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Original article

Serum paraoxonase-1 activity of dairy Holstein-Friesian cows in different lactation stages – preliminary study

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Abstract

The objective of this study was to investigate paraoxonase-1 (PON-1) activity in different lactation stages. The study was conducted on Holstein – Friesian dairy cows in 2nd and 3rd lactation. A significant decrease in paraoxonase activity was found in the postpartum period and during peak of lactation. Serum triglyceride and cholesterol concentration were also markedly reduced during postpartum period. The concentrations of uric acid in serum was 23% higher during lactation peak in comparison with dry and postpartum period. The results indicate that lower serum paraoxonase activity and higher concentration of uric acid are associated with oxidative character of transition period and lipid functional antioxidative protection during intensive milk production.

Key words: paraoxonase-1, lactation stages, HF cows

Introduction

Paraoxonase-1 (PON-1) or arylesterase (EC 3.1.8.1.) is the calcium-dependent esterase that catalyzes the hydrolysis of organophosphates and several aromatic carboxylic acid esters. PON1 is synthesized in the liver and most of it is released into the bloodstream, where the enzyme binds with high density lipoproteins (HDL). In human serum PON1 is closely associated with apolipoprotein A-I of HDL (La Du and Novais 1989). PON1 activity can be measured in either serum or heparinized plasma using two

synthetic substrates: paraoxon and phenyl acetate (Mackness 1998).

Paraoxonase is considered as a negative acute phase protein (-APP) which serum level and hepatic synthesis are reduced during infection (James and Deakin 2004). Administration of lipopolysaccharide (LPS) strongly decrease PON1 concentration (Feingold et al. 1998).

PON1 plays an important role in lipid metabolism and protection against oxidative stress. In particular, HDL-bound PON1 hydrolyzes lipid peroxidation products and protects other lipoprotein fractions as

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well as plasma membranes from oxidative modification. PON1 is to large extent responsible for the antioxidant, anti-inflammatory and antiatherosclerotic properties of HDL. Many studies indicate decreased PON1 activity in atherosclerosis, nephropathy, familial hypercholesterolemia and diabetes mellitus; all of them being major risk factors of atherosclerosis (Mackness et al. 1991). In addition, decrease in PON1 level is observed in both acute and chronic liver injury and the enzyme is suggested to be the additional marker of liver dysfunction in humans (Marsillach et al. 2009, 2010).

Most studies about PON1 focused on the human or rodent enzyme. However, PON1 was also identified in the bovine serum (Miyamoto et al. 2005). Processes such as pregnancy and intensive mid lactation have a great influence on lipid metabolism and liver function in dairy cows. These ruminants are highly vulnerable to oxidative stress commonly occurring around parturition period. Thus, changes in serum PON1 activity (as an enzyme involved in oxidative protection) could be a useful diagnostic marker of diseases and plasma oxidative-redox state in dairy cows. Recent studies have shown reduction of PON1 activity around transition period due to influence of inflammatory conditions in the reproduction (Turk et al. 2008).

The aim of this study was to measure PON-1 activity towards two egzogenic substrates in healthy dairy HF cows during dry period and two periods essential to production: during first week after partum and in peak of lactation. Also correlations of PON-1 activity with dynamics of changes in total cholesterol, triglycerides, beta-hydroxybutyrate, uric acid concentrations were examined.

Materials and Methods

Animals

Samples were taken from 10 clinical healthy Holstein – Friesian dairy cows in 2nd or 3rd lactation during 89-117 day period. During the whole lactation period nourishment, welfare and animal health were monitored. Daily average milk yield in peak lactation was from 38.2 kg to 53.6 kg. All animals during dry period, early postpartum and peak of lactation period were tested thrice using Edmonson's Body Condition Score (Edmonson et al. 1989).

Blood sampling

The blood samples were taken from *v. jugularis externa* in volume of 2 ml for EDTA-K2 and 5 ml for

clotting. Samples were taken in dry period (58-43 day before partum), 4-8 days postpartum and during lactation (46-59 days postpartum). Blood samples with anticoagulant were used for hematological analysis. Rest of the samples were allowed to clot at room temperature and centrifuged at 4000 rpm for 15 min. Serum samples were frozen and stored at -80°C for less than 9 months which enabled to receive reliable data (Zawiślak 2009). After that time paraoxonase and other biochemical activities were measured.

Hematological and biochemical blood analysis

Red, white blood cells parameters and platelets count were measured using Abacus Junior Vet hematological analyser. Plasma triglycerides (TG), total cholesterol, uric acid and beta-hydroxybutyrate (BHB) were assayed using Pointe Scientific kit with Point Miura biochemical analyser.

