Hepatic lipidosis in transition buffaloes in relation to back fat thickness, hemato-biochemical and mineral profile

Randhir Singh*, Sarnarinder Singh Randhawa and Charanjit Singh Randhawa

Department of Veterinary Medicine, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab, India.

Received: 14-09-2016 Accepted: 29-03-2017 DOI: 10.18805/ijar.B-3306

ABSTRACT
The study involved 140 buffaloes, 125 (87.41%) with ultrasonography (USG) features of normal liver and 18 (12.58%) with features of hepatic lipidosis (HL). Among the later 7 had grade I, 6 grade II and 5 grade III USG features of HL. The grouping of animals according to transition stage and evaluation of body condition score (BCS) showed that buffaloes with moderate to severe HL lost BCS from Far off dry (FOD) to fresh stage by more than one (1.0) increment whereas, healthy buffaloes lost BCS from FOD to fresh stage by less than 0.5 increment. Significantly low levels of haemoglobin, packed cell volume and total erythrocyte count was observed in buffaloes with severe HL. Significantly low levels of total plasma protein, glucose, calcium, potassium, copper and zinc along with increased levels of plasma urea nitrogen, beta hydroxyl butyric acid, non esterified fatty acid, aspartate amino transferase, alanine amino transferase, gamma glutamyl transferase, lactate dehydrogenase and Glutamic dehydrogenase were observed in buffaloes with HL. A strong positive association exists between the severity of BCS and back fat thickness (BFT) reduction and occurrence of HL in transition buffaloes.

Key words: Back fat thickness, Body condition score, Hemato-biochemical, Hepatic lipidosis, Mineral profile.

INTRODUCTION
Hepatic lipidosis (HL) is an increasingly recognized production disorder in high yielding dairy animals, constituting a major cause of liver related economic losses due to morbidity and mortality (Boke et al., 2004). The major risk factors for HL development in dairy cattle include negative energy balance (NEB), reduced dry matter intake (DMI) prior to calving, oxidative stress coupled with hormonal imbalance and parturition (Mohamed et al., 2004; Chandra et al., 2011; Singh et al., 2015a). Hepatic lipidosis is most common within first two weeks after parturition and affects almost half of the dairy herd immediately after calving (Jorritsma et al., 2001) usually in association with other production disorders. Excessive fat accumulation in the liver impairs normal liver function (Murondot et al., 2004), which may lead to hyperketonemia. Currently, the most reliable diagnostic test for HL is based on measurement of the amount of total lipids in liver by liver biopsy. Biopsy being an invasive technique involving risk of hemorrhage and infection is now a day’s widely replaced by use of ultrasonography (USG). In addition to this, the severity of fatty infiltration of liver can also be evaluated based on various hemato-biochemical and liver function tests.

The present study was designed to evaluate (1) the changes in BCS and BFT during transition period in relation to development of HL in water buffaloes (2) incidence of HL in water buffaloes of Punjab based on USG findings and various hemato-biochemical and liver function tests (3) to grade the severity of HL based on USG and laboratory findings.

MATERIALS AND METHODS
Animals: One hundred forty (140) multiparous buffaloes in advanced pregnancy were used and grouped according to transition stage as:
Far off dry (FOD): > 10 days following dry off and not < 30 days expected to calving
Close up dry (CUD): expected calving within 3 to 21 days
Fresh (F): 3 to 30 days in milk

Body composition: Body condition score (BCS) was estimated by the same individual using the five point visual BCS technique with 0.5 increment (Singh et al., 2015b).

Back fat thickness (BFT): Subcutaneous back fat thickness was measured by real time ultrasound using a portable Sonosite instrument at 7.5 MHz frequency. The transducer was placed vertically to an imaginary line between the pins (tuber Ischia) and hooks (tuber coxae) at the sacral examination site (≥9-11 cm cranial to the pins) (Nanda and Herdt 2009) after shaving of site and application of coupling gel. Images were measured at depth of 4.7.

