RESUMO. - [Dopplerfluxometria da veia portal em ovinos hígidos de acordo com a idade.] A ultrasonografia com Doppler pulsado foi utilizado para avaliar o fluxo sanguíneo portal, velocidade portal e índice de congestão portal em 24 ovinos saudáveis divididos em grupos (cordeiros, borregos e ovelhas), de acordo com a idade. As medições foram realizadas no 11º espaço intercostal direito utilizando ângulo de insonação ideal e método de inclusão uniforme. Os valores médios obtidos em cada grupo foram comparados com ANOVA, seguido pelo teste post-hoc de Tukey. A velocidade portal e o fluxo de sangue portal foram estatisticamente semelhantes entre os grupos (P>0.05). A velocidade portal média foram 17.75; 17.13 e 16.75; enquanto o fluxo de sangue portal médios foram 26.65; 31.04 e 24.32 para cordeiros, borregos e ovelhas, respectivamente. O índice de congestão portal foi estatisticamente diferente entre os grupos e os valores para cordeiros, novilhos e ovelhas foram 0.009; 0.058 e 0.09, respectivamente (P<0.01). Observaram-se diferenças estatísticas nos diâmetros da veia porta, na área da veia porta e nos índices de congestão portal entre os grupos, presumivelmente devido à influência do peso e não pela idade.

INDEX TERMS: Portal Vein Dopplerflowmetry, Doppler, liver, portal vein, sheep, ultrasonography.

INTRODUCTION
Sonographic examination in sheep has been widely used in research and clinical routine, in order to improve productivity of herds (Scott & Sargison 2010). Most studies in literature, however, refer to its use as a diagnostic tool in renal assessment due to the high rates of obstructive urolithiasis.
in this species (Scott 2013), for fetal sexing (Santos et al. 2007) and for estimating carcass composition (Leeds et al. 2008). A recent study provided reference data regarding sonographic appearance of the spleen in 60 healthy sheep (Floeck et al. 2013).


Although B-mode liver sonography presents satisfactory sensitivity for the detection of focal lesions, diagnosis of diffuse parenchymal liver diseases may be inefficient due to the large overlap of sonographic signals caused by different diseases (Feeney et al. 2008).

Sheep are not only predisposed to focal hepatic lesions but also to diffuse liver diseases, due to a variety of etiologies. Fatty liver infiltration (Kandeel et al. 2009), toxic and congestive liver diseases are of great clinical relevance and lead to significant losses in sheep production (Ulvdal 1990). Cobalt deficiency, vitamin E deficiency, pregnancy toxema, toxicosis and negative energy balance are some of the causes that can lead to fatty liver in sheep (Ulvdal 1990). Liver cirrhosis was reported in sheep (Kandeel et al., 2009). Furthermore, hepatic vascular anomalies, including portosystemic shunts, although infrequent in sheep, have been reported in goats (Humann-Ziehank et al. 2001). In veterinary literature, there is a single report of hepatic adenocarcinoma in a ewe (Loftsted et al. 1988).

Diffuse hepatocellular diseases, neoplasias and vascular anomalies often lead to significant changes in liver circulation, which can be detected by means of Doppler sonography (Kantrowitz et al. 1989). Portal vein (PV), as the carrying of a high percentage of total hepatic blood supply, is the main vessel that undergoes the effects of liver lesions (Nyland & Fisher 1990).

In human beings, as well as in companion animals, Doppler modality expanded clinical apply of sonographic exams, allowing detection of liver hemodynamic changes. In sheep, on the other hand, there has been little research due to the large overlap of sonographic signals caused by different diseases (Scott et al. 2008).

The aim of this study was to obtain normality ranges for portal vein diameter (PVD), area (PVA), velocity (PMV), flow volume (PBF) and congestion index (PCI) in healthy sheep of different ages. These indexes were also compared between the groups, to assess whether they are influenced by sheep age.

**MATERIALS AND METHODS**

**Animals.** The base population comprised 24 healthy crossbred (Ile de France x White Dorper) sheep from the Veterinary Hospital, School of Veterinary Medicine and Animal Science (FMVZ, São Paulo State University (Unesp), Botucatu, São Paulo, Brazil). The animals were allocated into three groups: lambs (7-28 days; n=8; 4 males and 4 females), yearlings (4-5 months; n=8; 8 males) and ewes (1.5-4 years; n=8; 8 females).

Lambs and ewes were maintained under semi-intensive management. Lambs grazed on pasture with creep feed and ewes were fed corn silage and commercial feed. Yearlings were confined in collective pens and were fed a commercial feed composed of 75% concentrate and 25% crushed coast-cross hay (18 – 20% crude protein and 75% total digestible nutrients). They were kept at approximately 3m² per animal.

