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ROLE OF ACUTE PHASE PROTEIN IN MASTITIS

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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. Acute-phase proteins (APPs) are serum molecules synthesized by many cell categories, especially hepatocytes. Usually, the structure of APPs and acute-phase responses are similar in all species, having universal character in animal kingdom. The concentration of APPs in blood plasma varies in response to infection or inflammation. In recent investigations, it was discovered that some APPs were secreted in bovine milk during clinical mastitis which can be used as a bio marker for early detection of mastitis. The objective of this review is to get an over view about APPs with its role in mastitis.

KEYWORDS: trauma, infection, tissue damage, inflammation, stress or neoplasia.

INTRODUCTION

The acute phase response (APR) is a prominent systemic reaction of the organism to local or systemic disturbances in its homeostasis caused by infection, tissue injury, trauma or surgery, neoplastic growth or immunological disorders (Gruys *et al*., 1999). At the site of invasion by a micro-organism and the place of tissue injury, a number of responses of the tissue itself are initiated. Proinflammatory cytokines are released, and the vascular system and inflammatory cells are activated. These responses in turn are associated with production of more cytokines and other inflammatory mediators which diffuse to the extracellular fluid compartment and circulate in the blood. The cytokines activate receptors on different target cells, leading to a

systemic reaction resulting in the activation of hypothalamic-pituitary-adrenal axis, reduction of growth hormone secretion (Gruys *et al*., 1999) and a number of physical changes clinically characterised by fever, anorexia, negative nitrogen balance and catabolism of muscle cells (Dinarello, 1983, 1989, Ingenbleek and Carpentier, 1985, Ingenbleek and Young, 1994, Kraft *et al*., 1992, Kushner *et al*., 1981, Langhans, 1996, van Miert, 1995). Furthermore, a series of changes can be measured such as (1) a decrease of blood plasma, low and high density lipoprotein-bound cholesterol and leukocyte numbers in blood, (2) increased values of adrenocorticotrophic hormone (ACTH) and glucocorticoids, (3) activation of the complement and blood coagulation systems, (4) decreased serum levels of calcium, zinc, iron, vitamin A and of α-tocopherol, and (5) a change in the concentration of several plasma proteins and acute phase proteins (APPs) (Dinarello, 1983, 1989, Gruys *et al*., 1994) largely due to a changed hepatic metabolism. When the receptor triggering has repeated pulses, the acute phase response can become chronic. Within a few hours of infection the pattern of protein synthesis by the liver is drastically altered, resulting in an increase of certain blood proteins and the positive APPs (Blackburn, 1994, Dinarello, 1983, 1989, Gruys *et al*., 1994, Ingenbleek and Young, 1994, Kushner *et al*., 1981). Hepatic mRNA up regulation of those APPs is associated with a decrease in synthesis of normal blood proteins like transthyretin (TTR, formerly called prealbumin), retinol binding protein (RBP), cortisol binding globulin, transferrin and albumin, which represent the negative APPs. The positive APPs are mainly the proteins, C-reactive protein (CRP), serum amyloid A (SAA) and haptoglobin (Hp) which are released by the hepatocyte after cytokine stimulation (Heinrich *et al*., 1990, 1998).

The acute phase response with its changes in blood plasma composition is thought to be beneficial to the organism by preventing microbial growth and helping to restore homeostasis. Some APPs opsonise microorganisms and activate complement, while others scavenge cellular remnants and free radicals, or neutralize proteolytic enzymes.

Acute phase reaction

Local inflammation is the major reaction of the body upon tissue injury caused by infection. However, infection may occur without inflammation e.g., in immune-compromised individuals. Inflammation may also develop due to non-infectious causes. Any tissue damage during these processes leads to release of pro-inflammatory cytokines (van Miert, 1995). These cytokines, nitric oxide and glucocorticoids trigger and modulate the systemic acute phase reaction and the hepatic acute phase protein response (Gruys *et al*., 1994, Heinrich *et*

al., 1990, 1998, van Miert, 1995). Protein-malnutrition and long-term starvation or anorexia, however can reduce or abrogate a full positive acute phase protein reaction, while reducing the negative acute phase reactants by the starvation process itself. The same holds for hepatic impairment. Bacterial infections usually lead to a strong systemic acute phase response (Alsemgeest, 1994, Alsemgeest *et al*., 1994), due to the strong reaction of the mononuclear-phagocytic system's cells. TNF-α and IL-1β are induced in response to endotoxin (Dinarello, 1983, Le and Vilcek, 1989, Monshouwer *et al*., 1996a, 1996b, Werling *et al*., 1996). In viral infections, generally the APR is milder (Alsemgeest, 1994, Höfner *et al*., 1994, Kimura *et al*., 1995, Nakayama *et al*., 1993). The main cytokines then released by infected cells are primarily interferon's (IFNs), especially IFNγ from mononuclear inflammatory cell

