Introduction

The abomasum displacement generally occurs in high producing dairy cows whatever their age, and especially in early lactation period [4]. It has been reported that displaced abomasum is closely associated with peri-parturient fatty liver (FL) [26] and, that fatty liver increases the incidence of displaced abomasum [4, 19]. Furthermore, the left sided displacement of abomasum was much more common than right sided displacement in dairy cows in the proportion of 8 to 1.

Routine biochemical parameters such as total bilirubin, glucose, total protein, albumin, triglyceride, cholesterol, HDL, LDL and VLDL blood concentrations and plasma AST/GGT activities are generally used for the evaluation of metabolic status [11]. The metabolic status should be evaluated in order to identify the incidence of fatty liver. GAAL et al. [7] has reported that determination of blood metabolites and enzyme activities could be useful for evaluation of metabolic disorders in dairy cows but parameters should be evaluated together for accurate diagnosis. On the other hand, abnormal lipid and lipoprotein concentrations are generally related with liver dysfunction and metabolic disorders [22]. In this respect, determination of apolipoprotein concentrations associated with the other routinely used biochemical parameters could be useful for the diagnosis of FL [28].

Apo B100 concentrations decrease in displaced abomasum cases as well in other metabolic disorders (ketosis, hypocalcaemia, downer cow syndrome and retained placenta) [13, 16, 18]. Furthermore, it has been recently reported that decrease of serum apo B100 concentrations are the obvious sign for FL [12, 14, 20]. Consequently, the objectives of the study are to examine the possible variations of the serum apo B100 concentrations during left sided displacement of the abomasum (LSDA cases) and to evaluate hepatic lipidosis in the affected cows.
Material and Method

1. Animals and samples: A total of 33 Holstein dairy cows, 3 to 8 year-old, calved 3-55 days prior to the study were used. Twenty-four cows were diagnosed for LSDA (LSDA group) whereas 9 clinically healthy cows served as control group. Milk production of cows was between 5000-6000 kg per annum. Systemic clinical examination and abdominal auscultatory percussion were performed for each animal. Cows with LSDA were examined transabdominally with ultrasonography using 3.5 MHz convex and 5 MHz sector (Pie Medical Scanner 250), when fluctuation and ping signs were positive [21]. Percutaneous needle aspiration was used in suspected cases, and abomasum content (fluid or gas) was aspirated where abomasum was displaced [8].

Blood samples were collected 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 were stored at -20°C until apo B100 assays were performed.

2. Biochemical and histological analyses: Serum apo B100 concentrations were determined by using commercially available bovine apo B100 plate SRID test kit (Bovine apolipoprotein measurement kit, Code no: P0116-1) according to manufacturer’s instructions. Each test sample, thought to contain bovine apo B, is placed in an individual test well. As the sample diffuses radially from the well into agar gel plate, a specific precipitin reaction occurs between bovine apo B and the specific antiserum to bovine apo B incorporated in the gel. A visible precipitin ring is formed. Since the area within this ring is directly proportional to the concentration of apo B in the test sample, measurement of the ring’s diameter allows calculation of hat apo B concentration, by comparison with the two known standard solutions.

Serum glucose, cholesterol, triglyceride, HDL, total protein, albumin, total bilirubin concentrations and serum AST (EC 2.6.1.1) /GGT (EC 2.3.2.2) activities were determined by using commercially available test kits. All serum samples were analyzed using an auto-analyser in one assay. On the other hand, VLDL and LDL were calculated according to the following equations:

VLDL = triglyceride / 5;
LDL = total cholesterol - (HDL cholesterol + triglyceride / 5) [1].

Hepatic biopsy samples were obtained from liver under local anaesthesia (Vilocain® (Lidocain HCL 20 mg/ml, Adrenalin 0.01 mg/ml, VILSAN, Ankara, Turkey, 1ml/cm²) with percutaneous biopsy needle (14-16g) using ultrasonography [24]. The samples were fixed in formaldehyde-calcium solution at 4°C in the dark for a day and then were delivered to laboratory for histopathological examination. Prepared cross-section samples (12 μm) were stained with Sudan Black [2] and Oil Red O [15]. After preparation, percentage of hepatic lipidosis was determined using ocular square micrometer (Olympus) in the slides (μm²/100μm²x1000 magnification). According to the degree of hepatic lipidosis, fatty liver was classified as mild (< 20%), moderate (20-40%) and severe (> 40%) [6].

