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THE INFLUENCE OF HOOF DISEASES ON THE CONCENTRATIONS OF SOME ACUTE PHASE PROTEINS AND OTHER VARIABLES OF THE PROTEIN PROFILE IN HEIFERS

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The aim of this study was to evaluate the possible influence of hoof diseases in heifers on the concentrations of selected acute phase proteins and some other variables related to protein metabolism in the blood. In the evaluation we included 35 heifers of a low-land black spotted breed and its crossbreeds, with pathological clinical findings on the hoofs. Blood samples were collected once, when the clinical signs of the disease were obvious. Blood samples were analyzed for haptoglobin (Hp), serum amyloid A (SAA), fibrinogen (Fbg), total proteins, albumin, creatinine, urea, and total immunoglobulins. The results obtained for sick animals were compared with those in 23 clinically healthy animals. In affected animals, the concentrations of Hp, SAA, and Fbg were significantly higher than in healthy animals ($p<0.05$, $p<0.001$, and $p<0.001$, respectively). Moreover, in heifers with hoof diseases we found significantly higher total protein concentrations ($p<0.05$). On the other hand, serum concentrations of creatinine and urea showed a trend of significantly lower values in heifers affected by lameness ($p<0.05$ and $p<0.001$, respectively). Concentrations of serum albumin and total immunoglobulins were not significantly different between healthy and sick animals. The presented data indicate an elevated production of acute phase proteins in heifers affected by hoof diseases and lameness, and suggest the usefulness of their measurement in the laboratory diagnosis of hoof diseases in cattle.

Key words: acute phase proteins, cattle, hoof diseases, lameness

INTRODUCTION

Bovine lameness and hoof diseases represent one of the major health problems for the dairy industry, and raise important questions about economic aspects and welfare issues in agriculture (Warnick *et al.,* 2001; Hernandez *et al.,* 2007). Economic losses arise from decreased milk production, poor performance, fertility problems, increasing culling rates, and treatment costs. The predominant hoof problems causing lameness in cows are sole ulcers, white line abscesses, interdigital phlegmons and digital dermatitis (Hoblet and Weiss, 2001;

Stokka *et al.,* 2001). Hoof diseases are generally accepted to be multifactorial, which comprise management, housing, nutrition, and physiological stage (Greenough and Vermunt, 1991). However, these disorders can occur by physical injury, heel warts, sole abscess, a stone in the hoof, or even digestive disorders (acidosis, displaced abomasum) (Lischer and Ossent, 1994). Some of these reasons may affect the concentrations of some biochemical variables (Parizi and Khalafizadeh, 2006). Stec *et al.* (2006) reported that the concentrations of globulins in cows with developed disorders in the locomotory system were higher than in healthy cows, while the concentrations of albumin decreased. However, there are only scarce data about the influence of hoof diseases on the concentrations of other parameters, recently used in veterinary clinical practice, e.g. acute phase proteins.

Acute phase proteins are serum proteins of hepatic origin that are synthesized and released in response to infection, inflammation, or tissue damage (Murata *et al.,* 2004). Acute phase proteins have numerous activities, by which they defend the host against pathological injury. They may minimize damage to normal tissues, help its regeneration, and assist in the restoration of homeostasis (Lomborg *et al.,* 2008). Measurements of acute phase proteins can detect or confirm the presence of infection or pathological lesions. In the clinical field, these biomarkers may serve as indicators of prognosis and efficacy of treatment (Eckersall, 2000). In cattle, the most sensitive acute phase proteins are haptoglobin and serum amyloid A. Fibrinogen, a precursor of fibrin, is also an acute phase protein, which in cattle is characterised by markedly increased synthesis in response to infection (Lowe *et al.,* 2004). Increases in the concentrations of the aforementioned acute phase proteins have been reported in response to various clinical and sub-clinical inflammatory conditions in cattle (mastitis, metritis, pneumonia) (Hirvonen *et al.,* 1999; Heegaard *et al.,* 2000). Disorders of the locomotory system (due to damaged tissues, painful processes and impaired homeostasis) may also lead to a systemic acute phase response characterised by higher concentrations of some acute phase proteins. However, little is known about the changes of acute phase proteins in cattle affected by hoof diseases and lameness.

