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# Oxidative Stress in Dairy Cows – Serum Paraoxonase Activity Related to Hepatomegaly*

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Association of serum paraoxonase (PON) activity and hepatomegaly in dairy cows as well as the relation between PON activity and HDL-cholesterol concentration were studied considering the role of oxidative stress in reproductive and metabolic disorders in dairy caws. A significantly lower PON activity ($P$<0.01) was found in cows with hepatomegaly compared to clinically healthy cows. Concentrations of serum glucose, triglyceride and cholesterol were also markedly reduced in cows with hepatomegaly ($P$<0.001, $P$<0.001 and $P$<0.05, respectively), whereas the serum HDL-cholesterol concentration showed no significant change. Paraoxonase/HDL-cholesterol ratio in hepatomegaly was significantly lower ($P$<0.05) as well. As part of antioxidative defence against lipid peroxidation, lower PON activity contributes to an increased risk of oxidative stress in cows with hepatomegaly.

*Keywords*
paraoxonase
HDL-cholesterol
oxidative stress
dairy cows
fatty liver

## INTRODUCTION

In recent years, the knowledge of fundamental processes involved in metabolic disorders in dairy cows has been mainly focussed on the impact of oxidative events. Increased production of reactive oxygen species can impair many vital functions, including oxidative cell injury by oxidation of macromolecule, in which lipid peroxidation is the major mechanism.[1]

Physiological conditions such as late pregnancy and early lactation pose great energy demands on the homeostasis of the body. The failure of metabolic adaptation to negative energy balance (NEB), which often occurs in transition from late pregnancy to early lactation, is the crucial moment in the development of oxidative stress.[2] In these reproduction states, the antioxidative capacity is frequently not sufficient to eliminate the increased amount of free oxygen radicals.[3] Adaptive mechanisms for

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glucose and amino-acid requirements involve mobilisation of stored energy and interconversion of metabolic fuels. Adipose and liver tissues are critical sites of these processes.[4] NEB results in the release of a large amount of non-esterified fatty acids (NEFA) from adipose tissue, resulting in increased production of ketone compounds and lipids accumulation in the liver, under the general name lipomobilisation syndrome. This causes hepatomegaly and development of fatty liver during lactation.[5] Cows with liver failure have raised hepatic lipoperoxidative processes and a low antioxidative status.[6]

Protection from oxidative stress in mammalian cells is due to a wide range of defence mechanisms, which include the activities of antioxidative enzymes.[7] Serum paraoxonase (PON, aryldialkylphosphatase, E.C. 3.1.8.1) is a mammalian high-density lipoprotein (HDL)-associated enzyme,[8] which catalyses hydrolyses of a broad spectrum of substrates, including organophosphorus compounds as well as oxidised lipids in the form of lipid hydroperoxides generated on low density lipoproteins (LDL).[9,10] Anti-oxidative/anti-inflammatory properties and activities of PON provide a relief from physiological oxidative stress as well as toxic environmental chemicals. Although improvement of the antioxidant status in dairy cows to prevent metabolic and reproductive disorders has been recognized for many years, the understanding of paraoxonase in veterinary research is still poor. In this field, we have recently demonstrated lower serum PON activity in dairy cows during dry pregnancy[11] and early lactation.[12]

Considering the role of oxidative stress in reproductive and metabolic disorders in dairy cows, the objective of this study was to investigate the association of serum paraoxonase activity and hepatomegaly in dairy cows as well as the relation between PON activity and HDL-cholesterol concentration.

## EXPERIMENTAL

### *Animals and Serum Sampling*

The study was carried out on 18 clinically healthy non-pregnant Holstein dairy cows from the region of Eastern Croatia in the middle of lactation and on 18 cows with enlarged liver (hepatomegaly) in the same reproduction period. Hepatomegaly was found by physical examination, *i.e.,* by palpation of the liver edge just behind the right costal margin. Blood samples were taken from *v. jugularis* or *v. coccygea* and after clotting for two hours at room temperature, they were centrifuged at 3000 pm for 15 min. Serum samples were stored at –20 °C for 6 weeks until analysis.

### *Reagents and Analysis Procedures*

In the collected sera, the paraoxonase activity was assayed by the slightly modified method of hydrolysis of paraoxon, previously described by Mackness *et al.*[13] and Schiavon *et al.*[14] Briefly, 10 μL serum was added to 350 μL 0.1 M Tris-HCl buffer, pH = 8.0, containing $2.0 \times 10^{-3}$ M paraoxon (*O*,*O*-diethyl-*O*-*p*-nitrophenylphosphate, Sigma Chemical Co., London, UK) as substrate, $2.0 \times 10^{-3}$ M $CaCl_2$ and $1 \times 10^{-3}$ M NaCl. Formation of *p*-nitrophenol was monitored at 405 nm and 37 °C using a Technicon RA-1000 autoanalyzer (Bayer, Milan, Italy).

