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Original article

# CHANGES IN CLINICAL AND BLOOD LIPID METABOLISM PARAMETERS IN HOLSTEIN DAIRY CATTLE DURING THE TRANSITION PERIOD

## A. KHALPHALLAH<sup>1</sup>, A. A. AAMER<sup>1</sup>, T. ABDELALL<sup>1</sup>, E. ELMELIGY<sup>2</sup>, S. OIKAWA<sup>3</sup> & K. NAKADA<sup>3</sup>

<sup>1</sup>Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Egypt; <sup>2</sup>Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University, Egypt; <sup>3</sup>Department of Veterinary Herd Health, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan

#### Summary

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The transition period is a critical period in the production cycle of the dairy cow. This study aimed to describe the clinical and biochemical changes, particularly lecithin: cholesterol acyltransferase (LCAT) and apolipoprotein B-100 (ApoB-100), during the transition period in dairy cattle. It was conducted on Holstein dairy cattle (n=80) classified into control cows (n=61) and diseased cows (n=19). They belonged to dairy farms in Ebetsu city, Hokkaido area, Japan. Body condition score (BCS) was significantly reduced in postfresh control cows when they were compared to those of close-up control group. Serum LCAT and apoB-100 were remarkably increased (P<0.05) at close-up period control group when they compared with those in close-up period diseased one. LCAT activities were also remarkably increased (P<0.05) while apoB-100 showed no significant changes at postfresh control cattle when they were compared to their values at post-fresh diseased cattle. Although apoB-100 was within the normal physiological reference values in the control and diseased cattle, it was lower than physiological reference values in 9.83% of the control cattle and in 26.32% of the diseased group. Blood glucose and packed cell volume were still within the physiological reference range in all cows. The study concluded that LCAT activities were a highly sensitive diagnostic marker of the transition period and of higher predictive value and diagnostic significance than the other lipid profile indicators of transition period disorders.

Key words: dairy cattle, fat metabolism, lecithin:cholesterol acyltransferase, transition period

#### INTRODUCTION

The management of the transition period of dairy cows from 3 weeks before parturition to 3 weeks postpartum is aimed at optimisation of animal health and productivity (Cook & Nordlund, 2004). The metabolic diseases are of greatest importance in dairy cows and pregnant ewes. In the other species, these diseases occur only sporadically. The high-producing dairy cow always verges on abnormal homeostasis as well as their breeding and feeding for high milk yields is etiologically associated with metabolic disease so common in these animals (Corohn, 1998; Radostits *et al.*, 2007).

Lipid metabolism is the key of transition cows biology (Drackley, 1999). Excessive lipolysis from adipose tissue is associated with greater incidences of periparturient diseases. Fatty livers have been reported in ketotic cows in the 1950s. Fatty liver infiltration was then noted in normal cows during early lactation. Later on, a fat mobilization syndrome in early lactation was described in cows, in which cows mobilized body lipids from adipose tissue and deposited lipids in the liver, muscle, and other tissues (Cameron *et al.*, 1998; LeBlanc *et al.*, 2005).

Elevated non esterified fatty acids (NEFAs) concentrations during the last 7 days before calving have been associated with a greater incidence of the three energy related diseases; ketosis, displaced abomasum (DA) and retained placenta but not milk fever (Cameron *et al.*, 1998; Le-Blanc *et al.*, 2005).

Many studies focused on the clinical and biochemical changes during transition period in dairy cows describing changes in blood NEFA (LeBlanc *et al.*, 2005), glucose (Yameogo *et al.*, 2008; Mokhber Dezfouli *et al.*, 2013), total protein (Yameogo *et al.*, 2008),  $\beta$ -hydroxybutyric acid (BHBA) (Sakha *et al.*, 2006) either in healthy cows or in cows with periparturient diseases.

Recently, the lipid profile has been reported an important indicator in heifers and cows. According to our knowledge, the serum biochemical changes of lecithin:cholesterol acyltransferase (LCAT) and apolipoprotein B-100 (apoB-100) in transition cows were not widely assessed periparturient diseases in cattle in disturbing the productivity of the herds. Diseases of periparturient period such as ketosis, DA and retained placenta mainly are associated with fatty liver. They also cause loss of milk production and poor reproductive performance as well as reduced immune competence that increase susceptibility to diseases and prolonged recovery (Whitaker et al., 1993; Goff & Horst, 1997; Drackley, 1999; Kehrli et al., 1999). Therefore, this study focused on evaluating the clinical and serum biochemical changes particularly those related to lipid metabolism such as LCAT and apoB-100 during the transition period in healthy and diseased Holstein dairy cattle and proving their diagnostic significance as predictors of periparturient diseases.

