

Evaluation of Liver Echotexture in Healthy Cows Using Computer-assisted Program

Key words

cow;
liver;
echotexture;
mean grey value;
portal vein depth

Ekin Emre Erkılıç^{1*}, Murat Can Demir², Mert Sezer¹, Semra Kaya², Enes Akyüz¹, Yusuf Umut Batı¹, Muhammet Ali Karadağ², Merve Sena Demir², Tahir Gezer¹, Ali Haydar Kırmızıgül¹, Cihan Kaçar², Gürbüz Gökce¹

¹Department of Internal Medicine, ²Department of Obstetrics and Gynecology, Kafkas University Faculty of Veterinary Medicine, TR-36100 Kars, Türkiye

*Corresponding author: ekin_emre_24@hotmail.com

Abstract: This study was aimed at the determination of the echotexture findings of the liver using computer-assisted programmes, and the investigation of the correlation between these findings and some biochemical parameters in healthy cattle. The study material comprised of 18 healthy Brown Swiss cows. The B-mode images acquired were used for the measurement of the mean grey value, homogeneity, contrast, liver depth, portal vein depth, portal vein diameter, hepatic vein diameter and hepatic vein depth. Contrast, homogeneity and mean grey value were determined as 52.23 ± 3.858 , 0.18 ± 0.006 and 102.27 ± 5.617 , respectively. Despite being statistically insignificant, the mean grey value was negatively correlated with the albumin level ($r = -0.311$, $p > 0.05$), and positively correlated with the BHBA concentration ($r = 0.328$, $p > 0.05$) and SDH activity ($r = 0.402$, $p > 0.05$). Liver depth and portal vein depth were measured as 129.35 ± 3.441 mm and 105.38 ± 3.263 mm, respectively. BHBA concentration was found to be significantly and positively correlated with liver depth and portal vein depth ($r = 0.523$, $p < 0.05$ and $r = 0.663$, $p < 0.01$, respectively). In result, an echotexture analysis of the liver was performed in healthy cattle using computer-assisted programmes, the correlation of the echotexture findings with some biochemical parameters was determined, and numeric data that could aid in assessment were generated using USG (ultrasonography) as a non-invasive method. While it is considered that mean grey values determined with computer-assisted programmes and hepatic and portal vein depths measured by USG could be used as indicative parameters for negative energy balance, there is need for further research on a greater number of animals.

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Introduction

Energy balance is explained as the difference between the energy intake from feed and the energy required for body maintenance, production and pregnancy. Negative energy balance occurs with the decrease in the amount of feed intake. As a result of negative energy balance, body condition decreases and animal welfare is impaired. Many physiological and metabolic changes occur in the animal (1). Negative energy balance causes excessive mobilization of body storage fats and excessive accumulation of these fats in liver parenchymal cells, causing fatty liver (2). Parameters such as glucose, beta hydroxy butyric acid (BHBA), and non-esterified fatty acids (NEFA) are used to evaluate energy balance in cattle (3). While the serum NEFA level indicates

the extent of fat mobilization from the body deposits, the serum BHBA level indicates the level of oxidation of fatty acids in the liver (4). In addition to these invasive diagnostic methods, non-invasive methods like ultrasonography have been used in recent years (5).

Ultrasonography, which is used especially in the management of reproductive conditions and diagnosis of diseases in cow breeding, has found an increasing use in the evaluation of many systems in recent years (6). Ultrasonography is also used in liver examination. The clinical findings associated with hepatic diseases being non-specific and liver enzyme tests not sufficing for diagnosis alone complicate

the diagnosis of hepatic diseases (7). It has been determined that the direct ultrasonographic examination of the liver significantly aids in the diagnosis of several hepatic diseases, including among others hepatic abscess, hepatic lipidosis, and fasciolosis (6).

The appearance of the tissue examined by ultrasonography is referred to as echotexture. Tissue echotexture is defined by histological structure, and thus, varies with histological structure (8, 9). Ultrasonographic examination enables the monitoring of tissue densities (10). Images acquired with ultrasonographic examination are assessed either visually (11) or by means of computer-assisted analyses (9). However, it is indicated that the visual assessment of tissue echogenicity is not reliable on its own (10, 12). This unreliability mainly arises from the perception of grey scale data differing among individuals (10). On the other hand, computer-assisted programmes quantitatively assess each pixel in the examined areas. Thereby, the subjectivity of visual assessment is able to be overcome (8). Depending on their brightness, each pixel may display 256 tones of grey (0: Black; 255: White). Homogeneity, contrast and mean grey value can be used for diagnosis once ultrasonographic images are transferred to the computer (13).

