

Acute phase proteins and their use in the diagnosis of diseases in ruminants: a review

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ABSTRACT: The acute phase response is a complex systemic early-defence system of reactions activated by trauma, infection, tissue damage, inflammation, stress or neoplasia. One of the most important elements of this response is the increased hepatic synthesis of some plasma proteins, collectively known as acute phase proteins. The discovery of these new biomarkers has allowed the clinical monitoring of different diseases; therefore, their clinical application has been studied widely in human medicine in order to improve the diagnosis, evaluation, treatment, prognosis and therapeutics of many diseases. Although a wide range of studies have been carried out to determine the usefulness of acute phase proteins in several diseases also in animals, they are still relatively under-utilised in veterinary medicine, predominantly in farm animals. The acute phase response and clinical application of acute phase proteins in ruminants are reviewed in this article, including their diagnostic use in clinical practice and application in the monitoring of treatment, which is one of the most promising practical uses of these proteins.

Keywords: cattle; small ruminants; acute phase proteins; biomarkers; diagnostics

Contents

- | | |
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| <ol style="list-style-type: none"> 1. Introduction 2. The acute phase response 3. Acute phase proteins <ol style="list-style-type: none"> 3.1. The diagnostic utility of acute phase proteins in veterinary practice 3.2. Acute phase proteins in ruminants <ol style="list-style-type: none"> 3.2.1. Positive acute phase proteins <ol style="list-style-type: none"> 3.2.1.1. Haptoglobin 3.2.1.2. Serum amyloid A 3.2.1.3. Fibrinogen 3.2.1.4. Ceruloplasmin 3.2.1.5. Alpha-1 acid glycoprotein 3.2.1.6. Alpha-1 antitrypsin 3.2.1.7. Lactoferrin 3.2.2. Negative acute phase proteins <ol style="list-style-type: none"> 3.2.2.1. Albumin 3.2.2.2. Transferrin 3.2.2.3. Transthyretin | <ol style="list-style-type: none"> 4. Acute phase proteins as biomarkers of diseases in cattle <ol style="list-style-type: none"> 4.1. Acute phase proteins in mastitis 4.2. Acute phase proteins in reproduction and peripartum reproductive disorders 4.3. Acute phase proteins in abdominal and cardiac disorders 4.4. Acute phase proteins in hoof diseases and lameness 4.5. Acute phase proteins in metabolic diseases 4.6. Acute phase proteins in calf diseases 4.7. Acute phase proteins and stress 5. Acute phase proteins in small ruminants 6. Conclusions 7. References |
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1. Introduction

Acute phase proteins (APPs) are blood proteins primarily synthesised by hepatocytes as part of the acute phase response. The acute phase response is a non-specific and complex reaction of an organism, triggered by different stimuli including injury, trauma, infection, stress, inflammation, as well as neoplasia (Cray et al. 2009). It comprises a wide variety of behavioural, physiological, biochemical and nutritional changes. The most important metabolic changes include the highly increased or decreased production of a large family of proteins from the liver, the acute phase proteins (Ceciliani et al. 2002; Murata et al. 2004).

Acute phase proteins have been well recognised for their application to human medicine and have been described to have value in the diagnosis and prognosis of various inflammatory and organ diseases, organ transplant, and cancer treatment (Deans and Wigmore 2005; Ridker 2007). Due to their altered levels in affected animals, APPs may provide an alternative means for monitoring animal health. However, the possible influence of inflammatory conditions on the concentrations of these proteins, and their use as indicators in the detection of diseases in veterinary clinical practice, especially in farm animal medicine is less well documented. For this reason, the main purpose of this article is to provide an overview on the diagnostically valuable APPs in cattle, and to discuss their usefulness in the detection and diagnosis of various economically important diseases of ruminants.

2. The acute phase response

The acute phase response represents a group of physiological processes occurring soon after the onset of infection, injury, trauma, inflammatory processes, and some malignant processes. It is a highly coordinated process, which consists of a large number of behavioural, physiological, biochemical, and nutritional changes (Ceciliani et al. 2012). The aim of these changes is to isolate and destroy the infectious agent(s), restore homeostasis and promote the healing processes (Janeway et al. 2001).

The acute phase response begins within inflammatory sites, where cells involved in the innate immune response (i.e. macrophages, monocytes) produce and release a vast number of inflammatory mediators, among which the cytokines (such

as interleukin-1, interleukin-6 and tumour necrosis factor- α) play very important roles (Bochsler and Slauson 2002). These cytokines influence organs involved in homeostasis, such as the central nervous system (CNS), the autonomic nervous system and the adrenal gland, to establish a rapid and intense protective or reactive response (Moshage 1997).

Cytokines induce a cascade of events which potentiate the appearance of characteristic clinical changes: fever, anorexia or weight loss (Gabay and Kushner 1999). In addition, cytokines activate receptors on different target cells leading to systemic inflammatory reactions, including hormonal or metabolic, resulting in a number of biochemical changes, such as increased production of adrenocorticotrophic hormone and glucocorticoids, activation of the blood coagulation system, decreased serum concentrations of calcium, zinc, iron, and vitamin A, and changes in the concentrations of some plasma proteins (Gruys et al. 2005). One of the most important metabolic changes is the strongly increased synthesis of a group of plasma proteins, namely the acute phase proteins, by the liver (Gabay and Kushner 1999).