3. Statistical analysis: Data was analyzed with two-sample t-test in Minitab 12 statistical program for Windows. Results are expressed as means ± standard deviations for clinical parameters and standard errors for biochemical parameters. Comparisons between two groups were determined significant at p<0.05 level. Correlations were calculated using the Pearson correlation method.

Results and illustrations

1. Clinical examination: The history includes a lack of appetite for grain, also slight weakness and decreased milk production (significant but not dramatic) for all the cows in LSDA group, while temperature (38.52 ± 0.42°C), heart rate (62.87 ± 14.40 Beats / min) and respiratory rate (36.17 ± 9.25 Breaths / min) were normal in all cows with LSDA. Furthermore, rumen motility was commonly reduced in frequency in the LSDA group, and hydration was subjectively abnormal only in one cow. Besides mentioned non-specific clinical signs, little amount of stool (n = 24) which turned into mud (n = 3) or no defecation (n = 1) were observed in the cows with LSDA. On the other hand, fluctuation (n = 24) and pings (n = 24) were detected simultaneously for all cows in the LSDA group in abdominal auscultatory percussion performed at 9-13º intercostal area. All the cases were confirmed by using ultrasonography. Also, percutaneous needle aspiration test gave positive results (pH less than 4.5) in suspected cases (n = 3).

2. Serum apo B100 determination and liver histology: The apo B100 concentrations were markedly decreased in the LSDA group (p < 0.001) and the lipid metabolism alterations in the affected cows were also confirmed by the measurements of the other biochemical markers. Serum triglyceride, cholesterol and lipoprotein (HDL, VLDL, LDL) concentrations were dramatically lowered compared to the control group (p < 0.001) (Table 1).

Significant increases of serum total bilirubin concentrations (p < 0.001), and of AST (p < 0.001) and GGT (p < 0.01) activities were also observed in the LSDA group compared to healthy cows. Moreover, the serum total protein concentration (p < 0.01) and the albuminemia (p < 0.05) were significantly decreased in the diseased cows. Slight but not significant decreases of glycaemia were also recorded in this group (Table 1).

While lesions of hepatic lipidosis were almost undetectable in the healthy cows, all the affected cows presented intense and extended histological signs of fatty liver (Table 2). In this group, the average score of hepatic lipidosis was 41.5 ± 2.5%. Severe fatty liver (from 41% to 68%) was detected in 13 cows (54%) (Figure 1) and moderate damage (from 22% to 37%) were evidenced in 11 cows (46%). Moreover, the degree of hepatic lipidosis was highly negat-
SERUM APOLIPOPROTEIN B100 CONCENTRATIONS IN DAIRY COWS WITH LEFT SIDED DISPLACED ABOMASUM

Generally correlated to apo B100 concentrations ($r = -0.591$, $p < 0.001$). Furthermore, the apo B100 concentrations positively correlated with serum cholesterol ($r = 0.506$, $p = 0.003$), serum HDL ($r = 0.543$, $p = 0.001$), serum VLDL ($r = 0.488$, $p = 0.002$) and serum triglyceride concentrations ($r = 0.406$, $p = 0.01$).

