Hemato-Biochemical Studies on Clinical Cases of Primary Ketosis in Buffaloes

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ABSTRACT

The study was conducted on 145 buffaloes brought to Teaching Veterinary Clinical Complex, LUVAS from Hisar and adjoining villages, with signs of anorexia and decreased milk yield. Urine samples from buffaloes were screened for ketosis using two tests (Rothera’s test and Keto-Diastix-strip test). The disease was confirmed in 24 buffaloes as primary ketosis on the basis of clinical signs (selective anorexia, drastic reduction in milk yield), absence of any other concurrent disease and two urine tests. Comparison of infected was made with eight apparently healthy buffaloes kept as control. Hematological findings in diseased animals revealed anemia, leucopenia, lower mean values of total erythrocyte count (TEC) and packed cell volume (PCV), eosinophilia and monocytosis whereas biochemical findings shows hypoglycemia, hypocalcemia, hypoproteinemia hypercholesterolemia, high triglycerides and enhanced alkaline phosphatase activity in affected animals as compared to control group.

Keywords: Primary ketosis, Rothera’s, Keto-Diastix, hematolo-biochemical.

Ketosis is an important production disease which is caused by negative energy balance in high producing animals and occurs usually within 2 months after calving (Radostits et al., 2000; Zhang et al., 2012). Ruminants are particularly vulnerable to ketosis just after calving; as direct supply of glucose is required and about 60 to 80% of glucose is utilized by mammary gland to produce lactose (Annison and Linzell, 1963). The disease is rare in pasture animals but occurs commonly in housed animals during summer and winter (Asrat et al., 2013). Shortened dry period (35 days or less) decreased the risk of ketosis with little to no effect on reproduction or production in the subsequent lactation (Rastani et al., 2005; Watters et al., 2008; Santschi et al., 2011). Animal of any age group may be affected but the incidence increases with age and peak incidence usually occurs in lactation 3rd-6th (Grohn et al., 1989). In addition to this, due to impaired liver function, there is also increase in the activity of liver specific enzymes i.e. aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in ketotic animals (Dann et al., 2005). In almost all clinically ketogenic animals, the most predominant sign is selective anorexia (animals refuses to feed on concentrates), drastic reduction in milk yield (Rajala-Schultz et al., 1999; Fleischer et al., 2001) and increase in the concentration of ketone bodies in urine, blood and milk (Radostits et al., 2000; Enjalbert et al., 2001; Tehrani-Sharif et al., 2011). Hemato-biochemical and pathophysiological changes in affected animals occur following the clinical disease leading to excessive lipolysis resulting in increased blood free fatty acids and ketone bodies i.e. beta-hydroxybutyrate (BHBA), acetoacetate (AcAc), and
acetone (Ac) at 70, 28 and 2%, respectively (Dar et al., 2014). As very limited literature is available on ketosis in buffaloes therefore, the present study was conducted to establish and figure out hematological and biochemical changes occurring in primary ketosis in buffaloes.

MATERIALS AND METHODS

Ethical approval

In the present study samples were collected from clinical cases. As per University rules for these samples approval of Institutional Animal Ethics Committee is not required.

Sample collection, history and testing

A total of 145 buffaloes from district Hisar and adjoining villages were screened in this study. Eight apparently healthy buffaloes from same areas were kept as control group. Clinical cases as of ketosis were diagnosed on the basis of two positive urine tests (Rothera’s test and Ketodiastix strip test) supported by clinical signs like selective anorexia, drastic reduction in milk yield and absence of any other concurrent diseases. All the ketotic buffaloes included in study were in the age group of 3 – 9 years and also in early lactation (0.5 – 3.5 months) while only one animal was pregnant.

Hematology

Blood samples were collected aseptically using EDTA/heparin coated sterile vials from jugular vein of the affected as well as healthy control group animals for hematology (hemoglobin, packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) as early as possible by standard procedures (Weiss and Wardrop, 2011).

Biochemical parameters

Fully automated Random Access Clinical Chemistry Analyzer (EM 200™ Erba Mannheim – Germany) was employed for estimation of biochemical parameters (glucose, total cholesterol, triglycerides, calcium, inorganic phosphorus, alkaline phosphatase and total proteins) using kits procured from Transasia Biomedical Limited.

Data recording and statistical analysis

Data was expressed as mean (±standard error of the mean) and was analyzed by applying Independent student’t’ test using SPSS computer software package to compare the significances of the differences of each parameter between the diseased animals and healthy control group.

