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*Review*

# The application of acute phase protein as biomarkers in bovine mastitis

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Acute phase proteins (APP) are components of the blood that plays a very significant role in maintaining or restoring homeostasis and inhibiting microbial growth in animal prior to the development of antibodies by the host during trauma, inflammation or infection process. The concentration of circulating APP in the blood is directly related to the degree and or severity of disease and the extent of tissue damage. Additionally, it is also useful in monitoring response to disease, in disease diagnosis, prognosis and general health screening and evaluation. Acute phase proteins are classified according to concentration of protein, mode of action and concentration in other animals. The description of APP in cases of disease will help in mapping out a profile of APP characteristics of that disease, thus providing valuable information with regards to the mechanism of the disease process and also serves as a very good diagnostic aid. This review focuses on the role of APP in determining the type of mastitis, severity of mastitis and determinant of herd health.

**Key Words:** Acute phase proteins, Bovine, Haptoglobulin, Mastitis, Serum Amyloid.

## INTRODUCTION

Acute phase proteins (APP) are protein component of the blood that are used to evaluate the response of innate immune system during inflammation, neoplasia, surgery, immunological disorders, trauma and infection (Murata et al., 2004; Eckersall and Bell, 2010; Salgado et al., 2011). Changes in serum concentration of more than 25% have been reported to have occurred in response to proinflammatory cytokines stimulated during disease process (Eckersall and Bell, 2010). In addition, the studies have shown that concentration of APP circulating in the blood is directly related to the degree or severity of the ongoing disease condition and tissue damage (Murata et al., 2004). Furthermore, it has also been observed that proper quantification of the APP in circulating blood will help in providing useful information with regards to diagnosis and prognosis of disease (Pyorala, 2003). Additionally, acute phase proteins have been reported to play a very significant role in maintaining or restoring homeostasis and inhibiting microbial growth in animal prior to the development of antibodies by the host during trauma, inflammation or infection process (Murata et al., 2004). In essence, they can be said to be elicited only following injuries to the system that have the potentials to trigger inflammatory response (Gånheim et al., 2007). In many animal species, APPs have been shown to express different response rate (major, moderate and minor) which in either case revealed a different serum concentration. In addition, acute phase protein is also useful in monitoring response to disease, disease diagnosis and prognosis in addition to general health screening or evaluation (Eckersall and Bell, 2010; Jain et al., 2011).

Acute phase protein is widely known to be stimulated when there is external or internal influence which in most instances includes trauma, surgery, neoplasia, inflammation and infection but in cattle, acute phase protein can be produce as a result of external physical stress which might not necessarily be the already existing known factors that triggers their production (Gånheim et al., 2007). The mechanism by which such response occurs is not yet fully established (Alsemgeest et al., 1995 ; Murata et al., 2004); however, the activation of the hypothalamic-pituitary-adrenal pathway by stress factors might trigger a localized (intra-pituitary) or systemic production of cytokines which may result in augmenting the liver acute phase protein and ultimate release into the blood stream (Alsemgeest et al., 1996). Furthermore, even though there are still some levels of doubts with regards to the level of the functional application of APP; however, preliminary information suggests that they contribute immensely in the control of inflammation and in host defense against a variety of pathogens. In addition, findings has shown that the level of circulating APP in serum is associated with the degree of severity of ongoing systemic infection and thus, provide a valuable information with regards to their application as potential indicators or biomarkers for detecting inflammatory process. With regards to the level of response to pathogens, bacterial infections have been observed to elicit strong response compared with viral infections which produces a weak response or a times no response at all (Glass et al., 2003).