In veterinary medicine, echotexture analyses are used in particular for the examination of the genital organs by means of computer-assisted programmes (9, 13-15). It is indicated that, echotexture analyses are also used for the examination of the kidneys in cats and dogs, and have an important place in the identification of chronic kidney damage in cats (16). While several literature reports are available on the visual assessment of ultrasonographic findings of the bovine liver (11, 17, 18), there is no previous study on the assessment of hepatic echotexture by computer-assisted programmes.

This study was aimed at the determination of the echotexture findings of the liver, which is critical to the energy metabolism in healthy cattle, using computer-assisted programmes, and at the investigation of the correlation between echotexture findings and some biochemical parameters.

Material and methods

Animal material

The study material comprised of 18 mid-lactating and non-pregnant Brown Swiss cows, which were aged 3-6 years and confirmed to be healthy based on routine clinical examinations and hematological and biochemical analyses. The animals were fed a mixed ration consisting of fescue, silage, and concentrated feed. The body condition of the animals was scored according to the method described by Edmonson et al. (19), on a scale from 1 (very thin) to 5 (very fat) at 0.25 point-intervals. The body condition score of

each animal was recorded. The animals were assigned to two groups, according to their body condition scores being below three (<3) or equal to and above three (≥ 3), and these groups were compared for ultrasonographic liver measurements, including liver depth, portal vein depth, homogeneity, and contrast values.

Blood sampling

Blood samples were collected from the coccygeal vein of each animal into gel- and EDTA-coated vacutainers. The blood samples collected into gel-coated vacutainers were centrifuged at 3000 rpm for 10 minutes, and the extracted sera were used for biochemical analyses. On the other hand, the blood samples collected into EDTA-coated vacutainers were used for hematological analyses. The hematological analyses were performed with an automated blood cell counter (VG-MS4e®, Melet Schloesing, France). Of the biochemical parameters investigated, BHBA, NEFA and sorbitol dehydrogenase (SDH) were determined using ELISA kits (BT LAB, China), and glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total bilirubin (TBIL) and albumin (ALB) were measured with an Abbott Architect (USA) biochemistry device.

Ultrasonographic examination

The animals were examined in a standing position and without being sedated. The skin was shaved at the level of the 12th intercostal space on the right side of each cow. After this area was degreased with 90% alcohol and cleaned with water, ultrasound gel was applied to perform examination. Ultrasonographic imaging was performed 1-8 MHz multifrequency convex probe (Esaote Biomedica®, Genova, Italy). The B-mode images acquired were stored in the computer in a format suitable for image analysis (BMP). For the standardization of the images, the frequency, depth, focus and gain were set as 3.5 MHz, 16 cm, 12 cm and 70, respectively. All examinations were performed by a single operator using these pre-set standards.

Echotexture analyses

The echotexture analyses of the stored images were performed in regions of interest (ROIs), which were established up to a depth of 5 cm in areas displaying homogeneous distribution. It was ensured that the ROIs did not contain any artifact. The images were analyzed using the Image J software. Once the software was activated, four ROIs, measuring 35x35 in size and 2 of which were positioned dorsally and 2 were positioned ventrally, were established (Figure 1). These regions were selected in a way that they provided a homogeneous and an objective representation of the hepatic tissue. For each animal, three liver images were used, and four ROIs were established for each image, such that 12 ROIs were evaluated per animal. The arithmetic means were calculated for homogeneity, contrast and



Figure 1: B-mode image of the liver and the definition of the ROI set. Yellow squares indicate regions of interest (ROIs) (35x35). 4 ROIs fields were used for each image. ROIs were placed up to a depth of 5 cm in areas displaying homogeneous distribution.

