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Activities of Aspartate Aminotransferase, Alanine Aminotransferase, Gamma-Glutamyltransferase, Alkaline Phosphatase in Plasma of Postpartum Holstein Cows

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Abstract: Depressed appetite and reduced Dry Matter Intake (DMI) and feeding energy-dense diets for a long time around parturition of cows may lead to excessive lipid mobilization which causes the liver damage. This study was meant to determine the effects of postpartun enzymes metabolic status in Holstein cows. In this study, blood samples were during the whole experimental period, obtained from the jugular venepuncture from each animal on 1 week prepartum (week 1), days delivery (week 0) and 1st 9 weeks postpartum (week 1-9). They were analyzed for examining Aspartate aminotransferase (AST), Alanine Aminotransferase (ALT), Gamma-Glutamyltransferase (GGT), Alkaline Phosphatase (ALP) activity. The resultes showed a higher activity of AST which was determined in the 1-3 weeks than other's. ALT activity indicated a statistically significant increase from the 5-7 weeks of lactation and activity in the 7th week postpartum periods significally reached to the peak. GGT activity in the antepartum 1 week until delivery day was significally lower in comparison with the first to reach the 9th weeks postpartum. ALP activity in the delivery day and 6-8 weeks significant increased in process. Therefore, the AST, ALT, GGT and ALP of enzyme activity which could be used significantly change in the blood plasma of Holstein.

Key words: Aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, alkaline phosphatase, cows, China

INTRODUCTION

Postpartum almost every cow experiences a period with high energy requirement related to milk production and frequently associated to an insufficient feed intake. In previous report, the dairy cows undergoes many metabolic changes in preparation for parturition and lactation. Following parturition, Dry Matter Intake (DMI) decreased (Grummer, 1993) and DMI need increased 4-6 fold in order to meet the high nutrient demands of milk production. However, the high yielding cows postpartum does not increase DMI as fast as the increased nutrient demands required for lactation (Roche *et al*., 2000; Hayirli *et al*., 2003; NRC, 2001) to deal with this nutrient shortage, the cow mobilises body reserves of fat and protein. This situation causes the Negative Energy Balance (NEBAL) and this imbalance increases fat mobilization (Rukkwamsuk *et al*., 2000) and can be a resust of cell structure damage or changes in enzyme activity such as AST, ALT, GGT and ALP in the liver. For example, it is reported that activities of AST, ALT and GGT during different lactation periods were statistically different in the blood plasma of dairy cows (Stojevic *et al*., 2005). Sato *et al*. (2005) found that serum ALP activity was higher in lactational periods in comparison with the dry period.

In comparison with other stages of the lactation cycle, cows in early lactation are of the highest possibility to suffer most metabolic diseases. Liver function is usually related to ketosis, abomasal displacement, mastitis, retain placenta, parturient paresis and endometritis which have been often associated with postpartum cows. This imbalance leads to increases of fat mobilization and hepatic ketogenesis. Under these conditions, it is practical to measure the plasma activities of the liver-specific enzymes. Sahinduran *et al*. (2010) reported significant increases in blood activities of AST, ALT, ALP and GGT in ketotic cows. GGT and AST concentrations is helpful in measuring liver function in cows with abomasal displacement (Sevinc *et al*., 2002;

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Zadnik, 2003). In Semacan and Sevinc (2005) report, GGT and AST study suggested that retained placenta might be associated with fatty live. According to biochemical analysis of serum, mastitis is accompanied by significant increase of enzymatic activities of ALT, AST and ALP (Amany *et al*., 2008). AST is more significantly correlated to the degree of endometritis (Sattler and Furll, 2004).

The objective of this research was to study the blood plasma AST, ALT, GGT and ALP ctivities of enzymes during lactation in Holstein cows postpartum. The achieved results may be helpful to understand biochemical processes in blood plasma of Holstein cows postpartum better, estimate their physiological status and diagnose diseases in cows postpartum.

MATERIALS AND METHODS

Experimental animal: A total of 32 Holstein cows (3-6 years old) which were randomly chosen from >1000 animals in lactating dairy in Guangxi area were used in this study. Samples were randomly collected from 32 postpartum cows at the time from antepartum the 1 week after calving until the 9th week. All herds were subjected to the same breeding and management programs that were housed in free stalls without grazing and all are healthy multiparous cows.

