\hat{y}=20.74(x^{0.26})$. These models were moderately accurate for estimating ALT, AST, GGT and ALP activities in blood plasma.

Table 1: Enzyme activities of ALT, AST, GGT and ALP in blood plasma and milk and their relative correlation

| Enzymes | Blood Plasma | | Milk | | Sperman's Rho(r_s) | P value |
|----------|-------------------------|-----|---------------------------|-----|------------------------|---------|
| | M±SE | n | M±SE | n | | |
| ALT(U/L) | 14.26±0.51 ^a | 246 | 13.61±0.49 ^b | 246 | 0.852*** | 0.000 |
| AST(U/L) | 76.99±1.59 ^A | 245 | 27.20±0.90 ^B | 245 | 0.341*** | 0.000 |
| GGT(U/L) | 23.17±0.32 ^B | 247 | 921.63±20.98 ^A | 247 | 0.628*** | 0.000 |
| ALP(U/L) | 79.23±1.95 ^B | 248 | 251.43±20.25 ^A | 248 | 0.707*** | 0.000 |

***Correlation was statistically significant at the $P<0.001$ level; ^{a,b}different superscript lower case letters ($P<0.05$) and ^{A,B}different superscript capital letters ($P<0.001$) within the same row means.

DISCUSSION

It has been demonstrated that liver function is frequently associated with abomasal displacement, ketosis, mastitis, parturient paresis, retain placenta and endometritis, which often occur in postpartum cows

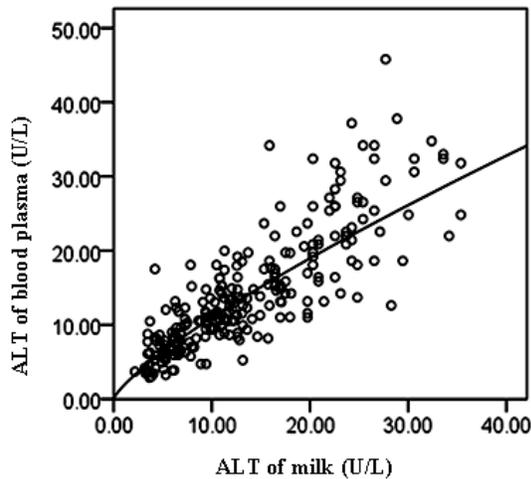


Fig 1: Regression of ALT enzymatic activity changes between blood plasma (\hat{y}) and milk (x). Regression equation: $\hat{y} = 1.78 (x^{0.79})$, $R^2 = 0.72$; $P < 0.001$.

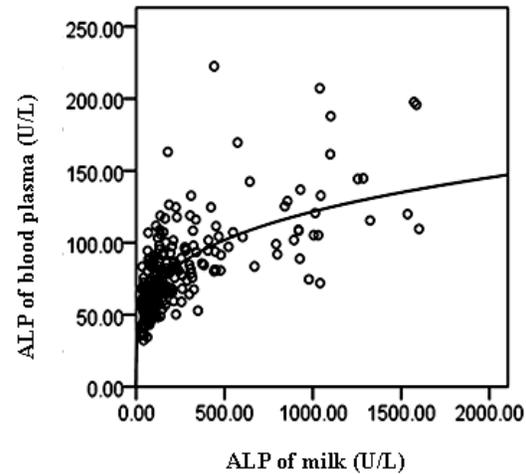


Fig 4: Regression of ALP enzymatic activity changes between the blood plasma (\hat{y}) and milk (x). Regression equation: $\hat{y} = 20.74 (x^{0.26})$, $R^2 = 0.52$; $P < 0.001$.

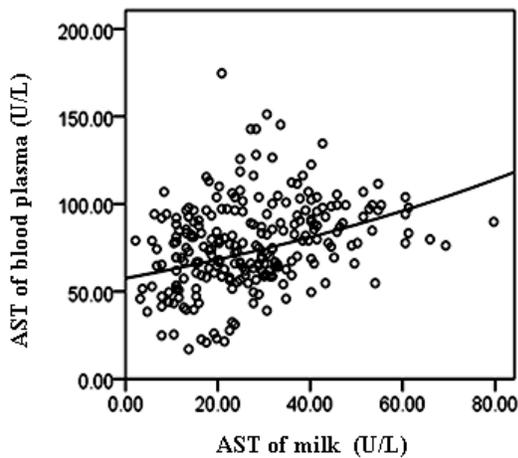


Fig 2: Regression of AST enzymatic activity changes between the blood plasma (\hat{y}) and milk (x). Regression equation: $\hat{y} = 57.48 e^{0.009x}$, $R^2 = 0.11$; $P < 0.001$.