Animals which entered the study were considered healthy based on physical examination and biochemical analysis (aspartate aminotransferase - AST, alkaline phosphatase - ALP and gamma glutamyl-transferase - GGT). Sheep were weighed during physical examination.

**Blood collection.** Blood samples were collected from the jugular vein by venipuncture into 4mL Serum Clot Activator tubes (BD Vacutainer®, BD Medical, Curitiba-BR, Brazil). After blood coagulation, samples were centrifuged at 3500g for 5 minutes. The plasmas were separated and transferred to Eppendorf tubes, frozen and stored at -20°C until moment of analysis.

Biochemical analysis and enzymatic activities of serum samples were measured through the use of colorimetric commercial kits (Ebram® Produtos Laboratoriais Ltda, São Paulo, SP, Brazil). Reading was carried out in a semi-automatic biochemistry analyzer Bio-2000 (Bioplus®, São Paulo, Brazil).

**Pulsed Doppler sonography.** Doppler measurements were performed by a single investigator. The ultrasound device was a My Lab™30 Vet Gold (Esaote Healthcare do Brasil®, São Paulo/SP, Brazil) with a 2MHz convex transducer.

Animals were held manually, in left lateral recumbency. Transcutaneous ultrasound examination was performed using the electronic cursors, at cross-sectional plane, as previously proposed (Néspoli et al. 2009) (Fig.1).

PVA could be then calculated using the following formula:

\[
A = \frac{(D)^2 \times \pi}{4}
\]

A = area; D = diameter; \( \pi = 3.14 \)

![Fig.1. B-mode ultrasound image showing caudal vena cava (CVC) and portal vein (PV) at 11th right intercostal space and their diameter measurements between calipers.](image-url)
rence was found between the groups. Statistical tests were performed for a significance level of P<0.05 (Zar 2009).

**Ethical aspects.** This project was approved by the Ethics Committee on Animal Use (CEUA) FMVZ-Unesp, Botucatu, under the Protocol 188/2014.

**RESULTS AND DISCUSSION**

The mean ALP and GGT in lambs were higher than the reference ranges for sheep (Table 1) (Kaneko et al. 2008). Nevertheless, intense osteoclastic activity can increase ALP values (Ramos et al. 1994) and, in addition, colostrum ingestion led to high GGT activity in lambs (Braun et al. 2010). Some lambs who had increased ALP and GGT levels were, therefore, included in this study. Mean GGT level in yearlings was also above reference ranges. Colostral antibodies, however, should be considered, due to yearlings' age (4 months). All liver enzymes levels of ewes were within normal ranges.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Reference value*</th>
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<tbody>
<tr>
<td>Lambs</td>
<td>67.93±21.44</td>
</tr>
<tr>
<td>Yearlings</td>
<td>109.85±44.02</td>
</tr>
<tr>
<td>Ewes</td>
<td>104.3±21.78</td>
</tr>
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</table>

AST = aspartate aminotransferase, ALP = alkaline phosphatase, GGT = gamma-glutamyl transferase. *Kaneko et al. (2008).

In the present study, PVD and PMV were easily measured in the 11th intercostal space of each animal by means of B-mode and pulsed Doppler sonography using manual restraint. A previous study also reported great accessibility of portal vein from 9th to 11th intercostal spaces with the animal positioned in left lateral recumbency (Kandeel et al. 2009, Néspoli et al. 2009). Some authors reported difficulties in locating liver vessels in pregnant sheep because of cranial displacement of the liver, what was not observed in the present study once none of the ewe were pregnant. PV could be differentiated from the other hepatic vessels due to its hyperechoic wall and location ventrally and laterally to the caudal vena cava, as previously reported (Kandeel et al. 2009).

In human patients, although liver biopsy is still the gold standard for definitive diagnosis, liver Doppler ultrasound parameters have been widely used to predict chronic liver diseases, liver fibrosis, fatty liver, cirrhosis and neoplasia (Gerstenmaier & Gibson 2014, Keddeas et al. 2016, Soker et al. 2016). Liver Doppler flowmetry is also considered an excellent tool in the assessment of severity of hepatic disease (Mukhopadyay & Saha 2015). The study of hepatic vascular hemodynamics is important once changes can be detected when parenchymal liver diseases lead to changes in hepatic compliance (O’Donohue et al. 2004) and in cases of vascular anomalies. Most of the studies reported in veterinary literature, however, are limited to Doppler sonography of the liver in healthy animals (Kantrowitz et al. 1989, Lamb & Mahoney, 1994, Sartor et al. 2010b) and for assessment of portosystemic shunts (D’Anjou et al. 2004) in dogs and...
cats. Therefore, there is a need to investigate the applicability of liver Doppler ultrasound in sheep. Doppler evaluation of PV and its intra-hepatic branches is based on morphology of spectral wave, flow direction and measurement of mean velocity, blood flow and congestion index (Sartor et al. 2010b). In the present study, PMV, PBF and PCI were accurately measured.