Therefore, the aim of this study was to evaluate the possible influence of hoof diseases on the concentrations of selected acute phase proteins, as well as some other variables related to the protein profile in the blood of heifers.

MATERIAL AND METHODS

The evaluation was carried out on 35 heifers of a low-land black spotted breed and its crossbreeds, which were clinical cases with various clinical findings on hoofs, and were hospitalized at the Clinic for Ruminants of the University of Veterinary Medicine and Pharmacy in Kosice (Slovak Republic). The animals were submitted to the clinic by a veterinarian from a private farmer. On the clinic, the heifers were housed individually, fed twice a day and had *ad libitum* access to water.

All heifers were examined clinically using standard clinical examination procedures oriented to the examination of the general health state (body temperature, food intake, behaviour, gait, and movement). Hoof disorders were diagnosed by orthopedic inspection performed according to the method described by Jackson and Cockcroft (2002). In the evaluated heifers pododermatitis, laminitis, sole ulcer, and digital dermatitis were the most often diagnosed diseases, and they did not show pathological lesions on other organ systems. Another 23 clinically healthy animals of the same age and breed, in good general health without any obvious disease, including lameness, as evaluated by routine clinical inspection were used as controls to compare the evaluated variables between sick and healthy animals.

Blood samples for the investigations were taken from animals after initial diagnosis and when the clincial signs of disease were obvious. Blood samples were collected by direct puncture of *v. jugularis* into plastic tubes with gel and clot activator for serum, and into special tubes with sodium citrate for plasma samples. Blood serum was used for the analyses of selected acute phase proteins – haptoglobin (Hp, mg/mL) and serum amyloid A (SAA, μg/mL), and variables of the protein profile – total proteins (TP, g/L), albumin (Alb, g/L), creatinine (CR, μmol/L), urea (U, mmol/L), and total immunoglobulins (TIg, U ZST – units of zinc-sulphate turbidimetric test). The concentrations of fibrinogen (Fbg, g/L) were measured in the blood plasma.

The concentrations of haptoglobin were assessed using a commercial colorimetric kit (Tridelta Development, Ireland) in microplates, based on Hp-haemoglobin binding and preservation of the peroxidase activity of the bound haemoglobin at low pH. Serum amyloid A was analysed by commercial ELISA kits (Tridelta Development, Ireland) in microplates. The optical densities were read on the automatic microplate reader Opsys MR (Dynex Technologies, USA). The determination of fibrinogen was performed on the semi-automatic 4-channel coagulometer Behnk CL-4 (Behnk Elektronik GmbH & Co., Germany) using commercial diagnostic kits (Diagon Kft, Hungary), based on the principle of electromagnetic detection of fibrin formation. Concentrations of TP, Alb, U, and CR were determined using commercial diagnostic kits (Randox) on an automatic biochemical analyser ALIZE (Lisabio, France). Total immunoglobulins were analysed by spectrophotometric turbidimetric method (zinc-sulphate test) (Slanina *et al*., 1976).

Statistical evaluation of the results was performed by assessment of average values (x), standard deviations (SD), medians, minimal (min) and maximal values (max). The significance of differences in the measured values (P) of corresponding variables between healthy and sick animals was evaluated by a Mann-Whitney non-parametric test. The relationships between the concentrations of evaluated acute phase proteins in heifers with clinical signs of hoof diseases and lameness were calculated by linear regression and Spearman (R) correlations coefficient, including the significance of the correlation. The data were analysed using the statistical programme GraphPad Prism V5.02 (GraphPad Software Inc.).

RESULTS

Results of the concentrations of evaluated acute phase proteins, and variables related to protein profile characterised by average values (x) and standard deviations (SD), as well as the evaluation of significance of differences in measured values (P) between two groups of heifers are given in Table 1. The comparison of the concentrations of acute phase proteins between healthy and sick animals is presented in Figure 1 – 3.

