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Variation factors of paraoxonase in blood and in HDL lipoproteins in dairy cow

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Fattori di variazione della paraoxonasi plasmatica e nelle lipoproteine HDL nella bovina da latte

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Summary
Paraoxonase-1 (PON) is an antioxidant enzyme synthesized by liver and mainly associated with high density lipoproteins (HDL). Aim of this study was to investigate the alterations of PON1 activity in plasma (pPON) or bound to HDL (HDL-PON) in relation to inflammatory conditions and physiological stages. Between -30 to +300 days from calving, 9 cows were monitored for metabolic and inflammatory profiles, milk yield, body condition score and feed intake. Cows were divided in 2 groups based on their response to *postpartum* inflammatory stimulus (high and low Liver Functionality Index: HI-LFI and LO-LFI). pPON decreased after calving ($P<0.05$) and was directly correlated with milk yield ($r=0.48$; $P<0.01$), albumins and other indirect indexes of negative acute phase proteins (-APP: cholesterol and retinol binding protein). On the contrary, pPON was inversely related with the +APP (haptoglobin, ceruloplasmin) and related indexes (bilirubin, ROM). The correlation between pPON and HDL-PON was low. After calving, the HI-LFI vs LO-LFI group had higher –APP and pPON levels, lower HDL-PON level and displayed better performances. Overall results demonstrated that pPON has a similar behavior in comparison to –APP, confirming previous data. The results suggested that pPON activity could represent an useful marker to assess the severity of *postpartum* inflammatory phenomena and their consequences.

Riassunto
La paraoxonasi-1 (PON1) è un enzima antiossidante di sintesi epatica associato principalmente alle lipoproteine ad alta densità (HDL). Scopo dello studio è stato quello di valutare le variazioni di PON1 nel plasma (pPON) o nelle HDL (HDL-PON) in relazione allo stato infiammatorio ed allo stadio fisiologico. Nove bovine sono state controllate tra -30 e +300 giorni dal parto per: marcatori ematici dello stato infiammatorio, produzione di latte, stato di ingrassamento, ingestione di alimenti. Le bovine sono state divise in 2 gruppi sulla base della loro risposta allo stimolo infiammatorio *post partum* (alto e basso Liver Functionality Index: HI-LFI e LO-LFI). La pPON ha mostrato un calo temporaneo dopo il parto ($P<0,05$) e la sua attività è risultata correlata con la produzione di

latte (r=0,48; P<0,01), le albumine ematiche ed altri indici indiretti delle proteine negative di fase acuta (-APP: colesterolo, retinolo). Invece correlazioni inverse sono state osservate con le +APP (apto-globina e ceruloplasmina) ed altri indici correlati (es. bilirubina, ROM). La correlazione tra pPON e HDL-PON è risultata modesta. Dopo il parto le bovine HI-LFI (*vs* LO-LFI), oltre a livelli più alti di -APP e di pPON, hanno mostrato migliori performance. Tali risultati confermano che nella bovina la pPON ha un andamento simile a quello delle –APP. Questa associazione supporta l'utilità della pPON per valutare la gravità della risposta infiammatoria nel postparto e le sue conseguenze.

Introduction

Paraoxonase-1 (PON1) is an antioxidant enzyme mainly synthesized by the liver and associated with high density lipoproteins (HDL) (1), however its production was recently reported also in other tissues (2). Plasma PON1 (pPON) falls during inflammation in mice and hamsters (3). In line with this finding, in dairy cow we previously observed a decrease of plasma paraoxonase level at calving and during the first 2 months of lactation, likewise the typical drop of negative acute phase proteins and their indexes (4). However, its trend following the first two months of lactation was not studied. Other authors (5, 6) reported higher pPON levels in dry period and in advanced lactation.
In dairy cow HDL fraction represents up to 80% of lipoproteins in plasma and its concentration greatly lowers during *peripartum* (7). Bionaz et al. (4) reported that pPON and HDL levels decreased in this period as well, but with a different trend as summarized by the PON/HDL ratio, suggesting a change in PON1 concentration in the HDL (HDL-PON).
Aim of this study was to further characterize in dairy cow, the effect of physiological stage and of inflammatory conditions typical of *peripartum* on pPON and HDL-PON activity. In particular, the effect of inflammation was assessed ranking cows according to a complex index based on consequences of inflammation around calving.