Table 1(a): Values of some hematological parameters in healthy and ketotic buffaloes:

<table>
<thead>
<tr>
<th></th>
<th>Hb (g/dL)</th>
<th>TLC (10^6/mm3)</th>
<th>N</th>
<th>Differential leukocyte count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diseased Animals (n=24)</td>
<td>10.62 ± 0.23**</td>
<td>9.53 ± 0.29**</td>
<td>34.83 ± 1.04</td>
<td>L 56.62 ± 1.41</td>
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<tr>
<td>Control Animals (n=8)</td>
<td>12.13 ± 0.27</td>
<td>11.65 ± 0.30</td>
<td>37.5 ± 0.90</td>
<td>60 ± 0.90</td>
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Significant (p < 0.05) = *
Significant (p < 0.01) = **

Table 1 (b): Values of some hematological parameters in healthy and ketotic buffaloes:

<table>
<thead>
<tr>
<th></th>
<th>TEC (10^6/mm^3)</th>
<th>PCV (%)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>MCV (fl)</th>
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</thead>
<tbody>
<tr>
<td>Diseased Animals (n=24)</td>
<td>4.91 ± 0.07**</td>
<td>30.92 ± 0.63**</td>
<td>21.35 ± 0.37</td>
<td>34.36 ± 0.32</td>
<td>62.95 ± 0.98</td>
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<tr>
<td>Control Animals (n=8)</td>
<td>5.87 ± 0.10</td>
<td>35 ± 0.70</td>
<td>22.52 ± 0.83</td>
<td>34.63 ± 0.40</td>
<td>65.80 ± 1.42</td>
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Significant (p < 0.05) = *
Significant (p < 0.01) = **
RESULTS AND DISCUSSION

Out of 145 animals screened for ketosis, 24 animals found to be clinically affected for ketosis on the basis of clinical signs and two sensitive tests (Rothera’s test and Keto-Diastix-strip test). The mean blood hemoglobin value (10.62 ± 0.23 g%), total leukocyte count (9.53 ± 0.29 × 10^3/mm^3), total erythrocyte count (4.91 ± 0.07 × 10^6/mm^3) and packed cell volume (30.92 ± 0.63%) in all the ketotic buffaloes was significantly lower as compared to healthy control animals. The mean blood values of eosinophils (5.83 ± 0.67%) and monocytes (2.58 ± 0.45%) in all primary ketotic buffaloes was significantly higher as compared to healthy control animals. However, there were no significant differences in the mean values of neutrophils, lymphocytes, basophils, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) between primary ketotic animals and control animals as shown in Tables 1(a) and 1(b).

Diagnosis of disease is done using two tests to reduce the chance of false positive result and to countercheck the sensitivity of the two tests. The mean levels of serum glucose (46.38 ± 2.00 mg %), serum calcium (6.65 ± 0.21 mg %) and serum total protein (7.17 ± 0.25 g %) in all primary ketotic buffaloes were signifi cantly lower while those of serum total cholesterol (155.38 ± 3.73 mg %), serum triglycerides (77.83 ± 2.36 mg %) and serum alkaline phosphatase (288.79 ± 16.62 IU/L) were significantly higher compared to healthy controls (Table 2). There was no significant difference in the levels of serum inorganic phosphorus (4.58 ± 0.29 mg %) as compared to healthy control animals (Table 2).

Hematological findings in diseased buffaloes when compared to healthy controls clearly indicated that there was no significant change in the individual hemoglobin content and volume of erythrocytes in this disease. Lower Hb, TEC and PCV values in diseased buffaloes were probably as a result of inappetence and selective feeding leading to poor body condition. Though TLC was found to be lower in diseased animals, it cannot be correlated to disease itself since leukocytic response to production diseases is not of much significance. The findings of present investigation are broadly in agreement with those reported by Devi (1997), Sahinduran et al. (2010) and Marutsova et al. (2015).

Hypoglycemia was consistently associated with clinical ketosis since the disease is caused by negative energy balance in the body (Radostitis et al., 2007). In the present study also, marked hypoglycemia was observed in all the clinical cases of disease. Shaw (1943) was first to report the changes in blood glucose in ketotic cows. Later, a number of workers have reported similar findings in ketotic buffaloes (Sharma et al., 2001; Rukkwamsuk et al., 2006; Sakha et al., 2006; Roy et al., 2007; Teli and Ali, 2007; Yameogo et al., 2008; González et al., 2009; Zhang et al., 2009; Djoković et al., 2010; Sahinduran et al., 2010; Youssef et al., 2010; Farag and Metwally, 2012; Górski and Saba, 2012; Simonov and Vlizlo, 2014; Shin et al., 2015). Asrat et al. (2013) also reported that clinical ketosis occurs in ruminants at times when they are subjected to heavier demands on their resources of glucose and glycogen that can be met from their digestive and metabolic activities. Moreover as the calving approaches, the blood level of progesterone starts decreasing, while

