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Bovine platelet-activating factor acetylhydrolase (PAF-AH) activity related to fertility

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Abstract

Plasma platelet-activating factor acetylhydrolase (PAF-AH), the enzyme characterized by the association with plasma lipoproteins, degrades platelet-activating factor (PAF) as well as PAF-like oxidatively fragmented phospholipids produced during oxidative stress. Apart from pro-inflammatory properties, PAF is also related to reproductive processes and successful fertility. In order to get a better insight into the involvement of PAF-AH in the fertility of cows, the aim of the study was to determine the PAF-AH activity as well as the C-reactive protein, cholesterol and high density lipoprotein-cholesterol (HDL-C) in the serum of dairy cows throughout the pregnancy and lactation, as well as in infertile cows. The results showed that serum PAF-AH activity changes throughout pregnancy and lactation with a lower level during periparturient period. It is also found higher PAF-AH activity in lactating cows with reproductive disorders compared to high lactating cows without reproductive disorders. Strong correlation between PAF-AH activity and HDL-C concentration indicates that HDL could have considerable influence on PAF-AH activity in bovine plasma. CRP concentration was also lower during transition period suggesting that lactation might stimulate CRP synthesis in bovine. A higher CRP concentration in cows with reproductive disorders compared to fertile cows at the peak of lactation, demonstrates that milk production is not the only factor influencing CRP in

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cows. A significant correlation between PAF-AH activity and CRP level shows that both parameters could be influenced by reproductive status of dairy cows.

Keywords: PAF-AH; Dairy cows; Fertility; CRP

1. Introduction

Platelet-activating factor acetylhydrolase (PAF-AH; E.C. 3.1.1.47) is an anti-inflammatory and anti-oxidative enzyme which is in mammalian plasma associated with low and high density lipoproteins (LDL and HDL) (Stafforini et al., 1999). The enzyme hydrolyzes the acetyl ester at the *sn*-2 position of the platelet-activating factor (PAF) producing acetate and biologically inactive lyso-PAF (1-*O*-alkyl-*sn*-glycero-3-phosphocholine) (Castro Faria Neto et al., 2005). PAF-AH also hydrolyzes PAF-like oxidatively fragmented phospholipids produced during oxidative stress. These compounds have a similar structure to PAF and provoke the same acute phase responses (Stafforini et al., 1997). Therefore, anti-oxidant properties of PAF-AH are involved in the protection against the harmful action of free radicals.

PAF is a bioactive phospholipid synthesized by a variety of mammalian cells. In addition to its first discovered physiological action—the activation of the platelets, PAF also activates polymorphonuclear leukocytes and monocytes, suggesting a pathophysiological action as being a mediator of inflammation and a well established activator of the immune system (Stafforini et al., 1987). On the other hand, the presence and function of PAF in the male and female reproductive systems of several species, including humans and bovines (Soubeyrand et al., 1998) demonstrates that PAF is an important mediator for physiological processes associated with reproduction, such as ovulation, sperm motility, fertilization, implantation, fetal tissue development and the initiation of parturition (Matsubara et al., 1997). Events during ovulation are associated with inflammatory-like changes (Espey, 1994) and PAF may influence this process (Abisoqun et al., 1989). Further, the reproductive tissues also contain PAF-AH (Matsubara et al., 1997). Moreover, in the bovine reproductive system, PAF-AH activity was demonstrated in endometrial cells of cows during early pregnancy (Tiemann et al., 2001). Additionally, it has been found the PAF-AH in the bovine endometrium is structurally identical to human and bovine serum enzyme. PAF-AH activity in the female reproductive system is under steroid control negatively correlating with estrogens (Narahara et al., 1996; OŃeill, 2005). During the last third of pregnancy, the enzyme activity decreases and promptly increases at the time of delivery (Yasuda and Johnson, 1992). To understand better, the potential roles of PAF in the processes of reproduction, it is essential to have information on the variations of PAF-acetylhydrolase during the reproductive cycle and pregnancy.

During transition period in dairy cows, low energy balance could induce metabolic disorders, especially lipid parameters changes, accompanied with subsequent disturbances in reproductive physiology (Butler, 2000; Lucy, 2003). The aim of the study was to determine the PAF-AH activity in the serum of dairy cows throughout the pregnancy and lactation as well as in cows with reproductive disorders. Taking into account the anti-inflammatory properties of PAF-AH, we also examined the C-reactive protein concentration, which is considered as an acute phase protein, in order to investigate the relationship between these two parameters. Total cholesterol and HDL-cholesterol (HDL-C) concentrations were also measured to get information of lipid metabolism changes during transition period and to examine the correlation of PAF-AH activity with total cholesterol and HDL-C concentrations, since PAF-AH is associated with lipoproteins in the blood.

