

## Study on the effect of left displacement of abomasum (LDA) on some serum minerals and biochemical changes in dairy cows

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### Abstract

Minerals and some biochemical parameters measured by blood serum analyses in dairy cows affected with left displacement of abomasum (LDA) to evaluate the effect of disease on mineral status, energy metabolism and liver function in animals. Samples were collected from 60 affected cows and 60 healthy control cows, based on herd, parity, and stage of lactation. Serum concentrations for nonesterified fatty acids (NEFA),  $\beta$ -hydroxybutyrate (BHB) and aspartate aminotransferase (AST) were significantly higher in cows with LDA as compared with control cows ( $P < 0.05$ ), whereas serum calcium concentration in cows with LDA was lower than in control group ( $P < 0.05$ ). Parity number and lactation had significant effect in energy metabolism and liver function of LDA-affected cows. Significant increases in mean serum concentrations of NEFA, BHBA and AST were observed in DA cows with three or more lactations compared with healthy control cows ( $P < 0.05$ ).

**Keywords:** LDA, Dairy cow, Biochemical parameters, Minerals.

### Introduction

A very common and economically important disease of dairy cows in early lactation is displacement of the abomasum (DA). The losses, due to disease, include lost milk production during the illness and postoperatively (economic Detilleux et al. 1997, Raizman & Santos 2002) and a higher culling rate in affected cows (Geishauser et al. 1998, Gröhn et al. 1998, Raizman & Santos 2002). One of the first described cases of DA was a Swedish right-sided DA found in a 15-year-old cow described by the farmer as having good appetite and production (Lagerlöf, 1925). The dislocation was

assumed to be congenital. Left-sided DA was first described in 1950 (Begg, 1950). Abomasal displacement and treatment was reported as early as the 1950s (Trought 1957, Wood & Allison 1957). In the current dairy practice, the incidence of DA is rising and it is estimated that 5 percent of the newly calved dairy cows will develop DA (Kelton et al. 1998).

The average incidence of abomasal displacement and ketosis in 2005/2006 in dairy herds of Sweden was 1.0 and 1.3 cases per 100 cows, respectively (Stengärde et al. 2008). Left displacement of the abomasum (LDA) occurs more

frequently than abomasal volvulus (AV), but AV is more compromising to the individual cow and leads to more severe disease or even death (Baird 2012). In one study, the ratio of LDA to AV was 7.4 to 1 (Constable et al. 2017). Significant associations were found between negative energy balance prepartum, as reflected by increased non-esterified fatty acid concentrations, and occurrence of LDA. High body condition score, suboptimal feed bunk management, prepartum diets containing more than 1.65 Mcal of NE1/kg of DM, high genetic merit and low parity were significant risk factors (Van Winden et al. 2004, Constable et al. 2017). The serum biochemical changes in abomasal displacement of cows were evaluated in different studies (Delgado-Lecaroz et al. 2000, El-Attar et al. 2007, de Cardoso 2008, Massey 1993). It had been shown that cows with hypocalcemia near the time of calving have increased risk of subsequent LDA (Massey 1993). In another study, low serum calcium in the second, but not the first, week postpartum was associated with an increased risk of LDA (Geishauser et al. 1999). Hypokalemic, hypochloremic and metabolic alkalosis are the main biochemical changes found in uncomplicated DA in dairy cows (Divers 2008), Geishauser et al. (2000) reported association of various metabolites with the risk of subsequent LDA. Cows with subclinical ketosis (serum BHBA  $\geq 1400$   $\mu\text{mol/L}$ ) and high serum aspartate aminotransferase activity (AST) in the first 2 weeks postpartum, had increased risk of LDA (Geishauser et al. 1999). Increased blood concentrations of NEFA, BHBA, haptoglobin, and increased enzyme activity of aspartate

aminotransferase (AST) and glutamate dehydrogenase (GD) (Itoh et al. 1998, Zadnik 2003), as well as decreased concentrations of total cholesterol have been reported in cows having a DA (Rehage et al. 1996, Komatsu et al. 2002).

The present study has been conducted to evaluate some mineral and biochemical changes in LDA-affected cows and compare them with the healthy ones in the same herd.

