



## DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE OF SERUM GASTRIN AND PEPSINOGEN IN DAIRY COWS WITH DISPLACED ABOMASUM

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### Summary

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The study aimed to describe the changes in clinical findings and serum levels of gastrin, pepsinogen and chloride (Cl) in dairy cattle with displacement of the abomasum (DA) from day 0 until day 30 after surgery and to evaluate their diagnostic and prognostic value in evaluation of the abomasal function. The study was conducted on DA cattle (n=25) belonging to dairy farms in Hokkaido area, Japan. Cows were examined and sampled at days 0 (surgery), 7 and 30. Based on blood  $\beta$ -hydroxybutyric acid (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). All DA groups had higher serum gastrin than their physiological reference values in cattle both before or after surgery. Serum gastrin was significantly increased ( $P<0.05$ ) in DA and DA SCK groups particularly at day 30 vs day 0. Serum pepsinogen and chlorides were not remarkably changed in any of the three diseased groups compared to reference values. Serum pepsinogen showed no significant within- and inter-group changes. The surgery and the 30-day follow-up period were not sufficient to serum gastrin to return to its physiological levels. In conclusion, further future studies may be required to investigate serum gastrin levels change in DA cattle. A longer follow up period up to 45 day is suggested.

**Key words:** dairy cattle, displaced abomasum, gastrin, pepsinogen

### INTRODUCTION

Displacement of the abomasum (DA) 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 & Santos 2002; Van Winden & Kuiper, 2003; Radostits *et al.*, 2007) and have a higher culling rate (Gröhn *et al.*, 1998; Raizman

& Santos 2002; Radostits *et al.*, 2007) that may reach 10% (Van Winden & Kuiper, 2003).

Current treatment costs range from \$100 to \$200 per case. 10% of the cows that are diagnosed with DA are culled or die before the next test day. Treated cows that remain in the herd produce 350 kg less milk the next month than cows without DA (Eicker, 1995).

Clinically, left displaced abomasum (LDA) characterised by gas accumulation in the abomasum resulting in a tympanic, resonant and high-toned ping sound (Breukink & Kroneman, 1963). Furthermore, diseased cows with DA were febrile with tachycardia, increased respiratory rates and ruminal hypomotility (El-Attar *et al.*, 2007).

Strong associations have 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 (Gröhn *et al.*, 1998; Gröhn, 2000; LeBlanc *et al.*, 2005).

Some studies reported the most common clinical findings and metabolic profiles associated with DA in dairy cattle (Stengärde *et al.*, 2010), DA and ketosis (Stengärde *et al.*, 2008). Furthermore, serum changes of pepsinogen (Mesarič *et al.*, 2002; Abouzeid *et al.*, 2008), gastrin (Ozturk *et al.*, 2013) and chloride (Cl) (Mokhber Dezfouli *et al.*, 2013; Ozturk *et al.*, 2013) in DA cows were also reported, however they did not discuss the clinical and biochemical changes associated with DA cows until the animals get recovered after surgical interference.

Accordingly, the current work described the pattern of changes through following up the clinical findings, milk losses, culling rate, complicated diseases and serum biochemicals including pepsinogen and gastrin in dairy cattle with DA

from day 0 before surgery until post operative day 30. The study also focused on the diagnostic and prognostic value of serum levels of gastrin, pepsinogen and chlorides in abomasal function evaluation in dairy cows with regard to their importance to recovery.

## MATERIALS AND METHODS

### *Experimental design*

The study was conducted on DA cattle (n=25) from dairy farms in Hokkaido area, Japan. DA cattle were treated surgically and by medicaments including i.v. fluid therapy with 1 L Ringer solution and 500 mL 25% glucose, and penicillin at surgery (day 0). Cows were sampled at days 0 (surgery), 7 and 30. Based on blood BHBA at day 0, DA cows were classified into three categories; DA only (n=8; <1.2 mmol/L), DA with subclinical ketosis (DA SCK; n=10; 1.2–2.4 mmol/L) and DA with clinical ketosis (DA CK; n=7; ≥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).