#### MATERIALS AND METHODS

#### Animals

This study was conducted on Holstein dairy cattle (n=80) belonging to dairy farms of Rakuno Gakuen University and Matshimora dairy farms in Ebetsu city, Hokkaido area, Japan. These cows were examined during the transition period at two different times; one is before calving during the close-up period: from -4 to -14DIM (days in milk) and the other after calving during the post-fresh period: between 1 and 50 DIM. The examined cows were classified into 2 main groups: healthy control cows (n=61) and diseased cows (n=19). The diseased cows were classified as diseased after calving (1 to 50 DIM). All cattle were treated under the Laboratory Animal Control Guidelines of Rakuno Gakuen University, which basiChanges in clinical and blood lipid metabolism parameters in Holstein dairy cattle during ...

cally conform to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health in the USA (NIH publication No. 86-23, rev. 1996).

#### Samples

All blood samples were obtained from the tail vein. Whole blood samples were collected with EDTA and stored at 4 °C until analysis. Samples collected in plain vacutainer tubes were then centrifuged at 3000 rpm for 15 min. Sera were separated and stored at -20 °C till analysis.

#### Methods

Clinical examination and estimation of serum biochemicals were conducted twice; at close-up (-4 to -14 DIM) and post-fresh (1 to 50 DIM). Clinical examination of all dairy cattle was done using a clinical chart (Rosenberger, 1990). Body condition score (BCS) of all cows was estimated based on a 5-point scale (Ferguson *et al.*, 1994).

Total plasma proteins were estimated by refractometry. PCV was measured by using the microhaematocrit method (Coles, 1986).

LCAT activity was determined spectrophotometrically with commercial kits (Sekisui medical, Tokyo, Japan) as per Uchida et al. (1995). Serum apoB-100 concentrations were estimated using single radial immunodiffusion assay and commercial kits (Bovis ApoB plate, the institute for metabolic Ecosystem, Osaka, Japan) according to Oikawa & Katoh (2002). Serum NEFAs (NEFA C, Wako Chemicals GmbH, Neuss, Germany) and blood glucose (Glucose, HK, Konelab, Thermo Electron Corporation) were determined on a Konelab 30 chemistry analyser (Thermo Electron Corporation). Serum NEFA and glucose were measured at Kishimoto Clinical Laboratory, Sapporo, Japan.

#### Statistical analysis

All statistical analyses were performed using computer software (SPSS version 17.0, Chicago, USA). The data obtained were analysed by independent-sample or paired *t*-test. The significance of differences between the means at control group and diseased group either in close-up period or post-fresh period was evaluated by Dunnett's test. Values were expressed as means±SD.

### RESULTS

The most common diseases of transition periods that were reported at the first 50 DIM in dairy cattle included retained placenta, ketosis, DA, milk fever, mastitis and dystocia. Retained placenta, ketosis and DA i.e. energy related diseases were sometimes associated with one or more diseases states such as milk fever, mastitis and dystocia.

During this study, clinical examinations revealed that the temperature, pulse and respiration showed no remarkable changes during the current study either in control or diseased cattle. The rumen movements were reduced in most of the diseased cases. Signs of dehydration were not observed in any of the examined cows.

BCS was significantly (P<0.05) reduced in post-fresh control cows when their values were compared with those of closeup control period. These remarkable changes were not reported between the diseased cattle. BCS was not remarkably changed in close-up or post-fresh periods when its values in control cattle were compared with those of diseased one, but BCS was still within the physiological reference values in control and diseased cattle (Table 1). A. Khalphallah, A. A. Aamer, T. AbdelAll, E. Elmeligy, S. Oikawa & K. Nakada

Serum LCAT activities and concentrations of apoB-100 were not considerably changed in cattle between close up and post-fresh periods either in healthy cows or in diseased one. On the other hand, although serum apoB-100 concentrations were within the normal physiological reference values in both control and diseased cattle, they were lower than normal reference values in 9.83% of the control group and in 26.32% of the diseased group.

Serum LCAT activities (P<0.01) and apoB-100 levels (P<0.05) were very reduced in close-up diseased cattle vs close-up controls. Serum LCAT activities were also remarkably (P<0.01) reduced in post-fresh diseased cattle compared to those of post-fresh control ones, while serum apoB-100 showed no significant changes between control and diseased groups at post-fresh period (Table 1).

