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## Acute Phase Proteins as Markers of Diseases in Farm Animals

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### 1. Introduction

Acute phase proteins have been studied widely in human medicine, especially as biomarkers of diseases, inflammatory processes and various infections, to diagnose and monitor the success of diseases, as well as to follow-up the treatment in clinical praxis (Hilliquin, 1995; Deans & Wigmore, 2005; Endre & Westhuyzen, 2008). However, the possible influence of inflammatory conditions on the concentrations of acute phase proteins, and the use of these indicators in the monitoring of animal health and detection of diseases in veterinary medicine, especially in farm animal medicine is less well documented. Therefore, an increased focus on the application of acute phase proteins in veterinary clinical practice has recently been developed. Recently studies show their functions, and influences on the organism (Murata et al., 2004; Petersen et al., 2004).

The acute phase response is in animal species, by which the organism respond to impaired homeostasis, caused by tissue injury or inflammation, leading to a range of metabolic activities and biochemical processes (Whicher & Westacott, 1992; Baumann & Gauldie, 1994). One of the most important metabolic changes during the acute phase response is the strongly plasma-increased or decreased liver production and secretion of the acute phase proteins. These biomarkers are non-specific but highly sensitive exhibiting strong differences in their production between different animal species (Eckersall & Bell, 2010).

For this reason, the objective of this article is provide an integrated overview about the diagnostic value of acute phase proteins levels in farm animals along with some clinical aspects of veterinary practices.

### 2. The acute phase response

All vertebrates demonstrate an early and non-specific complex of reactions to injury known as the acute phase response. The acute phase response is a cascade of host responses, which is induced by any process that leads to tissue damage e.g. bacterial and viral infection, parasite infestation, trauma, surgery, ischemic necrosis, burns, neoplastic growth (Baumann & Gauldie, 1994; Suffredini et al., 1999). The acute phase response is characterized by numerous local and systemic changes and involves a variety of cell types and organs (Koj, 1996; Gabay & Kushner, 1999). The reactions of the acute phase response are part of the non-

specific immune system and thus the first line of defense against invading pathogens. It is designed to hold the infection in check until the adaptive, highly specialized immune response is initiated (Fearon & Locksley, 1996).

Blood monocytes and tissue macrophages are central to initiating the acute phase response. After being activated by “alarm molecules” e.g. arachidonic acid metabolites and modified host proteins recognized as foreign, released from injured cells and tissues, monocytes and macrophages produce a vast number of inflammatory mediators, among which the cytokines play very important roles (Bellomo, 1992). The local, paracrine effects and the distant, endocrine effects of cytokines propagate the continuation of the acute phase response by stimulating various other cell types to the secondary release of cytokines responsible for the start of the systemic inflammatory response (Janeway et al., 2001; Cray et al., 2009).

During the acute phase response, the metabolic effort is directed at removal of the inflammatory stimulus, promotion of healing and repair processes and restoration of the homeostasis (Murata et al., 2004). However, the acute phase response is not uniformly beneficial. For example, iron sequestration and catabolism may result from prolonged cytokine production and cause anemia and impaired growth and weight loss (Jennings & Elia, 1996). Furthermore, when pro-inflammatory cytokines are produced in excessive amounts – as may occur during sepsis – shock and even death may ensue. The acute phase response thus needs to be tightly controlled.

### **2.1 Stimulation of the acute phase response**

The acute phase response is stimulated by the release of pro-inflammatory cytokines such as interleukin-1 beta (IL1- $\beta$ ), interleukin-6 (IL-6) and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) from macrophages and monocytes at the site of inflammatory lesions or infection (Heinrich et al., 1990). Cytokines are soluble proteins, which act at picomolar to nanomolar concentrations to regulate host inflammatory functions (Nathan & Sporn, 1991). Cytokines exert their effects mainly over shorter distances, but they may also affect cells far from their site of synthesis (Hagiwara et al., 2001). They have a short half-life span, their continue synthesis is a requisite for sustained elevated concentrations of cytokines. In addition, active inhibition through anti-inflammatory mediators such as IL-4, IL-10 and IL-13 is involved in termination of the acute phase response (Koj, 1996). The disease outcome depends on the balance between pro- and anti-inflammatory activities (Adrie & Pinsky, 2000). If their effects are not properly modulated or if they are synthesized in excessive amounts, the pro-inflammatory cytokines are potent enough to cause tissue injury, organ failure and death (Taniguchi et al., 1999).

### **2.2 Acute phase response and homeostasis**

Cytokines activate receptors on different target cells leading to systemic inflammatory reactions, including hormonal, metabolic or biochemical reactions, and resulting in a number of changes clinically characterized by fever, anorexia, weight loss or slow-wave sleep (Gruys et al., 2005). These symptoms reflect multiple changes in the homeostatic control of the diseased animals, such as increased production of adrenocorticotrophic hormone and glucocorticoids, activation of the complement cascade and blood coagulation system, decreased serum concentrations of calcium, zinc, iron, vitamin A

and  $\alpha$ -tocopherol, and changes in the concentrations of some plasma proteins (Pyörälä, 2000). One of the most important metabolic changes is the strongly increased synthesis of a group of plasma proteins, namely acute phase proteins, by the liver (Raynes, 1994).

### 3. Acute phase proteins

Acute phase proteins are phylogenetically old and may be found not only in mammals, but also in other vertebrates such as birds, marsupials, and fish. In general, acute phase proteins are a group of blood proteins that change in concentrations in animals subjected to external or internal challenges, such as infection, inflammation, trauma or stress (Murata et al., 2004).

By definition, the serum concentrations of acute phase proteins increase (positive acute phase proteins) or decrease (negative acute phase proteins) with at least 25 % during the acute phase response (Ceron et al., 2005; Eckersall & Bell, 2010). Moreover, there are large differences in the responsibility of various acute phase proteins, some of them respond markedly to inflammatory stimuli, another have moderate or minor responses (Petersen et al., 2004). Major acute phase proteins have low concentrations in the serum of healthy animals, but with their concentrations increase over 100- or 1000-fold on stimulation, reaching a peak 24 – 48 hours after the insult and fall rapidly during recovery (Niewold et al., 2003). Moderate acute phase proteins are present in the blood of healthy animals, but after stimulation their concentrations increase 5 – 10 fold, reaching a peak concentration 2 – 3 days after stimulation and decrease more slowly than major acute phase proteins (Eckersall, 2006). Minor acute phase proteins show a gradual increase of 50 – 100 % of normal values.

The functions of the acute phase proteins are varied and combined to defend the host against pathological damage and assist in the homeostasis restoration. A number of acute phase proteins are likely to participate directly in the protection of the host. Some of the acute phase proteins ( $\alpha_1$ -antitrypsin,  $\alpha_2$ -macroglobulin) have anti-protease activity designed to inhibit proteases released by phagocytes or pathogens to minimize damage to normal tissues (Pyörälä, 2000). Another acute phase proteins (haptoglobin, serum amyloid A, C-reactive protein) have scavenging activities and bind metabolites released from cellular degradation so they can re-enter host metabolic processes rather than be utilized by pathogen (Wagener et al., 2001). Other acute phase proteins ( $\alpha_1$ -acid glycoprotein) are characterized by anti-bacterial activity and by the ability to influence the course of the immune response (Eckersall, 2006a).

