



RESEARCH ARTICLE

Serum Non-Esterified Fatty Acids and Beta-Hydroxybutyrate in Dairy Cows with Retained Placenta

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ABSTRACT

The objective of this study was to establish serum concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA) in postpartum retained placenta in cattle. Moreover, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), cholesterol (CHOL), triglycerides (TG), total proteins (TP), albumin (ALB), glucose (GLU) and blood urea nitrogen (BUN) levels were evaluated. Blood samples were obtained from multiparous Holstein dairy cows (n=38) with retained placenta between 12 to 24 hours after calving (Group 2). Clinically healthy multiparous Holstein dairy cows (n=6) calved approximately 7 days prior to the study served as control (Group 1). Concentration of TG, LDL, VLDL, ALB, BUN, CHOL and GLU did not vary between groups. Cows with retained placenta (Group 2) had higher level of BHBA (P=0.041) and NEFA (P=0.05) than control group. HDL and TP serum levels in cows with retained placenta were significantly lower than control cows. It was concluded that retained placenta could be associated with energy metabolism imbalance and postpartum negative-energy balance.

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INTRODUCTION

One of the most common problems seen during the post-parturient period of high-yielding dairy cows is retained placenta (RP). It causes important economic losses due to decreased pregnancy rates, increased calving-conception intervals and a rapid drop in milk yield (Semacan and Sevinc, 2005; Seifi *et al.*, 2007; Rabbani *et al.*, 2010). Nutrition, age, heredity (Seifi *et al.*, 2007), body condition score (BCS), management and energy imbalance as various risk factors are possible causes of RP. RP is also closely associated with peri-parturient fatty liver and negative-energy balance (NEB) (Morrow *et al.*, 1990; Semacan and Sevinc, 2005). These associations indicated that peri-partum energy metabolism contributes to the development of RP. However, no single factor provides a sufficient explanation for why RP in dairy cows develop (Seifi *et al.*, 2007).

In the published literature, relationship of NEFA and BHBA with RP in Holstein dairy cattle in Turkey is sparse, therefore, the present study aimed to determine the

serum non-esterified fatty acids and beta-hydroxybutyrate levels in dairy cows with and without RP.

MATERIALS AND METHODS

Animals and samples

Six clinically healthy multiparous Holstein dairy cows, calved approximately 7 days prior to the study, served as control group (Group 1). A total of 38 Holstein dairy cows with RP, 3 to 6 years-old, calved 12 to 24 hours prior to the study were used (Group 2). The animals were selected from private dairy farms near Afyonkarahisar and Konya provinces in Turkey. Blood samples were taken from jugular vein, and after coagulation at room temperature (22-24°C) samples were centrifuged at 1073 g for 15 minutes at room temperature, then serum samples were stored at -20°C until analyzed.

Biochemical analysis

Serum triglycerides (TG) (Cat # 04657594), cholesterol (CHOL) (Cat # 04718917), high density

lipoprotein (HDL) (Cat # 04657560), low density lipoprotein (LDL) (Cat # 04657578), glucose (GLU) (Cat # 04657527), total proteins (TP) (Cat # 04657586), albumin (ALB) (Cat # 04657357) and blood urea nitrogen (BUN) (Cat # 04657616) levels were determined by using commercially available (Roche Diagnostic, Germany) test kits. Very low density lipoprotein (VLDL) was calculated according to the following equations: $VLDL = TG/5$. All sera samples were analyzed using an auto-analyzer one assay (Roche, Cobas C111). Serum non-esterified fatty acids (NEFA) (Randox Laboratories Ltd, UK, Cat # FA 115) and beta-hydroxybutyrate (BHBA) (Randox Laboratories Ltd, UK, Cat # RB 1008) concentrations were determined spectrophotometrically (Shimadzu UV-1601).

Statistical analysis

Data was analyzed with One-way ANOVA and Pearson correlation method in SPSS 15.0 statistical program for Windows. The significance level was set at $P < 0.05$.

RESULTS

The history included retained placenta between 12 to 24 h after calving, a lack of appetite and decreased milk production for all the cows in Group 2, while temperature, heart rate and respiratory rate were normal in all cows with RP. The feed intake was also decreased for the cows with RP. All the cows in Group 1 and 2 had an optimal BCS.

NEFA ($P=0.05$) and BHBA ($P=0.041$) levels in RP cows were higher than those in Group 1. Cows with RP were characterized by lower levels of HDL ($P=0.002$) and TP ($P=0.000$). CHOL and GLU levels were numerically decreased in Group 2. These were not statistically significant. In cows with RP a positive correlation was detected between NEFA and BHBA levels ($r=0.483$; $P=0.001$), and NEFA and TG ($r=0.382$; $P=0.009$) (Table 1).

DISCUSSION

The data presented here show that postpartum variations of serum NEFA and BHBA levels are important in the cows with RP. Metabolic events associated with energy insufficiency, increased fat mobilization (Kaneene *et al.*, 1993) and NEB postpartum developing at the end of gestation (Sevinc *et al.*, 2003; Seifi *et al.*, 2007) are frequently related to increased risk of RP (Kaneene *et al.*, 1993; Seifi *et al.*, 2007). Excessive lipid mobilization due to NEB causes change of serum NEFA and BHBA levels (Seifi *et al.*, 2007; Laszlo *et al.*, 2009). It was reported that prepartum NEFA (Laszlo *et al.*, 2009; Ospina *et al.*, 2010) and postpartum BHBA were both significantly associated with development of clinical diseases in dairy cows including RP, displaced abomasum, ketosis (Ospina *et al.*, 2010) and metritis (Hammon *et al.*, 1993). Seifi *et al.* (2007) showed higher concentrations of NEFA and BHBA in the cows with RP than cows without RP. Elevated NEFA and ketones are in all probability suitable metabolic indicators for

