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Relationship between paraoxonase-1 activity and lipid mobilisation in transition dairy cows

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ABSTRACT

The objectives of this study were to investigate the influence of the transition period on lipid mobilisation and paraoxonase-1 (PON1) activity, as well as the relationship between the indicators of lipid metabolism and PON1 activity. Twenty-four Holstein-Friesian dairy cows (aged 2-7 years) were included in the study. Parameters of lipid metabolism (triglycerides, total cholesterol, HDL-C, NEFA and BHB) and PON1 activity were monitored on days -30, -10, -2, 0, 5, 12, 19, 26 and 60 relative to parturition. Triglyceride concentration was significantly decreased from parturition until day 60 after calving, as compared to the values obtained before calving ($P<0.05$). Both total cholesterol and HDL-C concentrations significantly decreased at calving, with an increase during lactation ($P<0.05$). Serum NEFA concentrations significantly increased at calving ($P<0.05$) and stayed at the highest values up to day 19 after calving. Serum BHB concentrations increased significantly after calving on days 12 and 19 ($P<0.05$), which may be a consequence of increased NEFA around parturition. These changes indicate fat mobilisation from adipose tissue due to the energy deficit during the transition period. Serum PON1 activity decreased at calving but increased significantly on days 26 and 60 postpartum, suggesting a reduced antioxidant status in the postpartum period. Additionally, PON1 significantly positively correlated with total cholesterol ($r = 0.42$) and HDL-C ($r = 0.49$) and inversely correlated with NEFA ($r = -0.33$). The

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results suggested that PON1 activity is related to lipid metabolism and lipomobilisation syndrome and could be considered as a putative marker for metabolic and inflammatory-related disorders in transition dairy cows.

Key words: non-esterified fatty acids, β-hydroxybutirate, negative energy balance, oxidative stress, inflammation

Introduction

The transition from late pregnancy to early lactation in dairy cows is a critical period associated with physiological and metabolic adaptations to support foetal growth and milk production (GOFF and HORST, 1997). The energy balance is the key factor for maintaining the homeostasis of the body. Cows are dependent on gluconeogenesis as the major source of glucose. However, hepatic gluconeogenesis is insufficient to meet their energy requirements in this period, which results in a negative energy balance (NEB) with a high risk of diseases and decreased fertility (GOFF and HORST, 1997; DOEPEL et al., 2009). During early lactation, cows mobilize body fat reserves from adipose tissue, resulting in increased concentrations of non-esterified fatty acids (NEFA) in the blood. The majority of NEFA are metabolized in the liver into acetyl-coA and consequently into ketone bodies, such as β-hydroxybutirate (BHB) (DRACKLEY et al., 2001). Both NEFA and BHB are considered as good markers of excessive NEB and lipomobilisation syndrome (CHAPINAL et al., 2011).

The intensified process of NEFA oxidation in the liver results in increased production of reactive oxygen species (ROS) and oxidative stress during the transition period (MUDRON et al., 1999; TURK et al., 2013 and 2015). Paraoxonase-1 (PON1) is a HDL-associated antioxidant enzyme which protects lipoproteins against oxidative stress by hydrolysing lipid hydroperoxides and oxidatively fragmented phospholipids produced during oxidation by ROS (MacKNESS and DURRINGTON, 1995; TURK et al., 2008). In our previous studies, we found that PON1 activity is reduced during the transition period in dairy cows (TURK et al., 2004, 2005a, 2008, 2013 and 2015), as well as in cows with subclinical and clinical mastitis (TURK et al., 2012).

The aims of this study were to investigate the influence of the transition period on lipid mobilisation and PON1 activity, as well as the relationship between the indicators of lipid metabolism and PON1 activity.

