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# Acute phase proteins in dairy cows with mastitis

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## Abstract

In this study, to assess the diagnostic value for mastitis in dairy cows, change of acute phase proteins(haptoglobin and serum amyloid A) concentrations in milk and sera of dairy cows were measured. 50 dairy cows were used in this experiment and divided into two groups. The first group was the healthy dairy cow group whose milk contained less than $2.0 \times 10^5$ somatic cell counts(n=5). The second group was the mastitis-dairy cow group whose milk counted higher than $5.0 \times 10^5$ somatic cell counts(n=45). The concentration of haptoglobin and serum amyloid A in milk and sera from these two groups were determined by Tridelta range haptoglobin kit and serum amyloid A kit. The concentration of haptoglobin in the milk from first group was undetectable value and that of the second group was 124.0μg/mℓ. And the concentration of haptoglobin in serum of the first group was 32.0μg/mℓ and that of the second group was 214.4μg/mℓ. The concentration of serum amyloid A in the milk from first group was 0.32μg/mℓ and that of the second group was 17.7μg/mℓ. And the concentration of serum amyloid A in serum of the first group was 5.1μg/mℓ and that of the second group was 25.8μg/mℓ. It was concluded that concentration of haptoglobin and serum amyoid A in milk and serum may be was to discriminate between normal and mastitic milks.

Key words : Dairy cows, Mastitis, Haptoglobin, Serum Amyloid A

## Introduction

Bovine mastitis is the most costly disease in the dairy industry and can be caused by at least 135 different agents, mostly bacteria[1]. The majority of the infections is caused by the contagious pathogens *Staphylococcus aureus, Streptococcus agalactiae, Str dysgalactiae* and by the environmental pathogens *Str uberis* and *Escherichia coli.* Intramammary infections may also induce changes in the milk such as an increase in

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the numbers and types of somatic cells or inflammatory proteins, such as leutriens[2], or interleukins[3] which can occur in the absences of macroscopic changes in the milk, or may precede the onset of clinical signs.

Somatic cell count(SCC) is a recognized indicator of cow's health and milk quality. Milk SCC reflects the level of infection and resultant inflammation in the mammary gland of dairy cows, associated with mastitis[4,5]. Milk from healthy udders exhibits a SCC, which is less than $2.0 \times 10^5$ cells/㎖ of milk, while for cows with sub-clinical mastitis, excretion of SCC is usually higher than $2.0 \times 10^5$ cells/㎖. For milk with clinical mastitis, for example, SCC can reach a few million cells/㎖[6]. SCC is also an indicator of milk quality as shelf life is reduced for high SCC milk and the processing quality and yield of some milk products is reduced when SCC increases[7].

The acute phase response is the nonspecific early response of the organism to infection and inflammation[8~10]. It comprises a whole array of systemic reactions, including fever, increase in muscle protein catabolism, alterations in sleep patterns and appetite, and induction of a group of serum proteins called acute phase proteins[11] concurrent with a decrease in serum albumin. Monitoring progression of infection by acute phase protein measurements in blood samples is used extensively in human patients[10] and a similar clinical use as a diagnostic and prognostic aid has been proposed in veterinary medicine[4,9,12], as well as a means to monitor the health status of herds of production animals and of individual animals at slaughter[13,14].

The present investigation was undertaken to assess the value of acute phase proteins in the diagnosis of mastitis in dairy cows, to identify the most appropriate proteins for this purpose, and compare their diagnostic value with that of the somatic cell counts.

## Materials and Methods

### Milk samples, somatic cell counts and serum

Milk samples were collected separately from each udder of dairy cows in Jeonbuk area. Udders were washed, dried and teat orifices were swabbed with 70% ethanol solution. The first three streams of foremilk were discarded. About 10㎖ of milk was collected aseptically in sterile vials. The somatic cell count was performed by a automated Bently 2,500(USA) counter according to the manufacture's instruction. Dairy cows(n = 50) were used in this experiment and divided into two groups. The first group was the healthy dairy cow group whose milk contained less than $2.0 \times 10^5$ somatic cell counts(n=5). The second group was the mastitis-dairy cow group whose milk counted higher than $5.0 \times 10^5$ somatic cell counts(n=45).

And blood samples of same dairy cows were taken from a jugular vein in to vacutainers, serum was obtained after clotting by centrifugation and was stored at -20℃ until use.

### Assay acute phase proteins in milk and serum

The concentration of haptoglobin in serum and milk was determined accoding to the manufacture's instruction by the Phase haptoglobin Kit(Tridelta development), the basis of which is the haemoglobin binding method described by Eckersall et al[15] and Eckersall and Conner[16].

The concentration of haptoglobin in serum and milk was determined accoding to the manufacture's instruction by using an ELISA, based on that described by McDonald and others[17] and now available commercially(Tridelta development).