Figure 1. Comparison of the concentrations of Hp between groups of evaluated animals. The plots show the median (line within box), 25th and 75th percentiles (box), minimum and maximum values (whiskers)

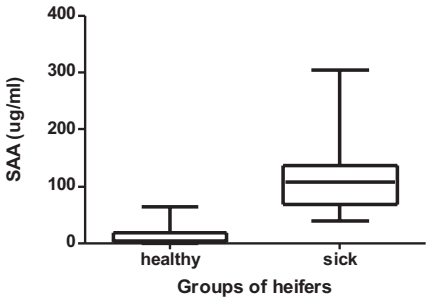

Figure 2. Comparison of the concentrations of SAA between groups of evaluated animals. The plots show the median (line within box), 25th and 75th percentiles (box), minimum and maximum values (whiskers)

Figure 3. Comparison of the concentrations of Fbg between groups of evaluated animals. The plots show the median (line within box), 25th and 75th percentiles (box), minimum and maximum values (whiskers)

In heifers with hoof diseases we found significantly higher serum concentrations of Hp than in healthy animals ($p<0.05$, Table 1). It is shown (Fig. 1) that while in healthy animals the median of Hp concentrations was 0.073 mg/mL and the individual values ranged from 0.001 to 0.296 mg/mL, in lame heifers we recorded a higher median of Hp concentrations (0.129 mg/mL), as well as a markedly wider range of individual values with a maximal concentration of 2.424 mg/mL. By more detailed analysis of individual Hp concentrations we found

that while in healthy animals 50 % of measured values ranged from 0.001 to 0.142 mg/mL, in sick animals this range was from 0.059 to 0.806 mg/mL.

In heifers suffering from hoof disorders, significantly higher serum concentrations were found also for SAA ($p<0.001$). The median concentration of SAA in animals without lamenenss was 3.40 μg/mL, and the individual values ranged from 0.30 to 64.50 μg/mL (Fig. 2). The median SAA concentration in lame heifers was higher (108.00 μg/mL), and we observed a wider range of individual values (from 39.20 to 304.00 μg/mL). More detailed analysis of serum SAA concentrations showed that in healthy cattle 50% of measured values were in the range of 0.98 – 19.30 μg/mL, in sick animals the values ranged from 68.50 to 136.00 μg/mL.

Significant differences between the two groups of animals were observed also in the plasma concentrations of fibrinogen ($p<0.001$). The median concentration of Fbg in clinically healthy cattle was 2.08 g/L, and the individual values ranged from 1.58 to 2.94 g/L (Fig. 3). In heifers affected by hoof diseases we found a higher median concentration (2.79 g/L), with a wider range of the measured individual values and a trend of higher Fbg concentrations (2.13 – 5.00 g/L).

The evaluation of the concentrations of other variables related to protein profile showed also significant differences between clinically healthy and sick animals (Table 1). In heifers with hoof disorders and lameness we observed significantly higher concentrations of total proteins than in animals without signs of hoof diseases ($p<0.05$). On the other hand, serum concentrations of creatinine and urea showed a trend of significantly lower values in heifers affected by lameness ($p<0.05$ and $p<0.001$, respectively). In the serum concentrations of albumin and total immunoglobulins we recorded a significant differences between healthy and sick animals.

Table 1. Comparison of the concentrations of selected acute phase proteins and parameters of the protein profile in healthy and sick animals ($x \pm SD$)

Variables	Groups of animals		p
	Healthy (n = 23)	Sick (n = 35)	
Hp (mg/mL)	0.094 ± 0.086	0.450 ± 0.601	<0.05
SAA (μg/mL)	12.700 ±16.80	113.900 ± 55.66	<0.001
Fbg (g/L)	2.190 ± 0.37	2.950 ± 0.65	<0.001
TP (g/L)	75.600 ± 5.5	81.200 ± 11.2	<0.05
Alb (g/L)	39.300 ± 3.2	38.500 ± 4.8	n.s.
CR (μmol/L)	126.300 ±16.7	109.900 ± 20.7	<0.01
U (mmol/L)	4.740 ± 1.01	2.610 ± 1.21	<0.001
TIg (U ZST)	32.200 ± 3.0	31.500 ± 6.9	n.s.

p – significance of the differences in measured values between healthy and sick animals
n. s. – non significant

Table 2. Correlation and regression analyses between the concentrations of evaluated acute phase proteins in heifers with clinical signs of hoof diseases

	Hp	SAA	Fbg
Hp	–	0.506[a]	0.571[b]
SAA	0.506[a]	–	0.581[b]
Fbg	0.571[b]	0.581[b]	–

[a, b] indexes mean statistical significance of correlations: a – $p<0.01$, b – $p<0.001$

By the assessment of correlations between the evaluated acute phase proteins in heifers with clinical signs of hoof diseases and lameness we recorded a significant positive correlation between Hp and SAA ($R = 0.506$, $p<0.01$), Hp and Fbg ($R = 0.571$, $p<0.001$), as well as between SAA and Fbg ($R = 0.581$, $p<0.001$) (Table 2).

