Evaluation of the hormones responsible for the gastrointestinal motility in cattle with displacement of the abomasum; ghrelin, motilin and gastrin

A. S. Ozturk, M. Guzel, T. K. Askar, I. Aytekin

This study provides the evidence of increased serum gastrointestinal motility hormone concentrations including ghrelin, motilin and gastrin in cattle with displacement of abomasum (DA). In this study, 38 cows with DA (21 left DA (LDA) and 17 right DA (RDA)) and 15 healthy controls were included. All cattle with DA were at the stage of postpartum one to eight weeks, and had clinical signs including anorexia, decreased milk yield and scanty, pasty faeces. Serum ghrelin, motilin and gastrin concentrations, and leptin concentration which is a functional antagonist of ghrelin, were determined by ELISA. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), Na, K, Cl, Ca and P concentrations were measured by spectrophotometer. In serum biochemical analysis, increases were seen on the serum ALT, AST and GGT activities; however, serum Na, K, Cl and P concentrations decreased in abomasal displacement compared with the control animals. The serum ghrelin, motilin and gastrin concentrations increased in the cattle with LDA and RDA, as compared with those in the healthy controls. On the other hand, serum leptin concentration decreased in the cattle with DA compared with the controls. Increases in the serum ghrelin, motilin and gastrin concentrations might be attributed to activation of gastrointestinal motility hormones to enhance of gastric emptying in impaired gastric motility and/or outlet occlusion in displaced abomasum.

Introduction
Displacement of abomasum (DA) is a common disease of dairy cattle in early lactation. Left displaced abomasum (LDA), right displaced abomasum (RDA), and abomasal volvulus are characterised by varying degrees of abomasal distension and displacement (Constable and others 1991). The aetiology is multifactorial, and a number of nutritional and management conditions are important risk factors. Impairment of abomasal motility and gas accumulation in the abomasum are two commonly known factors that contribute to the development of DA (Constable and others 1992, Van Winden and Kuiper 2003, Doll and others, 2009). Gastrointestinal motility depends on a complex interaction between neuromuscular and hormonal pathways (Koenig and Cote 2006). Several current studies have focused on abomasal motility in DA (Geishauser and others 1998b), abomasal emptying and prokinetic drugs use in cattle and calves (Constable and others 2006, Wittek and Constable 2005). However, there is little knowledge about gastrointestinal motility hormone concentrations except gastrin (Sen and others 2002) in cattle with DA.

Ghrelin is a 28-amino acid peptide, produced mainly in the oxyntic glands in the stomach of monogastrics and the abomasum of ruminants (Hayashida and others 2001). It was discovered in 1999 in rat stomach (Kojima and others 1999), and has been suggested as a hormone that stimulates gastrointestinal motility in a manner similar to gastrointestinal peptides, such as motilin, cholecystokinin and gastrin (Inui and others 2004, Poitras and Tomasetto 2009). Ghrelin and motilin, gastrointestinal peptides, have a number of common features. They are structurally similar, synthesised in the upper gastrointestinal tract, and have prokinetic activity on gastrointestinal motility (Ohno and others 2010). Similarly, gastrin is synthesised and secreted from neuroendocrine G cells which are principally located in the gastric antrum and stimulate gastrointestinal motility and gastric emptying (Misiewicz and others 1969, Zhang and others 2011). Leptin, is a functionally opposed action of ghrelin, acts as a major regulator of hormone for food intake and energy homeostasis (Delavaud and others 2000).

Since the impaired abomasal motility is suspected in the development of abomasal displacement, in this study, we aimed to evaluate serum gastrointestinal motility hormone concentrations including...
Materials and methods
A total of 53 Holstein-Friesian cattle, 21 cattle with LDA (LDA group), 17 cattle with RDA without volvulus (RDA group) and 15 clinically healthy adult cattle with no previous history of gastrointestinal disease (control group) were included in this study. All the cattle with DA were within the postpartum period varying from one to eight weeks (mean three weeks). The ages of the affected cattle varied from 2.5 to 8 years (mean 3.9 years). The cattle had been ill on average for three days (2–10 days). The control animals (two to five years old) were selected in the early lactation (two to six weeks) from the same environmental conditions.

Clinical examinations were performed, and the respiratory and pulse rates and body temperatures were recorded. Abdominal percussion and auscultation at the left or right rib cages were conducted. After simultaneous auscultation and percussion, rectal examination and abdominal ultrasonography were conducted. The diagnosis of DA was confirmed by laparotomy.