Cytokines and the acute phase response

At least 15 different low molecular weight peptide mediators are known to be secreted by activated leukocytes (interleukines) and other cells. They are collectively termed cytokines and are involved in triggering the acute phase response. Three main groups of cytokines corresponding to effect pathways can be distinguished (van Miert, 1995): (1) cytokines that primarily act as positive or negative growth factors for a variety of cells (IL-2, IL-3, IL-4, IL-7, IL-10, IL-11, IL-12 and granulocyte- macrophage colony stimulating factor), (2) cytokines with proinflammatory properties (TNF-α/β, IL-1α/β, IL-6, IFN-α/γ, IL-8, and macrophage inhibitory protein-1), and (3) factors with anti-inflammatory activity (IL-1 receptor antagonists, soluble IL-1 receptors, TNF-α binding protein and IL-1 binding protein). The proinflammatory cytokines (those of the second group) are responsible for induction of the fever and muscle catabolism, and they activate white blood cell precursors in the bone marrow, growth of inflammatory tissue fibroblasts and macrophages (Dinarello, 1983, 1989, Heinrich *et al*., 1990, Sehgal *et al*., 1989, van Miert, 1995). They are responsible for a broad spectrum of synergistic or antagonistic effects that influence the specific immune response of the stressed organism against foreign antigens and invading microorganisms (Pinelli, 1996, van Miert, 1995). TNF-α, IL-1β and IFNγ are crucial for the induction of other cytokines (IL-6 and IL-8) and agents such as platelet activating factor, prostaglandins, leukotrienes and nitric oxide (van Miert, 1995).

In the hepatic APR, TNF-α, IL-1 and IL-6 play a key role (Heinrich *et al*., 1990, 1998, Ingenbleek and Young, 1994, Le and Vilcek, 1989, Sehgal *et al*., 1989). They activate hepatocytic receptors, and synthesis of varying APPs starts. IL-6 is the major mediator for the

hepatocytic secretion of most of the APPs (Heinrich *et al*., 1998, Le and Vilcek, 1989, Sehgal *et al*., 1989). Furthermore, TNF-α causes muscle catabolism that is also mediated by glucocorticoids, as well as glucagon-induced hyperglycemia and amino acid uptake by the liver. IL-1 stimulates an increase in whole body amino-acid flux, and activation of the pituitary-adrenal system. It has been shown that Kupffer cells play an intermediate role (Knolle *et al*., 1995). After stimulation by the proinflammatory cytokines the Kupffer cells form IL-6 and present it to the hepatocytes. IL-6 depresses mononuclear phagocytic production of IL-1 and TNF-α thus mitigating the whole cascade reaction. Down-regulation of the hepatocytic APR is achieved by rapid hepatic removal of circulating cytokines (Heinrich *et al*., 1998), release of IL-10 by the Kupffer cells which results in suppression of the local IL-6 production (Knolle *et al*., 1995) and by gene suppression pathways co activated on receptor binding (Heinrich *et al*., 1990, 1998). Receptors for the proinflammatory cytokines may induce a janus-kinase effect resulting in activation of the APP formation pathway as well as several receptor inhibiting pathways (Heinrich *et al*., 1998). Moreover, parts of the hepatic APR are suppressed by IL-1 and IL-4 (Loyer *et al*., 1993) and some acute phase proteins can modulate monocyte cytokine production (Pue *et al*., 1996).

