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Hepatic Ultrasound Dimensional Characterization in Dairy Cattle - Relationship with Reproductive Status

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ABSTRACT

***Background*:** Ultrasound examination is an essential and accessible tool to evaluate the liver of dairy cows. Detailed ultrasound evaluation of the liver provides information on the size, position, morphology of the parenchyma, among other characteristics inherent to the organ. Morphological variations in hepatic ultrasound may be related to reproductive status, aiding in the differential diagnosis for liver alterations caused by diseases. However, the relationship between liver ultrasound measures and reproductive status is still unknown. The objective of this study was to evaluate the liver dimensions of lactating dairy cows and their associations with reproductive status.

***Materials, Methods & Results*:** This work utilized lactating cows and aimed to find any possible relationship between reproduction and liver measurable characteristics. No previous preparation of the cows was necessary, only the application of liquid alcohol at 70% during ultrasound scanning. The ultrasound evaluation of liver morphology was done at the right antimer, at the cranial abdominal region, between 12th and 5th intercostal espace (ICE). Liver measures evaluated: distances of spinous processes from dorsal vertebrae to liver apice (M1); distances of spinous processes from dorsal vertebrae to the furthest ventral limit of liver (M2); measures resulting from differences between 2 anterior vertebrae (M3), distances between lateral liver edge and portal vein (M4) and distances between lateral liver edge and vena cava (M5). In addition, we studied the diameter measures of caudal vena cava and portal vein. The organ showed regular contours and parenchyma with fine homogeneous echotexture of habitual echogenicity, regardless of reproductive status. The caudal vena cava presented triangular shape (shape related to the inlay in the hepatic sulcus) or slightly oval (shape that is not considered normal, correlated with thrombosis, heart failure or compression caused by the chest), with hypoechogenic wall, located at the hepatic edge. In addition, in animals with BCS greater than 3.25, it was more difficult to locate and evaluate the vena cava. The portal vein presented a characteristic rounded shape with branching, hyperechogenic wall and was easier to be localized and evaluated, in relation to the vena cava, even in cows with BCS greater than 3.25. Liver measures were: M1: 35.09 ± 5.92 cm, M2: 66.14 ± 10.71 cm, M3: 31.05 ± 7.96 cm, M4: 95.08 ± 12.66 mm, M5: 128.21 ± 13.64 mm and the diameter of portal vein and caudal portal vein, in mm, were respectively: 40.34 ± 5.79 and 37.83 ± 6.93. Pregnant cows showed M1 < 36 cm, M2 < 66 cm, M3 < 31 cm, M4 < 96 mm and M5 ≥ 128 mm, and empty cows M1 ≥ 36 cm, M2 ≥ 66 cm, M3 ≥ 31 cm, M4 ≥ 96 mm and M5 < 128 mm.

***Discussion*:** The distance of the liver thickness, in the position of the portal vein of pregnant cows, presented a lower value than that of empty cows. The use of reference values in different reproductive stages is recommended for comparison with animals affected by diseases. Liver dimensions and reproductive status of dairy cows showed a significant correlation and may be used in the future for prediction and aid in reproductive diagnosis, as well as for differential diagnoses of liver dysfunctions in these animals. There is enough scientific evidence in the present work to subsidize the presence of a correlation between the reproductive status and ultrasound measures evaluated in this research.

Keywords: liver, ultrasound, cattle, portal vein, vena cava.

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INTRODUCTION

Ultrasound examination can and should go beyond its use in reproduction. It can also be used as a complementary examination in the medical clinic of dairy cattle, maximizing its application and serving as a tool to assist the veterinarian in the diagnosis of the various conditions affecting these animals.

In this context, the great potential of using this diagnostic modality is understood and can be used to investigate organic alterations, helping satisfactorily the diagnosis of hepatic disorders [4,12], therefore minimizing the use of more invasive diagnostic methods [18].

Detailed ultrasound evaluation of the liver provides information on the size, position, morphology of the parenchyma, among other characteristics inherent to the organ [4]. This tool deserves to be highlighted as another viable device for monitoring liver health status [14,16] and can accurately predict, for example, the amount of triacyclglycerols [19] and other hematochemical indicators present in the organ [9].

Ultrasound aspects of the liver in dairy cows vary according to the clinical, physiological and reproductive condition of the animals. In addition, there is scientific evidence that correlates efficiently and reliably liver morphological aspects and reproductive status. In this context, morphological variations in hepatic ultrasound may be related to reproductive status of dairy cows, aiding in the differential diagnosis for liver alterations caused by diseases. The objective of this study was to evaluate the liver dimensions of lactating dairy cows and their associations with reproductive status.

