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MORPHOMETRIC CHARACTERISTICS OF LIVER TISSUE IN COWS DURING EARLY LACTATION*

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Summary: *Early lactation cows usually express negative energy balance (NEB), hypoglycemia and greater lipolysis in in adipose tissue. This factors are causing higher degree of lipogenesis and accumulation of triacylglycerol in fatty vacuoles in hepatocytes. The aim of this work is determination of dimensions and optical density of fatty vacuoles and nuclei in liver tissue during early lactation. Experiment was performed on 10 Holstein-Friesian cows. Hepatic tissue samples were collected by biopsy, 3-15 days after calving. Sampels were carefully processed, staind with HE and Sudan and examined microscopically. According to results of previously analysed metabolic profile two group of cows were determinated and confirmed by microscope evaluation of liver tissue. First group showed medical report which indicate fatty liver existence. Second one showed physiological report. Comparing dimensions and optical density of fatty vacuoles of both group, we have noted that first group have significanly greater dimensions of fatty vacuoles, reduced dimensions of nuclei, reduced level of optical density of nuclei and higher level of optical density of fatty vacuoles. Relationship between fatty vacuole and nuclei diameters was negative. This can be related with fatty degeneration, necrosis or apoptosis in fatty infiltrated hepatocytes.*

Keys words: *morphometry, liver, early lactation, cows.*

INTRODUCTION

In early lactation leads to large metabolic changes in cattle, a major change is a negative energy balance. Then it happens an increased lipid mobilization (Cincović et al., 2012; Đokovic, et al., 2014) in order to use fat for energy purposes, but the cows become prone to developing ketosis and fatty liver. As a consequence of lipolysis, increases concentration of the non esterified fatty acids (NEFA) in the bloodstream. This hairdryer occurs as a result of endocrine and metabolic changes, primarily due to the presence of insulin resistance (Cincović et al, 2014).They are deposited in hepatocytes in the form of triglycerides (TGC), forming fatty vacuoles, leading to fatty liver hepatocytes

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(Mihajlovic and Jovanovic, 2008), which changed its histological, morphometric and metabolic characteristics (Đokovic et al, 2011).

Size and dimensions of fat vacuoles, as well as their optical density indicates the degree of fatty liver. The flow of NEFA and fatty degeneration of hepatocytes caused a series of microscopically visible changes that are manifest in all organelles, and they can be seen in the nucleus in the form of changes in size, shape and optical density (Đoković i Šamanc, 2004). The basic structures of the liver unit are hepatocytes. Hepatocytes are liver lobules arranged radially, and in the spaces between them are hepatic sinusoid. The diameter of each of hepatocytes is 20-30 microns, eosinophilic cytoplasm, contains one and sometimes two nuclei, lysosomes, has abundant agranulirani and granular endoplasmic reticulum, which creates clusters of basophil-corpuscles. An important component of the hepatocyte of glycogen granules, involved in maintaining normoglycemia as well as the mitochondria. Lipid droplets are volatile component of the cytoplasm of hepatocytes, and their quantity is changed in accordance with the physiological state of the organism. Lipids in liver hepatocytes deposited in the form of triglyceride-TGC (Bobe et al, 2004).

The aim of our study was to examine the morphometric properties of liver tissue in early lactation as a function of fatty infiltration of hepatocytes.

MATERIAL AND METHODS

The experiment included 10 Holstein-Friesian cows: 5 cows healthy control group and 5 cows with a high concentration of ketones and bilirubin in the metabolic profile and a low concentration of triglycerides. The cows were in early lactation period of 3 to 15 days after calving.

To identify the site at which to insert the needle, first identify the 10th intercostal space. Remember there are 13 ribs and 12 intercostal spaces, so the 10th intercostal space is the third from the last intercostal space. Then draw an imaginary line from the tuber coxae (hook) to the elbow. The point at which this line intersects the 10th intercostal space is the point at which to insert the needle.

