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Evaluation of Imaging Techniques and Invasive Procedures in Diagnosis of Fatty Liver in Dairy Cows

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ABSTRACT

Background: Fatty liver is a major metabolic disorder of dairy cows with a high morbidity rate during early lactation. Diagnosis of mild and moderate fatty liver is difficult because affected cows often do not differ clinically from cows with normal liver. The need for the early identification of fatty liver cannot be overemphasized as it appears to be the root cause for occurrence of many post-partum metabolic diseases. A precise diagnosis of the different categories of fatty liver is possible by liver biopsies which entail a minor surgery. Spectral doppler is yet another milestone in identification of fatty infiltration of liver, without causing any trauma to the liver. With this backdrop this research study was carried out to document the ultrasonographic pattern of liver, to evaluate the Spectral Doppler derived indices of portal blood flow in fatty liver syndrome in cattle.

Methods: The study was conducted in clinical cases, presented to Large Animal Medicine Outpatient Unit of Madras Veterinary College Teaching Hospital over a period of 24 months. Dairy cows, with the history of recent calving, postpartum inappetance and brought for other routine check-ups were included for the study. Suspected cases were subjected to further examination. Concurrent diseases presented with Fatty liver syndrome were recorded. Animals were grouped into four categories based on the above investigations as group I, II, III and IV as control, mild fatty liver, moderate fatty liver and severe fatty liver respectively.

Result: B-Mode Ultrasonography was superior in grading the fatty liver, compared to spectral doppler. Spectral Doppler derived indices of portal blood flow in fatty liver syndrome revealed significant reduction in venous pulsatility index in severe cases. But grading the fatty liver was not possible with spectral doppler. Among clinical manifestations, Postparturient inappetance (98.3 per cent), reduced milk yield and weight loss were the prominent in fatty liver syndrome. Major concurrent diseases in fatty liver syndrome were ketosis, haemoprotozoan diseases, repeat breeder and mastitis. The present study will be helpful in field level diagnosis of fatty liver in cattle and supplements the knowledge on concurrent diseases that could influence the prognosis of individual animal. The study also opens gateway to do further research in considering alternates to the liver biopsy and histopathological studies.

Key words: B-mode ultrasonography, Bovine, Fatty liver, Spectral doppler.

INTRODUCTION

The occurrence of metabolic and infectious diseases recorded during the life span of a dairy cow is highest during the peripartum period, making it the most critical time for them. Fatty liver is one such major metabolic disorder of dairy cows with a high morbidity rate during early lactation. Fatty liver syndrome develops when the hepatic uptake of lipids exceeds the oxidation and secretion of lipids by the liver. Clinical signs of fatty liver include a decline in health status, well-being, productivity and reproductive performance of cows, which are non-specific signs. It is also associated with increased veterinary costs, longer calving intervals, decreased milk production and decreased average life span of cows. In extreme cases, cows develop hepatic encephalopathy, which is characterized by depressed consciousness, ataxia, somnolence, coma and death. The incidence of fatty liver is strongly associated with the incidence of other metabolic, infectious, digestive and reproductive disorders and share a common precipitating cause *viz*., 'a severe negative energy balance'.

Diagnosis of mild and moderate fatty liver is difficult because affected cows often do not differ clinically from cows with normal liver. Blood parameters are found to be poor indicators of the syndrome. A precise diagnosis of the different categories of fatty liver is possible by liver biopsies which entail a minor surgery. While B mode ultrasonography is performed as an advanced procedure to diagnose fatty liver, Spectral doppler is yet another milestone in identification of fatty infiltration of liver, without causing any trauma to the liver. With this backdrop this research study

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was carried out to document the ultrasonographic pattern of liver and to evaluate the Spectral Doppler derived indices of portal blood flow in diagnosing and staging the fatty liver syndrome.