## **Materials and methods**

### *Animals and Sampling*

Private practitioners at two ambulatory practices in Mashhad and Tehran, (Iran), were asked to examine and collect blood samples from cows diagnosed with LDA, on the day of diagnosis, and from 1 healthy control cow in the same herd. The cows had to be within 2 months postpartum; moreover, the healthy cows, from the same herd that presented age, lactation numbers, and milking days (DIM) similar to the animals with DA, were considered as the control animals. Samples of animals in the control group and the animals with DA were collected on the same day. The diagnosis of DA was based on a clinical examination by the veterinarian including auscultation to locate the characteristic metallic ping sounds found in cows with LDA and confirmation of diagnosis was carried out by surgery. Samples were collected from 60 cows with LDA and from 60 control cows from almost 10 dairy farms. Samples of blood were collected in the moment of the LDA diagnosis, prior to the surgery. All the animals belonged to 10 commercial herds

under intensive production system in the Mashhad and Tehran district, Iran. Blood from the jugular was collected from each cow in evacuated tubes, without additives (BD Vacutainer Systems, Plymouth, UK). Then, the blood samples were kept under cold condition until centrifugation, and after that, centrifuged (2500 rpm for 15 min) serum was withdrawn and the serum samples were stored at  $-20^{\circ}\text{C}$  until analysis.

#### Biochemical Analysis

Biochemical constituents of serum samples of candidate cows which have been measured in this study included: calcium (Ca), phosphorous (P), magnesium (Mg), AST (aspartate aminotransferase), NEFA and BHBA. All the samples were measured using commercial kits (Ca, P, Mg, AST: Pars Azmoon Co., Tehran, Iran; NEFA: FA 115 kit, Randox Laboratories Ltd., Crumlin, UK; BHBA: Ranbut kit, Randox Laboratories Ltd). The results were described as means  $\pm$  standard error (SE).

#### Statistical Analysis

All pair wise multiple comparisons of mean values of cattle with LDA and control were analyzed using one-way analysis of variance (ANOVA) followed by post hoc test using Sigma Stat software (version 3.1, SPSS, Chicago, IL, USA) according to (Gelfert et al. 2006) and a value of  $P < 0.05$  was considered to be significant.

#### Results

Parity number in LDA cows was  $2 \pm 1.1$  and in control cows was  $2.1 \pm 1.2$ . Serum calcium,

phosphorous and magnesium concentration of LDA affected and control cows are shown in fig 1. Serum calcium ( $7.45 \pm 0.37$  mg/dl) and phosphorous ( $6.11 \pm 0.87$  mg/dl) concentrations in affected cows were significantly lower than that of the control group ( $9.24 \pm 0.71$  mg/dl and  $7.91 \pm 0.47$  mg/dl respectively) ( $P < 0.05$ ). NEFA ( $1.35 \pm 0.23$  mEq/L), BHBA ( $2.7 \pm 0.68$  mmol/L) and activity of AST ( $97.62 \pm 13.5$  IU/l) significantly increased in LDA affected cows as compared with those of the control group ( $0.42 \pm 0.08$ ,  $0.68 \pm 0.13$  and  $72.93 \pm 8.76$  respectively) ( $P < 0.05$ ) (table 1).

Table 1. Serum NEFA, BHBA and AST concentrations of LDA affected and control cows (Mean  $\pm$  SE).

Group	NO	NEFA	BHBA	AST
LDA	60	$1.35 \pm 0.23^*$	$2.70 \pm 0.68^*$	$97.62 \pm 8.61^*$
Control	60	$0.42 \pm 0.08$	$0.68 \pm 0.13$	$72.93 \pm 8.76$

Table 2 shows the mean value of measured mineral parameters of LDA and control cows with different lactations. Serum calcium concentration in LDA-affected cows in all lactation groups in this study was significantly lower than those of the control group ( $P < 0.05$ ). Serum NEFA, BHBA and AST activities in LDA and control cows in different lactation groups are shown in table 3. As it is observed in table 2, LDA-affected cows that have had 1, 2 or 3 birth, had significantly higher serum NEFA, BHBA and AST level compared to the healthy cows with the same parity number ( $P < 0.05$ ).

Table 2. Mean serum concentrations of Ca, P and Mg of LDA affected and healthy control cows with different lactations (Mean± SE).