A total of 8 ml blood samples were collected via jugular venipuncture into the silicone gel coated tubes. Following clotting, serum samples were separated by centrifugation at 1550 g for 10 minutes at room temperature and stored at −80°C until biochemical analyses. Serum samples were analysed spectrophotometrically (Roche Diagnostics) for alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), Na, Cl, Ca and P concentrations. Serum ghrelin (Phoenix-EK, 031/30), motilin (USCN-E, 90575), gastrin (Raybiotech-EIA GAS-I) and leptin (DRG-EIA, 2395) concentrations were also analysed by commercial ELISA test kits according to the manufacturer’s instructions. To the authors’ knowledge, the activity of the peptide hormone analysis methods used in this study (ghrelin, motilin, gastrin and leptin) has not been validated in cattle and, as such, our results should be viewed as preliminary. The assays were assumed to be valid based on considerable sequence homology and validation of the peptide hormone analysis methods used in this study (ghrelin, motilin, gastrin and leptin) which is a functional antagonist of hipokalemiemia and hypophosphataemia were detected in cattle and other mammalian species as reported by researchers:

First, Shapiro-Wilk test was used for evaluating the normal distribution of the variables. Then, one-way analysis of variance (ANOVA) was performed in a completely randomised design: $Y_i = \mu + \alpha_i + e_i$ where $Y_i$ are observation values (motilin, gastrin, leptin, AST, ALT, Na, Cl, Ca and P); $\mu$ is the overall mean; $\alpha_i$ is the effect of the groups (LDA, RDA and control) and $e_i$ is residual error. TUKEY Multiple Range test was then used to separate these differences as well. Also, Kruskal-Wallis test, a non-parametric test, was performed to test whether there were any differences among the three groups for ghrelin and K concentrations. Then, Mann-Whitney test was applied to determine any further differences among the groups.

### Results
All the cattle with DA were afebrile with normal pulse and respiratory rates; however, poor appetite, sudden drop in milk yield, little defecation and scanty, pasty faeces, and decreased rumen contraction frequency were observed. In the simultaneous auscultation and percussion of the left and right abdominal wall, high-pitched resonant pings and splashing sounds were acquired over the last three ribs.

### Discussion
This study reported the evidence of increased serum gastrointestinal motility hormone concentrations including ghrelin, motilin and gastrin in cattle with DA. The clinical examination of the cattle with DA recorded within the early postpartum period indicated poor appetite, sudden drop in milk yield, reduction of rumination, scanty, pasty faeces, and high-pitched resonant pings were audible on simultaneous percussion and auscultation of the dorsal flank. All the affected cattle were afebrile with normal pulse and respiratory rates. The clinical findings observed in this study were similar to those previously reported by Constable and others (1991) and Sen and others (2002).

As can be seen from Table 1, serum biochemical changes in cattle with DA revealed significant decreases in serum Na, K, Cl and P concentrations. In this study, hyperkalemia, hypochloremia and hypophosphataemia were detected in cattle with DA compared with the control animals. They were agreeable with those of Zadnik (2003), Rohn and others (2004), Kalaitzakis and others (2010) and Sahinurkan and Albay (2006). Hyperkalemia, hypochloremia and hypophosphataemia are explained by alimentary tract stasis in cattle with DA (Rohn and others 2004). Potassium and chloride are trapped in the rumen and the abomasum, respectively (Geishausser and others 1996). Serum potassium concentrations are also reduced by renal K losses and alkalosis. Hiponatremia may be

| TABLE 1: Concentration of serum ghrelin, motilin, gastrin, leptin and other serum biochemical parameters in left displaced abomasum (LDA), right displaced abomasum (RDA) and control animals |
|---------------------------------|-------------|-------------|-------------|-------------|
| LDA (n:21)                      | RDA (n:17)  | Control (n:15)| P value    |
| Ghrelin (ng/l)                  | 173 ± 47  | 165 ± 42  | 115 ± 17  | < 0.001   |
| Motilin (ng/l)                  | 372 ± 64  | 350 ± 67  | 229 ± 23  | < 0.001   |
| Gastrin (ng/l)                  | 257 ± 49  | 226 ± 43  | 138 ± 23  | < 0.001   |
| Leptin (ng/l)                   | 3.28 ± 0.22 | 3.29 ± 0.24 | 3.63 ± 0.20 | < 0.001   |
| ALT (IU/l)                      | 51 ± 16   | 49 ± 15   | 22 ± 5    | < 0.001   |
| AST (IU/l)                      | 113 ± 10  | 139 ± 19  | 101 ± 19  | < 0.001   |
| GGT (IU/l)                      | 46 ± 11   | 49 ± 13   | 29 ± 7    | < 0.001   |
| Na (mmol/l)                     | 120.6 ± 10.7 | 122.0 ± 10.7 | 140.3 ± 7.0 | < 0.001   |
| K (mmol/l)                      | 3.4 ± 0.4  | 2.7 ± 0.6  | 4.8 ± 0.4  | < 0.001   |
| Cl (mmol/l)                     | 85.7 ± 13  | 96.2 ± 14.2 | 103.5 ± 7.7 | < 0.001   |
| Ca (mmol/l)                     | 1.95 ± 0.35 | 2.04 ± 0.38 | 2.27 ± 0.60 | < 0.090    |
| P (mmol/l)                      | 1.44 ± 0.31 | 1.43 ± 0.42 | 1.96 ± 0.41 | < 0.001    |

Superscript a, b and c indicate that differences are statistically significant among groups marked with different letters on the same line. The data were presented as mean ± sd and the significance concentration was set as $P < 0.05$. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.
caused by renal losses during the early alkalosis stage of disease. It is known that hypocalcaemia is an important risk factor for the left displacement of the abomasums (Van Winden and Kuiper 2005). However, in this study, there was no significant difference in serum Ca concentrations between DA and control groups. On serum hepatocellular enzyme activities, there were significant increases in ALT, AST and GGT in both groups. Liver damage is the most important risk factor in DA (Geihauser and others 1998a). The increased hepatocytic enzyme activities can be related to liver cell damage and possibly hepatic lipidosis.

