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**Αιματολογικές παράμετροι σε αγελάδες
γαλακτοπαραγωγής με μετατόπιση ηνύστρου
αριστερά και ηπατική στεάτωση**

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■ Haematological findings in dairy cows with concurrent left abomasal displacement and hepatic lipidosis (fatty liver)

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■ Αιματολογικές παράμετροι σε αγελάδες γαλαктоπαραγωγής με μετατόπιση ηνύστρου αριστερά και ηπατική στεάτωση

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ABSTRACT. The main aim of the present field study was to evaluate various haematological parameters in cows with left abomasal displacement (LDA) and concurrent hepatic lipidosis (fatty liver) of different severity. A further objective was to compare these haematological values among animals with concurrent LDA and fatty liver that finally died or recovered. Sixty eight Holstein dairy cows with LDA and 110 control cows were included in the study. Blood and liver biopsy samples were obtained during standing LDA surgery. Liver tissue was examined histologically and classified according to the severity of fatty liver. Haematological analysis and biochemical determination of total lipids and triglycerides concentration of liver tissue were performed. The majority of the haematological parameters evaluated were within normal range. Haematocrit and haemoglobin median values were significantly lower in all LDA cows compared to controls, but no difference was recorded among fatty liver severity groups. Haematological parameters showed no significant difference in animals that finally died compared to the ones that recovered. Haematological parameters were not significantly influenced by the concurrence of LDA and fatty liver in dairy cows.

Keywords: LDA, fatty liver, dairy cows, haematology

ΠΕΡΙΛΗΨΗ. Κατά το αρχικό στάδιο της γαλακτοπαραγωγής, οι περισσότερες γαλακτοπαραγωγές αγελάδες υψηλών αποδόσεων βρίσκονται σε αρνητικό ενεργειακό ισοζύγιο, καθώς οι ενεργειακές απαιτήσεις για τη γαλακτοπαραγωγή αδυνατούν να καλυφθούν από τη διατροφή, με συνέπεια την κινητοποίηση του λιπώδους ιστού και την απελευθέρωση λιπαρών οξέων. Η έντονη λιπώδης οδηγεί σε υπερευαισθησία λιπιδίων στο ήπαρ, κατάσταση που ονομάζεται ηπατική στεάτωση (HST) ή λιπώδης εκφύλιση του ήπατος. Μεταξύ των πολλών σχετιζομένων με την HST παθολογικών καταστάσεων συγκαταλέγεται

