

Alterations of pancreatic functions and lipid profiles in dairy cows with left displacement of the abomasum

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Abstract: The objectives of this study were to determine the serum activities of the pancreatic enzymes amylase, lipase, trypsinogen 1 and trypsinogen 2, serum concentrations of total cholesterol, high density lipoprotein, low-density lipoprotein and triglycerides and serum inflammatory indicators, namely C-reactive protein and procalcitonin, in Holstein-Friesian dairy cows with left displacement of the abomasum (LDA). A total of 60 cows (30 LDA-affected and 30 healthy) were included in the study. Laboratory analyses were performed using commercially available ELISA kits and chemical reagents according to the manufacturers' recommendations. There was a significant increase ($P \leq 0.05$) in the activities of lipase, trypsinogen 1 and trypsinogen 2 in LDA-affected cows compared to healthy cows. Amylase concentrations, however, remained unchanged. The serum concentrations of total cholesterol and high-density lipoprotein were significantly ($P \leq 0.05$) increased in LDA-affected cows while the concentrations of low-density lipoprotein and triglycerides were significantly ($P \leq 0.05$) decreased compared to healthy cows. Procalcitonin and C-reactive protein concentrations were significantly ($P \leq 0.05$) increased in LDA-affected cows compared to healthy cows. This study indicates that displacement of the abomasum may be associated with significant pathological effects in the pancreas that may affect cows in the post-operative period.

Keywords: abomasal disease; lipid metabolism; pancreatitis; systemic inflammatory response

Left displacement of the abomasum (LDA) remains one of the most common diseases of dairy cattle (Van Winden et al. 2003; Radostits et al. 2007; Doll et al. 2009; Leblanc 2010). The disease can be associated with high economic losses to the dairy herd due to the loss in milk production, cost of medical and surgical interventions and deterioration of the general health of the cow (Radostits et al. 2007; Doll et al. 2009; Ricciotti and FitzGerald 2011). LDA occurs early in lactation when the cow is abruptly introduced to low roughage-high concentrate rations (Radostits et al. 2007; Doll et al. 2009). Gas and fluid

accumulation in the abomasum lead to abomasal atony and migration (Radostits et al. 2007; Doll et al. 2009). Most high-producing dairy cows suffer from negative energy balance during early lactation due to decreased dry matter intake and the stress of parturition (Cameron et al. 1998). In this regard, scientific studies have documented a strong link between negative energy balance and the occurrence of LDA in the postpartum period in association with other metabolic diseases such as hepatic lipidosis and ketosis (Cameron et al. 1998). Many other factors such as anorexia due to other diseases, genetics and

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hypocalcaemia are known to predispose dairy cows to this condition (Holtenius et al. 2000).

Once displacement of the abomasum occurs, the cow's anorexia deepens and gastrointestinal passage time is increased leading to a state of partial to complete gastrointestinal obstruction and constipation (Sevinc et al. 2002; El-Attar et al. 2007; Duffield et al. 2009; Stengarde et al. 2010; Dezfouli et al. 2013). It is not known whether severe gas distension and abnormal position of the abomasum affect the function of the pancreas in cows with LDA. We hypothesised that pancreatic functions in cows affected with LDA might be deranged leading to pancreatic inflammation, changes in protein and lipid metabolism pathways and leakage of pancreatic enzymes into the circulation. Therefore, the objectives of this study were to determine the serum activities of the pancreatic enzymes amylase, lipase, trypsinogen 1 and trypsinogen 2, serum concentrations of total cholesterol (Chol), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TG) and serum inflammatory indicators, namely C-reactive protein (CRP) and procalcitonin, in Holstein-Friesian dairy cows affected with LDA.

MATERIAL AND METHODS

Ethical approval. All procedures undertaken in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Jordan University of Science and Technology (JUST-ACUC approval No. 115 of February 20, 2018). Written consent was obtained from all farmers and animal owners before cows were enrolled in the study.