MATERIALS AND METHODS

The study was conducted in clinical cases, presented to Large Animal Medicine Outpatient Unit of Madras Veterinary College Teaching Hospital, Chennai for a period of two years (September, 2021 to August, 2023). Dairy cows, reported with the history of recent calving, postpartum inappetance and dairy cows brought for other routine check-ups were included for the study. The complete signalment, past and present history and their feeding habits were recorded. Clinical signs for fatty liver such as inappetance, lethargy, reduced milk yield and drastic weight loss were taken in to consideration. Such cows were subjected to detailed clinical examination. The initial screening was done by checking for the presence of ketone bodies in urine, cow side blood glucose level and blood and urine Beta-hydroxybutyric acid levels. Other relevant hematobiochemical profiles were also done. Suspected cases were subjected to system wise examination, special diagnostic procedures and clinicopathological evaluations. Concurrent diseases presented with Fatty liver syndrome were recorded. Animals were grouped into four categories based on the above investigations as group I, II, III and IV as control, mild fatty liver, moderate fatty liver and severe fatty liver respectively. Ultrasonography was performed for screening and grading of fatty liver. Histopathology, buoyancy studies were done with liver biopsy specimen. Spectral Doppler indices and Portal vessel blood flow volume were studied.

Buoyancy test

Approximate fat percentage of liver was arrived based on floating or sinking nature of liver piece in various solutions (Herdt *et al*., 1983).

Liver fat - less than 13%	Normal	Sinks in all the three *i.e.*, Distilled water, 1.025 and 1.055 Copper sulfate solutions
Liver fat - 13-25%	Mild	Floats in 1.055 Copper sulfate solution and sinks in distilled water and 1.025 Copper sulfate solution
Liver fat - 25-34%	Moderate	Floats in 1.025 and 1.055 Copper sulfate solution, but sinks in Distilled water
Liver fat - more than 34%	Severe	Floats in all the three solutions

Histopathology

The samples were examined for their lipid content under light microscope. Liver fat content was classified histopathologically to 6 different degrees (Grades der Leberverfettung or GdL), ranging from 0 (no fat droplets visible, totally normal hepatocytes) to 5 (panlobular fatty infiltration).

GdL 2 and Below : Mild
GdL 3-4 : Moderate
GdL 5 : Severe

Ultrasonography

The liver was examined ultrasonographically from caudal to cranial, beginning caudal to the last rib and ending at the fifth intercostal space and from dorsal to ventral in every intercostal space. The echogenicity, attenuation pattern were documented.

Spectral doppler analysis

Transcutaneous Doppler study of portal blood flow was carried out in the pulsed wave (PW) mode from the 10^{th} or 11^{th} intercostal space on the right in the area of the portal vein (Plate 8), immediately before ramification (Starke *et al*., 2011). Doppler analysis was carried out off-line for assessment of blood flow velocities, peak maximum velocity (Vmax), peak minimum velocity (Vmin) and mean maximum blood flow velocity (Vmean). The diameter of the portal vein was measured, its area calculated (A) and the blood flow volume (BFV) was calculated using the formula:

$$BFV\ (L/h) = Vmean\ (cm/s) \times 3.6 \times A\ (cm^2)$$

Venous pulsatility index (VPI) of the portal vein was also calculated (Gallix *et al*., 1997) by using the formula:

$$VPI = \frac{Vmax - Vmin}{Vmax}$$

RESULTS AND DISCUSSION

Clinical observations

The most common clinical signs observed in the study were, postparturient inappetance (98.3 per cent), reduced milk yield (95.53 per cent) and weight loss (90 per cent). Those common signs were in concurrence with the observations of Nagarajan (1990). Other signs were dullness, reduced rumen motility, lameness and occasional vague signs of a central nervous system disturbance such as ataxia with an odd stare recorded in the present study were in agreement with Adewuyi *et al.* (2005). One of the common clinical signs of weight loss was in concurrence with the observations of Nagarajan (1990), which was reported to be due to the mobilization of fat store from body reserve, during early lactation to compensate energy requirement, as stated by Katoh (2002). The nervous sign recorded in the present study was in accordance with Rehage *et al.* (1999) who reported about the development of hepatic encephalopathy which was characterized by depressed consciousness, ataxia and coma in severe cases of fatty liver syndrome. The possible reason for the hepatic encephalopathy could be due to the increased levels of toxic metabolic products such as ammonia, uric acid and decreased levels of blood glucose, that occurred in fatty liver syndrome (Sevinc *et al*., 2003).

