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Studies on the Relationship Between Sub-Clinical Ketosis and Liver Injuries Within the First Two Months of Lactation Period in High Producing Iranian Holstein Cows

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Abstract: The relationship between Sub-Clinical Ketosis (SCK) and liver injuries within the first two months of lactation in three commercial dairy herds with rather constant routines in management and nutrition was studied. A total of 77 cows (38 cows in the first and 39 cows in the second months of lactation) were sampled for blood. The serum concentrations of glucose, beta-hydroxybutyrate (BHB), nonesterified fatty acid (NEFA), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), cholesterol, triglyceride and VLDL-cholesterol were measured at 30 and 60 days after calving. Sub-clinical ketosis was considered in cows with serum concentration of BHB>1000 μmol L^{-1}. The concentration of serum glucose in cows with SCK was significantly ($p<0.05$) lower than healthy cows after 30 days of calving. However, the concentrations of serum BHB, NEFA, triglyceride and VLDL-Cholesterol in SCK cows were significantly higher ($p<0.05$) than the healthy cows. In second month of lactation, the concentrations of serum BHB and NEFA in SCK cows were significantly higher than the healthy cows. The concentration of serum BHB, NEFA, triglyceride and VLDL-cholesterol in SCK cows were significantly higher ($p<0.05$) than the healthy cows at 30 and 60 days postpartum periods. In the first and second months of lactation, a positive significant correlation was observed between serum glucose and GGT ($R = 0.409$, $p<0.05$) in the healthy cows. However, significant correlations were observed between serum glucose and cholesterol ($R = 0.403$, $p<0.05$) and GGT and cholesterol ($R = 0.388$, $p<0.05$) in cows with SCK. Hepatic injuries were not observed in cows with SCK. In spite of negative energy balance in the first and second months of lactation, liver function tests were normal. The results of this study showed that the concentration of serum BHB and NEFA of SCK cows within the first two months of lactation was significantly higher than healthy cows, possibly due to higher energy demands of cows at this stage.

Key words: Sub-clinical ketosis, high producing Holstein cows, first two months of lactation

INTRODUCTION

Health and performance management systems for dairy cattle need to focus on early identification and subsequent prevention of production diseases such as clinical and sub-clinical ketosis (Ingvartsen *et al*., 2003) by treating individual cows or to improve the herd diet (Enjalbert *et al*., 2001). The current convention is to maximize DMI and energy intake prepartum and minimize the drop in DMI as parturition approaches (Mashek and Grummer, 2003). Dramatic increases in energy

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requirements during late gestation and early lactation superimposed an animal with a profound drop in DMI before calving and make the dairy cow highly susceptible to the metabolic diseases e.g., ketosis and hepatic lipidosis (Osborne, 2003). The animal attempts to supply the needs for milk production by drawing on body fat reserves. This release of free fatty acids results in the production of the major ketone bodies, acetone, acetoacetate and beta-hydroxybutyrate (BHB) (Dann *et al*., 2005; Padilla *et al*., 2005). These compounds are important source of energy when carbohydrate levels are reduced (Duffield, 2000). Sub-Clinical Ketosis (SCK) is defined as elevated concentrations of circulating ketone bodies in the absence of clinical signs of ketosis (Duffield, 2000). Cows are at risk of sub-clinical ketosis within the first two months postpartum (Duffield, 2000). SCK can affect milk production (Rajala-Schultz *et al*., 1999; McLauren *et al*., 2006), reproduction (Walsh *et al*., 2007a, b), increased frequency of left displaced abomasum (Grohn, 2000; LeBlanc *et al*., 2005) and decreased in nonspecific immunity (Sartorell *et al*., 2000). Reported overall incidence rates of SCK range from 6.9 to 14.1% in the first 2 months of lactation (Duffield *et al*., 1997) although prevalence as high as 34% has been reported (Duffield, 2000).

Test results could be used on a herd basis to determine the level of SCK and indicate the necessity for further investigations and management improvements. Blood glucose and BHB concentrations have been used as biological indicators reflecting the status of the dairy cow.