In this study, mean serum ghrelin, motilin and gastrin concentrations were significantly increased in cows with LDA and RDA compared with the control animals (Table 1). Several reasons may be attributed to the elevated ghrelin concentration. Ghrelin has an important role in regulating appetite, feeding and energy metabolism (Inui and others 2004). Serum ghrelin concentrations can change with acute as well as chronic eating status. Fasting causes elevation on the ghrelin concentration which decreases immediately after food intake (Korbonits and others 2004). The increase in serum ghrelin concentrations may also be linked to anorexia in DA. However, Chan and others (2004) have reported that prolonged fasting for three days has not changed ghrelin concentrations, suggesting that the eating-related changes are rather decreases after food intake than increases due to fasting. We also evaluated serum leptin concentration which has functionally an opposed action of ghrelin because of its regulating role on food intake and energy homeostasis. Plasma concentration of leptin is affected by nutrition and adiposity. Leptin can decrease in fasting and negative energy balance, and an elevation can be seen in obesity (Meier and Gressner 2004). In this study, serum leptin concentrations decreased in cattle with DA compared with the control animals. Similarly, complete food deprivation, or long-term food restriction, caused a rapid decrease in leptin concentration in ruminant (Delavaud and others 2006). However, in this study, mean serum leptin in DA may be due to anorexia and negative energy balance.

A reduced expression of motilin might lead to an insufficient gastrointestinal motility. Abomasal emptying rate is decreased in cows with LDA, and is further decreased immediately after surgical correction of LDA. This is also supported by results in cows undergoing surgical correction of LDA, the motilin agonist of erythromycin increases the abomasal emptying rate by binding to motilin receptors. (Wittek and Constable 2005). Momke and others (2012) recently reported that motilin plays an important role in the development of LDA, and the non-coding motilin transcription factor binding site mutation FN296874:g.90T>C is significantly associated with motilin expression concentrations and the incidence of LDA in German Holstein. However, in this study, mean serum motilin concentrations were significantly increased in cows with LDA and RDA compared with the control animals. The increased serum motilin concentrations with other gastrointestinal motility hormones, like ghrelin and gastrin, might be in hypomotility or outlet obstruction. Nishizawa and others (2006) reported an increase in the plasma concentrations of ghrelin in patients with dysmotility-like functional dyspepsia originating from delayed gastric emptying. Iwasaki and others (2012) recently reported that experimentally induced gastric outlet obstruction induces increased gastric ghelin production and plasma ghelin concentrations. In displaced abomasum, the passage of food to the intestines is partly or totally blocked due to outlet obstruction. Increased concentrations of gastric motility hormones can be stimulation for evacuation of abomasal content in displaced abomasum.

Gastrin is also a gastrointestinal motility-regulating hormone that causes increased motility in the stomach and stimulates gastric emptying (Zhang and others 2011). In the present study, serum gastrin concentration increased in the cattle with DA, like ghrelin and motilin. The findings hereby are in parallel with the previously reported results by Sen and others (2002). It was reported in human beings with Zollinger-Ellison syndrome, atrophic gastritis, autoimmune gastritis, gastric outlet obstruction, previous vagotomy, chronic renal failure and short-bowel syndrome (Arnold 2007). In this study, hypergastrinemia in DA may be attributable to abomasal hypomotility or outlet obstruction.

This study provides the evidence of increased serum gastrointestinal motility hormone concentrations including ghrelin, motilin and gastrin in cattle with DA. It was concluded that the increased serum ghrelin, serum motilin and gastrin concentrations in DA could be due to activation of these hormones to enhance of gastric emptying of impaired in abomasal motility and/or outlet occlusion in displaced abomasum.

**Funding**
This study was supported by the Scientific Research Projects Office of Mustafà Kemal University (Project No: 10GS1M101).


ZHANG, Q., YU, J. C., KANG, W. M. & ZHU, G. J. (2011) Effect of ω-3 fatty acid on gastrointestinal motility after abdominal operation in rats. Mediators of Inflammation 2011, 1–4
Evaluation of the hormones responsible for the gastrointestinal motility in cattle with displacement of the abomasum; ghrelin, motilin and gastrin

A. S. Ozturk, M. Guzel, T. K. Askar and I. Aytekin

Veterinary Record 2013 172: 636 originally published online May 30, 2013
doi: 10.1136/vr.101322

Updated information and services can be found at:
http://veterinaryrecord.bmj.com/content/172/24/636

These include:

References
This article cites 35 articles, 5 of which you can access for free at:
http://veterinaryrecord.bmj.com/content/172/24/636#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/