Haptoglobulin and serum amyloid A are the two most important acute phase proteins of cattle, which are produced in high amount following bacterial or viral disease but relatively low amount or absent in healthy cows (Murata et al., 2004; Gånheim et al., 2007). Similarly, reports of high levels of acute phase protein have been observed during parturition and also in cows with hepatic lipidosis (Alsemgeest et al., 1993; Nakagawa et al., 1997; Vannucchi et al., 2002); or in case of fasting cows following administration of dexamethasone (Murata et al., 2004) thus, indicating the possibility that it might actually occur as a result of liver hepatic acute protein phase production in response to glucocorticoid or estradiol. Additionally, an increase in the concentration of APP has been observed in cases of subclinical inflammatory diseases. For instance, increase in the concentration of plasma fibrinogen an important APP, have been reported following subclinical inflammatory disorders and have been reported as a good indicators for evaluating inflammatory conditions in cattle for many years (Eckersall and Conner, 1988; Gånheim et al., 2007). Moreover, Gånheim et al. (2007) reported the importance of acute phase proteins as good indicators of herd health when the author recorded a significantly lower mean weight gain in groups of animals with high incidence of disease. His finding further substantiates the fact that APP can serve as a good biomarker for determining herd health. Alsemgeest et al. (1994) and Murata et al. (2004) reported the clinical value of APP serving as a potential indicator of disease and health status of an individual animal and as a biomarker for herd health. Similarly, Eckersall and Bell, (2010), in their review highlighted the significance of both haptoglobulin and serum amyloid A in the diagnosis and prognosis of mastitis, endometritis, enteritis, peritonitis, pneumonia and endocarditis. The authors further reported that serum amyloid A is mostly associated with acute than chronic inflammatory conditions and a homologue of SAA (M-SAA3) have been reported to be secreted from milk samples obtained from ewes and cows with mastitis. In addition, studies using experimental models have also reported the secretion of SAA and Hp from infected mammary gland (Eckersall et al., 2006). The use of APP in serum and milk sample as potential indicators of mastitis in both natural and experimental conditions have also been reported Eckersall and Bell, (2006, 2010) thus, highlighting their significance as biomarkers of mastitis. Furthermore, an increase of Hp up to six fold were found in serum of dairy cow with metabolic and infectious diseases at the point of slaughter than in cows with minimal lesions and up to a 40 and 70 fold increase in Hp and SAA were reported in culled cattle with relatively minor lesions than in beef cows with similar lesions (Hirvonen and Pyörälä, 1998; Eckersall and Bell, 2010).This review focuses on the use of APP as potential biomarkers of bovine mastitis with a view to developing a conceptualized framework with regards to their usage as biomarkers of bovine mastitis.

## CLASSIFICATION OF ACUTE PHASE PROTEIN

Acute phase protein (APP) is known to play a very important role in the disease diagnosis, prognosis as well as serving as determinants of health both at individual and herd level (Murata et al., 2004). Their application as potential indicators of disease process are quite useful in veterinary medicine. Several method of classification of APP have been proposed. The purpose of this review we will restrict our classification to three, classification based on protein concentration, mode of action and concentration in different animal models (Gruys et al., 2005); Jain et al., 2011).

**Protein concentration**

On the basis of protein concentration, APP are classified into negative acute phase proteins and positive acute phase proteins (Murata et al., 2004; Jain et al., 2011). This occurs when the liver is triggered in response to stimulus and then initiate a number of acute phase reactions (APR) which resulted in down regulation or decrease in the production of other acute phase proteins such as albumin, transcortin, transthyretin, transferring and retinol-binding protein (Jain et al., 2011). While at the same time regulating the production of other acute phase protein which play a significant role as potential indicators of disease process. These acute phase proteins include, D-dimer protein, C-reactive proteins, alpha I antitrypsin, mannose- binding protein, alpha I antichymotrypisin, fibronectin, alpha II macroglobulin, prothrombin, factor VIII, plasminogen, von-willebrands factor, ferretin, complement factors ceruloplasmin, SAP complement, SAA and Haptoglobulin. The positive or up regulated acute phase protein demonstrate a unique physiological function with regards to host immune response. For instance, SAA and Hp have been reported to functions in bacterial growth inhibition while others produce negative feedback response to inflammatory process (Jain et al., 2011).

On the basis of their mode of action, acute phase protein are classified as follows;

(a) Proteins involve in coagulation (fibronectin and prothombin).
(b) Proteins serving as protease inhibitors (Alpha I antichymotrypsin and antitrypsin).
(c) Complement proteins (C2, C3, C4 and C5).
(d) Transport proteins (haptoglobolins, hemopexin and ceruloplasmin)
(e) Other proteins which include CRP, SAA, SAP and acid glycoprotein (Jain et al., 2011).