The gold standard diagnostic test for SCK is the measurement of BHB in serum or plasma because of its stability (Duffield, 2000; Herdt, 2000; Oetzel, 2004). There have been many thresholds that have been used to distinguish between healthy cows and cows with SCK (Duffield *et al*., 1998; Geishauser *et al*., 1998). However, BHB levels between 1000 μmol L^{-1} and 1400 μmol L^{-1} have been reported as thresholds that can be used for SCK (Duffield, 2000). A number of cowside tests have been evaluated for the detection of ketone levels in serum, milk or urine (Geishauser *et al*., 1997, 2000). Most of these tests lack sensitivity as compared to serum BHB, which remains the gold standard for studying ketosis.

Limited information is available regarding the prevalence of sub-clinical ketosis in dairy herds in Fars province, Iran. The objectives of this study were to determine the relationship between sub-clinical ketosis and liver injuries within the first two months of lactation in high producing Iranian Holstein cows. Results from this study would provide fundamental knowledge for improving dairy production in Fars province, Iran. Serum glucose, BHB and NEFA concentrations and absence of any signs of clinical disease were considered as sub-clinical ketosis and serum AST, GGT, triglyceride, VLDL-cholesterol and cholesterol concentrations were considered as liver function parameters.

MATERIALS AND METHODS

A total of 77 Holstein cows within the first two months of lactation (38 cows in the first month and 39 cows in the second month of lactation) with high-producing records were randomly selected from three commercial dairy herds that had a total of 530 cows in Fars province, Iran in year 2006. The animals were kept in free-stall housing.

All diets were based on alfalfa hay, corn silage and a combination of concentrates including barley, corn, beet pulp, soyameal, wheat bran, cotton seed meal, urea, fat powder and mineral and vitamin supplements.

Health and fertility records were maintained on all herds by the dairyman and their veterinarians. Signs of clinical diseases, including clinical ketosis, such as hard dry feces, diminished appetite, decreased milk production and loss of body weight were noted.

For the analysis of serum biochemical parameters, blood samples were collected from the coccygeal vein into plain vacutainers and the serum was separated after centrifugation for 15 min at 750 x g at room temperature. Any hemolyzed samples were discarded. Serum samples were stored at

-20°C until analyzed. Biochemical analysis including serum glucose was carried out using the glucose oxidase method, BHB by the Williamson-Melanbaye method (RANBUT Kit, RANDOX Com. UK), NEFA by the Matsubara method (NEFA Kit, RANDOX Com. UK), AST by the modified method of Reitman-Frankel, GGT by the modified method of Szasz, cholesterol by the modified method of Abell-Kendall/Levey-Brodie (A-K) and triglyceride by the McGowan method (Burtis and Ashwood, 1999). Serum VLDL-cholesterol was measured according to Friedewald *et al*. (1972). A cutoff point of 1000 μmol L^{-1} serum BHB (Radostitis and Blood, 2000) was used to distinguish healthy cows from cows with SCK.

The data in the first two months of lactation were analysed with independent t-test. The correlations between different parameters were determined with Spearman correlation test. All statistics were performed using SPSS software for windows, version 6.0 (Norusis, 1993).

RESULTS

In the first month of postpartum period, serum concentration of glucose in cows with SCK was significantly lower ($p<0.05$) than healthy cows but the concentrations of BHB, NEFA, triglyceride and VLDL were higher ($p<0.05$) in cows with SCK compared with healthy cows. In the second month of lactation the serum concentration of BHB and NEFA were higher ($p<0.05$) in cows with SCK compared with healthy cows (Table 1).

Within the first two months of lactation serum concentrations of BHB, NEFA, triglyceride and VLDL were higher ($p<0.05$) in cows with SCK.

In the first month of lactation, the serum GGT concentration in healthy cows was positively correlated with cholesterol ($R = 0.533$, $p<0.05$), triglyceride and VLDL ($R = 0.444$, $p<0.05$) and in SCK cows, there was a negative correlation ($R = -0.576$, $p<0.05$) between glucose and BHB but a positive correlation between cholesterol and GGT ($R = 0.761$, $p<0.05$) was noted. In the second month of lactation in healthy cows only a positive correlation ($R = 0.547$, $p<0.05$) was observed between glucose and GGT. Overall, in the first two month of lactation in healthy cows there was a positive correlation ($R = 0.409$, $p<0.05$) between glucose and GGT and in SCK cows positive correlations were observed between glucose and cholesterol ($r = 0.403$, $p<0.05$) and GGT and cholesterol ($R = 0.388$, $p<0.05$).