3. Acute phase proteins

Acute phase proteins are a large and varied group of plasma proteins, which are released into the blood stream in response to a variety of stressors. All the up-regulated proteins have been called positive APPs, in order to differentiate them from the other APPs (Petersen et al. 2004). They are further classified as major, moderate, or minor, depending on their concentration. Major proteins represent those that increase 10- to 100-fold, moderate proteins increase 2- to 10-fold, and minor proteins are characterised by only a slight increase (Ceron et al. 2005). Major proteins are often observed to increase markedly within the first 24–48 h after the triggering event and often exhibit a rapid decline due to their very short half-life. Moderate and minor proteins follow in the magnitude of their response and may both increase more slowly and persist for longer, depending on the triggering event (Niewold et al. 2003). Moderate and minor APPs may be observed more often during chronic inflammatory processes (Horadagoda et al. 1999). A minor decrease in the concentrations of negative acute phase proteins is observed during an acute phase response.

Acute phase proteins play important roles in various stages of the inflammatory reaction. In

general, the main function of APPs is to defend the host against pathological damage, assist in the restoration of homeostasis and in the regulation of different stages of inflammation (Petersen et al. 2004). Some of the acute phase proteins (α_1 -antitrypsin, α_2 -macroglobulin) have anti-protease activity designed to inhibit proteases released by phagocytes or pathogens to minimise damage to normal tissues. Other APPs (haptoglobin, serum amyloid A, C-reactive protein) have scavenging activities and bind metabolites released from cellular degradation (Wagener et al. 2002). Others (α_1 -acid glycoprotein) are characterised by anti-bacterial activity and by the ability to influence the course of the immune response (Fournier et al. 2000).

Despite the uniform nature of the acute phase response, there are numerous differences in the acute phase characteristics between different animal species (Eckersall and Bell 2010). Each animal species has its own major acute phase proteins that may provide important information for diagnostic purposes.

3.1. The diagnostic utility of acute phase proteins in veterinary practice

Acute phase protein concentrations are elevated in animals with many different diseases. Therefore, they have very poor diagnostic specificity in detecting the cause, and cannot be used as the primary test for the diagnosis of a particular disease. On the other hand, they have very high sensitivity in detecting many conditions that alter the health of the animal and in revealing subclinical inflammation or infections (Ceron et al. 2005). It was reported by Kent (1992) that APPs quickly and precisely demonstrate the presence of infectious and inflammatory conditions, but not the cause. Petersen et al. (2004) stated also that acute phase proteins can detect the presence of subclinical disease. In the clinical field, APPs may serve as indicators of prognosis and the effects of treatment. The magnitude and duration of the acute phase response reflects the severity of the infection and underlying tissue damage (Heegaard et al. 2000).

3.2. Acute phase proteins in ruminants

There are many acute phase proteins applicable as biomarkers in the detection of vari-

ous diseases and disorders in human medicine (Samols 2002). However, only some of these can be commonly used in ruminants for diagnostic purposes. Diagnostically, the most important acute phase proteins in ruminants are haptoglobin (Hp) and serum amyloid A (SAA) (Eckersall and Bell 2010).

3.2.1. Positive acute phase proteins

3.2.1.1. Haptoglobin

Haptoglobin (Hp) consists of two α and two β chains, connected by disulfide bridges (Morimatsu et al. 1991). In the circulation, Hp is highly polymerised with a molecular weight of approximately 1000–2000 kDa, and exists also as a polymer associated with albumin (Godson et al. 1996). The primary function of Hp is to bind free haemoglobin released from erythrocytes and to thereby inhibit its oxidative activity (Yang et al. 2003). The Hp-haemoglobin binding also reduces the availability of the haem residue for bacterial growth (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 various diseases (Eckersall 2000).

3.2.1.2. Serum amyloid A

Serum amyloid A (SAA) belongs to the family of apolipoproteins associated with high density lipoprotein (Uhlir et al. 1994). Different isoforms of SAA are expressed constitutively at different levels in response to inflammatory stimuli (Jensen and Whitehead 1998). During inflammation, SAA1 and SAA2 are expressed principally in the liver, whereas SAA3 is induced in many distinct tissues, including the mammary gland (Weber et al. 2006). The fourth isoform, SAA4, does not respond to external stimuli (de Beer et al. 1995). The main functions of SAA are the reverse transport of cholesterol from tissue to hepatocytes, opsonisation, inhibition of phagocyte oxidative bursts and platelet activation (Petersen et al. 2004). The M-SAA3 isoform found in colostrum stimulates the production of mucin from intestinal cells and thus helps to prevent bacterial colonisation (Mack et al. 2003).

3.2.1.3. Fibrinogen

Fibrinogen (Fbg), a precursor of fibrin, is also an acute phase protein, and in the coagulation cascade is the final substrate in the conversion of a clot to its insoluble fibrin form (Davalos and Akassoglou 2012). Fibrinogen is a β -globulin present in the plasma and is composed of three polypeptide chains linked by disulfide bridges and a glycoprotein (Gentry 1999). It 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). During an inflammatory reaction fibrinogen levels can increase two to three fold, which may significantly increase blood viscosity and cause red blood cell aggregation, as well as contribute to the growth of atherosclerotic plaques (Medcalf 2007). In humans, studies have shown an association between fibrinogen concentrations and subsequent cardiovascular disease risk, atherosclerosis and acute thrombosis (Eidelman and Hennekens 2003). In cattle, fibrinogen has been used for many years to evaluate inflammatory and traumatic diseases, and is characterised by markedly increased synthesis in response to infection (Hirvonen and Pyorala 1998).