The classification of APP according to their concentration in different animals is based on the knowledge that when the concentration of most acute phase proteins where observed at the base line, some APPs have been observed to have higher concentration than others (Jain et al., 2011). For instance, increase in concentration of up to 100 fold is observed with some APPs while at the same time maintaining a lower concentration at normal state a scenario is observed with CRP and SAA in humans. Different animals exhibit different concentration of APPs, for example the concentration of CRP in humans at normal state might not be the same with the concentration of CRP in cattle or cat (Murata et al., 2004). In cattle, major APPs includes Hp and SAA while fibrinogen, Cp, alpha-AGP and alpha I antitrypsin are the minor APPS; however, in all the APPs found in cattle SAA has been observed to be the most studied and reacts better than Hp following acute phase reaction in response to inflammation (Jain et al., 2011).

## TYPES OF ACUTE PHASE PROTEINS

The physiologic response of tissue to bacterial infections or trauma triggers a local inflammatory response and initiation of a cascade of system response. This multiplicity of events which occurs distant to the site of injury and includes leucocytes and quantitative and qualitative modification of other unrelated structural protein is called acute phase protein (Ceciliani et al., 2012). There are various types of APP who are known to elicited in response to inflammatory response, trauma or bacterial infection (Murata et al., 2004).

**Haptoglobulin**

This is a constituent of alpha globulin which binds specifically with toxic and proinflammatory free hemoglobin in the blood and initiates the reduction of oxidative damages associated with hemolysis (Yang et al, 2007; Murata et al., 2004). This type of APP are synthesized and secreted by large number of producing animals or pets and possesses antibacterial activity as well as inhibitory activity towards phagocytosis and granulocyte chemotaxis (Rossbacher et al., 1999). In normal animals, the serum concentration of Hp does not arouse much clinical interest, however, during infection, the concentration of Hp have been reported to increase up to 100 folds (Conner 1989; Murata et al., 2004). The applications of Hp as a useful diagnostic tool in cattle have been reported (Hirvonen and Pyörälä 1998; Eckersall et al., 2001). Godson et al. (1996), demonstrated in his studies when observing the serum concentration of Hp in cows challenged with bovine herpesvirus type-I and *Pasteurella haemolytica* and found out that there was a significant relationship between increase serum concentration of Hp and bacterial infection. This finding was further corroborated by the work of (Alsemgeest et al., 1996). In cattle, haptoglobin is considered a major APP with serum concentrations ranging from undetectable limit to an average of 1,400 mg/mL during acute inflammation process (Horadagoda et al., 1999).

**Serum amyloid A**

Serum amyloid A is group of proteins secreted by the liver which belongs to the apolipoproteins. They play a significant role in animals and are synthesized and secreted during the acute phase of inflammation (Jain et al., 2011). These groups of proteins are not widely used in veterinary medicine as compared with the haptoglobulin, this is probably due to the limitation arising from their quantification in the circulating blood (Murata et al., 2004). However, studies have shown that they are very useful potential indicators of inflammation in cattle (Alsemgeest et al., 1993; Alsemgeest et al., 1994). Furthermore, high levels of SAA have been reported in serum of cows and ewes with bovine mastitis and also during stress and at parturition (Eckersall et al., 2001). Thus indicating their clinical value in the diagnosis of mastitis and herd health.

### Alpha-l acid glycoprotein (AGP)

This is a moderate class acute phase proteins that are synthesized and secreted by the hepatocytes (Ceciliani et al., 2012). However, extrahepatic secretion and expression have also been reported (Murata et al., 2004). In cattle, AGP have been reported to play a significant role in monitoring inflammatory response (Carter et al., 2002; Eckersall et al., 2001).

### Fibrinogen

This is another moderate class acute phase protein that functions in homeostasis, tissue repair, fibrin formation and providing the medium for migration of inflammatory cells (Murata et al., 2004). The use of fibrinogen as a reliable marker in cattle in response to inflammatory processes, surgical trauma and bacterial infection has been reported (Hirvonen and Pyörälä, 1998; Ceciliani et al., 2012).

### Protease inhibitors

This belongs to the moderate class of acute phase proteins; their application in veterinary medicine is not widely reported. Hence, the reason why their diagnostic value in veterinary medicine is not fully established (Murata et al., 2004).Furthermore, Hirvonen et al. (1996) reported the in ability to establish the clinical significance of this type of APP in experimental mastitis. However, high levels of circulating serum protease inhibitors have been observed in inflammatory conditions in cattle (Conner, 1986).