2. Materials and methods

2.1. Animals and serum sampling

The study was conducted on a total of 133 Holstein-Frisian dairy cows located on farms in the region of northwest and eastern Croatia. Forty-eight of them were pregnant, i.e. 21 cows were in the first trimester of pregnancy (P1), 17 cows were in the second trimester of pregnancy (P2) and 10 cows were in dry period (P3). The remainder of the 85 animals were non-pregnant cows in lactation as follow: 23 cows in the early puerperium (1–15 days, L1), 27 cows were in the late puerperium (20–30 days, L2), 16 cows were in the mid-lactation between 40 and 60 days (L3) and 19 cows were in the late lactation longer than 150 days p.p. (L4). The last group (L4) comprised infertile cows with reproductive disorders, such as anoestrous, anovulatory cycle, ovulatory disturbance, cystic ovarian disease, luteal persistency and endometritis. The blood samples were taken from v. jugularis or v. coccygea and after clotting for 2 h at room temperature, they were centrifuged at 3000 rpm for 15 min. Serum samples were stored at −70 °C until analysis.

2.2. Reagents and analysis procedures

2.2.1. PAF-AH activity assay

Platelet-activating factor acetylhydrolase activity was determined by the spectrophotometric assay described by Kosaka et al. (2000). Briefly, 2 μL serum was added to 240 μL of 200 mmol/L HEPES (*N*-2-hydroxyethylpiperazine–*N*′-2-ethanesulfonic acid) buffer (Reagent 1), pH 7.6 and pre-incubated at 37 °C for 5 min. The reaction was started by adding 80 μL of 20 mmol/L citric acid monohydrate buffer, pH 4.5 containing 90 mmol/L 1-myristoyl-2-(4-nitrophenylsuccinyl)phosphatidylcholine (Reagent 2) (Azwell Inc., Osaka, Japan). The liberation of *p*-nitrophenol was monitored at 405 and 505 nm at 1 and 3 min after the addition of Reagent 2 using the automatic biochemical analyzer Olympus AU 600 (Olympus Optical Co., LTDL, Tokyo, Japan).

2.2.2. CRP, total cholesterol and HDL-C concentrations assays

The concentration of C-reactive protein was determined by the latex-enhanced immunoturbidimetric assay kit (Full range CRP, Randox Laboratories Ltd., Ardmore, UK). Considering marked cross-reactivity and amino acid sequence homology of CRP between human and other species, including bovine (Taylor et al., 1984; Maudsley and Pepys, 1987; Ying et al., 1992), human CRP assay was applied.

Total cholesterol and HDL-cholesterol were measured by standard commercial kits (Olympus Diagnostica GmbH, Hamburg, Germany) using automatic analyzer Olympus AU 600.

2.2.3. Quality control of measurements

The quality control for the measured parameters was based on the control of accuracy and the control of imprecision. The control of accuracy was performed for CRP, total cholesterol and HDL-C concentrations using commercial control sera (Roche Diagnostics, Olympus Diagnostica and Randox Laboratories Ltd.). The biases for these parameters were 3.8, 1.6 and 1.0%, respectively. Control of imprecision was performed using pool serum samples for the following parameters: CRP, total cholesterol and HDL-C concentrations as well as PAF-AH activity. The intra-assay CVs for these parameters were 1.1, 0.9, 0 and 0.8%, respectively, while the inter-assay CVs were 5.4, 6.3, 5.9 and 4.1%, respectively.

2.3. Statistical analysis

In order to assess significant differences between investigated groups the Student's *t*-test was applied, after testing data for normality (Kolmogorov–Smirnov test). Person correlation coefficients were used to examine the correlation between two parameters. SigmaStat 3.0 (SPSS Inc., Chicago, IL, USA) was applied for statistical analysis. Statistical significance was based on values $P < 0.05$.