Materials and methods

The study was carried out at the experimental barn of the Università Cattolica del Sacro Cuore (Piacenza, Italy), characterized by a constant monitoring and a systematic regulation of main environmental parameters [e.g, photoperiod (14 h/d of light), temperature (18-24°C), and relative humidity (60-70%)]. Nine pluriparous Holstein Friesian cows of medium-high genetic potential were adapted to the housing system and to frequent blood samples collection. Diets were composed by forages, dehydrated hay (alfalfa and grass) and corn silage, and were administered in two daily meals with a 12 hours interval (7:30 a.m. and p.m.). The concentrate was given by an automatic system with gaps of 12 hours in dry period and 3 hours during lactation. The concentrate, whose composition was changed after calving, gradually increased in order to reach 1 kg per 3 kg of milk

production (about 40 DIM). Diet at lactation peak was on average composed by 15 kg of concentrate, 20 kg of corn silage, 3 kg of alfalfa hay, and 2.5 kg of grass hay.

Blood samples were taken in vacuum tubes twice a week from jugular vein in the morning before forage meal. On blood samples were systematically determined hematocrit and a simple metabolic profile, to monitor metabolic conditions and health status. Samples collected at -30, 7, 30, 60, 90, 120, 180, 240, 300 days in milk (DIM) were selected (±3 days, but ±1 day for 7 and 30 DIM), far from health problems. A wide metabolic profile was determined, according to previously described methods (8), including: a) energy and proteins metabolism indexes (glucose, cholesterol, non-esterified fatty acids [NEFA], β-hydroxybutyric acid [BHB], urea and creatinine); b) inflammatory status indexes and related parameters [ceruloplasmin, haptoglobin, sialic acid, Ca, Zn, reactive oxygen metabolite (ROM), thiol groups; total proteins, albumins, globulins, total bilirubin, retinol, α-tocopherol, and β-carotene]; c) liver damage indexes (GOT/AST and GGT). Furthermore the activity of enzyme PON1 was assessed in plasma and in HDL isolated from cow plasma (9). During the trial, each cow was regularly monitored for milk production (every milking), dry matter intake (DMI) and health status (daily), body condition score (BCS) and body weight (every 14 days).

Cows were retrospectively divided in two groups (high = HI-LFI and low = LO-LFI) based on Liver Functionality Index (LFI; 10). This index is calculated according to plasma variations of albumins, cholesterol and bilirubin in the first month of lactation.

Statistical analysis was carried out using a repeated measure ANOVA (proc. Mixed, SAS Inst. Inc., Cary, NC; version 9.1), including in the model physiological stage (9 levels) and group (2 levels) as main factors and assessing the interaction between the two factors. Moreover simple Pearson's correlation was run considering the whole period, from -30 to +90, and from +120 to +300 DIM.

Results and discussion

Six out of 9 cows showed at least one health problem in the 1st month of lactation (3 mastitis, 2 ketosis and 1 retained placenta). After the 1st month of lactation, health issues concerned only 3 out of 9 cows (2 with mastitis and 1 with limb swelling). However, health problems were not serious, as shown by the low reduction in milk yield.

The LFI index takes higher values when hepatic functionality in *postpartum* is good, i.e., with a reduced deviation of liver synthesis (especially −APP) caused by inflammatory phenomena. The 9 cows displayed LFI values between -0.77 and +3.18; LO-LFI group included cows with negative index (4 cows, mean -0.42±0.28), while HI-LFI those with positive index (5 cows, +2.21±0.65).

DMI was overall high with an average of 11.2 kg in dry period and about 24 kg at lactation peak. The difference between the two LFI groups based on the whole period was statistically significant ($P<0.05$). In detail, HI-LFI cows ate more than LO-LFI (average 11.9±1.3 *vs* 10.6±1.5 kg DMI/d) in dry period; during lactation the difference remained (23.9±2.7 *vs* 22.3±2.5 kg DMI/d), with greater difference starting from the 2nd month.