### Ceruloplasmin

The clinical significance of this copper containing moderate to minor APP in the diagnosis of disease is not common when making comparison with other classes of APP (Ceciliani et al., 2012). However, a number of studies have reported their role in serving as a potential indicator of infection in cattle (Conner et al., 1986; Sheldon 2001).

C- Reactive proteins: this type APP plays a very significant role in host defenses against infection, prevention of autoimmune disease, clearance of damaged or dead tissue and regulation of inflammatory process (Murata et al., 2004). These group of APP have widely reported in ruminants, however, there was limitation regarding their usage as biomarkers of mastitis in cattle, this is because there are some level of doubts regarding nature as APP; furthermore, CRP of cattle is associated with lactation, rather than being synthesized and secreted by the liver cells. However, they are useful indicators of herd health and infection in pigs and mares (Yamashita 1991; Eckersall et al., 1996).

## APPLICATION OF ACUTE PHASE PROTEIN AS A DIAGNOSTIC TOOL IN BOVINE MASTITIS

Acute phase proteins are produced by the liver in response to inflammatory response, surgery or trauma. The concentration of these proteins in the circulating blood is directly related to the degree of severity of the ongoing disease process and quantifying their helps to provide useful diagnostic information necessary for determining the presence and stage of the disease processes eliciting such response (Nielsen, 2004; Eckersall and Bell 2010). In veterinary medicine, the accurate quantification of these proteins provides valuable clinical insight on inflammation and infection (Pyörälä, 2003). Bovine mastitis is a significant economic problem of the dairy farm as well as dairy industry (Vicente, 2014). This is as result in the reduction in milk yield and quality, mortality and high incidence of culling rate and the potential to serve as health risk to other cows in the farm (Boehmer et al., 2008; Viguier et al., 2009). In order to militate against this fall out as a result of mastitis, there is need to develop efficient and highly sensitivity and reliable diagnostic tool that will ensure wide coverage of mastitis detection at herd level.

In cattle, studies have shown that APP plays a very significant role in determining the type and severity of mastitis and also serves as a determinant of herd health (Murata et al., 2004). Furthermore, the serum concentration of the most sensitive acute phase proteins in cattle, haptoglobulin and serum amyloid A have been observed to increase significantly during acute stage of mastitis (Hirvonen et al., 1999; Pyorala, 2003). In addition, other studies have also proven that the two acute phase proteins serves as an efficient and reliable diagnostic tool of acute and chronic bovine mastitis (Grönlund et al., 2003; Petersen et al., 2004). Similarly, serum concentration of α1-acid glycoproteins have been

**Table 1.** Acute phase protein.

| Acute phase proteins | Category | Function | Concentration Normal (Mean±SEM) | Acute (Mean±SEM) | Disease/condition |
|---|---|---|---|---|---|
| Haptoglobulin | Major | Bind to Hg | <0.1g/l | 1.62±0.47g/l | Metritis |
| Serum amyloid A | Major | Opsonisation with cholesterol | 1.3±0.4mg/l Mean ± SEM | 115 +37 Mean ±SEM | Subclinical mastitis |
| Mammary associated serum amyloid A3 | Moderate | Milk, opsonin mucin stimulant | <0.3mg/l | 23,4.4103mg/lmMedian,Range | Clinical mastitis |
| A-acid glycoproteins | Moderate | Transport molecules in plasma, modulate innate adaptive immunity | 0.2-0.45g/l Ref range | 1.1±0.44g/l Mean ±SD | Acute disease |
| Lipopolysacharide binding proteins | Moderate | Binds to LPS and activate innate immune response | 1.7±0.3g/l Mean ±SEM | 11±1.2g/l Mean ± SEM | *M.hemolytica* |
| Ceruloplasmin | Moderate/ Minor | Oxidase activity, copper containing binding iron | 0.24g/l Mean ± SD | 0.36g/l Mean ±SD | Stress |
| Fibrinogen | Moderate | Form fibrin | 2.08;1.58-2.94g/l Median, Range | 2.79; 2.13-5.00g/l Median, Range | Hoof disease |
| Inter α-trypsin inhibitors(H4(ITIH4) | Moderate | Protease inhibitors | 0.75±0.25g/l | 6.0±1.5g/l | Mastitis |
| Fetuin(α2 Hs glycoprotein) | Moderate | Bone growth and fetal development | 0.57±0.04g/l | 0.89±0.213g/l | Trauma |
| α1antiproteinase(antitrypsin) | Minor | Protein inhibitors | 770±57IU/l | 1069±73IU/l | *M. hemolytica* |
| Albumin | Negative | Osmotic pressure, bind to fatty acid and bilirubin | 37±1g/l | 34±1g/l | Calving |
| Paraoxonase | Negative | Oxidase inhibitors | 83±7U/ml | 69±8U/ml | Calving |
| Lipoprotein | Negative | Cholesterol concentration assay and transport | 3.2±2mmol/l | 2.4±0.2mmol/l | Calving |
| Retinol binding protein | Negative | Vitamin A concentration assay and Transport | 32±2µg/100ml | 22±2µg/100ml | Calving |

