

Investigation of plasma pepsinogen level in calves with abomasal distention

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ABSTRACT

The aims of the present study was to determine whether or not abomasal damages occurs in calves with abdominal distention using by plasma pepsinogen levels and the positivity of the fecal occult blood test (FOBT). In the study, 30 calves with abdominal distention (experimental group) and 15 clinically healthy calves (control group), aged between 1-90 days, were used. Plasma samples were used to determine plasma pepsinogen levels, using bovine specific ELISA. Fresh stool samples used to determine occult blood in the samples. Melena and occult blood were detected in 11 and 19 of the stool samples, respectively. The plasma pepsinogen levels of calves with abdominal distention (18.06 ± 7.78 ng/ml) were significantly high compared to those of control group (6.70 ± 2.08 ng/ml), ($p < 0.001$). In addition, plasma pepsinogen levels were higher in melena (+) animals than both FOBT (+) animals and control animals ($p < 0.001$). It is suggestive that plasma pepsinogen levels could be used to determine abomasal damage in calves, especially with abdominal distention. It can be also useful biomarker to determine the severity of abomasal damage in calves.

INTRODUCTION

Abdominal distention is a general symptom of gastrointestinal disorders in cattle (Peek and Divers, 2018). The main cause of abdominal distention in ruminants is excessive gas production due to abnormal fermentation in the rumen, resulting in ruminal tympany. Certain disorders cause abdominal pain including abomasal ulcers, displacement of the abomasum, intestinal volvulus, and torsion also cause abdominal distention in cattle (Gouda et al., 2020; Radostits et al., 2007).

Abomasal ulcer is one of the causes of abomasal distension in cattle of all the ages. Signs of abomasal epithelial damage can range from the absence of clinical signs to hemorrhage followed by melena and peritonitis if erosive course spread all stratum of the abomasum. In recent years, the frequency of abomasal mucosal diseases in cattle is becoming more endemic with the transition to contemporary compact manufacture (Cable et al., 1998; Radostits et al., 2007).

Detection of abomasal ulcers is often confused with other causes of alimentary disorders with symptoms of indigestion. The clinical symptoms of abomasal ulcers are nonspecific and so variable, which are take place on a very wide scale (Cable, 1998; Radostits et al., 2007). Detection of abomasal ulcers is based on clinical symptoms and fecal occult blood test (FOBT) (Hajjimohammadi et al., 2017). However, clinical symptoms are not sufficient for its definitive diagnosis and some abomasum specific biomarkers such as pepsinogen and

gastrin need to be analysed.

Pepsinogen is an inactive form of pepsin, which is produced by parietal cells of the abomasal mucosa. Elevated serum pepsinogen activity indicate the presence of abomasal ulcer and also, in some cases, to the presence of other damage to the abomasal mucous membrane (Mesaric, 2005). The increase in plasma pepsinogen level occurs via leakage of pepsinogen from the damaged abomasal mucosa into the blood vessels (Harvey et al., 1983). It is well-known that, increased acidity of stomach contents increase activation of pepsinogen to pepsin, results in ulcers formation in humans and animals (Kataria, 2008; Mesaric, 2005). Biomarkers such as plasma gastrin and pepsinogen (Mesaric et al., 2000, Ok et al., 2001; Zadnik and Mesaric, 1999) have been studied in some gastrointestinal disorders such as abomasum displacement and abomasum ulcer in cattle. However, plasma pepsinogen levels have not been analysed in calves with abdominal distention, associated with abomasal ulcers or other gastrointestinal disorders. On the other hand, plasma pepsinogen levels has been suggested to be a suitable markers for evaluating the degree of gastrointestinal damages (Mesaric, 2005; Kataria, 2008).

The aims of the present study was to define whether or not abomasal damages occurs in calves with abdominal distention using by plasma pepsinogen levels and the positivity FOBT.

