



Diagnostic and Prognostic Significance of Lipid Profiles in Holstein Dairy Cattle with Displaced Abomasum: Before and After Surgical Operation

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ABSTRACT

The study aimed to describe the pattern of changes in clinical findings and lipid metabolism profiles in dairy cattle with displacement of the abomasum (DA) from day 0 until day 30 after operation. The study was conducted on DA cattle (n= 25) belonged to dairy farms in Hokkaido area, Japan. Cows were examined and sampled at days 0 (operation), 7 and 30. They were clinically and biochemically examined to estimate BCS and many serum biochemical constituents such as lecithin:cholesterol acyltransferase (LCAT) and apolipoprotein B-100 (apoB-100), β -hydroxybutyric acid (BHBA), non-esterified fatty acids (NEFAs) and aspartate amino transferase (AST). Based on blood BHBA at day 0, DA cows were classified into three categories; DA only (<1.2 mmol/l), DA with subclinical ketosis (DA SCK) (1.2-2.4 mmol/l) and DA with clinical ketosis (DA CK) (\geq 2.5 mmol/l). The changes in the pattern of serum biochemical constituents throughout this study indicated recovery of diseased cows and significant effect of surgical operation. Serum biochemical constituents returned to their physiological values indicating that these cows were restoring their normal physiological status. This was reflected through a significant ($P < 0.05$) elevation of LCAT, apoB-100, and cholesterol and a significant ($P < 0.05$) reduction in AST, NEFAs and BHBA (Not in DA group), in all DA groups particularly at day 30 when their values compared with those at day 0. The current study also recorded no remarkable changes ($P > 0.05$) between the diseased groups except for NEFA and BHBA (at day 0 between DA group and the other two groups) at any of the three sampling days.

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Introduction

Displacement of the abomasum (DA) in dairy cows is a multifactorial disease. Most of cases being diagnosed within the first week after calving (Stengärde and Pehrson, 2002; Doll *et al.*, 2009).

The metabolic load on the dairy cattle varies over time during the first month post-partum. Blood profiles in DA cows may therefore show differences due to time from calving. DA has also

been associated with other diseases such as retained placenta, metritis, and ketosis (Rohrbach *et al.*, 1999), as well as with hepatic lipidosis (Bobe *et al.*, 2004).

Clinically, left displaced abomasum (LDA) can be detected if gas is present in the abomasum resulting in a tympanic, resonant and high-toned ping sound (Breukink and Kroneman, 1963).

Diseased cows with DA were febrile with tachycardia and increased respiratory rates and ruminal hypomotility (Goetze and Müller, 1990; El-Attar *et al.*, 2007).

Many research articles reported DA cows where

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they described the most common clinical findings and metabolic profiles associated with DA in dairy cattle (Stengärde *et al.*, 2010), DA and ketosis (Nakagawa and Katoh, 1998; Civelek *et al.*, 2006; Stengärde *et al.*, 2008). Some authors described the clinical finding and serum biochemical changes in DA cows such as serum lecithin cholesterol acyltransferase (LCAT) (Nakagawa and Katoh, 1998), apolipoprotein B-100 (apoB-100) (Oikawa *et al.*, 1997; Civelek *et al.*, 2006), glucose (Pravettoni *et al.*, 2004), β -hydroxybutyric acid (BHBA) (LeBlanc *et al.*, 2005) and Non-esterified fatty acids (NEFA) (Cameron *et al.*, 1998). These studies did not discuss the changes associated with DA cows until the animals get recovered after surgical interference.

The current study aimed to describe the pattern of changes in clinical findings and serum lipid metabolism profiles mainly LCAT, apoB-100, BHBA and NEFA in dairy cattle with DA throughout a long term study from day 0 before surgery until day 30 after operation through following up of the treatment and assessing of DA prognosis. This study also classified DA cases into three groups based blood BHBA and could clarify the clinical findings and serum biochemical situation in each group and their importance to confirm the recovery of DA cows.