Concurrent diseases

The concurrent diseases recorded in fatty liver syndrome cases were, Ketosis (32.39 per cent), Anaplasmosis (17.80 per cent), Repeat breeder (16.40 per cent), Mastitis (12.67 per cent), Ruminal impaction (5.40 per cent), Abortion (5.40 per cent), Recumbency (4.10 per cent), Metritis (4.10 per cent), Theileriosis (2.70 per cent), Dystocia (1.40 per cent), Retained fetal membrane (1.40 per cent) and Ruminal acidosis (1.40 per cent).

Combination of ketosis and Mastitis (4.1 per cent), ketosis and Anaplasmosis (4.1 per cent), ketosis and Theileriosis (1.4 per cent), Mastitis and Metritis (1.4 per cent), Abortion and Anaplasmosis (1.4 per cent) and Repeat breeder and Anaplasmosis (4.1 per cent) were also recorded along with clinical cases of fatty liver.

The results of the present study were in accordance with the observations of Esposito *et al.* (2014), who also recorded the above-mentioned diseases to occur concurrently in fatty liver syndrome, which was attributed to the interplay between the immune, endocrine, metabolic systems and diminished immune competence at calving as proposed by Trevisi *et al*. (2011).

The occurrence of ketosis to be the major concurrent disease in fatty liver syndrome in the present study was supported by Brindle *et al.* (1985) who hypothesized that, the high demand for gluconeogenesis which was prominently increased during early lactation for the synthesis of milk lactose resulted in exhaustion of oxaloacetic acid and diversion of acetyl-CoA towards ketogenesis, resulting in ketosis. While Top (1995) proposed ketosis as a driving force for the occurrence of fatty liver syndrome through the evidence that lipolysis was not started before the blood glucose concentrations had decreased.

The incidence of infectious diseases in the present study might be attributed to suppression of immune function and increase in concentrations of pro-inflammatory cytokines as reported by Ametaj *et al*. (2002) and decreased capacity of leucocyte to migrate into the infected mammay gland or uterus as the reason for non-responsive mastitis in fatty liver syndrome (Zerbe *et al*., 2000).

B-mode ultrasonography

Normal study (Control group)

In the present study, liver could be visualized from 12th intercostal space to 7th intercostal space, which was in accordance with the observations of Braun (2009). The ultrasonographic patterns of homogenous echogenecity, clear visualization of intrahepatic vessel margins and diaphragm (Plate 1) were in agreement with the observations of Tharwat *et al.* (2012) who suggested the homogenous echogenecity to be caused mainly by a series of alternate collagen-water interfaces. The location of various intrahepatic structures and the anatomical proximity of liver margin to various visceral organs, as visualized through different intercostal spaces were also in concurrence with that of Braun (2009) and Starke *et al*. (2010).

There was a good correlation between B-mode ultrasonographic pattern of liver and the histopathological study of liver biopsy sample.

B-mode ultrasonographic pattern in mild fatty liver

Bright pattern, with visibility of intrahepatic vessel walls and visibility of diaphragm were the striking appearances of the B-mode ultrasonogram which correlates with the observations of Tharwat *et al.* (2012) and Besheer *et al*. (2023). The appearance of bright pattern could be attributed to the lower acoustic impedance of fat droplets, in contrast to the hypoechoic nature of normal liver parenchyma (Acorda *et al*., 1994). An earlier sign of deposition of fat droplets only around the intrahepatic vessels, with normal hepatic parenchyma was also recorded (Plate 2), which ranks the B-mode ultrasonography to be a more precise diagnostic tool.