#### 3.1 Acute phase proteins in cattle

Despite the uniform nature of the acute phase response, there are numerous differences in the acute phase characteristics between different animal species (Pyörälä, 2000). C-reactive protein is a good example of this phenomenon: in healthy humans it is practically negligible, but has a high relative increase during infections, whereas in healthy cattle it is present, but does not increase markedly during the acute phase response (Steel & Whitehead, 1994). Acute phase proteins have typically their representatives in different species (Table 1). In cattle, haptoglobin and serum amyloid A were identified as major acute phase proteins.

Animal species	Major APPs	Moderate APPs
Cat	SAA	AGP, Hp
Dog	CRP, SAA	Hp, AGP
Horse	SAA	Hp
Cattle	Hp, SAA	AGP
Pig	CRP, MAP, SAA	Hp

Table 1. Acute phase proteins in different animal species (Eckersall, 2007)

3.1.1 Haptoglobin

Haptoglobin (Hp) is a glycoprotein composed of 2  $\alpha$  and 2  $\beta$  subunits. The  $\alpha$  subunit has a molecular weight of 16 – 23 kDa and the  $\beta$  subunit 35 – 40 kDa. The subunits combine in the form of a  $\beta$ - $\alpha$ - $\alpha$ - $\beta$  tetramer chain. Human Hp has 3 subtypes known to be genetic polymorphism (Hp 1-1, Hp 1-2, Hp 2-2). In animals, Hp tetramers have noticeable species differences. Haptoglobin in carnivores and omnivores is thought to be similar to human Hp 1-1, while bovine Hp has closer similarities to Hp 2-2 (Morimatsu et al., 1991). In circulation, Hp is highly polymerized having a molecular weight of approximately 1000 – 2000 kDa, and exists also as polymer associated with albumin (Godson et al., 1996).

The primary function of Hp is to bind free hemoglobin in the blood. The affinity of Hp for hemoglobin is one of the major protein transporters (Bowman, 1992). By removing from the circulation any free hemoglobin, which has inherent peroxidase activity, Hp prevents oxidative damage of tissues (Yang et al., 2003). The Hp-hemoglobin binding also reduces the availability of the heme residue from bacterial growth and therefore Hp has an indirect anti-bacterial activity (Murata et al., 2004).

Many studies have indicated the significance of Hp as a clinically useful parameter for measuring the occurrence and severity of inflammatory responses in cattle with mastitis, pneumonia, enteritis, peritonitis, endocarditis, abscesses, endometritis and other natural or experimental infectious situations (Ohtsuka et al., 2001; Eckersall, 2006). Haptoglobin is used to monitor the treatment efficacy of antibiotics in cows with toxic puerperal metritis (Smith et al., 1998). Hp is also used to determine the effect of anti-inflammatory drugs following the castration of bull calves, the relative effects of bacterial contamination and involution of the uterus in dairy cows after calving, the effects of treatment in transport-stressed feedlot cattle, the effects of tail docking or surgical castration, and the changes in the blood profile of neonatal calves (Carter et al., 2002).

Haptoglobin is also induced in cows with fatty liver syndrome, by starvation, and in calves following stress associated with road transport (Kato et al., 2002).

3.1.2 Serum amyloid A

Serum amyloid A (SAA) is a small hydrophobic protein (9 – 14 kDa), which is found in serum associated with high density lipoprotein. In humans, four separate isoforms have been identified (Jensen & Whitehead, 1998). Of these, SAA1 and SAA2 respond to an acute phase reaction with increased production from the liver. In contrast, SAA4 is a constitutive protein that is produced normally at low concentrations and is not affected by the acute phase response. The SAA3 isoform is expressed in non-hepatic tissues during the acute phase response with increases found in lung, adipose tissue, ovarian granulosa, as well as in the mammary gland (Weber et al., 2006). The mammary isoform (M-SAA3) has also been detected in bovine colostrum (McDonald et al., 2001).



Serum amyloid A is the precursor of amyloid A and is therefore implicated in the pathogenesis of amyloidosis (Uhlir & Whitehead, 1999). Among the functions ascribed to SAA have been reverse transport of cholesterol from tissue to hepatocytes, inhibition of phagocyte oxidative burst and platelet activation (Petersen et al., 2004). Recently, a direct antibacterial action of SAA was identified, in which SAA was found to bind to Gram-negative bacteria leading to opsonisation of the target pathogen (Hari-Dass et al., 2005). The M-SAA3 isoform found in colostrum stimulates the production of mucin from intestinal cells assisting the initiation of secretions from the neonatal intestine and helping to prevent bacterial colonization (Mack et al., 2003).

Serum amyloid A is a valuable acute phase protein in diagnosing cattle with inflammation. Increased milk SAA concentrations can be detected in cows with mastitis (Eckersall et al., 2001). Moreover, SAA in conjunction with haptoglobin may be useful markers of milk quality. Elevated serum SAA concentrations are also found in cows at parturition or in cattle subjected to physical stress, suggesting that the acute phase response is also activated under conditions unrelated to inflammation (Alsemggest et al., 1993). In cattle, it was raised also by experimental infection with *Mannheimia haemolytica* and with bovine respiratory syncytial virus (Heegaard et al., 2000). The mammary isoform M-SAA3 is secreted in milk from the mammary gland of dairy cows with mastitis, which suggests a potential role of this biomarker for this condition (Jacobsen et al., 2005).

### 3.1.3 Fibrinogen

Fibrinogen (Fbg), a precursor of fibrin, is also an acute phase protein, which has been used for many years to evaluate inflammatory and traumatic diseases in cattle, and is characterized by markedly increased synthesis in response to infection (Hirvonen & Pyörälä, 1998). Fibrinogen is involved in homeostasis, providing a substrate for fibrin formation, and in tissue repair, providing a matrix for the migration of inflammatory-related cells (Thomas, 2000).

Fibrinogen is used in cattle and sheep as a reliable indicator of the presence of inflammation, bacterial infection or surgical trauma (Cheryk et al., 1998). It increases in various inflammatory conditions of cattle, such as peritonitis, endocarditis, pericarditis, pneumonia, and nephritis, or *E. coli* infection in calves (Jafarzadeh et al., 2004). However, plasma Fbg concentrations can also remain unchanged or decrease during acute inflammatory conditions of cattle. This may reflect consumption of the protein at the inflamed area which transiently can exceed the production (Welles et al., 1993).

### 3.1.4 Albumin

Serum albumin is the major negative acute phase protein. During the acute phase response the demand for amino acids for synthesis of the positive acute phase proteins is markedly increased, which necessitates reprioritization of the hepatic protein synthesis: albumin synthesis is down-regulated and amino acids are shunted into synthesis of positive acute phase proteins (Aldred & Schreiber, 1993). It has been reported that during the acute phase response 30 to 40 % of the hepatic protein synthesizing capacity is used for production of positive acute phase proteins, and the production of other proteins thus need to be diminished (Mackiewicz, 1997).

### 3.2 Acute phase proteins in small ruminants

The acute phase proteins in small ruminants have not been studied in as much detail as in cattle, but it appears that the acute phase response is similar. Experimental studies showed that caseous lymphadenitis in sheep can cause a marked increase of Hp and SAA concentrations, which is accompanied by lower and more lasting increase of the concentrations of  $\alpha_1$ -acid glycoprotein (Eckersall, 2007). Haptoglobin has proven useful as prognostic indicator of dystocia in sheep, and can be used to investigate the relationship between uterine involution and the presence of intrauterine bacteria in ewes (Scott et al., 1992). A recent study reports that vaccination of lambs causes an acute phase response that reaches a peak within 24-48 hours, and could have a role to play in the assessment of vaccine efficacy (Dowling et al., 2004).