Ultrasound for diagnosis of hepatic lipidosis (HL): All the buffaloes were examined after calving at fresh stage for fatty infiltration of liver by using portable ultrasound machine ‘Sonosite M-turbo’ having 2-5 MHz convex transducer. The 18 buffaloes showing characteristic ultrasonographic lesions

*Corresponding author’s e-mail: dr.randhirlo@gmail.com
of fatty infiltration of liver were subjected to routine hematobiochemistry and plasma mineral estimation.

The ultrasonographic features of HL were described according to Komeilian et al., 2011 i.e.,

I. Normal: homogenous granular echo texture of hepatic parenchyma with clear sharp obvious margins and absence of vessel blurring.

II. Grade I (Mild): bright pattern of liver and presence of vessel blurring but absence of marked deep attenuation.

III. Grade II (Moderate): bright pattern of liver, vessel blurring and presence of marked deep attenuation.

IV. Grade III (Severe): bright pattern of liver, vessel blurring and presence of marked deep attenuation. Hepatic vessels are difficult to identify and there is hyperechogenisity of the near field.

Hematology: Hematology was carried out on hematological analyser (ADVIA 2120, SIEMENS Hematology Analyzer, USA). Where as estimation of biochemical parameters viz. total plasma protein (TPP), albumin, plasma urea nitrogen (PUN), creatinine, glucose, sodium (Na), potassium (K) along with enzymes viz., aspartate amino transferase (AST), alanine amino transferase (ALP), triglyceride (TG), gamma glutamyl transferase (GGT) and lactate dehydrogenase (LDH) were analysed using Orthodiagnostic’s Vitros 350 biochemistry analyser using commercial kits. Glutamic dehydrogenase (GDH) was estimated manually as per the method of Ellen et al. (1992) whereas; arginase was estimated as per the method of Mia and Koger (1978). Both beta hydroxyl butyric acid (BHBA) and non esterified fatty acid (NEFA) were estimated on the ELISA plates from the plasma samples with the help of kits provided by Diasys Diagnostics systems, Germany.

Plasma minerals analysis: Plasma minerals analysis was done using Atomic absorption spectrophotometer (Perkin Elmer Analyst 700, USA). Digested plasma samples were used for estimation of copper (Cu), iron (Fe) and zinc (Zn) whereas, calcium and magnesium were estimated by mixing 0.1 ml plasma with 9.9 ml of 0.1 per cent of lanthanum chloride. Plasma inorganic phosphorus (Pi) was determined using method given by Tausky and Shorr (1953).

Statistical Analysis: The statistical analysis was carried out using SPSS (16.0). ANOVA followed by Duncan’s multiple range test (DMRT) was used to estimate significant difference at P < 0.05.

Ethical approval: All the procedures have been carried out in accordance with the guidelines laid down by the Institutional Ethics Committee and in accordance with local laws and regulations.

RESULTS AND DISCUSSION

Out of 140 examined, 125 (87.41%) had ultrasonographic features of normal liver and 18 (12.58%) had ultrasonographic features of HL. Among the latter 7 (38.88%) had grade I, 6 (33.33%) had grade II and 5 (27.77%) had grade III ultrasonographic features of HL (Fig 1a to 1d).

Body condition score and back fat thickness: The average BCS and BFT in all buffaloes was highest at FOD stage and reduced to lowest level after parturition at fresh stage. In healthy buffaloes BCS reduced significantly (P<0.05) from FOD to CUD and fresh stages (3.06±0.03 to 2.91±0.04, and to 2.61±0.04, respectively). Similar trend was observed for BFT (reduced from 1.73±0.03 to 1.61±0.04 to 1.32±0.03 cm, respectively). In mild HL, BCS and BFT were significantly lower at fresh (3.00±0.10 and 1.57±0.07 cm) and CUD stages (3.28±0.10 and 1.77±0.11 cm) as compared to FOD stage (3.64±0.09 and 2.16±0.15 cm) but did not differ significantly between CUD and fresh stages (Fig 2). In moderate HL, BCS reduced significantly from FOD to CUD and fresh stages (3.28±0.10 and 1.77±0.11 cm ) as compared to FOD stage (3.64±0.09 and 2.16±0.15 cm) but did not differ significantly between CUD and fresh stages (Fig 2). In severe HL, BCS and BFT were significantly lower at fresh (3.68±0.25 and 1.94±0.21 cm, respectively) as compared to FOD (4.70±0.20 and 3.26±0.29