B-mode ultrasonographic pattern in moderate fatty liver

The major pattern of B-mode ultrasonogram recorded in moderate fatty infiltration was heterogenous echogenecity



Plate 1: B-mode ultrasonographic patterns of normal liver.

of liver, either with blurred intrahepatic vessel margins or disappearance of minor intrahepatic vessels (Plate 3). The findings in the present study could be correlated to the findings of Acorda *et al.* (1994); Braun (2009) and Tharwat *et al.* (2012) who jointly opined that, heterogenicity and vascular blurring were due to coarsened liver parenchyma that occur due to infiltration of diffused micro fat droplets to macro-fatty bodies in liver.

B-mode ultrasonographic pattern in severe fatty liver

Deep attenuation was the major B-mode ultrasonographic finding in the present study (Plate 4). The findings of the present study were in agreement with the observations of Acorda *et al.* (1994) and Starke *et al.* (2010), who opined that deep attenuation to be a distinguished finding in severe fatty liver infiltration. These observations in the present study could be correlated with the panlobular distribution of macrovacuolar changes in hepatocytes, with alteration of the individual cells, as observed in the present study (Plate 5).

Liver biopsy

Buoyancy

In the present study, out of 75 clinical samples more than 88 per cent of samples from each category showed the floating or sinking properties in distilled water or copper sulfate solutions of 1.025 and 1.055 specific gravities when compared to the gold standard, histopathology and those were in agreement with the findings of Gowri *et al.* (2013).

Histopathology

The biopsy samples of severe fatty liver cows showed a panlobular distribution of fat droplets indicated by higher percentage of macrovacuoles in the cytoplasm of almost all the hepatocytes altering the shape of hepatocytes and pushing the nucleus towards the periphery, giving an

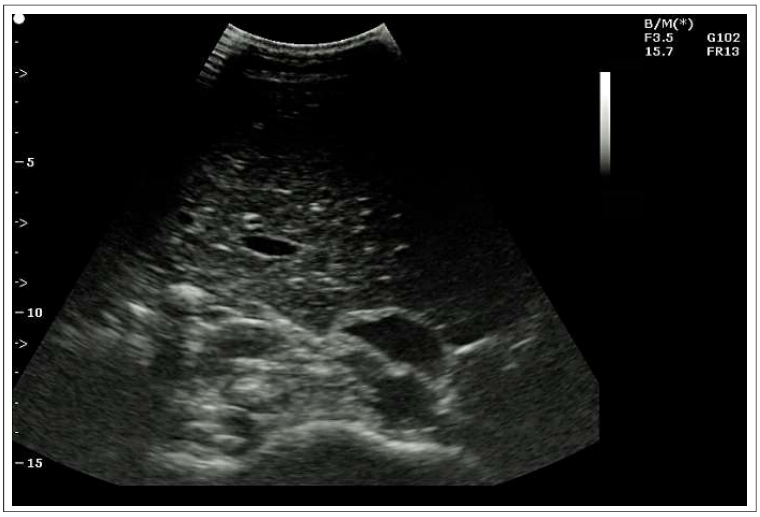


Plate 2: B-mode ultrasonographic view of mild fatty liver with bright pattern around the veins (3.5 Mhz).



Plate 3: B-mode ultrasonographic view of moderate fatty liver with heterogenous echogenecity and blurred vessel margins (3.5 Mhz).



Plate 4: B-mode ultrasonographic pattern of severe fatty liver syndrome with deep attenuation and near field vessel blurring (3.5 Mhz).