3.2.1.4. Ceruloplasmin

Ceruloplasmin (Cp) is a protein of the α -2 globulin fraction. It is a ferroxidase enzyme that is the major copper-carrying protein in the blood, and plays a role in iron metabolism (Lovstad 2006). Ceruloplasmin carries about 70% of the total copper in human plasma and may thus play a role in Cu homeostasis (Martinez-Subiela et al. 2007). Ceruloplasmin has been evaluated as a marker of animal health and welfare (Skinner 2001). Several studies in cattle indicate its diagnostic use with applications in many disease conditions (Sheldon et al. 2002; Szczubial et al. 2008). Chassagne et al. (1998) evaluated ceruloplasmin in dairy cows as a reliable indicator of early mastitis cases. Studies in young animals have shown that the concentrations of ceruloplasmin in the serum increase during induced pneumonic pasteurellosis, with the highest concentrations observed two and four hours after the inoculation (Fagliari et al., 2003).

3.2.1.5. Alpha-1 acid glycoprotein

Alpha-1 acid glycoprotein (AGP) or orosomucoid is a highly glycosylated protein of which about 45% is carbohydrate; the composition of the glycan residues is known to alter during an acute phase response (Fournier et al. 2000). AGP is considered to be a natural anti-inflammatory and immunomodulatory agent. It has also been suggested that AGP is required to maintain capillary permeability (Fournier et al. 2000). Moreover, AGP is one of the most important drug binding proteins in plasma with important pharmacokinetic implications (Huang and Ung 2013). It exhibits a moderate acute phase response in most species and is more likely to be associated with chronic conditions. The serum concentration of AGP is a valuable differential diagnostic analyte in the identification of infectious peritonitis (Bence et al. 2005).

3.2.1.6 Alpha-1 antitrypsin

Alpha-1 antitrypsin (AAT) is the most abundant circulating serine protease inhibitor (serpin) and is also an acute phase protease. It is the major inhibitor of serine proteases such as neutrophil elastase and proteinase-3 (Lomas 2006). In certain acute phase inflammatory reactions, AAT is elevated in order to limit the damage caused by activated neutrophil granulocytes and their enzyme elastase, limiting host tissue injury by proteases at the site of inflammation (Janciauskiene et al. 2011). The clinical importance of AAT is underlined in patients with AAT deficiency, a hereditary disorder that can lead to severe tissue breakdown during inflammation (Tuder et al. 2010). This may result in pulmonary emphysema, chronic obstructive lung disease, liver diseases and liver cirrhosis, in severe cases. In addition, an abnormal protein is made by the body and accumulates in the cells of the liver, leading to inflammation and/or cirrhosis of the liver (Fairbanks and Tavill 2008). Whether AAT aggregates could initiate liver disease was evaluated in dogs by Sevelius et al. (1994). In cattle, little is known about the diagnostic utility of alpha-1 antitrypsin.

3.2.1.7. Lactoferrin

Lactoferrin (Lf), also known as lactotransferrin, is a multifunctional transferrin protein capable of

binding and transferring Fe^{3+} ions. Lactoferrin is a globular glycoprotein with a molecular weight of about 80 kDa, which shows high affinity for iron (Metz-Boutigue et al. 1984). Although the overall structure of lactoferrin is very similar to that of transferrin, they differ in their relative affinities for Fe and the propensity for release of Fe (Moore et al. 1997). The capability of lactoferrin to bind iron is two times higher than that of transferrin (Adlerova et al. 2008). The lactoferrin-iron bond is very strong and can resist pH values of as low as 4 (Mazurier and Spik 1980). The ability to keep iron bound even at low pH is important, especially at sites of infection and inflammation where, due to the metabolic activity of bacteria, the pH may fall under 4.5 (Valenti and Antonini 2005). Seeing that most bacterial pathogens are dependent on Fe for their metabolic activities, growth, and proliferation, lactoferrin, through its Fe-binding capability, sequesters this essential metal limiting the growth of Fe-requiring pathogenic bacteria including enteropathogenic *Escherichia coli* (Brock 1980). Lactoferrin is a major component of the mammalian innate immune system and represents one of the first defense systems against microbial agents invading the organism mostly via mucosal tissues (Legrand et al. 2005). It affects the growth and proliferation of many infectious agents including both Gram-positive and -negative bacteria, viruses, protozoa, and fungi (Ward et al. 2002).

Lactoferrin is expressed in most biological fluids, including milk, saliva and nasal secretions. It is present in blood, plasma or serum in relatively low concentrations, but its concentrations increase during infection, inflammation, excessive intake of iron, or tumour growth (Levay and Viljoen 1995). Higher concentrations of lactoferrin were observed in bovine and human milk, or colostrum. The concentrations of lactoferrin in milk vary from 1.15 to 485.63 $\mu\text{g}/\text{ml}$ in healthy cows; however, its levels can rapidly increase in cows with subclinical and clinical mastitis and its concentrations are positively correlated with SCC, stage of lactation, and milk yield (Kawai et al. 1999; Hagiwara et al. 2003). Lactoferrin concentrations are higher in colostrum (varying between 1 mg/ml and 5 mg/ml), and during drying off and the early mammary involution period than during lactation (Kuttila et al. 2003; Stelwagen et al. 2009).

Lactoferrin plays a key role in the defense mechanisms of the mammary gland, contributing to the prevention of infectious microbiological diseases

(Lee et al. 2004). Therefore, the health status of a cow is a very important factor influencing the concentrations of lactoferrin in milk secretions. Harmon et al. (1975) induced *E. coli* infection in bovine mammary gland, which resulted in a 30-fold increase in the concentrations of lactoferrin in mammary secretion by 90 h post inoculation. Furthermore, they observed that during acute mastitis, lactoferrin concentrations in the milk increased up to 30-fold with the highest production in the infected quarter.