MATERIALS AND METHODS

Animals

In the study, 30 calves with abdominal distension (experimental group) and 15 clinically healthy calves (control group), aged between 1-90 days, were used. Routine clinical examinations including body temperature, heart rate, respiratory rate, skin elasticity test, and control of capillary filling time, mucous membranes, defecation and teeth grinding were performed in all the calves. Presence of rumen atony, abdominal tension, groaning sound on deep palpation over the abomasum from the right side were also examined.

According to the abdominal examination, right or both sided abdominal distension were diagnosed in 30 calves without enteritis and then they were used as experimental group. Furthermore, according to the macroscopic stool inspection and FOBT, 30 animals with abdominal distension were divided into two groups as melena positive (n=11) and FOBT (n=19).

Laboratory analysis

Hematology

Blood samples were collected into blood tubes with K₃E-DTA (Greiner Bio-one, Austria). These blood samples were used to establish total white blood cell, granulocyte, lymphocyte, monocyte and red blood cell counts and percentage of hematocrit (Abacus Junior, Diatron MI, Hungary)

Pepsinogen analysis

Blood samples were withdrawn from vena jugularis into heparinized blood tubes (Greiner Bio-one, Austria) and centrifuged to collect plasma samples. Collected plasma samples were kept at -80 °C until used. The pepsinogen levels were determined by using bovine specific ELISA kits (MyBiosource, San DiegoUSA) according to the producer directives.

The optical density (OD) of each well for pepsinogen was defined with a micro-ELISA plate reader (MR-96A, Mindray-China) at a test wavelength of 450 nm. The concentrations of plasma pepsinogen was calculated regression analysis on the basis of standard curve derived from two-fold dilutions of pepsinogen standard stock solution. The sensitivities of the ELISA kit for pepsinogen was 0.5 ng/ml.

Fecal occult blood test (FOBT)

Rectal fresh stool samples were collected from each animal and used to defined blood in stool samples, using FOBT (Gikan tets, Türklab, İzmir, Turkey) according to the manufacturer instructions.

Statistical analysis

Kolmogorov Smirnov test was used to define normality of distribution of the data. The significance of the differences in values between experimental and control groups were defined by Student's t test. One way Anova (posthoc Duncan) test was used to compare the differences in values between control, melena (+) and FOBT (+) calves. All the values were

Table 1. Hematologic values in control and experimental group (Mean±SD).

Parameter	Control Group (n:15)	Experimental Group (n:30)	p value
WBC (x10 ⁹ /l)	9,63±2,39	10,06±5,56	0,722
LYM (x10 ⁹ /l)	5,61±1,30	4,88±1,82	0,132
MID (x10 ⁹ /l)	0,17±0,17	0,24±0,31	0,290
GRA (x10 ⁹ /l)	6,17±8,67	4,94±4,44	0,612
RBC (x10 ¹² /l)	8,37±0,93	7,77±1,19	0,075
HGB (g/dl)	8,94±1,49	8,59±1,46	0,466
HCT (%)	31,03±5,39	29,89±4,72	0,491
MCV (fl)	36,93±4,62	38,60±4,23	0,252
MCH (pg)	10,70±1,02	11,15±0,97	0,175
MCHC (g/dl)	29,05±1,26	29,00±1,28	0,902
RDWc (%)	29,66±3,80	27,49±3,34	0,072
PLT (x ⁹ /l)	638,00±215,33	585,56±199,92	0,438
PCT (%)	0,43±0,14	0,40±0,14	0,604
MPV (fl)	6,74±0,47	6,91±0,53	0,267
PDWc (%)	33,12±2,09	33,08±2,06	0,944

WBC: total white blood cell count, LYM: lymphocytes count, MID: monocytes and eosinophils, GRA: granulocytes, RBC: red blood cell count, HGB: haemoglobin, HCT: haematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDWc: red cell distribution width, PLT: platelet count, PCT: platelet percentage, MPV: mean platelet volume, PDWc: platelet distribution width
Significant level was accepted as p<0.05.

indicated as mean and standard deviations of the mean (mean \pm SD). The level of significance was acknowledged as $p < 0.05$. SPSS software computer programme (version 14.01 for Windows, SPSS Inc. Chicago) was used to perform all the statistical analyses.

