

## Research Article

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### Assessment of Insulin and Insulin Resistance in Dairy Cattle with Displaced Abomasum Pre and Post-Surgery

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#### ABSTRACT

Displacement of the abomasum (DA) in dairy cattle is a multifactorial disease, with the majority of cases being diagnosed within the first week postpartum. The study aimed to describe the changes in clinical findings, serum levels of insulin, glucose and non-esterified fatty acids (NEFAs) as well as assess insulin resistance in Holstein dairy cattle with DA throughout a long term study from day 0 until day 30 post surgery. 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 (surgery), 7 and 30. They were clinically and biochemically examined to estimate Body Condition Score (BCS) and serum insulin, glucose and NEFAs. Insulin resistance was measured by using Quantitative Insulin Sensitivity Check Index (RQUICKI). Based on blood  $\beta$ -hydroxybutyric acid

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(BHBA) at day 0, DA cows were classified into three categories; DA only [ $<1.2 \text{ mmol L}^{-1}$ ], DA with subclinical ketosis (DA SCK) [ $1.2\text{-}2.4 \text{ mmol L}^{-1}$ ] and DA with clinical ketosis (DA CK) [ $\geq 2.5 \text{ mmol L}^{-1}$ ]. The clinical findings including body condition score (BCS) showed no significant changes either in between the three diseased groups or within the same diseased group at different sampling days (days 0, 7 and 30). Development of hypoinsulinaemia in all DA cases where the diseased cows need much more time than 30 days follow up to restore their physiological insulin level. RQUICKI values were not significantly reduced and were still within the physiological reference range throughout the present study in all DA groups. Changes in blood NEFA indicated significant effect of surgical operation on 30 day follow up period on the recovery of most diseased DA cattle.

## INTRODUCTION

Abomasal diseases of dairy cattle are mainly associated with stress conditions, nutritional disorders and metabolic disturbances. Highly lactating dairy cattle being fed large quantities of grain where exercise is limited may have abomasal atony (Lester and Bolton, 1994). Other contributing factors that can cause reducing abomasal motility include metabolic disorders (milk fever and ketosis), concurrent diseases (mastitis, metritis or retained placenta), changes of intra-abdominal organs (especially in late pregnancy) and genetic predisposition (Radostits *et al.*, 2007; Delgado-Lecaroz *et al.*, 2000).

Displacement of the abomasum (DA) in dairy cows is a multifactorial disease, with the majority of cases being diagnosed within the first week postpartum (Stengarde and Pehrson, 2002; Doll *et al.*, 2009). It is a common and economically important problem of dairy cattle in early lactation. Affected cows produce less milk at least in the short

term (Raizman and Santos, 2002 and Van Winden and Kuiper, 2003) and have a higher culling rate (Grohn *et al.*, 1998; Raizman and Santos, 2002; Radostits *et al.*, 2007) that may reach 10% (Van Winden and Kuiper, 2003).

Clinically, Left Displaced Abomasum (LDA) characterized by gas accumulation in the abomasum resulting in a tympanic, resonant and high-toned ping sound (Breukink and Kroneman, 1963). Furthermore, diseased cows with DA were febrile with tachycardia, increased respiratory rates and ruminal hypomotility (Goetze and Muller, 1990; El-Attar *et al.*, 2007).

Strong associations had been stated between many of the post partum diseases and increase the risk of DA. Several studies reported twins, dystocia, milk fever, retained placenta, metritis and ketosis as risk factors for LDA (Grohn *et al.*, 1989, Correa *et al.*, 1990; Rohrbach *et al.*, 1999; Grohn, 2000; LeBlanc *et al.*, 2005). DA has also been associated with other diseases such as retained placenta, metritis and ketosis (Rohrbach *et al.*, 1999).

Some studies reported the most common clinical findings and metabolic profiles associated with DA in dairy cattle (Mesaric *et al.*, 2002; Stengarde *et al.*, 2010; Ozturk *et al.*, 2013), DA and ketosis (Stengarde *et al.*, 2008).

Agenas *et al.* (2003) mentioned that DA may be associated with inconsistent changes in plasma glucose concentrations. A reduced feed intake leads to a rapid decrease in glucose and insulin concentrations, whereas stress may result in high glucose concentrations. Insulin sensitivity is difficult to evaluate in cows that are off feed and results in insulin and glucose concentrations do not contradict that DA cows may have reduced insulin sensitivity.