MATERIALS AND METHODS

Animals and experimental design

A total of 22 dairy cows from different genetic groups were used: 1 (3/4 Holstein-Gir), 1 (3/4 Holstein-Jersey), 2 (15/16 Holstein-Gir), 2 (31/32 Holstein-Gir), 2 Jerseys and 14 Holsteins. Average days on milk were (mean DOM) of 210 ± 76 days, mean age of 4.1 ± 1.8 years, average milk production of 31 ± 7.4 kg/day, mean body condition score (BCS) of 3.27 ± 0.33, average chest circumference (CC) of 199 ± 8 cm. Experimental animals were kept under the same conditions of environment, feeding and management.

The animals were divided into 2 groups: group of inseminated cows (I) and without early diagnosis of pregnancy, i.e., with < 30 days of bull exposure, non-inseminated cows, open cows (A) and the other group of cows with confirmation of pregnancy (P), pregnancy > 60 days: 113 ± 34 days of gestation.

Ultrasound evaluation

For the ultrasound examination of the liver, an ultrasound equipment model A6v[1], Sonoscape®, with multifrequency convex transducer (3-5MHz) was used. The cows were gently contained in the shoot, not requiring any chemical containment. No previous preparation was necessary, only the application of liquid alcohol at 70% during ultrasound scanning.

The ultrasound evaluation of liver morphology was done at the right antimer of the cows, at the cranial abdominal region, between 12th and 5th intercostal espace (ICE), with dorsoventral patterning at each ICE [4]. Within these accoustic windows, the ultrasonographic hepatic dimensions (hepatic variable pre determined), were evaluated through accoustic window of 10th ICE (Figure 1) [9]. Liver measures evaluated: distances of spinous processes from dorsal vertebrae to liver apice (M1); distances of spinous processes from dorsal vertebrae to the furthest ventral limit of liver (M2); measures resulting from differences between 2 anterior vertebrae (M3= M2-M1), distances between lateral liver edge and portal vein (M4) and distances between lateral liver edge and vena cava (M5); measured at the acoustic window of the 10th ICE. Measurements M1, M2 and M3 were made in centimeter. M4 and M5 were made in millimeter.

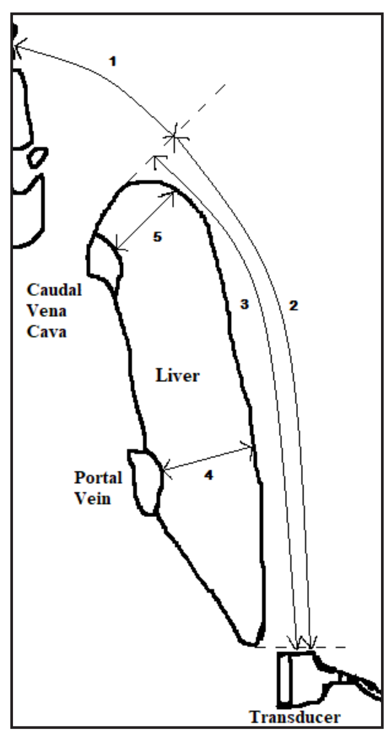

Figure 1. Schematic representation of a cross-section of the liver of a cow in the 10th ICE, with right caudal vision: 1 = dorsal midline to the dorsal end of the liver; 2 = thoracic spinous process to the ventral extremity of the liver; 3 = liver size (distance 2-1); 4 = distance from the lateral wall of the liver to the Portal vein; 5 = distance from the lateral wall of the liver to the caudal vein of the liver, adapted from Haudum [9].

Table 1. Structure of the contingency table used in the analysis of applied correspondence to determine the associations between the variables.

A*	B (Measurements)										Total
	> =36	<36	>=66	<66	>=31	<31	>=96	<96	>=128	<128	
P	2	7	2	7	3	6	3	6	5	3	44
I	2	2	2	2	2	2	3	1	1	3	20
A	5	4	3	6	4	5	5	4	4	4	44
Total	9	13	7	15	9	13	11	11	10	10	108

*A = reproductive status.

Data and statistical analysis

Statistical analysis was performed in order to verify the association of categorical variable levels, represented by liver morphology and hepatic ultrasound aspects. From the frequency tables obtained from each group of variables, the correspondence analysis technique was used to verify the associations between each variable and the groups (pregnant and empty). For each categorical variable, the counts for the structures according to layout (Table 1), where A and B, represented respectively the variables illustrated by A (i=1,...,I) and B (j=1,...,J), with their levels of association.