3. Results

3.1. PAF-AH activity

The serum PAF-AH activity was significantly lower in the last third of pregnancy (140 U/L) compared to the first (234 U/L) trimester of pregnancy (Fig. 1). Moreover, there was a significantly lower PAF-AH activity in both the early and late puerperium (131 and 130 U/L, respectively) compared to the mid-lactation (182 U/L). In the group of infertile cows, PAF-AH activity (232 U/L) was significantly higher than in cows in mid-lactation (Fig. 1).

3.2. CRP, total cholesterol and HDL-C levels

CRP concentration was significantly lower during the last trimester of pregnancy (2.3 mg/L) compared to the early pregnancy (3.8 mg/L). There was also a significantly lower CRP level in the early postpartum period (1.6 mg/L) compared to the late puerperium (2.9 mg/L) and the mid-lactation period (3.5 mg/L). There was no significant difference in the CRP level between infertile cows (4.5 mg/L) and mid-lactation cows (3.5 mg/L; Fig. 2).

The total cholesterol concentration was significantly lower in dry pregnancy (2.65 mmol/L) compared to the early pregnancy (3.83 mmol/L). During the postpartal period, both early and late

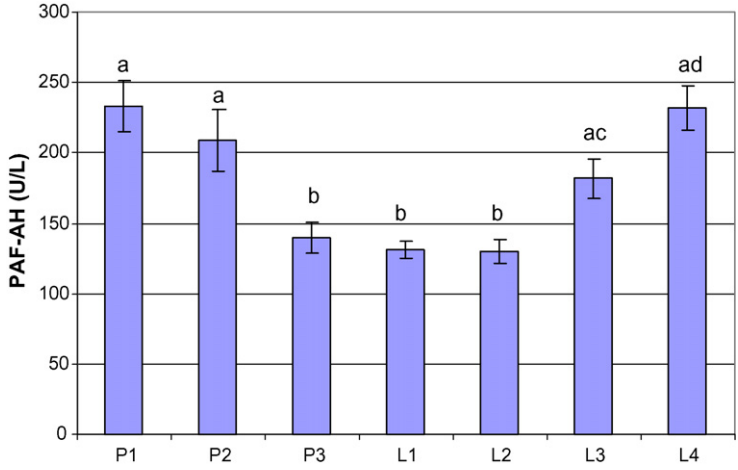

Fig. 1. Serum PAF-AH activity (U/L) in cows throughout the pregnancy and lactation. The means ± S.E. are presented. Bars with different letters (a, b, c, d) are significantly different ($P < 0.05$). P1: first trimester of pregnancy; P2: second trimester of pregnancy; P3: dry period; L1: early puerperium (1–15 days); L2: late puerperium (20–30 days); L3: mid-lactation (40–60 days); L4: lactation > 150 days (infertility).

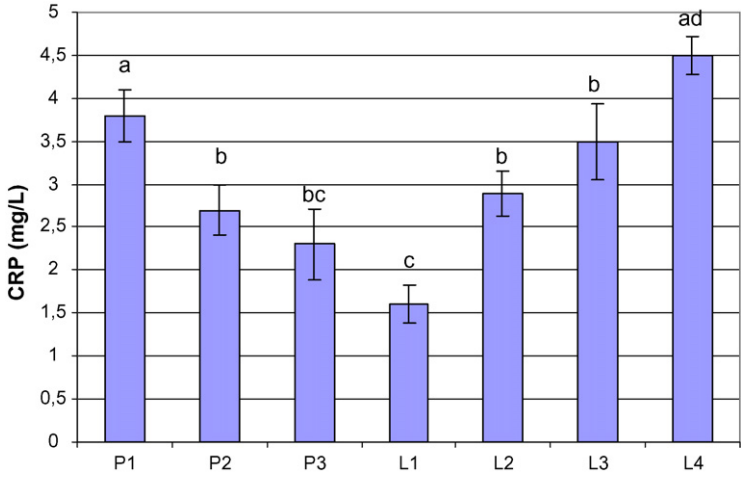

Fig. 2. Serum CRP concentration (mg/L) in cows throughout pregnancy and lactation. The means ± S.E. are presented. Bars with different letters (a, b, c, d) are significantly different ($P < 0.05$). P1: first trimester of pregnancy; P2: second trimester of pregnancy; P3: dry period; L1: early puerperium (1–15 days); L2: late puerperium (20–30 days); L3: mid-lactation (40–60 days); L4: lactation > 150 days (infertility).

puerperium, there were significantly lower values of total cholesterol concentrations (2.34 and 2.35 mmol/L, respectively) than in mid-lactation (3.48 mmol/L). In infertile cows (4.48 mmol/L), there was also a significantly lower cholesterol level than in cows in mid-lactation (Fig. 3).