3.2.2. Negative acute phase proteins

3.2.2.1. 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 reprioritisation of hepatic protein synthesis. Thus, albumin synthesis is down-regulated and amino acids are shunted into synthesis of positive acute phase proteins (Aldred and Schreiber 1993). It has been reported that during the acute phase response 30–40% of hepatic protein anabolic capacity is used for the production of positive acute phase proteins; thus, the production of other proteins needs to be curtailed (Mackiewicz, 1997). Albumin is responsible for about 75% of the osmotic pressure of plasma and is a major source of amino acids that can be utilised by an animal when necessary.

Albumin is a globular protein with a molecular weight of 69 kDa. Due to its small size and abundance albumin makes a large contribution to plasma colloid osmotic pressure. It also serves as a carrier protein for many insoluble organic substances (e.g. unconjugated bilirubin). Albumin has a relatively long half-life, approximately 14–20 days, and because of this, it has been touted as a marker of chronic nutritional status. Moreover, many studies have established albumin as an indicator of morbidity and mortality (Don and Kaysen 2004). The acute phase reaction triggers downregulation of albumin production (Gabay and Kushner 1999).

3.2.2.2. Transferrin

Transferrin (Tf), the iron-binding protein of serum has also been described as a negative acute

phase protein. It is a powerful chelator, capable of binding iron tightly but reversibly. A molecule of transferrin can bind two atoms of ferric iron (Fe^{3+}) with high affinity, which is higher in the extracellular pH of 7.4 and decreases in acidified endosomes, allowing the dissociation of Fe^{3+} (Gomme and McCann 2005). The primary role of transferrin is to transport iron safely around the body to supply growing cells (Huebers and Finch 1987). Essentially, all circulating plasma iron is normally bound to transferrin. It renders iron soluble under physiological conditions, prevents iron-mediated free radical toxicity, and facilitates transport into cells (Kaplan et al. 1991). Similar to lactoferrin, transferrin inhibits multiplication and growth of certain viral, bacterial and fungal organisms by limiting their access to iron.

Transferrin concentrations were determined by Moser et al. (1994) in cattle in various physiological states, in energy-deficient (ketotic) cows, in cases of several acute and chronic infections, as well as after endotoxin administration. The concentrations of transferrin in healthy animals were in the range of 2.0 and 6.6 g/l. While in animals with acute infections and ketosis the values varied between 1.5 and 8.5 g/l, chronic infectious diseases (such as paratuberculosis) were characterised by relatively low values (below 2 g/l). In addition, transferrin concentrations were observed to be lower in adult animals compared to young animals (Moser et al. 1994). Moreover, the concentrations of transferrin increased in veal calves with iron deficiency above 8 g/l, resulting in a negative correlation between haemoglobin and transferrin.

3.2.2.3. Transthyretin

Transthyretin (TTR), also known as thyroxin-binding protein, is a serum protein with a molecular mass of 55 kDa made up of four identical subunits, and belongs to the homotetrameric transport protein family (Foss et al. 2005). It is one of the three major thyroxin-binding proteins involved in the transport of thyroid hormones and forms a complex with retinol-binding protein to indirectly aid the transport of vitamin A in plasma (Ingenbleek and Young 1994). Transthyretin was originally called prealbumin because it ran faster than albumin on electrophoresis gels. TTR is also one of the precursor proteins commonly found in amyloid deposits, and its aggregation is known to be associated with

other amyloid diseases, including senile systemic amyloidosis and familial amyloid cardiomyopathy (Coelho 1996).

Transthyretin is a negative acute phase reactant in humans with decreased synthesis in inflammation and stress, and its concentrations are routinely measured as an indicator of health status (Beck and Rosenthal 2002). In pigs, following *Streptococcus suis* type 2 infection transthyretin showed a negative acute phase response with serum concentrations significantly lower at two days following infection (Campbell et al. 2005). However, its pathophysiology has not been fully elucidated in cattle.

4. Acute phase proteins as biomarkers of diseases in cattle

The possible use of acute phase proteins in cattle has been investigated in various inflammatory and non-inflammatory conditions, as well as in experimental infections and natural diseases which provide data for veterinarians and farmers regarding the possible use of APPs as biomarkers of diseases in the field (Ceciliani et al. 2012). Applications of acute phase proteins in bovine medicine have largely focused on diseases with economic importance. Bovine mastitis, reproductive and abdominal disorders are the most important of these. On the other hand, there are also applications for acute phase protein analyses in calf diseases, as well as in small ruminants.

4.1. Acute phase proteins in mastitis

Despite world-wide efforts, mastitis has remained economically the most important disease in dairy cattle. Therefore, the most important application of acute phase protein analyses in cattle is in the detection and monitoring of bovine mastitis (Ceciliani et al. 2012). The production and usefulness of APPs in cows with experimentally-induced mastitis and mastitis under field conditions were investigated by several authors. Conner et al. (1986) evaluated the concentrations of haptoglobin, ceruloplasmin and α -1 antitrypsin in cows with summer mastitis and in clinically healthy cows. In all cows with mastitis, they found elevated concentrations of Hp, ceruloplasmin and α -1 antitrypsin in comparison to cows without mastitis. The usefulness of APPs in the diagnosis of mastitis was investigated by Hirvonen

et al. (1996) in pregnant heifers experimentally infected with *Actinomyces pyogenes*, *Fusobacterium necrophorum* and *Peptostreptococcus indolicus*. They evaluated also the prognostic value of selected APPs (haptoglobin, fibrinogen, acid-soluble glycoproteins and α_1 -proteinase inhibitor) in the infected animals. According to the aforementioned authors, fibrinogen was a reliable indicator for detecting the presence of bacterial infection, but was not useful as a prognostic indicator for mastitis. They reported Hp and acid-soluble glycoproteins to be the most effective markers in the determination of the severity of infection and in predicting the final outcome of the disease in heifers with mastitis. In a later study, Hirvonen et al. (1999) examined the changes in some APPs in cows with acute experimental *E. coli* mastitis and their role in predicting the outcome of the disease. In this study, intramammary infection with *E. coli* induced an increase in the serum Hp and SAA concentrations in all cows. In addition, the concentrations of SAA were related to the severity of the disease. Similar findings were reported by Eckersall et al. (2001) in cows with clinical mastitis. They found significantly higher serum concentrations of Hp, as well as SAA in cows with both mild and moderate mastitis compared to healthy cows. However, these authors observed no significant differences between the cows suffering from mild and moderate mastitis.