Animals. The study was performed using 30 Holstein-Friesian dairy cows with confirmed diagnosis of left displacement of the abomasum and 30 healthy cows as control. The healthy control cows were selected from a dairy farm with similar housing and management conditions as those to which the LDA-affected cows were subjected. Control cows were matched for age, parity and stage of lactation to those affected with LDA. Control cows were subjected to a complete physical examination to ensure they were healthy before inclusion in the study.

LDA-affected cows were presented to the Veterinary Health Centre at Jordan University of

Science and Technology for evaluation of potential gastrointestinal problems. They belonged to several dairy farms that were served by this Centre as a referral clinic. At the clinic, cows were subjected to a complete physical examination. LDA was diagnosed by abdominal auscultation and percussion and then confirmed by laparotomy. Cows with any concurrent disease affecting other body organs or systems were excluded from analyses. Right paralumbar fossa omentopexy was performed to correct the position of the abomasum in cows affected with LDA (Steiner 2006). In the post-operative period, cows were subjected to a routine treatment protocol including ceftiofur hydrochloride (Excenel, Zoetis, New Jersey, USA) at 2.2 mg/kg intramuscularly once per day for three days, flunixin meglumine (Vet Tek, Inc., MO, USA) at 1.1 mg/kg intravenously once per day for three days, 500 ml of calcium borogluconate (Norbrook Inc., Kansas, USA) subcutaneously once, and 20 l of oral electrolyte solution once (100 g magnesium carbonate, 100 g yeast and 5 g calcium propionate).

Laboratory analyses. Approximately 10 ml of whole blood were collected from all cows by jugular venipuncture using a vacutainer tube and needle and placed in plain blood tubes. Blood collection was carried out at presentation and the samples were sent immediately to the laboratory. In the laboratory, serum was collected by centrifugation of blood tubes at 5000 g for 10 minutes. Serum was then stored at -20°C until used.

The following variables were measured using commercially available ELISA kits and chemical reagents according to the manufacturers' recommendations: amylase (Biolabo, France), lipase (Biolabo, France), trypsinogen 1 and trypsinogen 2 (MyBioSource, USA), triglycerides (Biosystems, Spain), total cholesterol (Biosystems, Spain), HDL (Arcomex, Jordan), LDL (Biolabo, France), procalcitonin (MyBioSource, USA), and C-reactive protein (CRP; MyBioSource, USA). All tests were previously validated for use in cows in our laboratory.

Statistical analysis. Collected data were coded and entered into an SPSS spread sheet (Version 23; Microsoft, USA). Initially, data were checked for normality using the Shapiro-Wilk test. Data were found to be of normal distribution; therefore, parametric tests were followed except for the categorical data. The data of the two groups (LDA and control) were examined for statistical differences

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using the independent *t*-test. The CRP data (categorical) were compared in the two groups using bivariate analysis with Spearman correction.

RESULTS

A total of 30 cows with confirmed diagnosis of LDA and 30 healthy control cows were used in the study. The average parity and days in milk in LDA-affected cows and healthy cows were 3.0 ± 0.5 , 22 and 2.75 ± 0.5 , 19, respectively. In LDA-affected cows, the history indicated normal vaginal delivery of a single foetus at parturition in all cows without any evidence of postpartum complications such as retained placenta or metritis. At presentation, the medical records of affected cows indicated that cows appeared bright and responsive with normal temperature, pulse rate and respiratory rate. The mean body condition score (BCS) in LDA-affected cows was 2 ± 0.25 (2.25 ± 0.5 in healthy cows). Haematology and serum biochemical analyses were performed in all cows at presentation in a routine manner. In the haematology analyses, no abnormalities could be identified and in the routine serum biochemical analyses, mild metabolic alkalosis characterised by hypochloraemia and hypokalaemia was present in LDA-affected cows. No such abnormalities were detected in healthy cows.