Table 1: Serum parameter concentration in the first, second and the first two months of lactation periods (Mean±SE)

Lactation period	Group	No. cows	BHB[1] (μmol L^{-1})	Glucose (mmol L^{-1})	AST[2] (U L^{-1})	GGT[3] (U L^{-1})
First month of lactation	Healthy	25	631.28±38.33	2.77±0.078	112.91±14.660	6.34±0.838
	SCK	13	1594.29±226.24*	2.35±0.130*	80.29±16.516	6.72±1.156
Second month of lactation	Healthy	21	713.77±18.72	2.81±0.120	106.90±23.370	8.51±1.288
	SCK	18	1385.44±89.99*	2.97±0.136	67.73±5.817	10.36±1.420
First two months of lactation	Healthy	46	668.94±28.23	2.79±0.069	110.17±13.16	7.33±0.730
	SCK	31	1473.02±107.72*	2.71±0.109	73.00±7.62	8.93±1.000
Lactation period	Group	No. cows	TG[4] (mmol L^{-1})	VLDL[5] (mmol L^{-1})	Cholesterol (mmol L^{-1})	NEFA[6] (mmol L^{-1})
First month of lactation	Healthy	25	0.14±0.008	0.028±0.001	2.78±0.226	0.35±0.036
	SCK	13	0.21±0.036*	0.042±0.007*	2.88±0.274	0.81±0.066*
Second month of lactation	Healthy	21	0.15±0.013	0.030±0.002	4.20±0.298	0.24±0.030
	SCK	18	0.15±0.010	0.030±0.002	4.68±0.205	0.84±0.044*
First two months of lactation	Healthy	46	0.14±0.007	0.028±0.001	3.43±0.209	0.29±0.023
	SCK	31	0.18±0.017*	0.036±0.003*	3.93±0.229	0.82±0.037*

1: Beta-hydroxybutyrate, 2: Aspartate aminotransferase, 3: Gamma-glutamyl transferase, 4: Triglyceride, 5: Very low density lipoprotein and 6: Nonesterified fatty acid, *: Significant at $p<0.05$, SE: Standard Error

DISCUSSION

The results of this study showed that the concentration of serum BHB in SCK cows within the first two months of lactation was significantly higher than that of healthy cows, possibly due to higher energy demands of cows at this stage which is in agreement with the results of Dann *et al*. (2005), LeBlanc *et al*. (2005) and Padilla *et al*. (2005). Ketosis is a disease related to the high rate of glucose utilization in the mammary gland and the inability of the cows to meet this glucose demand by normal physiology. On the other hand, SCK is defined as elevated concentrations of circulating ketone bodies (due to mobilization of NEFA) in the absence of clinical signs of ketosis (Dann *et al*., 2005). These compounds are important source of energy when carbohydrate levels are reduced (Duffield, 2000). SCK is important because it may remain undetected and yet have effects on productivity which parallel those elicited by clinical ketosis. Prevalence of SCK increases from primiparous to multiparous cows (Detilleux *et al*., 1994) and also other factors such as age (Andersson, 1988), season (Whitakar *et al*., 1993) and breed (Andersson, 1988) can affect its prevalence. SCK may start at serum BHB concentrations above 1000 μmol L^{-1} and clinical ketosis at about 2600 μmol L^{-1}. However, at exactly what level individual cow will show clinical sign is extremely variable (Andersson, 1984). Also it has been reported that a range of blood BHB concentrations from 1000 to 1400 μmol L^{-1} can be used for detecting SCK (Whitakar *et al*., 1993). At all sampling times, the serum BHB concentrations in SCK cow were significantly ($p<0.05$) higher than 1000 μmol L^{-1} (but less than 2600 μmol L^{-1}) which is considered as SCK condition (LeBlanc *et al*., 2005; Walsh *et al*., 2007b). BHB is synthesized from absorbed butyrate in the rumen epithelium and by the ketogenesis of hepatocytes in the conversion of long chain fatty acids during fat mobilization. In SCK cows, BHB is the predominant circulating ketone body and is relatively stable in whole body, plasma or serum (Dohoo and Martin, 1984). Blood glucose and ketone bodies can be used as a measure of energy status of the animal. There is relatively weaker degree of homeostatic regulation of BHB than glucose which means that BHB concentrations are less constrained physiologically and more likely is a reflection of nutritional status than blood glucose (Herdt, 2000).