Fig 1 (a): Ultrasonograms of normal liver of buffaloes; note the homogenous granular echo-texture of parenchyma and sharp obvious margins of vessels. (PV: Portal vein)
Fig 1 (b): Ultrasonograms of grade I fatty infiltration in buffaloes; note the bright pattern and vessel blurring, along with hyperecogeniety of near field but deep attenuation is not marked. (PV: Portal vein, OMA: Oesumum)

Fig 1 (c): Ultrasonograms of grade II fatty infiltration in buffaloes; note the bright pattern, vessel blurring and presence of obvious deep attenuation. (PV: Portal vein)

Fig 1 (d): Ultrasonograms of grade III fatty infiltration in buffaloes; note the bright pattern, vessel blurring and marked deep attenuation along with hyperecogeniety of near field. (PV: Portal vein)
cm, respectively) and CUD stages (4.60±0.18 and 2.80±0.24 cm, respectively) but did not differ significantly between FOD and CUD stages. Healthy buffaloes were having less BCS and BFT throughout the transition period as compared to buffaloes with moderate to severe HL. Buffaloes suffering from moderate to severe HL lost body condition from FOD to fresh stage by more than one (1.0) increment whereas, healthy buffaloes lost body condition from FOD to fresh stage by less than 0.5 increment. In the present study, the BCS was evaluated at three predefined transition stages in buffaloes on 1-5 scale with 0.5 increments, as a single BCS does not give any indication of whether a buffalo is gaining or losing body reserves over a period of time. Buffaloes suffering from HL with BCS > 4.0 prior to calving and they reduced significant amount of body condition and subcutaneous fat thickness after calving, suggesting a clear relation between the amount of adipose fat mobilization and development of HL. On the other hand, healthy buffaloes with BCS 3.0 prior to calving only reduced small amount of body condition and subcutaneous fat thickness after calving, suggesting that buffaloes calving at an average BCS of 3.0 are less likely to develop HL. Similar to our findings, previous studies (Bernabucci et al., 2005) also reported higher reduction in high BCS cows from late pregnancy to first 30 days in milk, than the cows with average and good BCS. This may be attributed to increased resistance of adipose tissue to insulin which predisposes the dairy animal to mobilize NEFA, thus potentially creating a vicious cycle of NEFA mobilization and DMI reduction during late prepartum period (Lucy et al., 2009).

**Ultrasonographic features of HL:** Out of 140 cases, portal vein (PV) with its stellate ramification was visible in 73(51.04%), whereas PV without stellate ramification was observed in 63(44.05%) cases. Portal vein was scarcely visible in 7(4.89%) cases. Caudal vena cava (CVC) was seen in only 12 (8.39%) cases. Bright pattern was present in all HL cases. Bright pattern along with vessel blurring and moderate deep attenuation of near field was characteristic of moderate HL cases. Bright pattern, vessel blurring along with marked deep attenuation and hyper-echogenesity was seen in severe HL cases. Hepatic ultrasonograms in the normal buffalo are characterized by numerous weak echoes distributed homogeneously over the entire liver parenchyma. The portal and hepatic veins can be seen within the normal echotexture, and the parenchymal edges are normally visible (Mohamed et al., 2004). In the present study, increased echogenicity of hepatic parenchyma in HL cases could be attributed to increased intensity of the internal echoes where the liver parenchyma appears white on ultrasonograms and is also difficult to differentiate from surrounding tissue. The liver and vessels contrast was also decreased. At many instances only large vessels were visible on ultrasonograms whereas, small vessels were poorly imaged or not seen at all. This may be due to the fact that the small vessels are compressed by infiltrated hepatic tissue (Mohamed et al., 2004; Braun, 2009 and Tharwat, 2012). Acoustic impedance due to fat containing hepatocytes leads to weakness of the echoes as the distance from the abdominal wall is increased, ultimately, leading to hyperechoic near field. This results in the hyperechogenicity of the region near the abdominal wall, whereas areas more distant from abdominal wall were imaged hypoechochogenic. The reason for such low percentage of CVC visibility in buffaloes was very little inter costal space which hinders the placement of transducer.