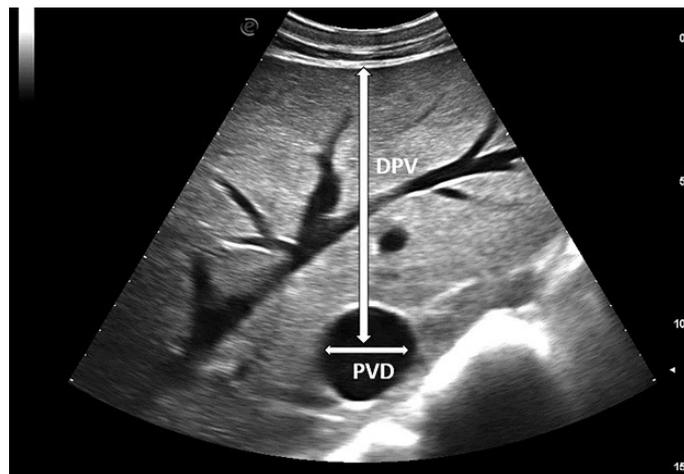


Figure 3: Depth of the portal vein (DPV) and diameter of the portal vein (PVD)

The white vertical arrow indicates the depth of the portal vein. Arrow is starting from the ventral of the innermost intercostal muscle and ends at the central of the portal vein. The smallest white horizontal arrow indicates the diameter of the portal vein.



Figure 2: Depth of the liver (DL)

The white arrow indicates the depth of the liver. Arrow is starting from the ventral border of the innermost intercostal muscle and ends at the dorsal border of the perivisceral fat tissue. In the measurement, the arrow passes through the center of the portal vein.



Figure 4: Depth of the hepatic vein (DHV) and diameter of the hepatic vein (HVD)

The white vertical arrow indicates the depth of the hepatic vein. Arrow is starting from the ventral of the innermost intercostal muscle and ends at the bifurcation of the hepatic vein. The small crossed arrow indicates the diameter of the hepatic vein.

mean grey values, such that a single value was provided for each parameter. Next, the data was analyzed using the selected statistical software programme.

Liver measurements

Measurements of the depth of the liver, diameter of the portal vein, depth of the portal vein, diameter of the hepatic vein and depth of the hepatic vein were performed ultrasonographically (20). The depth of the liver was calculated as the distance between the ventral border of the innermost intercostal muscle and the dorsal border of the perivisceral fat tissue, passing through the center of the portal vein (Figure 2). The depth of the portal vein (DPV) was calculated as the distance between the ventral border of the innermost intercostal muscle and the center of the portal vein (Figure 3). On the other hand, the depth of the hepatic

vein (DHV) was defined as the distance between the ventral border of the internal intercostal muscle and the bifurcation of the hepatic vein (Figure 4).

Statistical analysis

The data obtained was transferred to the SPSS25 software. Descriptive statistical analyses were performed and data were expressed as means±standard error. Correlations between the investigated data were determined with Spearman's correlation test. The groups established according to the body condition scores of the animals were analyzed by normality tests. Those that displayed normally distributed data were analyzed with the independent t-test, and the others were analyzed with the Mann-Whitney U-test. Statistical significance was set at $p < 0.05$.

Results

Vital and Hematological Parameters

The body condition scores of the animals included in the present study were determined to range between 2.5-3.5 (3.0±0.06). Body temperature, pulse and respiratory rate were within normal values and hematological parameters were within reference ranges.

Biochemical parameters

The biochemical analysis results of the animals included in the present study are shown in Table 1.

Ultrasonographic measurements

Data pertaining to the ultrasonographic measurement of the liver are presented in Table 1.

Table 1: Biochemical analysis results and liver measurements in all cows

Parameters	Mean ± Standard Error
Glucose (mg/dL)	70.05±2.259
AST (U/L)	100.78±4.431
ALT (U/L)	37.05±2.593
GGT (U/L)	18.16±1.375
TBIL (mg/dL)	0.26±0.025
ALB (g/L)	31.82±0.556
NEFA (mmol/L)	0.30±0.032
BHBA (mmol/L)	0.21±0.016
SDH (U/L)	12.27±0.543
Liver Depth (mm)	129.35±3.441
Portal Vein Diameter (mm)	40.10±1.253
Portal Vein Depth (mm)	105.38±3.263
Hepatic Vein Diameter (mm)	15.17±0.637
Hepatic Vein Depth (mm)	69.35±4.253

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; TBIL: Total bilirubin; ALB: Albumin; NEFA: Non-esterified fatty acid; BHBA: β-hydroxybutyric acid; SDH: Sorbitol dehydrogenase

The comparison of the cows with different body condition scores (<3 n=6, ≥3 n=12) for the liver depth, portal vein depth, homogeneity and contrast values demonstrated

statistically significant differences (Table 2). However, no statistically significant difference was determined between others. The mean grey value of the animals with a body condition score of <3 (115.68±7.49) was numerically higher than those with a BCS of ≥3 (95.57±6.94), yet no statistically significant difference was detected between the two groups for this parameter.

