The Study of Histopathological Changes upon Exposure to Vinegerized Copper Sulphate in Liver and Kidney of Broiler Chick

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Abstract: Copper sulfate is used as a fungicide in poultry feed and for the medication of drinking water. Sub acute copper poisoning is rare but chronic copper poisoning is frequently observed. Ninety chicks were used for the present study and they were divided into five groups (A, B, C, D and E) on the basis of various treatment schedule of vinegarized copper sulfate in feed (100mg, 500mg, 1gm and 2gm). At the end of each week weight gain, feed consumption, morphology and histopathology of liver and kidney were noted. Chicks showed various clinical symptoms including loose fecal material, poor feather growth, entheritus and coccidiosis disease. Major histopathological changes were degeneration of cells, infiltration of mononuclear cells and necrosis. The type and extent of lesions varied with the duration and age of chicks.

Key words: Copper sulphate · Vinegerized · Fungicide · Histopathology

INTRODUCTION

Copper is an essential trace mineral for poultry [1]. Copper (Cu) is plentiful in the environment and needed for the normal growth and metabolism of all living organisms [2-3]. Both deficiency and excess of copper sulphate lead to physiological disturbances and diseases [4]. The Cu requirement for broiler is 8ppm [1]. Level of Cu upto 125 to 250 ppm as CuSO₄ improves growth and feed efficiency in broiler [5]. In general up to about 250 mg Cu/kg diet tends to promote the growth of chicks [6-8]

Copper has been added to poultry diets in excess of its nutritional needs as an antimicrobial and growth promoter for many years [7-13]. But excess of Cu in diet depresses growth and feed efficiency in broiler, disturbs liver functions [14-15].

The concentration of copper in the liver increased rapidly at 600 and 800 mg/kg dietary level of copper [16]. Copper residues in the liver and excreta were significantly (p<0.05) increased as dietary Cu increased [17]. Chemical form of ingested copper has been shown to exert a definite affect on its bioavailability in the rat, in swine and in cattle [18].

MATERIALS AND METHODS

Experiment was carried out utilizing 1 day old broiler chicks during the period from 1-43 days of age. A total of 100 broiler chicks were reared up to 42 days during the period, March 20, 2009 to May 02, 2009. The animal used in the experiment was broiler (Gallus domesticus). Chicks were procured from Jamalpur Poultry Breeders Farm, Gujrat. Birds were kept in brooder room of Government Poultry Farm, Gujrat under standard conditions. We administrated the antibiotics and vaccines for prevention of infection and diseases following routine schedule.

On day 14 of age 90 chicks were randomly divided into 5 groups i.e. A through E, containing 18 chicks in each group. Each group was further subdivided into three replicates as A1, A2, A3, B1, B2, B3, C1, C2, C3, D1, D2, D3, E1, E2, E3 respectively containing six birds in each replicate. Group A was control group without any treatment, while all other groups were treatment groups. Treatment groups were A, B, C and D given 100mg, 500mg, 1gm and 2gm of vinegerized copper sulphate per kg of feed respectively. Treatment of copper sulphate was started at the beginning of third week and continued till the end of sixth week.

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Morphometric changes were noted. Feed conversion ratio (FCR) was calculated on day 21, 28, 35 and 42 of age. Liver and kidney of one bird of each group were eviscerated on day 28, 35 and 42 of experiment. Experimental chicks were slaughtered at random (one chick from each group) on day 28, 35 and 42 of age. Liver and kidney were removed. 3-4 mm² pieces were cut and rinsed with 0.085% saline solution kept in Bouin’s fluid for 8 hours. [19] The tissues were processed for routine histology, stained with hematoxylin and eosin and studied under microscope.

**Statistical Analysis:** Data was analyzed statistically by applying independent sample t test using SPSS 13.0 version.

**OBSERVATIONS AND RESULTS**

**Gross Features:** Obvious gross abnormalities were not present in control group. On gross examination liver and kidney were discolored, inflamed, friable and petechial and echymotic hemorrhages were present in treated groups.

**Histological Features:** Histopathological changes observed in liver of chicks fed 100 mg of vinegerized copper sulphate per kg of feed were degeneration of hepatocytes with necrosis and in kidney infiltration of mononuclear cells. Chicks given 500 mg per kg of feed showed minor (Petechial) hemorrhages on the liver and streaks of hemorrhages were also present on kidney.

![Fig. 1](image_url)

Fig. 1: A-Cross section of liver of A group (control) on day 28 of age showing normal hepatocytes. B-Cross section of liver of B group (100 mg/kg) on day 28 of age showing degeneration of hepatocytes. C-Cross section of liver of C group (500mg/kg) on day 35 of age showing degeneration of hepatocytes. D-Cross section of liver of D group (1gm/kg) on day 35 of age showing infiltration of mononuclear cells. E-Cross section of liver of E group (2gm/kg) on day 35 of age showing necrosis of hepatocytes.
Fig. 2: A-Cross section of kidney of A group (control) on day 28 of age showing normal nephrons. B-Cross section of kidney of B group (100 mg/kg) on day 35 of age showing infiltration of mononuclear cells. C-Cross section of kidney of C group (500mg/kg) on day 35 of age. D-Cross section of kidney of D group (1gm/kg) on day 35 of age showing degeneration of nephrons (disruption of parenchyma). E-Cross section of kidney of E group (2gm/kg) on day 42 of age.