Materials and methods

Animals and blood sampling. Twenty-four Holstein-Friesian dairy cows (aged 2-7 years), located on a commercial farm in North-western Croatia, were included in the study. The cows were fed a ration composed of haylage, corn silage, hay, and a complete feed mixture particularly for dry cows containing 16% of crude protein and for lactating cows with 19% of crude protein, respectively. All the cows were clinically healthy, with

an optimal body condition score between 3.25 and 3.75. The average milk yield per cow per lactation was 7350 kg.

Blood samples were taken before the morning feeding from the *v. coccygea* into Vacutainer tubes without anticoagulant, on days 30, 10 and 2 before parturition, on the day of calving and on days 5, 12, 19, 26 and 60 after parturition. After clotting at room temperature for 1 hour, blood samples were centrifuged at 1500 g for 15 minutes. The sera were separated and stored at -70 °C until used for analyses.

The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine University of Zagreb, and the Ministry of Agriculture of the Republic of Croatia.

Analytical procedures. Serum triglycerides (TG), total cholesterol and HDL-cholesterol (HDL-C) concentrations were assayed using standard commercial kits (Beckman Coulter Biomedical Limited, Lismeehan, O' Callaghan's Mills, Co. Clare, Ireland) on an automatic Beckman Coulter AU 680 analyser (Beckman Coulter Biomedical Ltd., Ireland).

Serum NEFA and BHB concentrations were measured by the automated clinical chemistry analyser, SABA 18 (AMS, Rome, Italy) using commercially available reagent kits (Randox Laboratories Ltd, Crumlin, UK).

Serum PON1 activity was measured by the hydrolysis of paraoxon method (MacKNESS et al., 1991; SCHIAVON et al. 1996). Serum was added in the reaction mixture of 0.1 M Tris-HCl buffer, pH 8.0 containing 2.0 mM paraoxon (O,O-diethyl-O-p-nitrophenylphosphate, Sigma Chemical Co, London, UK), 2.0 mM $CaCl_2$ and 1 mM NaCl at 37°C. P-nitrophenol generation was monitored at 405 nm on a Beckman Coulter AU 680. The enzyme activity was expressed in international units (U/L) as the amount of substrate hydrolysed per minute and per litre of serum (μmol/min/L).

Statistical analyses. Statistical analyses of data were performed using SAS 9.3. Software (SAS Institute Inc., Cary, NC, USA). The mixed model (PROC MIXED), with the repeated measure statement, was used to analyse the measured parameters. The cow was the subject variable on which repeated measurements were taken. The multiple comparison test of least-squares means was performed using the Tukey-Kramer correction The results are shown in graphs as the least squared means with a 95% confidence interval. Some variables were transformed before analysis to obtain normality and variance homogeneity, mostly by logarithm transformation, and after analysis the data were back transformed.

Pearson's correlation coefficient was calculated for the parameters measured (TG, cholesterol, HDL-C, NEFA, BHB and PON1) using the CORR module (PROC CORR). The correlation coefficient was interpreted according to PETZ (2004) with approximation of the level of the correlation - correlation coefficient: from ±0.00 to ±0.20 no or negligible

association, from ± 0.20 to ± 0.40 easy connectivity, from ± 0.40 to ± 0.70 significant correlation, and from ± 0.70 to ± 1.00 high or very high correlation. The values where P<0.05 were considered significant.

Results

Lipid metabolism. Triglyceride concentrations decreased significantly (P<0.05) from parturition (0.14 mmol/L) until day 60 (0.12 mmol/L) after calving, as compared to the values obtained before calving (Fig. 1A).

Total cholesterol concentrations significantly decreased (P<0.05) at calving (1.8 mmol/L) as compared to the values on day 30 before parturition (2.6 mmol/L). After parturition, their concentration gradually increased from day 12 (2.5 mmol/L) until day 60 (3.9 mmol/L) after parturition, compared with the day of calving (P<0.05, Fig. 1B).

