Plasma Haptoglobin Values in Cows With Different Somatic Cell Counting in Milk Samples

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ABSTRACT

Background: Subclinical mastitis is an important cause of economic losses in dairy cows in Rio Grande do Sul state (southern Brazil) because the deleterious effect on the health of the cow and on the quality of milk. The difficulty of early diagnosis of this problem had led to the study of early indicators of inflammatory conditions, as acute phase proteins, mainly haptoglobin and fibrinogen, in the case of bovines. The aim of this work was to study the relationship between plasma values of haptoglobin (Hp) and fibrinogen in dairy cows with somatic cell counting (SCC) in milk for testing the hypothesis that Hp or fibrinogen would be adequate early indicators of subclinical mastitis.

Materials, Methods & Results: Milk and blood samples were collected in cows of herds from Rio Grande do Sul. Three groups of 50 cows each were separated according to milk SCC. Samples of group 1 (healthy mammary gland) had SCC below 600,000 cell/mL, samples of group 2 (subclinical mastitis) had SCC above 600,000 cell/mL without any signal of clinical mastitis and group 3 (clinical mastitis) was compound by samples from cows showing evident clinical signs of mastitis. Additionally, total bacterial count (TBC) and concentration of lactose, fat, protein and total solids were determined in milk using infrared spectrophotometer. Blood samples were collected by venipunction of the coccygeal vein for analysis of fibrinogen and Hp. Fibrinogen was determined by the heat precipitation method and Hp by a spectrophometric method. The results showed lower concentration of lactose, fat and total solids and higher concentration of protein in milk samples of cows with mastitis. Fibrinogen and Hp values were higher in the mastitis group, but no differences were observed between healthy and subclinical mastitis groups. Significant correlation coefficients were observed between plasma fibrinogen and milk SCC (r = 0.27) and between plasma Hp and milk SCC (r = 0.357).

Discussion: The health condition of the mammary gland and therefore the somatic cell counting is determinant in the composition of milk so that an inflammatory condition may lead to decreasing values of lactose and fat as well as increment in total protein. Decrease in lactose and fat composition of milk have been reported in many works as a function of the deleterious effect of inflammation on the mammary tissue. Increase in milk total protein must be a direct effect of the inflammatory process, represented by somatic cells, bacteria and other substances leaking from the injured secretory tissue. The lack of difference of the plasma concentrations of haptoglobin and fibrinogen between subclinical mastitis and healthy mammary gland hinders the possibility of discriminating cows with the subclinical condition of the problem. Hp in plasma samples belonging to cows of low milk SCC is probably augmented by other factors than an inflammatory condition. The present study did not prove a clear usefulness for using plasma values of Hp for diagnosing subclinical mastitis in dairy cows. However, the significant correlation coefficient obtained between plasma Hp and milk SCC are worth of exploring more aspects of the relationships among acute phase proteins, clinical and subclinical mastitis condition and quality of milk.

Keywords: somatic cell count, haptoglobin, fibrinogen, mastitis, dairy cows.
INTRODUCTION

Mastitis is the main cause of economic losses in dairy herds [29] with a prevalence of 26% in milking cows [16]. In Brazil, the incidence of mastitis may reach 71% of the milking cows, as reported by Costa et al. [6] for the states of Minas Gerais and São Paulo. However, the major cause of losses by mastitis (70-80%) is represented by subclinical mastitis [30] which is more difficult for diagnosing. Pereira et al. [25] mentioned that in Brazilian dairy herds the incidence of subclinical mastitis could be 11.9 to 53.3%. Clinical or subclinical mastitis may produce deleterious effects on milk quality and composition [1].

The diagnosis of mastitis is done by routine on clinical examination basis, visual examination of milk, somatic cell count (SCC) directly by fluxometry or indirectly by the California Mastitis Test, total bacterial count (TBC) and culture of milk pathogens [13]. In the case of subclinical mastitis, the diagnosis becomes more difficult due to the absence of clinical signs and a sensitive laboratory indicator is necessary for discriminating healthy from mastitic cows in order to take appropriate measures in advance enough to avoid more economic losses.

Acute Phase Proteins (APPs) are secreted by the liver and other tissues in response to several stimuli that affect the homeostasis of the immune system, particularly infections, inflammations, stress and metabolic disorders [18]. In cows, haptoglobin (Hp), fibrinogen and serum amyloid A (SAA) are considered the main APPs with usefulness in the diagnosis of inflammatory process with high sensibility [19]. In dairy cows, monitoring APPs may be useful to identify subclinical situations, particularly in cases of mammary affections [26]. The recent discovery that APPs are synthesized in the mammary gland besides the liver [10], offered an interesting potential on the identification of problems related to mastitis in dairy cows.

The present work had the aim of relating plasma concentration of haptoglobin with somatic cell counting in milk samples obtained from dairy herds in Rio Grande do Sul state. The work hypothesis is that it would be possible to differentiate cows in a subclinical mastitis condition from healthy animals by the difference of plasma concentration of haptoglobin.