DISCUSSION

Presented results indicate that hoof diseases in cattle, accompanied by various local changes, lameness, as well as systemic reactions may induce increased production of some acute phase proteins. Smith *et al.* (2009) reported that sole ulcers, white line disease, and lameness in cows may cause not only decreased milk production and reproduction problems, but may have also a marked impact on the synthesis of acute phase proteins as a result of a generalized acute phase response. On the other hand, in the study of Laven *et al.* (2004), no increased concentrations of acute phase proteins were found in cattle with hoof haemorrhages.

In the presented study, significantly higher concentrations of the measured acute phase proteins in heifers affected by hoof disorders were found for haptoglobin, serum amyloid A, as well as fibrinogen. However, marked differences between the aforementioned inflammatory proteins were observed in the ability to react to an acute phase response causing event. The most marked differences between healthy and sick animals were recorded in the concentrations of serum amyloid A. The mean SAA concentration in heifers with hoof diseases was about nine fold higher than the average concentration recorded in the group of clinically healthy animals. On the other hand, the average serum concentration of Hp in heifers with clinical signs of hoof diseases and lameness was about fivefold higher compared with healthy ones. These findings correspond partially to the data presented by Kujala *et al.* (2010). The aforementioned authors showed a higher mean concentration of SAA in lame cows due to sole ulcer and white line disease than in healthy animals. However, in Hp concentrations they found no significant differences between healthy and lame cows. Therefore, the authors suggested that SAA is a better indicator for claw disorders than haptoglobin. Werling *et al.* (1996) reported also that SAA is a more sensitive acute phase protein than Hp in cattle with rapid increase in serum concentrations after the inflammatory stimulus. According to Muller-Doblies *et al.*

(2004), Hp requires a stronger stimulation to induce an increase in serum concentrations. On the other hand, Smith *et al.* (2009) described, similarly to our results, elevated serum Hp concentrations in lame cows with claw disorders.

The influence of hoof diseases and lameness on plasma Fbg concentrations in cattle is less well documented. The usefulness of the measurement of the Fbg concentrations in cattle has been demonstrated by monitoring of postoperative complications, e.g. peritonitis, as well as by the differentiation of traumatic reticuloperitonitis from other gastrointestinal disorders (Hirvonen and Pyörälä, 1998). The presented study suggest that the determination of the concentrations of fibrinogen may be also useful for the diagnosis of other diseases, as we found higher plasma Fbg concentrations in heifers affected by hoof disorders compared with clinically healthy animals. However, the differences in the concentrations of Fbg between the two groups of animals were less marked than the differences observed in the concentrations of Hp and SAA. Increased concentrations of Fbg have been recently described but only in 14 lame cows with clinical signs of claw diseases (Jawor *et al.,* 2008). Therefore, further investigations are needed to deepen our knowledge about the synthesis of some acute phase proteins in cattle with hoof disease and lameness.

To the best of our knowledge, there are scarce data describing the relationships between the concentrations of acute phase proteins, when determining some acute phase proteins simultaneously, e.g. Hp, SAA, and Fbg. The haptoglobin concentrations were demonstrated earlier by Tóthová *et al.* (2010) to correlate highly with the values of SAA in calves with respiratory diseases. Our study showed highly significant correlation also between the concentrations of Hp and Fbg, as well as SAA and Fbg, i.e. higher concentrations of Hp or SAA in animals with hoof diseases were associated with higher values of fibrinogen.