<table>
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<th>Table 2: Values of serum biochemical parameters in healthy and ketotic buffaloes:</th>
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<tr>
<td>Diseased Animals (n=24)</td>
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<tr>
<td>Control Animals (n=8)</td>
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Significant (p < 0.05) = *
Significant (p < 0.01) = **
estrogen content remain high or even increases (Grummer, 1995). High blood estrogen level in dairy bovines is considered a principle regulator decreasing appetite. Anorexia further pushes the animal towards more serious negative energy balance in this disease in which selective anorexia (refusal to feed on concentrates which is principal source of propionate, the glucogenic volatile fatty acid) is a prominent clinical feature. In the present study, serum triglycerides in clinical cases of ketosis in buffaloes were found to be significantly enhanced as compared to healthy control animals. Similar findings have been reported by several workers (Devi, 1997; Roy et al., 2007; Djoković et al., 2010 and Simonov and Vlizlo, 2014). Gordon (2013) stated that non-esterified fatty acid (NEFA) liberated from fat catabolism travel to the liver where these are re-esterified to triglycerides (TG) or oxidized to acetyl CoA. Beitz (2014) reported that hormonal imbalances (insulin: glucagon ratio) lead to increased lipolysis and subsequent increase in plasma NEFA levels. Serum cholesterol levels were found to be significantly higher in ketotic buffaloes compared to healthy control animals in the present study. Increase in cholesterol levels in ketotic buffaloes was again a result of gluconeogenesis which is a predominant feature in this disease. Utilization of adipose tissue and subsequent production of acetyl CoA leads to such a state in this disease, since utilization of acetyl CoA in TCA cycle is not optimum resulting in its accumulation and formation of FFA and ketone bodies. Several workers (Devi, 1997; Roy et al., 2007; Simonov and Vlizlo, 2014 and Lean and De Garis, 2011) too supported and observed that increased blood serum cholesterol concentration may be associated with activation of lipogenesis and gluconeogenesis, formation of intermediate products which might be used for synthesis of endogenous cholesterol. Sevinc et al. (2003) reported that concentration of cholesterol in blood serum depends on the state of liver. In the present investigation, a significant decrease in serum total protein was observed in ketotic buffaloes compared to healthy control animals, similar findings were reported by Roy et al. (2007) and González et al. (2009) in bovine ketosis. Most amino acids are glucogenic in nature except lysine and leucine (Lean et al., 1992). The gluconeogenic potential of protein may be as high as 70% and the level of gluogenic amino acids are reduced in ketotic bovines (DeBoer et al., 1985). These reports support the findings of a higher total protein levels in present study. In the present investigation, significant hypocalcemia was recorded in clinical cases of primary ketosis in buffaloes compared to control animals. Lower levels of serum calcium in such cases can be attributed to a general state of sub-clinical hypocalcemia which is a normal consequence of high metabolic demand of this mineral for high milk yield. Moreover, decreased serum calcium content was probably due to activation of compensatory reaction directed on reduction of the concentration of acidic metabolic products. Thus, binding of cations and acids takes place and they are excreted with urine in form of organic acids, hydrogen phosphates and calcium phosphates (Vlizlo et al., 2011). Since majority of clinical cases of primary ketosis are observed in early lactation only, this finding is amply justified. Findings of hypocalcemia in the present study are also in agreement with those of Biswal et al. (2006), Roy et al. (2007), Zhang et al. (2009), Sahinduran et al. (2010) and Simonov and Vlizlo (2014). No significant change was observed in the levels of serum inorganic phosphorus in animals suffering from primary ketosis compared to control animals. Hypophosphatemia is a prominent feature of many production diseases in buffaloes in Haryana state and adjoining areas but primary ketosis has never been linked to lower serum inorganic phosphorus levels in any investigation so far. However, hypophosphatemia has been reported in clinical cases of ketosis in bovines viz. Biswal et al. (2006) from Orissa (India) and Sahinduran et al. (2010) from Turkey. Findings of these workers are somewhat justified owing to the fact that recently parturited dairy bovines have been reported to be in consistent sub-clinical hypophosphatemic as well as hypocalcemic state as a consequence of negative energy balance and calcium-phosphorus homeostasis mechanism which maintains these two in a ratio of 2.3:1 under all circumstances except when the demand of calcium is overwhelming and overcomes it. Probably the animals selected for the present study were better managed and provided minerals in ample quantity during pregnancy as well as during post-partum period in early lactation. This assumption gains more credence from the fact that primary ketosis is usually encountered in those animals which are well fed and kept in good management condition, that’s why the disease is also known as estate acetonemia. Significantly increased activity of liver/skeletal system related enzyme alkaline phosphatase (ALP) was observed in buffaloes suffering from primary ketosis in the present investigation compared to control buffaloes which indicated some degree of hepatic insufficiency. Moreover, the results of
the investigation in relation to calcium homeostasis status (serum total calcium and serum alkaline phosphatase activities) in ketotic buffaloes pointed out the development of secondary osteodystrophy which is confirmed by increased alkaline phosphatase activity.

CONCLUSION

Significant changes in haemato- biochemical findings in diseased animals included anemia, leukopenia, lower mean values of TEC and PCV, eosinophilia, monocytosis, hypoglycemia, hypocalcemia, hypoproteinemia, hypercholesterolemia, high triglycerides and enhanced alkaline phosphatase activity confirming negative energy balance and its consequences whereas no significant difference were seen in mean values of neutrophils, lymphocytes, basophils, MCH, MCV, MCHC and inorganic phosphorus.

REFERENCES