Enzyme activity

Paraoxonase activity was assayed using a slightly modified method described by Mackness et al. (1991). Serum (20 µl) was added to 800 µl of 100 mM Tris-HCl buffer pH 8.0 containing: 2 mM paraoxon (O,O-diethyl-O-p-nitrophenylphosphate, Sigma Chemical Co.) and 2 mM CaCl₂. The generation of p-nitrophenol was monitored at 412 nm at 25°C. The molar extinction coefficient used to calculate the rate of hydrolysis was 18.290 M⁻¹ cm⁻¹. One unit of paraoxonase activity produced 1 nmol of p-nitrophenol per min. The blank sample (incubation mixture without serum) was run simultaneously to correct for spontaneous substrate breakdown. Activity toward phenyl acetate was measured in the reaction mixture (3 ml) containing 1 mM substrate and 2 mM CaCl₂ in 100 mM Tris-HCl buffer pH 8.0. After adding 10 µl of serum to the mixture increase in absorbance at 270 nm was monitored for 1 min at 37°C. The results are expressed in U/ml; 1 U hydrolyzes 1 µmol of phenyl acetate/min. The molar extinction coefficient used to calculate the rate of hydrolysis was 1310 M⁻¹ cm⁻¹.

Statistic analysis

Data were analyzed by Student's test and the values $p \leq 0.05$ was considered significant.

Table 1. Serum PON-1 activity toward paraoxon and phenyl acetate in different lactation stages of HF cows. Values are mean $\chi \pm$ SD, U/ml.

Substrate	PON-1 activity in different lactation stages		
	Dry period	Postpartum	Peak of lactation
Paraoxon	97.17 \pm 22.5	83.17 \pm 30.7	90.93 \pm 29.4
Phenyl acetate	207.56 \pm 48.7 ^a	168.26 \pm 23.8 ^{a,b}	184.00 \pm 50.0 ^{a,b}

^a – values of PON-1 activity towards phenyl acetate, are significantly different ($p \leq 0.05$) in comparison to paraoxon.

^b – values of PON-1 activity towards phenyl acetate are significantly different at $p \leq 0.05$, in comparison to cows during dry period.

Table 2. Serum concentration of biochemical parameters in different lactation stages of HF cows. Values are mean $\chi \pm$ SD, mmol/l and μ mol/l.

Parameter	Dry period	Postpartum	Peak of lactation
Total cholesterol (mmol/l)	4.03 \pm 0.97	2.36 \pm 0.33 ^a	6.03 \pm 1.03 ^a
Triglycerides (mmol/l)	0.25 \pm 0.11	0.08 \pm 0.02 ^a	0.12 \pm 0.05 ^a
Uric acid (μ mol/l)	55.93 \pm 17.85	55.34 \pm 11.86	71.99 \pm 23.8
Beta-hydroxybutyrate (mmol/l)	0.53 \pm 0.1	1.06 \pm 0.3 ^a	0.67 \pm 0.1

^a – values are significantly different at $p \leq 0.05$ towards values of parameters measured during dry period.

Results

Hematological parameters

All morphological blood parameter's values were within the reference range in postpartum, dry period as well as during lactation. Body Condition Score (BCS) during dry period and after calving was 3.25 ± 0.05 . During lactation peak mean score was 3.0 ± 0.1 .

The bovine serum PON1 activity

The results of our studies showed no significant differences in PON1 activity towards paraoxon between postpartum, dry period and lactation peak, that is between 46th and 59th day after partum ($p > 0.05$). Results are shown in Table 1.

PON1 activity towards phenyl acetate significantly differs between stages of milk production. The highest activity occurred during dry period, on the other hand, it decreased over 19% one week after partum and 11.4% during peak of lactation in comparison with dry period ($p \leq 0.05$) (Table 1).

The serum total cholesterol concentrations

There were significant differences in different lactation stages ($p \leq 0.05$). The highest concentration occurred in lactation peak and was 2.6 times higher than during the first week after partum and 1.5 times higher than during dry period (Table 2).

Triglycerides concentrations

Triglycerides concentrations were significantly different in postpartum and lactation in comparison with dry period ($p \leq 0.05$). The highest concentration occurred in serum of dry cows, and was almost 3 times lower in postpartum (Table 2). In lactation peak increased concentration of triglycerides occurred. Nevertheless, there were no significant differences in mean value of this parameter in comparison to values after first week of partum.

Beta-hydroxybutyrate concentrations

The highest concentration of beta-hydroxybutyrate in serum was one week after partum.