### 3.3 Acute phase proteins in pigs

In pigs, C-reactive protein, haptoglobin,  $\alpha_1$ -acid glycoprotein, and pig specific major acute phase protein were identified as the diagnostically most important acute phase proteins.

#### 3.3.1 C-reactive protein

In pigs, as in dogs and humans, C-reactive protein (CRP) is the prototypical acute phase protein with major diagnostic value. Tillett and Francis (1930) discovered CRP over 70 years ago in the blood of patients with *Streptococcus pneumoniae* infection, as a substance that precipitated the C-polysaccharide of the cell wall of the pneumococcus and they called it C-reactive substance, which was later changed to C-reactive protein (Du Clos, 2004). C-reactive protein plays important roles in the protection against infection, clearance of damaged tissues, and regulation of the inflammatory response (Mold et al., 2002). Structurally, CRP is a cyclic pentamer which binds with a variety of pathogenic bacteria or intracellular antigens of damaged cells, thus recognizing foreign molecules and altered self (Murata et al., 2004).

In pigs, CRP is considered to be one of the best markers for the identification of inflammatory lesions. It can be used as a parameter for monitoring a pig's general state of health, including for stress assessment (Burger et al., 1998). Serum CRP concentrations increased following aseptic inflammation and during the experimental infection with *Actinobacillus pleuropneumoniae* (Lampreave et al., 1994). Porcine CRP has also been found to rise in experimental models of *Mycoplasma hyorhinis*, *Toxoplasma gondii*, *Streptococcus suis* and porcine reproductive and respiratory syndrome virus infection (Eckersall, 2006).

#### 3.3.2 Alpha<sub>1</sub>-acid glycoprotein

The precise function of  $\alpha_1$ -acid glycoprotein (AGP) is not yet clear, but it does bind to a number of metabolites such as heparin, histamine and serotonin, steroids and catecholamines (Israili and Dayton, 2001). It is also known to bind to pharmacological compounds which may have therapeutic implications as the amount bound can affect the metabolically active fraction of the drug. Increased AGP due to an acute phase response thus may reduce the concentration of free drugs, thus affecting their pharmacokinetics.

#### 3.3.3 Pig specific major acute phase protein

Specifically in pigs, a specific acute phase protein (pig MAP) of unknown function has been reported as a sensitive indicator of infection. Increased levels in pig MAP have been shown during infections with *Actinobacillus pleuropneumoniae*, in post weaning multisystemic wasting disorder as well as following animal transportation (Segales et al., 2004).

#### **4. The usefulness of the evaluation of acute phase proteins in the veterinary clinical practice**

The measurement of the concentrations of acute phase proteins can detect or confirm the presence of infection or pathological lesion, but a major role for these analytes in farm animal medicine could be in the monitoring of the health status of animals in production. Acute phase proteins can detect the presence of sub-clinical disease which is the cause of reduced growth rate and losses in the production (Petersen et al., 2004). Use of an acute phase index, by combining the results of both positive and negative acute phase proteins has been suggested as a means to increase the sensitivity of detection of sub-clinical disease (Toussaint et al., 2000). In the clinical field, acute phase proteins may serve as indicators of prognosis and effect of treatment. The magnitude and duration of the acute phase response reflect the severity of the infection and underlying tissue damage (Heegaard et al., 2000).

Acute phase proteins have been extensively investigated in various inflammatory and non-inflammatory conditions. However, there are many more areas of enquiry which can be pursued to deepen our knowledge about the acute phase response and also to develop novel applications for the acute phase proteins, e.g. in various physiological conditions (after birth), during some less frequently studied diseases of young (diarrhoea, omphalophlebitis) and adult cattle (laminitis, mastitis), as well as not only in acute infections, but also in chronic inflammatory conditions.

#### **5. Material and methods**

##### **5.1 Animals used in the study, clinical examination, sample collection, and the evaluated parameters**

###### **5.1.1 Acute phase proteins in relation to the growth and development of calves**

The evaluation of the dynamics of age-dependent changes in the concentrations of selected acute phase proteins during the first 6 months of life was performed in seven clinically healthy calves (three males, four females) on a farm near to the University of Veterinary Medicine and Pharmacy in Kosice. The calves were of a low-land black spotted breed and its crossbreeds. The observation of the calves started at the age of 1 month and body weight of 45 – 51 kg. The animals were kept loosely in individual pens and fed ordinary whole milk 2 times a day. The transition to a solid diet lasted until the age of 2.5 month. During this period, the calves were fed gradually decreasing amounts of milk, and increasing amounts of meadow hay, and concentrates (transitional feeding period). At the age of 3 months, they were moved to a stable and housed loosely in larger groups of animals, and fed hay and grain with free access to water. Before each sample collection, the calves were examined clinically using standard clinical examination procedures (Jackson & Cockcroft, 2002). The evaluated calves showed no health disorders during the whole time of observation.

The analyses of evaluated parameters were performed in blood samples. Blood samples were taken monthly by jugular venipuncture during the first 6 months of life. The first collection was established at the age of 1 month. Blood samples were collected into plastic tubes with gel and clot activator. Serum was stored at -20 °C until analysis of haptoglobin (Hp, mg/ml) and serum amyloid A (SAA, µg/ml). Moreover, blood samples were collected also into tubes with sodium citrate, and the separated plasma was used for the analysis of the concentrations of fibrinogen (Fbg, g/l) immediately after the separation without storage.



### 5.1.2 Acute phase proteins in calves suffering from various inflammatory diseases

Sixty-nine sick calves with clinical signs of various inflammatory diseases such as respiratory diseases, diarrhoea or omphalophlebitis were used in this study. The calves were of a Slovak spotted breed, low-land black spotted breed, or their crossbreeds at the age from 2 weeks to 6 months. The evaluated calves were sent to the Clinic for Ruminants of the University of Veterinary Medicine and Pharmacy in Kosice (Slovak Republic) by privat veterinarian from three different conventional dairy farms. On the clinic, the animals were housed individually, fed twice a day with free access to water.

After the arrival to the clinic, all calves were thoroughly clinically examined using standard clinical examination procedures, oriented to the examination of general health state (body temperature, food intake, behaviour), and then specially to the respiratory system, gastrointestinal tract and umbilicus, including the recording of the clinical signs of the diseases (Jackson & Cockcroft, 2002). The evaluation of acute phase proteins was performed in 4 groups of calves grouped on the basis of the clinical examination: Group A – calves with clinical signs of respiratory diseases ( $n = 46$ ); Group B – calves with diarrhoea ( $n = 10$ ); Group C – calves with omphalophlebitis ( $n = 5$ ); Group D – calves with multisystemic diseases ( $n = 8$ ). This group of calves consisted of animals with more than one affected organ (respiratory system, digestive tract, navel, joints). To compare the evaluated variables between sick and healthy animals, twenty-eight clinically healthy calves (Group H) of the same age and breed, in good general health without any obvious disease were used as a group of controls.