characterizing the increased risk of RP (Laszlo *et al.*, 2009). Leblanc *et al.* (2005) similarly found that evaluation of NEFA and BHBA were more strategic to monitor transition dairy cows. Serum levels of NEFA may be more useful to identify cows with a metabolic abnormality or energy imbalance that might predispose them to RP (Quiroz-Rocha *et al.*, 2009). Ospina *et al.* (2010) informed that postpartum NEFA concentration was most associated with developing of RP. However, Kaczmarowski *et al.*, (1993) reported similar levels of ketone bodies and free fatty acids in cows with and without RP after calving. Our study showed higher postpartum levels of NEFA and BHBA in cows with RP. These results might be associated with postpartum NEB (Seifi *et al.* 2007), disrupted energy metabolism and adaptation problems. Adaptation for nutrient partitioning for fetal needs, onset of lactogenesis and stress of calving are some of the other factors that influence the serum NEFA (Adewuyi *et al.*, 2005) and BHBA production after calving. But, these findings did not reveal a cause and effect relationship.

Abnormal lipid and lipoprotein levels are often associated with liver dysfunction (Rayssiguier *et al.*, 1988), fatty liver and related diseases (Grummer, 1993; Katoh *et al.*, 1993; Van Den Top *et al.*, 1995; Civelek *et al.*, 2006a; 2006b). Serum CHOL levels have potential as indicators of disease risk in dairy cows (Kaneene *et al.*, 1993). In this study, CHOL level (not statistically but numerically decreased) was below average reference values in the cows with RP. Macak *et al.* (1999) produced similar data in RP cows. Semacan and Sevinc (2005) have also shown that serum levels of CHOL and HDL in cows with RP were lower than control cows. It may be affected by esterification of cholesterol, unsaturated fatty acids, or rising lactation (Macak *et al.* 1999). However, Kaczmarowski *et al.* (1993) reported similar levels of CHOL in cows with and without RP. In the present study, we found lower level of HDL in RP cows. HDL decrease might be associated with low concentrations of CHOL, because 60% of HDL was constituted by cholesterol (Rayssiguier *et al.*, 1988; Sevinc *et al.*, 2003). Furthermore, changes in serum CHOL concentrations could be simply due to decrease of feed intake, because most cholesterol in ruminants is of intestinal origin (Uchide *et al.*, 1997). It is well known that feeding problems during the peri-parturient period may affect susceptibility of cows to metabolic disorders (Dann *et al.*, 2005) including RP.

Marcos *et al.* (1990) reported that steatosis, a metabolic disorder, leads to damage in hepatocytes, resulting in dysfunction of protein synthesis, but concerned mechanisms are not fully understood (Gruffat *et al.*, 1996). In the present study, we have shown that the serum level of TP in cows with RP was lower than those of control cows ($P=0.000$). ALB also had numerically decreased in Group 2 (but not statistically important). ALB serum level is a marker of liver function (Basoglu *et al.*, 1998). These results suggest a better body protein status in cows without RP. Strang *et al.* (1998) reported that hepatocytes loaded with TG were less sensitive to the hormonal stimulation of albumin and protein synthesis than normal hepatocytes.

Table 1: Serum concentrations (Means±SE) of biochemical substrates in cows of the retained placenta and control group

Parameter	Group 1 (n=6)	Group 2 (n=38)	P value
Non-esterified fatty acids (mmol/L)	0.53±0.06 ^a	0.84±0.06 ^b	0.050
Beta-hydroxybutyrate (mmol/L)	0.08±0.02 ^a	0.51±0.08 ^b	0.041
High density lipoprotein (mg/dL)	93.35±8.94 ^a	59.12±3.77 ^b	0.002
Total protein (g/dl)	8.15±0.36 ^a	6.62±0.13 ^b	0.000
Cholesterol (mg/dL)	106.00±10.23	86.29±4.82	0.132
Triglycerides (mg/dL)	10.17±2.20	12.42±0.68	0.242
Low density lipoprotein (mg/dL)	14.88±2.85	22.52±2.24	0.195
Very low density lipoprotein (mg/dL)	2.03±0.44	2.48±0.14	0.242
Glucose (mg/dL)	59.00±4.44	49.79±7.43	0.630
Albumin (g/dL)	3.25±0.13	3.07±0.07	0.352
Blood urea nitrogen (mg/dL)	11.18±0.82	13.98±0.85	0.210

Values bearing different letters in a row differ significantly.

We found numerically higher TG levels in RP cows (but not statistically important) compared with Group 1. The higher concentrations of TG in RP cows might have been resulted from more energy needs of the cows (Seifi *et al.*, 2007). On the other hand, Van Den Top *et al.* (1995) showed that mean plasma TG concentrations were not different between healthy and fatty liver cows after calving.

Our study also showed numerically lower postpartum levels of glucose (but not statistically important). According to Kaczmarowski *et al.* (1993), Sevinc *et al.* (2003) and Civelek *et al.* (2006b), liver dysfunctioning associated with fat accumulation would also lead to a slight reduction of gluconeogenesis. Furthermore, the dropped serum GLU concentration could be simply due to decrease of feed intake, thus deficient propionate.

These associations indicate that energy metabolism imbalance and postpartum NEB may contribute to the development of RP. Serum levels of NEFA and BHBA may be more useful to evaluate postpartum metabolic status of the cows and energy imbalance that might predispose them to RP.

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