In accordance with the changes of the total cholesterol concentration, HDL-C concentration was significantly lower in late pregnancy (1.60 mmol/L) than in the beginning of the pregnancy (2.33 mmol/L). In both early and late puerperium (1.53 and 1.58 mmol/L, respectively), there was a significantly lower HDL-C concentration as well, compared to mid-lactation (2.18 mmol/L). In

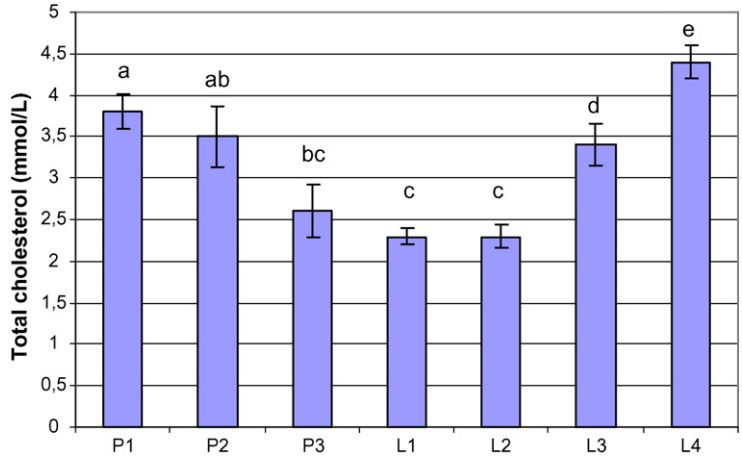

Fig. 3. Serum total cholesterol concentration in cows throughout pregnancy and lactation. The means ± S.E. are presented. Bars with different letters (a, b, c, d) are significantly different ($P < 0.05$). P1: first trimester of pregnancy; P2: second trimester of pregnancy; P3: dry period; L1: early puerperium (1–15 days); L2: late puerperium (20–30 days); L3: mid-lactation (40–60 days); L4: lactation > 150 days (infertility).

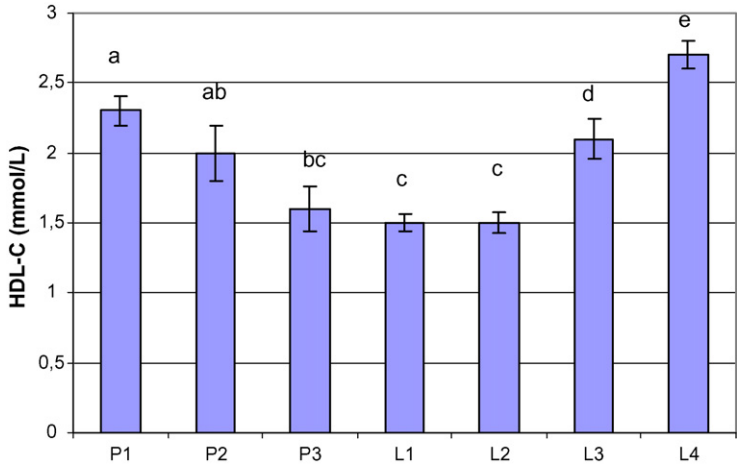

Fig. 4. Serum HDL-C concentration in cows throughout pregnancy and lactation. The means ± S.E. are presented. Bars with different letters (a, b, c, d) are significantly different ($P < 0.05$). P1: first trimester of pregnancy; P2: second trimester of pregnancy; P3: dry period; L1: early puerperium (1–15 days); L2: late puerperium (20–30 days); L3: mid-lactation (40–60 days); L4: lactation > 150 days (infertility).

infertile cows (2.70 mmol/L), there was also a significantly lower HDL-C level than in cows in mid-lactation (Fig. 4).