Materials and methods

Animals

The study was conducted on DA cattle (n= 25) belonged to dairy farms in Hokkaido area, Japan. DA cattle were treated surgically and by using medicaments including I.V. fluid therapy i.e. ringer solution 1 liter and 25% glucose 500 ml, and penicillin at operation day (day 0). Cows were sampled at days 0 (operation), 7 and 30. Based on blood BHBA at day 0, DA cows were classified into three categories; DA only (<1.2 mmol/l), DA with sub-clinical ketosis (DA SCK) (1.2-2.4 mmol/l) and DA with clinical ketosis (DA CK) (\geq 2.5 mmol/l). All cattle were treated under the Laboratory Animal Control Guidelines of Rakuno Gakuen University, which basically 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, revised 1996). Clinical examination of all dairy cattle was conducted using clinical chart ac-

ording to Rosenberger (1990). Body condition score (BCS) of all cows was estimated based on a 5-point scale (Ferguson *et al.*, 1994).

Samples

All blood samples were collected from the jugular vein into plain vacutainer tubes, and then were centrifuged at 3000 rpm for 15 min. Sera were separated and stored at -20°C till analysis.

Serum biochemical analysis

LCAT activity was determined using spectrophotometer with commercial kits (Sekisui medical, Tokyo, Japan) according to the method of Manabe *et al.* (1987); Uchida *et al.* (1995) and Nakagawa and Katoh (1999).

Serum apoB-100 concentrations were estimated using single radial immunodiffusion assay and commercial kits (Bovis ApoB plate, Institute for Metabolic Ecosystem, Osaka, Japan) according to Marcos *et al.* (1989); Katoh *et al.* (1993) and Oikawa and Katoh (2002).

Spectrophotometric assay of serum total cholesterol (TC) and free cholesterol (FC). They were analyzed colorimetrically using a selective chemistry analyzer (Abbott Alcyon 3001, USA) with reagent kits (Wako Pure Chemical, Osaka, Japan) according to Nakagawa and Katoh (1998). Cholesteryl ester (CE) was estimated by subtracting the FC concentration from that of TC (Nakagawa and Katoh, 1998).

Serum BHBA was measured by using an automated chemistry analyzer (VITALAB Selectra 2, Merck, Germany) that used BHB dehydrogenase (Ranbut, Randox, UK) assays (Bruss, 1997).

Serum NEFAs (NEFA C, Wako Chemicals GmbH, Neuss, Germany), blood glucose (Glucose, HK, Konelab, Thermo Electron Corporation) and serum activities of aspartate aminotransferase (AST) (AST/GOT, IFCC, Konelab, Thermo Electron Corporation, Vantaa, Finland), were determined on a Konelab 30 chemistry analyzer (Thermo Electron Corporation).

Statistical analysis

All statistical analyses were performed using Computer Software (SPSS version 17.0, Chicago, USA). The data obtained from clinical examination

and biochemical analyses were analyzed by analysis of variance (ANOVA). The significance of differences between the means at selected sampling days (days 7 and 30) and day 0 was in each DA group evaluated by Dunnett's test. The significance of differences between the means at diseased groups (DA SCK and DA CK) at sampling days; 0, 7 and 30, and DA group in the same parallel days evaluated by Dunnett's test were expressed as means \pm SD. Correlation coefficient was calculated for NEFA and apoB-100 in all DA groups using Pearson Correlation at $P < 0.05$ (Spsswin, 1997).

Results

DA is a common and economically important problem of dairy cattle in early lactation where 44% (11 of 25) of DA cases were reported in the 1st week after calving (1-7 Days in milk (DIM)), 52% (13 of 25) of cases were occurred in the 2nd and 3rd weeks post calving (8-21 DIM) and only one cow (4%) was diagnosed at 128 DIM with Right displaced abomasum with abomasal volvulus (RDA with AV). About 84% (21 of 25) of the DA cases were LDA and 16% (4 of 25) was RDA.

The clinical findings in all DA cases including BCS (Tables 2 and 4) showed no significant changes either between the diseased groups (DA SCK or DA CK) when their values compared with those of DA group at days 0, 7 and 30, or within the same diseased group at days 7 and 30 when their values compared with those at day 0. BCS was still within the physiological reference values. Temperature, pulse and respiration indices were within the physiological reference range. Ruminant movement was reduced in the diseased cows at day 0 then they improved after the surgical correction of DA.