Insulin is an anabolic peptide hormone, synthesized and stored in beta cells located in the islets of Langerhans in the pancreas (Hayirli, 2006). Insulin facilitates the cell uptake of glucose and

lipolysis in adipose tissue. Insulin concentration is high before parturition and decreases to low levels after parturition (van Kneegsel *et al.*, 2007; Kokkonen *et al.*, 2005), so the low concentration of insulin after calving stimulates lipid mobilization from adipose tissue to counteract the Negative Energy Balance (NEB) in early lactation.

Insulin resistance was defined as the state in which a physiological level of insulin produces a lower than normal biological response (Kahn, 1978). Berson and Yalow (1970); Hayirli (2006) also defined insulin resistance as a condition in which a greater amount of insulin is required to produce a normal response.

In cattle with high concentrations of glucose, reduced insulin sensitivity has been considered as a prerequisite for DA (van Meirhaeghe *et al.*, 1988; Pravettoni *et al.*, 2004). In late pregnancy, decreased insulin sensitivity in peripheral tissue is part of this physiological response. Glucose is used for the growing foetus during late gestation and for milk production in early lactation (Bell, 1995). In another study, Inconsistent findings about glucose and insulin concentrations (van Meirhaeghe *et al.*, 1988; Itoh *et al.*, 1998; Komatsu *et al.*, 2002; Stengarde and Pehrson, 2002; Van Winden *et al.*, 2003; Zadnik, 2003a; Pravettoni *et al.*, 2004) have been reported in cows having a DA.

In primary ketosis, the affected cows have hyperketonemia and low concentrations of both glucose and insulin in plasma. Secondary ketosis occurs in concurrent with other diseases (Holtenius and Holtenius, 1996). In Secondary ketosis, cows have hyperketonemia and varying serum concentrations of glucose and insulin. Affected cows are often overconditioned. The cows with DA had lower concentrations of insulin compared with the control cows, most likely due to a reduced feed intake (Agenas *et al.*, 2003; Stengarde *et al.*, 2010).

Cows with ketosis have reduced tissue responsiveness to insulin (Sakai *et al.*, 1993) and

ketoacidosis is one of the causes of insulin resistance (Holtenius, 1993; Steen *et al.*, 1997).

Lower Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) referred to decreased insulin sensitivity in calves (Bossaert *et al.*, 2009). A negative linear relationship between body condition and RQUICKI in lactating dairy cows was found (Holtenius and Holtenius, 2007) and overconditioned dairy cattle was reported to have low insulin sensitivity or a reduced insulin response (Holtenius *et al.*, 2003; Rukkamsuk *et al.*, 1998).

Insulin-resistant cows did not have lowered RQUICKI values. The RQUICKI values were not correlated with insulin sensitivity in cows with ketosis and signs of puerperal metritis (Kerestes *et al.*, 2009).

Insulin resistance has been proposed as part of the etiology of DA (van Meirhaeghe *et al.*, 1988). Stengarde *et al.* (2010) reported lower RQUICKI values in DA cows compared with controls which indicated that DA cows had reduced insulin sensitivity.

Therefore, this study aimed to estimate milk losses, culling rate, complicated diseases in dairy cattle with DA. It also described the pattern of changes in clinical findings and serum biochemicals including insulin, non-esterified fatty acids (NEFA), glucose and RQUICKI 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. Insulin sensitivity was evaluated in all DA groups.

## MATERIALS AND METHODS

**Materials:** 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 L 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  $\beta$ -hydroxybutyric acid (BHBA) at day 0, DA cows were classified into three categories; DA only [ $<1.2 \text{ mmol L}^{-1}$ ], DA with subclinical ketosis (DA SCK) [ $1.2\text{-}2.4 \text{ mmol L}^{-1}$ ] and DA with clinical ketosis (DA CK) [ $\geq 2.5 \text{ mmol L}^{-1}$ ]. 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).

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  $-20^{\circ}\text{C}$  till analysis.

**Methods:** Clinical examination of all dairy cattle using clinical chart according to Rosenberger (1990) was done. Body Condition Score (BCS) of all cows was estimated based on a 5-point scale. Rates of milk losses, culling rate and predisposing factors and complicated diseases associated with DA cases in the three diseased groups were investigated (Ferguson *et al.*, 1994).

Serum insulin was analyzed with a porcine insulin radioimmunoassay (Porcine Insulin RIA KIT, Linco research, St. Charles, MO., US), previously evaluated for bovine samples, using a Cobra II Auto-Gamma counter (Packard Instrument Company, Meriden, CT., US).

