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EVALUATION OF LIVER FUNCTION TESTS IN COWS DURING PERIPARTURIENT PERIOD

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ABSTRACT

The objective of the study was to determine various liver metabolic changes associated with periparturient period (1 week before calving and 3 weeks after parturition) in dairy cows.

Twenty-one Holstein study cows and eight control from one commercial herd were used all the animals were monitored for bromosulphthalein (BSP) clearence test, liver biopsy and metabolic profile at 1st week before calving, on calving day (day 0), and at 1st, 2nd and 3rd weeks after parturition. Single intravenous injections of BSP were given to cows and the change in plasma BSP concentration with time was analysed for each measurement point. Additionally, metabolic profile as determined at each time during the study.

Analyses of variant indicated that most dramatic changes especially occured at wk 1 before parturition prior to wk 2 after parturition when compared to the other study groups ($p<0.05$) and non-pregnant cows ($p<0.05$). Results of the study showed that the periparturient period is very important in cattle life and only one diagnostic test may be mislead for evaluating the changes in the liver functions in the periparturient period. Therefore, performing all the diagnostic tests together are give us more valuable data about during the period in cows.

Key Words: *Cattle, Periparturient period, Liver*

ÖZET

Doğum Öncesi ve Doğum Sonrası Dönemdeki İneklerde Karaciğer Fonksiyon Testlerinin Değerlendirilmesi

Bu çalışmanın amacı süt ineklerinde periparturient dönemde (doğum önceki 1 hafta ile doğumdan sonraki 3 haftalık periyod) karaciğerde meydana gelen metabolik değişiklikleri saptamak amacıyla yapıldı.

Bu çalışma periparturient dönemde bulunan 21 baş Holstein inek (çalışma grubunu) ve kuru dönemde bulunan 8 inekte (kontrol grubu) yapıldı. Bütün hayvanlar doğum öncesi 1. hafta, doğum günü (0. gün) ve doğumdan sonraki 1, 2 ve 3. haftalarda bromosulphthalein (BSP) clearence test, karaciğer biyopsisi ve metabolik profil yönünden gözlendi. Ölçüm yapılan tüm zamanlarda intravenöz olarak tek doz BSP verilerek, plazma BSP konsantrasyonlarındaki değişiklikler belirlendi. Ayrıca çalışma süresince ölçüm yapılan her zamana ait metabolik profil de saptandı.

Analizleri yapılan değişkenlerdeki en dikkat çekici değişikliklerin diğer gruplar ($p<0.05$) ve gebe olmayanlarla karşılaştırıldığında ($p<0.05$) özellikle doğumdan önceki 1. hafta ile doğumdan sonraki 2. haftaya kadarki dönemde oluştuğu gözlendi. Bu çalışmada periparturient dönemin sığırların yaşamında oldukça önemli bir dönem olduğu ve bu dönemde bulunan sığırların karaciğer işlevlerinin değerlendirilmesinde sadece bir testin yanıltıcı sonuçlar verebileceği sonucuna varıldı. Bu yüzden bu dönemdeki ineklerin karaciğer işlevlerinin değerlendirilmesinde bütün diagnostik testlerin birlikte uygulanması daha yararlı sonuçlar vermektedir.

Anahtar Kelimeler: *Sığır, Doğum öncesi ve doğum sonrası dönem, Karaciğer.*

INTRODUCTION

The periparturient period for a dairy cow begins two to three weeks prepartum and continues until two to three weeks postpartum (1-3). The measurement of liver functions in this period is a much important advantage for detecting prognose and choosen theraphy appropiately. BSP clearence, one of the most important parameter evaluating liver flow function, is a relatively nontoxic organic anion used as an in vivo indicator of liver performance (4). Following parturition and initiation of lactation, there is a significant increase in the splanchnic tissue mass concomitant with an increase in liver blood flow (LBF) (5). This is probably due to both hypertrophy and hyperplasia of the liver and the organs of the gastrointestinal tract in response to the increase in nutrient intake (6). Further, the concentrations of a number of blood constituents are significantly altered during periparturient period in cattle (7). Serum metabolites, suche as TB, TP, GGT, AST and SDH, seems to be most useful in identifying animals with hepatic disease (4). The dehidrogenases (GLDH) have the shortest half-lives in serum and may not increase in cattle with chronic hepatic disease (4, 8). AST comes from tissue other than liver and is not specific for hepatic function. Valuable information concerning dietary protein content and utilization can be detected from herd urea levels (9, 10). Blood glucose is an insensitive measure of energy status because it is subject to tight homoeostatic regulation (5). Most of the net accumulation of TG in the liver occurs following calving in response to endocrin changes, depressed feed intake during the week prior to calving [11]. One of the most important diagnostic method for liver damages was liver biopsy.