Therefore, considering I rows and J columns, the notations used to obtain distances and coordinates were defined by: nij which represented the observed frequency of the categories of rows and columns denoted respectively, by the ith category of variable A and j-th category of variable B; ni+ corresponded to the marginal totals of the rows of category A; analogously, n+j indicated the marginal totals of the columns of category B and finally, n ++ the total sum of frequencies.

The obtained scores and correlations at each axis, illustrated in the exception maps, were obtained based on the p-correspondence matrix, in which each element was denoted by (1)

$$p_{ij} = \frac{n_{ij}}{n_{++}} \quad (1)$$

The formulation of marginal proportions for the variable with its levels, arranged in rows and columns, respectively, were calculated according to expressions (2) and (3)

$$r = \left(\frac{n_{1+}}{n_{++}}, \frac{n_{2+}}{n_{++}}, \ldots, \frac{n_{I+}}{n_{++}} \right) \quad (2)$$

$$c = \left(\frac{n_{+1}}{n_{++}}, \frac{n_{+2}}{n_{++}}, \ldots, \frac{n_{+J}}{n_{++}} \right) \quad (3)$$

Following these specifications, given the mathematical procedure described by Blasius [3], coordinates and correlations were calculated, which allowed identifying which levels of categorical variables were similar, which were identified by circles in each quadrant in the constructed perceptual maps. For this, the Software R Core Team [15] was used.

RESULTS

The liver was located and evaluated between the 5th and 12th ICE of the right antimer. During ultrasound examination, the organ showed regular contours and parenchyma with fine homogeneous echotexture of habitual echogenicity, regardless of reproductive status. Focal or diffuse alterations that indicated any disease condition were identified in any of the cows and would lead to removing that animal from the experiment. It was also possible to obtain images of the caudal vena cava and the portal vena, using the 11th and 10th ICE, respectively. The hepatic dimensions can be seen in the Table 2.

The vena cava was located dorsally in relation to the portal vein. It was possible to evaluate the diameter of both vessels. The caudal vena cava presented triangular shape (shape related to the inlay in the hepatic sulcus) or slightly oval (shape that is not considered normal, correlated with thrombosis, heart failure or compression caused by the chest), with hypoechogenic wall, located at the hepatic edge. In addition, in animals with BCS greater than 3.25, it was more difficult to locate and evaluate the vena cava. The portal vein presented a characteristic rounded shape with branching, hyperechogenic wall and was easier to be localized and evaluated, in relation to the vena cava, even in animals with BCS greater than 3.25. In addition, it was easily differentiated from the hepatic veins by the shape of the branching and echogenicity of the walls. The lumen of the cava and porta veins was equally anechogenic, referring to the fluid content, the blood.

The diameters of the caudal vena cava and portal vein were not associated with reproductive status. The diameters of the caudal vena cava of empty cows and pregnant cows presented values of 36.91 ± 7.39 and 38.50 ± 6.87 mm (mean ± standard deviation), respectively. The diameters of the portal vein of empty cows and pregnant cows presented values of 38.46 ± 4.71 and 41.64 ± 6.27 mm (mean ± standard deviation), respectively.

In order to investigate the association between the metric variables of the group of pregnant cows and the group of empty cows, the 2nd group of liver measurements were calculated (Table 3) from the percentage maps (Figure 2).

All liver measurements: distances of spinous processes from dorsal vertebrae to liver apice (M1); distances of spinous processes from dorsal vertebrae to the furthest ventral limit of liver (M2); measures resulting from differences M2 and M1 (M2-M1) (M3), distances between lateral liver edge and portal vein (M4) [Figure 3] showed higher values in empty cows compared to pregnant cows.

The distances between the hepatic lateral edge to the caudal vena cava (M5 - Figure 4), were the only measurements that presented higher value in pregnant cows when compared to empty cows. These variables showed similarity, and were identified by circles (Figure 2) in each quadrant in the constructed perceptual maps.

Table 2. Hepatic dimensions of all cows evaluated.

Measure 1	Measure 2	Measure 3	Measure 4	Measure 5	D[1] Portal Vein	D[1] Vena Cava
	cm				mm	
35.09 ± 5.92	66.14 ± 10.71	31.05 ± 7.96	95.08 ± 12.66	128.21 ± 13.64	40.34 ± 5.79	37.83 ± 6.93

D[1]= diameter.