It was significantly higher in comparison with peak of lactation and dry period concentrations at $p \leq 0.05$.

Uric acid concentrations. The concentration of uric acid in serum was 23% higher during lactation peak in comparison with dry and postpartum period (Table 2).

Discussion

Our data suggest that there are differences in PON-1 serum activity of Holstein-Friesian dairy cows depending on substrate and stage of lactation (Table 1). PON-1 paraoxonase activity towards egzogenic substrate, paraoxon, was lower than its arylesterase activity towards another egzogenic substrate – fenyl acetate. Measured human PON-1 activities using these two substrates were inverted. As Zawislak (2009) has shown PON-1 mean paraoxonase activity in human serum was 117.16 ± 101.88 U/ml, and PON-1 arylesterase activity was 52.48 ± 26.25 U/ml. Furthermore, high standard deviation of enzyme activity indicates variation within the human population. Similar results were obtained by Costa et al. 2005. Serum PON-1 activities standard deviations in examined cows were lower which may lead to conclusion that cattle population is more homogenic. In our preliminary studies we didn't use NaCl which stimulates paraoxonase activity of B phenotype in human serum, thus concentrating on distinguishing changes in activity during different lactation stages. For observing potential polimorfism bigger group of animals should be examined, because it's possible that all of our cows might have the same genotype.

PON-1 arylesterase activity in Holstein-Friesian dairy cows was two times higher in comparison to its activity towards paraoxon substrate and significantly changed in different lactation stages. The lowest values were measured postpartum and during peak of lactation, which may suggest decreased detoxication abilities of examined animals during high milk production period. Variability of PON-1 activity towards fenyl acetate was present in cows with good Body Condition Score 3.25 during dry period and 3.0 during postpartum, simultaneously taking under consideration very intensive milk production. Monitored BHB values (Table 2) confirm no negative energy balance in animals with such BCS.

Results of Bernabucci et al. (2005) studies seemed to confirm that our animals, with such values of BCS, have optimal proantioxidative state. As well as increased plasma concentration of uric acid. Its dissociated form, monoanion urate plays an important role in antioxidative protection especially

during periparturient time and lactation when dynamic metabolic changes in cow organisms take place. Enhanced oxygen metabolic reactions generate large amount of free oxygen radicals, which may lead to tissue and cell damage by peroxidation of proteins, nucleic acids and cell membranes (Castillo et al. 2006).

Lowered concentration of triglycerides and cholesterol in blood may be caused by several factors. After calving, fatty acids are released, mobilized in large amounts from adipose tissue to plasma. Nonesterified fatty acids (NEFA) can be taken up by liver in proportion to concentration in blood. The dairy cattle rate of triglycerides hepatic synthesis can be compared with nonruminants, unlike cow's very low density lipoprotein (VLDL) secretion rate. Measured rate of VLDL liver secretion was 5 times lower in calves than in humans (Bauchart 1993). Similar situation occurs during fasting of Holstein – Friesian cows, where intensive NEFA mobilization from adipose tissue to the liver via the portal vein takes place. On the fourth day of fasting the VLDL-TG concentration in hepatic blood to the NEFA concentration in portal blood ratio decreased in comparison with the first day baseline. This decrease was observed in all cows (Oikawa et al. 2010) validating the thesis of slow hepatic VLDL secretion rate in ruminants and the same rate of TG synthesis in the liver compared with nonruminants (Pullen et al. 1990). Lower concentration of triglyceride and cholesterol postpartum was also observed by Turk et al. (2004) and cholesterol levels reaching the minimum value after 3rd day of calving by Bernabucci et al. (2004). Very low density lipoproteins VLDL are synthesized in liver, secreted into blood stream, take part in hepatocyte triacylglycerol export. In cattle triacylglycerols are the main component of VLDL protein, being over 60% of its weight (Stead and Welch 1975). Apolipoprotein B₁₀₀ is part of VLDL particle (also can be C-I, C-II, C-III, apo E, less A-series) (Bauchart 1993). Rates of apoB production, synthesis and secretion from the membrane of endoplasmic reticulum, control hepatic secretion of VLDL (Emery et al. 1992). Lower mRNA levels after calving could influence lowering VLDL concentrations (Bernabucci et al. 2004) and this way decrease TG and cholesterol concentrations. The decrease of cholesterol concentration may also be due to accumulation in hepatocytes and acceleration of metabolism at the beginning of milk production (Markiewicz et al. 2001).

Our experiments showed that measuring serum PON-1 activity of dairy cattle during different lactation stages could be a useful diagnostic tool, helping to obtain better health assessment during high milk production.

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