PON activity was expressed as the amount of substrate hydrolized per minute and per litre of serum (μmol $min^{-1}$/L) or per HDL-cholesterol concentration in serum (μmol $min^{-1}$/mmol). The unit of PON/HDL-cholesterol ratio is μmol $min^{-1}$/mmol.

The serum glucose and triglyceride concentrations were measured using the enzymatic GOD-PAP and GPO-PAP method, respectively (Herbos Diagnostics, Sisak, Croatia). The serum total cholesterol concentration was measured by the enzymatic CHOD-PAP method, while the HDL-cholesterol concentration was determined by the same procedure after selective precipitation of lower density lipoproteins using a mixture of phosphortungstic acid and magnesium chloride (Herbos Diagnostics, Sisak, Croatia). All measurements were performed with a Technicon RA-1000 autoanalyzer (Bayer, Milan, Italy).

Quality control for biochemical parameters was based on the control of accuracy and control of imprecision using commercial control sera (Roche Diagnostics GmbH, Mannheim, Germany) and the pool serum sample. The within-run coefficients of variation (CVs) for glucose, triglyceride, cholesterol and HDL-cholesterol concentrations were 0.7 %, 1.5 %, 1.6 % and 1.0 %, respectively, while the between-run CVs were 5.0 %, 4.8 %, 5.6 % and 5.4 %, respectively.

### *Statistical Analysis*

Statistical differences between the investigated groups were assessed by either the Mann-Whitney rank sum-test or Student´s *t* test after testing the data for normality and equal variance using SigmaStat 2.0 (Systat Softwere Inc., Richmond, California, USA). Values $P<0.05$ were considered statistically significant.

## RESULTS AND DISCUSSION

PON activity and PON/HDL-cholesterol ratio as well as glucose, triglyceride, cholesterol and HDL-cholesterol concentrations are presented in Table I. A significantly lower PON activity ($P<0.01$) was found in cows with hepatomegaly compared to clinically healthy cows (Figure 1). The serum glucose, triglyceride and cholesterol concentrations were also markedly reduced in cows with hepatomegaly ($P<0.001$, $P<0.001$ and $P<0.05$, respectively), while the serum HDL-cholesterol concentration showed no significant change. PON/HDL-cholesterol ratio in cows with hepatomegaly was significantly lower ($P<0.05$) as well (Figure 2).

Taking into account the values of metabolic parameters, we observed a characteristic pattern of metabolic

TABLE I. Serum PON activity, PON/HDL-cholesterol ratio and glucose, triglyceride, cholesterol and HDL-cholesterol concentrations in clinically healthy cows (control group)[a] and cows with hepatomegaly[b]

| Parameter | Mean ± SD | Median (Range) | *P* |
|---|---|---|---|
| PON activity / (μmol min$^{-1}$/L) | | | |
| Control group | 758 ± 266 | 752 (400–1255) | <0.01 |
| Cows with hepatomegaly | 576 ± 161 | 565 (315–895) | |
| PON/HDL-cholesterol ratio / (μmol min$^{-1}$/mmol) | | | |
| Control group | 732 ± 234 | 666 (297–1568) | <0.05 |
| Cows with hepatomegaly | 504 ± 383 | 403 (262–1006) | |
| Glucose / (mmol/L) | | | |
| Control group | 4.87 ± 1.42 | 4.88 (3.12–9.40) | <0.001 |
| Cows with hepatomegaly | 2.94 ± 1.28 | 3.19 (0.37–5.82) | |
| Triglyceride / (mmol/L) | | | |
| Control group | 0.23 ± 0.10 | 0.23 (0.05–0.47) | <0.001 |
| Cows with hepatomegaly | 0.09 ± 0.06 | 0.07 (0.05–0.31) | |
| Cholesterol / (mmol/L) | | | |
| Control group | 4.94 ± 1.63 | 4.75 (2.7–8.0) | <0.05 |
| Cows with hepatomegaly | 3.83 ± 1.37 | 3.60 (1.4–5.8) | |
| HDL-cholesterol / (mmol/L) | | | |
| Control group | 1.13 ± 0.27 | 1.20 (0.8–1.6) | >0.05 |
| Cows with hepatomegaly | 1.27 ± 0.40 | 1.30 (0.6–2.0) | |