In this study, 44% (11 of 25) of DA cases were reported in the 1<sup>st</sup> week after calving or 1–7 days in milk (DIM), 52% (13 of 25) occurred in the 2<sup>nd</sup> and 3<sup>rd</sup> 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 with left displaced abomasum (LDA) and 16% (4 of 25) – with RDA.

### *Blood samples and analyses*

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 (Ferguson *et al.*, 1994). Rates of milk losses, culling rate and predisposing factors and complicated diseases associated with DA cases in the three diseased groups were investigated.

Blood samples were collected from the jugular vein into plain vacutainer tubes, and then were centrifuged at 3000×g for 15 min. Sera were separated and stored at -20 °C until analysis. Clinical examination and estimation of serum biochemicals were conducted 3 times; days 0, 7 and 30.

Serum gastrin levels were determined by double antibody gastrin <sup>125</sup>I radioimmunoassay (RIA Kit, DPC, USA) according to Kataria *et al.* (2008).

The serum pepsinogen value was measured by the colorimetric method of Edwards *et al.* (1960) with modifications (Mylrea & Hotson, 1969; Berghen *et al.*, 1987). Plasma was added to a substrate mixture (0.9 % bovine albumin fraction V in glycine buffer, pH 2.0) and mixed thoroughly. An aliquot of this mixture was incubated in a water bath at 37 °C for 24 h. Then reaction was stopped by adding 4% trichloroacetic acid solution (Mylrea & Hotson, 1969). After filtration of the protein precipitate the concentration of acid soluble tyrosine was determined by Folin Coicalteau's reagent. For this purpose, 0.5 mL of Folin Coicalteau's reagent and 10 mL of 0.25N sodium hydroxide were added to 2 mL of filtrate. Two mL of tyrosine standard was also treated in the same way. The optical density was read at 680 nm wavelength using a spectrophotometer (GeneQuant 100, GE healthcare, Japan). The enzyme activity

was expressed as milli-units (mU) tyrosine (Reid *et al.*, 1967).

Serum chlorides were determined by atomic absorption spectrophotometer with an autoanalyzer (Shimadzu Model AA 6200, Tokyo, Japan).

### *Statistical analysis*

All statistical analyses were performed using Computer Soft Ware (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±SD at P<0.05 (Spsswin, 1997).

## RESULTS

The obtained results revealed that 29.41% and 70.59 % of LDA cases happened in the 1<sup>st</sup>, and the 2<sup>nd</sup> and 3<sup>rd</sup> weeks of lactation, respectively. 50%, 25 % and 25% of the RDA cases were reported in the 1<sup>st</sup> week, the 2<sup>nd</sup> and 3<sup>rd</sup> weeks of lactation, and 128 DIM, respectively.

The culling rate of the examined cows was 16% (4 of 25). The rates of milk loss were 233.45±73.53, 217.91±81.38 and 291±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 – ketosis, retained placenta, milk fever, mastitis and metritis. They were also considered as complication of DA (56%; 14 of 25).

The clinical parameters (temperature, pulse and respiratory rate) in DA groups were within the physiological reference range. Ruminal movements were reduced in the diseased cows at day 0 then after the surgical correction of DA they improved at days 7 and 30. BCS (Table 1) showed no significant changes neither between the diseased groups (DA SCK or DA CK) when their values compared with those of DA group at days 0, 7 and 30, nor within each DA group when their values at days 7 and 30 compared with those at day 0. BCS was still within the physiological reference values.

Serum pepsinogen concentrations (Table 2) were not remarkably changed neither at days 7 or 30 in each of DA groups vs day 0, nor between the diseased groups. They were within the physiological reference values.