Blood samples were taken from both healthy and sick animals once after initial clinical examination, when the clinical signs of the disease in sick animals were apparent. Blood samples were collected by direct puncture of *v. jugularis*, subsequently haptoglobin (Hp, mg/ml), serum amyloid A (SAA,  $\mu\text{g/ml}$ ), and fibrinogen (Fbg, g/l) were assessed.

### 5.1.3 The influence of chronic respiratory diseases on the concentrations of acute phase proteins in calves

The influence of chronic respiratory diseases on the concentrations of selected acute phase proteins was investigated in twenty-seven sick calves of a low-land black spotted breed and its crossbreeds at the age of 3 – 6 months, which were clinical cases suffering from chronic respiratory diseases of various degree, and were hospitalized on the Clinic for Ruminants of the University of Veterinary Medicine in Kosice (Slovak Republic). The animals were submitted to the clinic by privat veterinarian from three dairy farms localized in the district of the university. The feeding regime of the animals on these farms was similar. On the clinic, the animals were kept in individual pens, fed twice a day with free access to water. The body weight of the calves was 85 – 140 kg.

In this study, calves with clinical signs manifested for more than 2 weeks despite antimicrobial, antiinflammatory, and supportive therapy done by private veterinarians of the farm were analyzed. The duration of the disease in animals was estimated from the history of the disease process. Diagnosis was done by clinical examination and in some cases by ultrasound as well as endoscope examination by the same veterinarian. Clinical examination was oriented predominantly to the examination of general health state (body temperature, food intake, behaviour), and the respiratory system by visual inspection (breathing rate, nasal discharges, type of breathing, dyspnoe, coughing) and auscultation

(increased or decreased loudness of the breathing sounds, abnormal sounds). The calves did not show pathological lesions on other organ systems. On the clinic we performed further therapy of sick calves by antibiotics (marbofloxacin, amoxicillin, tetracycline, benzylpenicillin, streptomycin), antiinflammatory drugs (flunixin-meglumine), supportive remedies (vitamins), and infusions.

Blood samples for the investigations were taken from calves once during the study period, after the clinical examination and including the animals into the study. Blood samples were collected by direct puncture of *v. jugularis*. subsequently Hp and SAA, were assessed.

Calves were divided into two groups according to their health state during the treatment: Group A (n = 16) – calves with observable response to treatment and improvement of general health state (normal body temperature, improved appetite, intermittent coughing, less marked abnormal breathing sounds, but not in a comparable general condition as healthy calves); Group B (n = 11) – calves with no response to treatment, which despite long lasting antibiotic, anti-inflammatory, and supportive therapy during the treatment died, or because of poor prognosis (as a consequence of persistent disturbance of general health state and marked changes on the respiratory system) were euthanised. The control group consist in fifteen clinically healthy calves of the same age, nutrition and breed.

#### **5.1.4 Acute phase proteins in the laboratory diagnosis of mastitis in dairy cows**

To assess the relationship between clinical and sub-clinical mastitis and concentrations of milk amyloid A in milk samples, and selected acute phase proteins in blood serum 41 dairy cows (of a low-land black spotted breed and its crossbreeds with various clinical findings on the mammary gland) were studied. These cows were in the 3rd – 4th lactation, but not in the period shortly after parturition. Clinical examination of the mammary gland was performed by visual inspection and palpation, using standard physical methods of examination. Clinical mastitis was diagnosed by the presence of observable signs of inflammation in the infected quarter such as swelling, heat, pain or redness, and by the presence of clots and flakes in the milk, or by its abnormal color or consistency. To detect sub-clinical mastitis the Californian Mastitis Test (CMT) was performed. According to the results of the clinical examination of the udder and to the results of CMT the animals were divided into 4 groups: Group I – cows without clinical changes on the mammary gland and with negative CMT (n = 7), Group II – cows without clinical changes on the mammary gland and with weakly positive CMT (n = 12), Group III – cows without clinical changes on the mammary gland and with strongly positive CMT (n = 13), Group IV – cows with clinical changes on the mammary gland and changes in milk appearance (n = 9).

Milk samples were collected into plastic tubes by hand-stripping. Blood samples were collected by direct puncture of *v. jugularis*. Milk samples were used to assess concentrations of milk amyloid A (M-SAA, ng/ml) and Hp and SAA were assessed in blood samples.

#### **5.1.5 Acute phase proteins in heifers affected by hoof diseases**

Selected acute phase proteins were assessed in 35 heifers of a low-land black spotted breed and its crossbreeds, with various clinical findings on hoofs. These animals were hospitalized on the Clinic for Ruminants of the University of Veterinary Medicine and Pharmacy in Kosice (Slovak Republic). The animals were submitted to the clinic by private 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 clinically examined using standard clinical examination procedures, oriented to the examination of 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 & 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 were collected by direct puncture of *v. jugularis* into plastic tubes with gel and clot activator for serum (analysis of haptoglobin and serum amyloid A), and into special tubes with sodium citrate for plasma (analysis of fibrinogen).

## 5.2 APP analyses

### 5.2.1 Haptoglobin

Haptoglobin was assessed using commercial colorimetric kits (Tridelta Development, Ireland) in microplates, based on Hp-haemoglobin binding and preservation of the peroxidase activity of the bound haemoglobin at low pH. The optical densities were read on automatic microplate reader Opsys MR (Dynex Technologies, USA) at an optical density of 630 nm.

### 5.2.2 Serum amyloid A

Serum amyloid A was analysed by a commercial ELISA kit (Tridelta Development, Ireland). The optical densities were read on automatic microplate reader Opsys MR (Dynex Technologies, USA) at 450 nm using 630 nm as reference.

### 5.2.3 Milk amyloid A

The concentrations of M-SAA were analyzed according to the method described in the section 5.2.2, modified by the manufacturer for the determination of amyloid A in milk samples.

### 5.2.4 Fibrinogen

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.

### 5.2.5 Californian Mastitis Test

The Californian Mastitis Test was performed using equal volumes of milk and alkyl-aryl-sulphonate by the same person in each cow.

## 5.3 Statistical analyses

Statistical analyses were done in the programme GraphPad Prism V5.02 (GraphPad Software Inc.) by assessment of arithmetic means ( $\bar{x}$ ) and standard deviations (SD) for each

evaluated parameter and each group of animals, calculated using descriptive statistical procedures.

The significance of the influence of age (P) on the evaluated variables during the whole monitored period was analyzed by the non-parametric Friedman's rank sum test. The significance of the differences in values between the sample collections was evaluated by the Dunn's Multiple Comparisons Test.

The analysis of the significance of differences in measured values between calves with various inflammatory diseases was performed by Kruskal-Wallis nonparametric ANOVA test and Dunn's Multiple Comparisons Test. The aforementioned statistical methods were used also by the evaluation of the differences between cows with various clinical findings on the mammary gland.

The assessment of the significance of differences in measured values of the evaluated variables between healthy animals and calves affected by chronic respiratory diseases was performed by Mann-Whitney non-parametric test. Similar statistical methods were used for the analyses of differences between healthy animals and heifers with hoof diseases.

6. Results

6.1 Acute phase proteins in relation to the growth and development of calves

Concentrations of acute phase proteins during the first six months of calves life were monitored. The results showed significant changes in haptoglobin, serum amyloid A, as well as fibrinogen concentrations ( $P < 0.05$ ,  $P < 0.05$ , and  $P < 0.01$ , respectively). The serum concentrations of Hp during the first three months of life and in the 5th month of age were roughly uniform. More marked changes in the mean Hp concentrations were recorded in the 4-month-old calves (increase) and in the 6-month-old ones (decrease).