3.3. Correlation between PAF-AH activity and the concentrations of CRP, total cholesterol and HDL-C

The correlation between PAF-AH activity and the concentrations of CRP, total cholesterol and HDL-C was examined on the total number of animals ($n = 133$). A significant positive correlation ($P < 0.0001$) was found between PAF-AH activity and CRP concentration ($r = 0.409$; Fig. 5). A

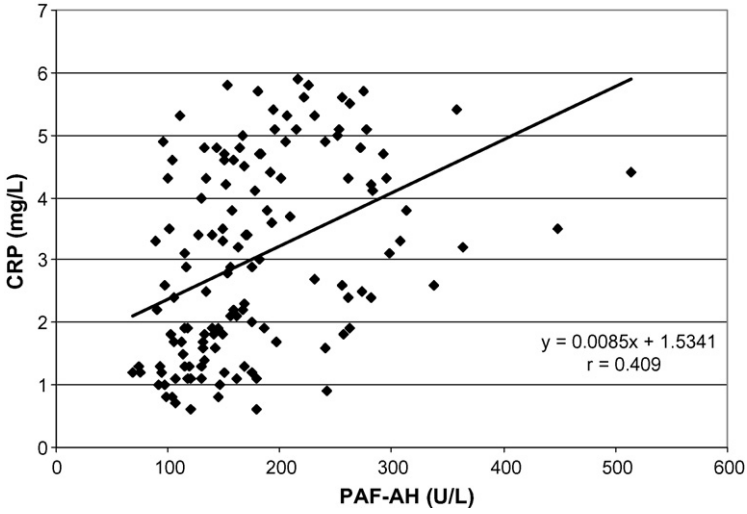

Fig. 5. Correlation between PAF-AH activity and CRP concentration in all cows.

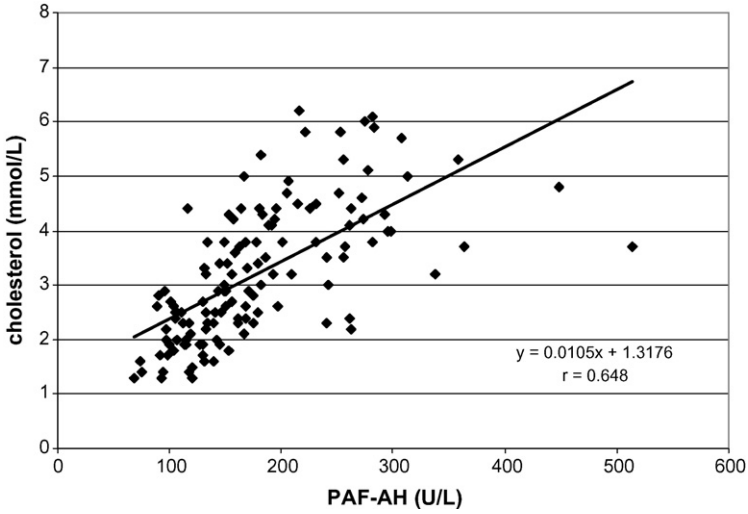

Fig. 6. Correlation between PAF-AH activity and total cholesterol concentration in all cows.

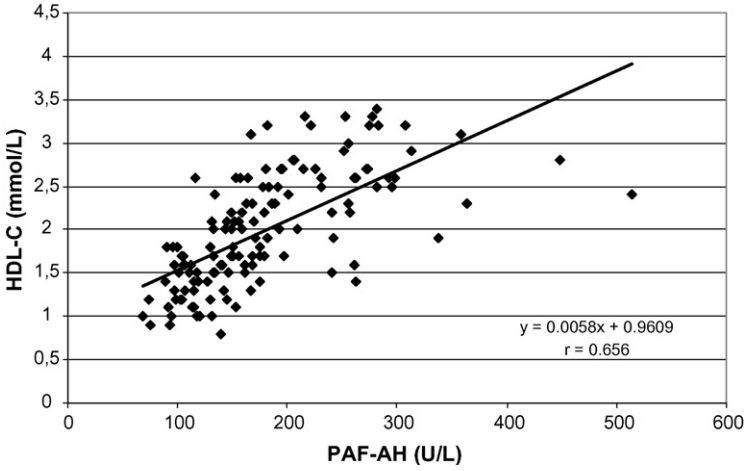

Fig. 7. Correlation between PAF-AH activity and HDL-C concentration in all cows.

significant positive correlation ($P < 0.0001$) was also found between PAF-AH activity and total cholesterol as well as the HDL-C concentration ($r = 0.650$ and 0.656, respectively; Figs. 6 and 7).

4. Discussion

Although the exact mechanism of the role of PAF in reproductive physiology is still uncertain, there is some evidence on influence of PAF and PAF-AH on fertility (Tiemann et al., 2001; Bücher et al., 2006). PAF-AH contributes to the regulation of the PAF level in reproductive tissues by its inactivation to lyso-PAF. In the present study, the results show that serum PAF-AH activity changes throughout the pregnancy and lactation. The results also present higher PAF-AH activity in cows with reproductive disorders than in fertile cows.

