




## Original Article



## Evaluating Oxidative Stress Parameters, Pepsinogen, and Insulin-like Growth Factor-1 in Dairy Cows with Displacement of Abomasum

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## ABSTRACT

**Introduction:** Displacement of the abomasum (DA) frequently occurs in high-producing dairy cows. It is a multifactorial disease and has an economic impact on dairy farms. This study aimed to investigate the levels of pepsinogen, Insulin-Like Growth Factor-1, and oxidative stress parameters (malondialdehyde and nitric oxide) in DA cases.

**Materials and methods:** Blood samples were taken from 51 dairy cows suspected of DA, referring to the Veterinary Clinic of Shiraz University, Shiraz, Iran, in the summer of 2021. Twenty of them had Left DA (LDA), 6 had Right DA (RDA), 13 had LDA with clinical signs of ulcer of the abomasum, 3 had RDA with clinical signs of ulcer of the abomasum, and 9 of them (3-5 years old) were clinically healthy as control. The serum concentration of pepsinogen, Insulin-Like Growth Factor-1, malondialdehyde, and nitric oxide were measured.

**Results:** The result of the current study showed that the concentration of pepsinogen was higher in cows with DA compared to healthy cows. The concentrations of malonaldehyde, nitric oxide, and IGF-1 were higher in LDA with the abomasal ulcer, compared to other groups.

**Conclusion:** Pepsinogen can be suggested as a predictor parameter for DA. Malonaldehyde, nitric oxide, and IGF-1 can be considered biomarkers in LDA with the abomasal ulcer. However, further studies are needed to find other effective parameters for predicting DA.

## 1. Introduction

Displacement of the abomasum (DA) can be frequently observed in high-producing dairy cows. It is a multifactorial disease and has a negative economic impact on dairy farms, mostly 3-4 weeks after parturition<sup>1</sup>. The etiology of DA has not clearly been understood; however, some diseases, such as fatty liver, ketosis, and hypocalcemia, are associated with DA<sup>2,3</sup>. Reduction in dry matter intake in the transitional period in dairy cows is one of the contributing factors to DA<sup>4</sup>. Moreover, diseases such as rumen atony and decreased rumen contraction, hypocalcemia, endotoxemia, alkalemia, and hyperinsulinemia might predispose to DA<sup>5</sup>.

Displacement of the abomasum is characterized by

the accumulation of gas in the abomasum and displacement of the abomasum to the dorsal part of the abdomen. The hypothetical cause of gas accumulation in the abomasum is increased gas production and a hypomotility of the abomasum<sup>1</sup>. Clinical signs of DA are colic, anorexia, milk yield drop, cow discomfort, and death in some cases<sup>4</sup>.

The growth hormone axis is an important endocrine control center that plays a vital role in metabolic adaptation in dairy cows<sup>6</sup>. According to some studies, Insulin-Like Growth Factor-1 (IGF1) may be useful as a predictive marker for postpartum production diseases<sup>3,6</sup>. Some researchers reported that oxidative stress parameters, such as malondialdehyde and nitric oxide, can

be used to evaluate inflammatory conditions, ischemic effects, and tissue damage caused by obstruction, strangulation, or both in gastrointestinal disorders<sup>7-9</sup>. Pepsinogen levels in the blood could be useful for diagnosing DA in cattle<sup>10</sup>. Pepsinogen is a proenzyme produced by the parietal cells of the abomasal mucosa. In humans, serum pepsinogen is elevated in different diseases, including gastric and duodenal ulcers<sup>11</sup>. Therefore, the current study aimed to investigate pepsinogen levels, Insulin-Like Growth Factor-1, and oxidative stress parameters (malondialdehyde and nitric oxide) in referral DA cases.

## 2. Materials and Methods

### 2.1. Ethical approval

All animals were treated by the regulations on the guidelines of the Iranian Council of Animal Care (1995), and the experiment was approved by the Iranian Ministry of Agriculture (experimental permission no. 1828).