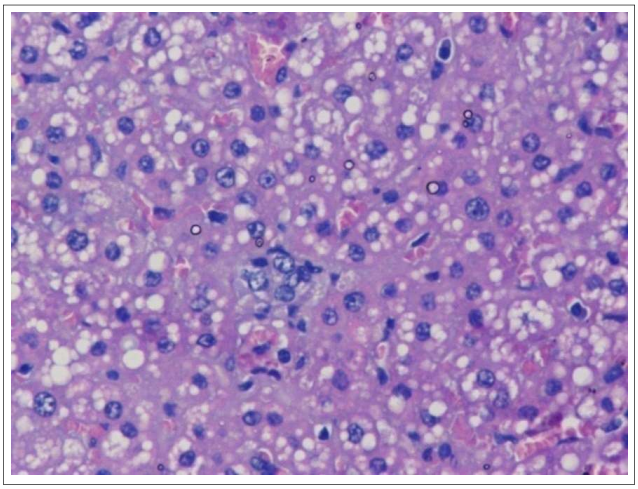


Plate 5: Grade IV-Numerous micro and macrovacuolar changes (40×).

indication of cellular damage (Plate 5). This stage was in correlation with the increased levels of leakage enzymes such as AST. The histopathological findings of the present study were in correlation with the findings of Bobe *et al.* (2004).

Spectral doppler study of portal vessel

Spectral doppler wave forms

In the present study, irregular and bizarre wave patterns, with irregular amplitudes and reduction in Vmax were recorded in various degrees of fatty infiltration (Plate 6-9). Dampening of the wave with reduction in amplitude was the only consistent quality against the severity of fatty infiltration in liver. The change in the wave patterns in fatty liver cases in the present study could be attributed to the accumulation of fat in hepatocytes in fatty liver (Orrego *et al*., 1981), loss of elasticity of portal vessel (Johannsen *et al*., 1993), due to compression over the vasculature posed by the intra hepatic TAG accumulation (Erdogmus *et al*., 2008), that led to the dampened wave forms (Starke *et al*., 2011).

Grading of fatty liver based on the wave patterns was not possible as there was overlapping of the wave patterns between mild and moderate groups of fatty liver syndrome.

Spectral doppler derived indices

In the present study, a significant reduction in venous pulsatility index was recorded in clinical cases of fatty liver syndrome (Table 1) with an insignificant reduction in portal vessel blood flow volume (PBFV) which was in accordance with the findings of Reynolds *et al*. (2003). The negative correlation recorded in the present study was in concurrence with the findings of Starke *et al*. (2011).

The insignificant reduction in blood flow volume in the present study could be attributed to the increase in compensatory pressure as postulated by Erdogmus *et al.* (2008).

Sensitivity and specificity of various diagnostic tests

In the present study the sensitivity and specificity of B-mode ultrasonography was found to be closer to histopathology when compared to the sensitivity and specificity of buoyancy and spectral doppler studies (Table 2).

The comparatively reduced sensitivity and specificity of spectral doppler wave in the present study, in comparison to B-mode ultrasonography was due to the non-specific changes in the wave pattern such as irregular amplitudes being shown in mild and moderate cases, insignificant reduction in the maximum velocity between the various clinical groups and lack specific waveform for a particular group as proposed by Haudum *et al.* (2011).

