CONCENTRATION OF ASCORBIC ACID IN THE BLOOD OF COWS AFFECTED WITH MASTITIS

1,2 MIROSŁAW KLECKOWSKI, 1 WŁODZIMIERZ KLUCIŃSKI, 1AMJAD SHAKTUR AND 1JACEK SIKORA

1Department of Clinical Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, 02-776 Warsaw, Poland 2Veterinary Hygiene Laboratory, 18402 Łomża, Poland e-mail: zhwlomza@op.pl

Received for publication November 26, 2004.

Abstract

The research was conducted on 56 cows. The cows were divided into 4 groups: A, B, C with clinical form of mastitis caused by Staphylococcus aureus, Streptococcus agalactiae and Escherichia coli and D control. A markedly low level of ascorbic acid concentration in the serum of cows affected with mastitis (29.4 µmol/dm³) as compared with the control group (64.9 µmol/dm³) was observed.

Key words: cows, mastitis, ascorbic acid, antioxidants.

The status of good health and high milk production of cows results from the balance between prooxidative and antioxidative processes in the body fluids, and in the cells. In Poland the first investigations concerning oxidative balance in cows were done in 1987 by Kleczkowski (10). Oxidative stress can be stopped by the defence activity of antioxidative systems that consist of antioxidants inhibiting activated molecules (1, 4). The most important antioxidant is ascorbic acid (AA). The compound remains an interesting topic of much scientific research even though it was discovered many years ago. The ability of AA to minimize the harmful environmental influence on the metabolism is of special interest (16, 17).

AA protects DNA of the cells from the free radicals damage, prevents infections by strengthening cell membranes, and helps protecting phagocytic cells from oxidative damage. As it has been found in recent studies, white blood cell DNA damage was repair with the use of several antioxidants (5). In the presence of AA, bovine erythrocytes haemolysis was significantly lower (23). Some antioxidants were found out to be able to protect the white blood cells in different degree. There was 41% protection with AA, 55% protection with α-tocopherol and 50% protection with β-carotene (19). Neutrophils can defend themselves against the oxidant stress through a potent antioxidant system, for example: AA, α-tocopherol, and superoxide dismutase (8).

AA may indirectly improve immune responses by helping to maintain tissue levels of vitamin E. It is important for immune system factors and has anti-inflammatory properties. It has been found in some studies that AA is helpful for boosting immune system function (4, 6). Vitamin C deficiency can lead to decreased ability of body to fight infection. AA is needed for the growth and recovery of tissues in all parts of the body. Therefore, antioxidant therapy is a focus of recent research.

The unique properties of AA result from its ability to change easily from the reduced to oxidized form and vice versa. Therefore, this chemical molecule plays an important role in ensuring the oxidation – reduction potential in cells. It is very important, that this molecule, when found at small concentration, acts as an antioxidant in cell metabolism. However, AA has also the prooxidative function. As an antioxidant, AA is oxidized to dehydroascorbic acid in presence of copper ions. As prooxidant it can accelerate the formation of hydroxyl radicals from hydrogen peroxide in the presence of reduced metal ions. It is proven that AA together with Fe ions, Cu or hydrogen peroxide can influence the start of lipid peroxidation (1, 9, 11, 12).

The assessment of antioxidative activity in the cow blood may be performed by measuring the direct and indirect antioxidant metabolic coefficients. One of them is ascorbic acid. The antioxidative activity in the cow blood in the course of inflammation of the mammary gland is not well known. That is why knowledge of this phenomenon is very important both for cognitive and practical aims. Taking into consideration the suggestions presented above, the present investigations focused on the evaluation of concentration of ascorbic acid in the serum of cows affected by the clinical form of mastitis caused by Staphylococcus aureus, Streptococcus agalactiae, and Escherichia coli.
Material and Methods

The search was conducted on 56 cows, Black-and-White breed, 3-8 years old, with average annual milk yield 4450 kg. During winter, all the cows were housed in highly tethered stalls and fed hay, feed concentrates, and beet pulp *ad libitum*. During summer, the cows were on pasture and additionally were given green grass forage and concentrates. Depending on the season of the year, the feeds originated from the local area. Daily feeding dose was balanced with regard to energy and protein, according to commonly acceptable standards (20). The investigations were conducted for 2 years. During the first year 28 cows were used, whereas in the following year the other 28 cows.