Lipid metabolism profiles showed several changes along the study. Some of them were significant ($P < 0.05$), which indicated the significant effect of operation, recovery of the animal and a sufficient follow up period of 30 days, while some of them were unremarkable. Serum LCAT activities and concentrations of apoB-100, TC, CE and FC (Table 1) were significantly ($P < 0.05$) increased in the three diseased groups particularly in day 30 when their values compared with those in day 0. Their serum levels started to increase gradually after operation at day 7 and reach maximum at day 30 where it reached to the standard reference values particularly for apoB-100 and TC. LCAT activities

returned to physiological reference values only in DA group; however they were less than the physiological reference values in the other two groups. Serum BHBA (Table 2) was not significantly changed ($P > 0.05$) in DA group while they were remarkably reduced ($P < 0.05$) in DA SCK and DA CK groups at days 7 and 30 after operation when their values compared with those at day 0, however they reached the physiological standard reference values only in DA group and DA SCK while they were still higher than the reference values in DA CK. Serum NEFAs concentration and Serum AST activities (Table 2) were significantly decreased ($P < 0.05$) in all DA groups at days 7 and 30 when their values in each DA group compared with those at day 0 where they reached their reference values.

The significant changes in serum activities of LCAT and AST, and serum concentrations of apoB-100 and cholesterol were not reported ($P > 0.05$) between DA group and the other two groups either in Days 0, 7 or 30 (Tables 3 and 4). On the other hand, these significant changes in lipid profiles indices (Table 4) were reported in serum BHBA and NEFA, which were significantly increased ($P < 0.05$) in DA SCK group and DA CK group at day 0 when their values compared with those of DA group, but these findings were not reported ($P > 0.05$) between these groups and DA group after operation in days 7 and 30.

Blood glucose levels (Tables 2 and 4) showed no significant changes ($P > 0.05$) throughout this study either within the same diseased group when their values at day 0 (Before operation) compared with those at days 7 or 30 (after surgery), or between the diseased groups when their values in DA group compared with those in the other two groups. All glucose values were within the physiological reference range.

The current study mentioned a negative relationship between NEFA and apoB-100 where reduction in serum NEFA after operation was associated with increased serum apoB-100. This relationship was reported in both DA group at days 0 ($r = -0.341$, $p > 0.05$), 7 ($r = -0.074$, $p > 0.05$) and 30 ($r = -0.484$, $p > 0.05$) and DA CK group at days 7 ($r = -0.452$, $p > 0.05$) and 30 ($r = -0.538$, $p > 0.05$).

Table 1. Mean values of serum profiles of lipid metabolism in each DA group

Type	No	BHBA (mmol/l) (0.35-0.47)≠			NEFA (mmol/l) (0.21-0.62)≠≠			Glucose (mmol/l) (4.50-6.71)≠≠≠			AST (U/l) (78-132)≠			BCS (2.5-4)≠≠≠		
		Day 0	Day 7	Day 30	Day 0	Day 7	Day 30	Day 0	Day 7	Day 30	Day 0	Day 7	Day 30	Day 0	Day 7	Day 30
DA	8	186.29±52	271.5±67.5	708.7±281.5**	0.09±0.0	0.2±0.0*	0.2±0.1**	6.9±1.9	6.2±1.31	11.5±2.7**	5.7±1.6	4.9±0.9	9.5±2.1**	1.3±0.3	1.2±0.4	1.9±0.7*
DASCK	10	261.6±96.9	340.8±85.6	510.9±59.9**	0.1±0.0	0.13±0.0	0.22±0.1**	6.3±2.7	6.2±1.5	11.5±2.3**	5.1±2.3	4.9±1.2	9.3±1.9**	1.2±0.4	1.4±0.3	2.2±0.5**
DA CK	7	246.2±46.8	396.8±78.8*	626.6±156.9**	0.10±0.0	0.2±0.0*	0.2±0.1**	7.25±2.2	8.2±2.5	13.6±5.5*	5.9±1.8	6.6±0.01	11.0±4.5*	1.4±0.4	1.6±0.5	2.6±1.02*

DA: displacement of the abomasum. SCK: subclinical ketosis. CK: clinical ketosis. LCAT: lecithin cholesterol acyltransferase. ApoB-100: apolipoprotein B-100. TC: total cholesterol. CE: cholesteryl ester. FC: free cholesterol. * Significant when compared with the value at day 0 (* P<0.05; **P<0.01) in each DA group. # Reference value according to Nakagawa and Katoh (1998), ## Reference value according to Mahley et al. (1984), ### Reference value according to Itoh et al. (1997) #### Reference value according to Radostits et al. (2000).