Sample collection: Samples of jugular blood were obtained weekly for 32 cows from week 1-9 postpartum at 1-3 h after the am. Blood samples (10 mL) were taken from each one and the plasma was separated from heparinised blood samples by centrifugation at normal temperature (1,500×g, 10 min). Plasma were stored at -20°C until being used for biochemical measurements.

Laboratory analyses: Samples were analyzed at the Laboratory of Clinical Institution of Animal Science and Technolog Guangxi University. They were measured by Semi-automatic biochemical. The activities of AST, ALT, GGT and ALP in plasma were examined with commercial kits using autoanalyzer.

Data analysis: The data were processed and analyzed using the statistical package SPSS Version 17.0. The data were summariaed with descriptive statistics: Mean (M)±Standard Error (±SE) and Student's t-test was used to evaluate differences between enzymes in plasma.

RESULTS AND DISCUSSION

The experiment was conducted from prepartum week 1 before the expected parturition (varying between 5-10 days prior to the real day of parturition) until the 9th week postpartum. From the 1st week up to day delivery period ($p<0.001$), the measured activities showed a constant decrease of AST values in the blood plasma of observed animals in the 2nd week postpartum, AST activity in blood plasma was statistically far higher than in the other weeks ($p<0.001$), a statistically significant decrease of AST activities demonstrated both in the results obtained from animals which were in week the 1st, 3rd, 8th and 9th ($p<0.05$) and in the day delivery and 4-7 weeks ($p<0.001$) comparing to the results during the 1st week postpartum lactation and the day delivery and 4-7 weeks postpartum values were lower than the perpartum week 1st, 2nd, 3rd and 8-9th postpartum period ($p<0.001$), 7th week posrtpartum value was lower than perpartum 1st week ($p<0.05$) (Table 1). However, all cows' AST activity showed a significant 2 peak periods at 2nd and 8th week. The average AST activity increased with 55.39 from 64.19±3.46-119.48±3.49 U L^{-1} at 1st peak period from day delivery up to 2nd week from 2-5 week postpartum decreased with 51.77, from 119.48±3.49-67.77±3.47 U L^{-1}; it increased with 22.06 from 67.77±3.47-89.77±3.18 U L^{-1} at 2nd peak period from 5th-8th weeks (Fig. 1).

Table 1: Plasma enzyme activity AST, ALT, GGT, ALP in Holstein cows (M±SE)

Weeks	AST (U L^{-1})	ALT (U L^{-1})	GGT (U L^{-1})	ALP (U L^{-1})
-1	88.27 ± 2.68^{cdBC}	6.80 ± 0.40^{D}	20.99 ± 1.22^{bcAB}	59.89 ± 2.21^{dCDE}
0	64.19 ± 3.46^{eD}	7.00 ± 0.36^{D}	20.26 ± 1.05^{cB}	73.30 ± 3.52^{bcAB}
1	100.52 ± 3.49^{bB}	5.50 ± 0.39^{D}	23.28 ± 1.21^{abcAB}	69.87 ± 2.23^{cBC}
2	119.48 ± 3.98^{aA}	6.00 ± 0.41^{D}	24.58 ± 1.39^{aAB}	67.17 ± 3.13^{cBCDE}
3	90.60 ± 3.49^{cB}	6.65 ± 0.33^{D}	24.12 ± 1.13^{abAB}	59.57 ± 1.79^{dCDE}
4	72.47 ± 4.30^{eD}	7.00 ± 0.49^{D}	24.12 ± 0.83^{abAB}	56.88 ± 2.65^{dE}
5	67.71 ± 3.47^{eD}	10.94 ± 0.38^{C}	25.77 ± 1.39^{aA}	57.97 ± 1.70^{dDE}
6	72.35 ± 3.42^{eD}	16.06 ± 1.02^{B}	24.12 ± 0.78^{abAB}	68.43 ± 2.46^{cBCD}
7	73.06 ± 2.81^{deCD}	27.56 ± 1.03^{A}	24.12 ± 0.78^{abAB}	82.26 ± 2.13^{aA}
8	89.77 ± 3.18^{cB}	15.08 ± 1.04^{B}	24.77 ± 1.22^{aAB}	82.69 ± 2.94^{aA}
9	89.97 ± 3.60^{cB}	14.35 ± 0.66^{B}	24.96 ± 1.27^{aAB}	77.83 ± 2.38^{abAB}
Total	84.40±1.33	11.18±0.39	23.73±0.35	68.71±0.89