Although cows may develope fatty liver during calving period mild or moderate fatty liver do not have clinical signs and the condition has been associated with other health and production problems. Therefore, the purpose of the study was to establish any changes that may ocur in LBF, and the other liver function in periparturient dairy cows in order to aid identification of the cows which are likely to develop liver damage.

MATERIAL and METHOD

Animals and study procedures: Twelve pregnant, Holstein (aged between 3 and 7 years; mean 4.4 year) cows were monitored 1 wk before predicated calving and 3 wks (one wk intervals) for BSP clearence, blood chemistry and liver histopathologic changes, while six non-pregnant non-lactating cows were kept as control group. Plasma BSP clearence, serum chemistry and liver biopsies were obtained from the animals at 1 week prior to calving, on calving day (day 0), and at 1st, 2nd and 3rd weeks after calving. The cows were and had complated one and six lactations. The mean milk yields of the cows in the previous citations had been 6200 kg. The cows calved during March and April and were fed a diet of corn silage, hay and concentrates containing 13% crude protein.

Measurement of BSP clearence: Analyses of plasma samples for BSP clearence was as described by Cornelius (1980) (4). Blood samples were taken before one gram of dye was injected intravenously. Following 5th and 30 rd minutes, two heparinized blood samples were taken from cows. The BSP concentrations of sample was determined spectrophotmetrically as follows: 2 ml plasma was tranferred to 12x105 mm cuvettes containing 3 ml 0.1 N NaOH. The blank consisted of 2 ml plasma, 3 ml 0.1 N HCl, and 1 ml distilated water. Any spectrophotometer with a wavellength of 565 nm can be used for the BSP deteminations. Standard curves for BSP determinations should be made using 2 m bovine plasma. The BSP concentrations of the samples (miligrams per 100 ml) are next plotted on semilog paper, and the $T_{1/2}$ for BSP clearence calculated. The $T_{1/2}$ is that time required for the BSP concentration to be halved in the plasma. The BSP clearence can be expressed clinically in $T_{1/2}$ unit (minutes).

Fractional clearence K, or the percentage of dye cleared from the plasma per minute, can easily be calculated from the following formula:

$$K = \frac{\text{Ln } 2}{T1/2} \quad \text{where } \ln 2 = 0.693$$

Liver Biopsy: Liver tissue samples were obtained by biopsy method described by Craig et al. (8). The method was performed as follows: A sample of liver was taken from the animal by percutaneous neddle biopsy under local anaesthesia. The site was in the 11 th intercostal space on the rightchest wall about 20 cm below the rip of the transverse process. Up to 500 mg of liver may be obtained in this way. The sample was placed in a cup with a 10% formalin solution and sent to the laboratory immediately for subsequent determination of structural changes in liver tisue. Tissue sample cut and stained with oil red O stain to demonstrate neutral fat under light microscopy.

Blood Variables: Blood samples were taken from the jugular vein before the liver biopses were obtained. The serum and plasma samples were harvested within an hour by centrifugation 4°C for 15 minutes at 3,000 rpm and stored in plastic tubes at −20°C. Serum biochemical analyses included TP, urea, TB, TG, GLU concentration, and AST, CK, GGT and SDH activities were measured by standart methods using commerical kits supplied from RoscheDiagnostics (D-68298, Mannheim, Germany) in Roche/Hitachi 917 Clinical Chemistry Analyzer.