Table 2. Mean values of serum profiles of lipid metabolism and BCS in each DA group

Type	No	BHBA (mmol/l) (0.35-0.47)≠			NEFA (mmol/l) (0.21-0.62)≠≠			Glucose (mmol/l) (4.50-6.71)≠≠			AST (U/l) (78-132)≠			BCS (2.5-4)≠≠≠		
		Day 0	Day 7	Day 30	Day 0	Day 7	Day 30	Day 0	Day 7	Day 30	Day 0	Day 7	Day 30	Day 0	Day 7	Day 30
DA	8	0.5±0.1	0.7±0.5	0.8±0.2	0.8±0.4	0.3±0.2**	0.2±0.1**	5.8±0.6	5.4±1.0	5.6±0.7	221.9±170.1	88.6±21.9*	77.6±18.9*	3.1±0.5	3.0±0.4	2.9±0.4
DASCK	10	1.8±0.4	0.7±0.2**	0.8±0.4**	1.8±0.3	0.4±0.1**	0.24±0.1**	5.9±0.8	5.6±0.6	5.6±0.7	344.8±291.8	112.1±18.7*	72.1±26.2**	3.2±0.5	3.1±0.3	3.0±0.2
DA CK	7	4.6±1.5	1.3±0.6**	1.4±1.2**	1.8±0.5	0.5±0.4**	0.3±0.3**	4.8±1.9	4.5±1.6	5.2±0.8	295.2±121.3	175.8±79.3*	62.2±12.5**	3.1±0.4	2.9±0.2	2.8±0.2

DA: displacement of the abomasum. SCK: subclinical ketosis. CK: clinical ketosis. BHBA: β-hydroxy butyric acid. NEFA: non-esterified fatty acid. AST: Aspartate Aminotransferase. BCS: body condition score. * Significant when compared with the value at day 0 (* P<0.05; **P<0.01) in each DA group. # Reference value according to Radostits et al. (2000), ## Reference value according to Oikawa and Katoh (2002), ### Reference value according to Zadnik (2003a), #### Reference value according to Ferguson et al. (1994).

Table 3. Mean values of serum profiles of lipid metabolism between DA groups

Type	No	LCAT (U) (929-1059)#		ApoB-100 (g/l) (0.01-0.2)# or (0.2-0.32)##		TC (mmol/l) (6.24-22.13)###		CE		FC						
		DA SCK	DA CK	DA SCK	DA CK	DA SCK	DA CK	DA SCK	DA CK	DA SCK	DA CK					
Day 0	25	186.3±54.2	261.6±96.9	246.2±46.8	0.1±0.0	0.1±0.0	0.1±0.0	6.9±1.9	6.3±2.7	7.3±2.2	5.7±1.6	5.1±2.3	5.9±1.8	1.3±0.3	1.2±0.4	1.4±0.4
Day 7	25	271.5±67.5	340.8±85.6	396.8±78.8	0.2±0.0	0.1±0.0	0.2±0.0	6.2±1.3	6.2±1.5	8.2±2.5	4.9±0.9	4.9±1.2	6.6±0.0	1.2±0.4	1.4±0.3	1.6±0.5
Day 30	25	708.7±281.5	510.9±59.9	626.5±156.9	0.2±0.0	0.2±0.1	0.2±0.0	11.5±2.7	11.5±2.3	13.6±5.5	9.5±2.1	9.2±1.9	11.0±4.5	1.9±0.7	2.2±0.5	2.6±1.0

DA: displacement of the abomasum. SCK: subclinical ketosis. CK: clinical ketosis. LCAT: lecithin cholesterol acyltransferase. ApoB-100: apolipoprotein B-100. TC: total cholesterol. CE: cholesteryl ester. FC: free cholesterol.
Reference value according to Nakagawa and Katoh (1998), ## Reference value according to Mahley et al. (1984), ### Reference value according to Itoh et al. (1997) #### Reference value according to Radostits et al. (2000).