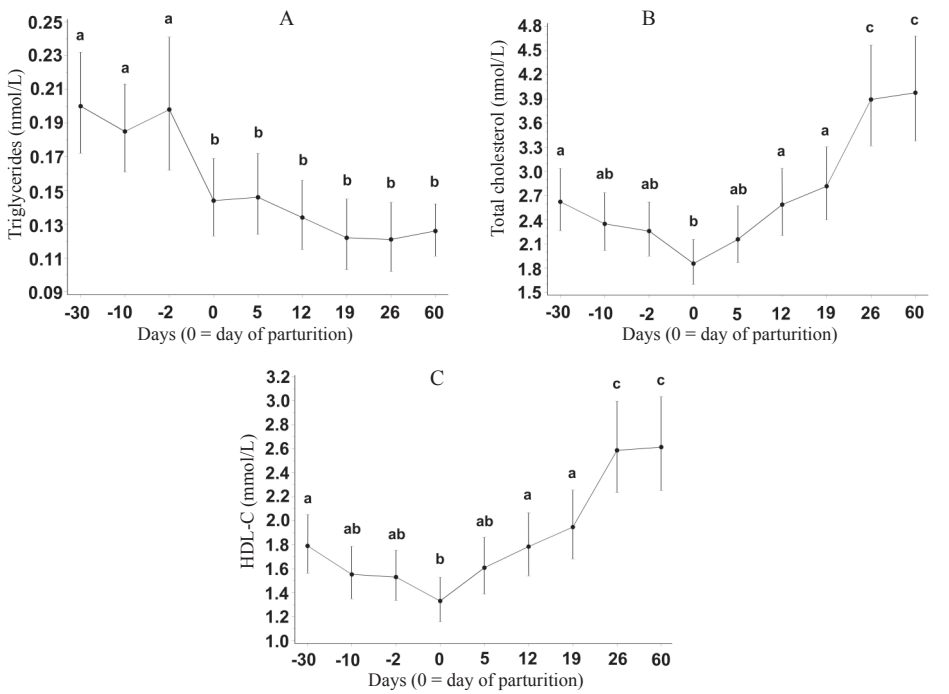

Fig. 1. Triglycerides (A), total cholesterol (B) and HDL-C (C) concentrations (lsmeans ± 95% CI) in the serum of cows from day 30 before parturition until day 60 after parturition. Values with different superscripts are statistically different (P<0.05).

Serum HDL-C concentrations had a similar pattern to total cholesterol concentrations, with the lowest value at calving (1.3 mmol/L). They increased after calving, having significantly higher values on days 12 (1.7 mmol/L) and 19 (1.9 mmol/L) after parturition compared to the value at calving (P<0.05); and on days 26 and 60 postpartum (2.5 and 2.6 mmol/L, respectively) as compared to the values on day -30 relative to calving (1.7 mmol/L, P<0.05, Fig. 1C).

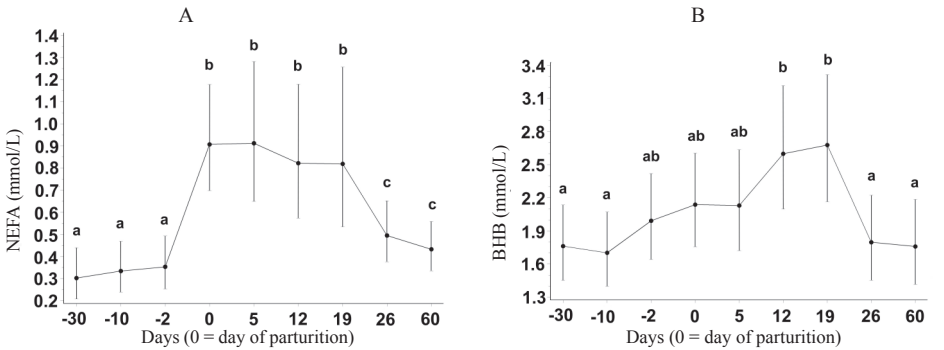

Fig. 2. NEFA (A) and BHB (B) concentrations (lsmeans ± 95% CI) in the serum of cows from day 30 before parturition until day 60 after parturition. Values with different superscripts are statistically different (P<0.05).