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 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 (Spsswin, 1997). Insulin resistance was measured by RQUICKI that was based on fasting plasma glucose, insulin and NEFA and a low value is indicative of decreased insulin sensitivity. A metabolic index was calculated as  $RQUICKI = 1 / [\log_{10}(\text{glucose, mg dL}^{-1}) + \log_{10}(\text{insulin, } \mu\text{U m L}^{-1}) + \log_{10}(\text{NEFA, mmol L}^{-1})]$  (Perseghin *et al.*, 2001; Rabasa-Lhoret *et al.*, 2003).

## 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) occurred in the 2nd and 3rd weeks post calving (8-21 DIM) and only one cow (4%) was diagnosed at 128 DIM [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 culling rate of the examined cows was 16% (4 of 25). The rates of milk loss were  $233.45 \pm 73.53$ ,  $217.91 \pm 81.38$  and  $291 \pm 72.52$  kg in DA, DA SCK and DA CK, respectively.

Many parturient diseases [64% (16 of 25)] were considered as predisposing factors for DA in dairy cows. These diseases included ketosis, retained placenta, milk fever, mastitis and metritis. These diseases were also considered as complication of DA [56% (14 of 25)].

The clinical findings in all DA cases including BCS ( $p > 0.05$ ) showed no significant changes either within the same diseased group at days 7 and 30 when their values compared with those at day 0 (Table 1) or between the diseased groups (DA SCK or DA CK) (Table 2) when their values compared with those of DA group at days 0, 7 and 30. BCS was still within the physiological reference values. Temperature, pulse and respiration indices were within the physiological reference range. Ruminal movement was reduced in the diseased cows at day 0 then they improved after the surgical correction of DA.

Blood insulin showed unremarkable changes ( $p > 0.05$ ) in all DA cases either within the same diseased group (Table 1) when their values at day 0 compared with those at days 7 or 30, or between the diseased groups (Table 2) when their values in DA group compared with those in the other two groups. On the other hand, although blood insulin increased after operation in the three DA groups at days 7 and 30 comparing with those in day 0, this increase was insignificant and less than the reference values.

Serum NEFAs concentrations (Table 1) were significantly ( $p > 0.05$ ) decreased 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. serum NEFA (Table 2) were significantly ( $p > 0.05$ ) increased in DA SCK group and DA CK group at day 0 when their values compared with those of DA group at day 0, but these findings were not reported between these groups and DA group after operation in days 7 and 30.

The obtained results revealed that blood glucose levels showed no significant ( $p > 0.05$ ) changes throughout this study either within the same diseased group (Table 1) when their values at day 0 (before operation) compared with those at days 7 or 30 (after surgery), or between the diseased groups (Table 2) when their values in DA group compared with those in the other two groups. All glucose values were within the physiological reference range.

The RQUICKI was remarkably ( $p > 0.05$ ) increased (Table 1) in DA SCK and in DA CK at days 7 and 30 when their values compared with those at day 0. These significant changes were not found in DA group. RQUICKI (Table 2) showed no significant ( $p > 0.05$ ) changes between the diseased groups when their values in DA group compared

Table 1: Mean values of serum insulin, NEFA, chloride, glucose and RQUICKI and BCS in each DA group

Type	No.	Insulin			NEFA ( $\mu\text{U mL}^{-1}$ )			Glucose			RQUICKI [0.45–0.48 (0.46)] <sup>#####</sup>			BCS (2.5–4) <sup>#####</sup>		
		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	1.89	2.09	2.86	0.81	0.25	0.19	5.77	5.39	5.60	0.54	0.70	0.68	3.06	3.0	2.88
		±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		1.36	1.46	1.99	0.40	0.17**	0.11**	0.62	1.03	0.69	0.09	0.17	0.09	0.50	0.40	0.38
DA SCK	10	1.81	3.76	3.19	1.76	0.35	0.24	5.98	5.55	5.57	0.46	0.59	0.61	3.20	3.13	3.00
		±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		1.57	2.32	2.02	0.34	0.13**	0.11**	0.81	0.62	0.71	0.05	0.12*	0.11**	0.48	0.32	0.24
DA CK	7	1.84	1.98	2.09	1.75	0.52	0.34	4.82	4.49	5.16	0.50	0.68	0.67	3.07	2.96	2.82
		±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		1.38	1.37	2.35	0.45	0.43**	0.33**	1.89	1.61	0.77	0.11	0.16*	0.13*	0.37	0.17	0.24