Table 4. Mean values of serum profiles of lipid metabolism and BCS between DA groups

Type	No	BHBA (mmol/l) (0.35-0.47)#		NEFA (mmol/l) (0.21-0.62)##		Glucose (mmol/l) (4.50-6.71)###		AST (U/l) (78-132)#		BCS (2.5-4)###						
		DA SCK	DA CK	DA SCK	DA CK	DA SCK	DA CK	DA SCK	DA CK	DA SCK	DA CK					
Day 0	25	0.5±0.1	1.8±0.4**	4.6±1.5**	0.8±0.4	1.8±0.3**	1.8±0.5**	5.8±0.6	5.9±0.8	4.8±1.9	221.9±170.1	344.8±291.8	295.2±213	3.1±0.5	3.2±0.5	3.1±0.4
Day 7	25	0.7±0.5	0.7±0.2	1.3±0.6	0.3±0.2	0.4±0.1	0.5±0.4	5.4±1.0	5.6±0.6	4.5±1.6	88.6±21.9	112.1±18.7	175.8±79.3	3.0±0.4	3.1±0.3	2.9±0.2
Day 30	25	0.8±0.2	0.8±0.4	1.4±1.2	0.2±0.1	0.2±0.1	0.3±0.3	5.6±0.7	5.6±0.7	5.2±0.8	77.6±18.9	72.1±26.2	62.2±12.5	2.9±0.4	3.0±0.2	2.8±0.2

DA: displacement of the abomasum. SCK: subclinical ketosis. CK: clinical ketosis. BHBA: β-hydroxy butyric acid. NEFA: non-esterified fatty acid. AST: Aspartate Aminotransferase. BCS: body condition score.

* Significant when compared with the value at DA case (* P<0.05; **P<0.01).

Reference value according to Radostits et al. (2000), ## Reference value according to Oikawa and Katoh (2002), ### Reference value according to Zadnik (2003a), #### Reference value according to Ferguson et al. (1994).

Discussion

DA is a common disease of dairy cattle in early lactation with in the first 3 weeks post-calving; 44% in the 1st week, 52 % occurred in the 2nd and 3rd weeks. The previous reports mentioned that LDA commonly occurred after calving (Rohn *et al.*, 2004) and most frequently in highly lactating cows during early lactation (Veysi *et al.*, 2003). DA cases were also reported within a period from 3 to 7 weeks after parturition (Constable *et al.*, 1991; Zadnik, 2003a; El-Attar *et al.*, 2007). Inanition during this period leads to serious metabolic consequences where the postpartum energy balance is mostly influenced by feed uptake. Prolonged periods of reduced appetite lead to the same consequences (Rohn *et al.*, 2004).

The clinical findings in all DA cases showed no significant changes in temperature, pulse, respiration and BCS either between the diseased groups or within the same diseased group. All these findings were within the physiological reference range reported by Ferguson *et al.* (1994) and Radostits *et al.* (2000). On the other hand, the previous reports about DA stated that diseased cows with DA were febrile with increased heart and respiratory rates and ruminal hypomotility (Goetze and Müller, 1990; El-Attar *et al.*, 2007). Cows with DA had anorexia, reduced milk yield (Ozturk *et al.*, 2013) defecate less frequently, and the feces were scanty and pasty (Radostits *et al.*, 2007; Ozturk *et al.*, 2013) because of the failure of abomasal emptying (Zadnik, 2003b; El-Attar *et al.*, 2007).

The obtained results stated no significant changes in BCS in all DA cases either before or treatment by surgical correction of DA throughout the study period. BCS was not associated with the risk of LDA. LeBlanc *et al.* (2005) found that prepartum BCS was not associated with LDA risk. Other studies reported that cows with excess BCS at parturition are at increased risk for LDA. The increased incidence rate of LDA for cows with high BCS may be associated with increased ketosis and fatty liver, greater reduction of prepartum intake, and slower increases in postpartum intake for over conditioned cows at parturition (Grummer, 1995).