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και η μετατόπιση του ηνύστρου αριστερά (ΜΗΑ), κατάσταση αρκετά συχνή και με σημαντικές οικονομικές επιπτώσεις. Η ανορεξία που προκαλεί η ΜΗΑ οδηγεί σε περαιτέρω επιδείνωση του αρνητικού ενεργειακού ισοζυγίου, η οποία καταλήγει σε σοβαρού βαθμού ΗΣΤ. Ακόμα και μετά την επιτυχημένη χειρουργική αποκατάσταση της ΜΗΑ, σε πολλές αγελάδες η κλινική εικόνα δε βελτιώνεται και αυτό σχετίζεται και με τη σοβαρότητα της ΗΣΤ. Η ΜΗΑ επηρεάζει το ισοζύγιο υγρών του ζώου, την κλινική και αιματολογική του εικόνα, ενώ η συνύπαρξη διαφόρου βαθμού ΗΣΤ αποτελεί βασική παράμετρο που επιπλέκει τη νόσο και επηρεάζει την πρόγνωση. Στη βιβλιογραφία υπάρχουν μελέτες σχετικές με τα ευρήματα της αιματολογικής εξέτασης σε περιστατικά με ΜΗΑ ή με ΗΣΤ. Υπάρχει όμως σημαντική έλλειψη δεδομένων που να αφορούν περιπτώσεις ταυτόχρονης προσβολής των αγελάδων από ΜΗΑ και ΗΣΤ. Σκοπός της παρούσας μελέτης ήταν ο προσδιορισμός των αιματολογικών παραμέτρων σε περιστατικά αγελάδων με συνύπαρξη ΜΗΑ και ΗΣΤ διαφόρου βαθμού, και η σύγκριση των ευρημάτων μεταξύ των ζώων που ιάθηκαν μετεγχειρητικά κι εκείνων που πέθαναν. Στη μελέτη συμπεριλήφθηκαν 68 αγελάδες γαλακτοπαραγωγής, φυλής Holstein, με ΜΗΑ και 110 ζώα ίδιας φυλής ως μάρτυρες. Από κάθε ζώο λήφθηκαν δείγματα αίματος, από τη σφαγίτιδα φλέβα και ήπατος, μέσω λαπαροτομής κατά τη χειρουργική αποκατάσταση της ΜΗΑ και μέσω διαδερμικής βιοψίας στους μάρτυρες. Η αιματολογική εξέταση έγινε με αυτόματο αναλυτή. Στα δείγματα ηπατικού ιστού έγινε ιστοπαθολογική εξέταση και υπολογίστηκε ημιοσοτικά, με κατάλληλη μέθοδο κατά τη μικροσκοπηση, η σοβαρότητα της ΗΣΤ. Επιπλέον, στον ηπατικό ιστό έγινε και βιοχημικός προσδιορισμός των ολικών λιπιδίων και των τριγλυκεριδίων. Όλα τα ζώα με ΜΗΑ παρουσίασαν ΗΣΤ, ενώ σοβαρού βαθμού ΗΣΤ παρατηρήθηκε σε όλα τα ζώα που τελικά πέθαναν. Στην πλειονότητά τους, οι αιματολογικές παράμετροι είχαν τιμές εντός των φυσιολογικών ορίων. Με εξαίρεση τις τιμές του αιματοκρίτη και της αιμοσφαιρίνης, δεν παρατηρήθηκαν στατιστικά σημαντικές διαφορές ανάμεσα στα ζώα με ΜΗΑ και ΗΣΤ και στους μάρτυρες. Επίσης, δεν καταγράφηκαν σημαντικές διαφορές στις αιματολογικές παραμέτρους ανάμεσα στα ζώα που ιάθηκαν και σε αυτά που πέθαναν.

Λέξεις ευρετηρίασης: αιματολογία, ηπατική στεάτωση, μετατόπιση ηνύστρου αριστερά, αγελάδες γαλακτοπαραγωγής.

INTRODUCTION

Nearly all and especially high-producing dairy cows remain in energy-deficient state during the early stage of lactation because energy requirements for milk production exceed the capacity for energy intake (Collins and Reid 1980). This situation results in the mobilization of the body's fat stores (Morrow et al. 1979). Excessive lipomobilization increases the fat accumulation in liver cells (Cebra et al. 1997) and leads to development of hepatic lipidosis, a condition associated with health and production problems (Reid and Roberts 1983). The risk of disease appears to increase in parallel with liver fat concentration (Zerbe et al. 2000). Among hepatic lipidosis associated diseases is left displacement of abomasum (LDA) (Holtenius and Niskanen 1985; Rehage et al. 1996; Van Winden et al. 2003), a common problem in high-producing dairy cows in early lactation (Van Winden et al. 2003), with important economic effects (Detilleux et al. 1997). A typical clinical sign of LDA is the gradual loss of appetite, which further aggravates energy deficit and results in enhanced lipomobilization and severe fatty liver (Holtenius and Niskanen 1985). Despite the successful surgical treatment, the general condition of cows with LDA may remain poor during

the post-operative period (Rohn et al. 2004a), because post-surgical convalescence of cows with LDA is related to the severity of hepatic lipidosis (Rehage et al. 1996; Kalaitzakis et al. 2006).

LDA, also, affects the hydration status, the blood cells counts and the health and, therefore, productivity of the cows (Rohn et al. 2004a). Reports for the haematological findings in LDA (Rohn et al. 2004a), as well as in fatty liver cows separately (Reid et al. 1984) have been published. However, the coexistence of LDA and various degree of fatty liver may have different impact on haematology of dairy cows, even though there has not been adequate research in this.

Taking all the above information into account, the main aim of the present field study was to evaluate various haematological parameters in cows with LDA and concurrent fatty liver of different severity. A further objective was to compare these haematological values in the animals with concurrent LDA and fatty liver, which finally either died or were recovered.