MATERIALS AND METHODS

A total of 150 milk samples and the corresponding blood samples were collected from Holstein cows belonging to different herds, regardless of age, lactation number or days in milking. The choice criterion of milk samples was strictly the somatic cells count (SCC) as follows:

Group 1 (healthy animals): SCC <600,000 cell/mL
Group 2 (subclinical mastitis): SCC >600,000 cell/mL without any clinical signal
Group 3 (clinical mastitis): any SCC from cows with evident signal of mastitis.

The milk samples were collected in appropriate recipients of 50 mL capacity using conservative substances (bronopol for somatic cell count and azidol for bacterial count). The determination of SCC in milk samples was done by fluxometry (Somacount, Bentley Instruments), as well as total bacterial count (TBC) (Bactocount, Bentley Instruments). The chemical components of milk (lactose, fat, protein and total solids) were determined by infra-red technique (Somacount, Bentley Instruments).

Blood samples were collected using EDTA vacutainer tubes (5 mL) by puncturing the coccygeal vein, conditioned in thermal boxes and transported to the laboratory. Fibrinogen was determined immediately by the heat precipitation method [14]. The blood samples were centrifuged (920 g, 10 min) and the plasma conditioned in eppendorf tubes for freezing until determination of biochemical analysis. Serum concentrations of Hp were quantified by a spectrophotometric method using a commercial kit1. A statistical analysis was done using the software SPSS 13.0, applying ANOVA test and multiple comparison test of Tukey. The total data were used for a Pearson correlation test to study the relationship between Hp and fibrinogen concentrations with SCC.

RESULTS

The values of SCC in the three groups were significantly different as expected by the criteria of selection of the animals. The mean values of SCC in healthy cows was 130 x 10³ cell/mL, in cows with subclinical mastitis was 1,073 x 10³ cell/mL and in cows with clinical mastitis was 4,350 x 10³ cell/mL. Also, the TBC were different among the 3 groups, with mean values of 90 x 10³ CFU/mL (colony-forming unit) for healthy cows, 1,077 x 10³ CFU/mL for cows with subclinical mastitis and 5,783 x 10³ CFU/mL for cows with clinical mastitis.
As a result of the different health status in the mammary gland, a difference in the milk components was found (Table 1). Lactose and fat concentrations in milk were significantly decreased, while protein concentration increased, as the values of SCC were higher.

The plasma concentration of haptoglobin (Hp) was higher ($P < 0.05$) in the group of mastitic cows comparing the other two groups, but no difference was found between healthy cows and cows with subclinical mastitis (Table 2). The concentration of fibrinogen (Table 2) was significantly higher in mastitic cows (6.50 g/L) compared to cows with subclinical mastitis (5.48 g/L) or healthy cows (5.16 g/L).

**DISCUSSION**

In spite of considering the SCC as a gold standard indicator of the mammary gland inflammation [1, 22], the great variability of this parameter in healthy animals by factors as age [2], milking onset [8], milking end [7] and seasonality, among others [31], may diminished its efficiency in the decision of discriminating a cow with subclinical mastitis. Efforts in research have been directed for searching a biomarker of subclinical mastitis, not only to identify affected animals but also to evaluate and to prevent alterations in milk quality [15].

A polemic aspect in dairy husbandry refers to the number of SCC in milk for considering a subclinical mastitis. In European countries this value is established as $>300,000$ cell/mL [11, 13]. However, in the Brazilian conditions, several works show higher means of SCC in the different states for Holstein cows: 400,000 cell/mL in Rio Grande do Sul [21], 641,000 cell/mL in São Paulo [17] and 530,000 cell/mL in Minas Gerais [33]. Significant changes in milk composition, especially for protein and lactose, were found with SCC $>500,000$ cell/mL [17]. Official regulations of the Brazilian Ministry of Agriculture [3] establish a maximum SCC of 600,000 cell/mL in milk classified as type A. Therefore, taking in consideration the latter data for the Brazilian conditions, the SCC value of 600,000 cell/mL seems to be an adequate threshold for considering subclinical mastitis. This value was the

**Table 1.** Values of milk components in cows with different somatic cell counts (SCC).

<table>
<thead>
<tr>
<th>Mean SCC ($10^3$ cell/mL)</th>
<th>N</th>
<th>Lactose (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Total solids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>130</td>
<td>50</td>
<td>4,52</td>
<td>0,26</td>
<td>3,64</td>
<td>0,89</td>
</tr>
<tr>
<td>1,073</td>
<td>50</td>
<td>4,26</td>
<td>0,31</td>
<td>3,63</td>
<td>0,85</td>
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<td>4,350</td>
<td>50</td>
<td>3,80</td>
<td>0,56</td>
<td>3,18</td>
<td>1,44</td>
</tr>
</tbody>
</table>

Different letters mean significant difference by Tukey test ($P < 0.05$). SD = Standard deviation.