Eckersall et al. (2001) reported that most serum proteins leak into milk across the blood-mammary barrier as a result of the disruption caused by the inflammation due to mastitis. Moreover, milk seems to be a better sample material than serum for testing the concentrations of APPs during mastitis (easier and quicker sample collection without stressing the animals). According to the results presented by Eckersall et al. (2001), milk samples from cows with both mild and moderate mastitis had significantly higher Hp, as well as SAA concentrations than the milk from healthy cows. Moreover, in milk samples from cows with moderate mastitis, the SAA concentrations were significantly higher than in cows with mild mastitis. On the other hand, there were no significant differences between the infected cows in terms of Hp concentrations in milk. Thus, SAA concentrations in milk seem to have greater potential usefulness for the detection of the severity of mastitis.

The SAA response in milk and plasma to experimental intramammary inoculation of *E. coli* in cows was examined by Jacobsen et al. (2005). All

cows, regardless of the severity of infection, showed elevated SAA concentrations in milk and plasma after inoculation. Milk SAA concentrations began to increase between 6 h and 12 h after inoculation, and plasma SAA values increased between 12 h and 24 h *post inoculation*. Cows with severe mastitis had higher milk SAA concentrations than cows with moderate or mild mastitis. SAA may therefore serve as an indicator of the degree of tissue damage. The rapid increase in milk concentrations after intramammary inoculation of mastitis pathogens suggests that SAA may be particularly suited for early detection of mastitis (Pedersen et al. 2003). The fast return towards baseline values after bacterial clearance suggests that milk SAA measurements may also be used as indicators of treatment efficacy. Higher concentrations of Hp and SAA in the serum and milk of cows with clinical mastitis were observed also by Nielsen et al. (2004). Gronlund et al. (2005) examined the concentrations of Hp and SAA in cows with naturally occurring chronic subclinical mastitis. In cows with chronic subclinical mastitis, increased concentrations of both measured APPs in milk were observed, indicating an activation of the acute phase response also in cows with chronic mastitis.

Further investigations showed an extrahepatic synthesis of a specific isoform of serum amyloid A directly from mammary epithelial cells (M-SAA) (McDonald et al. 2001). Therefore, M-SAA is believed to be a more sensitive indicator of mastitis, which accumulates in milk only during mammary inflammation. The usefulness of M-SAA in the diagnosis of clinical and subclinical mastitis was investigated by Kovac et al. (2011). Their results showed markedly higher M-SAA concentrations in milk samples from quarters with clinical changes, as well as from quarters without clinical signs of mastitis, but with strongly positive Californian Mastitis Tests (CMT). In addition, the concentrations of M-SAA found in samples from mammary quarters without clinical changes were also relatively high, as the uninfected mammary quarters had to have very low or undetectable concentrations of M-SAA. These results suggest that some quarters might be affected by inflammatory processes, while returning negative results in the CMT. Elevated concentrations of M-SAA in quarters with mastitis compared to healthy quarters were also reported by Nazifi et al. (2008).

The usefulness of acute phase proteins in milk in the determination of milk quality was examined by

Akerstedt et al. (2008). These authors determined the relationship between APPs in milk and additional biomarkers of milk quality, such as total protein, casein, whey protein, fat and lactose content in milk. The presence of Hp and M-SAA in milk was associated with lower total protein, casein, lactose content and higher proteolysis activity, which are signs of poor milk quality.

4.2. Acute phase proteins in reproduction and peripartum reproductive disorders

The usefulness of acute phase protein analyses was shown also in some reproductive disorders in cattle, predominantly in the detection and monitoring of metritis, as well as around the periparturient period. According to Gymnich et al. (2003) haptoglobin concentrations undergo significant changes around parturition, with the highest values in cows one day *post partum*. Uchida et al. (1993) evaluated the concentration of Hp in cows in the periparturient period and observed significantly higher values around parturition than before and after parturition. Similarly, Ametaj (2005) and Tothova et al. (2008) reported an increase of the two main acute phase proteins, Hp and SAA, in cows after parturition. According to the results presented by Chan et al. (2010), the SAA concentrations in healthy cows reach their highest values within three days after the delivery.

Post partum endometritis is a common problem in cattle, because uterine contamination following calving is frequent. Skinner et al. (1991) reported that Hp concentrations are high in cows with metritis. Chan et al. (2004) evaluated clinically healthy cows and cows with *post partum* reproductive diseases after calving, and observed that Hp concentrations in clinically diseased cows were significantly higher than in clinically healthy cows. In a later study of Chan et al. (2010), cows with acute puerperal metritis had significantly higher Hp concentrations than healthy cows. The highest Hp concentration was found in the period of three days after parturition, and for SAA concentrations four to seven days *post partum*. Low Hp concentrations were found by Smith et al. (1998) in cows with toxic puerperal metritis. Hirvonen et al. (1999) reported also that the Hp concentrations remained low or moderate in most cows with acute *post partum metritis*. However, the data obtained in recent years suggest that acute phase proteins

may be used as early predictors or risk factors for metritis. Huzzey et al. (2009) showed that cows with Hp concentrations higher than 1 g/l at day 3 *post-partum* were 6.7 times more likely to develop mild or severe metritis. The usefulness of APPs in evaluating the efficacy of therapy was investigated by Mordak (2008) in cows with retained placenta (with or without manual removal of the membrane). The highest Hp values were found in cows where the placenta had been expelled after four days, and the lowest in cows where the placenta had been easily removed manually.