From the beginning of the experiment, all the cows were subjected to clinical examination. After clinical examination, the milk samples were taken simultaneously both for testing of somatic cell counts and with the aim to identify the pathogenic microorganisms.

The clinical examination of cows, including the mammary gland examination, evaluation of macroscopic changes of mammary gland secretion and somatic cells count in milk, were conducted according to rules developed by Malicki and Binek (13) and Malinowski and Klósowska (14). The organoleptic properties of the secretion of the mammary gland were estimated using a black Schalm plate. The initial tests determining the somatic cell count in milk were conducted with the help of California Mastitis Test using a Schalm reagent plate and Mastirapid. The somatic cells counts were determined by microscopic method (14). The isolation and identification of such microorganisms as: *Staphylococcus aureus, Streptococcus agalactiae*, and *Escherichia coli* were performed using bacteriological methods (3, 14).

Blood samples were collected, at the same time for all the groups (from 5^th^ to 6^th^ a.m.), from the external jugular vein. The ascorbic acid concentration was measured by the hydrazine method (7, 18).

On the basis of clinical examination, somatic cell counts and results of bacteriological examination, the cows were divided into 4 equal groups (A, B, C, D). Groups A, B, and C were the experimental groups, whereas group D served as a control. In the experimental groups there were cows affected by clinical form of mastitis (A-29 quarters, B-39 quarters, C-36 quarters). Pure culture of *Staphylococcus aureus* was isolated from milk of group A cows, *Streptococcus agalactiae* from milk of group B cows, and *Escherichia coli* from milk of group C cows. Among the cows from group D no diseases, including mastitis, occurred and no pathogenic microorganisms were isolated from their milk.

The organoleptic properties of the secretion of the mammary gland were estimated using a black Schalm plate. The initial tests determining the somatic cell count in milk were conducted with the help of California Mastitis Test using a Schalm reagent plate and Mastirapid. The somatic cells counts were determined by microscopic method (14). The isolation and identification of such microorganisms as: *Staphylococcus aureus, Streptococcus agalactiae*, and *Escherichia coli* were performed using bacteriological methods (3, 14).

Blood samples were collected, at the same time for all the groups (from 5^th^ to 6^th^ a.m.), from the external jugular vein. The ascorbic acid concentration was measured by the hydrazine method (7, 18).

The statistical analysis of the results was performed with the use of Tukey post-hoc test, correlation coefficient. The data were expressed as mean and standard deviation of mean (mean±SD), and *P*≤0.05, *P*≤0.01, and *P*≤0.001 were taken as the level of significance.

Results

Selected clinical symptoms occurring in cows are presented in the Table 1.

<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Udder swelling</td>
<td>14</td>
<td>100</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td>Rubor of udder skin</td>
<td>14</td>
<td>100</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td>Light painfulness</td>
<td>10</td>
<td>71</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td>Hard painfulness</td>
<td>4</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Induration</td>
<td>3</td>
<td>21</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>Constipation</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Indigestion</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Increase in body temperature</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The organoleptic evaluation of mammary gland secretion is an important part of the entire examination. From the presented results we could find out that the most often occurring organoleptic changes were milk aqosity, presence of flakes, clots, and shreds, while other changes, such as colour change, serous, purulent or bloody discharge, were rather rare. The information obtained during organoleptic evaluation indicates that the most important changes were in the milk of cows only from A, B and C experimental groups. There were
no organoleptic changes among the cows of the control group D. The positive California Mastitis Test results were obtained only in milk of cows from experimental groups. In group D, the test gave negative results. The average number of somatic cells is presented in the Fig. 1. The average AA concentration in the serum is presented in Fig. 2.

**Fig. 1.** Average number of somatic cells in the milk.

Differences statistically significant (P≤0.001) between experimental groups (A, B, C) and control (D).

**Fig. 2.** Average concentration of ascorbic acid in the serum.

Differences statistically significant (P≤0.05) between groups A, C and D.

The highest serum concentration of ascorbic acid was found in cows from the control group D (60.8±16.4 μmol/dm³); a less than that was demonstrated in cows from group B (33.3±7.9 μmol/dm³); a still lower was found in cows from group C (29.4±7.8 μmol/dm³) and the lowest was in group A (25.6 ± 7.9 μmol/dm³).