Serum NEFA concentrations significantly increased (P<0.05) at calving (0.90 mmol/L) as compared to the values before parturition, from days -30 to -2 relative to calving (0.30 to 0.35 mmol/L), and remained at higher levels until day 19 postpartum (0.8 mmol/L). On days 26 and 60 postpartum, NEFA concentrations declined significantly (0.49 and 0.43 mmol/L, respectively) to the values recorded before parturition (Fig. 2A).

Serum BHB concentrations increased significantly (P<0.05) on days 12 and 19 after calving (2.60 and 2.68 mmol/L, respectively) compared to the values on days -30 to -10 and 26 to 60 relative to calving (Fig. 2B).

Paraoxonase-1 activity. Serum PON1 activity decreased at calving (227 U/L) and gradually increased after parturition until days 26 and 60 postpartum (298 and 290 U/L, respectively) when its activity was significantly higher (P<0.05) compared to the values at calving (Fig. 3).

Correlations between the parameters of lipid metabolism and PON1 activity. The correlations between the parameters of lipid metabolism and PON1 activity are shown in Table 1. Significant positive correlations between total cholesterol and HDL-C concentrations (r = 0.95; P<0.0001) and an inverse correlation between TG and HDL-C (r = -0.24; 0.01) were found. Both cholesterol and HDL-C inversely correlated with NEFA

concentration (r = -0.27; P<0.01 and r = -0.24; P<0.001, respectively). Additionally, a significant positive correlation between NEFA and BHB concentrations was observed (r = 0.38; P<0.0001). Serum PON1 activity significantly positively correlated with total cholesterol and HDL-C (r = 0.42; P<0.001 and r = 0.49; P<0.0001, respectively) and inversely correlated with NEFA concentration (r = -0.33; P<0.0001).

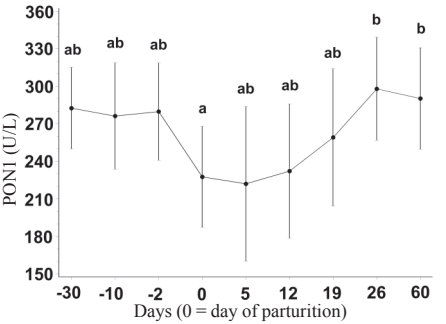

Fig. 3. PON1 activity (lsmeans ± 95% CI) in the serum of cows from day 30 before parturition until day 60 after parturition. Values with different superscripts are statistically different (P<0.05).

Table 1. Correlations (Pearson's correlation coefficient and P value) between parameters of lipid metabolism and PON1 activity.

	PON1	NEFA	BHB	HDL-C	TG	Cholesterol
PON1		-0.33 <0.0001	n.s.	0.49 <0.0001	-0.16 <0.05	0.42 <0.001
NEFA	-0.33 <0.0001		0.38 <0.0001	-0.24 <0.001	-0.16 <0.05	-0.27 <0.01
BHB	n.s.	0.38 <0.0001		n.s.	n.s.	n.s.
HDL-C	0.49 <0.0001	-0.24 <0.001	n.s.		-0.24 <0.01	0.95 <0.0001
TG	-0.16 <0.05	-0.16 <0.05	n.s.	-0.24 <0.01		-0.14 <0.05
Cholesterol	0.42 <0.001	-0.27 <0.01	n.s.	0.95 <0.0001	-0.14 <0.05	

n.s. - non-significant

Discussion

The present study demonstrated the changes in lipid metabolism and PON1 activity during the transition period in dairy cows. Moreover, PON1 activity significantly correlated with lipid parameters and indicators of lipomobilisation syndrome.