**Hematological indices:** Significantly low levels of Hb, PCV and TEC were observed in severe HL cases (8.82±0.15 g/dl, 26.40±0.30%, 4.80±0.09 x10^6/µl, respectively) as compared to healthy counter parts (12.50±0.33 g/dl, 33.22±1.37%, 6.19±0.11 x10^6/µl, respectively). PCV was significantly lower in mild (29.79±0.37%) and moderate (26.84±0.43%) HL as compared to healthy buffaloes while TEC did not differ significantly from healthy buffaloes. Significantly higher average values of TLC (x10^3/µl) were observed in moderate (11.03±0.19) and severe (11.98±0.14) HL as compared to mild HL (9.41±0.09) and healthy buffaloes (8.79±0.37). Leukocytosis may be attributed to intravascular hemolysis.
and in cases of less severe damage, the cells may be destroyed within the monocyte-macrophage system in the spleen, liver, bone marrow, and lymph nodes.

**Biochemical profile:** Detailed biochemical profile of buffaloes is presented in Table 1 and shows significantly low levels of TPP in all three groups of HL affected buffaloes as compared to healthy control. Plasma urea nitrogen was significantly increased in animals with moderate to severe HL as compared to healthy control. Hypoglycaemia was the feature of buffaloes with moderate to severe HL. Significant increase was observed in BHBA and NEFA levels in moderate to severe HL affected buffaloes. Mean AST, ALP, GGT, LDH and GDH levels were significantly higher in moderate and severe HL cases with enzyme levels crossing the upper critical limit. Triglyceride recorded decrease in moderate to severe HL cases whereas, arginase recorded increase in mild to severe HL cases. Hepatic lipidosis in the present study was associated with elevated concentrations of NEFA and BHBA, both of which can be cytotoxic at high concentrations and can decrease the physiological functions of organs leading to adverse metabolic consequences (Tharwat, 2012). An elevated concentration of NEFA increases lipogenesis and ketogenesis in hepatocytes (Cadorniga et al., 1997) and high concentrations of BHBA decreases rate of gluconeogenesis. Hepatocellular enzymes, particularly AST and GDH may be useful in monitoring the HL in buffaloes that commonly occurs at parturition. Glutamate dehydrogenase is more liver-specific enzyme. Increased serum GDH activity is suggestive of either hepatocyte death or sub-lethal hepatocyte trauma. Previous studies reported that GDH and GGT concentrations in dairy animals with liver tumors (Braun et al., 2005). Likewise, other researchers mentioned that AST, SDH and GDH activity was increased in cases of fatty liver syndrome because of liver cell destruction (Kalaitzakis et al., 2007). The activities of cholestatic enzymes such as GGT and hepato-cellular leakage enzymes such as AST have been used to evaluate liver function in dairy animals. In present study, serum GDH and AST concentrations were increased in buffaloes with moderate and severe HL. Although higher GDH levels are generally reported in acute liver damage (within 4 to 24 hours of hepatic injury), our findings suggest that higher levels of GDH can also be found in buffaloes with HL, which may either develop within hours or take weeks. A similar finding of an increased level of GDH in HL cases was previously documented (Ok et al., 2013). There was significant decline in serum triglyceride levels in moderate and severe HL cases as compared to healthy controls. Previous studies by several researchers (Kalaitzakis et al., 2007; Ok et al., 2013; Drackley et al., 2001 and Sevinc et al., 2001) reported that triglyceride, cholesterol and HDL-cholesterol concentrations were decreased in the cows with fatty liver.

**Plasma minerals profile:** Calcium, K, Cu and Zn levels were significantly lower in HL groups as compared to healthy control (Table 1). The mean values of phosphorus, magnesium and sodium did not differ significantly between three forms of HL or from healthy buffaloes.

**CONCLUSION**

The present study highlights a strong positive association between severity of BCS and BFT reduction and occurrence of HL in transition buffaloes. Further, ultrasound can be used with promising results for diagnosis and grading of HL in buffaloes at farm level.