Blood glucose and PCV were significantly (P<0.05) decreased in post-fresh control cows than in the close-up control group. Total plasma proteins were significantly (P<0.05) increased in post-fresh control cows when their values were compared with those of close-up control group. Such changes were not reported for blood glucose, total proteins and PCV between close-up and post-fresh diseased cattle. Blood glucose, total proteins and PCV were still within the physiological reference values in both groups (control and diseased cows) during the period before and after calving. Blood glucose concentrations (Table 1) were substantially (P<0.05) reduced in close-up diseased animals compared with close-up

Table 1. Mean values of body condition score (BCS) and blood lecithin cholesterol acyltransferase
(LCAT), apolipoprotein B-100 (ApoB-100), non-esterified fatty acids (NEFA), glucose (GLU), total
protein (TP) and packed cell volume (PCV) in cattle during the transition period

	Close up cattle		Post-fresh cattle	
	Control	Diseased	Control	Diseased
BCS $(2.5-4)^1$	3.39±0.23	3.11±0.29	3.23±0.24	3.01±0.34
LCAT (U) (929–1059) <sup>2</sup>	891.25±123.79	386.19±87.71**	898.39±120.89	403.04±90.21**
ApoB-100 (g/L) (0.01-0.2) <sup>3</sup>	0.18±0.08	0.13±0.08*	0.17±0.07	0.17±0.10
NEFA (mmol/L) (0.21–0.62) <sup>4</sup>	0.22±0.17	0.17±0.11	_	_
GLU (mmol/L) (4.50–6.71) <sup>5</sup>	6.08±0.71	5.47±0.57*	5.04±1.04	4.89±0.70
TP (g/L) (57–81) <sup>6</sup>	63.98±4.22	64.94±5.92	65.95±5.11	68.00±4.00
PCV (%) (30–40) <sup>7</sup>	30.58±1.74	31.37±2.01	29.18±2.36	30.37±4.50

\*P<0.05; \*\*P<0.01 vs control animals. Reference values according to: <sup>1</sup>Furgson *et al.* (1994), <sup>2</sup>Nakagawa & Katoh (1998), <sup>3</sup>Mahley *et al.* (1984), <sup>4</sup>Oikawa & Katoh (2002), <sup>5</sup>Zadnik (2003), <sup>6</sup>Radostits *et al.* (2000), <sup>7</sup>Rosenberger (1990). control ones. Blood glucose showed no significant changes between control and diseased cows at post-fresh period. On the other hand, total proteins and PCV were not significantly changed in both close-up and post-fresh periods.

Serum NEFA (Table 1) showed no significant changes at the close-up period between healthy and diseased cows and were within the physiological reference ranges.

#### DISCUSSION

The current study mentioned that the most common diseases of transition periods that were reported at the first 50 DIM in dairy cattle included retained placenta, ketosis, The retained placenta, ketosis and DA (energy related diseases) were reported to be occasionally associated with one or more of milk fever, mastitis and dystocia. Kehrli *et al.* (1999) added that maladaptive response to negative energy balance (NEB) and other stressors during periparturient period may be associated with impaired immune function resulting in increased susceptibility to different diseases such as metritis, retained placenta, DA.

Clinical examination revealed no remarkable changes during the current study neither in control nor in diseased cattle. They were within the physiological reference values as reported by Rosenberger (1990). Some other studies mentioned different clinical findings associated with diseases of transition cows such as those related to DA cases where diseased cows with DA were febrile with increased heart and respiratory rates and reduced ruminal movements (El-Attar et al., 2007). Ketosis has been found to be associated with a gradual loss in body condition over several days or even weeks and a moderate to marked decline in milk yield over five

to six days before the onset of obvious clinical signs (Edwards & Tozer, 2004) which can persist (up to 5 liters per day) for up to two weeks after diagnosis (Rajala-Schultz *et al.*, 1999).

The results of this study showed that BCS was significantly reduced in postfresh control cows when their values were compared with those of close-up ones. This value is still considered within the physiological references in dairy cows, as mentioned by Ferguson et al. (1994). This change in BCS values was not noticed in diseased cattle. Also no changes were reported between control and diseased cow either during close-up or post-fresh periods. Previous studies reported that most dairy cows face NEB in early lactation; postpartum feed intake is lower in cows with higher BCS prepartum, leaving them in NEB for a longer period than cows with normal or low BCS (Hayirli et al., 2002). Edwards & Tozer (2004) reported that ketosis was usually associated with gradual loss of body condition over several days or even weeks.