Table 1: Effect of vinegarized CuSO4 on weight gain and feed consumption ratio in broiler chicks

<table>
<thead>
<tr>
<th>Age in weeks</th>
<th>No. of chicks used</th>
<th>Feed consumed (gm)</th>
<th>Weight gain (gm)</th>
<th>Feed conversion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control CuSO4</td>
<td>100mg of CuSO4</td>
<td>500mg of CuSO4</td>
<td>1gm of CuSO4</td>
</tr>
<tr>
<td>3rd week 18</td>
<td>1300</td>
<td>18 ± 5.4</td>
<td>7309.6 ± 730.96</td>
<td>7308.0 ± 4.93</td>
</tr>
<tr>
<td>4th week 17</td>
<td>2350</td>
<td>22 ± 5.3</td>
<td>12766.6 ± 3.74</td>
<td>12053.0 ± 3.48</td>
</tr>
<tr>
<td>5th week 16</td>
<td>3300</td>
<td>22 ± 5.3</td>
<td>17121.0 ± 3.43</td>
<td>17233.3 ± 4.35</td>
</tr>
<tr>
<td>6th week 15</td>
<td>4450</td>
<td>22 ± 5.3</td>
<td>22281.6 ± 3.77</td>
<td>22258.6 ± 3.77</td>
</tr>
</tbody>
</table>
On exposure to 1gm of vinegerized copper sulphate per kg of feed histopathological changes observed in liver of chicks includes infiltration of mononuclear cells, necrosis and degeneration of hepatocytes. Kidneys of chicks were necrosed and infiltration of mononuclear cells and degeneration of nephrons was seen. The study demonstrated that 2gm of vinegerized copper sulphate per kg of feed caused hepatocytes degeneration and infiltration of mononuclear cells were observed in liver. Infiltration of mononuclear cells, degeneration of nephrons and necrosis were seen in kidney in fourth week.

**FCR and Weight Gain:** In third week the difference between weight gain of A and B group was found to be statistically significant as shown by the p value i.e. 0.034. Feed consumption of B group was significantly different from A group (p 0.000), in fourth and fifth week. FCR of B group was significantly different from A group as shown by p 0.014 in third week. Feed intake of birds was significantly different in fourth, fifth and sixth weeks between A and C group (0.000). There was no significant difference between weight gain and FCR of A and C group in all weeks (p>0.05). The exposure to 2gm/kg of vinegerized CuSO₄ showed no significant difference in weight gain i.e. p > 0.05 in all weeks. In fourth, fifth and sixth week feed intake of E group showed significant difference from A group (p 0.000). The difference between weight gain and FCR of group A and E was non-significant (p > 0.05) in all weeks.

**DISCUSSION**

Copper has received considerable attention due to its antimicrobial properties that improve performance in animals when fed over the minimum requirement [20,7,8,21]. The successive non-toxic doses of the copper showed the accumulative effect i.e. manifestation of chronic poisoning. In present study when vinegerized copper sulphate was mixed in the feed of chicks, birds showed various clinical symptoms and reduction in weight gain as shown in table (Table 1). Similar results were obtained by [22] no significant effect on live body weight of laying hens,feed intake and egg weight.[29-31] fed diets supplemented with CuSO₄,[23-25]. When CuSO₄ was added into feed of birds at level of 500 mg/kg, feed intake increased significantly, however weight gain and feed conversion ratio showed no significant change. Our results are contrary to the other scientists. The body weight gain was reduced significantly by the supplementation of Cu at 500 mg/kg in the diet of country chicken and layer pullets [5,26,27].

The type and extent of lesions in liver and kidney varied with the age of chicks. Liver was discolored, hypertrophic and hemorrhagic. Kidney was pale, hypertrophic, hemorrhagic and deposition of urates was present on the kidney. The liver colour varied from pale yellow to orange, while that of the kidneys from brown to black. Pre-haemolytic changes in liver included vacuolation and swelling of parenchymal cells and parenchymal cell nuclei, parenchymal cell necrosis and swelling of the Kupffer cells [28-29]. During the haemolytic crisis, extensive focal necrosis of liver tissue was seen. The most striking features of hepatic biopsies from sheep that survived the haemolytic crisis were the large amounts of bile pigment in canaliculi and small bile ducts and the occurrence of periportal fibrosis. While parenchymal cells showed fatty change and nuclear enlargement and vacuolation, focal necrosis was no longer evident. Changes in hepatic enzyme activities were also quite pronounced and related to the phase of copper intoxication.

Histological studies of liver showed degeneration of hepatocytes, necrosis and infiltration of mononuclear cells. The varying degrees of pigmentation, cirrhosis and necrosis of the liver in the copper-exposed animals [30]. Necropsy and histological examinations of swine revealed abnormal liver pigmentation (yellow-brown to orange coloration), hepatic centrilobular necrosis, ulcers of the gastric cardia, watery blood, reddened bone marrow and splenic myeloid metaplasia [31].

Histopathological changes of kidney included necrosis, degeneration of nephrons, infiltration of mononuclear cells and deposition of urates. Copper-induced histopathological changes included centrilobular necrosis and perilobular sclerosis with nuclear oedema of the liver and tubular necrosis as well as nuclear pycnosis and cell proliferation in the medullary region of the kidneys [32]. During the haemolytic crisis, degeneration, necrosis, decreased enzyme activities and reduced function of the proximal convoluted tubules occurred. These renal changes were accompanied by an increase in blood urea levels[33]. The hepatic damage, somewhat like that of liver cirrhosis in humans, was observed in the copper-dosed animals [34].
The birds upon exposure to 1gm/kg and 2gm/kg of vinegerized CuSO₄ showed more pronounced gross pathological changes. The changes included discoloration, enlarged size, hemorrhages and deposition of fat, fibrin, or urates in liver and kidney of broiler chicks. The histopathological changes i.e. necrosis, degeneration of cells and infiltration of mononuclear cells in liver and kidney were more obvious in birds fed 1gm/kg and 2gm/kg of vinegerized CuSO₄ in diet.

REFERENCES