The pattern of changes in serum lipid metabolism throughout the study indicating recovery of most diseased cows and significant effect of both of operation and 30 day follow up period which reflected through a significant elevation ($P < 0.05$) of

serum LCAT, apoB-100, and cholesterol (DA and DA SCK), and a significant reduction ($P < 0.05$) in serum AST, NEFAs and BHBA (DA SCK or DA CK) in all diseased groups particularly at day 30 following surgery when their values were compared with those at day 0 whereas most of these parameters returned to their physiological values as well as most of cows restored their physiological status. Only in DA group, LCAT activity returned to physiological reference values reported by Nakagawa and Katoh (1998). These significant changes were not reported ($P > 0.05$) between the 3 diseased groups at any of the sampling days. The previous reports said that LCAT activity, together with the CE and FC concentration, is reduced in cows with ketosis and LDA (Nakagawa and Katoh, 1998).

The current study proved that TC was remarkably reduced ($P < 0.05$) in all DA groups at day 0. The previous studies mentioned that concentrations of TC (Rehage *et al.*, 1999; Komatsu *et al.*, 2002; Stengärde *et al.*, 2010) were remarkably reduced in cows having a DA. This may be due to the reduced feed intake (Stengärde *et al.*, 2010). Serum cholesterol concentrations usually change with feed intake (Janovick Guretzky *et al.*, 2006) and with fat intake (Duske *et al.*, 2009). The blood TC concentrations were significantly decreased in puerperal ketotic cows (1-15 days prepartum) (Đoković *et al.*, 2012). It was thought that estimation of the LCAT activity and of the CE concentration during the non-lactating stage would be useful in discovering cattle that are susceptible to postparturient disorders such as ketosis (Nakagawa and Katoh, 1998). The reduction in LCAT activity was detected prior to diagnosis of ketosis or milk fever (Nakagawa and Katoh, 2000).

Serum apoB-100 concentrations were not reduced in diseased DA cows throughout the present study either before surgery at day 0 or after operation at days 7 and 30 when they compared with their reference values mentioned by Mahley *et al.* (1984) and Itoh *et al.* (1997) in healthy cattle, however serum apoB-100 concentrations were significantly ($P < 0.05$) increased in all DA groups particularly at day 30 when they compared with their values at day 0. In contrast, the apoB-100 concentration decreased in cows with ketosis (Oikawa *et al.*, 1997), LDA (Oikawa *et al.*, 1997), retained placenta (Oikawa *et al.*, 1997), milk fever (Oikawa and Katoh, 2002).

The current study also reported similar changes

in serum apoB-100 concentrations between the 3 diseased DA groups at any of the three sampling days as well as apoB-100 concentrations were similar to the physiological reference values. This may be explained that the previous reports indicated that the decreased apoB-100 concentrations were similar among all diseased cows (40 to 60% of healthy controls during early lactation) and are not largely different from that in cows with fatty liver. Same decreased rates suggest that decreases of apoB-100 concentrations in ketosis, LDA, retained placenta, milk fever and downer cow syndrome are primarily due to fatty liver, thereby supporting the hypothesis that these diseases are related to fatty liver (Gerloff *et al.*, 1986; Herdt, 1988; Morrow, 1976; Morrow *et al.*, 1979; Reid, 1980). Some research articles reported that serum LCAT activity as a diagnostic marker for fatty liver-related diseases such as DA and ketosis, is more diagnostic than apoB-100 concentrations because the reduction in its serum activity precedes clinical signs and also because the activity is not changed during the peripartum period, at least in some healthy cows (Nakagawa and Katoh, 1998). On the other hand, the apoB-100 concentration is low during early lactation, relative to the other stages (Marcos *et al.*, 1990; Yamamoto *et al.*, 1995). However, the hepatic apoB-100 mRNA does not remarkably decrease during early lactation (Gruffat *et al.*, 1997), suggesting that the apoB-100 concentration during early lactation is regulated at posttranslational levels such as the intracellular proteolytic degradation.