$^{a-e}$Different significantly superscript lower case letters ($p<0.05$) and $^{A-E}$Different significantly superscript capital letters ($p<0.001$) within the same row means

The ALT activity in cows which were in lactation periods also showed significant differences. In the 7th week, there was an increase of activity values up to a peak period, from 1st week up to the 7th, the average ALT activity increased with 22.06, from 5.50±0.39-27.56±1.03 U L^{-1} (Fig. 1) and the values showed statistically higher than in the other weeks ($p<0.001$) through interactive comparison between results of each period, researchers noticed that ALT activities prepartum week 1 prior until the 5th week postpartun demonstrated a statistically significant decrease comparing to the week 6th, 8th, 9th postpartum of lactation period ($p<0.001$) meanwhile the activities of this enzyme in the 5th week periods of lactation were statistically significantly higher than those measured from week 1-4 postpartum period ($p<0.001$) (Table 1).

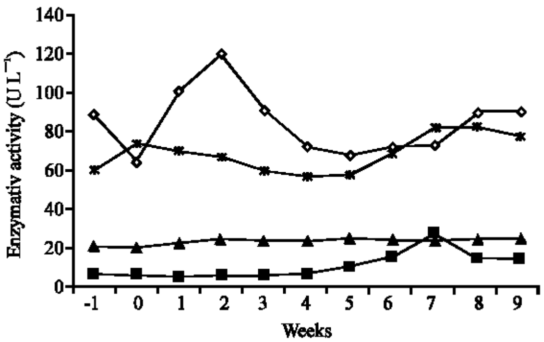

Fig. 1: Activities of enzymes AST (◆), ALT (■), ALP (✱) and GGT (▲) in plasma postpartum dairy cows from antepartum 1 week until postpartum 9 weeks

The results of comparing GGT activities in plasma during different production periods were statistically different. Mean values of GGT activities in peak period which is at 5th week (Fig. 1) postparum of lactation period were far higher than the day delivery of lactation ($p<0.001$) and perpartum 1st week ($p<0.05$) during the perpartum 1st week, activity values were significantly lower than at the 2nd, 5th, 8th and 9th weeks postpartum lactation ($p<0.05$) day delivery of lactation were significantly lower than at the postpartum weeks 2-4 and 6-9 (Table 1).

ALP activity had two significant peak periods at day delivery and the 7th week, respectively. At 1st peak period from the 1st week up to day delivery, the average ALP activity increased with 13.41 from 59.89±2.21-73.30±3.52 U L^{-1}; from 0-4 weeks postpartum, it decreased with 16.42; from 73.30±3.52-56.88±2.65 U L^{-1}; it increased with 25.81; from 56.88±2.65-82.69±2.94 U L^{-1} at 2nd peak period from the 4th-8th weeks (Fig. 1). The measured activities of ALP values in the blood plasma on the week 1st and 3rd period were lower than the day delivery and 7th-9th week ($p<0.001$) and than 1st, 2nd, 6th week ($p<0.05$) in the 7-8 weeks of postpartum, ALP activity in the blood plasma was statistically much higher than in the other weeks such as 1 and 1-6 week ($p<0.001$) and 0 week ($p<0.05$); furthermore, the 4th week showed a statistically significant decrease of ALP activities comparing to the results during weeks 0, 1 and 6 postpartum lactation ($p<0.001$) and 2nd week ($p<0.05$) and values at the 5th week were lower than the day delivery and the 1st week postpartum ($p<0.001$) and 2nd week and 6th week ($p<0.05$) the value at the 9th week posrtpartum was higer than the perpartum 4-5 weeks ($p<0.001$) and the week 2 and 6 ($p<0.05$) (Table 1).