Materials and methods

Animal selection

For the purpose of this study a total of 68 Holstein dairy cows (55 cows and 13 first calved heifers) from 28

dairy farms in Northern Greece were selected. The animals were derived from 28 dairy farms which had a total population of 2,950 (mean 101 cows per farm) dairy cows; no herd contributed more than 3 cows. Samples were collected during a 15-month period. On-farm visits took place after the veterinarians' diagnosis of LDA and at the earliest possible time after LDA diagnosis. The patient's history was taken (age, parity number, days in milk- DIM), recent health and production problems, days from the onset of clinical signs of LDA), the body condition score (BCS) (scale 1-5) was recorded and a clinical examination was carried out, always by the same veterinarian. Then, surgical correction of LDA was performed on all cows after local anaesthesia via laparotomy in the right flank, in a standing position. Reposition of the abomasum and omentopexy were done according to Dirksen (1967). The same protocol was followed on all of the cows right after surgery by the herd veterinarians. It included (once daily, for 3 consecutive days) 500 ml of calcium borogluconate 20% intravenously (IV), 500 ml of dextrose 35% IV, 5 l of NaCl 0.9% IV, rumen stimulating powders, ceftiofur (Excenel®, Pfizer) and flunixin meglumine (Finixin®, Intervet / Schering - Plough). All of the cases mentioned above with evidence of infectious or overt disease not related to LDA or liver disease (such as fever, vaginal discharge, metritis and mastitis) were excluded from the study; finally, a total of 68 Holstein dairy cows were included. The study protocol was performed in compliance with institutional guidelines and the European Union legislation for research on animals. All owners were informed and gave consent for the cows to be included in the study and to undergo the testing procedures.

Definitions

Groups

Cows with LDA were divided into different groups based on the histologically determined degree of fatty liver. None of the cows was classified in the first two Grades of Fatty Liver (GFL), GFL 0 and GFL 1, in which there were no histologically visible lipids. The GFL, after taking into consideration the triglyceride (TG) hepatic content concentrations in the liver tissue, was classified as follows: GFL 2 as mild fatty liver, GFL 3 as moderate, GFL 4 as moderate to severe and GFL 5 as severe fatty liver. All animals were followed up for their post-surgical outcome, health and productivity. All of the animals that did not show any improvement

after surgical correction of the LDA and finally died within the next two weeks had severe fatty liver (classified as GFL 5). So, cows of the study were finally divided into the following 4 groups according to the GFL degree and post-surgical convalescence: Group 1 (GFL 2 and GFL 3, all animals recovered), Group 2 (GFL 4, all animals recovered), Group 3 (GFL 5, all animals recovered) and Group 4 (GFL 5, all animals died).

Control animals

In order to obtain reference values of the tested parameters, blood and liver samples were obtained from 184 cows. The source was the same 28 dairy farms that provided the LDA cows for the study. In each farm, for every referred LDA cow, 3 cows were selected as controls and were sampled. The selection of the control group was based on the following criteria: 1. there was no disease history during the current milking period; 2. they were clinically healthy at the time of sampling; 3. they were at the same lactation stage (<60 DIM) as the LDA cows; 4. they were over 2.5 years old; and 5. there was no histologically visible fat in their liver (GFL<2), as was revealed during the histopathological examination that followed the biopsy. All samples were obtained and evaluated with exactly the same methods as the LDA cows of the study, with the exception of liver biopsy which was performed transcutaneously. After histological examination, from the total sample, 74 cows were excluded due to histologically detected liver fat (classified as GFL=2). Consequently, a total of 110 healthy Holstein dairy cows (mean 2.4 cows per herd and no more than 4 cows from each herd), which fulfilled all the aforementioned criteria, were included as control group in the study. Their liver was classified as GFL 0 and 1.

Sample collection

Blood samples were taken from each cow just before the operation, by jugular vein puncture using an 18-gauge needle in vacuum glass tubes containing sodium citrate 3.8% as anticoagulant, supplied with the haematological diagnostic kit (IDEXX, Bovine Sample Preparation Kit, QBC® Vet Autoread TM Hematology Analyzer, Sample Preparation Procedures for Bovine Venous Blood. Idexx LaBor). Whole blood samples were transferred to the laboratory and evaluated within 6 hours after collection.

