ABSTRACT

Nutritional haemoglobinuria is a disease of economic importance in buffalo-rearing countries especially India, Pakistan and Egypt. Generally, it is a sporadic disease of high producing dairy animals and is more commonly a disease of buffaloes. Buffaloes either in advanced pregnancy or in early lactation, with the majority of animals in their 3rd to 5th lactations, are more susceptible (Singh, 1990; Chugh et al., 1996). Severity of the disease is more pronounced in buffaloes as compared to cows (Singh, 1990). The exact etiology and pathogenesis of nutritional haemoglobinuria is not clearly known though hypophosphataemia is consistently reported (Chugh et al., 1996). A variety of risk factors have been reported to be associated with the disease in different parts of the world. Dietary phosphorus deficiency and/or diets containing cruciferous plants are the suspected causes of hypophosphataemia (MacWilliams et al., 1982). In the present study, addition of maize husk and barley grit to the diet caused an outbreak of nutritional haemoglobinuria in recently calved buffaloes at a farm.

Keywords: buffalo herd, Bubalus bubalis, nutritional haemoglobinuria, maize husk, barley grit

MATERIAL AND METHODS

The study was performed at a dairy farm with 110 animals where an outbreak of coffee coloured urine occurred in 19 buffaloes. Out of these, three animals died, and the rest of the animals were treated. Six buffaloes suffering from haemoglobinuria (Group 1) were randomly selected from a farm where addition of maize husk and barley grit to the fodder diet led to an outbreak of hemoglobinuria in recently (2-3 months ago) parturited buffaloes. The animals were being fed a total of 20 kg green fodder per animal and 1:1 mixture of maize husk and barley grit 10 kg/animal. Animals of the farm were not very high (4-9 litres per day) milk yielders. The outbreak occurred in the months of winter when the environmental temperature was less than 10°C and the animals were being kept indoors and were deprived of sunlight. The dairy farm had a total of 110 animals out of which six clinically healthy pregnant (Group 2) and six healthy non-pregnant buffaloes (Group 3) of same breed from the same farm were included in the study. Blood samples were analyzed for micro and macro mineral elements and biochemical parameters using standard methods as below.
Chemical analyses

Blood

Blood samples from the selected buffaloes were collected in mineral-free (heparinised) glass vials. The samples were centrifuged at 3000 rpm for 30 minutes at room temperature to separate plasma. The plasma samples were stored in small aliquots in mineral-free glass vials at -10°C until analysis. Two milliliters of plasma was digested with nitric acid and perchloric acid and after digestion the volume was made up to 10 ml with double distilled water for micro-mineral analysis. Concentrations of various plasma minerals viz. Ca, Zn, Cu, and Mn were measured with an atomic absorption spectrophotometer (SpectraAA 20 plus, Varian, Melbourne, Australia) and Plasma Pi was estimated by method of Taussky and Shorr (1953).

Fodder which was being fed to these animals was collected, oven dried (overnight at 65°C) and ground fodder samples were digested on a hot plate with sulphuric acid and hydrogen peroxide according to the method of Wolf (1982) and Ca was estimated with an atomic absorption spectrophotometer. Fodder P estimated by the vanado molybdate phosphoric yellow colour method (Jackson 1967) using a spectrophotometer.

Statistical analysis

Statistical analysis of the data was done by the method described by Singh et al. (1998).

RESULTS AND CONCLUSION

In a dairy farm, addition of maize husk and barley grit and to the fodder diet caused an outbreak of hemoglobinuria in recently parturited buffaloes. Out of a total of 110 animals, nineteen animals (17.27%) suffered from nutritional haemoglobinuria. All the affected animals were in their 3rd to 4th lactation. Khan and Akhtar (2007) also recorded highest affection in 4th lactation in buffaloes.

