ACTA VET. BRNO 2024, 93: S69-S74; doi: 10.2754/avb202493S11S69

Protein profile in dairy cows with left displaced abomasum

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Received April 29, 2024
Accepted December 12, 2024

Abstract

The goal of this study was to compare and evaluate the protein profiles of healthy cows and cows diagnosed with left displaced abomasum (LDA) (at the first phase of lactation). Cows were admitted to the Clinic of Ruminants, University of Veterinary Medicine and Pharmacy in Košice, Slovakia (UVMP) between 2018 and 2020. Blood samples were collected from the jugular vein to an EDTA tube from 26 cows, 13 healthy ones and 13 diagnosed with LDA. Biochemical indices were determined in a UVMP laboratory and included total protein, albumin, creatinine, urea and total immunoglobulins. Statistical analysis was performed using GraphPad Prism 9.0.0 and two-tailed Student's *t*-test. The mean value of total protein was 75.38 ± 14.93 g/l, and 77.84 ± 10.97 g/l for control group and for LDA affected cows, respectively. The mean albumin concentration for the control group was 37.27 ± 6.02 g/l, while for LDA group it was 32.84 ± 8.7 g/l. The mean creatinine and urea concentrations were 99.72 ± 66.88 µmol/l and 4.44 ± 4.47 mmol/l, respectively, in the control group, and 91.67 ± 25.44 µmol/l and 5.09 ± 2.65 mmol/l, respectively, in cows diagnosed with LDA. Total immunoglobulins were at the level of 37.13 ± 7.65 zinc sulphate turbidity test (ZST) in healthy cows whereas LDA group had 32.8 ± 5.2 ZST. Although the difference was not significant ($P > 0.05$), protein profiles varied between healthy cows and cows diagnosed with LDA. Further research is needed to determine the differences in protein profiles between groups of larger numbers of individuals.

Ruminants, abomasum, biochemical indices

Abomasal displacement (AD) is a common disorder diagnosed in dairy cattle. It can occur either as the right (RDA) or the left (LDA) displaced abomasum within the abdominal cavity. Prior to the appearance of the first symptoms an enlargement of the abomasum occurs due to gas accumulation (Coppock 1973). In the early 1960s it was hypothesized that the distention of the abomasum is a result of its impaired motility, which prevents gases (methane and CO_2) from expulsion (Doll at al. 2009). According to the statistics, LDA occurs more frequently than RDA with the LDA:RDA ratio at 2.5–7:1. Additionally, most cases of LDA occur around the time of parturition, with significantly higher incidence during the 3rd to 5th parturition. While AD most often affects dairy cows, it can also occur, though less frequently, in calves and young cattle regardless of sex. Furthermore, the worldwide incidence of AD was found to be 0.05–5.80% (Mueller 2011; Zerbin at al. 2015). Aetiological factors that can contribute to AD development can be generally divided into the following groups: dietary, due to concurrent disease, negative energy balance, and calcium related effect on abomasum function (Breukink 1990; Van Winden and Kuiper 2003). Decreased appetite (mainly for concentrate) and reduced milk production are commonly observed in cows affected with AD. In cases of volvulus, anorexia is more severe and milk production more reduced with a faster general clinical deterioration. During AD, the vital signs (temperature, heart, and respiratory rates) are usually within the normal range or slightly increased, similar to rumen activity. In some cases, cows affected by AD may experience limited defecation or diarrhoea. As the rumen

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is pushed medially during AD, the flank may appear sunken, and a small swelling can be palpated caudally to the last rib. Furthermore, with the progression of the disease, pain symptoms can appear as visibly arched back posture (Mueller 2011; Wittek 2021).

Although several analyses of the energetic profiles in cows affected by LDA have been conducted, to the authors' knowledge there is limited information regarding the protein profile. The protein profile has been shown to significantly impact production and reproductive indices in dairy cows and may also play an important role in the context of LDA.

The aim of this study was to compare and evaluate the protein profiles of healthy cows and cows that were diagnosed with LDA (all at the first phase of lactation).

Materials and Methods

The study analysed dairy cows admitted to the Clinic of Ruminats, University of Veterinary Medicine and Pharmacy in Košice, Slovakia (UVMP) between 2018 and 2020. The mean age and weight of dairy cows was 3.5 years and 500 kg, respectively. All cows included in the study were kept in a free-stall barn and fed a total mixed ration. Their productivity ranged from 15 to 25 litres per day. A total of 13 cows (group A) were admitted to the clinic with a history of long period indigestion, diarrhoea or scanty faeces and decreased milk yield. According to the anamnesis, the analysed cows were in the first phase of lactation (4 to 21 days after parturition). All these cows were diagnosed with LDA and underwent surgical correction of abomasum using the right paralumbar omentopexy technique. Diagnosis of LAD was confirmed by clinical examination. Auscultation and percussion revealed an area of high resonance sound - ping sound. Ping sound was presented at the line from tuber coxae to elbow on the left side. Control group (group B) of 13 healthy cows (of the same age, breed, and calving date as group A) were selected from the same herd as affected cows.