4.3. Acute phase proteins in abdominal and cardiac disorders

The usefulness of the measurement of acute phase protein concentrations in cattle, predominantly of fibrinogen, has been described in traumatic pericarditis, reticuloperitonitis, abomasal displacement, in the monitoring of postoperative complications, as well as in the differentiation of reticuloperitonitis from other gastrointestinal disorders (Hirvonen and Pyorala 1998; Jafarzadeh et al. 2004). Hirvonen and Pyorala (1998) evaluated the usefulness of Fbg and Hp in the diagnosis of traumatic reticuloperitonitis in dairy cows. In addition, they studied how abdominal surgery affects these parameters, and whether they can be used to predict recovery from abdominal disorders. In their study, the preoperative Fbg and Hp concentrations in cows with traumatic reticuloperitonitis were significantly higher than those for cows with abomasal displacement or explorative laparotomy. The plasma Fbg values in cows with traumatic reticuloperitonitis remained high for about two days after surgery. The Hp concentrations in these cows showed only a small increase, which was followed by a steady decrease during the late hospitalisation phase. Moreover, the values measured correlated well with the clinical findings from those cows with traumatic reticuloperitonitis. According to McSherry et al. (1970), displacement of the abomasum does not usually induce a significant fibrinogen response. In the study presented by Jawor et al. (2009), the Fbg concentrations in cows with displaced abomasum were within normal values. Significant changes during post-operative monitoring were found only for SAA concentrations.

Nazifi et al. (2009) evaluated the relationships between cardiac diseases (functional murmurs,

pathological murmurs, endocarditis, and pericarditis) and the concentrations of APPs in dairy cattle. In this study, cases with pericarditis and endocarditis had higher Hp and SAA concentrations than cows with murmurs. In addition, the concentrations of both measured APPs were lower in cows with endocarditis than those measured in cows with pericarditis, which suggests that the measurement of APPs can be helpful in differentiating an acute inflammatory condition like pericarditis from other cardiac disorders.

4.4. Acute phase proteins in hoof diseases and lameness

The usefulness of acute phase proteins in the detection of lame cows was evaluated by Kujala et al. (2010). Their results showed higher concentrations of SAA in lame cows than in healthy animals, with values elevated from day 0 until days 7–8. In the serum Hp concentrations, no significant differences between healthy and lame cows were found. Significantly higher concentrations of Hp, SAA, as well as fibrinogen were found by Tothova et al. (2011) in heifers with hoof diseases compared to healthy animals. Laven et al. (2004) evaluated the concentrations of Hp, Fbg, ceruloplasmin and serumucoid in first lactation heifers with hoof horn haemorrhage to determine the relationships with the development of the disease. However, they did not find relationships between the presence of an acute phase response and the development of hoof horn haemorrhages in heifers after calving.

The presence of an acute phase response in association with lameness due to claw disorders was investigated by Smith et al. (2010) in dairy cattle. In addition, they evaluated the effect of treatment on acute phase protein concentrations and thus measured the effectiveness of the treatment. Lame cows with claw disorders were found to have increased serum Hp concentrations. In animals with pododermatitis septica and interdigital necrobacillosis, the Hp concentrations decreased after the treatment between days 1 to 5, which indicated effective treatment for these disorders. In contrast, treatment did not affect the concentrations of Hp in animals with pododermatitis circumscripta. Jawor et al. (2008) evaluated the concentrations of APPs at selected time points during the treatment of cows with limb diseases as a tool to monitor treatment and as an early predictive marker of pos-

sible complications. The highest concentrations of Hp, SAA and Fbg were recorded at the beginning of the treatment. In cows, in which the treatment process ran without complications, a gradual decrease of acute phase protein concentrations was observed. In cows with further complications (e.g. wound infections, other inflammatory states of the limbs), they found increases in one or two of the measured APPs at the next blood collection.

4.5. Acute phase proteins in metabolic diseases

Recent epidemiological studies have reported that despite world-wide efforts, the incidence of metabolic diseases in commercial dairy farms is still high (Goff 2006). It has been described in human medicine that metabolic changes and disorders may initiate and promote uncontrolled systemic inflammation (Gatzka et al. 2002; Sordillo et al. 2009). Yaqoob and Calder (2007) reported that in humans, altered lipid metabolism, increased circulating concentrations of non-esterified fatty acids (NEFA) and oxidative stress are significant contributing factors to systemic inflammation and the development of inflammatory-based diseases. Dairy cows may undergo similar metabolic disorders and changes in homeostasis, e.g. during the periparturient period, in lipomobilisation syndrome and subacute ruminal acidosis. Changes in homeostasis and physiological challenges occurring in animals with metabolic disorders may contribute to the activation of the host immune system, including the initiation of inflammatory responses.