Group		No	Ca	P	Mg
LDA	Lact 1	24	6.96± 0.40*	6.28± 1.54*	2.09± 0.65
	Lact 2	18	7.64± 0.45*	6.47± 1.87*	2.94± 0.45
	Lact ≥ 3	18	7.31± 0.54*	5.55± 0.82*	3.19± 0.73
Control	Lact 1	24	9.11± 0.77	8.46± 0.55	2.31± 0.65
	Lact 2	18	9.15± 1.22	8.00± 0.93	2.81± 1.24
	Lact ≥ 3	18	9.98± 1.74	7.08± 0.88	2.84± 1.34

Table 3. Serum BHBA, NEFA and AST concentrations of LDA affected and control cows with different lactations (Mean± SE).

Group		No	BHBA	NEFA	AST
LDA	Lact 1	24	2.18± 1.04*	0.99± 0.25*	95.00± 15.99*
	Lact 2	18	2.57± 0.95*	1.70± 0.51*	91.12± 24.41*
	Lact ≥ 3	18	4.11± 1.46*	1.55± 0.39*	125.85± 6.30*
Control	Lact 1	24	0.67± 0.27	0.36± 0.12	69.75± 16.61
	Lact 2	18	0.67± 0.23	0.40± 0.18	68.44± 7.15
	Lact ≥ 3	18	0.70± 0.19	0.51± 0.14	81.66± 17.82

## Discussion

Delgado-Lecaroz et al. (2000) reported that cows with DA or RTA had significantly lower Ca, P, Mg, K, and Cl concentrations and higher anion gap at the time of diagnosis compared to the healthy cows from the same herds ( $P<0.05$ ). It has been found that 70% of the affected cows had total serum Ca concentrations below the lower limit of the laboratory reference range (8.3 mg/dL) compared to 23% of the control cows with values below normal. In another study a mild hypocalcemia ( $2.2\pm 0.21$

mmol/L) has been found in LDA-affected cows compared to the control group ( $2.36\pm 0.18$  mmol/L) (Zadnik 2003). In the present study, the mean serum calcium concentration of cows with LDA was significantly lower than that of the control cows ( $P<0.05$ ) and 84% of LDA-affected cows had total serum Ca concentrations below the lower limit of laboratory reference range compared to 26% of matched control group with values below normal. The range of total serum calcium concentration for the 25 LDA-affected cases with hypocalcemia, in the present study was from 5.99 to 8.19 mg/dl and for 8 matched control cows was from 5.46 to 8.11 mg/dl. It means that the majority of our cows with LDA had a mild hypocalcemia similar to the results reported by Delgado-Lecaroz et al. (2000). Significant reduction in serum calcium concentration in cows with LDA and RDA has been shown by El-Attar et al (2007).

Post calving hypocalcaemia ( $<1.97$  mmol/L) is a significant risk factor for the development of abomasal displacement (Massey et al. 1993, Østergaard & Gröhn, 1999, Houe et al. 2001). The risk for the development of left-sided dislocation in cows suffering from hypocalcaemia two weeks after parturition was high (Massey et al. 1993, Geishauser et al. 1998, Geishauser et al. 1999). The abomasal and rumenoreticular wall tone adversely affects by hypocalcaemia (Huber et al. 1981, Delgado-Lecaroz et al. 2000).

On the contrary, Geishauser and Okentorp (1997) found that calcium is not significantly associated with displaced abomasum in cows diagnosed with LDA. In another study 20 LDA-

affected cows and 20 healthy cows were studied in southern Brazil (de Cardoso et al. 2008). They reported that serum calcium concentration of cows with LDA ( $11.86 \pm 3.6$  mg/dl) in dairy farms of southern Brazil were not significantly different from calcium concentration of normal healthy cows ( $11.35 \pm 1.76$  mg/dl) (Constable et al. 2017).

In addition to calcium concentration, phosphorous and magnesium concentrations of LDA cows were lower than those of the matched control cows, but only values for phosphorous concentration were statistically significant ( $P < 0.05$ ). Non-significant increase of serum magnesium concentration in LDA cows ( $3.15 \pm 1.49$  mg/dl) versus normal cows ( $3.02 \pm 0.53$  mg/dl) was also reported by de Cardoso et al. (2008).