Serum concentrations of SAA in calves gradually and significantly decrease ( $P < 0.05$ ). The mean concentrations of SAA in the first three months of life were more than two-fold higher than those in older animals. The highest mean concentration of Fbg (3.14 g/l) in blood plasma of calves was recorded in the 2nd month of age. In the 3rd month of age a repeated decrease of the values measured with the lowest mean Fbg concentrations (2.17 g/l) was observed in the 6-month-old calves.

Variable		Age of the calves (months)						P
		1	2	3	4	5	6	
Hp (mg/ml)	x	0.068 <sup>A</sup>	0.064	0.056	0.213	0.062	0.021 <sup>A</sup>	< 0.05
	± SD	0.021	0.026	0.024	0.344	0.081	0.018	
SAA (µg/ml)	x	59.12 <sup>a</sup>	53.37	39.71	21.20	10.51 <sup>a</sup>	19.86	< 0.05
	± SD	35.61	21.43	26.65	19.97	10.36	27.42	
Fbg (g/l)	x	2.31	3.14 <sup>a</sup>	2.55	2.87	2.82 <sup>b</sup>	2.17 <sup>a,b</sup>	< 0.01
	± SD	0.76	0.75	0.32	0.57	0.40	0.20	

The same superscripts in rows mean statistical significance of differences in concentrations between the columns: a, b –  $P < 0.05$ , A –  $P < 0.01$   
P – significance of the differences

Table 2. Age-related changes in the concentrations of evaluated APPs in clinically healthy calves from the 1st till 6th month of age

6.2 Acute phase proteins in calves suffering from various inflammatory diseases

Hp exhibited significant differences between clinically healthy calves and calves suffering from various inflammatory diseases ( $P < 0.001$ , Table 3). The highest mean value among the sick animals was found in calves with clinical signs of respiratory diseases (Group A). The Hp concentrations obtained in calves with respiratory signs were significantly higher than the values recorded in clinically healthy animals (Group H,  $P < 0.001$ ). Higher mean concentration we found also in calves with multisystemic diseases (Group D), but the obtained results were not significantly different compared with values recorded in clinically healthy animals. The mean concentrations found in calves with diarrhoea (Group B) and in calves with omphalophlebitis (Group C) were roughly uniform and lower than in the above mentioned animals from the Group A and D.

Significant differences between the evaluated groups of calves were found also for SAA ( $P < 0.001$ , Table 3), with concentrations in samples from calves with clinical signs of respiratory diseases (Group A) being significantly higher than in clinically healthy calves (Group A,  $P < 0.001$ ). Markedly higher mean concentration of SAA compared with healthy animals was found also in calves with omphalophlebitis (Group C), with the maximum individual concentration of 106.00  $\mu\text{g/ml}$ . The SAA concentrations recorded in calves with signs of diarrhoea (Group B) and in calves affected by multisystemic diseases (Group D) were the lowest among the evaluated groups of calves, and were nearly similar.

Variables		Groups of calves					K-W <i>P</i>
		H	A	B	C	D	
Hp (mg/ml)	x	0.04 <sup>a</sup>	0.73 <sup>a</sup>	0.10	0.13	0.43	< 0.001
	SD	0.03	0.78	0.16	0.16	0.78	
SAA ( $\mu\text{g/ml}$ )	x	29.78 <sup>a</sup>	93.38 <sup>a</sup>	51.47	74.94	42.02	< 0.001
	SD	24.62	50.96	25.05	26.16	28.21	
Fbg (g/l)	x	2.31 <sup>a</sup>	3.86 <sup>a</sup>	2.84	2.81	3.16	< 0.001
	SD	0.41	1.55	0.77	0.51	1.22	

The same superscripts in rows mean statistical significance of differences in measured concentrations between the groups of calves: a -  $P < 0.001$

K-W - Kruskal-Wallis analysis; P - significance of the analysis

Groups of calves: H - clinically healthy calves, A - calves with clinical signs of respiratory diseases, B - diarrhoeic calves, C - calves with omphalophlebitis, D - calves with multisystemic diseases

Table 3. Comparison of the concentrations of Hp, SAA and Fbg between clinically healthy calves and calves affected by various inflammatory diseases

Similarly, the concentrations of Fbg in blood plasma differed significantly between the evaluated groups of calves ( $P < 0.001$ ). Significantly higher concentrations of Fbg compared with clinically healthy calves we observed in calves suffering from respiratory diseases (Group A,  $P < 0.001$ ). Trend of higher Fbg concentrations was observed also in calves affected by multisystemic diseases (Group D) with the maximum individual concentration of 5.04 g/l in this group of calves. Higher values of Fbg were found also in calves suffering from diarrhoea (Group B), as well as in calves with navel inflammation (Group C). However, the differences between these groups of animals and the healthy ones were not significant.



6.3 The influence of chronic respiratory diseases on the concentrations of acute phase proteins in calves

The average Hp concentration was significantly higher ( $P < 0.001$ ) in calves suffering from chronic respiratory diseases as compared with healthy animals (Table 4). Moreover, the analyses of results in sick animals showed significantly higher serum concentration of Hp in died, or euthanised calves (Group B;  $P < 0.05$ ) as compared with those in improved health state (Group A, Table 5).

Variables	Group of calves		P
	Healthy (n = 15)	Sick (n = 27)	
Hp (mg/ml)	0.05 ± 0.06	1.11 ± 0.80	< 0.001
SAA (lg/ml)	28.02 ± 20.60	63.19 ± 39.42	< 0.01

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

Table 4. Concentrations of Hp, and SAA in healthy animals and calves suffering from chronic respiratory diseases (x ± SD)

SAA levels ( $P < 0.01$ ) in sick calves were significantly higher than in healthy individuals. The mean value of this variable in calves with poor prognosis (Group B) was about two-fold higher ( $P < 0.01$ ) compared with the mean in the Group A (Table 5).

Variables	Group of sick calves		P
	A (n = 16)	B (n = 11)	
Hp (mg/ml)	0.81 ± 0.60	1.56 ± 0.86	< 0.05
SAA (µg/ml)	44.70 ± 30.78	90.07 ± 35.73	< 0.01

Groups of calves: A – group of calves with improved general health state; B – group of died or euthanised calves

P – significance of the differences in measured values between two groups of sick calves, n. s. – non significant

Table 5. Comparison of concentrations of Hp, and SAA between two groups of sick calves (x ± SD)

6.4 Acute phase proteins in the laboratory diagnosis of mastitis in dairy cows

M-SAA concentrations in milk samples differed significantly between the groups ( $P < 0.001$ ), with concentrations in samples from cows with clinical mastitis (group IV) being significantly higher than in samples from groups I and II ( $P < 0.001$  and  $P < 0.05$ , respectively, Table 6). The concentrations of M-SAA in milk samples increased with increasing CMT score.

The serum concentrations of Hp showed also tendency of gradual significant increase with increasing CMT score and clinical changes on the mammary gland ( $P < 0.05$ , Table 6). The highest mean Hp concentration we found in cows with clinically manifested signs of mastitis. Similarly, SAA concentrations differed significantly between the evaluated groups of cows ( $P < 0.05$ ), with the highest mean concentration in animals with clinical signs of mastitis. However, the differences in the obtained results of Hp and SAA concentrations between the evaluated groups of cows were less significant ( $P < 0.05$ ) compared with M-

SAA concentrations. The mean SAA concentrations found in cows from group I and group II were roughly uniform.