Table 2: Comparison of some parameters between groups of cows with different body condition scores (BCSs)

Parameters	BCS <3 (n=6)	BCS ≥3 (n=12)	p Value
BHBA (mmol/L)	0.19±0.023	0.22±0.021	0.290
NEFA (mmol/L)	0.27±0.036	0.32±0.045	0.399
Liver Depth (mm)	118.07±6.183	134.99±3.176	0.042
Portal Vein Diameter (mm)	40.07±2.222	40.12±1.588	0.986
Portal Vein Depth (mm)	93.95±5.568	111.09±2.956	0.026
Hepatic Vein Diameter (mm)	15.13±0.997	15.19±0.845	0.962
Hepatic Vein Depth (mm)	59.77±6.497	74.14±5.106	0.146
Contrast	63.83±5.482	39.61±6.038	0.010
Homogeneity	0.16±0.006	0.19±0.007	0.011
Mean Grey Value	115.68±7.486	95.57±6.939	0.071

NEFA: Non-esterified fatty acid; BHBA: β-hydroxybutyric acid; Bold p values denote statistical significance at the p < 0.05 level

Visual and Echotexture Analysis Results

Ultrasonographic imaging showed that the liver was homogeneous and hypoechoic. The blood vessels of the liver were clearly viewed. The results of the liver echotexture analysis of the animals included in the present study are given in Table 3.

Table 3: Liver echotexture findings

Parameters	Mean ± Standard Error
Contrast	52.23±3.858
Homogeneity	0.18±0.006
Mean Grey Value	102.27±5.617

Ultrasonographic liver measurements, echotexture parameters and their correlation with some biochemical parameters

Although statistically insignificant, mean grey value was negatively correlated with albumin level ($r=-0.311$, $p=0.209$), and positively correlated with BHBA concentration ($r=0.328$, $p=0.184$) and SDH activity ($r=0.402$, $p=0.098$). Contrast was positively correlated with SDH activity ($r=0.319$, $p=0.197$), and negatively correlated with albumin level ($r=-0.431$, $p=0.074$). A positive correlation was determined between homogeneity and albumin level ($r=0.390$, $p=0.110$). The correlation of BHBA concentration with liver depth and portal vein depth was positive and statistically significant ($r=0.523$, $p=0.026$ and $r=0.663$, $p=0.003$, respectively).

Discussion

Hematological and biochemical analyses have an important place in evaluating the health status (21). Hematological and biochemical data need to be assessed together with vital values (22). It was determined that the body temperature, heart rate and respiration rate of the clinically healthy animals included in the present study fell within the normal reference ranges (23). Similarly, the investigated hematological parameters and biochemical parameters also fell within the reference ranges reported for healthy animals (22, 24, 25).

Animals require the dietary intake of certain energy levels for survival and production. An insufficient dietary energy intake leads to a negative energy balance, which may cause the development of some metabolic diseases. BHBA and NEFA are two parameters commonly used to assess the energy balance (3). In a previous study conducted during the transition period, it was determined that BHBA and NEFA levels were lower 3 weeks before parturition and at the time of parturition, when compared to the 3rd week postpartum (26). While the targeted serum BHBA levels for cows are below 0.6 mmol/l in the dry period and below 1 mmol/L during lactation, the targeted NEFA levels are below 0.7 mmol/L during lactation and below 0.4 mmol/L before parturition (3). The animals included in the present study were mid-lactating cows, and their mean BHBA and NEFA levels were determined as 0.21 ± 0.02 mmol/L and 0.30 ± 0.03 mmol/L, respectively. These levels show that the study material did not suffer from a negative energy balance.

