EFFECT OF DIFFERENT DOSES OF DIETARY ARSENIC (As) ON BIOCHEMICAL, GROSS AND HISTO-MORPHOLOGICAL CHANGES IN DIFFERENT ORGANS OF COMMERCIAL BROILER

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EFFECT OF DIFFERENT DOSES OF DIETARY ARSENIC (As) ON BIOCHEMICAL, GROSS AND HISTO-MORPHOLOGICAL CHANGES IN DIFFERENT ORGANS OF COMMERCIAL BROILER

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ABSTRACT
The study was aimed to investigate the toxic effects of arsenic(As) exposure in a commercial broiler. A total number of 72 broiler chicks (12 days of old) were assigned in four dietary treatments with three replicates. Control group T0 received only basal diet and the other groups T1, T2 and T3 received feed supplemented with As at a dose level of 100, 200 and 300 ppm/kg feed respectively. The body weight of each bird was weighed at 3 days interval and found decreases in weight gain significantly (P<0.01) among the As treated groups. Elevated ALT (P<0.01) and serum creatinine in treated birds was attributed to gross and histopathological changes in liver and kidney respectively. Gross pathological changes showed diffuse congestion, haemorrhage, presence of necrotic foci on liver and congestion in kidney. Microscopical examination of liver from control and T1 groups revealed normal histological picture. However, liver of higher treatment group birds showed fatty changes and congestion. Severe congestion and dilatation of hepatic vessels were the common histological in T2 group. Cirrhosis, severe congestion of hepatic vessels and fatty changes were observed in T3. Microscopically, kidneys from control (T0), T1 & T2 (200ppm) showed normal architecture with normal glomeruli, proximal convoluted tubules (PCT) and distal convoluted tubules (DCT). Fatty degeneration, cytoplasmic vacuoles were observed in the kidneys of birds from T3 group.

Keywords:
Arsenic, toxicity, Body weight, Alanine Transaminase (ALT), serum creatinine, Morphological and Histopathology

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1. INTRODUCTION

Bangladesh is a densely populated developing country and its economy is very much dependent on agriculture. Poultry is a part of agricultural farming system in Bangladesh. The poultry sub-sector is an important avenue in fostering agricultural growth and reduce malnutrition for the people in Bangladesh (FAO. 2009). It is an integral part of the farming system in Bangladesh and has created direct, indirect employment opportunity including support services for about 6 million people (Ansarey et al., 2012). This sub-sector has proved as an attractive economic activity, thereby indicating its importance for the entire economy. The sector accounts for 14% of the total value of livestock output and is growing rapidly (Raihan and Mahmud, 2008). Poultry contributes about 22-27% of the total animal protein supply in the country (Prabakaran, 2003). Broiler rearing is becoming more popular in Bangladesh to meet up the growing demand of animal protein in the country. Now a days commercial broiler farming provides employment opportunities for unemployed family members, improve socio-economic conditions and women empowerment among rural people in Bangladesh (Rahman et al., 2006). Poultry meat is a high-quality animal protein source plays significant role in maintaining the health and nutrition of the people (Shahzad et al., 2012). But the poultry meat might be a threat to public health when it is contaminated with arsenic. The use of insecticides and pesticides, veterinary drugs and feed additives are the major sources for the exposure of arsenic to animals (Friberget et al., 1986). Contamination of drinking water with arsenic is the principal source of exposure to livestock and human (Bode and Dong, 2002; Yihet al., 2002). If the recommended levels of arsenic in broiler feed are not observed strictly then it can accumulate in poultry flesh which might be detrimental to the consumers. Arsenic is one of the major culprits that contaminates food chain and makes significant contribution to induce arsenic-related diseases in Bangladesh (Khan et al., 2010). Arsenic-based drugs (roxarsone, used in chickens) were deliberately used in U.S. poultry production for decades, potentially representing an unnecessary and easily controllable source of exposure to Arsenic (Liu et al. 2016) Arsenic pollution in natural water resources has become a great challenge throughout the world which poses serious human health problems, being the potent toxic agent in the ecosystem (Aruljothi et al., 2013; Khan et al., 2014). It was estimated that 59 out of 64 districts of Bangladesh are contaminated with arsenic (Chakraborti et al., 2010). Due to serious consequence, WHO leveled the arsenic disaster of Bangladesh as “the largest mass poisoning of a population in history” (Smith et al., 2000). In Bangladesh most of the poultry farms are maintaining with shallow tubewell water, which contains relatively more arsenic than deep tube well water. Feeding broilers with arsenic-rich food and/or water contribute to the accumulation of arsenic loads in its meat and excreta (Islam et al., 2009). In addition, uses of some arsenic-containing feed additives, like roxarsone, add the arsenic loads in broiler feed and thereby in its meat & by products (Wallinga, 2006). Nevertheless, cooking of arsenic intoxicated meat may create additional arsenic-rich toxic by-products for consumers (Hanaoka et al., 2001). Researchers from the National Institutes of Health and the USDA’s Food Safety Inspection Service reported alarmingly high levels of arsenic contamination in the broiler flesh (Lasky et al., 2004). The arsenic doesn’t disappear from body once broiler eat arsenic (Roxarsone) preparation and it’s distributed throughout the body tissue including the breast, thigh and leg muscles. The rest is excreted unchanged in poultry waste hence huge amount of arsenic is present in the excreta.
and 90% of this manure is later converted into fertilizers that can contaminate crop near lakes, river and eventually drinking water (Wenner, 2006). Therefore, arsenic has been identified as a roadblock to potential animal waste management solutions (Nachmanet al., 2005).