The tissue was processed and stained with HE stain and Sudan, according to standard procedures. From each sample of the liver is made two histological blocks and from each blocks 2 micrograms preparations. At each micrograms was analyzed 100 hepatocytes, selected randomly. Calibration was performed by microscopic measurement. All samples were photographed, and the photographs processed in ImageJ software. Stereological procedures were analyst according to basic principles described by Reid (1980).

The optical density and the dimension of the nucleus and of fatty vacuoles were calculated by the software. We examined the correlation between measured parameters.

RESULTS AND DISCUSSION

Cows were grouped into two main groups based on histological findings. The physiological findings with the presence of small fat droplets in the cell, which visually does not exceed the size of the sail and make up less than 10-15% of the cells. Another finding is the finding of typical fatty liver, which is histologically characterized by infiltration of centrolobular fatty vacuoles, while in one case there was infiltration through the entire lobulus, it is dominated by vacuoles filled with grease that completely alter cell histology. These two characteristic findings are shown in Figure 1 and 2. The optical density of nuclei was 401477.2 at physiological findings, ie 171361.3 in preparations with fatty liver ($p < 0.001$). In cows with fatty liver found significantly lower optical density of the nucleus. The diameter of the nucleus was also significantly lower in samples with fatty liver disease compared to healthy (7353.3: 5194.8 nm) ($p < 0.001$). The results are shown in Table 1. The optical density of the fatty vacuoles was 2258360.2 with fatty liver, or in the physiological findings 708578.1. The diameter of fat vacuoles with physiological findings amounted to 4658.7 nm, while the mixture with a fatty liver amounted to 19901.9 nm. The results are shown in Table 2. The correlation between the diameter of the core and of fatty vacuoles is negative: $r = -0.85$ ($p < 0.01$). Regression analysis is shown in Figure 3 and Table 3 and 4. It was also found negative correlation between the optical density of nucleus and fat droplets, and this relationship towards to linearity ($p < 0.1$) (results not shown).

Liver biopsy is the only reliable method to determine severity of fatty liver in dairy cattle in the transitional period. Blood, urine and milk metabolites or blood enzyme activity have been proposed as diagnostic tools. Our previously investigation demonstrated that in healthy transitional cows a mild fatty infiltration occurred in liver during the late pregnancy and early lactation. The histopathological examination showed a moderate to severe degree of fatty liver in ketotic cows. The lipomobilisation markers, serum BHB and NEFA concentrations were markedly enhanced in puerperal ketotic cows. However, liver steatosis compromised hepatocyte metabolism, leading to significantly weaker circulating concentrations of glucose, TG and total cholesterol, and induced some cellular lesions as evidenced by significant increases in the serum bilirubin concentrations and in the AST enzyme activities in puerperal ketotic cows. All these biochemical metabolites may be used as important biochemical indicators in the determination of the functional status of the liver in high-yielding dairy cows during the transition period (González ET AL, 2011; Djokovic et al, 2013; Đoković et al., 2014).

The negative relation between the diameter and the optical density of nucleus with the same characteristics of fat vacuoles indicates that hepatocytes due to fatty infiltration may enter into a process of degeneration, apoptosis and necrosis. These processes are shown by Hübscher (2006), Wang et al. (2008) and Alkhouri et al. (2011). It is known that in tissues that are subject to necrosis, can simultaneously take place and apoptosis in cells with minor damage. Cells during hepatocyte fatty infiltration and / or degeneration may die (cell death) and necrosis and apoptosis depending on how much damage cells. Regardless of the type of cell death, reducing the dimension of of nucleus-karyopyknosis, is characteristic of cells whose viability is compromised (Knezevic and Jovanovic, 2008).