A blood sample was collected from the jugular vein to an ethylenediaminetetraacetic acid (EDTA) tube form 26 cows, including healthy ones and those diagnosed with LDA. Samples were collected from all cows within 4 h from the first symptoms of disease. Biochemical indices were determined at the laboratory of Clinic of Ruminants, UVMP. Laboratory processing of the samples began by centrifuging each sample at 3,000 *g*. After centrifugation, plasma was collected and analysed. Analysed biochemical indices included total protein (TP), albumin (ALB), creatinine (Creat), and urea (U) that were recorded using an automated biochemical analyser Alizé (Lisabio, Pouilly-en-Aixois, France) and diagnostic kits (Randox Laboratories Ltd., Crumlin, United Kingdom).

The levels of total immunoglobulins (TIg) were measured by a Specord 210 Plus spectrophotometer (Analytik Jena AG, Jena, Germany) using Zinc Sulphate Turbidity (ZST) test modified by Slanina et al. (1976).

Statistical analyses were performed using GraphPad Prism 9.0.0. Comparison between the two groups of cows was performed with two-tailed Student's *t*-test to achieve a statistical significance between P value ($P < 0.05$; $P < 0.001$; $P < 0.0001$).

Results

Results of blood analysis of the indices (TP, ALB, Create, U, TIg) obtained from plasma are presented in Table 1, showing the mean and standard deviation values. Although we did not find any significant differences between the groups ($P > 0.05$), a trend was evident in two results. In cows affected by LDA, concentrations of TP and U increased, whereas ALB, Creat, and TIg decreased.

Table 1. Biochemical profile: Total protein (TP), albumin (ALB), creatinine (Creat), urea (U) and total immunoglobulins (TIg) in healthy and affected with left displacement abomasum cows.

Group		TP (g/l)	ALB (g/l)	Creat (μmol/l)	U (mmol/l)	TIg (ZST)
Control	Mean	75.38	37.27	99.72	4.44	37.13
	SD (±)	14.93	6.02	66.88	4.47	7.65
LDA	Mean	77.84	32.46	91.67	5.09	32.8
	SD (±)	10.97	8.7	25.44	2.65	5.2

Levels of total immunoglobulins (TIg) were measured using the Zinc Sulphate Turbidity (ZST) test modified by Slanina et al. (1976).

Discussion

The time during late gestation to early lactation is a transitional period for all animals including dairy cows due to physiological and metabolic changes (Drackley et al. 2005). Abomasum displacement typically occurs between 4 and 6 weeks after parturition (Constable et al. 1992). Rarely, it can also occur a few to several weeks before birth (Radostits et al. 2007). During the first weeks of lactation, high yielding dairy cows suffer from negative energy balance due to high energy demand for milk production and limited feed intake (Nielen et al. 1994). Postpartum negative energy balance can lead to increased incidence of metabolic disorders (Melendez et al. 2009). Clinical examination of LDA affected cows revealed reduction in appetite, sudden decrease of milk yield, and pasty faeces. Moreover, simultaneous percussion and auscultation of the left flank, particularly in the upper third of the abdomen, determined a high-pitched resonant ping audible between the 9th and 11th intercostal space, typical for LDA cases (Mokhber-Dezfouli et al. 2013).

Major differences have been determined in blood profiles of cows diagnosed with LDA within the 2nd to 56th day postpartum compared to healthy cows. These differences indicate a negative energy balance as well as liver cell damage and an inflammatory response (Stengärde et al. 2010). In many studies serious changes during the periparturient period were observed in blood metabolites including mineral, enzymatic, and hormonal profiles, as well as some variables related to protein metabolism (Tóthová et al. 2008; Hagawane et al. 2009; Piccione et al. 2011).

Determination of serum protein concentrations and the evaluation of their changes during the disease process is fundamental for valid biomarkers (Okutucu et al. 2007). Quantification of the TP concentration represents a basic step in general biochemistry and routine clinical laboratory practice. Several methods have been developed for the determination of TP in serum or plasma, which are based on various analytical methods (Zaia et al. 1998). Both chemical and physical methods are available to determine TP in biological fluids. In clinical biochemistry, chemical methodologies are more frequently used because of the possibility to adapt these techniques to automated analysers (Eckersall et al. 1999). Tóthová et al. (2018) confirmed dynamic changes in the concentrations of serum TP. The lowest concentration was observed 1 week before and 1 week after calving followed by an increase until the end of week 6 postpartum. Lower concentration of serum TP in cows before parturition may occur due to utilization of amino acids from the maternal circulation needed for protein synthesis in foetal muscles (Antunovic et al. 2002). In contrast, lower TP concentration in cows after parturition reflects the transfer of immunoglobulins from the bloodstream to the mammary gland that are required for the synthesis of colostrum and milk (Roubies et al. 2006). The TP values showed higher, though non-significant concentrations in LDA-affected cows compared to control. According to research, serum concentration is lower in cows with LDA, which can be explained by the alimentary privation of the affected animals (de Cardoso et al. 2008; Song et al. 2020). In a case of displaced abomasum, increased total serum proteins may occur due to raised haemoconcentration (Abd El-Raof and Ghanem 2007).