Cows were characterized by an excellent milk yield level (peak at about 50 DIM and average total production of 12,776±841 kg in 305 days). HI-LFI group showed a higher total production compared to LO-LFI (13,008±712 *vs* 12,486±1003 kg in 305 days; $P<0.05$). The BCS (average 2.64±0.25 points at -30 DIM) displayed the typical *postpartum* reduction. The minimum value was in average reached at 60 DIM (-0.68 points *vs* -30 DIM). HI-LFI group, despite a higher milk production, showed a nu-

merical lower decrease compared to LO-LFI (-0.66 *vs* -0.72 points; NS).

Plasma indexes of inflammatory status

Trends of these indexes reflected the typical variations of *peripartum* already described in previous studies (4, 8, 11). In particular, +APP increased their levels immediately after calving. Haptoglobin (fig. 1) and ceruloplasmin showed their peak at 7 DIM followed by a gradual decrease. Albumins and indirect indices of other -APP, including cholesterol (index of lipoproteins; fig. 1) and retinol (index of its carrier protein, Retinol Binding Protein), specularly to +APP, had their minimum at 7 DIM. Subsequently had a progressive increase and reached at 30 DIM levels similar or higher to those observed at the end of pregnancy. Among other parameters related to inflammatory status noteworthy were the bilirubin, index of hepatic synthesis of enzymes involved in its clearance, and ROM; both had a trend similar to +APP. As expected, due to our partition system, HI-LFI group showed less marked variations of inflammatory process indexes compared to LO-LFI. In detail, HI-LFI had less pronounced haptoglobin and ceruloplasmin increase after calving, lower levels of ROM and ceruloplasmin already at -30 DIM, and a faster cholesterol and albumins recovery after *postpartum*.

Plasma indexes of energy balance

The sudden energy request by organism after calving caused a plasma glycaemia drop, while the contextual lipomobilization caused an increase of NEFA and BHB. The trend of energy balance indexes reflected this situation, with some differences between the two groups. HI-LFI, in respect to LO-LFI, showed a faster glucose recovery after calving and a less marked NEFA increase (peak at 7 DIM; 0.69±0.23 *vs* 0.88±0.31 mmol/L), in agreement with the smaller BCS drop. All the parameters recovered *prepartum* levels at 90 DIM. BHB showed a trend similar to NEFA, with a peak at 7 DIM followed by a decrease to steady values, but with numerically higher values in HI-LFI (1.83±0.58 *vs* 1.34±0.47 mmol/L).

Plasma-PON and HDL-PON

pPON activity (Fig. 1) showed a marked decrease immediately after calving (P<0.05) followed by a fast recovery, reaching levels higher than *prepartum* at 30 DIM. Later pPON showed a reduction, becoming steady from 120 DIM (Fig. 2). These data partly confirm observations of Turk (5), who found the highest levels at about 100 DIM (defined "advanced lactation"). However, in comparison to Turk's experiment the difference exists in value magnitude, maybe due to analytical aspects, already mentioned by Bionaz et al. (4), and probably even to other causes, like genetic, health and body conditions (2, 14). In our study sampling frequency for the whole lactation allowed to outline PON1 trend more accurately than in Turk (5) and Turk et al. (6), who provided only one value for the whole lactation. LO-LFI cows, which presented higher overall inflammation *postpartum*, showed also a slower *postpartum* increase of pPON (P<0.07 *vs* HI-LFI at 30 DIM; Fig. 2).

HDL-PON activity during lactation (Fig. 1) showed a trend similar to pPON, however its values in dry period were the highest in all the cows, unlike pPON, which peaked at 30 DIM. The correlation between pPON and HDL-PON activity was low (r=0.24; P<0.05), but it was stronger in LO-LFI (r=0.43; P<0.001) than in HI-LFI group (r=0.06; NS). Some hypotheses could be advanced to explain these differences. PON-1 is mostly associated with the surface of HDL and its activity is modulated by compositional and physicochemical properties of lipoproteins. Several studies

Figure 1 - Left: pattern of changes of the overall pPON (Standard Error [ES]=11.13) and HDL-PON (ES=13.04) from -30 to +90 DIM. Right: comparison between haptoglobin (ES=0.32) and total cholesterol (ES=1.24) variations in the two LFI groups from -30 to +90 DIM