The current study mentioned a negative relationship between NEFA and apoB-100 where reduction in serum NEFA after operation was associated with increased serum apoB-100. This was reported in both of DA group and DA CK group. This may be explained that an elevation of serum NEFA associated with development of fatty liver that reduce ability of the liver for VLDL secretion to circulation that finally led to lowered concentration of serum apoB-100. The other reports found that Very low-density lipoproteins (VLDL) formation depends on apolipoproteins and cholesterol. Because apolipoprotein supply is limited in cattle, the ruminant liver has a low capacity to secrete VLDL (Grummer, 1993), and overproduction of TGs will accumulate in the hepatocytes cytosol, causing hepatic lipidosis (Cavestany *et al.*, 2005; Ingvarsten *et al.*, 2003).

Serum BHBA were not significantly changed in

DA group while they were remarkably reduced ($P < 0.05$) at days 7 and 30 after operation in DA SCK and DA CK groups when they compared with their values at day 0, however they reached their reference values reported by Radostits *et al.* (2000) only in DA group and in DA SCK group while they were still higher than the reference values in DA CK. The other studies reported that serum BHBA concentrations are a less specific indicator of energy balance than plasma NEFA. High values are associated with decreased milk production, increased CK and LDA and reduced fertility (Herdt *et al.*, 2001). SCK may start at serum BHBA concentrations in the first 2 weeks after calving above 1200 $\mu\text{mol/l}$ (11.7 mg/dl) (Sakha *et al.*, 2006; 2007). Cows with increased serum BHBA ($\geq 1400 \mu\text{mol/l}$ or 14.4 mg/dl) in the first 2 weeks post calving were at higher risk to subsequently develop either CK or LDA (Duffield, 2000; Oetzel, 2004; Duffield *et al.*, 2009). CK may start at Blood BHB about 2600 $\mu\text{mol/l}$ (Andersson, 1984) or 3000 $\mu\text{mol/l}$ (29 mg/dl) or more (Oetzel, 2004). The lipid content in the liver and the blood BHBA concentrations were significantly higher in puerperal ketotic cows (1-15 days prepartum) (Đoković *et al.*, 2012). Increased blood concentrations of BHBA have been reported in cows having DA (Komatsu *et al.*, 2002; Zadnik, 2003a).

Serum NEFA concentration and AST activities were significantly ($P < 0.05$) decreased in all DA groups at days 7 and 30 when they compared with their values at day 0. They reached their physiological reference values reported by Radostits *et al.* (2000) and Oikawa and Katoh (2002) after operation at days 7 and 30. The changes in serum NEFAs a sensitive indicator of energy metabolism through reduction of their serum concentrations throughout the current study referred to correction of the Negative energy balance (NEB) which was associated with DA cases. The previous studies stated that NEFAs are a sensitive indicator of energy balance (Herdt, 2000). The NEB is expected in milking cows, so blood NEFA concentrations are high after calving is clearly variable and very difficult to assess after calving. On the other side, postpartum serum BHBA was a more sensitive and specific test than NEFA concentration (Cameron *et al.*, 1998). Increased blood concentrations of NEFA and increased enzyme activity of AST (Muyllle *et al.*, 1990; Itoh *et al.*, 1998; Komatsu *et al.*, 2002; Zadnik, 2003a) have been reported in cows having DA.

The blood NEFAs concentrations and the AST activities were significantly higher in puerperal ketotic cows (1-15 days prepartum) (Đoković *et al.*, 2012). Cattle with DA were at the stage of postpartum one to eight weeks, and had increased serum AST activities in abomasal displacement (Ozturk *et al.*, 2013). Elevated NEFA concentration in plasma is also a prerequisite for development of hepatic lipidosis that occurs in DA cows (Rehage *et al.*, 1999). LeBlanc *et al.* (2005) also added that between 1 and 7 days post partum, increasing serum concentrations of BHBA and NEFA were associated with increased risk of subsequent LDA. Other studies also mentioned that alkaline phosphatase, AST (O'Zkan and Poulsen, 1986; Zadnik, 2003a; Mokhber Dezfouli *et al.*, 2013), total leukocytic, neutrophils, total protein count, urea, and glucose concentrations were significantly increased in the LDA cases (Mokhber Dezfouli *et al.*, 2013).