Species specific APP response during APR

Several plasma proteins are known as APPs, however, depending on the species the protein pattern of each single APP during the APR i highly variable. In cattle, Hp and serum amyloid A (SAA) are considered as the most prominent APPs, whereas C-reactive protein (CRP) is normally present in circulation and its concentration remains unchanged during an acute phase (Eckersall and Conner, 1988, Gronlund et al., 2003,). In contrast, CRP is recognized as a major reactant in the pig together with pig-Map (pig major acute phase protein) and also Hp. In man, CRP besides SAA shows the highest increases during an APR, whereas Hp increases only moderately (Heinrich et al., 1990). Similarly in the dog, CRP is classified as a major APP, whereas in the rat, α2-2013 macroglobulin and α1-acid glycoprotein are the APPs with the greatest increase of concentration during the APR (Eckersall and Conner, 1988, Heinrich et al., 1990).

Acute phase proteins

Negative acute phase proteins

In addition to decrease in serum zinc, iron and albumin, a decrease in transferrin, cortisol-binding globulin, transthyretin (TTR) and retinol-binding protein (retinol=vitamin A) have

been described (Ingenbleek and Young, 1994). Their decrease indicates a temporarily increased availability of free hormones bound to these proteins. The negative acute phase proteins are therefore described by some authors as 'acute booster reactants (Ingenbleek and Young, 1994). In malnutrition and chronic infections the response of positive acute phase variables may be less evident (Morlese *et al*., 1998, Stephensen, 1999). Changes in blood protein profiles partly depend on starvation and muscle catabolism (Reeds *et al*., 1994). In chronic infestation and inflammatory states of children and during pregnancy in developing countries in addition to malnutrition, vitamin A deficiency is worsened (Stephensen, 2001, Stephensen and Gildengorin, 2000). The latter has a well-known negative feedback effect on immunity (Baeten *et al*., 2004, El Beitune *et al*., 2003, Stephensen, 2001,West, 2004).

Positive acute phase proteins

Although species-differences exist for separate proteins and especially are known between mammals and birds, the positive APPs of man and domestic animals (Dowton and Colten, 1988, Kushner *et al*., 1981, McGuire *et al*., 1996) can generally be listed in three major groups: (1) with an increase of about 50%: ceruloplasmin and complement factor-3 (C3), (2) with an increase of two-three fold: haptoglobin, fibrinogen, α-globulins with antiprotease-activity and lipopolysaccharide binding protein, and (3) with a rapid increase of up to 5-fold to 1000-fold: CRP and SAA. For the pig, a kallikrein-related 'major acute phase protein' (pigMAP) has to be added to this latter group (Alava *et al*., 1997). Some of the APPs are foetal proteins normally not found in large quantities in sera of adult subjects, e.g., α-macrofoetoprotein in the rat (van Gool *et al*., 1984) and α1-acid glycoprotein (AGP) in most animal species. Positive acute phase proteins are formed during the acute phase response associated with anorexia and changed metabolism. This indicates that rather than the role of protein absorption in the digestive tract, muscle protein functions as major storage for the amino acids required for APP synthesis. Since the amino acid composition of the APPs differs from that of muscle protein, the demands for phenylalanine, tryptophan and tyrosine together necessitates the mobilization of an amount of muscle protein that is considerably in exceeding (thrice) the quantity of the APP synthesized (Reeds *et al*., 1994). To minimize muscular catabolism for hospitalized acute phase patient's, protein diets have been recommended (Alexander *et al*., 1980) which are now beginning to be given to pigs and chickens as well. Distinct positive APPs from some species do not react in the same way in other species, serum amyloid P-component (SAP) is an APP in the mouse, but not in man, and CRP reacts as APP in several monogastric species, but not very well in small ruminants

(Gruys *et al*., 1994). Transferrin, which is a negative APP of most mammalian species, reacts as positive APP in chicken (Hallquist and Klasing, 1994, Tohjo *et al*., 1996).

Haptoglobin – acute phase protein

Hp belongs to the group of Acute Phase Proteins (APPs) which come into play during the Acute Phase Reaction (APR). It is initiated by macrophages of the affected tissue or by blood monocytes which release a wide range of mediators including cytokines. These cytokines act on fibroblasts and endothelial cells in the near vicinity causing a second release of cytokines. Only this second wave of cytokines triggers the actual cascade of complex reactions as part of the APR occurring locally and systemically. Locally, cytokines mediate leukocyte recruitment, in particular neutrophils and mononuclear cells, to the sites of inflammation. Systemically, they act on the immune system, bone marrow, brain and liver, and the reaction comprises the generation of a febrile response, an increase in adrenocorticotropic hormone (ACTH) secretion, leukocytosis and alteration of the hepatic APP gene expression. This change of hepatic APP expression leads to increases as well as decreases of APP plasma concentrations dividing them into positive and negative APPs, respectively (Heinrich *et al*., 1990, Baumann and Gauldie, 1994). Since Hp is produced at elevated levels during the APR, it is categorised as a positive APP (Skinner *et al*., 1991, Dobryszycka, 1997).