observed to have increase substantially during chronic mastitis an indication that it can serve as a potential biomarker in the diagnosis of bovine mastitis (Pyorala, 2003).

## ACUTE PHASE PROTEINS ASSOCIATED WITH BOVINE MASTITIS AND THEIR CLINICAL SIGNIFICANCE

The description of acute phase protein in cases of disease will help in mapping out a profile of APP characteristic of that disease, thus providing valuable information with regards to the mechanisms of disease process and also serves as a very good diagnostic aid (Table 1). Studies have revealed that, the application of acute phase protein as a diagnostic tool or marker in bovine mastitis is focused on those bovine conditions that will elicit acute phase response when there is inflammation, infection and traumas as well as the stimulation of cytokines induced responses of which bovine mastitis have been observed to possess these qualities, where acute phase proteins found in the milk and blood (Ceciliani et al., 2012; Petersen et al., 2004). In addition, it was observed that there was a variation in the serum profile of acute phase proteins between different species of animals and the nature of inflammatory response elicited (Godson et al., 1996; Salgado et al., 2011; Ceciliani et al., 2012). In cattle, serum concentration of ceruloplasmin, haptoglobulin, protease inhibitors, Alpha-I acid glycoprotein and fibrinogen have observed to increase during acute inflammatory response (El-Deeb and Elmoslemany, 2016; Skinner et al., 1991; Motoi et al., 1992; Morimatsu et al., 1992; Godson et al., 1996) indicating their significance as an aid to diagnosis of mastitis. In addition, high levels of protease inhibitors, haptoglobulin and serumucoid have been observed during infection with *Pasteurella haemolytica* **(**Godson et al., 1996; Eckersall, 2000). Similarly, high levels of ceruloplasmin were observed in cows with suppurative inflammation of the mammary gland and infection with *Salmonella dublin* (Godson et al. 1996).In his work, Alsemgeest et al. (1994), was able to establish the significance of HP and SAA as an important biomarker in the diagnosis of bovine mastitis and also to differentiate between acute and chronic inflammation, this he was able to achieve when he observe a significant difference in the level of serum concentration measured both during acute and chronic inflammation. This finding is unique in the sense that, apart from establishing the usefulness of Hp and SAA as a diagnostic marker in bovine mastitis it went further to differentiate between acute and chronic mastitis. In addition, Grönlund et al. (2003) and Eckersall and Bell, (2010) also reported that during acute inflammation, high levels of serum bovine SAA were observed than in chronic inflammation. This finding was further corroborated by Petersen et al. (2004) who in his review of acute phase proteins as a diagnostic tool in bovine mastitis reported that concurrent analysis of the serum Hp and SAA were able to distinguish between acute and chronic mastitis. This finding was further corroborated by Juhani et al. (1996) where he observed high level of serum acute phase proteins in experimentally induced mastitis in heifers. Similarly, Baeker et al. (2002) also reported the secretion of a new form of APP called lipocalin-type prostaglandin D synthase (L-PGDS; prostaglandin-H2 D-isomerase, EC 5.3.99.2) into mastitis milk. Furthermore, Ceciliani et al. (2012) gave a detailed description of bovine acute phase proteins, the category, function as well as concentration as depicted in the (Table 1).

## CONCLUSION

The significance of acute phase protein as an important diagnostic marker in bovine mastitis cannot be under estimated. The information provided in this review affirmed the importance of different acute phase protein as a potential biomarkers in the diagnosis of bovine mastitis. This is because many studies have provided useful information with regards to its usage as a diagnostic tool both in natural and experimentally induced mastitis in cattle. We therefore, recommend determination of the accurate correlation of each acute phase proteins with etiologic agent of bovine mastitis.

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