Metabolic diseases, including hepatic lipidosis, cause major economic losses. The primary method used for the diagnosis of hepatic lipidosis is biopsy. On the other hand, ultrasonography is an alternative method that offers significant potential. However, it is indicated that the use of this method requires specific standardized data (27). The fattening of the liver leads to the constriction of the hepatic blood vessels. Furthermore, an increased fat level in the

liver increases the reflectivity of the organ, and thus, results in brighter images being generated during ultrasonographic examination (28). The animals included in the present study were healthy and their liver echogenicity was determined to be homogeneous hypoechoic, as reported by Streeter and Step (6).

In cows, the 10th to 12th intercostal spaces are preferred for the imaging of the portal vein diameter (7, 20, 29). In a previous study by Fiore et al. (20), the diameter of the portal vein of healthy cows was measured on various prepartum (day 15 ± 5) and postpartum days (day 10 ± 2 , day 30 ± 2 and day 50 ± 2) at the level of the 10th intercostal space by ultrasonographic examination. The diameters measured on these specific days were reported as 43.69 ± 8.31 mm, 41.45 ± 5.88 mm, 37.35 ± 5.41 mm and 39.85 ± 7.59 mm, respectively. On the other hand, portal vein diameters measured ultrasonographically at the level of the 12th intercostal space have been reported as 3.2-5.3 cm by Braun (7) and 3.11 ± 0.16 cm by Imran et al. (29). When measured at the level of the 12th intercostal space by ultrasonographic examination, the portal vein diameter of buffaloes was determined as 3.12 ± 0.17 cm (30). The portal vein diameters measured in the present study were similar to those reported by Braun (7), but higher than those reported by Imran et al. (29) and Tadagani et al. (30). Furthermore, the portal vein diameters measured in the present study were higher than those reported by Fiore et al. (20) for postpartum days 30 and 50, and lower than the values reported by these researchers for prepartum day 15 and postpartum day 10. These differences are attributed to pregnancy, different prepartum and postpartum measurement days, animal breed, and the ultrasonographic imaging site being different.

Fiore et al. (20) reported a positive correlation between BHBA concentration and portal vein diameter in periparturient animals with metritis and mastitis and healthy animals, and between BHBA concentration and portal vein depth in sick animals. Similarly, in the present study, BHBA concentration was found to be positively correlated with liver depth and portal vein depth in healthy animals ($r=0.523$, $p=0.026$; $r=0.663$, $p=0.003$, respectively). We consider that portal vein depth and liver depth could be used for the non-invasive assessment of the liver.

There are several studies available on the visual evaluation of ultrasonographic liver findings (11, 17, 18, 31). Reports indicate that while hepatic lipidosis is associated with increased echogenicity (6, 11) the normal liver has a homogeneous hypoechoic appearance (6). In agreement with literature reports, the liver of the healthy animals included in the present study also displayed a homogeneous hypoechoic appearance. As visual evaluation is subjective, computer-assisted analyses are used to overcome this disadvantage (8). Several literature reports are available on the echotexture analysis of various tissues and organs with the use of computer-assisted programmes (9, 13, 14, 16, 32). In a study on the endometrial echotexture of goats, significant

alterations were determined to occur in echotexture parameters during estrus and early gestation, and these were reported to be related to the hormone progesterone (9). In a study on the nuchal ligament of horses, it was ascertained that the mean grey value varied with age (32). It has been reported that bovine endometrial echotexture could be used for the evaluation of endometrial changes that occur during and after endometritis treatment as well as for the evaluation of recovery (13). Zonturlu et al. (14) suggested that echotexture parameters could be used for the differentiation of early gestation and endometrial cysts in mares. It has been indicated that renal cortical echotexture has a significant place in the identification of major renal damage in cats (16). In the present study, we determined the mean grey value, homogeneity and contrast values of the liver of healthy cows using a computer-assisted programme. Differences between our results and echotexture data reported in previous studies were attributed to differences in the tissues analyzed and the software used for the analyses. The mean grey value having been determined to be insignificantly yet positively correlated with the BHBA concentration ($r=0.328$, $p>0.05$) and SDH activity ($r=0.402$, $p>0.05$), and negatively correlated with the albumin level ($r=-0.311$, $p>0.05$) suggests that this parameter could be used for the evaluation of both the liver and negative energy balance.