[a]$n$ = 18, [b]$n$ = 18.

changes associated with liver failure. Liver is the key regulator of blood glucose concentration and the only site of gluconeogenesis.[5] The observed lower serum glucose concentration could be caused by lower early lactation energy balance, which is related to subsequent health problems, leading to an intense mobilisation of non-esterified fatty acids (NEFA) from adipose tissue and to synthesis of large amounts of triglycerides in the liver.[15,16,17] Thus, the output of lipoproteins from the liver is markedly reduced, causing lower serum triglycerides and cholesterol concentrations.[18] Lipids accumulation in the liver results in liver failure and fatty liver development.[5] NEFA in the liver are susceptible to peroxidative processes causing a rise in the formation of lipoperoxide products.[19] The obtained lower serum PON activity in cows with hepatomegaly could be considered as a result of the metabolic imbalance taking place mainly in the liver. Raised oxidative processes in the liver as a consequence of lipomobilisation syndrome might diminish the serum PON activity, indicating the involvement of PON in hepatocellular oxidative stress. Furthermore, a significantly lower PON activity without decreasing the HDL-cholesterol concentration (which is presented as PON/HDL-cholesterol ratio) indicates that the PON activity can decrease independently of the HDL-cholesterol concentration. This indicates that metabolic adaptations and activities might also influence enzyme synthesis in the liver. As part of the antioxidative defence against lipid peroxidation, lower PON activity contributes to an increased risk of oxidative stress in cows with hepatomegaly.

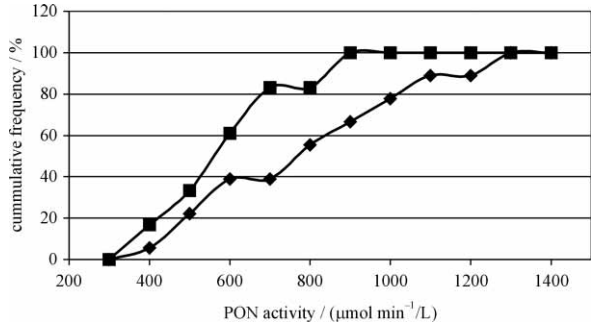


Figure 1. Cumulative frequency distribution profile of PON activity in control group (–◆–) and cows with hepatomegaly (–■–).

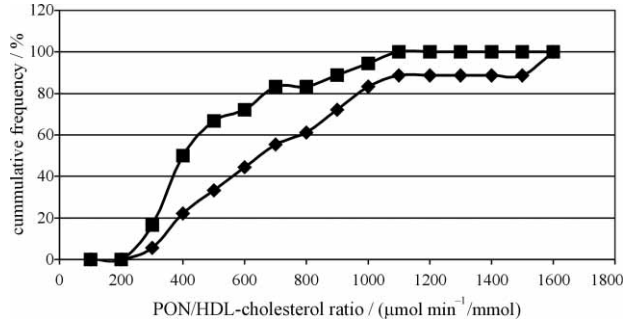


Figure 2. Cumulative frequency distribution profile of PON/HDL-cholesterol ratio in control cows (–◆–) and cows with hepatomegaly (–■–).

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## SAŽETAK

### Oksidacijski stres u mliječnih krava – povezanost aktivnosti paraoksonaze u serumu i povećanja jetre

**Romana Turk, Dubravka Juretić, Darko Gereš, Nenad Turk, Vera Simeon-Rudolf, Branka Rekić i Ante Svetina**

Uzimajući u obzir ulogu oksidacijskoga stresa u reprodukcijskim i metaboličkim poremećajima, istražena je aktivnost paraoksonaze (PON) u serumu mliječnih krava s povećanom jetrom te odnos aktivnosti PON i koncentracije HDL-kolesterola. Utvrđena je značajno manja aktivnost PON u skupini krava s povećanom jetrom u usporedbi s kontrolnom skupinom. Koncentracije glukoze ($P$<0,001), triglicerida ($P$<0,001) i kolesterola ($P$<0,05) bile su također značajno manje u skupini krava s povećanom jetrom nego u skupini zdravih krava. Koncentracija HDL-kolesterola nije se statistički razlikovala između skupina, dok je omjer PON/HDL-kolesterol bio značajno manji ($P$<0.05) u krava s povećanom jetrom. Smatrajući PON kao dio antioksidacijskoga sustava u sprečavanju peroksidacije lipida, njena niža aktivnost može doprinijeti povećanom riziku od oksidacijskoga stresa u mliječnih krava s povećanom jetrom.