Energy homeostasis and metabolism in general is altered in cows affected by lipomobilisation syndrome. Tothova et al. (2013) evaluated dairy cows one to two weeks after parturition, and in cows with serum concentrations of non-esterified fatty acids above 0.35 mmol/l they found significantly higher mean values of Hp and SAA than in cows with concentrations of non-esterified fatty acids below 0.35 mmol/l. Moreover, the study showed a highly positive correlation between the concentrations of both measured acute phase proteins, Hp and SAA, and between the concentrations of NEFA in dairy cows after calving. According to Bernabucci et al. (2005) and Sordillo et al. (2009) increased circulating NEFA concentrations are directly associated with increased systemic inflammatory conditions, and large amounts of adipose stores during times

of energy deficiency are linked with adverse health effects on the transition cow.

Sub-acute ruminal acidosis (SARA) is another significant disorder in ruminants that increases the morbidity and mortality of animals, especially for dairy cattle and sheep (Krause and Oetzel 2006). Repeated bouts of rumen acidosis can damage the surface of the rumen wall, allowing bacteria and toxins produced by bacteria to enter the portal circulation and invoke an inflammatory response (Gozho et al. 2005). These translocated endotoxins stimulate the release of pro-inflammatory cytokines, resulting in enhanced secretion of acute phase proteins (Emmanuel et al. 2008). Khafipour et al. (2009) evaluated the effect of sub-acute ruminal acidosis on the concentrations of some acute phase proteins in dairy cows, and observed increased values of SAA, Hp and lipopolysaccharide-binding protein during the challenge. On the other hand, Gozho et al. (2007) reported that the induction of sub-acute ruminal acidosis in lactating dairy cows increased the concentrations of SAA, but did not affect other markers of inflammation, including haptoglobin and fibrinogen. According to Plaizier et al. (2008) increases in acute phase proteins vary among methods of SARA induction, even when the methods result in similar rumen pH depression. They suggest that the inflammatory response might not be solely due to bacterial endotoxins in the rumen.

4.6. Acute phase proteins in calf diseases

Most of the investigations on the synthesis of acute phase proteins in calves have been focused on their evaluation in animals with respiratory diseases, as one of the leading causes of morbidity and mortality in calves and young cattle. Conner et al. (1989) showed that intra-tracheal aerosol inoculation with *Mannheimia haemolytica* in calves raised the levels of haptoglobin, α_1 -antitrypsin, and seromuroid. Horadagoda and Eckersall (1994) also evaluated calves intra-tracheally infected with *M. haemolytica*. The results showed a small, insignificant increase in Hp concentrations within 10 h post-inoculation. In contrast, the concentrations of SAA increased progressively from undetectable values at inoculation to 18 mg/l measured 10 h after the infection.

Godson et al. (1996) investigated APPs in animals with bovine respiratory diseases. They found that only a few animals responded to the viral challenge

by increasing their Hp concentrations. Moreover, the induction of haptoglobin production was temporally associated with the development of the disease, as well as the disease severity (fever, sick score, weight loss). Furthermore, Godson et al. (1996) reported significantly higher Hp concentrations in animals that subsequently died compared to those that recovered. Similar findings were reported by Tothova et al. (2010) in calves suffering from bovine respiratory disease under field conditions. Moreover, Tothova et al. (2010) showed that not only acute diseases of the respiratory tract, but also chronic cases are characterised with increased production of some APPs. In addition, in the aforementioned study, cases with severe clinical signs and poor prognosis were associated with markedly higher Hp and SAA concentrations. The usefulness of APPs in determining the response to therapy and making the right treatment decisions was evaluated by Carter et al. (2002). They found higher Hp concentrations in calves requiring more than one treatment compared to calves with one treatment.

Diarrhoea in calves is another multifactorial disease that can have serious financial implications in dairy herds. Piercy (1979) investigated the production of ceruloplasmin in response to experimental infection with *Salmonella* Dublin. In the infected calves, they found a significant increase in this acute phase protein between three and four days after infection, which decreased to normal values on day 7. Deignan et al. (2000) examined the serum concentrations of Hp in young calves in response to experimental infection with a mixture of three *Salmonella* serotypes (*S. Dublin*, *S. Enteritidis*, *S. Heidelberg*), and compared these levels with clinical markers of infection. Following the infection, the Hp concentrations increased significantly within three days of challenge. This increase in serum Hp values showed a statistical correlation with other more subjective clinical markers of infection, such as diarrhoeal scores, morbidity scores and temperature. On day 5 post-challenge, the serum Hp concentrations in *Salmonella* – challenged calves had returned to the normal values in all animals analysed, despite the persistence of clinical symptoms of infection in most of the these animals. These obtained data indicate that Hp concentrations reflect the severity of infection, and may aid in predicting the prognosis of the infection. Similarly, Skinner et al. (1991) indicated the significance of Hp as a clinically useful parameter for measuring the occurrence of enteritis in cattle.

The usefulness of the assessment of Hp, SAA and fibrinogen concentrations for the monitoring of treatment in calves with diarrhoea was evaluated by Jawor and Stefaniak (2006). In this study, calves with diarrhoea had higher concentrations of Hp, Fbg and SAA at the beginning of the treatment. During the treatment, their concentrations decreased gradually. Similarly, Tothova et al. (2012) reported higher mean SAA concentrations in calves with diarrhoea compared with clinically healthy calves. However, Hp and Fbg concentrations were not markedly different between healthy and diarrhoeic calves. According to Muller-Doblies et al. (2004), stronger stimulation is required to induce an increase in serum Hp concentrations.

Tothova et al. (2012) evaluated calves with omphalophlebitis to determine if this disease may affect the concentrations of major APPs. In calves with clinical signs of omphalophlebitis concentrations of SAA were markedly higher than in healthy calves. There were no marked differences in the concentrations of Hp and Fbg between healthy and sick animals. An opposite trend, with markedly higher concentrations of Hp and Fbg, was observed by Tothova et al. (2012) in calves affected by multi-systemic diseases (with more than one affected organ – navel, joints, digestive tract, and respiratory system). The SAA values obtained in this group was only slightly higher compared with healthy calves. Similar findings were reported by Ganheim et al. (2007), who found higher concentrations of Hp and fibrinogen in calves with diarrhoea with concurrent respiratory symptoms compared to those that had signs of only respiratory diseases or diarrhoea.