The reported results mentioned that serum gastrin levels (Table 3) in all DA cases throughout the present study either before or after operation were higher than the physiological reference values of blood gastrin in cows. Serum gastrin was significantly increased ( $P<0.05$ ) in DA group at days 7 and 30, and DA SCK group at day 30 as compared with those in

**Table 1.** Body condition scores in cows with displaced abomasum (DA), displaced abomasum with subclinical ketosis (DA SCK) and displaced abomasum with clinical ketosis (DA CK) at surgery (day 0) and at post operative days 7 and 30. Data are presented as mean±SD

	Day 0	Day 7	Day 30
DA group (n=8)	3.06±0.50	3.0±0.40	2.88±0.38
DA SCK group (n=10)	3.20±0.48	3.13±0.32	3.00±0.24
DA CK group (n=7)	3.07±0.37	2.96±0.17	2.82±0.24

**Table 2.** Serum pepsinogen (mU) in cows with displaced abomasum (DA), displaced abomasum with subclinical ketosis (DA SCK) and displaced abomasum with clinical ketosis (DA CK) at surgery (day 0) and at post operative days 7 and 30. Data are presented as mean±SD

	Day 0	Day 7	Day 30
DA group (n=8)	1193.33±263.67	960.15±319.30	1102.76±257.70
DA SCK group (n=10)	1002.50±260.16	1002.32±318.53	885.62±178.07
DA CK group (n=7)	878.15±251.83	915.98±184.89	1056.08±273.03

**Table 3.** Serum gastrin (ng/L) in cows with displaced abomasum (DA), displaced abomasum with subclinical ketosis (DA SCK) and displaced abomasum with clinical ketosis (DA CK) at surgery (day 0) and at post operative days 7 and 30. Data are presented as mean±SD

	Day 0	Day 7	Day 30
DA group (n=8)	195.00±30.88	323.00±91.63**	434.25±73.81**
DA SCK group (n=10)	199.56±90.86	262.25±77.43	337.78±59.24**
DA CK group (n=7)	239.00±91.24	463.50±263.07	499.83±206.94

\*  $P<0.05$ ; \*\* $P<0.01$  compared with the value at day 0 in each DA group.

**Table 4.** Serum chlorides (mmol/L) in cows with displaced abomasum (DA), displaced abomasum with subclinical ketosis (DA SCK) and displaced abomasum with clinical ketosis (DA CK) at surgery (day 0) and at post operative days 7 and 30. Data are presented as mean±SD

	Day 0	Day 7	Day 30
DA group (n=8)	97.50±6.41	99.38±3.11	93.63±14.09
DA SCK group (n=10)	97.40±7.37	101.30±4.85	95.80±13.91
DA CK group (n=7)	96.57±3.05	101.00±3.83	101.67±5.05

day 0. Gastrin concentrations were not remarkably changed in DA CK group throughout the study, however they started to increase gradually after the surgery until day 30 but this increase was insignificant. Serum gastrin showed no significant changes between DA groups when their values in DA group were compared with those in the other two groups on days 0, 7 or 30.

There were no significant changes in serum levels of chlorides (Table 4) which were within the reference ranges during the entire period of the study.

## DISCUSSION

The current study stated that DA caused economic losses in DA, DA SCK and DA CK groups, due to milk loss, increased treatment costs and culling rates. Other studies also report that 10% of cows with DA are culled or die (Van Winden & Kuiper, 2003). The DA cattle produce less milk at least in the short term (Raizman & Santos, 2002) and have a higher culling rate (Raizman & Santos, 2002; Van Winden & Kuiper, 2003).

DA in the present study is a common disease of dairy cattle in early lactation with in the first 3 weeks post-calving; 44% in the 1<sup>st</sup> week, 52 % occurred in the next 2 weeks. Previous reports mention that LDA commonly occurs in the post-partum period (3 to 7 weeks postpartum)

(Rohn *et al.*, 2004) and most frequently in highly lactating dairy cows during early lactation (Veysi *et al.*, 2003; El-Attar *et al.*, 2007).

The obtained results revealed that occurrence rates of LDA and RDA were about 84% and 16%, respectively in agreement with reports indicating that 80 to 90% of all DA are left-sided (Guard, 1990; Pehrson & Shaver, 1992). Also, 80 to 90% of LDA are diagnosed within 1<sup>st</sup> month after calving (Pehrson & Shaver, 1992).

The current study stated that 29.41% and 70.59 % of LDA cases happened in the 1<sup>st</sup>, and in the 2<sup>nd</sup> and 3<sup>rd</sup> weeks of lactation, respectively. 50%, 25% and 25% of the RDA cases were reported in the 1<sup>st</sup> week, the 2<sup>nd</sup> and 3<sup>rd</sup> weeks of lactation, and 128 DIM, respectively. It has been acknowledged that the LDA and RDA were encountered within 6 weeks (Radostits *et al.*, 2007) and 3–6 weeks after parturition (Delgado-Lecaroz *et al.*, 2000), respectively.