In the present study significant differences were found between cows with DA and controls in blood parameters of NEFA, BHBA and AST indicating substantial alterations in metabolism. Also, elevated NEFA and BHBA concentration in LDA cows showed negative energy balance in these animals. Similar results have been reported by other researchers (Itah et al. 1998, Zadnik 2003, Van Winden & Kuiper 2003, Van Winden et al. 2003, Le Blanc et al. 2005). In the current study, the increased mean levels of BHBA and NEFA confirmed that cow with DA suffered from obvious and prolonged disturbance in energy (glucose and lipid) metabolism due to the combined pathological influences of energy imbalance (ketosis) and hepatic lipidosis. Desired BHBA concentration in dairy cows is below 1.0 mmol/L. BHBA concentration begins to increase when the animal is

submitted to enhanced energy stress (Whitaker 1997). In the present study, 76% of DA cows had serum BHBA concentration higher than laboratory reference value compared to 13% of the control cows with values above normal reference range. NEFA is a better indicator of lipid metabolism than BHBA and the optimal serum NEFA concentration is below 0.7 mmol/L (Whitaker 1997). Seventy eight percent of LDA-affected cows in the present study had serum NEFA concentrations above the optimal limit of laboratory reference range, compared to 17% of the control cows with values above normal. Elevated NEFA concentration in plasma is a prerequisite for development of hepatic lipidosis that occurs in DA cows (Rehage et al. 1996). Insulin resistance has been suggested as part of the etiology of DA (van Meirhaeghe et al. 1988). In the present study, the increased enzyme activity of AST was found in the DA cows compared to the control cows, indicating liver cell damage. A significant increase of serum AST activity ( $130 \pm 20.97$  IU/L), in LDA cows compared to the control group ( $75.13 \pm 5.67$  IU/L) in the 10-day period before development of LDA, was shown by Van Winden et al. (2003). Increased AST concentration in LDA-affected cows was reported in other studies (Zadnik, 2003, El- Attar et al. 2007, Stengärde et al. 2010).

As seen in table 2, serum calcium concentration of LDA cases with different lactations was significantly lower than those of the control cows ( $P < 0.05$ ). It is interesting that the results for the first lactation cows were similar to those of older cows. In the present study the Ca, P,

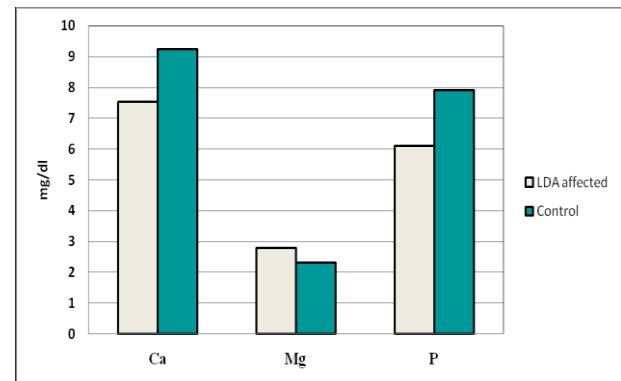
concentrations of LDA cows with different lactation were significantly lower and correspondingly BHBA, NEFA and AST concentrations in these animals were significantly higher as compared to those of the control cows (tables 2 & 3) ( $P < 0.05$ ). The difference between serum Ca concentrations for LDA cases and the control cows was not significantly affected by lactation group at the time of diagnosis. It is noteworthy that the results for 1st lactation cows were similar to those of older cows (table 1). As indicated in table 1, the serum Ca concentration of LDA cows in first lactation group was lower than the other two groups. This may be due to experiencing more stress in this group of animals.

Calving and lactation in heifer that has no previous experience in these cases will lead to greater stress tolerance. The BHBA, NEFA and AST concentration in LDA-affected cows with three or more lactations were significantly higher than those concentrations in LDA-affected cows of the other two groups ( $P < 0.05$ ). It means that the degree of negative energy balance and hepatic lipidosis in this group of cases were significantly higher than the other groups and care should be taken when treating LDA disease in these animals.

#### Conclusion

LDA is a multifactorial disease in dairy cows soon after parturition and in the first days of lactation. It causes many economic losses to herds with high incidence of disease. There are knowing the biochemical changes in cows with disease can help to better understand the disease and improve pathophysiology knowledge of disease and also

therapies. In the present study it was found that primiparous cows with LDA had worse serum calcium changes than multiparous cows. So, essential support is needed even after the surgery. Increased serum NEFA and BHBA concentration in affected cows emphasizes the need to pay attention to the energy balance and preventing the lipolysis especially in the fat cows.



**Fig 1.** Serum Ca, Mg and P concentrations of LDA affected and matched control cows.

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