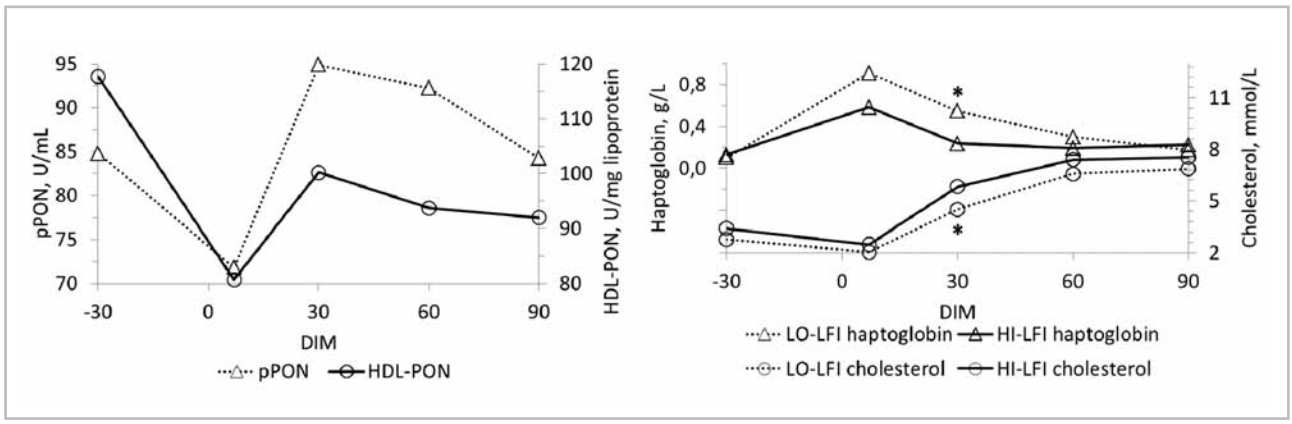
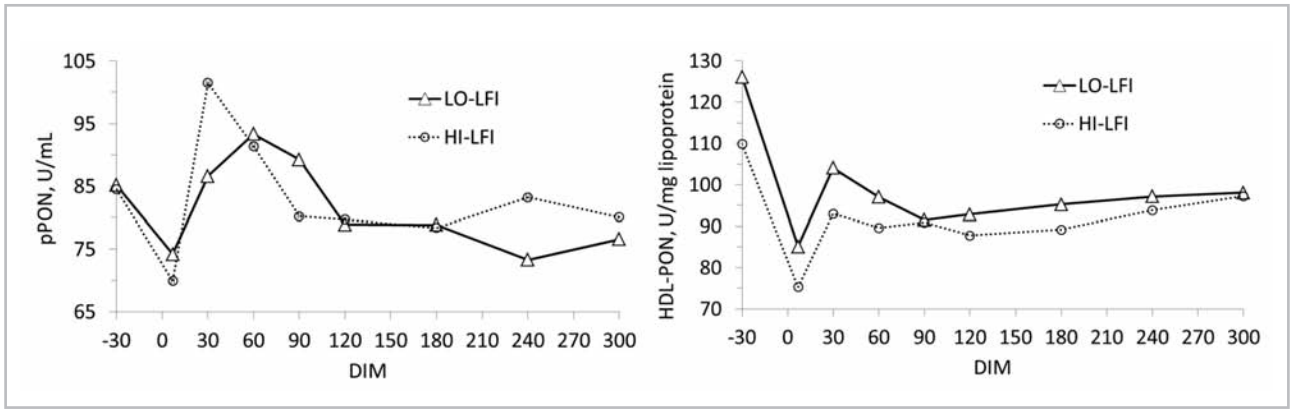