The changes of PAF-AH activity during pregnancy and postpartum period in this study indicate the steroid regulation of the enzyme activity. Miyaura et al. (1991) showed an inverse relationship between the plasma estrogens concentration and PAF-AH activity in rats. Yasuda and Johnson (1992) also found a decrease in plasma PAF-AH by administration of estrogens. A lower PAF-AH activity in late pregnancy in this study might be caused by the increased estrogen level near upon to parturition. Thus, in late pregnancy, when the PAF-AH activity is low, the PAF level is allowed to be high. A significant decrease in PAF-AH activity during the late gestation was also found in maternal plasma in several species (Maki et al., 1988). In addition, Narahara et al. (2003) demonstrated that the PAF-analogue inhibited PAF-AH secretion by human decidual macrophages, indicating a central role of PAF in periparturient events.

Tiemann et al. (2001) found the changes in PAF-like activity in bovine endometrium during the oestrous cycle and early pregnancy. They also found the decrease in PAF-AH activity in the endometrium of early pregnant cows compared to cyclic cows, suggesting the involvement of PAF and PAF-AH in the implantation process of cattle. Consequently, the higher PAF-AH activity in infertile cows in this study demonstrates increased degree of PAF degradation and a decline in PAF concentration, which is important in the process of fertilization and implantation. In addition, Tiemann et al. (2001) found that the bovine PAF-AH isolated from the cytosolic fraction of endometrium is identical to plasma form of the enzyme. This indicates that variation in serum PAF-AH activity might reflect the changes in endometrial enzyme activity and negatively influence fertility in cows.

The observed decrease in serum CRP concentration in cows during pregnancy and an increase during lactation has been also found in previous studies (Morimatsu et al., 1991; Lee et al., 2003). Morimatsu et al. (1991) found a high correlation between milk yield and serum CRP level suggesting that lactation might stimulate CRP synthesis in the bovine liver as an acute phase reaction. However, CRP concentration was significantly higher in subfertile cows (L4) in lactation longer than 150 days when the milk yield is declined, compared to cows in mid-lactation between 40 and 60 days (L3), when the milk yield is the highest. This indicates that, apart from milk production, reproductive status could also influence the CRP level in cows. In accordance to our results, Lee et al. (2003) also demonstrated a higher CRP level in cows with reproductive disorders and in cows with inflammatory diseases considering that CRP level is due to health status in cows. A significant correlation between PAF-AH activity and CRP level shows that both parameters could be influenced by reproductive status of dairy cows.

In human plasma, 70% of PAF-AH is associated with LDL (containing apoB) and the remainder is associated with HDL. These enzyme activities appear to be the same protein with identical behaviour and could be transferred between these two lipoproteins (Stafforini et al., 1989). Guerra et al. (1997) showed a strong correlation between plasma PAF-AH activity and LDL-C among individuals with normolipidemia. Surya et al. (1991) found that in abetalipoproteinemia, a disorder characterized by the absence of apoB-containing lipoproteins (LDL, VLDL and chylomicrons), all the enzyme activity is bound to HDL indicating that LDL is not necessary for plasma PAF-AH activity. Accordingly, bovine serum contains a very small amount of LDL containing apoB, while HDL comprehends around 80% among all lipoproteins in bovine plasma (Ferreri and Gleockler, 1979). From this reason, the strong correlation between PAF-AH activity and total cholesterol and HDL-C concentrations in the present study indicate that plasma PAF-AH activity could be associated mostly with HDL rather than with LDL. In addition, Maki et al. (1988) found that in rabbits plasma enzyme activity is entirely associated with HDL, since in some vertebrates the HDL fraction is more metabolically active than LDL.

5. Conclusion

To conclude, the results of the present study show the variation in serum PAF-AH activity during pregnancy and lactation, as well as in cows with reproductive disorders indicating that the enzyme activity is due to reproductive status in cows. A higher CRP concentration in cows with reproductive disorders compared to fertile cows at the peak of lactation demonstrates that milk production is not the only factor influencing CRP in cows. Additionally, a marked correlation between PAF-AH activity and CRP concentration shows that both parameters could be related to reproductive health of dairy cows.

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