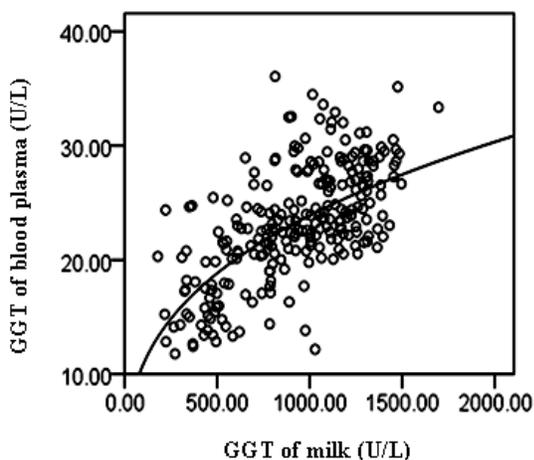


Fig 3: Regression of GGT enzymatic activity changes between the blood plasma (\hat{y}) and milk (x). Regression equation: $\hat{y} = 2.22 (x^{0.34})$, $R^2 = 0.43$; $P < 0.001$.

(Sevinc *et al.*, 2002; Faculty *et al.*; 2003; Sattler and Füll, 2004; Semacan and Sevinc, 2005; Amany *et al.*, 2008; Sahinduran *et al.*, 2010). Previously, diseases mentioned above can be diagnosed by detecting enzymatic activities in blood of dairy cows. For example, determination of GGT and AST concentrations is helpful for assessing the liver function of cows with abomasal displacement (Sevinc *et al.*, 2002; Faculty *et al.*; 2003). Biochemical analysis of serum declared that mastitis was accompanied by significant increase of enzymatic activities of ALT, AST and ALP (Amany *et al.*, 2008). Although the measurement of activities of hepatic enzymes in blood is generally carried out, it has some disadvantages. First, blood samples were taken by jugular venipuncture which can cause stress on animals, which turned out to induce pathological changes, including feeding behavior changes, hypertension, reproductive dysfunction, gastric and intestinal ulcers, inefficient feed conversion, electrolyte imbalance and immune deficiency (Mudroň *et al.*, 2005). Second, it is inefficient and time-exhausted. In the present study, it is intended to make contribution to measure the metabolic status of liver with milk enzymes, because it is not only more economic but also can avoid hurting animals. In addition, it is easier to carry out collection of milk samples. Not only in science but also in public discussion, there is increasing interest in animal welfare and minimizing losses in product yield and quality. Therefore, with more researches conducted in this field, milk is expected to play a decisive role in the process of disease diagnosis.

In the present study, the enzyme activities of ALT, AST, GGT and ALP in blood plasma and milk from postpartum cows were measured and compared. The enzyme activity of ALT in blood plasma from dairy cows in our study is in agreement with that reported by Stojević *et al.* (2005) (14.89 ± 5.88 U/L), in which the values of AST (43.35 ± 13.56 U/L) and GGT (17.11 ± 4.52 U/L) appeared to be lower than that obtained in this study (AST, 76.99 ± 1.59 U/L; GGT, 23.17 ± 0.32 U/L), but the value of these two enzymes is consistent with that reported by Civelek *et al.* (2006) (AST, 72.33 ± 1.90 U/L; GGT, 22.44 ± 1.60 U/L) and Sevinc *et al.* (2002) (AST,

78.5±9.00 U/L; GGT, 22.33±2.10 U/L). Furthermore, the AST activity in this study is also in agreement with that reported by Farzaneh *et al.* (2006) (76.55±1.86 U/L), in which the reported activity of ALP (92.88±4.77) was higher than that obtained in our study (ALP, 79.23±1.95 U/L). But the discrepancy between their results and ours is unavoidable because of the differences in the management, nutrition, breed, lactation period, flock size, season, case definition and the diagnostic criteria. Furthermore, detection of enzymes in milk showed that the activities of ALT ($P<0.05$) and AST ($P<0.001$) were significantly lower, while the activities of both GGT and ALP were significantly higher ($P<0.001$) than that in blood plasma.

Based on the comparison of enzymatic activity in blood plasma with that in milk, the results of correlation analysis and regressive models showed a close relation between them. With the same analysis, Britti demonstrated that the enzyme activity of GGT in blood plasma is closely related to the concentration of IgG in the same sample (Britti *et al.*, 2005). This indicates that detection of enzymes ALT, AST, GGT, and ALP in milk may be an alternative way to measure the liver function of dairy cow, which potentially plays a vital role in detecting diseases of lactating animals after calving. To make this promising detection method be practical, it still needs further clinical studies to prove that.

Conclusion: In the present study, the activities of enzymes ALT, AST, GGT and ALP were measured in samples of blood plasma and milk from healthy Holstein cows, respectively. The results showed that the enzymes activities of GGT and ALP in milk were higher than that in blood plasma. For ALT, the enzyme activity in milk was the same as that in blood plasma. Taken together, it seems that detection of these enzymes in milk may be an alternative way to measure the liver function of dairy cows. To further understand the relationship between the enzymes in milk and liver function, more experiments need to be conducted to confirm a direct correlation between them.

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