DA: Displacement of the abomasum. SCK: subclinical ketosis. CK: clinical ketosis. NEFA: non-esterified fatty acid. RQUICKI: revised Quantitative Insulin Sensitivity Check Index. BCS: body condition score. \*Significant when compared with the value at day 0 (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ) in each DA group. <sup>#</sup> Reference value according to Holtenius and Holtenius (2007); <sup>a</sup>1-3 weeks of lactation, <sup>b</sup>4-6 weeks of lactation. <sup>###</sup> Reference value according to Oikawa and Katoh (2002), <sup>####</sup> Reference value according to Zadnik (2003a). <sup>#####</sup> Reference value according to Stengarde *et al.* (2010). <sup>#####</sup> Reference value according to Ferguson *et al.* (1994).

Table 2: Mean values of serum insulin, NEFA, chloride, glucose and RQUICKI and BCS between DA groups

Days after surgery	No.	Insulin			NEFA ( $\mu\text{U mL}^{-1}$ )			Glucose			RQUICKI [0.45-0.48 (0.46)] <sup>#####</sup>			BCS (2.5-4) <sup>#####</sup>		
		DA	SCK	DA CK	DA	SCK	DA CK	DA	SCK	DA CK	DA	SCK	DA CK	DA	SCK	DA CK
Day 0	25	1.89	1.81	1.84	0.81	1.76	1.75	5.77	5.98	4.82	0.54	0.46	0.50	3.06	3.20	3.07
		±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		1.36	1.57	1.38	0.40	0.34**	0.45**	0.62	0.81	1.89	0.09	0.05	0.11	0.50	0.48	0.37
Day 7	25	2.09	3.76	1.98	0.25	0.35	0.52	5.39	5.55	4.49	0.70	0.59	0.68	3.0	3.13	2.96
		±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		1.46	2.32	1.37	0.17	0.13	0.43	1.03	0.62	1.61	0.17	0.12	0.16	0.40	0.32	0.17
Day 30	25	2.86	3.19	2.09	0.19	0.24	0.34	5.60	5.57	5.16	0.68	0.61	0.67	2.88	3.00	2.82
		±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
		1.99	2.02	2.35	0.11	0.11	0.33	0.69	0.71	0.77	0.09	0.11	0.13	0.38	0.24	0.24

DA: displacement of the abomasum. SCK: subclinical ketosis. CK: clinical ketosis. NEFA: non-esterified fatty acid. RQUICKI: revised Quantitative Insulin Sensitivity Check Index. 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 Holtenius and Holtenius (2007); <sup>a</sup>1-3 weeks of lactation, <sup>b</sup>4-6 weeks of lactation. <sup>##</sup> Reference value according to Oikawa and Katoh (2002), <sup>###</sup> Reference value according to Zadnik (2003a), <sup>####</sup> Reference value according to Stengarde *et al.* (2010), <sup>#####</sup> Reference value according to Ferguson *et al.* (1994).

with those in the other two groups group at either day 0, 7 or 30. The RQUICKI values in the three diseased groups either before (day 0) or after operation (days 7 and 30) were higher than the physiological reference values.

## 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 in the postpartum period (Rohn *et al.*, 2004) and most frequently in highly lactating dairy cows during early lactation (Veysi *et al.*, 2003). DA cases were also recorded within a period from 3 to 7 weeks after parturition (Zadnik, 2003a; El-Attar *et al.*, 2007).

The current study stated that DA caused economic losses due to milk loss either in DA, DA SCK or DA CK, cost of treatment and increased culling rate. The other studies also revealed that 10% of cows with DA are culled or died (Van Winden and Kuiper, 2003). The DA cattle produce less milk at least in the short term and have a

higher culling rate (Raizman and Santos, 2002). It has been reported that 10% of cows with DA are culled or died (Van Winden and Kuiper, 2003). Constable *et al.* (1992) reported that the case fatality rate of LDA was 5.6%. Abouzeid *et al.* (2008) mentioned that the percentages of dead and disused animal were higher for cows with AV (28.6%) and LDA (23.6%) than for those with RDA (5.0%).