The depths of the liver and portal vein provide data on the size of the liver in healthy animals by means of their anatomical position. The depth of the portal vein is particularly important for the assessment of the shape and thickness of the dorsal region of the liver in relation to the nutritional status and BCS of the animal (33). It was reported that, in cows with a BCS of 3.48, the depth of the liver was 159.60 ± 19.91 mm in the prepartum period and 145.36 ± 11.06 mm on the 30th day postpartum (20). There is a relationship between the depth of the liver and the depth of the portal vein with BCS. Previous research has shown that, in healthy cattle, as the BCS decreases, the depth of the liver and portal vein as well as the diameter of the portal vein also decrease (20,33). In agreement with these reports, the present study demonstrated that the depth of the liver and portal vein had significantly decreased as the body condition score decreased. No alteration was determined in the diameter of the portal vein. These results show that the ultrasonographic results of the liver are strongly correlated with the BCS, which reflects alterations in the mobilization of the body reserves.

As a result, a significant positive correlation was determined between liver depth and portal vein depth with BHBA in healthy cows. It has also been determined that liver depth and portal vein depth vary according to BCS. It is thought that USG examination, which is a non-invasive method, and portal vein and liver depth measurements may be an indicator of the existence and severity of negative energy balance. Since only healthy animals were evaluated in the presented study, it is unknown how the liver echotexture will be affected in patients. Based on the results of this research, it is not

possible to say whether there is a relationship between liver echotexture and the evaluated parameters. Therefore, more comprehensive studies in which sick and healthy animals are evaluated together are needed.

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Conflict of interest: The authors have declared no conflict of interest.

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Vrednotenje ehogenosti jeter pri zdravih kravah z uporabo računalniško podprtega programa

E. E. Erkiçiç, M. C. Demir, M. Sezer, S. Kaya, E. Akyüz, Y. U. Batı, M. A. Karadağ, M. S. Demir, T. Gezer, A. H. Kırmızıgül, C. Kaçar, G. Gökce

Izveleček: Namen raziskave je bil določiti ehogenosti jeter z uporabo računalniško podprtih programov ter raziskati povezavo med takšnimi ugotovitvami in nekaterimi biokemičnimi parametri pri zdravem govedu. V raziskavo je bilo vključenih 18 zdravih krav rjave pasme. Pridobljene slike v B-načinu so bile uporabljene za merjenje srednje sive vrednosti, homogenosti, kontrasta, globine jeter, globine portalne vene, premera portalne vene, premera jetrne vene in globine jetrne vene. Kontrast, homogenost in srednja siva vrednost so bile določene kot $52,23 \pm 3,858$, $0,18 \pm 0,006$ oziroma $102,27 \pm 5,617$. Kljub statistično neznačilni vrednosti je bila srednja siva vrednost negativno povezana z ravnjo albumina ($r = -0,311$, $p > 0,05$), pozitivno pa s koncentracijo BHBA ($r = 0,328$, $p > 0,05$) in aktivnostjo SDH ($r = 0,402$, $p > 0,05$). Izmerjeni sta bili globina jeter in globina portalne vene, ki sta znašali $129,35 \pm 3,441$ oziroma $105,38 \pm 3,263$ mm. Ugotovljeno je bilo, da je koncentracija BHBA značilno in pozitivno povezana z globino jeter in globino portalne vene ($r = 0,523$, $p < 0,05$ oziroma $r = 0,663$, $p < 0,01$). Na podlagi tega je bila pri zdravem govedu z uporabo računalniško podprtih programov opravljena analiza ehogenosti jeter, določena je bila korelacija ehogenosti z nekaterimi biokemičnimi parametri in pridobljeni so bili številčni podatki, ki bi lahko pomagali pri oceni z uporabo ultrazvoka (USG) kot neinvazivne metode. Čeprav velja, da bi se povprečne sive vrednosti, določene z računalniško podprtimi programi, ter globine jetrne in portalne vene, izmerjene z USG, lahko uporabile kot indikativni parametri za negativno energijsko bilanco, so potrebne nadaljnje raziskave na večjem številu živali.

Ključne besede: krava; jetra; ehogenost; srednja siva vrednost; globina portalne vene