Although the evaluated heifers with clinical signs of hoof disorders and lameness were found to have higher concentrations of measured acute phase proteins compared with healthy cattle, in sick animals we observed a markedly wider range of individual values of haptoglobin, serum amyloid A, as well as fibrinogen. Similar findings were reported by Jawor *et al.* (2008) and Smith *et al.* (2009), when lame cows were found having either high or undetectable serum Hp concentrations. The higher values of standard deviations recorded in our study may reflect the different reactivities of various acute phase proteins during impaired homeostasis. According to Jacobsen *et al.* (2004), the ability to produce haptoglobin and serum amyloid A is an innate characteristic of the individual. Stengärde *et al.* (2008) and Lomborg *et al.* (2008) reported also that animals can vary in their acute phase response to the same exposure. Different diseases severity (i.e. more severe diseases are accompanied by higher concentrations of acute phase proteins) might be another reason for higher values of standard deviations of measured acute phase proteins (Young *et al.,* 1996).

Seeing that tissue damage and other pathologic lesions in locomotory disorders may cause disturbances in homeostasis and changes in metabolism, we expected that hoof diseases and lameness may affect the concentrations of blood biochemical parameters. Yaylak *et al.* (2009) found no significant

differences in the values of blood parameters between lame and non-lame cows. However, the presented study indicates that hoof diseases and lameness in heifers may influence the concentrations of some variables related to protein metabolism, predominantly the values of total proteins. Higher concentrations of total proteins observed in heifers with clinical signs of hoof diseases and lameness may reflect the response of the organism to inflammation. Murray *et al.* (2001) reported that serum protein concentrations, and albumin and globulin ratios are generally considered indicators of some pathologic processes and inflammatory conditions. According to Whitaker *et al.* (1999), albumin and globulin values are used in evaluating inflammatory diseases and stated that in illness cases, globulin increases while albumin decreases. Moreover, albumin is a typical negative acute phase protein, the concentrations of which decrease in the case of activated acute phase response (Eckersall, 2000). However, our study showed no significant differences in the concentrations of albumin and total immunoglobulins between clinically healthy animals and animals suffering from hoof disorders. Because of scarce data about metabolic changes in cattle affected by hoof diseases and lameness, further investigations are needed to yield satisfactory results.

In conclusion, the presented results indicate that hoof diseases and lameness in heifers may be associated with a systemic acute phase response characterized by elevated concentrations of some acute phase proteins. Thus, our data suggest that the measurement of major bovine acute phase proteins in the blood, besides the clinical diagnosis, would be a valuable contribution to laboratory diagnostics of hoof diseases in cattle.

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UTICAJ BOLESTI PAPAKA NA KONCENTRACIJU NEKIH PROTEINA AKUTNE FAZE I DRUGIH PROTEINA KOD JUNICA

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SADRŽAJ

Cilj ovog istraživanja je bio da se proceni mogući uticaj oboljenja papaka junica na koncentraciju nekih proteina akutne faze i drugih parametara povezanih sa metabolizmom proteina krvi. U ispitivanje je bilo uključeno 35 junica crno-bele šarene nizijske rase i njihovih meleza sa različitim kliničkim nalazima na papcima. Krv je uzorkovana jednom, kada su klinički znaci bolesti bili očigledni. U krvnom serumu su određivane koncentracije: haptoglobina (Hp), serumskog amiloida A (SAA), fibrinogena (Fbg), ukupnih proteina, albumina, kreatinina, uree i ukupnih imunoglobulina. Ovi rezultati su zatim upoređeni sa nalazima 23 klinički zdrave životinje. Kod obolelih životinja, koncentracija Hp, SAA i Fbg je bila značajno veća nego kod zdravih jedinki ($p<0,05$, $p<0,01$ i $p<0,001$). Kod junica sa obolelim papcima dokazana je značajno veća koncentracija ukupnih proteina ($p<0,05$). Istovremeno, koncentracija kreatinina i uree, u serumu obolelih junica, je bila značajno niža u odnosu na vrednosti registrovane kod zdravih jedinki ($p<0,05$ i $p<0,001$). Koncentracija albumina i ukupnih imunoglobulina nije se značajno razlikovala kod zdravih i obolelih životinja. Dobijeni podaci ukazuju na povezanost koncentracije proteina akutne faze kod junica sa obolelim papcima i hromošću i mogu biti od koristi za laboratorijsku dijagnostiku.