OXIDATIVE STRESS BIOMARKERS IN CROSS BREED COWS
AFFECTED WITH FOOT AND MOUTH DISEASE

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ABSTRACT

The aim of the study was to measure the oxidative stress biomarkers and also elucidate the hematological and biochemical picture of cross breed cow with foot and mouth disease. From the study it was observed that among the oxidative stress biomarkers, superoxidase dismutase, glucose-6-phosphatase, ascorbic acid and uric acid showed a significant (P< 0.05) decrease in the FMD affected animals. Among the hematological and biochemical parameters, the RBC count, serum total protein, albumin, cholesterol, urea, BUN, and calcium showed a highly significant (P< 0.01) decrease in the FMD affected animals in comparison to the healthy animals but indirect serum bilirubin and AST activity increased significantly (P< 0.05) in the FMD affected animals. These results demonstrate that the cows with FMD were under oxidative stress and there was a significant change in some of the blood metabolites. Thus, during FMD the antioxidant vitamins (eg Vit A, Vit C, Vit E) would be beneficial for the antioxidant defense system of the animals.

Key words: Blood metabolites parameters, Cattle, FMD disease, Oxidative stress biomarkers.

When an organism has oxidants or free radicals more than the body can neutralize them, oxidative stress (OS) condition occurs. The oxidants have a negative effect on the animal’s health and reproduction and also initiate tissue damage. To counteract the oxidants, the body has the antioxidant system. OS is extremely dangerous as it does not exhibit any symptoms and is recognizable with great difficulty by means of laboratory methods. So by estimating the OS biomarkers can be saved the animal population. Blood examination is performed for screening and assessing the general health status. The epidemiology and etiology of FMD have been extensively investigated (Yang et al., 1993). However, little work has been done and documented regarding the status of biomarkers of OS during FMD and a few published reports on hematological and biochemical parameters of cattle with FMD are available (Gokce et al., 2004). Keeping this in view, the present work was designed to run in cattle so that FMD of Indian origin is better understood and a more effective treatment can be given.

The study was performed with two groups of animals — group 1 consisted of 10 crossbred cattle (Holstein Friesian X local) from farm of Ranchi Veterinary College and group 2 consisted of 30 crossbred cattle (Holstein Friesian X local) having clinical signs of FMD. The control animals were screened on the basis that they were clinically healthy and free of disease since last 6 months and were non pregnant. A complete case history and owner complain were recorded for each animal under group 2(test). They were clinically examined and there was presence of excessive secretion of stringy or foamy saliva, fever as well as blisters on the feet that had ruptured and caused lameness. Presence of vesicles in the mouth and on the muzzle...
and teats were also noticed in the animals. Animals without characteristic lesions of FMD were not considered for the study. FMD was confirmed by performing Sandwich ELISA. All the animals under study were not vaccinated against FMD. Blood samples were collected, taking all aseptic precautions, from the animal by jugular vein puncture. The samples collected in sterile test tubes were left undisturbed for 4–6 h to separate the serum. The separated serum samples were cleared by centrifugation at 3000 rpm for 5 min. The hemolysate was prepared by centrifuging blood at 3000 rpm for 15 minutes to remove plasma. Plasma were separated and erythrocytes were washed and centrifuged thrice at 3000 rpm for 5 minutes with chilled normal saline solution and the supernatant were discarded including the buffy layer of WBC each time. Then distilled water was added to erythrocyte pellet slowly and with constant stirring upto the marked level to prepare hemolysate. The samples were then stored at -20°C in different aliquots for the analysis of various biochemical constituents.

Oxidative stress was assessed by quantification of malonyl dialdehyde (MDA) and the activities of antioxidant enzymes such as superoxidase dismutase (SOD), glutathione peroxidase (GSH-Px) and glucose-6-phosphate dehydrogenase (G6PD) in hemosylate (Ahmed et al., 2005). The antioxidant vitamins β-carotene and ascorbic acid were estimated in plasma (Sies et al., 1991). All hematological and biochemical parameters were estimated by spectrophotometric methods (Nath et al., 2007). The results of animal experiments are expressed as mean ± S.E and P< 0.01 and P< 0.05 are taken as the level of significance. The different parameters were analyzed by analysis of variance (ANOVA) as per Snedecor and Cochran (2004).

Correlations between the parameters were determined by the Pearson’s correlation coefficient (r).

Mean ± S.E of different oxidative stress markers are presented in Table I. The results of the present investigation revealed that the FMD group showed an increase in MDA concentration compared to the control but was not significant. Ghanem and Hamid (2010) reported a significant (P < 0.05) increase in MDA level during FMD in cattle. Erythrocyte SOD and G6PO activity showed a significant (P< 0.05) decrease whereas erythrocyte glutathione peroxidase (GSH-Px) showed a decrease in group2 but was not significant. During FMD due to less feed intake there is less glucose and thus less glucose -6-phosphate. Our result are in good agreement with the results of Ahmed (2007). During FMD, there may be an increased production of oxidants and GSH-Px may have reacted with them to protect the animals from severe tissue damage. Li and Nan (1989) stated that an increased MDA concentration has been associated with a decrease in the activity of GSH-Px concentration and is parallel to the present findings. Ascorbic acid and serum uric acid concentration decreased significantly (P< 0.05) in the FMD affected animals from that of the healthy animals while the concentration of β-carotene was almost same in both the groups. The decrease ascorbic acid concentration maybe firstly, by excessive utilization and intense consumption for neutralizing the ROS overproduction, and additionally by transient lack of appetite during the stress condition (Kizil et al., 2010) during FMD.