Plasma inorganic phosphorus was very low in animals of Group 1 as compared to those of Group 3 (Table 1). Mean Ca concentration was identical in all the groups. Micro-minerals viz., Cu, Zn and Mn varied non-significantly among the three groups and were within the normal range as recommended by Underwood (1999). Normal serum calcium concentrations in the animals suffering from post parturient haemoglobinuria have also been reported by MacWilliams, 1982 whereas Ali (1991) recorded 1.89% increase in Ca levels and 44.01% decline in Pi levels in affected animals. In a study conducted in healthy cows, concentrations of calcium and inorganic phosphorous did not change during the period of late pregnancy and early lactation (Ciaramella et al., 2000). So the present change in P levels in the affected animals was supposed to be due to deficient diet fed to these animals although stress of early lactation might have been an additional factor.

Difference in blood glucose levels was non-significant in these groups (Table 2). The mean level of serum alkaline phosphatase (ALP) was significantly higher in affected buffaloes (155.08±14.5 U/L) as compared to the control groups. The values of 378.00±36.51 and 232.50±58.68 U/L reported by Singh et al. (1992) and Akhtar et al. (2008), respectively were higher than the present findings. Whereas a significantly decreased activity of alkaline phosphatase at calving and in the 1st week of lactation has been recorded in healthy cows (Ciaramella et al., 2000).

Increased serum alkaline phosphatase
Table 1. Comparison of various minerals (Mean ± SD) in different groups of buffaloes in the farm.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pi (mg%)</th>
<th>Ca (mg%)</th>
<th>Cu (ppm)</th>
<th>Zn (ppm)</th>
<th>Mn (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>2.93±0.78a</td>
<td>11.58±0.49</td>
<td>1.27±0.08</td>
<td>2.05±0.33</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>Group 2</td>
<td>4.88±0.90ab</td>
<td>11.62±0.22</td>
<td>1.24±0.11</td>
<td>1.68±0.16</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>Group 3</td>
<td>5.85±0.49b</td>
<td>11.20±0.35</td>
<td>1.21±0.10</td>
<td>1.51±0.18</td>
<td>0.04±0.01</td>
</tr>
</tbody>
</table>

a, b - different superscripts within one column indicate statistically significant difference (P<0.05).

Table 2. Comparison of various minerals (Mean ± SD) in different groups of buffaloes in the farm.

<table>
<thead>
<tr>
<th></th>
<th>AKP</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>155.08±14.5a</td>
<td>68.6±5.26</td>
</tr>
<tr>
<td>Group 2</td>
<td>107.05±19.83ab</td>
<td>58.3±4.93</td>
</tr>
<tr>
<td>Group 3</td>
<td>112.78±29.49b</td>
<td>60.5±3.66</td>
</tr>
</tbody>
</table>

a, b - different superscripts within one column indicate statistically significant difference (P<0.05).

Table 3. Comparison of macro-minerals (Mean ± SD) in different feeds (on DM basis) being fed in the farm.

<table>
<thead>
<tr>
<th></th>
<th>Green fodder</th>
<th>Maize husk</th>
<th>Barley grit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (%)</td>
<td>1.34</td>
<td>0.06</td>
<td>0.02</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.25</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

concentration in the affected animals could be attributed to anaemia as a result of intravascular haemolysis which leads to generalized hypoxia. This hypoxic condition developed in the liver, heart and kidneys damages cell membranes, results in leakage of ALP (Cornelius, 1980).

Levels of calcium in green fodder were in normal range whereas levels of P (Table 3) were lower as per McDowell (2003). In maize husk and barley grit, both Ca and P were much below the normal dietary requirements of the ruminants.

In conclusion, a deficiency of phosphorus in the diet due to inclusion of maize husk and barley grit, which is considered as a good protein source, could be responsible for the development of nutritional haemoglobinuria in these buffaloes.

Three years after this study, clinical signs of haemoglobinuria were again recorded in the animals of that farm as the owner was again using the same diet (maize husk and barley grit).

REFERENCES