Although few researches have been conducted on the detrimental effect of arsenic on poultry tissue, various grade of contamination of poultry feed with arsenic & its consequence in histopathological & biochemical parameters has not been well explained. Therefore the present study has been designed to investigate the effect of different label of arsenic trioxide intoxication in broiler feed on the morphological & biochemical parameters.

2. MATERIALS AND METHODS

In this study seventy-two 12th day old broiler chicks (Cobb-500 strain) were collected from a farm of Ranigonj, Dinajpur under CP Company Ltd. Bangladesh. The experiment was performed in poultry shed of VAS faculty, HSTU, Dinajpur. For this purpose, the chicks were randomly allotted into four groups T0, T1, T2 and T3 (6 chicks in each group with 3 replicates). The chicks were maintained for 3 days for acclimatization. All were maintained in the identical management and are given adlibitum water and respective feed. The birds of group T0 were kept as control, received only basal diet from CP feed whereas birds of group T1, T2 and T3 were given arsenic trioxide (AS3O3, Loba Company limited) at deferent dose labels in feed for a period of four weeks.

2.1 Site of the experiment

The birds were reared in isolated poultry shed, under the department of Poultry Science, Faculty of Veterinary Science, HSTU, Dinajpur, Bangladesh. The length and width of the shed were 25 and 15 feet.

2.2 Cleaning and disinfection of the house

The room was thoroughly washed by sweeping and washing with tap water using a hose pipe. The room was disinfected with a phenolic disinfectant (phenyl) and allowed to dry. Then the shed was again disinfected by spraying a quaternary ammonium derivative containing 40% N-alkyl dim ethyl benzyl ammonium chloride (Timsen TM) from Eon Animal Health Products Ltd., Dhaka, Bangladesh @ 1gm/4 litre of water. New required wire cages, water and feed trays were placed in the poultry shed. All windows were closed and then the shed was fumigated with formalin (Emark) and potassium permanganate (Ronas Chemicals Ind. Co. Ltd., China) @ 40 ml formalin in 20 gm KMnO4 for each 100 cubic feet area). Two days before placing the chicks the shed was properly ventilated.