Table 3. Liver measurements calculated in all cows and in both categories: pregnant and empty.

	Liver measurements				
	M1 (cm)	M2 (cm)	M3 (cm)	M4 (mm)	M5 (mm)
All cows	35.1 ± 5.9	66.1 ± 10.7	31.1 ± 8.0	95.1 ± 12.7	128.2 ± 13.6
Pregnant cows	< 36	< 66	< 31	< 96	≥ 128
Empty cows	≥ 36	≥ 66	≥ 31	≥ 96	< 128

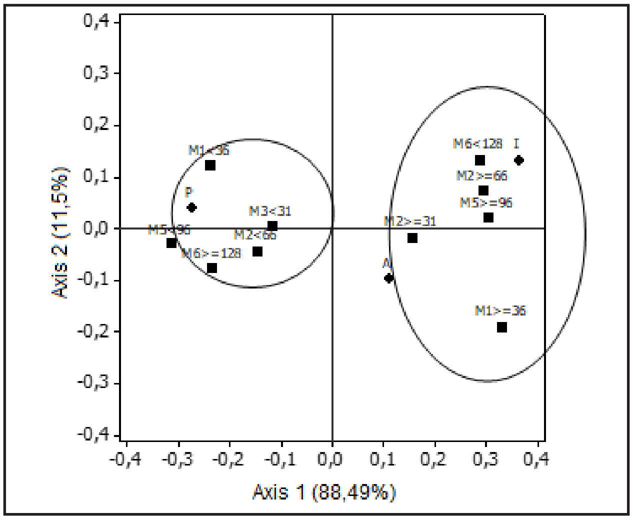

Figure 2. Perceptual map of liver measurements.

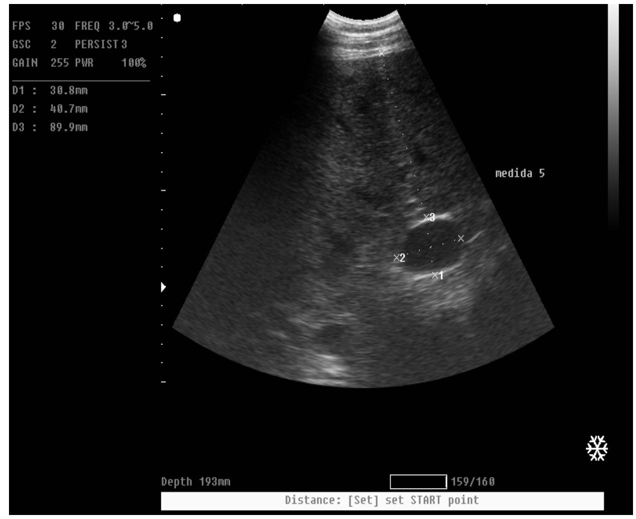

Figure 3. Distance between the hepatic lateral border to the portal vein (dotted line). The portal vein with hyperechogenic walls stands out.

Figure 4. Distance between the hepatic lateral border to the vena cava (dotted line). The vena cava with hypoechogenic walls stands out.

DISCUSSION

The functionality of the ultrasound technique in the liver evaluation of dairy cows was proven to be relevant in this study. However, it is worth mentioning, the greatest difficulty in obtaining images in cows with BCS >3.25, perhaps due to the greater acoustic impedance of adipose tissue, especially in the evaluation of the caudal vena cava.

The ultrasound findings are in agreement with what was previously described, showing evident hepatic characteristics and easy identification [4]. The liver characteristics found in the present study are similar to those reported in ewes [8], goats [17], and in heifers [6]

As previously proposed, it was possible to measure the liver of dairy cows [10], thus allowing us to state that the reproducibility of the model is feasible and adequate. In addition, the measures characterized by the present study can be used as hepatic reference dimensions (Table 2) of Brazilian cows with physical and productive characteristics similar to those studied.

The diameter of the vena cava increases with the advance of pregnancy and the diameter of the portal vein decreases [4,5] influencing the distance of the hepatic lateral wall (thickness) in relation to the portal vein. These findings are in agreement with previously reported results, showing a positive correlation (0.40 to 0.56, variation due to the ICE evaluated) [5]. Both diameters of the veins did not present correlation with reproductive status, which can be explained by the form of evaluation performed, where these diameters were measured in a certain space of time and not repeated over time as described in the current literature.