The cut-off value for pepsinogen was determined by adding twice the standard deviation of the control group to the mean of the control group. Concentrations greater than the cut off point (10.87ng/ml) were considered increases for each

animal. Thus, the cut off value for pepsinogen was then used to define the number of animals with increased plasma pepsinogen levels (Rastawicki et al. 2011, Sharma and Jain 2013).

RESULTS

Clinical findings

The clinical symptoms were partial or complete anorexia, depression, eye recession into the orbit, increase or decrease

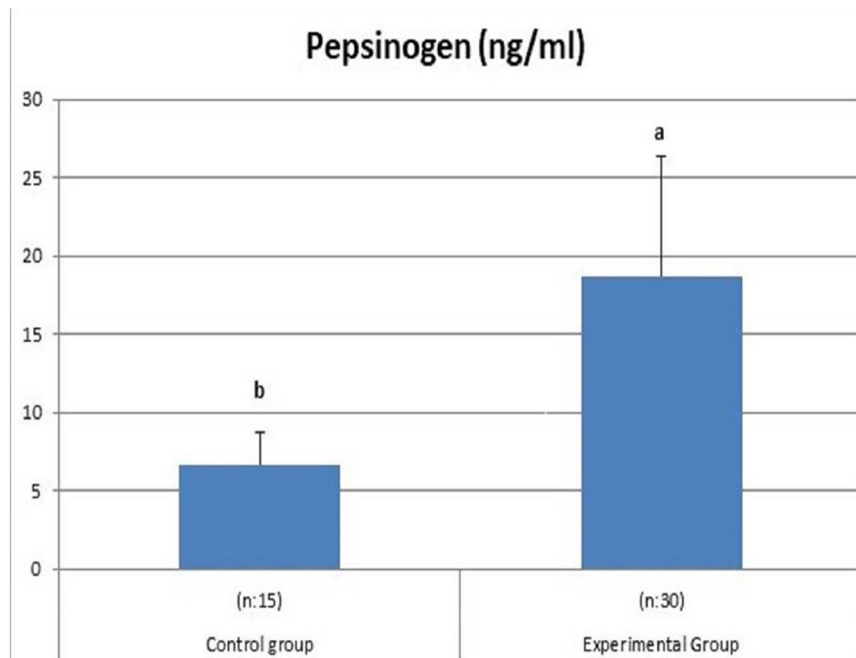


Figure 1. Plasma pepsinogen concentrations of control and experimental groups (Mean \pm SD).