### 2.2. Animals

Clinical and paraclinical examinations were performed on 51 adult dairy cows suspected of DA and referred to the Veterinary Clinic of Shiraz University, Shiraz, Iran, in the summer of 2021. Of the samples, 20 had LDA, 6 had RDA, 13 had LDA with clinical signs of ulcer of the abomasum, 3 had RDA with clinical signs of ulcer of the abomasum, and 9 were clinically healthy as control. Each animal was subjected to a clinical examination and routine hematological investigation.

### 2.3. Diagnosis of displacement of the abomasum

The occult blood test was done according to the method proposed by Geishhauser<sup>12</sup>. All cows enrolled in the study were physically examined. The specific DA tests included ping on auscultation and percussion, fluid splashing, Liptak test, and ultrasonography. The lost percussion area of the liver in RDA was examined according to Van Winden and Kuiper<sup>4</sup>.

### 2.4. Laboratory analysis

Blood samples (10 ml) were collected from the tail vein. The samples were centrifuged at 3,000 × g at 4°C for 5 minutes, and the serum was frozen at -20°C and stored until analysis. Serum pepsinogen, malonaldehyde, nitric oxide, and IGF-1 were measured. Serum pepsinogen levels were determined using a micro-method described by Dorny and Vercruyse<sup>13</sup>.

Malonaldehyde, nitric oxide, and IGF-1 concentrations were determined using commercial ELISA kits (Pars Azmoon, Iran) following manufacturers' instructions.

### 2.5. Statistical analysis

The resulting data were statistically processed using the statistical package for the social sciences (SPSS 22, Chicago, USA). One-way ANOVA test was performed, followed by the LSD test to determine the mean significant differences between treatments. The  $p < 0.05$  was considered a significant difference between the groups.

## 3. Results

### 3.1. Pepsinogen

Serum pepsinogen activity increased significantly ( $p < 0.05$ ) in all DA cases. Moreover, pepsinogen activity was higher in LDA and RDA groups with ulcers of the abomasum, compared to other groups ( $p < 0.05$ ). In the present study, the serum activity of pepsinogen, in all cases of LDA plus RDA with signs of ulcer of the abomasum (melena and positive fecal occult blood test) was higher than in LDA, RDA, and control groups (Table 1).

### 3.2. Malonaldehyde and nitric oxide

Malonaldehyde and nitric oxide activity (Table 1) were higher in group LDA with abomasal ulcer ( $p < 0.05$ ). The increase of malonaldehyde positively correlated with nitric oxide activity in LDA, RDA, RDA with abomasal ulcers, and LDA with abomasal ulcer groups (Table 2).

**Table 1.** Pepsinogen, malondialdehyde, nitric oxide, and IGF-1 serum activities in dairy cows' displacement of the abomasum (Mean ± SE).

| Groups (n = 51)                  | Pepsinogen (IU/liter)    | Malondialdehyde (nmol/liter) | Nitric oxide (µmol/liter)  | IGF-1 (µg/liter)            |
|----------------------------------|--------------------------|------------------------------|----------------------------|-----------------------------|
| LDA (n = 20)                     | 4.64 ± 1.24 <sup>b</sup> | 15.55 ± 6.16 <sup>a</sup>    | 47.50 ± 22.37 <sup>a</sup> | 69.90 ± 35.26 <sup>a</sup>  |
| RDA (n = 6)                      | 5.68 ± 1.21 <sup>b</sup> | 19.35 ± 7.66 <sup>a</sup>    | 67.33 ± 34.91 <sup>a</sup> | 79.83 ± 40.50 <sup>a</sup>  |
| LDA with abomasal ulcer (n = 13) | 8.26 ± 1.14 <sup>c</sup> | 23.40 ± 9.02 <sup>b</sup>    | 96.69 ± 49.56 <sup>b</sup> | 112.15 ± 50.22 <sup>b</sup> |
| RDA with abomasal ulcer (n = 3)  | 8.02 ± 1.69 <sup>c</sup> | 18.96 ± 10.34 <sup>a</sup>   | 63.33 ± 43.03 <sup>a</sup> | 83.33 ± 48.16 <sup>a</sup>  |
| Control (n = 9)                  | 2.43 ± 0.32 <sup>a</sup> | 15.15 ± 4.60 <sup>a</sup>    | 50.11 ± 14.02 <sup>a</sup> | 49.11 ± 24.03 <sup>a</sup>  |