PMV was measured using uniform insonation method, which is easier to use and produces higher amplitude Doppler signal in comparison with other methods (Lamb & Mahoney 1994). Weight, portal vein diameter and area were significantly different between lambs, yearlings and ewes (P<0.01) (Table 2). Higher values were determined in adult sheep, while lower values were observed in lambs, presumably due to variations in body weight. This result is in agreement with that reported by other authors (Sartor et al. 2010a), who verified significantly smaller values for PVD and PVA in small-sized dogs in comparison with medium-sized dogs.

Although some authors described morphologic aspects of hepatic B-mode sonography in sheep (Néspoli et al. 2009), there are no reports on values for ovine PV in the veterinary literature. It is important to measure PV to calculate portal blood flow and because some diseases, including cirrhosis, can change portal vein diameter (Anda et al. 2016). In healthy calves, aged from birth to 104 days, portal vein diameter ranged from 1.4cm to 1.8cm (Braun & Krüger 2013). Considering age ranges, sheep PVDs in the present study were lower than the values obtained in calves.

There was no statistical difference in the mean values of PMV and PBF between the groups. Although there exists a difference in the arrangement of the tributaries of the portal vein in sheep in comparison with dogs and cats (Heath 1967), values obtained for portal vein velocity in the present study are close to the ones obtained in previously published studies of healthy dogs of varying sizes (Nyland & Fisher 1990). A lack of weight influence on PMV was observed in the present study, as described for dogs (Sartor et al. 2010b). Although there are no reports of the behavior of portal velocity in sheep with liver disease, in dogs portal velocity may reduce in cases of portal hypertension and cirrhosis (Sartor & Mamprim 2014), and increase in patients with intra-hepatic portosystemic shunts (D’Anjou et al. 2004).

Although the lowest PBF was observed in ewes and the highest in yearlings, no statistical difference was found between the groups. Mean flow is close to the values previously obtained in healthy medium-sized dogs (Nyland & Fisher 1990). On the other hand, the PBF of the sheep of the present study is lower than that reported by Sartor et al. (2010b). Sartor et al. (2010b) also described that body weight can influence portal blood flow in dogs (Sartor et al 2010b), what was not observed in this study. Measurement of PBF is of great importance because portal vein contributes with two thirds of total hepatic blood supply (Kantrowitz et al. 1989) and a significant reduction in this value could, therefore, suggest an important compromise in hepatic hemodynamics.

PCI reduction was observed in human patients presenting chronic liver disease, cirrhosis and portal hypertension (Moriyasu et al. 1986). In this study, PCI was significantly different between the groups, with the lower mean value seen in lambs and the higher mean value in ewes. This presumably occurred due to lower W and, consequently, DVP, in the lambs, as described by others, for healthy dogs (Sartor et al. 2010b).

### CONCLUSIONS

The present study provided data that can be used as reference ranges during sonographic evaluation of sheep at different ages in clinical routine.

Distant hemodynamic values from these described, in association to liver changes on B-mode sonography, suggest the presence of liver disease and may increase diagnostic sensitivity.

Statistical differences were observed in DVP, AVP and PCI between the groups, presumably due to influence of weight and not to age.

### ACKNOWLEDGMENT

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### CONFLICT OF INTEREST STATEMENT

The authors have no competing interests.

### REFERENCES


<table>
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<tr>
<th>Table 2. Mean (± standard deviation) W, PVD, PVA, PMV, PBF and PCI in lambs, yearlings and ewes</th>
</tr>
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<tbody>
<tr>
<td>Groups</td>
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<tr>
<td>-------</td>
</tr>
<tr>
<td>W (kg)</td>
</tr>
<tr>
<td>PVD (cm)</td>
</tr>
<tr>
<td>PVA (cm²)</td>
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<tr>
<td>PMV (cm/s)</td>
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<tr>
<td>PBF (ml/min/kg)</td>
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<td>PCI (cm s)</td>
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</tbody>
</table>

*W = weight, PVD = portal vein diameter, PVA = portal vein area, PMV = portal mean velocity, PBF = portal blood flow, PCI = portal congestion index. a, b, c Means in the same row with different letters are significantly different (P<0.01).*
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