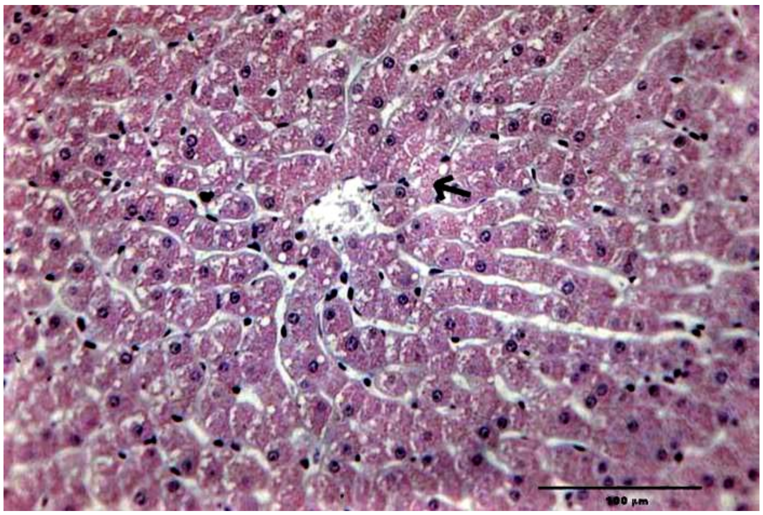

Figure 1. Histological finding of normal liver tissue

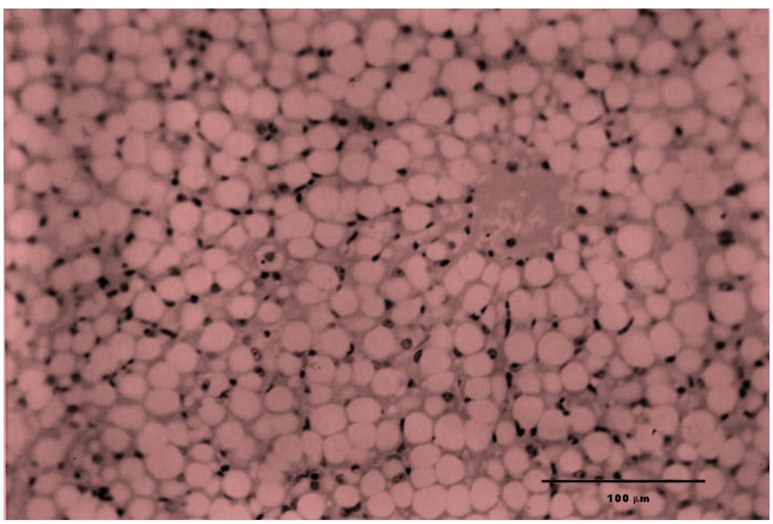

Figure 2. Histological finding of fatty liver tissue

Table 1. Optical density of nucleus and nucleus diameter in cows with normal liver tissue (control) and fatty liver infiltration

	Optical density of nucleus		Nucleus diameter (nm)	
	Control	Fatty liver	Control	Fatty liver
Average	401477.2	171361.3	7335.3	5194.8
SD	64484.1	51201.9	999.9	1219.8
CV	16.06%	29.88%	13.63%	23.48%
p	P <0.001		P <0.001	

Table 2. Optical density and diameter of fatty vacuoles in cows with normal liver tissue (control) and fatty liver infiltration

	Optical density of fatty vacuoles		Diameter of fatty vauoles (nm)	
	Control	Fatty liver	Control	Fatty liver
Average	708578.1	2258360.2	4658.7	19901.9
SD	155922.2	309406.3	1116.4	3165.3
CV	22%	13.7%	23.9%	15.9%
p	P <0.001		P <0.001	