## Results and Discussion

Mastitis is defined as an inflammatory reaction of the parenchyma of the mammary gland that can be of an infectious, traumatic or toxic nature[18]. Mastitis is characterized by physical, chemical and usually bacteriological changes in the milk and by pathological changes in the glandular udder tissue. The diagnosis of mastitis is based on clinical signs, eg swelling of the udder, tender to the touch, fever, and depression. In many cases a reduced milk production can be observed. Because of the large number of subclinical mastitis cases, the diagnosis of mastitis can also depend on indirect tests which in turn depends on, eg the leukocyte numbers in the milk[19]. Bovine mastitis is generally considered to be of infectious nature leading to inflammation of one or more quarters of the mammary gland and it is often affecting not only the individual animal but the whole herd or at least several animals within the herd. If left untreated, the condition can lead to deterioration of animal welfare resulting in culling of affected cows, or even death. Mastitis is recognized as one of the most important disease affecting dairy cows worldwide. Mastitis in dairy cow is caused by numerous bactetia such as *S aureus, Str agalactiae* and *E coli*. Intramammary infection may induce changes in an increase of numbers and types of somatic cells, inflammatory proteins.

The acute phase serum protein response is a well-known general indicator of inflammation, trauma or other pathological conditions and its relevance for the monitoring of the health status of domestic animals is being increasingly realized. Acute phase proteins are a group of liver-derived serum proteins whose concentrations change in response to infection, inflammation or injury[11,20,21]. The changes in serum protein composition which occur after tissue damage represent a part of the systemic response of the injured animals which is mediated by pro-inflammatory cytokines such as TNF-, IL-6 and IL-1. These responses play a vital role in containing the tissue damage and enhancing the processes of repair and resolution. From a clinical perspective, the assay of acute phase proteins can provide a method for detecting inflammation. In cattle, the most sensitive acute phase proteins are haptoglogin, serum amyloid A and $\alpha_1$ acid glycoprotein in response to inflammatory condition[16].

Haptoglobin is one of a series of acute phase proteins that is found in the blood of both humans and animals. Under normal conditions, it is either absent from the blood or present at very low levels, dependent on the species, ranging from less than 0.05㎎/㎖ in cattle to 1-3㎎/㎖ in humans. However, haptoglobin can increase significantly in response to acute infection, inflammation or trauma. The rise in serum haptoglobin and the continuous monitoring of this during the acute phase response gives valuable information to the clinician in both human and veterinary medicine. Conversely, decreased haptoglobin is used as a marker to indicate the extent of haemolysis. Decreases in haptoglobin levels are observed if free haemoglobin is present in the blood arising from haemolysis of the red blood cells. Free

haemoglobin binds to haptoglobin with subsequent removal of the complex by the liver[22]. 50 dairy cows were used in this experiment and divided into two groups. The first group was the healthy dairy cow group whose milk contained less than $2.0\times10^5$ somatic cell counts. The second group was the mastitis-dairy cow group whose milk counted higher than $5.0\times10^5$ somatic cell counts. The concentration of haptoglobin milk and serum from these two groups were determined by Tridelta range haptoglobin kit. The concentration of haptoglobin in the milk from first group was undetectable value and that of the second group was 124.0㎍/㎖ (Table 1). And the concentration of haptoglobin in serum of the first group was 32.0㎍/㎖ and that of the second group was 214.4 ㎍/㎖(Table 2). Haptoglobin is one of the most reactive acute phase protein in cattle[23]. It has a low constitutive level and exhibits a high relative increase during acute phase has, in several disorder of cattle, also been shown to increase[24]. It major function is to bind free haemoglobin and thus protect the host form the oxidative activity of haemoglobin.

Haptoglobin binds free hemoglobin, eg, released by hemolysis, which is thought to be of importance in the host response to infection[25]. It is a strongly reacting acute phase protein in most species studied, including man, mouse, rat, pig, cattle, and rabbit[14,25,26]. In cattle and in mice the level of haptoglobin in healthy animals is very low and the protein is induced 50~100 times by the acute phase reaction[4,9,22]. In clinical medicine, the rapid assay of haptoglobin could provide the following ; (1) a sign of microbial infection ; (2) an objective index of disease activity and response to therapy ; (3) a screening test for organic disease[22].

The serum amyloid A family of acute phase proteins are named because of their

Table 1. Concentration of haptoglobin in milk of healthy cows and cows with mastitis

| Groups | SCC/㎖* | | HP (㎍/㎖)** | |
|---|---|---|---|---|
| | Mean | Range | Mean ± SE | Range |
| Healthy (n = 5) | $2.0\times10^5$ | $1.2\times10^5$ - $2.2\times10^5$ | NA*** | all < 20.0 |
| Mastitis (n = 45) | $2.5\times10^6$ | $5.0\times10^5$ - $9.3\times10^6$ | 124.0 ± 8.4 | 80.0 - 270.0 |

* SCC : Somatic cell counts
** HP : Haptoglobin
*** NA : Not applicable owing to small number with a detectable concentration of haptoglobin.