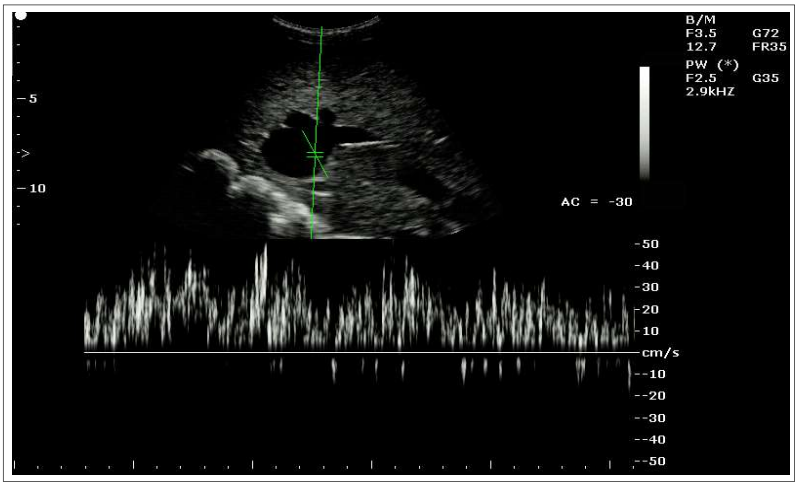


Plate 6: Normal venous pulsatility wave pattern over portal vein.

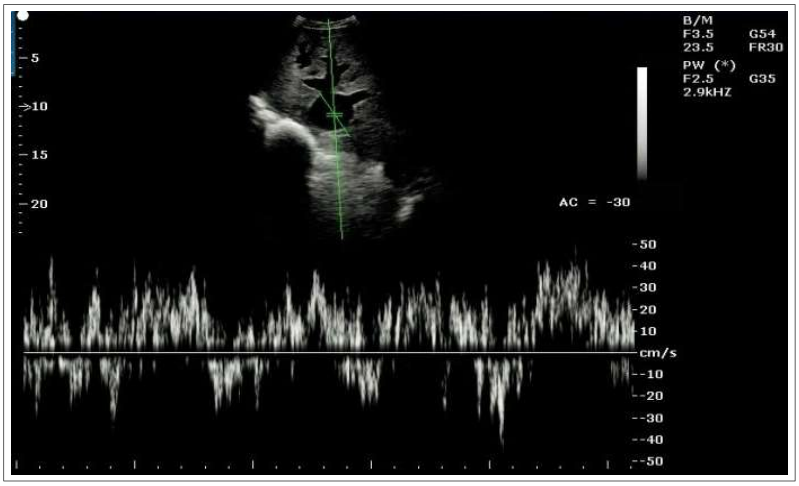


Plate 7: Spectral doppler wave of portal vessel in mild fatty liver showing irregular amplitudes of venous pulsatility.



Plate 8: Spectral doppler wave of portal vessel in moderate fatty liver cases showing bizarre pattern.

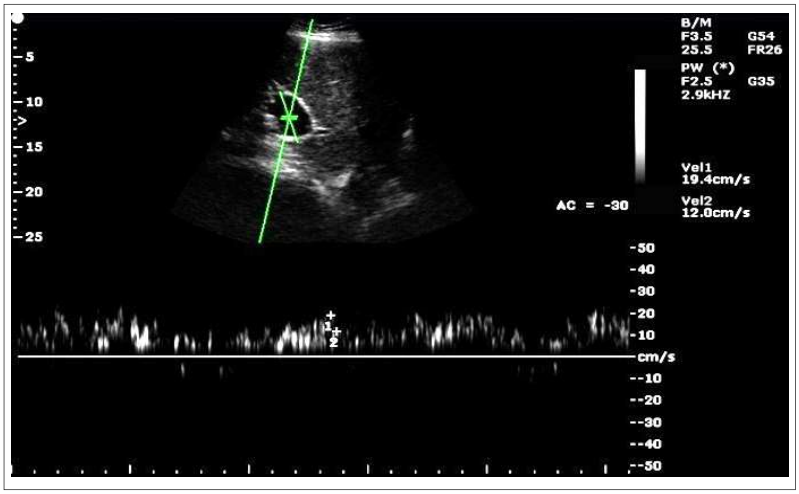


Plate 9: Dampened waves in severe fatty liver cases.

Table 1: Spectral doppler indices of portal vessel in various groups of fatty liver.