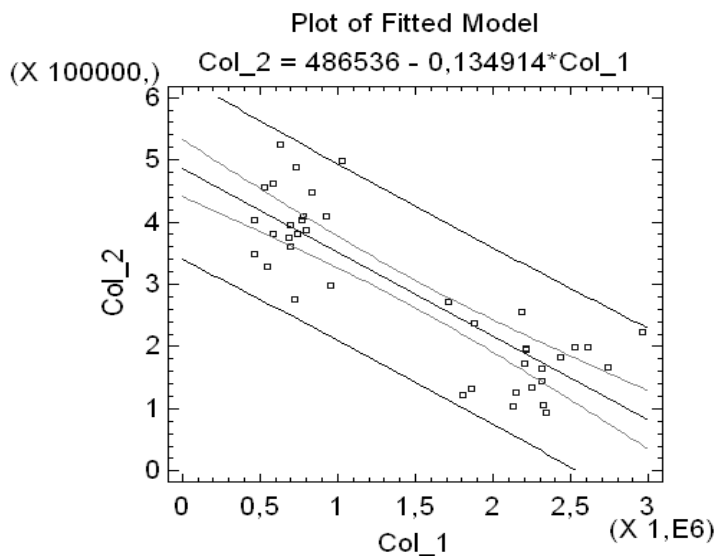

Figure 3. Correlation between diameter of nusleus and fatty vacuoles

Table 3 and 4. Regresion analysis of relation between diameter of nusleus and fatty vacuoles

Coefficients

	Least Squares	*Standard*	*T*	
Parameter	*Estimate*	*Error*	*Statistic*	*P-Value*
Intercept	486536,	22681,8	21,4505	0,0000
Slope	-0,134914	0,0134183	-10,0545	0,0000

Analysis of Variance

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
Model	4,78488E11	1	4,78488E11	101,09	0,0000
Residual	1,79859E11	38	4,73314E9		
Total (Corr.)	6,58348E11	39			

Correlation Coefficient = -0,852527
R-squared = 72,6802 percent
R-squared (adjusted for d.f.) = 71,9612 percent

CONCLUSION

Morphometric characteristics of liver tissue comprising diameter and optical density cores and fatty vacuoles in hepatocytes were significantly different in histologic finding derived from cows with fatty liver compared to control. In the fatty liver tissue there was founded fat vacuoles with large diameter, and the dimensions of the nucleus was significantly lower. Also, there is a higher optical density of fat globulesvacuoles and the lower the optical density of the nucleus in relation with normal histological findings. The negative correlation between the dimensions of fat vacuoles and size of nucleus confirm that fatty infiltration significantly endanger the viability of hepatocytes.

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MORFOMETRIJSKE KARAKTERISTIKE TKIVA JETRE KOD KRAVA U RANOJ LAKTACIJI

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Izvod: Krave u ranoj laktaciji su podložne nastanku negativnog energetskog bilansa, hipoglikemiji i pojačanoj lipolizi u masnom tkivu, što dovodi do višeg stepena lipogeneze i akumulacije triglicerida u masnim vakuolama hepatocita. Cilj ovog rada je određivanje dimenzija i optičke gustine masnih vakuola i jedra krava u ranoj laktaciji. U eksperiment je uključeno 10 krava Holštajn-frizijske rase, čiji su uzorci jetrinog tkiva uzeti biopsijom 3-15 dana nakon teljenja (5 krava kontrolne grupe i 5 krava sa metaboličkim profilom koji ukazuje na masnu jetru). Uzorci su pažljivo obrađeni, obojeni hematoksilin-eozin i Sudan tehnikom i mikroskopski pregledani. Upoređivanjem veličina i optičkih gustina masnih vakuola kod ove dve grupe krava zaključili smo da grupa krava sa masnom jetrom ima značajno veće dimenzije masnih vakuola, manje dimenzije jedra, znatno manju volumetrijsku gustinu jedra i veću volumetrijsku gustinu masnih vakuola. Korelacija između promera masnih vakuola i jedra je bila negativna. Ovo možemo povezati sa masnom degeneracijom i procesima nekroze i apoptoze koji otpočinju u hepatocitima krava sa masnom jetrom.

Ključne reči: morfometrija, jetra, rana laktacija, krave.

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