Table 2. Concentration of haptoglobin in serum of healthy cows and cows with mastitis

| Groups | SCC/㎖* | | HP(㎍/㎖)** | |
|---|---|---|---|---|
| | Mean | Range | Mean ± SE | Range |
| Healthy (n = 5) | $2.0\times10^5$ | $1.2\times10^5$ - $2.2\times10^5$ | 32.0 ± 9.3 | 23.3 - 99.8 |
| Mastitis (n = 45) | $2.5\times10^6$ | $5.0\times10^5$ - $9.3\times10^6$ | 214.4 ± 15.3 | 80.7 - 900.0 |

* SCC : Somatic cell counts
** HP : Haptoglobin

response[30]. Serum haptoglobin concentration

immunological and biochemical similarity to amyloid A, the fibril protein in reactive systemic amyloidosis. The liver produces several different isoforms of SAA following stimulation by immune system modulators including interleukin-1, interleukin-6 and tumor necrosis factor. In its native form, SAA generally consists of a 104-amino-acid polypeptide(12kd) in association with the HDL 3 subclass of plasma lipoproteins. SAA is an acute phase reactant that is synthesised in the liver and is released into the plasma in a variety of inflammatory conditions. It is also released into plasma in response to major trauma or, as in the present study, major surgery. Within plasma most of the SAA is transported as a component of HDL[27~29] for which it has a high affinity. SAA displaces apoA1 and, to a lesser extent, also apoAII from HDL in a process that leaves the HDL increased in both particle size and density[7,30,31]. Thus, a typical SAA-enriched HDL circulating in plasma during times of inflammation has the size of HDL2 but the density of HDL[8,27,30]. As in the present study, this association of SAA with HDL is also associated with a depletion of HDL cholesteryl esters and an enrichment in HDL triglyceride and phospholipids[7,8,27~30]. 50 dairy cows were used in this experiment and divided into two groups. The first group was the healthy dairy cow group whose milk contained less than $2.0\times10^5$ somatic cell counts. The second group was the mastitis-dairy cow group whose milk counted higher than $5.0\times10^5$ somatic cell counts. The concentration of serum amyloid A in milk and sera from these two groups were determined by Tridelta range serum amyloid A kit. The concentration of serum amyloid A in the milk from first group was 0.3㎍/㎖ and that of the second group was 17.7㎍/㎖(Table 3). And the concentration of serum amyloid A in serum of the first group was 5.1㎍/㎖ and that of the second group was 25.8㎍/㎖ (Table 4).

The bovine acute phase protein reaction

Table 3. Concentration of serum amyloid A in milk of healthy cows and cows with mastitis

| Groups | SCC/㎖* | | SAA(㎍/㎖)** | |
|---|---|---|---|---|
| | Mean | Range | Mean±SE | Range |
| Healthy (n=5) | $2.0\times10^5$ | $1.2\times10^5$-$2.2\times10^5$ | 3.2±0.4 | 2.1- 5.6 |
| Mastitis (n=45) | $2.5\times10^6$ | $5.0\times10^5$-$9.3\times10^6$ | 17.7±3.7 | 8.6-80.8 |

* SCC : Somatic cell counts
** SAA : Serum amyloid A

Table 4. Concentration of serum amyloid A in serum of healthy cows and cows with mastitis

| Groups | SCC/㎖* | | SAA(㎍/㎖)** | |
|---|---|---|---|---|
| | Mean | Range | Mean ± SE | Range |
| Healthy (n = 5) | $2.0\times10^5$ | $1.2\times10^5$ - $2.2\times10^5$ | 5.1 ± 3.2 | 3.50 - 10.0 |
| Mastitis (n = 45) | $2.5\times10^6$ | $5.0\times10^5$ - $9.3\times10^6$ | 25.8 ± 6.5 | 8.82 - 50.0 |

* SCC : Somatic cell counts
** SAA : Serum amyloid A

has been reviewed by Eckersall and Conner[16] and Gruys et al[20]. Haptoglobin concentrations in healthy cattle are often undetectable[24,32] but during an acute phase response bovine haptoglobin can increase 50~100 times[32], making it the most prominent acute phase protein in cattle[33]. On the other hand, SAA is a remarkably moderate acute phase protein in cattle increasing around 2~5 times during an acute phase response[33,34]. Nevertheless, SAA seems to react faster than haptoglobin in response to an acute phase protein inducing event[35] and the difference between serum concentrations induced by acute and chronic inflammation, respectively, was found to be bigger for SAA than for haptoglobin in naturally infected cattle[21,33].

It was concluded that concentration of haptoglobin and serum amyloid A in milk and serum may be was to discriminate between normal and mastitic milks. However, further investigations are required of the potential of these test as marker of mastitis in cases encountered in clinical practice.

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