In this study, it is found that the enzyme activity of AST and GGT during parturition period showed to be higher than that obtained by Stojevic *et al*. (2005). But the AST values is nearly consistent with report that AST: 78.5±9.00; 72.33±1.90; 76.55±1.86 U L^{-1} (Sevinc *et al*., 2002; Civelek *et al*., 2006; Farzaneh *et al*., 2006) and is within the reference normal rang value of cows 60-150 and 78-132 U L^{-1} (Sahinduran and Albay, 2006; Altug *et al*., 2007). The GGT values is in agreement with that reported by Sevinc *et al*. (2002) and Civelek *et al*. 2006 (GGT: 22.33±2.10; 22.44±1.60 U L^{-1}). The values of ALT during parturition period in the study is similar to that reported by Stojevic *et al*. (2005) and is almost within the reference normal rang value of cows 11-40 U L^{-1} (Altug *et al*., 2007). Activity values of ALP reported by Farzaneh *et al*. (2006) (92.88±4.77) was higher than that obtained in the study which is totally within the reference range of ALP activity values (Sato *et al*., 2005, 0-488 U L^{-1}).

AST activity in blood peaked on postpartum week 2, at which the values was similar to previous report (Park *et al*., 2002; Jaakson *et al*., 2007). The highest AST activity was reported during early lactation but as lactation progressed the activity of this enzyme decreased (Stojevic *et al*., 2005). According to the study result, the AST activities were the lowest on postpartum 5-7 weeks and a significant increase occured on postpartum 8th week with 2nd peak, the related report of AST activity on 8th week is unknown so far. AST in liver, skeletal muscle and heart of cows is sensitive (Sattler and Furll, 2004) and the increases of serum AST activities is a indicator of soft tissue damage, probably live damage. Hoedemaker *et al*. (2004) indicated that increase of AST activity postpartum is related to extensive muscle breakdown and increased amino acid catabolism; besides, Bobe *et al*. (2004) and Xu *et al*. (1998) point out a corelation between elevated AST activity and postpartum Nonesterified Fatty Acids (NEFA) accumulation into liver.

In previous report, ALT activity in cattle is not specific for the liver in order to have a diagnostic significance (Kramer and Hoffman, 1997) but in the study, it is found that ALT's increase in the blood plasma is of significant changes in the cows postpartum. The results showed ALT is a significally increased of cows in 7th week postpartum period. In Tainturier *et al*. (1984) study's, ALT has a significant change of cows in the parturition period and ALT activity had a decrease in the 7th and 8th months of pregnancy and it remained the same up to the end of pregnancy and 1st month of lactation period. The highest activitiy of ALT was confirmed in the third period lactation (Stojevic *et al*., 2005).

In the experimental observations, GGT did not change as significant as other three enzymes in the postpartum period of cows and significally increased in 5th week postpartum. El-ghoul *et al.* (2000) reported that GGT activity in late pregnancy was far <1st week after delivery and the activity increased on 6th week after calving. GGT activity in the 2nd and 3rd periods was statistically lower than in the 1st production period and in the dry period by Stojevic *et al.* (2005).

Previously reporting that ALP activities increased on 5th day postpartum (Peter *et al.*, 1987) and was not same as in the present study. The results confirm that the values of ALP increased in the day delivery which is bascically consistent with Peter *et al.* (1987) who reported the activity of ALP began to increase significantly in 1 day before parturition and reach a peak on the day of parturition. The second peak period of ALP occured a week earlier than AST.

In short in terms of the speculation, changes of these enzymes were mainly caused by the negative energy balance in postpartum periods cows. In dairy cows peaks of yielding milk were usually between postpartum weeks 4 and 8 but maximum dry matter intake usually between postpartum weeks 10 and 14 (Ensminger *et al.*, 1990). Another report about peaks of milk yield and dry matter intake in dairy cows were usually from postpartum weeks 4-10 but peaks of the lowest energy balance were in postpartum 2nd week (Rastani *et al.*, 2005), researchers concluded that the difference of milk yield and DMI may mainly result in the damage of liver funtions which caused changes of enzymes. More researches need to be conducted to reveal whether there are other factors related to the enzymes changes.

CONCLUSION

In the study, the four enzymes were found to change significantly in postpartum cows. The periods of significant increase for them are: AST was on 2nd and 8th week; ALT, 7th week; GGT, 5th week; ALP, the day delivery and 7th week. These values could be used as valuable reference for diseases diagnosis of postpartum cows.

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