Sites of haptoglobin synthesis

Hp is mainly found in plasma, but is also present in many other body fluids in mammals such as milk, urine, cord serum, cerebrospinal fluid, amniotic fluid and saliva (Katnik and Dobryszycka, 1990, Hiss *et al*., 2003, Hiss *et al*., 2004).

Liver is the primary site of Hp synthesis (Miller *et al*., 1951). In addition, Hp expression has been reported in a variety of extrahepatic tissues. Hp mRNA could be detected in spleen, thymus, heart, lung and kidney of the rat after lipopolysaccharide (LPS) challenge (Kalmovarin *et al*., 1991). Similarly, Hp mRNA was also found in murine adipocytes at a basal level and at elevated levels after LPS challenge (Friedrichs *et al*., 1995). These researchers estimated the basal level of Hp mRNA in adipose tissue to be 10-15% of the levels in liver. Moreover, murine lung epithelial cells express Hp mRNA (Yang *et al*., 2000). There is also evidence of Hp mRNA in the reproductive tract. Hp mRNA expression was shown in rabbit oviductal tissue from 6 h post-conception to day 3 and in the uterus on 5 and 6 days post-conception (Herrler *et al*., 2004), in human endometrium (Sharpe-Timms *et al*., 2000) and in bovine oviduct (Lavery *et al*., 2004). In addition, macrophages and eosinophils

as well as epidermal keratinocytes express Hp in humans (Yang *et al*., 2000, Li *et al*., 2005). Finally, Hp mRNA was identified in the mammary gland of cows (Hiss *et al*., 2004). Summarising for cattle, liver, oviduct and mammary gland are the only sites currently recognised of Hp mRNA expression.

The studies on the investigation of the expression of mRNA for Hp suggest that mammary tissue can be a source of APP in bovine milk (Hiss *et al*., 2004). Hp mRNA expression was shown in bovine circulating leukocytes, thus identifying these immune cells as one possible source of Hp mRNA transcripts found in homogenates of healthy and diseased quarters (Thielen *et al*., 2005, Thielen *et al*., 2007). Hp mRNA has also been found in mammary tissue and leukocytes in healthy cattle (Cooray *et al*., 2007). Also Hp mRNA synthesis in the mammary gland has been supported by quantitative RT-PCR (Eckersall *et al*., 2006). Further, it has been stated that neutrophils and epithelial cells may play an essential role in elevating milk Hp (Lai *et al*., 2009).

Physiological function of haptoglobin

The main physiological tasks assigned to Hp are transport and immunomodulatory properties. The best recognised function of haptoglobin is to bind free haemoglobin (Hb) and to transport Hb to the liver. More specifically, after the release of Hb into plasma, a physiological phenomenon associated with haemolysis or apoptosis of erythrocytes occurs. Hp can attach to Hb by non-covalent binding at a ratio of 1:1 (Fraser and Smith, 1971). This Hp-Hb complex cannot pass the glomerular filtration in the kidney due to its large molecular size, thereby preventing renal losses of the small Hb molecule (Fagoonee *et al*., 2005). Instead, the Hp-Hb complex is metabolised by CD163-positive monocytes/macrophages making the Hb-iron available for new Hb synthesis (Kristiansen *et al*., 2001). Besides the recycling of Hb-iron, the formation of the Hp-Hb complex possesses two additional benefits. On the one hand, Hp has a bacteriostatic effect by hampering the iron requiring process of bacterial replication as shown in rats inoculated with pathogenic *Escherichia coli* (Eaton *et al*., 1982). On the other hand, Hp was assigned an anti-oxidative role by inhibiting Hb-driven free radical oxidative tissue damage (Miller *et al*., 1997).