N: Number, <sup>abc</sup> Superscript letters in columns show significant differences in the groups ( $p < 0.05$ )

**Table 2.** Correlation between malondialdehyde and nitric oxide in dairy cows with the displacement of the abomasum

| Group                            | Correlation |
|----------------------------------|-------------|
| LDA (n = 20)                     | 0.920       |
| RDA (n = 6)                      | 0.812       |
| LDA with abomasal ulcer (n = 13) | 0.911       |
| RDA with abomasal ulcer (n = 3)  | 0.977       |
| Control (n = 9)                  | -0.0533     |

**Table 3.** Correlation between IGF-1 and pepsinogen in dairy cows with the displacement of the abomasum

| Group                            | IGF-1(µg/liter) |
|----------------------------------|-----------------|
| LDA (n = 20)                     | -0.182          |
| RDA (n = 6)                      | 0.444           |
| LDA with abomasal ulcer (n = 13) | -0.512          |
| RDA with abomasal ulcer (n = 3)  | 0.866           |
| Control (n = 9)                  | -0.143          |

### 3.3. IGF-1

IGF-1 in the LDA with clinical signs of the abomasal ulcer (Table 1) was significantly higher ( $p < 0.05$ ). There were negative relationships between pepsinogen and IGF-1 in LDA with clinical signs of abomasal ulcer and LDA groups. A strong positive correlation (Table 3) was indicated between pepsinogen and IGF-1 in terms of RDA with clinical signs of abomasal ulcers ( $p < 0.05$ ).

## 4. Discussion

The result of the current study showed that the concentration of pepsinogen was higher in cows with DA. Moreover, malonaldehyde, nitric oxide, and IGF-1 were higher in LDA with the abomasal ulcer.

Although pepsinogen activity in the blood is commonly measured for evaluating gastrointestinal parasitism, it should not be ignored that a certain physiological level of pepsinogen is normal in ruminants<sup>13</sup>. The normal range of pepsinogen activity in ruminants' blood is 0-5 IU/l and is not associated with any damage to the abomasum mucosal<sup>11</sup>. Low or normal serum pepsinogen levels ( $< 5.0$  IU/l) could predict low susceptibility to major changes to the abomasum mucous membrane of the abomasum<sup>10</sup>.

In dairy cows, the transition from late pregnancy to early lactation is critical, with many interacting endocrine adaptations. If these adaptations are inadequate, dairy cows are susceptible to metabolic and infectious diseases after calving<sup>14-17</sup>. Hepatic-derived IGF1 might serve as an indicative marker for ketosis<sup>6</sup>. Ketosis and fatty liver in cows around parturition are associated with DA. Oxidative stress might contribute to some periparturient infectious and metabolic diseases and abomasal tissue damage due to DA<sup>18,19</sup>. Oxidative stress increases liver steatosis and could be an important factor in the pathogenesis of fatty liver<sup>20,21</sup>.

Oxidative stress could play an important role in the etiology and pathogenesis of periparturient metabolic diseases in dairy cows. Further studies are necessary to establish the role of oxidative stress in fatty liver and related metabolic diseases in cows.

## 5. Conclusion

In conclusion, the obtained results of the current study indicated that pepsinogen concentration was higher in cows with DA; moreover, malonaldehyde, nitric oxide, and IGF-1 were higher in LDA with the abomasal ulcer. Pepsinogen can be suggested as a predictor parameter for DA. Malonaldehyde, nitric oxide, and IGF-1 can be considered biomarkers in LDA with the abomasal ulcer.

However, further studies are needed to find other effective parameters for predicting DA.

## Declarations

### Competing interests

There is no conflict of interest.

### Authors' contribution

The final manuscript draft was reviewed by all authors, who also approved it.

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### Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and falsification, double publication and submission, and redundancy) have been checked by all the authors.

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