The serum activities of the amylase ($\mu\text{mol/l}$), lipase ($\mu\text{mol/l}$), trypsinogen 1 (nmol/l) and trypsinogen 2 (nmol/l) pancreatic enzymes in dairy cows

Table 1. Serum concentrations of pancreatic enzymes and procalcitonin in dairy cows with left displacement of the abomasum ($n = 30$)

Parameters	LDA-affected cows			Healthy cows		
	mean \pm SD	max	min	mean \pm SD	max	min
Lipase ($\mu\text{mol/l}$)	$1.1 \pm 0.3^*$	1.6	0.67	0.56 ± 0.2	0.87	0.15
Amylase ($\mu\text{mol/l}$)	0.32 ± 0.1	0.57	0.17	0.33 ± 0.12	0.50	0.13
Trypsinogen 1 (nmol/l)	$28 \pm 13^*$	75	16	6.67 ± 5.0	11.70	0.50
Trypsinogen 2 (nmol/l)	$250 \pm 0.83^*$	6.20	2.80	1.0 ± 0.50	2.5	0.33
Procalcitonin (ng/l)	$27 \pm 7.0^*$	40	16	6.4 ± 2.0	9.50	3.0

* $P \leq 0.05$

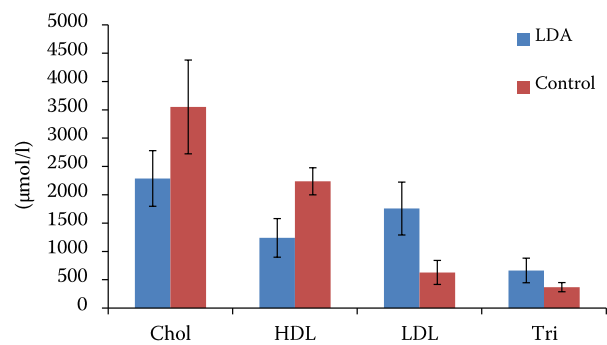


Figure 1. Serum concentrations ($\mu\text{mol/l}$) of total cholesterol (Chol), high-density lipoprotein (HDL), low-density lipoprotein (LDH) and triglycerides (Tri) in dairy cows with LDA and healthy cows ($n = 30$)

LDA = left displacement of the abomasum

affected with LDA are presented in Table 1. There was a significant increase ($P \leq 0.05$) in the activities of lipase, trypsinogen 1 and trypsinogen 2 in LDA-affected cows compared to healthy cows. Amylase activities, however, were not significantly changed in the two groups of cows.

The serum concentrations ($\mu\text{mol/l}$) of total cholesterol (Chol), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (Tri) in dairy cows with left displacement of the abomasum are presented in Figure 1. The serum concentrations of total Chol and HDL were significantly ($P \leq 0.05$) increased in LDA-affected cows while the concentrations of LDL and triglycerides were significantly ($P \leq 0.05$) decreased compared to healthy cows.

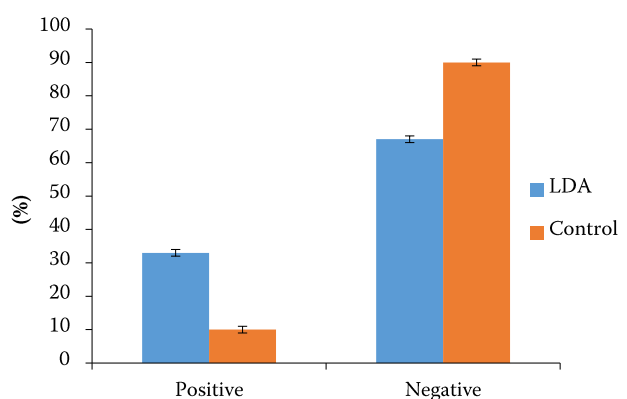


Figure 2. Percentage of LDA-affected and healthy cows with increased serum concentrations of C-reactive protein (CRP) ($n = 30$). % – positive value indicates CRP ≥ 500 nmol/l according to the manufacturer's recommendations

LDA = left displacement of the abomasum

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Serum concentrations of procalcitonin (ng/l) in dairy cows affected with LDA are presented in Table 1. Procalcitonin concentrations were significantly ($P \leq 0.05$) elevated in LDA-affected cows compared to healthy cows.