Liver biopsies were performed during the surgical operation, through the open abdominal wall after reposition of the abomasum, using a liver biopsy needle (Berlin Model, 2.5 mm X 25 cm, Eickemeyer Medizintechnik für Tierärzte, Tuttlingen, Germany). Liver puncture was performed once and the obtained sample (150-350 mg) was divided into 2 parts; the first was fixed in 10% buffered formalin for histopathological examination and the other had been kept frozen (-20°C) until used for biochemical determination of total lipids (tLPD) and triglycerides (TG).

Liver biochemical analysis

tLPD concentration in the liver tissue was determined after chlorophorm - methanol - water extraction (Folch et al. 1957). For TG concentration, the total lipids extracted were saponified (1 ml KOH 0.5 N and 1 ml absolute Ethanol), 60 min in 70°C, and the triacylglycerol produced was measured by the method of Eggstein and Kuhlmann (1974), using a spectrophotometer (Hitachi U-2000, Hitachi Ltd, Tokyo, Japan).

Haematological analysis

Haematological analysis included the determination of haematocrit (HCT), haemoglobin (Hb), white blood cells count (WBC), granular cells count (GRAN), lymphocytes count (LM) and platelets count (PLT) with the aid of the veterinary haematology analyzer IDEXX QBC®, using the specific setup for bovine samples.

Histological examination

Biopsy specimens were fixed in neutral-buffered 10% formalin solution, cut into sections of 3 to 4 µm in thickness and stained with Hematoxylin and Eosin. Specimens were examined via light microscopy for lipid content. Liver fat content was classified according to a 6-point scale of severity of fatty infiltration (i.e., GFL) (Mertens 1992). The range of GFL scores varied from 0 (no fat droplets visible) to 5 (panlobular fatty infiltration). The liver tissue was evaluated in 3 concentric regions in the area from the central vein to the portal triad of the hepatic lobule. In each of those regions, scores were assigned according to the following guidelines: no lesion = 0 points; cloudy swelling = 0.5 points; cloudy swelling with small vacuoles (representing lipids washed out by alcohol during staining) = 1.0 point; many small vacuoles =

2.0 points; medium-sized vacuoles = 3.0 points; large vacuoles = 4.0 points; and appearance of stamp cells (hepatic cells that contain a large volume of lipid to the extent that cell contour is altered and nuclei are displaced) = 5.0 points. The most substantial lesion was used to assign the score for each region of the hepatic lobule.

For example, if in a region large vacuoles (4.0 points) were observed in some cells, but small vacuoles and cloudy swelling (1.0 point) were observed in others, a score of 4.0 points was assigned. Points were summed up and a score for the lobule was obtained. For every specimen, 5 lobules were scored and the mean score was determined. From the mean scores, each cow was classified as having 1 of 6 degrees of fatty liver (GFL 0 to 5) according to the following index: GFL 0 = 0 points; GFL 1 = 0.5 to 1.0 points; GFL 2 = 1.5 to 4.5 points; GFL 3 = 5.0 to 7.0 points; GFL 4 = 7.5 to 9.5 points; and GFL 5 > 9.5 points.

Statistical Analysis

Analysis was performed using a commercial software program (SPSS, version 16.0, SPSS Inc, Chicago, IL). Because the assumptions of ANOVA (homogeneity of variances was tested by Levene test and normality by Kolmogorov-Smirnov test) were not satisfied by the fatty liver-LDA groups (even after transformations of data), the values of parameter among the control animals and the four groups of fatty liver-LDA cows were compared using the Kruskal-Wallis non-parametrical test. When significant differences were observed among the groups, the Mann-Whitney test was used for pair-wise comparisons, in order to identify which group medians were significantly different. For all tests, values of $p < 0.05$ were considered significant.