In the present study, NEFA concentrations began to be elevated at calving, reaching the value of 0.9 mmol/L, and they remained at the highest values up to day 19 after calving. It has been suggested in previous studies that NEFA concentrations above 0.6 or 0.7 mmol/L after calving are associated with a higher risk of post-calving metabolic and infectious disease, decreased milk production or decreased reproductive performance (VAN SAUN, 2004; OETZEL, 2004; OSPINA et al., 2010a,b and 2013). Other studies have also observed an increased NEFA concentration around parturition, indicating adipose tissue breakdown (BUTLER, 2000; CASTILLO et al., 2006; BIONAZ et al., 2007; TURK et al. 2013). A large part of NEFA is metabolized by β-oxidation in the liver to acetyl-coenzyme A (acetyl-CoA), to produce energy. The excessive amount of acetyl-CoA is shunted to *de novo* cholesterol synthesis or via ketogenesis to acetoacetate, and subsequently to acetone and BHB (LASSEN and FETTMAN, 2004). In the present study, BHB concentrations increased significantly on days 12 and 19 after calving, which was a consequence of increased NEFA around parturition, as observed in our previous study and also by others (VANHOLDER et al., 2005; BIONAZ et al., 2007; TURK et al., 2013). In addition, a significant correlation was found between BHB and NEFA, indicating that a considerable portion of NEFA was metabolized to BHB. However, OSPINA et al. (2010a) found only a weak correlation between NEFA and BHB, probably because a smaller amount of NEFA was metabolized to BHB.

Triglyceride concentrations significantly declined at calving and remained at lower values until day 60 of lactation. Similar results were obtained in our previous studies (TURK et al., 2004, 2005a and 2013) and by MANTOVANI et al. (2010), as a consequence of either the mammary gland taking up the triglycerides for milk fat synthesis (BERNARD et al., 2008) or triglyceride accumulation in the liver (RUKKWAMSUK et al., 1998; TURK et al., 2005b). Both total cholesterol and HDL-C significantly decreased at calving, with an increase during lactation. These lipid changes are typical during the transition period, as was previously reported (GRUMMER, 1993; TURK et al., 2008).

Serum PON1 activity decreased at calving and increased significantly on days 26 and 60 postpartum, suggesting a lower antioxidant status in the postpartal period. Oxidative stress is considered to be a notable component in the signalling processes involved in inflammatory responses, including stimulation of cell adhesion molecules and production of chemo-attractant. Reactive oxygen species and oxidatively fragmented lipids, which are generated during oxidative stress, are pro-inflammatory compounds and provoke an acute phase response, APR (STEINBERG, 1997), suggesting a strong link between