Statistical analyses: The BSP clearence and clinical chemistry data were analyzed by paired t tests and compared with those obtained in non-pregnant non-lactating cows by one way analysis of variance. Continuous variables were analyzed as a repeated-measures randomized complete block design, using a general linear mixed models procedures. For all pairwise comparisons, a value of $p<0.05$ was considered significant. Subsequently, all analyses were carried out using the SPSS (1999) software package.

RESULTS

First of all, the actual calving dates were very close to the predicated times. The BSP retention half time both at 5 and 30 min was significantly higher and fractional clearence (K) significantly lower in cows on day 0 and at 1st wk after calving when compared to the other study groups ($p<0.05$) and non-pregnant cows ($p<0.05$) (Table 1). The each parameters mentioned above tended to fall back to nearly normal levels at 3 wk after calving. Sampling liver from the cows at time-points before and after calving, higher fat levels were found in the liver tissue at 1st wk prior to calving, higher fat consistency was determined on day calving and 1st wk after calving, and fallen slowly back to the normal level as well as that of non-pregnant, non-lactating cows. TB concentrations in periparturient cows on calving day, at 1 wk were higher than in non-pregnant, non-lactating healthy cows ($p<0.05$), although there was quite wide individual variation. Furthermore, there were significant higher levels in serum AST and SDH activites at 1st wk before calving, on day calving and 1st wk after calving. The GGT values were slightly increased before parturition, although a significance increase was observed on day 0, at 1st wk after parturition ($p<0.05$). While the serum GLU values in cows after calving were significantly lower when compared with non-pregnant, non-lactating cows ($p<0.05$), and peaked at 1st wk, the serum urea concentration was significantly reduced 2 wks after calving when compared with non-pregnant, non-lactating cows ($p<0.05$).

Table 1. Comparison of results of BSP excretion in periparturient cow wih those from non-pregnant non-lactating cows.

Items	Control	1 wk before calving	Calving	1 wk after calving	2 wk after calving	3 wk after calving
Half time (min)$T_{1/2}$	3.8±0.16	4.7±0.23^{a}	4.6±0.17^{a}	4.8±0.22^{a}	4.0±0.30^{ab}	3.6±0.21b
Fractional clearence, K (min)	0.264±0.016^{a}	0.168±0.012^{c}	0.136±0.011^{d}	0.102±0.028^{e}	0.144±0.008^{d}	0.196±0.014^{b}
5 min retention, %	6.4±0.5^{e}	19.4±1.8^{bc}	21.3±1.6^{b}	25.3±2.4^{a}	17.2±1.7^{c}	8.4±2.1^{d}
30 min retention, %	1.6±0.3^{d}	4.1±0.7^{c}	5.4±0.6^{b}	5.9±0.3^{a}	4.6±0.6^{c}	1.7±0.3^{d}

$^{a-f}$ In each row, values with different letter superscripts are significantly ($p<0.05$) different.

Table 2. Comparison of blood chemistry results in periparturient and non-pregnant non-lactating cows.

Items	Control	1 wk before calving	calving	1 wk after calving	2 wk after calving	3 wk after calving
AST, U/L	24±1.5^{d}	27±1.2^{c}	34±3.2^{b}	42±2.4^{a}	32±2.6^{b}	23±5.1
GLDH, U/L	3.6 ±0.42^{d}	5.6±0.37^{b}	5.9±0.63^{b}	6.8 ±0.32^{a}	4.9±0.45^{c}	3.8±0.41^{d}
GGT, U/L	16.9±1.0^{d}	17.2±0.68^{c}	18.4±1.74^{b}	19.1±0.69^{a}	16.5±0.48^{d}	14.3±1.37^{e}
CK, U/L	16.1±1.4^{e}	25.0±4.2^{d}	48.3±8.41^{b}	54±6.3^{a}	36±5.4^{c}	18.8±7.6^{e}
Glucose, mg/dL	65.1±2.24^{a}	47.0±1.32^{b}	34.0±1.21^{c}	25.4±1.16^{d}	37.2±1.34^{c}	62.5±2.14^{a}
Urea, mg/dL	16.2±1.22^{e}	23.4±0.58^{b}	31.3±0.78^{a}	20.8±0.46^{c}	18.1±0.69^{de}	15.9±0.71^{e}
TP, g/dL	6.8±1.48^{c}	5.9±1.36^{d}	6.2±1.27^{c}	6.8±1.45^{b}	7.1±2.26^{ab}	7.3±1.64^{a}
TG, mg/dL	16.3±1.12^{d}	18.4±1.38^{d}	22.0±1.56^{c}	29.3±1.65^{a}	25.1±1.34^{b}	17.0±2.11^{d}
TB, μmlo/l	4.0±0.32^{f}	6.7±0.74^{d}	8.0±0.23^{b}	8.9±0.89^{a}	7.4±0.72^{c}	5.1±0.44^{e}