The significant negative correlation between BHB and glucose concentration (Padilla *et al*., 2005) in the first month of lactation is in the line of the fact that hypoglycemia is the driving force in bovine sub-clinical and clinical ketosis, which ends to ketonemia (Bruss, 1997). It has been shown that in SCK, cows can become ketonemic without the presence of significant hypoglycemia (Grohn *et al*., 1983), as was seen in the second and first two months of lactation in the present study.

The significantly ($p<0.05$) higher triglyceride concentration in SCK cows in present study is in agreement with the findings of Holtenius and Hjort (1990) and Drackley *et al*. (1992). The higher serum triglyceride concentration in SCK cows in this study is not in agreement with other studies (Reichel and Sokoi, 1987) with fatty liver syndrome cows and liver injuries. This shows the normal function of liver in this study in SCK cows. The higher liver lipoprotein synthesis will decrease the incidence of fatty liver syndrome and liver injuries (Grummer, 1995). The lower serum glucose and higher serum BHB concentrations in cows with SCK in the first month of lactation in the present study is in agreement with the findings of Dann *et al*. (2005), Radostits and Blood (2000) and Padilla *et al*. (2005). Regarding the circulating glucose, there is conflicting data in the literature. Bremmer *et al*. (2000) reported that glucose concentration is decreased in response to energy restriction in the diet, while Canfield and Butler (1991) concluded that there is little influence of the energetic status of the animal on the blood glucose concentration. Overall blood glucose is an insensitive measure because it is subjected to tight homeostatic regulation. The same trend for BHB was observed for cows with SCK in the first, second and within two months of lactation period.

Circulating levels of non-esterified fatty acids (NEFA) and BHB are valid measurements of energy metabolism (Dann *et al*., 2005). Serum NEFA greater than 0.4 mmol L^{-1} has been proposed to identify

the negative energy balance and SCK (Stokol and Nydam, 2005). In the present study, the concentration of serum NEFA in SCK cows at all stages of sampling were higher than 0.4 mmol L^{-1}. The SCK cows mobilized adipose lipid reserves to support the negative energy balance and had elevated concentrations of BHB and NEFA in serum and had lower concentrations of glucose (Dann *et al*., 2005; Padilla *et al*., 2005). Since the serum concentration of VLDL is correlated with serum triglyceride (Friedewald *et al*., 1972) both can be used as indicators of energy status of the cow. A decrease in serum triglyceride has been reported in liver injuries and fatty liver syndrome conditions (Reichel and Sokoi, 1987), which is due to low capacity of liver lipoprotein synthesis (Grummer, 1995). In the present study, the serum triglyceride and VLDL concentrations were higher ($p<0.05$) in SCK cows compared with healthy cows which is another sign of the absence of liver injuries in the former cows. Concentrations of triacylglycerol usually increase in parallel with those of total lipid (Grum *et al*., 1996). There was no significant differences between healthy and SCK cows for serum AST, GGT and cholesterol concentrations at all sampling times. Liver injuries is associated with higher serum hepatic enzymes e.g., AST and GGT (Smith, 1996).The serum concentration of GGT increases in liver and bile duct malfunctions (Steen *et al*., 1997) and liver is the main source of serum GGT (Kaneko, 1989), while serum concentration of AST increases due to fat accumulation in the liver which results in high hepatocytes membrane permeability and is a good tool for detection of early metabolic liver diseases (Karsai and Schafer, 1984). In the present study, fatty infiltration did not cause liver damage as indicated by liver-specific enzymes (AST and GGT) measured in serum at all sampling times which is in the line of the findings of Dann *et al*. (2005). Steen *et al*. (1997) reported that AST activity was greater in cows with ketosis and hepatic lipidosis than in cows that were healthy.

In conclusion, the results of this study show that the prevalence of SCK in Fars province is considerable and measuring serum BHB as a routine monitoring program could be beneficial for dairy herds. In order to prevent the economic loss due to SCK, early treatment of SCK cows is important and prevention of the disease has to be achieved through good nutritional programs in the dry and early lactation periods.

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