The results showed that serum LCAT activities and concentrations of apoB-100 were not remarkably changed between close-up and post-fresh periods either in healthy cows or in diseased one. Serum LCAT activities were reported to be within the normal physiological range in all healthy cows (Nakagawa & Katoh, 1998; Katoh, 2002). Nakagawa & Katoh (1998) reported that serum LCAT activities in cows with ketosis and in those with LDA were significantly decreased compared to those in healthy cows. On the other hand, although serum apoB-100 concentrations were within the normal physiological reference values that were mentioned by Mahley et al. (1984) in some control or diseased cattle, they were lower than normal reference values in 9.83% of the control cattle and in 26.32% of the diseased group. Previous reports mentioned that the apoB-100 concentration is low during early lactation, relative to the other stages (Yamamoto et al., 1995). The decreased apoB-100 concentrations are similar among all diseased cows and also in 40 to 60% of healthy controls during early lactation and, moreover, are not largely different from those in cows with fatty liver (Gerloff et al., 1986; Herdt, 1988). The present study also revealed that serum LCAT activities and concentrations of apoB-100 were markedly reduced in diseased cattle at close-up period when their values were compared to those in diseased cattle. The previous reports indicated that LCAT activity, together with the cholesteryl ester concentration, was decreased in cows affected with ketosis and LDA (Nakagawa & Katoh, 1998) and with milk fever (Nakagawa & Katoh, 2000). The apoB-100 concentration is decreased in cows with ketosis, LDA, retained placenta (Oikawa et al., 1997) or milk fever (Oikawa & Katoh, 2002). The current work found that serum LCAT activities were also remarkably reduced in diseased cattle at post-fresh period compared to those of the control ones while serum apoB-100 showed no significant changes between control and diseased groups at post-fresh period. These results are in line with those of Nakagawa & Katoh (2000) who confirmed that the reduction in LCAT activity was detected prior to the occurrence of ketosis or milk fever. They also mentioned that as a diagnostic marker for fatty liver-related diseases, LCAT activity is more useful than apoB-100 and apolipoprotein C-III concentrations because the reduction precedes clinical signs such as ketonuria or recumbency and also because the activity is not altered during the peripartum period, at least in some healthy cows.

Blood glucose and PCV were significantly decreased in post-fresh control cows compared to close-up ones. These remarkable changes were not reported in the diseased cattle. However, blood glucose and PCV values in all examined cattle were still within the physiological reference values that were reported by Zadnik (2003) and Rosenberger (1990), respectively. The obtained results in this study also stated that blood glucose concentrations were significantly reduced in the diseased cows when their values were compared to those of the control group in the close-up period. These changes were not reported between control and the diseased cows at post-fresh period. Some other literature reports revealed that glucose concentration is reduced and NEFA concentration is increased during the prepartum period in primiparous cows that developed clinical mastitis in the postpartum period compared to healthy cows (Schwegler et al., 2013). Moyes et al. (2009) observed that a higher prepartum glucose concentration is associated with the occurrence of postpartum diseases. Blood glucose concentrations in cows with subclinical ketosis (SCK) were significantly lower than in cows without SCK (Sakha et al., 2007). PCV, haemoglobin and serum iron are consistently higher in non-lactating cows than in lactating cows. PCV varies with lactation stage, being highest in dry cows and lowest in early lactation (Radostits et al., 2007). Mokhber Dezfouli et al. (2013) reported that haemoglobin, PCV, total leukocyes, neutrophils and total protein concentrations were significantly increased in the LDA cases compared to the control group. The present study found that total proteins were significantly Changes in clinical and blood lipid metabolism parameters in Holstein dairy cattle during ...

increased in post-fresh control cows compared to those of the close-up period. Such changes were not reported in diseased cattle. The total proteins values in control and diseased cattle in the current study were still within the physiological reference values which were similar to those reported by Radostits et al. (2000). On the other hand, total proteins in the present study had not significantly changed between control and diseased cattle neither in close-up nor in post-fresh periods. In contrast, the concentrations of total proteins and globulins were significantly higher in cows with clinical ketosis compared with those in healthy cattle (Yameogo et al., 2008). Wolter (1992) mentioned that ketosis is characterised by a liver steatosis which causes hypoproteinaemia. Mokhber Dezfouli et al. (2013) reported that total protein and glucose concentrations were significantly higher in the LDA cases compared to controls.

In this study, serum NEFA showed no significant changes at the close-up period between healthy and diseased cows. Serum NEFA concentrations in all examined dairy cattle were within the physiological reference ranges which is close to the results reported by Oikawa & Katoh (2002). Elevated NEFA concentration in plasma is a prerequisite for development of hepatic lipidosis that occurs in DA cows (Rehage et al., 1996). Schwegler et al. (2013) reported a reduced serum glucose concentration and increased serum NEFA concentration during the prepartum period in primiparous cows that developed clinical mastitis in the postpartum period compared to healthy cows. Cameron et al. (1998) stated that NEB is expected in milking cows, so blood NEFA levels are high after calving and can be hardly evaluated.

The study concluded that serum LCAT activities were a highly sensitive indicator (diagnostic marker) of the transition period. Further, it was of a higher predictive value and hence diagnostic relevance than the other studied lipid metabolism profile indicators such as apoB-100, glucose and NEFA, in diseases of the transition period. The changes in serum LCAT activities were of high diagnostic importance as they enabled the cattle practitioners to anticipate metabolic diseases and to establish certain nutritional, management and medical strategies to avoid their occurrence.

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#### Correspondence:

Arafat Khalphallah, DVM, PhD Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt tel: +2021283888020, fax: +20882366503, email: arafatvet2003@yahoo.com