Variable		Groups of cows				K-W P
		I. (n=7)	II. (n=12)	III. (n=13)	IV. (n=9)	
M-SAA ng/ml	x	325.7 <sup>A,B</sup>	1433.1 <sup>a</sup>	3910.4 <sup>A</sup>	6073.8 <sup>B,a</sup>	< 0.001
	± SD	173.8	949.2	2145.8	4414.0	
Hp mg/ml	x	0.046	0.122	0.299	0.329	< 0.05
	± SD	0.053	0.263	0.314	0.339	
SAA µg/ml	x	29.7	27.6 <sup>a</sup>	48.2	71.5 <sup>a</sup>	< 0.05
	± SD	27.6	28.0	42.5	31.5	

The same superscripts in rows mean statistical significance of differences in measured concentrations between the groups of cows: a – P < 0.05; A, B – P < 0.001  
K-W – Kruskal-Wallis analysis; P – significance of the analysis  
Groups of cows: I – cows without clinical findings on the mammary gland and with negative CMT, II – cows without clinical findings on the mammary gland and with weakly positive CMT, III – cows without clinical findings on the mammary gland and with strongly positive CMT, IV – cows with clinical changes and changes in the milk appearance

Table 6. The concentrations of M-SAA, Hp, and SAA in dairy cows with various findings on the mammary gland

6.5 Acute phase proteins in heifers affected by hoof diseases

The data referring to the concentrations of evaluated acute phase proteins in healthy animals and heifers with hoof diseases are presented in Table 7. In affected animals, the concentrations of Hp, SAA, as well as Fbg were significantly higher than in healthy animals (P < 0.05, P < 0.001, and P < 0.001, respectively).

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)	12.70 ± 16.80	113.90 ± 55.66	< 0.001
Fbg (g/l)	2.19 ± 0.37	2.95 ± 0.65	< 0.001

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

Table 7. Comparison of the concentrations of evaluated acute phase proteins in healthy animals and heifers with hoof diseases (x ± SD)

7. Discussion

7.1 Acute phase proteins in relation to the growth and development of calves

A high number of biochemical parameters in calves have been investigated earlier by several authors, stating that growth and development of the organism is accompanied by dynamic changes in the values of various haematological parameters (Hugi & Blum, 1997; Knowles et al., 2000). The evaluation of metabolic disorders in calves is helpful for interpreting results of biochemical parameters regarding physiological processes. These

processes include development, nutrition, functional immaturity of various organs, unstableness of the homeostasis, and immunological reactions. However, there are only scarce data about the possible influence of age on the concentrations of other parameters, including APP.

After birth, newborns and young animals go through a period of rapid growth and development, and adapt their life outside the uterus. This transition from foetal to neonatal life and then from newborn to young animal necessitates major physiological adjustments (Bittrich et al., 2004). Young calves must adapt to various environmental factors, including nutrition which changes from a primarily carbohydrate-based energy supply during the foetal period to a high fat and relatively low carbohydrate nutritional energy supply in colostrum and milk, and then from milk to solid diet (Odle, 1997). The exposure to the new environment and foreign antigens requires the establishment of appropriate defence responses. The neonate is immunocompetent, but the adaptive immune system is immature (Morein et al., 2002). Non-specific defence mechanisms, including the reactions of the acute phase response may thus be important for the adaptation to complicated physiological processes during growth and development of calves. Therefore, the concentrations of acute phase proteins were expected to be also influenced by the age of evaluated animals. The concentrations measured in young calves thus may differ from the values in adult cattle.

The results presented here indicate that there are significant changes in the concentrations of the evaluated acute phase proteins in calves. The most pronounced changes were observed in the concentrations of SAA. The highest mean of SAA concentration was observed at the age of 1 month followed by a gradual decrease up to 5th month of life. Orro et al. (2008) reported higher mean serum concentrations of SAA shortly after birth, being the highest at the age of 7 days (112.0 mg/l), decreasing after 10 days of age. According to the aforementioned authors, possible factors contributing to the higher serum SAA concentrations in newborn and young calves include neonatal synthesis of acute phase proteins in the liver due to the birth trauma or intake of colostral inducers such as cytokines. Studies performed by Hagiwara et al. (2001) have shown that colostrum contains high amounts of pro-inflammatory cytokines, which are the main inducers of the APP production by the liver. These inflammatory mediators present in colostrum may stimulate the hepatic production of acute phase proteins. On the other hand, the higher serum concentrations of SAA in newborn and young calves could also be caused by direct transfer of this protein from the colostrum to the calf, similarly to immunoglobulins, as colostrum of healthy cows contains mammary-associated amyloid A (McDonald et al., 2001). These age-dependent changes of SAA concentrations in young calves may reflect physiological adaptation mechanisms to the new environment, that are not necessarily a sign of a disease. The higher values of standard deviations obtained in calves reflect the different reactivity of acute phase proteins to various external or internal stimuli. The wider range of individual values of measured acute phase proteins, predominantly SAA, also suggests the differences in the variability of animals reacting to impaired homeostasis. Pyörälä (2000) reported a significant variation between the different APP profiles and that the production of these proteins varies not only among different animal species, but also within them.

There are only few studies available about the serum concentrations of Hp in newborn and young calves, and the data are contradictory. The results obtained in calves showed less pronounced changes in the Hp concentrations during the first three months of life than those observed in the serum SAA concentrations. The concentrations of Hp in the blood

serum of calves in the first three months after birth were roughly uniform, and the values were comparable with the concentrations measured in healthy adult cattle. Similar findings were reported by Hyvönen et al. (2006). Orro et al. (2008) stated also that serum Hp concentrations after birth were more stable compared with serum amyloid A. Slightly higher mean Hp concentration was observed by above-mentioned authors at the age of 3 days, and then (after a small decrease) the serum concentrations of Hp remained relatively stable. On the other hand, Knowles et al. (2000) reported considerable fluctuation and high Hp concentrations during the first two weeks of life. According to Dobryszczyka (1997) lower concentrations of Hp shortly after birth may be related to the increased consumption of Hp due to haemolysis of foetal red cells and the functional immaturity of the neonatal liver to compensate for this. A more pronounced increase in serum Hp concentrations was observed in calves aged of 4 months with repeated decrease of values approximately to the initial concentrations. These higher Hp concentrations can be explained by sub-clinical infections, other stressors (e.g. displacement of calves from individual pens to stable, larger groups of animals), or possible effect of exposure to changing environmental factors.

Studies performed by Knowles et al. (2000) showed that mean Fbg concentrations in calves increased during the first 2 weeks after birth, although the rise was relatively small, and the concentrations did not exceed the general reference limit used for healthy cattle. Very similar transient and relatively small increases in Fbg concentrations during the first 2 weeks of life in calves have been reported by Gentry et al. (1994). Our results showed a transient increase in the plasma concentrations of fibrinogen at the age of 2 months, which was followed by a repeated decrease of mean Fbg concentrations, and the obtained values were similar to those usually measured in healthy adult cattle. Higher concentrations of Fbg in calves, obtained in our study at the age of 2 months, may be related to the exposure of calves to changing nutritional and rearing factors and may be associated with the normal process of growth.