Within this context, the distance from the hepatic lateral wall to the portal vein (M4), or, thickness of the liver, in the position of the portal vein of pregnant cows in the present study, presented a lower value than that of empty cows (< 96 mm versus ≥ 96 mm, respectively). These results agree with previously reported data, which described values between 71 and 109 mm in a study involving 186 dairy cows [5]. These authors also found, in 3 cows of different breeds (Simental, Swiss Brown and Crossbred with Holstein), evaluated 8 times each, a negative correlation between the diameter of the portal vein (-0.41 to -0.23, with this variation due to the ICE evaluated) and the gestational stage.

There is a hemodynamic and endocrine change in pregnant animals, providing a protective hemodynamic intake to the pregnant female and better perfusion to promote fetal growth, through the supply of oxygen and nutrients, increasing the diameter of intra- and extrahepatic veins, which flow into the portal vein [2]. In this phase there is also a generalized plasma increase, providing portal hypertension [1,2], which will support physiological needs, providing decreased portal thickness and improvement in blood perfusion [13]. In human females, portal hypertension that occurs in this phase was proven with the use of the Doppler tool [7]. A positive correlation was found between liver thickness in the portal vein region, live weight, and age of lactating Holstein cows of Germanic origin [10]. These same authors justified that the correlation could be explained by the fact that younger animals have not yet reached adult size. This explanation helps us in the evaluation of the measures obtained in the present study, because the animals evaluated had heterogeneous ages and genetic lineage different from the author.

Liver thickness values (5.8 ± 0.6 to 8.0 ± 0.7 cm) of Holstein calves were obtained, evaluated from the 2nd day until 104 days of life, and the use of reference values for comparison with animals affected by diseases was recommended [6]. In this context, we can make the same recommendations, in this case, for Brazilian dairy cows in different reproductive stages. An observation that should be taken into account concerns live weight, age, and withers height, for which a positive correlation with liver size was found previously [10]. In the present study, these correlations were not observed, which can be explained by the different genetic groups and especially the lineage of the animals.

The values found for M1 (distance from the spinous processes of the dorsal vertebrae to the hepatic apex), 35.1 ± 5.9 cm in all cows regardless of other variables, show similarities with previously reported values of 32.0 ± 2.15 cm for this variable in Jersey X Sindi cross-breed cows, non-lactating and healthy, weighing 300-450 kg and aged 4 to 12 years [11]. The hepatic thicknesses in the portal vein region presented mean values of 95.1 ± 12.7 mm, similar to the values found by other authors, who described measurements between 79 and 136 mm in 133 Holstein cows with 100 days of lactation [10].

The hepatic reference values are similar to those found in foreign animals, but there is a caveat in the use of values in animals in different reproductive status. An observation should be taken into account in relation to the measures of this study, as they were unique and punctual measures, and no repeated measurements were performed in the animals, during a certain period of time, which could demonstrate any correlation with reproductive status. A clear demonstration of this aspect is the non-correlation found between caudal vena cava diameter and reproductive status, contrary to previously reported findings [5]. These authors found variation in the diameter of the caudal vena cava as a function of reproductive status, but the evaluation was performed in animals of 3 different breeds to the study in question and performed in 8 shots, during a determined period of time. These results demonstrate a positive correlation (0.40 to 0.56, variation due to the ICE evaluated).

In the present study, cows with different reproductive stages were evaluated and in the group of cows considered pregnant, cows with gestational age higher than those in previous studies (113 ± 34 days versus 28 to 70 days of gestation) [5] were allocated. In this context, because it is a punctual evaluation and with a higher gestational age, no correlations were found with the progress of pregnancy, but the values found for caudal vena cava diameter were higher for pregnant animals compared to non-pregnant animals (36.91 ± 7.39 and 38.50 ± 6.87 mm). Therefore, the diameter of the vena cava was larger, but in the present experiment, because it is only a measure in time, it was not successful in determining such correlation. As the gestational period progresses, higher amounts of blood from uterine veins flow into the vena cava, overloading it [5].

CONCLUSIONS

The ultrasound examination of the liver in cows is simple, feasible and we can use it as a tool to determine the size of this organ, with restrictions on animals of high BCS. Liver dimensions and reproductive status of dairy cows showed a significant correlation and may be used in the future for prediction and aid in reproductive diagnosis, as well as for differential diagnoses of liver dysfunctions in these animals.

MANUFACTURER

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***Ethical approval*.** The project was approved by Animal Ethics committee from Federal University of Lavras under protocol number 015/13.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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