The significant changes in lipid profiles indices were also reported in serum BHBA and NEFA which were highly increased in DA SCK and DA CK at day 0 compared to their values in DA group at day 0, because serum NEFA usually increase after parturition either physiologically (Cameron *et al.*, 1998) or due to diseased condition such as DA (LeBlanc *et al.*, 2005). BHBA concentration remarkably increased in the first 2 weeks after calving (Oetzel, 2004; Sakha *et al.*, 2006; 2007) which was associated with increased risk of DA and CK. In case of DA SCK or DA CK cases in the current study, blood NEFAs were dramatically elevated and were usually associated with remarkable elevation of serum BHBA as well as SCK and CK were usually associated with higher increase of blood BHBA and NEFA in the 1st 2 weeks after calving, but these findings were not remarkable between these groups and DA group after operation in days 7 and 30 because all animals started to recover and restore their physiological status. Serum AST activities were not significantly changed in between the diseased groups at days 0, 7 or 30 compared with those at DA group. Geishauser *et al.* (1997); LeBlanc *et al.* (2005) and Stengärde *et al.* (2010) reported increases in concentration of NEFA, BHBA, and AST in cattle 1 to 2 wk before DA. This implies that some of the changes may last over sometime, related to other diseases foregoing the DA or to early stages in the development of DA. Also, Mokhber Dezfouli *et al.* (2013) also reported that AST and glucose concentrations were

significantly increased in the LDA cases.

DA cases showed no significant ($P > 0.05$) changes in blood glucose levels throughout this study either within the same diseased category or between the diseased groups. All glucose values were within the physiological reference ranges mentioned by Dubreuil and Lapierre (1997); Radostits *et al.* (2000) and Zadnik (2003a). These findings agreed with the previous reports which stated that blood glucose levels showed no significant changes in DA cows compared with those in healthy group (Komatsu *et al.*, 2002; Stengärde and Pehrson, 2002; Van Winden *et al.*, 2003; Zadnik, 2003a; Pravettoni *et al.*, 2004). The same results reported by Agenäs *et al.* (2003) who added that a reduced feed intake lead to a rapid decrease in glucose and insulin concentrations, whereas stress may result in high glucose concentrations. Stengärde *et al.* (2010) reported that there were not any significant changes in glucose concentration between DA cows and controls, even though the DA cows were most likely in a more pronounced NEB, as reflected by elevated concentrations of NEFA and BHBA. In contrast, results from several previous studies show elevated plasma concentrations of glucose in cows with DA (van Meirhaeghe *et al.*, 1988; Muylle *et al.*, 1990; Cupere *et al.* 1991; Rehage *et al.*, 1999; Itoh *et al.*, 1998). Constable *et al.* (2013) reported that dairy cattle with DA showed low feed intake with high amount of milk produced, hypovolemia, and hyperglycemia. Abouzeid *et al.* (2008) also observed hyperglycemia in cows with RDA and AV. Mokhber Dezfouli *et al.* (2013) reported that glucose concentrations were significantly increased in the LDA cases.

The present work reported that DA SCK or DA CK cases showed no significant ($P < 0.05$) changes in blood glucose. In contrast, Sakha *et al.* (2007) and Tehrani-Sharif *et al.* (2012) stated that blood glucose concentrations in subclinical ketotic cows were significantly lower than in non ketotic cows.

Conclusion

The pattern of changes in serum lipid metabolism indicators proved recovery of most diseased cows and the significant effect both of surgical operation and 30 day follow up period which reflected through a significant elevation of serum LCAT, apoB-100 and cholesterol and a significant reduc-

tion in serum levels of AST, NEFAs and BHBA (particularly in DA SCK and DA CK) in all DA groups particularly at day 30 following surgery comparing to their values at day 0 whereas most of these parameters returned to their physiological reference values. These significant changes were not reported between the 3 diseased groups except for NEFA and BHBA (at day 0 between DA group and the other two groups) at any of the three sampling days. The current study mentioned a negative relationship between NEFA and apoB-100 where reduction in serum NEFA after operation was associated with increased serum apoB-100.

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