## **7.2 Acute phase proteins in calves suffering from various inflammatory diseases**

In this study results suggest that among the evaluated diseases of calves, respiratory diseases induce the most marked acute phase response as measured by significantly higher concentrations of haptoglobin, serum amyloid A, as well as fibrinogen compared with clinically healthy animals. Higher concentrations of Hp, SAA and Fbg in blood samples observed in this study agree with previous findings which reported that the concentrations of these proteins rise in cattle with respiratory diseases (Godson et al., 1996; Wittum et al., 1996). Carter et al. (2002) indicated also the usefulness of APP as important diagnostic factors in calves with respiratory infections, and suggested them as valuable markers for differentiating animals with respiratory signs from healthy ones.

In the presented study, significantly higher concentrations of the evaluated acute phase proteins in calves with clinical signs of respiratory diseases were found for Hp, SAA, as well as fibrinogen, although there was a considerable variation between the aforementioned inflammatory proteins in the ability to react to an acute phase response causing event. The most marked differences between healthy calves and calves suffering from respiratory diseases we found in the concentrations of haptoglobin. While the mean serum Hp concentration in calves affected by respiratory diseases was more than eighteen fold higher than the average concentration recorded in the group of clinically healthy animals, the average concentration of SAA in calves with respiratory signs was about three fold higher

compared with healthy calves. The findings correspond to the data presented by Angen et al. (2009), who obtained higher concentrations of Hp, as well as SAA in calves affected by harmful agents in the respiratory tract compared with healthy animals, but the serum SAA concentrations in diseased calves were much closer to those of the healthy calves than what was found for haptoglobin. Therefore, the aforementioned authors concluded that Hp is a more sensitive indicator of diseases in the investigated herds. Similarly, Carter et al. (2002) suggested that even if SAA is more sensitive and rapidly reacting biomarker, haptoglobin might be preferable in the field, its bigger and more prolonged response is giving rise to its usefulness to detecting disease.

The usefulness of the measurement of plasma Fbg concentrations in cattle has been demonstrated mostly by the diagnosis of traumatic conditions, monitoring of postoperative complications, e.g. peritonitis, as well as by the differentiation of traumatic reticuloperitonitis from other gastrointestinal disorders (Jafarzadeh et al., 2004). The results presented show that the determination of the concentrations of Fbg may be useful also in the monitoring of respiratory diseases, as in calves with clinical signs of respiratory diseases we found significantly higher concentrations of Fbg than in clinically healthy calves. However, the differences in the concentrations of Fbg between healthy calves and calves with respiratory signs were in means less marked than the differences observed in the concentrations of Hp and SAA. On the other hand, marked increase in plasma Fbg concentrations was found in calves after infection with *Dictyocaulus viviparus*, *Mannheimia haemolytica* or bovine viral diarrhoea virus (Gänheim et al., 2003). Therefore, in this area of research, further investigations are needed to deepen our knowledge about the synthesis of fibrinogen in calves suffering from respiratory diseases.

Although the evaluated calves with clinical signs of respiratory disorders were found to have higher concentrations of measured acute phase proteins compared with healthy calves, in sick animals we observed a markedly wider range of individual values of Hp, SAA, as well as Fbg. The higher values of standard deviations recorded in our study may reflect the different reactivity of various acute phase proteins to impaired homeostasis. Similarly, considerable individual variations in acute phase response to respiratory tract infections were reported by Wittum et al. (1996). Lomborg et al. (2008) reported also that animals can vary in their acute phase response to the same exposure. Different disease 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).

The influence of other disease conditions on the concentrations of acute phase proteins in calves is less well documented, and there are only scarce data reporting some results in this area of interest. Experimental *Salmonella* infection in young calves has been shown to cause an increase in the production of haptoglobin (Deignan et al., 2000). However, the Hp values following *Salmonella* infection, obtained by the aforementioned authors, did not reach the concentrations seen in other disease conditions in cattle. In our study, presented results showed in calves with clinical signs of diarrhoea a slightly higher mean SAA concentration compared with clinically healthy calves. However, in Hp and Fbg concentrations we found no marked differences between healthy and diarrhoeic calves. Thus, these findings indicate that the disturbances in the homeostasis, inflammatory reactions of the organism, and tissue damage caused by diarrhoea did not evoke sufficient inflammatory response giving a more



marked systemic increase in the concentrations of measured acute phase proteins. Similarly, according to Muller-Doblies et al. (2004), Hp requires a stronger stimulation to induce an increase in serum concentrations.

Seeing that the inflammation of the navel, the tissue damage and other pathologic lesions in the associated structures may cause inflammatory reactions, we expected that omphalophlebitis in calves may affect the concentrations of major acute phase proteins. However, to the best of our knowledge, there are no published reports describing the influence of omphalophlebitis on the concentrations of acute phase proteins. In our study, the presented results showed in calves with clinical signs of omphalophlebitis, similarly to the calves with diarrhoea, more markedly higher mean concentration of SAA than in clinically healthy calves, but in the concentrations of Hp and Fbg we did not find marked differences between these two groups of calves. These findings might be a consequence of a different initiation of the production of various acute phase proteins, seeing that SAA is a more sensitive acute phase protein than Hp in cattle, with rapid increase in serum concentrations after the inflammatory stimulus (Werling et al., 1996). An opposite trend with more markedly higher mean concentrations of Hp and Fbg was observed in calves affected by multisystemic diseases, while the mean SAA concentration obtained in this group was only slightly higher compared with clinically healthy calves. Similar findings were reported by Gänheim et al. (2007), who found higher concentrations of Hp and Fbg in calves with diarrhoea at the same time as respiratory symptoms compared to those that had signs of only respiratory diseases or diarrhoea. Because of only scarce data available about the changes in the concentrations of acute phase proteins in calves affected by diarrhoea, omphalophlebitis, as well as multisystemic diseases, further investigations in larger animal groups are needed to yield satisfactory results.

### **7.3 The influence of chronic respiratory diseases on the concentrations of acute phase proteins in calves**

Most of the investigations about the synthesis of acute phase proteins in respiratory diseases have been focused on the immediate or acute phase response to the infection (Wittum et al., 1996; Carter et al., 2002). However, only a few reports on the acute phase protein production in chronic inflammatory conditions have been published, and the data are not uniform. Horadagoda et al. (1999) found that the concentrations of Hp, SAA, and  $\alpha_1$ -acid glycoprotein were higher in cases of acute compared with chronic inflammation. In contrast, Alsemgeest et al. (1994) indicated that serum Hp concentrations and Hp:SAA ratios were elevated in cases of chronic rather than acute inflammation. Our results indicate that in chronic inflammatory processes, especially chronic diseases of the respiratory tract, increased serum concentrations of Hp and SAA are generally observed. However, the response to chronic inflammation varies from one protein to another. Similarly, in general, Horadagoda et al. (1999) observed differences in the inducibility of SAA and haptoglobin by chronic disorders. Our results suggest that Hp is more typical indicator for chronic conditions than SAA, as in calves suffering from chronic respiratory diseases we found more than twentyfold higher mean Hp concentration compared with healthy animals, whereas average concentration of SAA in sick calves were significantly higher, but compared with Hp only about twofold higher than in healthy calves. Alsemgeest et al. (1994) found that Hp did not increase in very acute inflammatory conditions such as peracute pneumonia. They found the largest Hp concentrations in animals with serious,

often chronic inflammatory diseases, at which bovine SAA was a more sensitive indicator of acute disease than haptoglobin. This may be consequence of a different initiation of the production of various APPs, as Hp is characterised by a later increase in serum concentration after stimulus remaining elevated for longer period. On the other hand, SAA is rapidly reacting acute phase protein characterised by a dramatic increase in serum concentration after the inflammatory stimulus and a relatively rapid normalisation (Petersen et al., 2004).