Many postparturient diseases in the present study were considered as predisposing factors for DA in dairy cows. 64 % of DA cases were associated with these diseases. These diseases included ketosis, retained placenta, milk fever, mastitis and metritis. Oikawa *et al.* (1997) stated the association of ketosis with DA. Massey *et al.* (1993) mentioned that hypocalcemia at parturition was also assumed as a predisposing factor for LDA where this was reported from one herd, but there were conflicting data from field studies on the effect of oral supplementation of Calcium around parturition on the incidence of LDA (Melendez *et al.*, 2003). Milk fever usually precedes LDA (Care *et al.*, 1999). Strong associations had been stated between many of the post partum diseases and increase the risk of DA. Twins, dystocia, milk fever, retained placenta,

metritis and ketosis have been considered as risk factors for LDA (Grohn *et al.*, 1989; Correa *et al.*, 1990; Rohrbach *et al.*, 1999; Grohn, 2000; LeBlanc *et al.*, 2005). Curtis *et al.* (1985) found that cows with uncomplicated ketosis were at increased risk for LDA. Markusfeld (1985) reported that Cows with retained placenta or metritis were also at increased risk for LDA. Duffield and Bagg (2002) also added that herds with ketosis in early lactation cows were at risk of increased incidence of DA (>8%) and increased herd removals in the first 60 DIM (<8%). The current study also added that the previous diseases were also considered as complications of DA whereas 56 % of DA cases were complicated by ketosis, milk fever, retained placenta, metritis and/or mastitis. DA has been found to increase the risk of other postpartum disorders. This agreed with Curtis *et al.* (1985) who mentioned that dairy cattle with LDA are at increased risk for ketosis and metritis. Thus, feeding and management practices that prevent LDA reduce the incidence of some other postpartum disorders. Radostits *et al.* (2007) also reported that cows with LDA are at increased risk of ketosis and metritis.

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

The obtained results stated no significant changes ( $p > 0.05$ ) in BCS either in each DA group

or between DA groups. All these findings were within the physiological reference range reported by Ferguson *et al.* (1994). LeBlanc *et al.* (2005) referred to that prepartum BCS was not associated with the risk of LDA. These findings do not refute the importance of body condition. Other studies reported that cows with excess BCS at calving are at increased risk for LDA; incidence rates of LDA for cows with low (2.75 to 3.25), medium (3.25 to 4) and high (over 4) BCS at parturition were 3.1, 6.3 and 8.2%, respectively (Dyk, 1995). The increased incidence rate for cows with high BCS may be related to increased ketosis and fatty liver, greater depression of prepartum intake and slower increases in postpartum intake for cows that are overconditioned at parturition (Grummer, 1995).

Blood insulin concentrations showed unremarkable changes ( $p > 0.05$ ) in all DA cases either within each DA group (DA, DA SCK or DA CK) or between the three diseased groups. On the other hand, although blood insulin increased after operation in the three DA groups at days 7 and 30 compared with those in day 0 but this increase was insignificant and less than its reference values reported by Holtenius and Holtenius (2007). This may indicate hypoinsulinaemia. The previous studies stated lower concentrations of insulin in cows with DA when compared with those of control cows, most commonly due to a reduced feed intake (Agenas *et al.*, 2003; Stengarde *et al.*, 2010). Some articles added that along with high concentrations of glucose, decreased insulin sensitivity has been suggested as a prerequisite for DA (Van Meirhaeghe *et al.*, 1988; Pravettoni *et al.*, 2004). Glucose and insulin concentrations Findings in cows having DA have been inconsistent (van Meirhaeghe *et al.*, 1988; Itoh *et al.*, 1998; Komatsu *et al.*, 2002; Stengarde and Pehrson, 2002; Van Winden *et al.*, 2003; Zadnik, 2003a; Pravettoni *et al.*, 2004). The metabolic pattern in cases of induced or spontaneous hepatic lipodosis

and ketosis included low concentrations of serum insulin, plasma glucose and liver glycogen and high concentrations of serum glucagon, adrenaline and growth hormone, plasma BHBA and NEFA and liver triglycerides (DeBoer *et al.*, 1985; Veenhuizen *et al.*, 1991; Drackley *et al.*, 1992). Cows with primary ketosis have low concentrations of both plasma glucose and insulin (Holtenius and Holtenius, 1996). Cows with secondary ketosis have varying serum concentrations of glucose and insulin. Some authors added that cows with ketosis have low tissue responsiveness to insulin (Sakai *et al.*, 1993).

Serum NEFA concentration 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); 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 NEB which was associated with DA cases. The previous studies reported 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 highly variable and very difficult to evaluate after calving (Cameron *et al.*, 1998). Increased blood concentrations of NEFA have been reported in cows having DA (Itoh *et al.*, 1998; Komatsu *et al.*, 2002; Zadnik, 2003a). The blood NEFAs concentrations were significantly higher in puerperal ketotic cows (1-15 days prepartum) (Dokovic *et al.*, 2012). Cow with increased plasma NEFA concentration is at high risk for development of hepatic lipidosis 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.