Serum concentrations of C-reactive protein (CRP) in dairy cows with left displacement of the abomasum (%; positive value indicates $\text{CRP} \geq 500$ nmol/l according to the manufacturer's recommendations) are presented in Figure 2. A significantly ($P \leq 0.05$) higher number of LDA-affected cows (ten cows) exhibited elevated serum concentrations of CRP compared to healthy cows (three cows).

DISCUSSION

This study aimed at investigating possible pathological alterations in pancreatic functions, lipid metabolism and systemic inflammatory responses in dairy cows with left displacement of the abomasum. As expected, results showed a significant elevation in serum activities of pancreatic lipase, trypsinogen 1 and trypsinogen 2. Although there is paucity of data in the recent literature that indicate pancreatic damage associated with LDA in cows, severe histopathological pancreatic lesions including fatty pancreas and pancreatitis were found in dairy cows with abdominal fat necrosis and obesity (Tani et al. 2017). Only serum amylase has previously been studied in cows affected with LDA (Aly et al. 2016). While in this study, there were no changes in serum amylase concentrations, Aly et al. (2016) reported a significant increase in the activity of this enzyme in dairy cows affected with LDA (Aly et al. 2016). The difference in amylase activities between this study and the previously reported values could be related to the different methodologies employed.

A link between hepatic metabolic and inflammatory responses in dairy cows with LDA was established previously (Klevenhusen et al. 2015; Al-Rawashdeh et al. 2017). In dairy cows affected with LDA, the serum activities of aspartate aminotransferase, gamma glutamate transferase, and concentrations of serum amyloid A were increased significantly (Klevenhusen et al. 2015). In a similar fashion, the results presented here indicate possible substantial pathological changes affecting the pancreas with a significant inflammatory response associated with increased C-reactive protein, and procalcitonin.

C-reactive protein is considered to be an important local and systemic natural defence element in many body organs including the pancreas (Kaya et al. 2016). Kaya et al. (2016) reported a significant elevation of CRP in dairy cows affected with endometritis. In diseased cows, the serum concentration of CRP increased 3–3.5 times compared to healthy cattle as reported by Lee et al. (2003).

Procalcitonin is another important element of systemic inflammation (Simon et al. 2004). It has been used in the detection and monitoring of systemic inflammatory responses associated with many infectious and non-infectious diseases such as sepsis and acute pancreatitis, and as a prognostic marker following major trauma and surgeries (Simon et al. 2004; Rau et al. 2007; Maruna et al. 2008). In this study, serum concentrations of procalcitonin were significantly increased in dairy cows with LDA which may indicate marked systemic inflammation associated with the condition.

In this study, the serum concentrations of total cholesterol and HDL were significantly increased in LDA-affected cows while the concentrations of LDL and triglycerides were significantly decreased compared to healthy cows. Previous studies in high-yielding dairy cows affected with LDA and energy deficiency have shown that HDL, LDL and total cholesterol concentrations were significantly decreased when compared to healthy controls (Durgut et al. 2016).

The discrepancy in the lipid profiles between the current study and previous studies could be explained by different methodologies and by the nutritional and general health status of cows included in the studies. Nevertheless, impaired lipid metabolism, fatty liver and ketosis are commonly diagnosed in postpartum cows with LDA (Durgut et al. 2016).

In conclusion, a possible association could be detected between displacement of the abomasum and pancreatic pathology and systemic inflammatory response in dairy cows that may affect cows in the post-operative period.

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