**Discussion**

It was thought that multidirectional interaction of individual microorganisms, which jointly can result in making up of a stimulating factor or inhibitory antioxidant activity in body fluids or in tissues and have modulating influence on inflammation states. With regard to the fact that in literature there are few publications concerning clearly the AA antioxidant activity in blood of cows affected by inflammation of the mammary gland, some sentences below will be dedicated to this fascinating phenomenon.

As a result of the conducted investigations, it was possible to confirm that the cows from groups A, B and C were affected with clinical form of inflammation of the mammary gland. The confirmation of the clinical form of mastitis was the occurrence of the clinical symptoms of the disease listed in table 1, macroscopic changes in milk (Table 2), and increased number of somatic cells (Fig. 1). Taking into consideration the kind and intensity of individual clinical symptoms, as well as
the results of additional investigations it was possible to state that the inflammation of the mammary gland in cows from the group C had the most acute course and was caused by *Escherichia coli*. Typical signs include fever, severe depression, shivering, hard painfulness, rubor of udder skin, udder swelling, and anorexia (21). It was found that associations between clinical signs and changes in serum AA concentration were weaker than changes in the concentration of AA in the milk. However, the decrease in serum AA concentration was associated with peak body temperature and changes in milk yield (22). The similar relationship was observed in group C (Fig. 2). The disease in cows from the group A was somewhat milder and inflammation of their mammary glands was caused by *Staphylococcus aureus*. However, swelling, rubor, induration, subcutaneous oedema, heat, and pain could extend along the mammary vein (21). The mildest course of mastitis was found out in cows from group B, where the etiologic factor was *Streptococcus agalactiae*. Clinical signs were swelling, rubor of udder skin, and light painfulness (21). Relatively small number of somatic cells in the milk from affected quarters can be a result of nutritional antioxidative factors and initial phase of the inflammation. The cows from the control group D showed no clinical symptoms of mastitis and the results of additional examinations were negative.

AA concentration in the serum of dairy cows ranges from 40 to 70 µmol/dm³ (7). After the analysis of our results, we can observe decrease in AA concentration in the serum of cows in the groups A, C and B (clinical form of mastitis) when compared with control group D (Fig. 2). Similar results were observed after intramammary infusion of *Escherichia coli*. Plasma AA concentration decreased to 39% and the content of the vitamin in milk from affected quarters declined to 52-62%. No change was observed in AA concentration in plasma and milk from unaffected quarters. However, peak body temperature and other clinical signs during clinical mastitis were associated with a considerable decrease in the concentration of AA in milk and plasma from the affected quarters. It has been found in some studies, that the infection may stimulate the inflammatory process and release oxygen free radicals, which deplete the body’s resource of AA. Moreover, the decrease in serum AA concentration can reduce its synthesis, increase the uptake by cells, and increase the oxidation. Especially AA within neutrophils can be oxidized by reactive oxygen species found within the cells. Monocytes activated the production of free radicals. The level of AA might be low in peripheral arterial net in the state of inflammation and oxidative stress. There has been observed, that AA is needed for recovery of tissues in all parts of the body. It is suggested that AA provides potential benefit for recovery from acute form of mastitis (13, 22). An increased level of AA can lead to increased ability of the body to fight infection. It has been found in some studies that vitamin C is helpful for boosting immune system function. Due to that, when we treat the infected cows, the level of AA increases. It appears from our results when we compared the concentration of AA in infected cows with that in normal ones. Some researchers observed a negative correlation between AA and interleukin-6 (IL-6) and tumour necrosis factor (TNF) (15). Ascorbic acid is essential for the formation of collagen and fibrous tissue for normal intercellular matrices in the mammary gland, teeth, bones, cartilage, connective tissue and skin. It also interacts with other nutrients (1) and vital molecules acting as a detoxifier helping to cleanse the body from toxins (6, 17).

Activity of antioxidant mechanisms preventing the oxidant stress during inflammation depends on various etiological factors, and inflammatory mediators. The conducted investigations confirmed the influence of bacterial factors in clinical form of mastitis on the antioxidative activity, including AA, in blood of cows. It is obvious that a decrease in serum AA concentration of cows with mastitis is the result of the disease.

### References

11. Kleczykowski M.: Kwas askorbowy jego właściwości oraz udział w przemianach. Materiały XX Krajowej Konferencji Naukowej Pracowni Biochemicznych ZHW. PIWet, Puławy, 1993, pp. 6-35.