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. Other studies also reported an association of ketosis, hypocalcaemia and milk fever with DA (Massey *et al.*, 1993; Oikawa *et al.*, 1997; Care *et al.*, 1999).

Duffield & 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 found out that 56% of DA cases were complicated by ketosis, milk fever, retained placenta, metritis and/or mastitis. DA increases the risk of other postpartum disorders. This agreed with Curtis *et al.* (1985), Radostits *et al.* (2007) who mentioned that dairy cattle with LDA were at increased risk for ketosis and metritis.

The clinical findings in all DA cases showed no significant changes in temperature, pulse, respiratory rates and BCS neither between the diseased groups nor within each DA 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, cows with DA were reported to be febrile with increased heart and respiratory rates, ruminal hypomotility (El-Attar *et al.*, 2007), anorexia and reduced milk yield (Ozturk *et al.*, 2013).

The obtained results stated no significant changes in BCS in all DA cases neither before or after surgical treatment of DA. BCS was not associated with a risk of LDA. The same was demonstrated by LeBlanc *et al.* (2005) who observed that prepartum BCS was not associated with a risk of LDA. Other studies reported that cows with excess BCS at calving are at increased risk for LDA (Dyk, 1995).

Serum pepsinogen levels in the three DA groups were within the physiological reference values mentioned by Ohwada *et al.* (2002). The present study also indicated that serum pepsinogen showed no significant changes between the diseased DA groups. On the other hand, cows with abomasitis, LDA, RDA and abomasal

ulcers have increased serum pepsinogen levels (Mesarič *et al.*, 2002). Hajimohammadi *et al.* (2010) confirmed that serum pepsinogen levels were significantly higher in LDA and RDA affected sheep (investigated as a model for cattle). In contrast, Ohwada *et al.* (2002) proved that serum pepsinogen concentrations in LDA, RDA and AV cows were significantly decreased compared with healthy cows. Abouzeid *et al.* (2008) also mentioned that the pepsinogen values in all cows with DA at day 0 were remarkably decreased compared to the reference range. Moreover, there were no significant differences among cows with LDA, RDA and AV.

All DA cases throughout this study had higher serum gastrin concentrations than physiological reference values mentioned by Ok *et al.* (2001). both before or after surgery. This may indicate that the operation and 30-day follow-up period were not long enough until for restoration of gastrin to its physiological levels. Serum gastrin was significantly increased in DA group and DA SCK group particularly at day 30 vs day 0, but not remarkably changed in DA CK although it started to increase gradually but insignificantly until day 30. Ozturk *et al.* (2013) reported proofs for increased serum gastrointestinal motility and hormone concentrations including ghrelin, motilin and gastrin in cattle with DA.

The current study reported no significant changes in serum chlorides neither between the diseased DA groups nor within each DA group. Chloride concentrations were within the physiological reference ranges (Radostits *et al.*, 2007). In contrast, it was reported that cows with DA or AV were associated with hypokalemia (Constable *et al.*, 2013; Mokhber Dezfouli *et al.*, 2013), hypochloremia

(Radostits *et al.*, 2000; Abouzeid *et al.*, 2008; Mokhber Dezfouli *et al.*, 2013) and low feed intake (Constable *et al.*, 2013). Ozturk *et al.* (2013) mentioned that cattle with DA had low serum Cl concentrations.

## CONCLUSION

In this study there were no other prominent significant changes either clinically (BCS) or biochemically (serum pepsinogen and chlorides) either in each DA group or between the diseased groups. Pepsinogen and chloride levels were within the physiological reference ranges. Serum gastrin concentration were higher than the physiological reference values throughout the current study indicating that the 30 day follow up period was not long enough to allow for full restoration of the physiological gastrin level and for complete recovery of the abomasal mucosa. Further studies may be required to investigate serum gastrin levels in cattle with displaced abomasum, suggesting a longer follow-up period up to the 45<sup>th</sup> post operative day.

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