2.3 Clinical observation

Birds of each group were observed for clinical symptoms and mortality the pattern if any during experimental period of 4 weeks

2.4 Body Weights

Initial body weight of individual bird from each group was recorded and subsequently three days interval. Body weight for each group was recorded up to 42 days of experimental period to evaluate effect of arsenic trioxide.

2.5 Biochemical observations

At the end of the experiment, 12 birds (4 bird from each replicate) for each treatment group were randomly selected and blood samples were collected from the wing vein into labelled EDTA bottles for blood analysis [Serum glutamic pyruvic transaminase (SGPT)/ Alanine
Transaminase (ALT) and serum creatinine.

2.6 Gross Pathological Examination
At the end of 4 weeks of experimental period, six birds from each group were sacrificed to study gross pathological changes in various visceral organs. During necropsy, lesions observed on various organs i.e. liver, kidney, pancreas, heart and duodenum were recorded to know pathological alterations, if any.

2.7 Histopathological Examination
For histopathological examination the collected samples were preserved for fixation in the Bouin’s fluid for 24 hours. The tissues were then dehydrated by using ascending graded of alcohol (70%, 80%, 90%, 95%, 100% and 100%) and kept for one hour in each grade of alcohol. The tissues were then transferred to the xylene-1 and xylene-2 each for ninety minutes. Then the tissues were infiltrated in the liquid paraffin at 60ºC temperature for ninety minutes and repeated again. Finally the tissues were embedded in paraffin and paraffin blocks were made. The paraffin blocks were cut at 6 µm thickness using microtome machine (Mu 509, Euromex, Japan). After sectioning of paraffin block, the slices were floated on warm water in a water bath at 45ºC for stretching. The sections with glass slides were stained with Hematoxylin and Eosin (H & E) stain for general histological study. Observations of the slides were done by using a light microscope and photographs were taken with an automatic photo micrographic system.

2.8 Statistical Analysis
Data were expressed as mean ± standard error (SE) and analyzed using one-way analysis of variance (ANOVA) followed by Duncan’s test as a post-doc test using IBM SPSS Statistics 20.0 software package and the chart was created by Microsoft Excel 2007 software. Results were considered to be statistically significant when P values are less than 0.01 (P<0.01).

3. RESULTS

3.1 Clinical observation
Various clinical signs including depression, dullness, emaciation, open mouth breathing, ruffled feathers, pale comb and hyper-excitability were observed in all treated groups. The control group show no clinical sign. No mortality was observed in any experimental group.

3.2 Average body weight (gm)
Average body weight of broiler at three days interval in different groups from 12 to 42 days of age broiler is given in Table 1 and graph 1. Average body weight of broiler at the end of the experiment (i.e. in 6 weeks) was 2453.555±3.188gm, 2248.389±4.529gm, 2142.22±3.616gm and 2033.50±2.98gm for the group T0, T1, T2 and T3 respectively.

3.3 Gross Pathological Observations
After completion of 6 weeks of the experiment, six birds from each group were sacrificed to study detailed gross pathological observations. During necropsy, control group birds did not reveal gross pathological changes (Fig. 4.1). The visceral organs appeared normal. However, birds of T2 & T3 group revealed congestion in visceral organs especially in the liver (Fig. 4.2).

3.4 Histopathological observations
Six birds from each group were sacrificed at the end of the 6th week of experiment. The organs viz. liver, kidney, muscle, heart, gizzard and spleen were collected and preserved. Liver and kidney were examined under microscope after processing. Histopathological
changes in different groups are described below:

**3.4.1 Liver**
Microscopical examination of liver from a control group and birds treated with 100ppm (T1 group) arsenic revealed normal histological picture (Fig. 4.3), however liver of higher treatment groups birds showed fatty changes and congestion. Severe congestion and dilatation of hepatic vessels were the common histological changes observed in liver from birds of T2 group (Fig. 4.4). Cirrhosis (Fibrous tissue proliferation in the liver parenchyma), severe congestion of hepatic vessels and fatty changes were observed in the T3 groups (Fig. 4.5) i.e, (300 ppm arsenic trioxide in the diet).