Discussion

In the present study, with fluctuation and ping sings, the main observed clinical signs of LSDA were a decreased feed intake, a low milk production and also slight weakness. The percutaneous needle aspiration test has confirmed the diagnosis in 12.5% of cows. Biochemical analyses evidenced hepatic cell injury (significant increases of serum AST and GGT activities) and liver dysfunction (changes in serum bilirubin, protein and albumin concentrations and slight, but not significant, decrease of glycaemia) in cows with LSDA. Furthermore, marked decreases of serum triglyceride and cholesterol concentrations were observed in diseased animals, suggesting a severe impairment of lipid metabolism. The dramatically lowered concentrations of lipoproteins (HDL, VLDL and LDL) and of serum apo B100 concentrations confirmed that lipid transport (decreased lipoprotein synthesis or release from the liver) was deeply affected during abomasum displacement. Liver injury in LSDA group was also confirmed by histological analysis which showed moderate to severe fatty liver, whereas no or very few hepatic lipidosis was observed in the healthy cows. All these results showed that abomasal displacement on the left side was associated with fatty liver in the post-parturient cows and that the observed liver damage was severe in the most cases.

A strong relationship between FL and displaced abomasum has been already reported by HERDT et al. [9]. Furthermore, SEVINCE et al. [26] found the average FL was 31.5 ± 6.1% in the displaced abomasum cases. Fatty liver would directly lead to the alteration of lipid profiles observed in LSDA affected cows. Indeed, the decrease of the release of triglyceride as VLDL from the liver may be the result of severe FL [22]. The decrease of serum LDL could directly resulted from the reduction of VLDL synthesis and / or release by the liver and indirectly from increased catalolism, whereas HDL decrease might be associated with low

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<th>Table I. — Serum concentrations of biochemical substrates (Apo B100, Cholesterol (CHOL), Triglyceride (TG), Glucose (GLU), Total Bilirubin (TBIL), Total Protein (TP), Albumin (ALB) and serum enzyme (AST, GGT) activities in the left sided displaced abomasum (LSDA) group and in the control group of post–parturient cows. Results are expressed as Means ± Standard errors (SEM).</th>
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<td><strong>Control</strong></td>
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<td><strong>n = 9</strong></td>
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<td>Apo B100 (g/L)</td>
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<th>Table II. — Rate of hepatic lipidosis in LSDA cows and in the healthy cows. Results are expressed as means ± Standard errors (SEM).</th>
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concentrations of cholesterol because 60% of HDL was constituted by cholesterol [22]. However, changes in serum triglyceride and cholesterol concentrations could be simply due to decrease of feed intake, because most triglycerides and cholesterol in ruminants are of intestinal origin [27]. KATOH [13] and MAZUR et al. [17] have shown that important decreases of serum concentrations of HDL, LDL and VLDL occurred naturally after birth and after experimental FL cases. But in the present study, lipoprotein concentrations remained elevated in the control group compared to the LSDA affected cows. The decreases of lipoprotein concentrations was one of the most important metabolic feature occurring in FL and related diseases in cows [14]. Serum apolipoprotein concentrations fairly decreased in cows with FL and related metabolic disorders [13, 16]. Moreover, it has been found that apo B100 concentrations were significantly lower (average 56 ± 8 μg/ml) in postpartum dairy cows with displaced abomasum [19], retained placenta and ketosis [10]. In the present study, there was an important decrease (p < 0.001) for serum apo B100 concentrations in dairy cows with LSDA. It could be caused by the loss of apo B100 synthesizing and releasing capability of the liver due to severe FL in LSDA cases [18, 19]. Also a high negative correlation between apo B100 concentrations and degree of hepatic lipodosis was evidenced. However, CIVELEK et al. [5] reported significant decreases of serum apo B100 concentrations in obese heifers which were not associated with liver fat infiltration.

The other biochemical markers also emphasized liver injury. Albeit not specific for liver in cow, the increases of serum AST activities indicated soft tissue damage, probably liver damage. As a great amount of GGT is in bile duct epithelia, this enzyme is a sensitive and specific marker for cholestasis. Serum bilirubin concentrations are inversely related to the severity of hepatic disease. Although individual changes in serum apo B100 concentrations could be related to the severity of hepatic lipodosis. Although individual changes in serum apo B100 concentrations should be interpreted carefully, the determination of serum apo B100 concentrations would avoid liver biopsy and provide a non invasive approach of hepatic lipodosis.

Acknowledgement

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References

19. — OIKAWA S. and KATOH N.: Reduced concentrations of apolipo-