Another important property associated with Hp is the modulation of the inflammatory response by acting on different immune cells. Hp suppresses the production of proinflammatory, but not anti-inflammatory cytokines in human monocytes and inhibits the respiratory burst activity of human neutrophils (Oh *et al*., 1990, Arredouani *et al*., 2005). In

addition, lymphocyte proliferation normally occurring after stimulation with concanavalin A or LPS in rabbits reduced in the presence of Hp (Baseler and Burrell, 1983). There is evidence that Hp stimulates angiogenesis, thus supporting tissue repair under inflammatory conditions (Cid *et al*., 1993). In addition, Hp appears to act as a systemic regulator of dendritic cell function by preventing functional maturation of epidermal Langerhans cells, i.e. their transformation to cells capable of presenting antigens to T-cells (Xie *et al*., 2000).

Cytokine control of Hp production

The APP synthesis is controlled by cytokines as mentioned above. They act directly upon specific receptors of hepatocytes prompting APP production (Peters *et al*., 1997). APPs can be divided into two major categories according to their regulators: type 1 APP production is induced by interleukin (IL)-1 and tumour necrosis factor-alpha (TNF-α), whereas type 2 APP synthesis is elicited by IL-6 (Baumann and Gauldie, 1994). IL-6 is believed to be the primary stimulator of most APP genes, however, there is evidence that IL-1 and TNF-α can amplify the effects of IL-6 (Heinrich *et al*., 1990). In cattle, IL-6 could be established as the principal regulator of Hp production in hepatocytes (Yoshioka *et al*., 2002), hence, it can be classified as type 2 APP in this species. Similarly, Hp is ranked as type 2 APP in man, however, as type 1 in the rat (Baumann and Gauldie, 1994).

Induced by IL-6, the actual Hp gene transcription within a cell is mediated by signal transducers and activators of transcription proteins (STAT) of which STAT3 has been described as the main signalling protein in mice hepatocytes *in vitro* (Kim and Baumann, 1997). After binding of IL-6 to its receptor, STAT3 is activated at the cytoplasmic side of the IL-6 receptor by phosphorylation. Once activated it translocates to the nucleus. In mice, the three main regulatory elements of the Hp gene promoter are two recognition sites for the transcription factor CCAAT/enhancer binding protein beta (C/EBPβ) flanking a STAT interaction site. Binding of STAT3 to this interaction site has been identified as the key up regulator of murine Hp gene transcription induced by IL-6, whereas, binding of other STAT proteins, e.g. STAT5, exerts inhibitory effects (Kim and Baumann, 1997, Wang *et al*., 2001).

Role of haptoglobin in diagnosis

In certain investigations, it has been established that Hp and SAA were secreted in bovine milk during clinical mastitis. It has also been shown that experimentally induced mastitis can stimulate expression of these proteins in milk (Eckersall *et al*., 2001, Gronlund *et al*., 2003). Particularly in the case of mastitis, considering it is the second major reason for dairy cows

leaving the herd (Rinderzüchter, 2005), an objective and rapidly assessable indicator of the disease is desirable that allows the effective discrimination between healthy and diseased animals, preferably even quarters. In the event of naturally occurring clinical mastitis, serum Hp levels provided sensitivities and specificities for differentiating between healthy cows and cows affected by mastitis of 82% and 94%, respectively, for milk Hp the corresponding values were assessed at 86% and 100% (Eckersall *et al*., 2001). Analyses of Hp in milk from cows with naturally occurring subclinical mastitis yielded a sensitivity of 85% and a specificity of 94% (Hiss *et al*., 2005). Gronlund *et al*. (2005) concluded from their study with cows suffering from natural chronic subclinical mastitis that the absence of any detectable Hp as well as SAA in milk was a good indicator of healthy quarters. Nazifi *et al*. (2008) observed increased level of haptoglobin concentration in cases of pericarditis and endocarditis indicating its diagnostic value in case of bovine heart disease. Haptoglobin has also been reported as a potential biomarker for the preclinical diagnosis of Parkinson's disease (Arguelles *et al*., 2009).