**3.4.2 Kidney**
Microscopically, kidneys from control (T0), T1 (100ppm) &T2 (200ppm) showed normal architecture with normal glomeruli, proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) (Fig. 4.6). Fatty degeneration, cytoplasmic vacuoles were observed in the section of kidneys from the birds receiving 300 ppm arsenic trioxide/kg in feed i.e. T3 group (Fig. 4.7).

**3.5 Biochemical Observation**
Average SGPT & Serum creatinine of broiler in different groups after 42 days of an experiment is given in Table 2. The average SGPT & Serum creatinine of broiler was found comparable in different groups. Average Serum creatinine at the end of experiment (i.e. in 42 days) was 0.10 ± .0, .20 ± .026, 0.30± .026 and 0.383 ± .03073mg/dl whereas average SGPT was 17.3667±.28363, 21.2333±.37476, 26.2833± .36735 and 38.0167 ±.40449µ/L for T0, T1, T2 & T3 respectively.
Table 1: The effects of different levels of Arsenic on body weight (gm) of broiler from 12\textsuperscript{nd} days to 42\textsuperscript{nd} days of experiment

<table>
<thead>
<tr>
<th>Days</th>
<th>Various treatment groups showing mean ± SE values</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T\textsubscript{0} (100ppm arsenic)</td>
<td>T\textsubscript{1} (200ppm arsenic)</td>
</tr>
<tr>
<td>D\textsubscript{12}</td>
<td>349.3889±1.14372</td>
<td>348.2222±1.30998</td>
</tr>
<tr>
<td>D\textsubscript{15}</td>
<td>485.0000\textsuperscript{b}±4.05276</td>
<td>481.0556\textsuperscript{b}±1.36834</td>
</tr>
<tr>
<td>D\textsubscript{18}</td>
<td>660.8333\textsuperscript{c}±2.58610</td>
<td>653.9444\textsuperscript{bc}±2.45412</td>
</tr>
<tr>
<td>D\textsubscript{21}</td>
<td>855.1667\textsuperscript{d}±7.43699</td>
<td>840.1111\textsuperscript{bc}±2.93713</td>
</tr>
<tr>
<td>D\textsubscript{24}</td>
<td>1070.6111\textsuperscript{d}±4.03914</td>
<td>1032.0556\textsuperscript{c}±3.67332</td>
</tr>
<tr>
<td>D\textsubscript{27}</td>
<td>1300.7778\textsuperscript{d}±2.54830</td>
<td>1248.7778\textsuperscript{c}±2.49123</td>
</tr>
<tr>
<td>D\textsubscript{30}</td>
<td>1544.6667\textsuperscript{d}±4.10333</td>
<td>1476.1667\textsuperscript{c}±5.18246</td>
</tr>
<tr>
<td>D\textsubscript{33}</td>
<td>1802.5000\textsuperscript{d}±3.25421</td>
<td>1739.5556\textsuperscript{c}±3.10199</td>
</tr>
<tr>
<td>D\textsubscript{36}</td>
<td>2078.6111\textsuperscript{d}±3.92090</td>
<td>2004.2222\textsuperscript{c}±3.11811</td>
</tr>
<tr>
<td>D\textsubscript{39}</td>
<td>2208.3333\textsuperscript{d}±5.93978</td>
<td>2131.0556\textsuperscript{c}±3.73070</td>
</tr>
<tr>
<td>D\textsubscript{42}</td>
<td>2453.5556\textsuperscript{d}±3.18824</td>
<td>2248.3889\textsuperscript{c}±4.52960</td>
</tr>
</tbody>
</table>

Means on the same row with different superscripts are significantly different (P < 0.01).