Parameter	Group I (n=10)	Group II (n=42)	Group III (n=27)	Group IV (n=6)	F value
Vmean (cm/sec)	47.92^{a}±1.98	46.29^{a}±0.61	45.25^{a}±0.96	41.01^{b}±1.36	3.16*
VPI	0.19^{a}±0.005	0.17^{ab}±0.002	0.16^{bc}±0.004	0.15^{c}±0.01	6.47**
PBFV (L/h)	1240.16±54.07	1163.73±24.62	1102.44±34.98	1043.66±29.86	2.64^{NS}

** = P<0.01; * = P,<0.05; NS= Non-significant.

Parameters bearing same superscript do not differ significantly.

Table 2: Sensitivity and Specificity of various diagnostic tests in diagnosing fatty liver, compared to histopathology.

Parameter	Sensitivity (%)	Specificity (%)
B mode ultrasonography	90.14	100
Buoyancy	88.41	90
Spectral doppler study	83.3	80

CONCLUSION

Major clinical signs observed in fatty liver syndrome were inappetance (98.3 per cent), reduced milk yield (95.53 per cent) and weight loss (90 per cent) and vague signs included dullness, reduced rumen motility, ataxia and lameness. The major concurrent diseases recorded were Ketosis (32.39 per cent), Hemoprotozoan diseases (20.50 per cent), Repeat breeder (16.40 per cent) and Mastitis (12.67 per cent).

The sensitivity and specificity of B mode ultrasonography was found to be superior and closer to histopathology when compared to sensitivity and specificity of buoyancy and spectral doppler results.

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Conflict of interest

The authors declare that there are no conflicts of interest.

REFERENCES

Acorda, J.A., Yamada, H. and Ghamsari, S.M. (1994). Ultrasonographic features of diffuse hepatocellular disorders in dairy cattle. Vet. Radiol. and U.S. 35(3): 196-200.

Adewuyi, A.A., Gruysi, E. and Van Eerdenburg, F.J.C.M. (2005). Non esterified fatty acids (NEFA) in dairy cattle. A review. Vet. Q. 27(3): 117-126.

Ametaj, B.N., Bradford, B.J., Bobe, G. and Beitz, D.C. (2002). Acute phase response indicates inflammatory conditions may play a role in the pathogenesis of fatty liver in dairy cows. J. Dairy Sci. 85(Suppl.1): 189.

Besheer, G., Elshafey, Elfadadny, A., Metwally, S., Saleh, A.G., Ragab, R.F. *et al*. (2023). Association between biochemical parameters and ultrasonographic measurement for the assessment of hepatic lipidosis in dairy cows. Italian Journal of Animal Science. 22(1): 136-147. doi: 10.1080/1828051X.2023.2170284.

Bobe, G., Young, J.W. and Beitz, D.C. (2004). Invited review: Pathology, etiology, prevention and treatment of fatty liver in dairy cows. J. Dairy Sci. 87(10): 3105-3124.

Braun, U. (2009). Ultrasonography of the liver in cattle. Vet. Clin. North Am. Food Anim. Pract. 25(3): 591-609.

Brindle, N.P.J., Zammit, V.A. and Pogson, C.I. (1985). Regulation of carnitine palmitoyltransferase activity by malonyl CoA from mitochondria in sheep liver, a tissue with low capacity for fatty acid synthesis. J. Biol. Chem. 249(5): 177-182.

Erdogmus, B., Tamer, A., Buyukkaya, R., Yazici, B., Buyukkaya, A., Korkut, E., Alcelik, A. and Korkmaz, U. (2008). Portal vein hemodynamics in patients with non-alcoholic fatty liver disease. Tohoku J. Exp. Med. 215(1): 89-93.

Esposito, G., Irons, P.C., Webb, E.C. and Chapwanya, A. (2014). Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim. Reprod. Sci. 144(3): 60-71.

Gallix, B., Taourel, P., Dauzat, M., Bruel, J. and Lafortune, M. (1997). Flow pulsatility in the portal venous system: A study of Doppler sonography in healthy adults. American Journal of Roentgenology. 169(1): 141-144. doi: 10.2214/ajr.169.1.9207514.