RESULTS

Histological examination

All the 68 selected cows had an increased lipid concentration, visible as vacuoles in light microscope, consequently classified in the fatty liver degree above 1 (GFL 2-5). The 68 cases (13 heifers among them) were classified as follows: 4 in GFL 2, 10 (4 heifers) in GFL 3, 13 in GFL 4 and 41 (9 heifers) in GFL 5. Particularly in GFL 5, 20 animals recovered (15 cows - 5 heifers) and 21 died (17 cows - 4 heifers) within the next 4 weeks following surgery (Table).

Table. Values of blood, liver, and clinical variables from healthy lactating reference cows and lactating Holstein cows with LDA and various degrees of fatty liver.

Parameter	Control Group		Group 1		Group 2		Group 3		Group 4	
	n=110		(GFL 2 + GFL 3) Recovered, n = 14		(GFL 4) Recovered, n = 13		(GFL 5) Recovered, n = 20		(GFL 5) Died, n = 21	
	Values in the 2.5 th - 97.5 th percentiles	Median	Mean (SE)	Median	Mean (SE)	Median	Mean (SE)	Median	Mean (SE)	Median
HCT (%)	24.0-42.4	31.95 ^x	27.37 (1.09)	27.30 ^y	28.64 (1.23)	26.80 ^y	26.74 (0.87)	26.60 ^y	29.44 (1.22)	27.70 ^y
Hb (g/L)	82-141	109 ^x	91.0 (0.36)	88.5 ^y	94.0 (0.39)	87.0 ^y	90.5 (0.29)	92.5 ^y	99.6 (0.39)	94.0 ^y
WBC (x10⁹/L)	3.9-13.4	7.0 ^x	9.57 (2.53)	5.20 ^x	7.31 (1.03)	6.90 ^x	8.31 (0.88)	7.10 ^x	10.68 (2.40)	6.50 ^x
GRAN (x10⁹/L)	1.00-8.58	3.2 ^x	3.12 (0.76)	1.70 ^x	4.18 (0.94)	3.10 ^x	4.60 (0.92)	3.30 ^x	5.019 (1.71)	2.80 ^x
GRAN (%)	20.42-74.8	44.0 ^{x,y}	35.07 (5.02)	29.00 ^{x,z}	47.92 (6.31)	56.00 ^x	48.35 (14.87)	48.00 ^x	40.71 (5.19)	31.00 ^x
LM (x10⁹/L)	2.20-8.25	3.55 ^{x,y}	6.44 (2.28)	3.60 ^x	3.23 (0.28)	3.30 ^{x,z}	3.70 (0.29)	3.60 ^x	5.65 (1.67)	3.50 ^x
LM %	25.20-79.6	56 ^{x,y}	64.36 (4.96)	68.50 ^{x,z}	52.85 (6.17)	44.00 ^x	51.70 (4.85)	52.00 ^x	59.29 (5.19)	69.00 ^x
PLT (x10⁹/L)	172.1-628.	387.5 ^x	428.1 (63.9)	354.5 ^x	324.9 (37.6)	359.0 ^x	376.6 (32.9)	346.5 ^x	406.2 (40.3)	423.0 ^x
tLPD (mg/g)	8.58-22.17	14.89 ^x	202.5 (14.1)	203.80 ^y	278.78 (15.9)	270.0 ^z	202.50 (14.1)	289.5 ^y	352.46 (9.5)	366 ^w
TG (mg/g)	4.57-19.0	12.05 ^x	24.60 (1.63)	24.46 ^y	40.51 (4.55)	36.00 ^z	75.15 (7.12)	69.45 ^y	114.24 (8.89)	106.70 ^w

Within a row, different superscripts (x,y,z,v,w) denote significant ($p < 0.05$) differences among groups' medians. (GFL; Grades of Fatty Liver, WBC; White blood count, GRAN; Granular cell count, LM; Lymphocytes count, PLT; Platelets count, TG; Triglyceride concentration in wet hepatic tissue, tLPD; Total lipid concentration in wet hepatic tissue).

Liver biochemistry

Total lipids and triglycerides concentration of the hepatic tissue showed an increase proportionally to the severity of the fatty liver, increasing in parallel with the ascending degrees of fatty liver ($p < 0.05$) (Table). Especially within GFL 5, TG values of the animals that died (Group 4) (maximum values over 200 mg/g) were higher compared to those that recovered (Group 3) ($p < 0.05$).