D: day
NS: Not Significant
S: Significant
Table 2: Serum biochemical parameters of broilers at 42nd days fed varying levels of As.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Various treatment groups showing mean ± SE values</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_0$</td>
<td>$T_1$</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.1000$^a$$\pm$ 0.0000</td>
<td>0.2000$^b$$\pm$ 0.02582</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td>17.3667$^a$$\pm$ 2.8363</td>
<td>21.2333$^b$$\pm$ 3.7476</td>
</tr>
</tbody>
</table>

Means on the same row with different superscripts are significantly different (P < 0.01).
SGPT: Serum glutamic pyruvic Transaminase
SE: Standard Error
** Means: Significant
Fig. 4.1: Normal liver, and heart of broiler (T₀ group)

Fig. 4.2: Congested liver of broiler (T₃ group)
Fig. 4.3: Microscopic view of the liver: showing regular pattern of hepatic cord in group T₀ and T₁ (H and E, Dimension- 947×619)

Fig.4.4: Microscopic view of the liver: group T₂ showed congestion in liver (H and E, Dimension- 947×619)
Fig.4.5: Microscopic view of the liver: group T3 showed cirrhosis in liver (H and E, Dimension-947×619)
Fig. 4.6: Microscopic view of the kidney: Group T₀, T₁ & T₂ showed no remarkable change in kidney tubules (proximal and distal convoluted tubules & Henle’s loop (H and E, Dimension-947×619).

Fig. 4.7: Microscopic view of the kidney: Group T₃ showed fatty changes in kidney tubules (H and E, Dimension-947×619).

4. DISCUSSION

Various clinical signs including depression, dullness, emaciation, open mouth breathing; ruffled feathers, pale comb were observed in all treated groups. The control group showed no clinical signs. The above findings were similar to that of other workers as Sharaf et al., (2013), Ghaffar et al., (2017) who also observed decreased body weight and feed intake, dullness, open mouth breathing, increased thirst, ruffled feathers, pale comb and skin irritation. These physical alterations could be due to increased and impaired permeability of blood vessels and intestinal functions leading to poor absorption of nutrients ultimately resulting in dullness and depression (Khan et al., 2013). Previously respiratory distress due to arsenic in birds, fish and goats has been reported by Khan et al., 2013; Ghaffar et al., 2015 and Ghaffar et al., 2016.

Average body weight of broiler at three days interval in different groups from 12 to 42 days of age broiler is given in Table 1. Average body weight of broiler at the end of the experiment (i.e. in 6 weeks) was 2453.555±3.188gm, 2248.389±4.529gm, 2142.22±3.616gm and 2033.50±2.98gm for the group T₀, T₁, T₂ and T₃ respectively which was proportionate to the severity of dosage. Similar reduction in body weight gain and feed intake have also been recorded after oral administration of different levels of As in broiler chicks in previous studies by Vodela et al., (1997), Chio et al., (1997), Desheng et al., (2006), Chen et al., (2000), and Ghaffar et al., (2017). Lower body weight in higher dose treatment group birds could be due to digestive disturbances. Arsenic may produce inflammation in proventriculus and gizzard, gelatinous exudate beneath the horny lines of gizzard causing sloughing of horny lines.
Microscopical examination of liver from a control group and birds treated with 100 ppm arsenic revealed normal histological picture, however liver of higher treatment groups birds showed fatty changes and congestion. Severe congestion and dilatation of hepatic vessels were the common histological changes observed in liver from birds of T2 group i.e., (200 ppm arsenic trioxide in the diet). Cirrhosis (Fibrous tissue proliferation in the liver parenchyma), severe congestion of hepatic vessels and fatty changes were observed in the T3 groups i.e., (300 ppm arsenic trioxide in the diet). Cirrhosis and congestion in the blood vessels of liver also reported by PK. Singh et al., (2011) in albino rat treated by As containing ground water. Dutta (2004) also observed fatty changes, congestion of hepatic vessels, thrombi in central vein and coagulative necrosis in his study carried out on rat with As treatment. M.S. Islam et al., (2013) observed that the distribution of arsenic concentration was highest in liver and lowest in faces of chickens. Such histological changes in the liver of treatment group birds might be due to toxic effect of arsenic trioxide on liver because most of the toxicants (like arsenic) are metabolized in liver & thereby affect its morphology. Microscopically, kidneys from control (T0), T1 (100 ppm) & T2 (200 ppm) showed normal architecture with normal glomeruli, proximal convoluted tubules (PCT) and distal convoluted tubules (DCT). Fatty degeneration, cytoplasmic vacuoles were observed in the section of kidneys from the birds receiving 300 ppm arsenic trioxide/kg in feed i.e. T3 group. These findings are in agreement with the previous study of Dutta et al., (2004). Since kidney is involved with ultra-filtration, selective reabsorption and tubular secretion, As may be accumulated in the kidney. The histopathological changes in treatment group birds could be due to accumulation of arsenic in kidney & thereby exert harmful effect.