oxidative stress and inflammation. Furthermore, during inflammatory conditions, phagocytes produce ROS that are needed for killing pathogens (THANNICKAL and FANBURG, 2000). Thus, an increased amount of ROS may overcome the antioxidant system and compromise the immune function of cows (SORDILLO and AITKEN, 2009). High-density lipoprotein plays an important role as an anti-oxidative/anti-inflammatory particle, providing non-specific defence of a host. This action of HDL is mediated by several structural proteins and enzymes carried on the particle, including PON1 and other proteins, such as platelet-activating factor acetylhydrolase (PAF-AH), apolipoprotein AI (Apo AI) and haptoglobin (LINK et al., 2007; TURK, 2009). The APR leads to the depletion of anti-inflammatory/anti-oxidative proteins from HDL, such as PON1 and Apo AI, suggesting the remodelling of HDL particles during inflammatory and oxidative stress conditions (CABANA et al., 1996; FEINGOLD and GRUNDFELD, 2010). In addition, FEINGOLD et al. (1998) found decreased PON1 mRNA expression in the liver and reduced serum PON1 activity in rodents challenged by cytokines which mediate the APR, which might indicate that PON1 responds to inflammatory conditions as a negative acute phase protein (APP). Thus, lower PON1 and increased oxidative stress might be an important mechanism by which NEB mediates inflammatory responses, and increases the incidence of reproduction and production diseases in the onset of lactation. Several studies in animals, including cows, pigs, dogs and cats, have found decreased PON1 activity related to inflammatory conditions (TURK et al., 2012; TVARIJONAVICIUTE et al., 2014; ESCRIBANO et al., 2015; TECLES et al., 2015) proposing PON1 as a negative APP. In horses with subclinical leptospirosis, PON1 activity was not changed according to the positive antibody titre on *Leptospira* spp. (TURK et al., 2011). Previous studies in dairy cows have observed decreased PON1 activity during the transition period, suggesting inflammatory conditions around parturition (BIONAZ et al., 2007; BOSSAERT et al., 2012). Furthermore, it has been found that *in vitro* exposure to NEFA mixture increased the expression of proinflammatory cytokines and ROS concentration in bovine endothelial cells, indicating alterations in inflammatory responses during the transition period in response to lipid mobilisation (CONTRERAS et al., 2012). Additionally, in the present study, PON1 significantly positively correlated with total cholesterol and HDL-C, and inversely correlated with NEFA concentrations, indicating the relationship of PON1 with lipid metabolism and lipomobilisation syndrome, as well as the involvement of PON1 in immune-related disorders in dairy cows during the periparturient period. Considering all the above, these results indicate that PON1 should be considered as a putative biomarker for metabolic and inflammatory-related disorders in transition dairy cows.

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SAŽETAK

Ciljevi su ovog rada bili istražiti utjecaj prijelaznog razdoblja na mobilizaciju lipida i aktivnost paraoksonaze-1 (PON1) te odnos između pokazatelja metabolizma lipida i aktivnosti PON1. U istraživanje su bile uključene 24 mliječne krave holštajnsko-frizijske pasmine u dobi od dvije do sedam godina. U serumu su određivani pokazatelji metabolizma lipida (trigliceridi, ukupni kolesterol, HDL-C, slobodne masne kiseline i beta-hidroksibutirat) i aktivnost PON1 i to 30, 10 i 2 dana prije teljenja, na dan teljenja te 5., 12., 19., 26. i 60. dana nakon teljenja. Koncentracija triglicerida bila je značajno snižena od porođaja do 60. dana laktacije u usporedbi s vrijednostima dobivenim prije teljenja ($P<0,05$). Koncentracije kolesterola i HDL-C bile su značajno niže kod teljenja s postupnim porastom tijekom laktacije ($P<0,05$). Koncentracija slobodnih masnih kiselina bila je značajno veća kod teljenja ($P<0,05$) i zadržala se na većim vrijednostima do 19. dana laktacije. Koncentracija beta-hidroksibutirata bila je značajno veća 12. i 19. dana nakon teljenja ($P<0,05$), vjerojatno kao posljedica povećane koncentracije slobodnih masnih kiselina oko teljenja. Ove promjene ukazuju na mobilizaciju masti iz masnoga tkiva kao posljedica nedostatka energije tijekom prijelaznog razdoblja. Aktivnost PON1 bila je smanjena kod teljenja i značajno se povećala 26. i 60. dana laktacije što ukazuje na smanjeni antioksidacijski status u postpartalnom razdoblju. Također, aktivnost PON1 značajno je pozitivno korelirala s ukupnim kolesterolom ($r = 0,42$) i HDL-C ($r = 0,49$) i obrnuto korelirala s koncentracijom slobodnih masnih kiselina ($r = -0,33$). Rezultati ukazuju da je PON1 povezana s metabolizmom lipida i lipomobilizacijskim sindromom te da bi se mogla smatrati mogućim pokazateljem metaboličkih i upalnih poremećaja mliječnih krava u prijelaznom razdoblju.

Ključne riječi: slobodne masne kiseline, beta-hidroksibutirat, negativni energetski balans, oksidacijski stres, upala