The aforementioned results indicate that serum concentrations of acute phase proteins, particularly Hp, may reflect the severity of the disease and may be useful as a prognostic indicator, as in calves, which during the treatment died or were euthanised, we found significantly higher mean concentrations of measured acute phase proteins, compared with those with good response to treatment and improved general health state. Eckersall et al. (2007) concluded that investigation of a range of acute phase proteins could provide additional diagnostic information on the progress of the disease, and could be sensitive marker of respiratory infections in calves. Similar findings for haptoglobin are reported by Godson et al. (1996). On the other hand, Berry et al. (2004) questioned the efficacy of SAA as a diagnostic tool because it may be elevated under stressful situations. Heegaard et al. (2000) found that the magnitude and duration of serum Hp concentration correlated well with the severity of experimental respiratory infection, whereas serum SAA concentrations increased more rapidly following infection. Carter et al. (2002) and Gånheim et al. (2007) concluded that measurement of blood Hp concentrations is a better way to predict morbidity in calves than SAA. However, our results obtained in serum concentrations of Hp and SAA in calves affected by chronic respiratory diseases indicate that both indices may be useful in the determination of the severity and prognosis of the disease. The aforementioned results suggest that Hp concentrations in the range of 1 – 3 mg/ml, and SAA concentrations around 100 µg/ml predict severe course of the disease with poor prognosis. Similar findings in calves are reported by Heegaard et al. (2000). Skinner et al. (1991) found that haptoglobin concentrations of more than 0.2 mg/ml indicated mild infection, values above 0.4 mg/ml diagnosed severe infection, while extended pathological conditions were typically associated with Hp concentrations in the range of 1 – 2 mg/ml. These results closely correlate with our observations in sick calves.

#### **7.4 Acute phase proteins in the laboratory diagnosis of mastitis in dairy cows**

The results of our study indicate that inflammatory diseases of the mammary gland lead to an increase in the concentrations of M-SAA. Raised levels of Hp and SAA have previously been shown in milk from cows with clinical mastitis as a result of the leakage of these proteins from the blood to the milk (Hirvonen et al., 1999; Eckersall et al., 2001). On the other hand, M-SAA is synthesized directly in the mammary epithelia of the udder in response to infection (Jacobsen et al., 2005). Therefore, M-SAA is believed to be a more sensitive indicator of mastitis; it accumulates in milk only during mammary inflammation. Petersen et al. (2005) reported that M-SAA concentrations, similarly to our results, were higher in quarters with mastitis compared to healthy quarters. On the other hand, Nazifi et al. (2008) presented markedly higher mean M-SAA concentrations for clinically healthy cows and for cows with sub-clinical mastitis (6.96 and 54.53 mg/ml, respectively). These contradictory data indicate that further studies are necessary to deepen our knowledge about the behavior of M-SAA in such conditions.

Mastitis can be caused by different microbial agents, mostly bacteria. Some bacteria invading a cow's mammary gland absorb milk nutrients, and some of them can produce endotoxins that destroy mammary tissue (Haltia et al., 2006). If these toxins escape the gland and spread throughout the cow's body, they may activate systemic inflammatory reactions. Moreover, other inflammatory mediators, e.g. cytokines released in response to infection and injury may activate systemic inflammatory reactions, including the induction of the synthesis of acute phase proteins by the liver (Baumann & Gauldie, 1994). The results of our study showed that the concentrations of Hp and SAA were higher in serum from cows with clinical mastitis, and increased with increasing CMT score. The increases observed in the concentrations of these proteins in serum of cows with mastitis are in line with several previous studies (Hirvonen et al., 1999; Eckersall et al., 2001). It appears that localized severe inflammation of the udder is sufficiently intense to induce a measurable systemic acute phase response. The concentrations of measured acute phase proteins had a tendency to be higher in the serum from the cows with local signs of mastitis and also in cows without clinical changes on the mammary gland, but with positive CMT. However, the finding that the differences in Hp and SAA concentrations observed between the groups of cows were less significant than the differences in M-SAA concentrations means that the measuring of serum concentrations of some acute phase proteins would be less useful to the evaluation of the severity of mastitis than the measuring of the concentrations of M-SAA directly in milk samples.

### **7.5 Acute phase proteins in heifers affected by hoof diseases**

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 Hp, SAA, as well as Fbg. 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 we 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 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 the plasma Fbg concentrations in cattle is less well documented. The usefulness of the measurement of the Fbg concentrations in cattle has been demonstrated by other, predominantly gastrointestinal disorders (Jafarzadeh et al., 2004). The presented study suggest that the determination of the concentrations of fibrinogen may be also useful by 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, in this area of research, further investigations are needed to deepen our knowledge about the synthesis of some acute phase proteins in cattle with hoof diseases and lameness.

## 8. Conclusion

In the study presented results indicate that most of the evaluated acute phase proteins were pronouncedly related to the age of clinically healthy calves. Therefore, these data suggest that the age of evaluated animals should be taken into consideration during the diagnostic procedure, and by the interpretation of measured values of acute phase proteins when using these proteins as disease markers. Our data support the usefulness of APP assessments to monitor animals with respiratory diseases, and indicate their use as sensitive markers to identify calves with various inflammatory diseases.

Our findings also indicate that not only acute diseases of the respiratory tract, but also chronic cases are characterized by an increased production of some acute phase proteins. Moreover, diseases with severe clinical signs and poor prognosis (death or euthanasia) are associated with markedly higher Hp and SAA concentrations. Therefore, the aforementioned data suggest that the measurement of some acute phase proteins may serve as prognostic indicator in respiratory diseases.

Generated data also suggest the usefulness of milk amyloid A for diagnosis of bovine sub-clinical mastitis, as well as in the determination of the severity of mastitis. Moreover, presented results indicate that hoof diseases and lameness in heifers may be also associated with a systemic acute phase response characterized by elevated concentrations of some APP, suggesting that their assessment as a part of the laboratory diagnosis would be a valuable supplementation to the proper clinical diagnosis and determination of other blood laboratory parameters regarding to a better evaluation of the systemic status.

Although at present, the routine use of APP to monitor the herd health state, as well as healthy individual has not been a current veterinary diagnosis, presented data suggest their usefulness also in the veterinary medicine. The aforementioned findings may deepen our knowledge about the production of these proteins in a variety of physiological conditions, as well as during some inflammatory disease conditions in farm animals.



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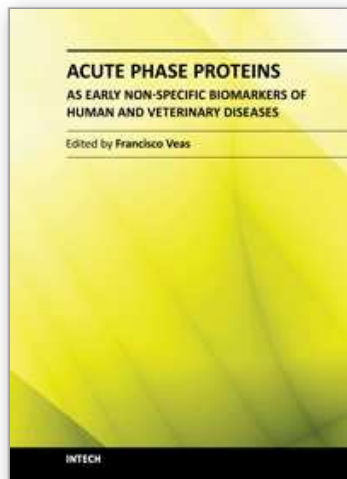


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