Figure 2 - pPON (ES =10.75) and HDL-PON pattern of changes (ES=12.33) from -30 to +300 DIM in LO-LFI and HI-LFI groups



demonstrated that HDL-PON is a lipid-dependent enzyme and that its conformation within the hydrophobic environment of HDL is crucial for its stability and activity (12). Also HDL subclasses show different capacity to stabilize PON1 on lipoprotein surface (13). Therefore, we suggest that compositional factors of HDL in the two groups could affect the binding of paraoxonase to the surface of HDL (9, 15). In human, Ferretti et al. (9) reported a reduction in the affinity of this bond in obese subjects. This possible cause is unlikely in our study because all cows showed an acceptable body condition at calving (about 2.4 points). In dairy cows, Miyamoto et al. (15) investigated the concentration of PON1 in different subclasses of HDL and reported a higher PON1 concentration in the heaviest HDL fraction synthesized by the liver, and a lower one in the lighter fraction synthesized by the

gut. According to these results, an increase of PON-HDL would reflect an increase of the liver HDL subclass or a reduction of the gut HDL subclass (e.g. for a reduced feed intake). In our conditions PON1 affinity to different HDL fractions does not seem crucial to explain different correlations between pPON and HDL-PON in cows characterized by more or less serious postpartum inflammatory phenomena (e.g. different LFI). Indeed DMI difference between LFI groups was not so high in the weeks around calving, so even differences in the synthesis of lighter HDL fraction should have been slight. On the contrary, in our study the hepatic synthesis of lipoproteins (that we measured indirectly through plasma total cholesterol), was significantly lower in LO-LFI *vs* HI-LFI cows at 30 DIM ($P<0.05$); therefore, it can be suggested that the low correlation between pPON and HDL-PON could be also due to a different liver synthesis rate of PON1 and of lipoproteins (which are mostly HDL in *peripartum*) as previously suggested by Bionaz et al. (4).

Correlations

pPON and milk production showed in the 1st part of lactation (until 90 DIM) a positive and rather high correlation ($r=0.48$; $P<0.01$), confirming a link between the overall performance and health. Among plasma parameters, the most interesting correlations were observed during the 1st part of lactation (from +7 to +90 DIM), in which inflammatory processes show the most important variations. In this phase, pPON was positively correlated to –APP: cholesterol ($r=0.31$; $P<0.1$), albumins ($r=0.54$; $P<0.001$), and retinol ($r=0.40$; $P<0.05$). These data are in agreement with Turk et al. (16), although in our study the correlation is stronger with albumins and lower with cholesterol. On the contrary, negative correlations were observed between pPON and +APP and related indexes: haptoglobin ($r=-0.34$; $P<0.05$), ceruloplasmin ($r=-0.41$; $P<0.05$), bilirubin ($r=-0.45$; $P<0.01$) and ROM ($r=-0.55$; $P<0.001$). During advanced lactation (from +120 to +330 DIM), in which +APP and –APP variations are less pronounced, pPON showed correlations only with albumins ($r=0.60$; $P<0.001$) and retinol ($r=0.42$; $P<0.05$). Finally, considering HDL-PON in the period from +7 to +90 DIM, negative correlations were observed with ceruloplasmin ($r=-0.40$; $P<0.05$), bilirubin ($r=-0.36$; $P<0.05$), and ROM ($r=-0.49$; $P<0.01$), while among –APP only albumins were correlated ($r=0.44$; $P<0.01$).

Conclusions

Our study confirms the inverse relationship between PON1 and inflammatory status in *peripartum* of dairy cow. These results can be partly explained by the HDL reduction *postpartum*, and partly by the reduced PON1 synthesis. Our data, in agreement with previous studies (4), support that PON1 is a -APP because it behaves as a negative acute phase protein (e.g., positive correlation with albumins and Retinol Binding Protein). This hypothesis was already advanced by Feingold et al. (17) in hamster and by Mackness et al. (18) in man. These studies however considered only serious inflammatory events, while in our data this relationship is evident also in subclinical cases. The relationship with some inflammatory indexes (+APP and –APP) appears to be stronger considering the plasma PON1 (pPON), and to be weaker with PON1 associated with HDL (HDL-PON).

In conclusion, pPON can be considered a good index for severity of *postpartum* inflammatory phenomena and their consequences, so it could be useful to better define inflammatory profile in addition to Liver Functionality Index parameters. Other studies are needed to better investigate the factors involved in PON1 variation in dairy cow.

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