**Table 2.** Plasma values of fibrinogen and haptoglobin in cows with different SCC.

<table>
<thead>
<tr>
<th>Mean SCC ($10^3$ cell/mL)</th>
<th>N</th>
<th>Fibrinogen (g/L)</th>
<th>Haptoglobin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>130</td>
<td>50</td>
<td>5,16</td>
<td>2,35</td>
</tr>
<tr>
<td>1,073</td>
<td>50</td>
<td>5,48</td>
<td>2,43</td>
</tr>
<tr>
<td>4,350</td>
<td>50</td>
<td>6,50</td>
<td>2,72</td>
</tr>
</tbody>
</table>

Different letters mean significant difference by Tukey test ($P < 0.05$). SD = Standard deviation.
original limit proposed in Europe for consider a subclinical mastitis [28] and it was considered in the present work as the cut-off point for subclinical mastitis condition.

The present work had the objective of testing the hypothesis that haptoglobin (Hp), an acute phase protein, may appear augmented in the plasma of cows with subclinical mastitis, in order to identify the animals before the condition get worse and take the adequate measures of husbandry. The previous analysis of the milk composition in the studied groups showed differences as a function of the health condition of the mammary gland. The amount of lactose had a gradual and significant decrease with the increment of somatic cell count (SCC). Prada e Silva et al. [27] and Zafalon et al. [34] mentioned that lactose content in the milk decrease gradually as the inflammatory condition of mammary gland becomes more severe as a result of the diminished synthesis by the secretory tissue, the increase of permeability of the cell membrane and the utilization of lactose by the intramammary pathogens. Ogola et al. [23] reported a significant decrease in lactose content in milk samples with SCC above 500,000 cell/mL suggesting that cows with subclinical mastitis may have a fall in the milk components. The fat content in milk showed significant decrease in cows with clinical mastitis. The fall in lactose and fat contents led to a diminished value of the total solids in the group of cows with clinical mastitis. Less quantity of total solids in milk of mastitic cows may be attributed mainly to the less amount of lactose and fat [4,5].

Milk protein content was higher in cows with mastitis, probably due to the high amount of somatic cells and bacteria. The inflammatory process causes an increase in cell permeability due to the lesion of the secretory tissue [12]. The results of the present work are in disagreement with Park et al. [24] who found a fall in the milk protein of cows with SCC above 351 x 10³ cell/mL.

Cows with clinical mastitis had a significant altered value of haptoglobin, but the hypothesis that different concentrations of haptoglobin may exist between milk samples from subclinical mastitis and healthy mammary gland could not be proved. This finding revealed that, in the conditions of the present work (a survey study), plasma Hp would not be useful as a diagnosis indicator of subclinical mastitis and that the Hp levels may not differentiate among cows with different values of SCC. The results suggest that, at field conditions, other causes different from the mammary gland status may be influencing the plasma Hp values either in apparently healthy cows or in cows with subclinical conditions. In controlled conditions of groups of animals with experimentally induced mastitis, it seems plausible that Hp may discriminate healthy cows from cows with subclinical mastitis and even among cows with different values of SCC [33]. Eckersall et al. [9] studied the plasma Hp concentration in cows with mastitis and found values of 0.47 g/L in cows with mean SCC of 6,400 x 10³ cell/mL and 0.74 g/L of Hp in cows with SCC of 8,700 x 10³ cell/mL. Nevertheless, those authors mentioned that in healthy cows the values of plasma Hp were negligible. Nielsen et al. [20] also detected higher plasma Hp values in cows with mastitis compared with healthy cows. However, in the present work, the group of healthy cows had a secretion of Hp not compatible with the aforementioned results, which is probably due to other uncontrolled causes.

Nevertheless, a significant correlation was found between SCC and Hp values (r = 0.357) showing that SCC may be determinant for the increment of blood Hp concentration, especially in cows with high values of SCC. This finding revealed that in controlled situations Hp may be a promissory indicator of the mammary health condition.

Fibrinogen is another acute phase protein that may be considered in inflammatory conditions. The values of fibrinogen obtained in the present work were in agree with the finding of Tabrizi et al. [32] who confirmed an increase in plasma fibrinogen in cows with clinical and subclinical mastitis compared to healthy animals, demonstrating a response of the organism to the injury on the udder. Unfortunately, similar to the finding for Hp, fibrinogen was not sensible for discriminating clinical from subclinical mastitis although the values showed progressive augmented values as SCC were higher.

CONCLUSION

The plasma concentrations of the two main acute phase proteins in cows, fibrinogen and haptoglobin, increase in cows with clinical mastitis, but their values did not distinguish between cows in subclinical condition and healthy cows. The data suggest that other factors different from the inflammatory condition of the udder contribute for stimulating the secretion of those acute phase proteins in dairy cows. However, the significant relationship between SCC and haptoglobin, may be worth of exploring more aspects about the possible role of haptoglobin in the diagnosis of subclinical conditions of
the mammary gland or even in the evaluation of the milk quality.

SOURCE AND MANUFACTURER
1 Phase Range Haptoglobin Assay, Tridelta Development Ltd, Maynooth, Ireland.

REFERENCES


