

## ACUTE PHASE PROTEINS AND MASTITIS IN COWS

BOŽIĆ TATJANA,\* DANICA OBRADOVIĆ,\*\* ZORA MIJAČEVIĆ\* and D. GVOZDIĆ\*

\*Faculty of Veterinary Medicine, University of Belgrade, Bulevar JNA 18, 11000 Belgrade, Yugoslavia

\*\*Department of Biochemistry, Clinical-hospital Centar, Belgrade.

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*Tissue damage or inflammation in mammals leads to systemic changes known as the acute phase response. Among the varied physiological alterations, which together produce this response, is a change in the circulating level of a number of liver produced proteins, the so-called "acute phase proteins". In this work the concentrations of two serum proteins, ceruloplasmin (Cp) and C-reactive protein (CRP), which are classed as acute phase reactants, were compared in a group of cows which suffered from mastitis with those in a group of cows which were clinically normal. The serum level of Cp and CRP did not differ in the two examined groups, so it appears that Cp and CRP are not acute phase proteins in cattle.*

*Key words: Acute phase proteins; C-reactive protein; ceruloplasmin; acute mastitis; cattle.*

### INTRODUCTION

Acute phase proteins are serum proteins derived from the liver, whose concentrations increase during the acute phase response to inflammation or infection. The response occurs in all animals, but it is significantly different in different species (Bingley et al., 1969; Conner et al., 1986; Gordon et al., 1985; Koj, 1974; Eckersall et al., 1988). Assay of the acute phase proteins in humans has become accepted as a useful diagnostic aid for the detection and monitoring of conditions leading to the acute phase response (Kohn et al., 1980). Some of the acute phase proteins such as ceruloplasmin (Cp) and C-reactive protein (CRP) have been extensively examined in humans and laboratory animals, but very little is known about acute phase reactants in cattle, despite their importance in the diagnosis of inflammatory diseases, particularly acute mastitis.

Ceruloplasmin is a metalloenzyme with oxidase activity, that is associated with iron and copper homeostasis (Kincaid, 1986). Each molecule of Cp contains six to eight atoms of copper, which influence its biological activity (Calabrese, 1983). A number of functions have been proposed for Cp, but none of them

is definitive (Laurell, 1985). It may function as a scavenger of oxygen derived free radicals secreted by phagocytes (Broadley et al., 1988).

C-reactive protein was discovered in 1930 by Tillet and Francis. It was the first recognized protein of the acute phase response, which increased in concentration within a few days at the beginning of inflammation or infection. CRP is a pentraxin (Pepys, 1981), formed from five identical subunits. Its major function is the degradation of the nuclear contents of damaged cells (Robey et al., 1985). The purpose of this study was to measure Cp and CRP concentrations in cattle with acute mastitis and to assess their usefulness as a diagnostic tool in differentiating infected from uninfected animals.

#### MATERIAL AND METHODS

Normal serum Cp and CRP concentrations were determined in 20 mature, lactating, nonpregnant Holstein cows on pasture in the area of Bečej. At the time of blood sampling the animals did not show any signs of acute inflammation (normal appetite, pulse rate, body temperature, rumen contractions and negative mastitis test).

Acute mastitis was diagnosed in twelve adult lactating dairy cows using a mastitis test. The somatic cell count (SCC) from at least one quarter was higher than  $1 \times 10^7$ . *S. aureus* was isolated from milk on blood agar plates and confirmed by the coagulase test.

The oxidase activity of Cp in serum was obtained using a colorimetric enzyme assay (Holmberg and Laurell, 1951). The oxidation of p-phenylenediamine dihydrochloride (PPD) by ceruloplasmin was measured in samples incubated for 30 minutes at 37°C at pH 5.45. After the incubation period enzyme activity was stopped by the addition of sodium azide solution. The extent of oxidation was determined by measuring the absorbance of the purple oxidation product of PPD at 530 nm on a spectrophotometer (Karl Zeiss-Jena). The oxidase activity determined from the absorbance was converted to the concentration of Cp and expressed in mg/dL (Sunderman and Shozo Nomoto, 1970).

The CRP concentrations were determined by liquid immunochemistry, where antibody-antigen complexes were measured nephelometrically (Turbox reagent kits - Orion Diagnostica).

Statistical analysis of data for Cp and CRP concentrations included calculation of mean value, standard error (SE), standard deviation (SD), Student's t-test and linear regression analysis.

#### RESULTS AND DISCUSSION

The average Cp (mg/dL) and CRP (mg/L) concentrations found in samples of sera from cows with clinically defined mastitis and healthy cows are given in Table 1. Cp and CRP concentrations in the healthy and infected groups of animals were not significantly different ( $p > 0.05$ ).



Table 1. Cp (mg/dL) and CRP (mg/L) concentrations in healthy and infected cows. (Mean and estimates of variation)

Statistical parameters	Healthy animals n = 20		Infected animals n = 12	
	Cp	CRP	Cp	CRP
$\bar{X}$	14.12	10.55	13.72	11.92
SD	6.51	10.16	5.64	11.02
SE	1.46	2.27	1.63	3.18

The results of linear regression analysis revealed no significant correlation ( $p > 0.05$ ) between C-reactive protein and ceruloplasmin concentrations in either healthy cows or in cows with acute mastitis. (Figures 1 and 2).