Mean ± S.E of different haematological and biochemical parameters are presented in Table 2. A significant (P<0.05) decrease in erythrocyte count was observed in the infected group. The report corroborates with the findings of Gokce et

### TABLE 1: Mean ± S.E. of different Oxidative stress biomarkers in cows during FMD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control cows</th>
<th>FMD affected cows</th>
</tr>
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<tbody>
<tr>
<td>MDA nmol/gm Hb</td>
<td>1.904±0.132a</td>
<td>2.287±0.371a</td>
</tr>
<tr>
<td>SOD U/L</td>
<td>391.023±25.930a</td>
<td>337.756±9.502b</td>
</tr>
<tr>
<td>GSH-Px U/mg Hb</td>
<td>123.556±5.208a</td>
<td>113.224±7.005a</td>
</tr>
<tr>
<td>G-6-PD U/gm Hb</td>
<td>11.907±0.128a</td>
<td>8.872±0.687b</td>
</tr>
<tr>
<td>β-carotene(mg/dl)</td>
<td>1.075±0.134a</td>
<td>0.814±0.130a</td>
</tr>
<tr>
<td>Ascorbic acid(mg/dl)</td>
<td>11.229±0.814a</td>
<td>8.856±0.603b</td>
</tr>
<tr>
<td>Serum uric acid(mg/dl)</td>
<td>2.927±0.159a</td>
<td>2.525±0.166b</td>
</tr>
</tbody>
</table>

Mean with different superscripts denotes significance (P<0.05) difference between the rows.
al. (2004) and Ghanem and Hamid (2010). Immune-mediated mechanism like erythrophagocytosis might be responsible for the destruction of erythrocytes. During FMD there maybe depression of erythropoiesis and so there maybe decrease production of RBC (Mohapatra et al., 2005). In cow, sheep and goat ESR is 0 because of very low fall of erythrocyte in one hour (Brar et al., 2004). Reductions in serum total protein and albumin concentrations have been reported to be associated with hepatic and renal damage and starvation resulting in protein loss, parasitic infestation and chronic organ diseases (Gokce et al., 2004). Albumin is mainly synthesized in the liver and its production is regulated by the physiological conditions of the liver. Significant decrease in total serum protein during FMD may be due to the result of liver damage (Mohapatra et al., 2005) as there was a significant (P < 0.05) increase in AST activity. Protein requirement as well as protein catabolism increases during anorexia and in the presence of any lesions on the body (Kaneko et al., 2004). Anorexia and off fed due to mouth lesions that characterize cattle with FMD may be in a part a possible cause as stated by Gokce et al., (2004). Serum cholesterol, BUN and serum urea concentration decreased significantly (P < 0.01) in the diseased animals. The decrease in serum cholesterol level maybe due to the decline in the lipogenesis due to the viral infection or the decrease maybe due to inhibition of the metabolism of lipid in the liver and its disruption in transport from liver. The decrease in serum cholesterol maybe also due to hepatic dysfunction. Gokce et al., (2004) also observed serum cholesterol (P < 0.01) level significantly low in the FMD group compared to those in the control group. But Nahed (2010) in his work observed a significantly higher (P < 0.05) concentration of serum cholesterol in the FMD affected animals which is contradiction to the present findings. Gokce et al., (2004) also recorded a decrease in serum urea in FMD affected animals but was not significant. The concentration of urea depends on diet. The affected animals were suffering from anorexia and off-fed and ultimately the protein intake was less which caused less transaminase of protein to L-glutamate and ultimately less amount of urea was synthesized as amino nitrogen waste in the liver.
Again the urea cycle takes place in the liver and as during FMD there may be liver dysfunction so there was less synthesis of urea.

Since the group 2 animals may have dysfunction liver and it impairs the liver's ability to conjugate bilirubin. Thus, the unconjugated (indirect) serum bilirubin increased significantly (P< 0.05) from the control animals. The significant (P<0.05) decrease in serum calcium content maybe the result of off fed of the animal and decrease in protein bound content of calcium (Mohapatra et al., 2005). Kaneko et al. (2004) stated that hypocalcemia level maybe due to inappetence and hypoproteinemia and hypoalbumine of the diseased animals. The finding correlates with the findings of Gokce et al. (2004), Mohapatra, et al. (2005) and Nahed (2010). There may be liver damage and so AST activity increased significantly (P< 0.05). Kumar and Roy (2009) recorded a significant (P < 0.05) increased activity in the FMD affected animals.

Among the oxidative stress biomarkers, there was significant (P< 0.05) negative correlation between MDA and SOD and between uric acid and ascorbic acid and a significant (P< 0.05) positive correlation between MDA and ascorbic. The rest did not show any significant correlation. Among the hematological parameters, there was a significant (P<0.01) positive correlation between Hb and MCH and between Hb and MCHC, between PCV and MCV and between MCH and MCHC and a significant (P<0.01) negative correlation between RBC and MCV. Among the serum biochemical variables, there was positive correlation between urea and BUN, between total bilirubin and indirect bilirubin and between total bilirubin and glucose, between direct bilirubin and calcium and between indirect bilirubin and glucose. A significant (P<0.05) negative correlation was observed between cholesterol and direct bilirubin, between urea and glucose and between BUN and glucose. Among the serum protein fractions and liver enzymes, there was a significant (P< 0.05) positive correlation between total protein and albumin and between total protein and A:G, between albumin and A:G and between AST and ALT and between ALT and ALP. A significant (P<0.05) negative correlation was observed between albumin and globulin and between globulin and A: G. The rest of them had no significant correlation among themselves.

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