Gowri, B., Prathaban, S., Srinivasan, S.R. and Balachandran, S. (2013). Clinicopathology of fatty liver in cows. Indian Vet J. 19(4): 138-139.

Haudum, A., Starke, A., Beyerbach, M., Wohlsein, P. and Rehage, J. (2011). Ultrasonographic assessment of liver dimensions in dairy cows with different hepatic triacylglycerol content. J. Anim Sci. 89(5): 1392-1400.

Herdt, T.H., Goeders, L., Liesman, J.S. and Emery, R.S. (1983). Test for estimation of bovine hepatic lipid content. JAVMA. 182(9): 953-955.

Johannsen, U., Menger, S., Staufenbiel, R. and Klukas, H. (1993). Studies on the morphology and function of the liver of high performance cows 2 weeks post partum. Dtsch. Tierarztl. Wochenschr. 100(2): 177-181.

Katoh, N. (2002). Relevance of apolipoproteins in the development of fatty liver and fatty liver-related peripartum diseases in dairy cows. J. Vet. Med. Sci. 64(4): 293-307.

Nagarajan, B. (1990). Liver biopsy in bovines. Thesis submitted to Tamil Nadu Veterinary and Animal Sciences University.

Orrego, H., Blendis, L.M., Crossley, I.R., Medline, A., Macdonald, A., Ritchie, S. and Israel, Y. (1981). Correlation of intrahepatic pressure with collagen in the disse space and hepatomegaly in humans and in the rat. Gastroenterol. 80(3): 546-556.

Rehage, J., Qualmann, K., Meier, C., Stockhofe-Zurwieden, N., Hoeltershinken, M. and Pohlenz, J. (1999). Total serum bile acid concentrations in dairy cows with fatty liver and liver failure. Dtsch Tierarztl Wochenschr. 106(1): 126-129.

Reynolds, C.K., Aikman, P.C., Lupoli, B., Humphries, D.J. and Beever, D.E. (2003). Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. J. Dairy Sci. 86(4): 1201-1217.

Sevinc, M., Basoglu, A., Huzelbektas, H. and Boydak, M. (2003). Lipid and lipoprotein levels in dairy cows with fatty liver. Turk. J. Vet. Anim. Sci. 27(2): 295-299.

Starke, A., Haudum, A., Weijers, G., Herzog, K., Wohlsein, P., Beyerbach, M., De Korte, C.L., Thijssen, J.M. and Rehage, J. (2010). Noninvasive detection of hepatic lipidosis in dairy cows with calibrated ultrasonographic image analysis. J. Dairy Sci. 93(7): 2952-2965.

Starke, A., Schmidt, S., Haudum, A., Scholbach, T., Wohlsein, P., Beyerbach, M. and Rehage, J. (2011). Evaluation of portal blood flow using transcutaneous and intraoperative. Doppler ultrasonography in dairy cows with fatty liver. J. Dairy Sci. 94(6): 2964-2971.

Tharwat, M., Oikaw, S. and Buczinski, S. (2012). Ultrasonographic prediction of hepatic fat content in dairy cows during the transition period. J. Vet. Sci. Technol. 3(1): 111. doi: 10.4172/2157-7579.1000111.

Top, A.M. (1995). Diet and lipid metabolism in ruminants with particular reference to fatty liver development. Vet. Sci. Commun. 3(4): 231-236.

Trevisi, E., Bertoni, G., Archetti, I., Amadori, M. and Lacetera, N. (2011). Inflammatory response and acute phase proteins in the transition period of high-yielding dairy cows. Intech, Open Access Publisher. pp. 355-379.

Zerbe, H., Schneider, N., Leibold, W., Wensing, T., Kruip, T.A.M. and Schuberth, H.J. (2000). Altered functional and immunophenotypical properties of neutrophilic granulocytes in postpartum cows associated with fatty liver. Theriogenol. 54(5): 771-786.