Albumin is often considered as the only discrete and homogenous protein fraction discernible on the electrophoretogram. Animal total serum protein consists in 35–50% of ALB (Kaneko et al. 1997). Albumin plays an important role in maintaining homeostasis and the transport of substances, and it acts as a free-radical scavenger (Hankins 2006). It is responsible for about 75% of the osmotic pressure of plasma and is a major source of amino acids that can be utilised by the animal's body when necessary (Mackiewicz 1997). Catabolism of ALB occurs in various tissues, where it enters cells by pinocytosis and is then degraded by proteases (Evans 2002). Reduced concentrations of serum ALB indicate decreased amino acid synthesis from diets, poor liver health or loss of proteins in plasma.

Serum ALB plays a significant part in maintaining the redox potential in extracellular fluids by regulating the osmotic pressure of plasma in the vascular system (Majorek et al. 2012). Several factors must be considered to appropriately interpret serum proteins as animal health indicator. Cows in high producing herds, characterized by presence of high yielding cows and by larger use of dietary concentrates, had higher serum ALB concentrations (Bobbo et al. 2017). Strydom et al. (2008) reported serum ALB concentrations ranging from 18.1 g/l to 35.9 g/l in undernourished cattle and 31.3 g/l to 41.1 g/l in adequately nourished animals, showing that ALB is the best predictor of malnourishment in cattle in South Africa. These dairy cows were at the beginning of lactation, and although statistical significance was not confirmed in ALB values, the average concentration was lower compared to the control group, which confirms the already discussed information about reduced liver metabolism, low synthesis of amino acids and, at the same time, reduced ability to transport fatty acids, hormones, nutrients, drugs and metabolites (Hamerly et al. 2014). Urea and Creat are the final products of protein metabolism. Both are often regarded as indicators of renal function (Song et al. 2020). When glomerular filtration rate falls below 50%, U and Creat concentrations have an increasing character. The rising concentration character of U and Creat can result from increased protein decomposition or dehydration (Braun et al. 2003).

Urea is a product of protein breakdown, and its concentration reflects protein metabolism in dairy cows (Butler 1998). Increased concentration of U is influenced by raised amount of rumen degradable proteins. Energy deficit can stimulate the catabolism of tissues containing proteins (turning into amino acids), leading to an increase in U production (Van Saun 2006). Our results demonstrate increased U concentration in dairy cows diagnosed with LDA. This finding explains the fact that the dairy cows included in the study were in the first phase of lactation, which is characterized by a negative energy balance in combination with reduced food intake. That is the reason of degradation of the body protein, which was manifested by a slight increase in the U concentration compared to the control group.

In mammals, Creat is constantly produced from the spontaneous, irreversible, non-enzymatic breakdown of Creat in skeletal muscle (Braun et al. 2003). Muscle Creat is therefore the main source of plasma and urinary Creat. Change in skeletal muscle mass in healthy animals with normal renal function affects plasma Creat concentration (Wyss and Kaddurah-Daouk 2000). A study published by Megahed et al. (2019) monitored Creat concentration and compared it with sonographic measurement of subcutaneous fat and muscle thickness confirmed a decrease of both plasma Creat concentration and thickness of the gluteus medius muscle. Reduced concentration of Creat indicates mobilization of proteins from body reserves. This study determined increased U concentration and decreased Creat concentration due to reduced feed intake and degradation of body protein in the group of dairy cows with LDA.

Although the results of the authors' study were non-significant, similar to other studies a dropping trend in globulin was detected. A significant decrease of globulin in LDA affected cows was also reported by El-Deen and Abouelnasr (2014), Aly et al. (2016), and Ismael et al. (2018). According to Ismael et al. (2018), changes in globulin concentration can occur due to hypoalbuminaemia which resulted from anorexia with decreased food consumption and decreased albumin synthesis in liver.

Although the results of the authors' study were not statistically significant, the trends in the values are consistent with those observed in other studies. Due to the small sample size and variability in the results, the study has notable limitations. Therefore, further analysis with a larger sample of cows from various environments is needed to determine the significance of the changes between groups.

The presented analysis evaluated the protein profile in dairy cows with LDA and compared it with the control group of healthy cows. Although results showed differences between concentrations of indices, their statistical significance was not confirmed. Further research on a larger number of individuals is needed to determine the differences between groups.

Acknowledgement

This work was supported by the VEGA project 1/0177/22.

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