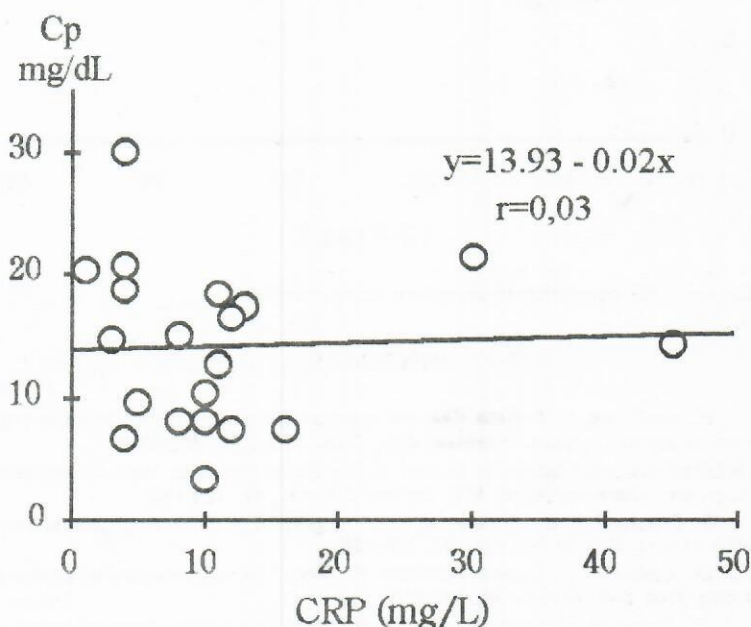


Figure 1. Cp and CRP correlation in healthy cows.

CRP is present in normal ruminant serum (Maudsley, 1985), contrary to humans, where it is the acute phase protein of choice to use as a diagnostic indicator of acute infections. In many species ceruloplasmin can be put in to the group of slow responding proteins (Conner et al, 1988), while in humans it is not a reliable acute phase reactant (Laurell, 1985.) Our results did not show any significant increase in serum Cp and CRP levels in the infected group of cows compared to the controls. Thus according to our investigation neither C-reactive protein nor Cp is a valuable aid in the prognosis and early diagnosis of mastitis in cattle.

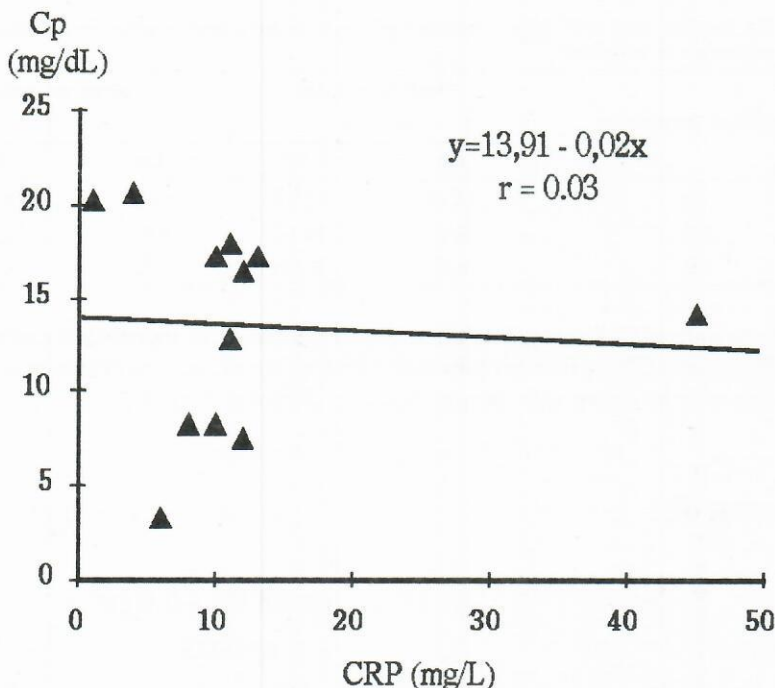


Figure 2. Cp and CRP correlation in cows with acute mastitis.

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#### PROTEINI AKUTNE FAZE I MASTITIS KRAVA

BOŽIĆ TATJANA, DANICA OBRADOVIĆ, ZORA MIJAČEVIĆ I D. GVOZDIĆ

#### SADRŽAJ

Oštećenje tkiva ili zapaljenje kod sisara dovodi do sistemskih promena poznatih kao odgovor akutne faze. Pored mnogih fizioloških promena, koje zajedno čine ovaj odgovor, dolazi i do promene nivoa u cirkulaciji većeg broja proteina poreklom iz jetre, tzv. "proteina akutne faze". U ovom radu smo poredili koncentracije dva proteina krvnog seruma, ceruloplazmina (Cp) i C-reaktivnog proteina (CRP), koji su klasifikovani kao proteini akutne faze, kod grupe klinički zdravih krava i grupe krava obolelih od akutnog mastita. Nivo Cp i CRP nije se razlikovao kod obe ispitivane grupe, te stoga smatramo da kod krava ovi proteini ne spadaju u proteine akutne faze, i ne mogu se koristiti za ranu dijagnozu akutnih zapaljenskih procesa.