The significant changes in lipid profiles indices were also reported in serum NEFA concentrations which were highly increased ( $P < 0.05$ ) 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 (Agreed with Cameron *et al.*, 1998) or due to diseased condition such as DA (agreed with LeBlanc *et al.*, 2005). These findings were not remarkable ( $p > 0.05$ ) between DA SCK and DA CK groups and DA group after operation in days 7 and 30 because all animals started to recover and restore their physiological status. LeBlanc *et al.* (2005); Stengarde *et al.* (2010) reported increases in concentration of NEFA in cattle 1 to 2 wk before DA. This referred to that some of the changes may last over sometime, related to other diseases foregoing the DA or to early stages of DA development.

NEFA concentrations were highly increased in all DA groups at day 0 before operation indicated NEB. At the same time, insulin concentration was reduced and glucose level was not changed when they compared with their references values that were reported by Oikawa and Katoh (2002) for NEFA; Zadnik (2003a) for insulin; Holtenius and Holtenius (2007) for glucose. The other articles revealed that elevated NEFA concentrations caused inhibition of insulin-stimulated glucose uptake by peripheral tissues, lowered the number of Glucose transporter 4 (GLUT 4) and disturbed intracellular insulin signalling pathways in the liver and peripheral tissues. Defects in intracellular signalling pathways include abnormality of GLUT 4 translocation, reduced Insulin receptor substrate (IRS-1) phosphorylation (Zierath *et al.*, 1998; Le Marchand-Brustel *et al.*, 1999), high risks for receptor down regulation and decreased coupling efficiency between occupied receptors and stimulated glucose transport (Garvey *et al.*, 1986).

Furthermore, glucose utilization by insulin-sensitive tissues was competed by free fatty acids (Koopmans *et al.*, 1996) and had toxic effects on peripheral tissues (Spector and Fletcher, 1978). Van Epps-Fung *et al.* (1997) also demonstrated the adverse effect of elevated NEFA concentration on adipose tissue insulin sensitivity.

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); 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; Stengarde and Pehrson, 2002; Van Winden *et al.*, 2003; Zadnik, 2003a; Pravettoni *et al.*, 2004). The same results reported by Agenas *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. Stengarde *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); Tehrani-Sharif *et al.* (2012) stated that blood glucose concentrations in subclinical ketotic cows were significantly lower than in non ketotic cows.

The obtained results revealed that RQUICKI was remarkably increased ( $P 0.05$ ) in DA SCK and in DA CK at days 7 and 30 when they compared with those at day 0. The RQUICKI values in all DA groups were usually higher than the physiological reference values reported by Stengarde *et al.* (2010). The previous reports mentioned that low RQUICKI value is indicative of decreased insulin sensitivity (Perseghin *et al.*, 2001; Rabasa-Lhoret *et al.*, 2003; Bossaert *et al.*, 2009). A negative linear relationship was proved between RQUICKI and body condition in lactating dairy cows (Holtenius and Holtenius, 2007) and overconditioned cows have been shown to have decreased insulin sensitivity or a reduced insulin response (Holtenius *et al.*, 2003; Rukkamsuk *et al.*, 1998). In another study, insulin-resistant cows did not have lowered RQUICKI values. The RQUICKI values were not correlated with insulin sensitivity in cows with ketosis and signs of puerperal metritis (Kerestes *et al.*, 2009).

In the present study, DA cows did not have lowered RQUICKI values. on contrast, Stengarde *et al.* (2010) showed a tendency for lower RQUICKI values in DA cows compared with controls. This may imply that DA cows had reduced insulin sensitivity. The current article mentioned that the RQUICKI showed no significant ( $p > 0.05$ ) changes between the three DA groups.

## CONCLUSION

This study concluded development of hypoinsulinaemia in all DA cases and the diseased cows need much more time than 30 days follow up

to restore their physiological insulin level. RQUICKI values were not significantly reduced and were still within the physiological reference range throughout the present study in all DA groups. This implied that DA, DA SCK and DA CK cases were not associated with insulin resistance. Changes in blood NEFA indicated significant effect of surgical operation and 30 day follow up period on the recovery of most diseased DA cattle. NEFA concentrations were highly increased in all DA groups at day 0 before operation indicated NEB while insulin was reduced and glucose was not changed when they compared with their references values so the elevated NEFA concentrations caused inhibition of insulin-stimulated glucose uptake by peripheral tissues.

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