**Table 1: Biochemical parameters and mineral profile in healthy buffaloes (control) and buffaloes with mild, moderate and severe hepatic lipidosis**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy(n=08)</th>
<th>Mild(n=07)</th>
<th>Moderate(n=06)</th>
<th>Severe(n=05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPP (g/dl)</td>
<td>7.23±0.09w</td>
<td>6.98±0.07w</td>
<td>6.31±0.06y</td>
<td>5.54±0.08w</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.86±0.03w</td>
<td>2.78±0.01w</td>
<td>2.48±0.06x</td>
<td>2.22±0.04x</td>
</tr>
<tr>
<td>PUN (mg/dl)</td>
<td>9.94±0.20w</td>
<td>9.93±0.11w</td>
<td>11.89±0.31y</td>
<td>16.00±0.46y</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.16±0.02w</td>
<td>1.12±0.03x</td>
<td>1.15±0.04y</td>
<td>1.18±0.05y</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>70.90±0.59w</td>
<td>69.38±0.58w</td>
<td>60.30±0.53y</td>
<td>52.00±0.73y</td>
</tr>
<tr>
<td>BHBA (mmol/L)</td>
<td>0.36±0.03w</td>
<td>0.38±0.04x</td>
<td>0.64±0.03y</td>
<td>0.93±0.02y</td>
</tr>
<tr>
<td>NEFA (mmol/L)</td>
<td>0.28±0.03x</td>
<td>0.31±0.04x</td>
<td>0.50±0.02y</td>
<td>0.63±0.02y</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>65.72±0.81w</td>
<td>71.96±1.26w</td>
<td>100.60±3.13y</td>
<td>164.02±6.26y</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>27.66±0.64w</td>
<td>33.58±0.90x</td>
<td>122.38±3.65y</td>
<td>160.89±1.52y</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>35.19±0.78w</td>
<td>34.91±0.59x</td>
<td>32.64±3.44y</td>
<td>29.18±0.49y</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>25.33±0.75y</td>
<td>37.39±2.14y</td>
<td>75.25±1.01x</td>
<td>98.72±2.36y</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>1093.90±29.00w</td>
<td>1381.50±38.77w</td>
<td>1851.70±29.48w</td>
<td>2150.00±35.35w</td>
</tr>
<tr>
<td>GDH (u/ml)</td>
<td>10.00±0.20x</td>
<td>13.41±0.64y</td>
<td>23.11±0.61y</td>
<td>28.65±0.21y</td>
</tr>
<tr>
<td>Arginase (IU/L)</td>
<td>4.23±0.07w</td>
<td>4.70±0.13x</td>
<td>5.59±0.07y</td>
<td>5.67±0.07y</td>
</tr>
<tr>
<td>Ca (mmol/l)</td>
<td>2.42±0.04w</td>
<td>2.23±0.03x</td>
<td>1.85±0.04y</td>
<td>1.78±0.04y</td>
</tr>
<tr>
<td>K (mmol/l)</td>
<td>4.20±0.04x</td>
<td>4.34±0.05x</td>
<td>4.55±0.05y</td>
<td>4.74±0.05y</td>
</tr>
<tr>
<td>Cu (µmol/l)</td>
<td>11.18±0.06z</td>
<td>10.97±0.05x</td>
<td>10.84±0.04y</td>
<td>10.69±0.04x</td>
</tr>
<tr>
<td>Fe (µmol/l)</td>
<td>141.45±1.40x</td>
<td>139.4±1.12x</td>
<td>139.02±1.05y</td>
<td>134.15±1.10y</td>
</tr>
<tr>
<td>Zn (µmol/l)</td>
<td>14.95±0.04x</td>
<td>14.81±0.08x</td>
<td>13.72±0.12y</td>
<td>11.91±0.17y</td>
</tr>
</tbody>
</table>

Values bearing different superscripts (w, x, y) across the rows differ significantly (P<0.05)
ACKNOWLEDGEMENT

The authors are highly thankful to the Director of Research and the Head, Department of Veterinary Medicine, GADVASU for providing the necessary facilities. The authors thank the participating buffalo farm owner for his cooperation throughout the study period.

REFERENCES