DISCUSSION

Measurement of BSP clearence T ½ is useful in evaluating liver excretory function in adult cattle (6). In the present study, clearence of BSP was prolonged from 1 st wk before calving to 1st wk post partum cows at 1st wk compared to that in animals at other time-point. The increase in half-time and retention of BSP and decrease in fractional clearence before calving suggested that LBF changes were occuring in the liver during peripartum period. Reid and collins (7) reported that the ultrastructural changes in postpaturient cows particularly the decrease in the volume of rough endoplasmic reticulum and mitochondrial damage, have functional consequences and could account for alterations in the pattern of BSP excretion and in blood chemistry in periparturient cows. In the study, the marked infiltration during periparturient period was similar to results of this study and a previous study performed by Collins et al (2). Furthermore, the serum and liver TG accumulation levels were higher in the study. According to Sevinc et al (13), the accumulation of fat in liver cells and development of fatty liver are caused by reduced synthesis of VLDL (very low density lipoprotein).

The results of this study were in agreement with previous studies indicating that activities of liver enzymes are sensitive indicators of liver damage in cattle (14, 15). Changes in blood concentrations of GLU, TB, TP and AST enzyme activity may all reflect alterations in liver function associated with fat accumulation (15). The AST activity was high 8 of 12 (66%) in cows around parturition in this study, while the highest level was observed on day calving and at 1st wk after parturition. The enzyme is not, however, exclusively a liver enzyme, can come from muscle, as well as from liver, so it is not liver-specific (4). It is possible that the rise in AST activity in plasma around parturition was attributable to liver and muscle damage as evidenced by increase in CK aktivities at this time as well as observed in our study. The SDH and GGT values were high in only 4 (30%) of 12 cattle in periparturient period. SDH is a liver specific enzyme in the cattle, but it is not an extremely sensitive test because of serum half-life time is very short. GGT is a fairly sensitive and specific indicator of cholestasis or liver damage (7).

Our results showed that, as parturition approached, the serum glucose concentration decreased exponently, probably reflecting the increasing demands of the fetus and the mammary gland for glucose. Back out of the parturition (at wk 1 prior to calving and on day calving), plasma glucose concentration increased sharply. Herdt (9) claims that this condition is associated with possibly reflecting hormonal changes at calving that promote gluconeogenesis and glycogenolysis. The result of the study is similar to the previous studies (16-18) which declared during the 1 st wk of lactation, plasma glucose concentration decreased 25%, but by the 2nd wk in action, started to increase exponentially. The same authors claim that the increase may reflect the recovery of feed intake and improving energy status of the cow and higher than those observed in previous studies.

The low urea concentration in cows after calving in this study could be a reflection of the reduced anabolism of proteins due to fatty infiltration (7). Slightly higher serum bilirubin concentration in the study was determined especially within 2 wk after parturition. Although serum bilirubin level is neither a specific nor a sensitive test for hepatic disease (14), high concentration of bilirubin may compete with BSP for excretion, but there was not an appereant correlation between results of BSP clearence test and bilirubin concentrations in the present study

As regards to all of the data, subclinical liver damage occurs well at 1 wk before calving and reached peak at 1 wk after parturition, then slowly fallens back to normal. None of the results of the common tests for liver damage or function were clinically usefull when used alone for detection hepatic disease (2, 14), the BSP clearence test associated with the other variables studied in the trial give us important data about liver damage in cattle within periparturient period. Despite therapy is not completely effective in the period, early diagnosing may make prevent possible.

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