Average SGPT & Serum creatinine of broiler in different groups after 42 days of an experiment is given in Table 2. The average SGPT & Serum creatinine of broiler was found comparable in different groups. Average Serum creatinine at the end of experiment (i.e. in 42 days) was 0.10 ± .0, .20 ± .026, 0.30 ± .026 and 0.383 ± .03073 mg/dl whereas average SGPT was 17.3667 ± .28363, 21.2333 ± .37476, 26.2833 ± .36735 and 38.0167 ± .40449 µ/L for T0, T1, T2 & T3 respectively. The elevated serum levels of transaminases, which are located primarily in the cytosol of hepatocytes, is a sign of damage which leads to liver dysfunction in treated birds. This finding is similar to Ghaffar et al., (2017), Islam et al., (2012), Devaraju et al., (2010) & Gaim et al., (2015). Such increased SGPT may be attributed to the histopathological changes in liver which has been mentioned earlier. The increased level of serum creatinine after arsenic trioxide intoxication may result from the enhanced formation of metabolic waste product of muscle metabolism. Further, creatinine is anhydride of creatine. Muscle contains phosphocreatine which undergoes spontaneous cyclization with loss of inorganic phosphorous to form creatine. Conversion of creatine to creatinine is a non-enzymeatic irreversible process. Due to affinity for thiol group of various proteins found in the cell membrane of muscles, arsenic damages the cells due to which the enzyme CPK (Creatine phosphokinase) gets released from the cells which is responsible for the conversion of phosphocreatine into creatine.
5. CONCLUSIONS

Poultry, a promising sector in Bangladesh is now under the thread of environmental heavy metal. The exposure of heavy metals like arsenic may affect the physiology of poultry which in turn may be the issue of public health through feed chain. It was observed from the current study that supplementation of arsenic in chicken diets at 100, 200 and 300 mg/kg feed produces various deleterious effects on growth performance, gross and microscopic study of different organs as well as biochemical parameters. Decreased body weight in arsenic-treated birds is due to malabsorption and altered metabolism. The present study revealed various degrees of histological changes that accompanied the biochemical changes in the liver and kidney tissues in experimental groups as compared with those of control group. Supplemented As at all doses had a noteworthy effect on microscopic structure of liver and kidney such as congestion and cirrhosis in liver, fatty degeneration and vacuolation in glomerulus of kidney. Increased SGPT/ALT in blood of all treated groups resulted from altered permeability of plasma membrane, cellular damage and altered metabolism which was a specific indicator of hepatocellular damage. However, the accumulation rate of arsenic in various organs was not recorded due to lack of technical facilities. Therefore, it is recommended for further study to determine the affinity of as in different organs. Moreover, the economic losses in the farming sector due to as exposure & its preventive strategies should also be undertaken.

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REFERENCES


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