Haematology

HCT and Hb median values were significantly lower in all LDA cows compared to controls, but no difference was recorded among fatty liver severity groups (Table).

White blood cells' numbers were not significantly

different between the various groups and the controls; similar results occurred for LM and GRAN numbers (Table). Regarding LM and GRAN, some very high values (up to $38.1 \times 10^9/L$ and $17.6 \times 10^9/L$ for LM and GRAN respectively) were recorded in animals that died (Group 4), but, overall, there was no median significant difference between the groups and controls.

Platelets' number, which varied extensively, were of wide range in all groups, but no significant difference among them was recorded.

DISCUSSION

This study aimed to evaluate various haematological parameters of LDA cows with concurrent fatty liver. All the animals included were field cases and the sample collection was done at the earliest

possible time after LDA diagnosis. Most of the literature on LDA cases is based on animals that were hospitalized in clinics (Holtenius and Niskanen 1985, Geishauser et al. 1996, Rehage et al. 1996, Rohn et al. 2004a, Ahmed 2004, Grünberg et al. 2005). Generally, since the majority of cows with LDA are referred cases, the time from diagnosis or onset of clinical symptoms to admission is about 2-5 days (Rohn et al. 2004a). Moreover, usually only the better and younger ones are referred to clinics, due to the overall cost and transportation (Rohn et al. 2004b). Due to this "selection", only a few studies deal with field LDA cases and, therefore, there is a relevant shortage of published data concerning haematology of field-cases.

The haematological analysis was performed with an automatic analyzer that is easy and practical to handle and can be used by practitioners or small clinics. For HCT, no significant difference between fatty liver groups was evident in the present study. On the contrary, in another research, haemoconcentration and increase in HCT and Hb were recorded in LDA cows, as liver triglyceride content was increasing (Ahmed 2004). HCT was, in the majority of cases, within normal (reference) range (Howard and Smith 1999, Panousis et al. 2001) and only some sporadic cases of elevated HCT were recorded, due to dehydration, in cows with severe fatty liver that finally died. The normal or subnormal HCT is in accordance with the earlier observations in LDA cows (Rohn et al. 2004a).

Similar results with HCT were recorded for Hb. The early lactation stage of the LDA cows, also, provides the possible explanation for these findings. During late pregnancy and onset of lactation, slight anaemia is likely to occur (Stöber and Gründer 1979). In a former study of hepatic lipidosis, without LDA, no significant impact of liver triglyceride content on Hb was recorded (Reid et al. 1984). In another study with LDA cows with hepatic lipidosis, Hb appeared to be

increased due to haemoconcentration (Ahmed 2004).

The WBC, GRAN and LM were not significantly different in LDA- fatty liver cows compared to the control ones. The median values of all groups were more or less within the normal values (Howard and Smith 1999, Panousis et al. 2001). There are previous reports concerning decreased WBC and simultaneous decrease in both GRAN and LM in cows with fatty liver (Morrow et al. 1979; Reid et al. 1984) and fatty liver with LDA (Ahmed 2004). There are studies on fatty liver cows without LDA which revealed increased WBC (Padilla-Arenalles et al. 2007), while others reported no significant difference in WBC between cows with and without fatty liver (Wentick et al. 1997, Zerbe et al. 2000). In the present study, only a few leukocytosis cases were recorded, due to the selection criteria of the animals, as eligible for the study were only those without any profound infectious disease. On the other hand, some low WBC values were recorded in all groups. However, no significant difference was evident among groups, probably due to the wide variation of values. Given that cattle, in general, show unreliable variations with respect to the numbers of white cells and alteration of the ratio of granulocytes to lymphocytes (Taylor 2006), it is obvious that the assessment of WBC cannot explain sufficiently the difference between various degrees of fatty liver in field cases where fatty liver concurs with LDA.

Conclusion

Haematological values were not significantly influenced by the concurrence of LDA and hepatic lipidosis in dairy cows. Haematocrit and haemoglobin median values were significantly lower in all LDA cows compared to controls, but no difference was recorded between fatty liver severity groups. Haematological values showed no significant difference in animals that finally died compared to the ones that recovered. ■

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