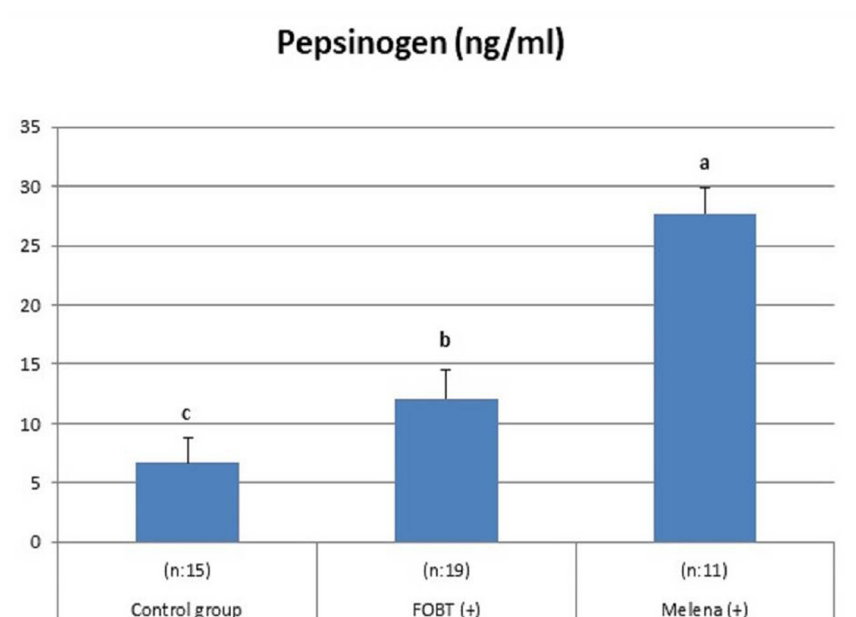


Figure 2. Plasma levels of pepsinogen in control, FOBT (+) and melena (+) calves (Mean \pm SD).

in appetite and variable heart rate, increase in respiratory rate, decrease in skin elasticity, prolongation of capillary filling time, pale mucous membranes, cold extremities, decreased defecation, and teeth grinding in calves with abdominal distention. In some of these animals, melena, raising their feet towards the abdomen, tachypnea, rumen atony, abdominal tension, groaning sound on deep palpation over the abomasum from the right side and tremors in the muscles were detected.

Laboratory findings

No statistically significant difference was found in the hematological parameters of the experimental and control groups (Table 1). Red blood cell count and hematocrit percentage were tented to be decrease in calves with abdominal distention compared to those of control group. However, red blood cell count and hematocrit percentages of calves with abdominal distention were not statistically different compared to that of control group (Table 1).

In the study, plasma pepsinogen levels were 6.70 ± 2.08 ng/ml and 18.48 ± 7.86 ng/ml in control calves and calves with abdominal distention, respectively. The pepsinogen values of calves with abdominal distention were defined to be significantly higher than control group ($p < 0.001$) (Figure 1). In addition, 19 calves with abdominal distention were found to be positive for FOBT while, other 11 calves were melena positive. The plasma pepsinogen values in melena (+) calves were significantly high compared to those of FOBT (+) calves and control calves ($p < 0.001$) (Figure 2). According to the cut-off value, plasma pepsinogen levels increased in 24 calves with abdominal distention.

DISCUSSION

In the present study, plasma concentrations of pepsinogen were significantly higher in calves with abdominal distention than control group ($p < 0,001$) (Figure 1). In addition, 24 calves with abdominal distention had high plasma pepsinogen concentration compared to the cut-off value. Furthermore, plasma pepsinogen values were significantly high in melena pozitiv calves compared to those of both FOBT positive and control calves $p < 0.001$ (Figure 2). High plasma pepsinogen concentrations are thought to be associated with vascular permeability in the abomasum, such as the melena group in this study, and any damage to the gastric mucosa allows the diffusion of hydrogen ions from the lumen to the mucosal tissues similarly to previous studies. The studies report that the diffusion of pepsin and pepsinogen to the mucosa causes injury to the gastric mucosa (Hajimohammadi et al., 2010; Voros et al., 1984; Zadnik and Mesaric, 1999). These reports also support our results (Figure 2).

Abomasal damage was not confirmed with necropsy in the present study but based on the previous studies in cattle (Hajimohammadi et al., 2010; Voros et al., 1984; Zadnik and Mesaric, 1999), the increase in plasma pepsinogen concentrations detected in this study may indicate the occurrence of abomasal damages in calves.

Abdominal distention is a common manifestation of many gastrointestinal disorders in cattle (Peek and Divers, 2018). Ab-

dominal distention occurs due to abnormal accumulations of food, gas or fluid in the abdominal cavity. This may occur due to accumulation of gases resulting in abnormal fermentation or food retention in case of impaction (Radostits et al., 2007). Furthermore, displacement of the abomasum, volvulus and torsion may also be effective in inducing abdominal distention in cattle (Radostits et al., 2007). Abomasal dilatation can result from mechanical obstruction of the exit from the abomasum at the level of the pylorus (eg, ulcer, foreign body) as well as from the accumulation of feeding, fluid, or gas, or from disruption of the muscular activity of the abomasum by damage to the part of the vagus nerve. Increases in abomasal lumen pressure reduce blood flow to the abomasal mucosa and submucosa. Thus, this pressure predisposes the gastric mucosa to damage and ulceration resulting from the back diffusion of hydrogen ions. (Constable, 1992; Marshall, 2009; Panciera, 2007; Songer, 2005).