Concentration of Hp in serum increases following abscess formation, endotoxin administration and post-operation (Alsemgeest, 1994). Hp is a prominent acute phase protein in most species studied, but the serum concentration can be influenced by other factors besides the acute phase response. Increased levels of free Hb in the serum are followed by decreased serum concentration of free Hp. In cattle, during an acute hemolytic crisis due to babesiosis (Bremner, 1964), Hp disappeared from the circulation. In addition to the acute phase response, non-acute renal disease and obstructive jaundice may cause hyperhaptoglobulinemia. Increased serum or plasma Hp concentration in cattle was found after trauma (Earley and Crowe, 2002, Fisher *et al*., 2001), experimental local aseptic inflammation (Conner and Eckersall, 1988), various acute infections under field conditions (Alsemgeest *et al*., 1994, Skinner *et al*., 1991), acute inflammation (Lipperheide *et al*., 1997), mastitis (Gronlund *et al*., 2003, Gronlund *et al*., 2005, Hirvonen *et al*., 1999, Nielsen *et al*., 2004, Ohtsuka *et al*., 2001), castration (Earley and Crowe, 2002, Fisher *et al*., 2001) and metritis.

Bovine Haptoglobin was correlated to the severity of experimental infections with bovine respiratory syncytial virus (Heegaard *et al*., 2000), and spontaneous natural infections with foot and mouth disease virus (Hofner *et al*., 1994). It has also proved useful in distinguishing between acute and chronic inflammation when combined with SAA (Horadagoda *et al*.,

1999). In a field study, the bovine metabolic disorders hypocalcemia and ketosis were not associated with increased Haptoglobin serum concentration (Skinner *et el.*, 1991). In dairy cows with toxic puerperal metritis, anti-microbial therapy is associated with a decrease in serum Haptoglobin

Acute phase proteins for diagnosis of bovine mastitis

Acute phase proteins may provide an alternative means of monitoring animal health. Due to a relatively short halflife in serum and high response in diseased animals (Mackiewicz, 1997), APP serum response constitute a valid measure of a systemic response to an initiating stimulus at the time of blood sampling. Like rectal temperature, APP levels are not suitable for establishing a specific diagnosis but can provide objective information about the extent of on-going lesions in individual's animals. At the herd level, APP might be useful for determining where the spread of the disease is taking place, by providing information about the prevalence of ongoing clinical and subclinical infections indicated by the high serum concentration of selected APP (Petersen *et al*., 2002) and by serving as a prognostic tool, with the magnitude and duration of the acute phase response reflecting the severity of infection (Hirvonen *et al*., 1999, Hulten *et al*., 2002, Peltola, 1982, Skinner *et al*., 1991). Haptoglobin, C-reative protein and serum amyloid A (SAA), which are the among the strongly reacting acute phase protein in animals.

C- reactive protein was discovered in the blood of patients during the acute phase of pneumococcal pneumonia (Tillet and Francis, 1930). In bacterial meningitis the CRP concentration was elevated, whereas no changes are seen in viral meningitis (Peltola, 1982). CRP is also reported to be useful for distinguishing between viral and bacterial pneumonia (McCarthy *et al*., 1978). Also, recent research has shown that slightly elevated CRP concentration might be a valid marker for increased risk of cardiac disease in humans (Ledue *et al*., 2003, Sellmayer *et al*., 2003). Even though increased concentration of bovine CRP during naturally occurring infections and a correlation with herd health status have been reported (Lee *et al*., 2003), CRP is generally not considered an acute phase protein in cattle (Nakajima *et al*., 1993).

In cattle, an increased SAA serum and plasma concentration has been found following experimentally induced (Bremner, 1964, Conner and Eckersall, 1988) and naturally occurring inflammation (Alsemgeest *et al*., 1995) as well as experimental and natural infections. The SAA response during viral respiratory disease is well described (Ganheim *et al*., 2003,

Heegaard *et al*., 2000). After inoculation with Pasteurella multocida the SAA concentration increased (Horadagoda *et al*., 1994). SAA has been suggerted to be more useful in distinguishing between acute and chronic inflammation than neutrophil counts and white blood cells (Horadagoda *et al*., 1999).

CONCLUSION

The acute phase reaction is a natural response to tissue injury and includes a range of metabolic activities which include alterations in the rate of synthesis of several proteins produced by the liver. It is established that the cytokines play a key role in mediating this response. Measurement of the proteins in serum is of considerable value in the diagnosis, management and prognosis of many diseases hat exhibit an acute phase response such as mastitis. Though little information is available about the APP in relation to mastitis, A detailed study may be needed to establish a strong correlation between the two.

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