4.7. Acute phase proteins and stress

The potential use of acute phase proteins as indicators of general health and the effects of stress on cattle has been investigated by many researchers. Ganheim et al. (2007) investigated the concentrations of APPs in relation to health status, and found that calves with a higher incidence of diseases had more days on which they exhibited elevated concentrations of acute phase proteins suggesting that APPs could be a useful tool for evaluation of health status in calves. Conner et al. (1988) described an increase in acute phase proteins in response to stress stimuli in calves. Alsemgeest et al. (1995) reported that in calves, stress caused by housing on a slippery floor had no effect on the concentra-

tions of Hp, but that the concentrations of SAA increased.

With respect to transport stress on cattle, it has shown that after four to six hours of transport in solitary tie stalls the serum concentrations of Hp and SAA were significantly increased indicating that they could serve as markers of stress in cattle (Lomborg et al. 2008). The effect of long distance (1600 km over one day) shipping and pre-shipping management on acute phase protein concentrations in calves was investigated by Arthington et al. (2008). Plasma Hp concentrations showed a non-significant rise after transport, while ceruloplasmin values showed a significant increase on the first day after transport. On the other hand, in feedlot calves serum haptoglobin concentrations increase after transport (1400 km over two days; Murata and Miyamoto 1993).

5. Acute phase proteins in small ruminants

Acute phase proteins have not been evaluated in such detail in small ruminants as in cattle, but it appears that the acute phase response is similar. Despite the clinical value of the determination of acute phase proteins also in small ruminants, there is a lack of information about their use in ovine and caprine practice. In these species, Hp and SAA are considered the major acute phase proteins, with 80 and 22 fold increases in experimentally induced inflammatory conditions, respectively. Concentrations of AGP and fibrinogen in this experiment showed only 2- and 3.5-fold increases, while albumin values decreased significantly (Gonzalez et al. 2008; Lephherd et al. 2009).

In the last several years, acute phase proteins were studied in sheep with certain disease conditions. Eckersall et al. (2007) evaluated the acute phase protein response in sheep during experimental caseous lymphadenitis infection, in order to determine their potential value in monitoring the progress of the disease. The serum concentrations of Hp, SAA and AGP were elevated in this condition. For AGP, an extended response was found, which occurred at a point when the infection is transforming from an acute to a chronic phase. Thus, AGP could have a role as a marker for chronic conditions in sheep. A rapid increase in acute phase protein concentrations was determined also by Chalmeh et al. (2013) in sheep following the induction of endotoxaemia by *E. coli*.

Another study showed higher levels of Hp, SAA, as well as fibrinogen in ewes with pregnancy toxemia (El-Deeb 2012). In addition, acute phase proteins were detected in ewes with intrauterine bacterial contamination *post partum*, and were used as prognostic indicators of ovine dystocia (Scott et al. 1992; Regassa and Noakes 1999).

Further studies showed that acute phase protein measurements could be successfully applied also in goats. Hashemnia et al. (2011) assessed the changing pattern of APPs in experimental caprine coccidiosis and found markedly higher concentrations of Hp and SAA at day 7 after inoculation. Moreover, the magnitude and duration of the acute phase responses correlated well with the severity of the clinical signs and diarrhoea in goat kids. Acute phase proteins were evaluated by Gonzalez et al. (2010) as markers of sub-acute ruminal acidosis in goats. In this study, serum Hp exhibited a moderate increase during the induction period, while SAA levels did not change. In a further study, Gonzalez et al. (2011) evaluated the behaviour of acute phase proteins in fasting-induced pregnancy toxemia in goats. However, they found a significant increase only in the concentrations of Hp, but not in other acute phase proteins. Trevisi et al. (2005) monitored the changes in some inflammatory indices in goats around kidding. Their results suggest that an increase in inflammatory indices (mainly Hp) before kidding could underlie the typical problems in energy balance status around parturition.

6. CONCLUSIONS

As non-specific markers of inflammation, acute phase protein testing is a useful tool for the assessment of health in general, the pathogenesis of various diseases in animals, the spread of infection or the efficacy of treatment. The measurement of acute phase proteins may also be useful in defining the objective health status of an animal or a herd. They are reliable biomarkers that can be used both in diagnostic approaches and for research purposes.

Practical uses and advantages of acute phase protein assays have been described in a large number of scientific reports published in the last few years. Clinical application of acute phase proteins has not been extensive in routine clinical animal practice due to practical limitations associated with their analysis. Most methods available for measuring specific acute phase proteins are immunological

techniques, which are time-consuming and relatively expensive, and so limit the wide-scale use of acute phase proteins in routine practice. As there is a broad spectrum of possible applications of acute phase protein-based diagnostics in ruminants, it is necessary to develop and optimise rapid field tests that allow the measurement of acute phase protein concentrations in a short time period. Despite the challenges associated with their detection, acute phase proteins may be used in the diagnosis and prognosis of diseases also in farm animal medicine. Acute phase proteins have proven to be very useful in the early detection of subclinical diseases or alterations in the health status of an animal, with predictive information regarding the development of disease. Changes in the serum concentrations of APPs indicate the need for a more detailed clinical evaluation of a patient. In addition, acute phase proteins can be a powerful tool in the monitoring of treatment.

7. REFERENCES

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