In calves, abdominal distention is most frequently seen in the first 1-2 weeks of their life (Jonathan et al., 1987). Common clinical signs in these calves include refusing to drink milk, distending abdomen, grinding their teeth, kicking at the belly, depression, letargy, dropping ears and colic. If these animals are not treated they may die in less than four hours. Abdominal distention may cause various factors and it may be a common indicator of many gastrointestinal disorders in cattle (Peek and Divers, 2018). However, its differential and diagnosis is very difficult.

Abomasal ulcers in calves are one of the common complication of abdominal distention, resulting in death in a short period if not treated. Clinical manifestations of abomasal ulcers may be absent or range from haemorrhages and subsequent melena to peritonitis (Cable, 1998; Radostits et al., 2007). The diagnosis of abomasum ulcer is based on clinical symptoms and FOBT. However, clinical symptoms are often quite and non-specific (Mesaric, 2005). Thus, these signs are not sufficient for differential diagnosis of abdominal distention and abomasal ulcers.

Plasma gastrin (Ok et al., 2001) and pepsinogen (Mesaric et al., 2000; Zadnik and Mesaric, 1999) have been studied in some gastrointestinal disorders such as abomasum displacement and abomasum ulcer in cattle. In studies, increases in pepsinogen levels have been detected in cattle and sheep with abomasal ulcers (Hajimohammadi et al., 2010; Mesaric, 2005; Zadnik and Mesaric, 1999). Furthermore, plasma pepsinogen increases have also been shown in cattle with abomasal displacement (Voros et al., 1984) and ostertagia infection (Pitt, 1988). Mesaric (2005) reported that pepsinogen measurement can be a simple serum test for the diagnosis of subclinical abomasal ulcer in cows. Some studies have reported that high pepsinogen concentration may be related to vascular permeability in the displaced abomasum. It has been stated that any damage to the gastric mucosa undergoes diffusion of hydrogen ions from the lumen into the mucosal tissues. In addition, diffusion of pepsin and pepsinogen to the rest of the mucosa has been reported to cause more damage to the mucosa (Hajimohammadi et al., 2010; Hajimohammadi et al., 2017). The increase in abomasum pH is an important stimulant for gastrin secretion

and causes an increase in pepsinogen secretion (Kataria et al., 2008). It is known that increased plasma pepsinogen and gastrin levels indicate abomasal damages in cattle. Thus, these biomarkers have been suggested to be a useful tool for detection of abomasal damages in cattle (Mesaric et al., 2002). However, the role of pepsinogen in the detection of ulcers or damages has not been demonstrated in calves with abdominal distention related to abomasal or other gastrointestinal disorders.

CONCLUSION

In conclusion, high plasma pepsinogen concentrations, melena and a positive FOBT can be used to confirm the suspicion of abomasal damage in calves with abomasal distension. It was found that plasma pepsinogen level could be a useful biomarker to diagnose abomasal damage in calves with abdominal distension.

DECLARATIONS

Ethics Approval

This study was approved by the Local Ethics Committee for Animal Care of the Burdur Mehmet Akif Ersoy University (15/08/2018- decision number: 396). Consent forms were signed by the animal owners.

Conflict of Interest

The authors declare that they have no conflict of interests.

Author Contribution

Idea, concept, and design: NM, RY, HİG, TA

Data collection and analysis: NM, RY, HİG, TA

Drafting of the manuscript: NM, RY, HİG, TA

Critical review: NM, RY, HİG, TA

Data Availability

The data used to prepare this manuscript are available from the corresponding author when requested.

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