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## Effect of Spirulina on Toxic Signs, Body Weight and Hematological Parameters in Arsenic Induced Toxicities in Ducks

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**Abstract:** The present study, was undertaken for the effect of spirulina on toxic signs, body weight and hematological parameters in arsenic induced toxicities in ducks. One hundred and 75 ducklings were divided into 5 equal groups separately. One group  $(T_0)$  of ducklings was kept as control. One group  $(T_1)$  of ducklings were given arsenic trioxide @ 100 mg/L drinking water and rest three groups of ducklings  $(T_2, T_3)$  and  $(T_4)$  were given arsenic trioxide @ 100 mg/L plus spirulina in three different doses i.e. 30, 60 and 120 mg/L in drinking water daily for 90 days starting from day 15. Five birds were sacrificed from each group in every 15 day intervals and toxic signs, body weight and hematological parameters were recorded. Ducks of  $(T_1)$  group (only arsenic trioxide) showed depression, reduced feed intake, dullness and ruffled feathers which were in mild in nature in other groups i.e. arsenic plus spirulina. In arsenic treated groups  $(T_1)$  the not gained body weight was maximum (14.93%), whereas in arsenic plus spirulina treated groups  $(T_2, T_3)$  and  $(T_4)$  the not gained body weight in ducks (4.08-11.26%) were better than only arsenic treated groups. Reduction of TEC, Hb and PCV values and rise of ESR values were significant (P<0.01) in  $(T_1)$  (arsenic treated) groups. However, in arsenic plus spirulina treated rest groups reduction of TEC, Hb and PCV were less than arsenic treated groups. The present study reveals that spirulina may be helpful for reducing the body burden of arsenic in ducks.

Key words: Spirulina, toxic signs, body weight, hematological parameters, arsenic toxicities, ducks

### INTRODUCTION

The peoples of Bangladesh have been suffering from serious public health problem arising from groundwater drinking arsenic contaminated (Khaleguzzaman et al., 2005). Nearly 62, out of 64 districts of the country's tube wells contain dangerous levels of inorganic arsenic, tube wells, which are serving as main sources for drinking and cooking purposes. The general populations are exposed to arsenic through drinking water, dust, fumes and dietary sources. The highest concentrations of arsenic were reported in seafood, rice, mushrooms and poultry in U.S.A. (Tao and Bolger, 1999).

An organic arsenical compound Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) is used widely in poultry production to control coccidial intestinal parasites. It is excreted unchanged in the manure and introduced into the environment when litter is applied to farmland as fertilizer. Although, the toxicity of roxarsone is less than that of inorganic arsenic, roxarsone can be degraded biotically and abiotically, to produce more toxic inorganic forms of arsenic, such as arsenite and arsenate (Bednar *et al.*, 2003). It has been reported that every U.S people may ingest 3.6-5.2 µg/inorganic arsenic daily from chicken alone consuming in an average 60 g chickens/day. Drinking water, dust, fumes and diet represent other forms of exposure. Inorganic forms of arsenic are classified as carcinogens, with chronic

exposure (10-40 µg day<sup>-1</sup>) associated with skin, respiratory and bladder cancers (Lasky et al., 2004). Spirulina, microscopic blue-green algae, has a property of reducing heavy metals and nephrotoxic substance from the body. It is not only a whole food, but it seems to be an ideal therapeutic supplement. So far, no other natural food is found with such a combination and amazing concentration of so many unusual nutrients like protein, amino acid, iron, betacarotene, phycocyanin, gama lenolenic acid, vitamin B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>6</sub>, B<sub>12</sub>, essential fatty acid etc. In fact it is the highest known source of protein, beta-carotene which is a precursor of vitamin A and only vegetable source of vitamin B 12 (Robert, 1989). Beta-carotene concentration of spirulina is ten times higher than carrot. It was evident that food rich in betacarotene can reduce the risk of cancer (Peto et al., 1981). It was found in the laboratory that the natural carotene of spirulina could inhibit, shrink and destroy oral cancer cells. Phycocyanin of spirulina also prevents cancer and its growth (Peto et al., 1981; Shekelle et al., 1981). In spirulina extract plus zinc-treated group, the clinical scores for keratosis before and after treatment was statistically significant (p<0.05) (Misbahuddin et al., 2006). The beta-carotene in algae and leafy green vegetables has greater anti-oxidant effects than synthetic beta-carotene (Amotz, 1987).

Ducks are one of the the main source of meat in our Bangladesh. Duck meats may contain arsenic through

arsenic contaminated water, growth promoters containing arsenicals and through arsenic medication. Arsenic is concentrated by many species of fish and shellfish and is used as a feed additive for poultry and livestock; fish and meat are therefore the main sources of dietary intake of almost 78.9%, according to a recent U.S. survey (Gartrell *et al.*, 1986). In Canada, arsenic levels ranging from 0.4-118 mg kg<sup>-1</sup> have been reported in marine fish sold for human consumption, whereas concentrations in meat and poultry range up to 0.44 mg kg<sup>-1</sup> (Department of National Health and Welfare, Ottawa, 1983).

In the context of the above situation, the present study was undertaken with the following objectives:

- Study the effect of spirulina on toxic signs and body weight in arsenic induced toxicities in ducks.
- Study the effect of spirulina on hematological parameters i.e. TEC, Hb, ESR and PCV in arsenic induced toxicities in ducks.

#### **MATERIALS AND METHODS**

The present study, was undertaken to perform the effect of spirulina in reducing toxic signs, body weight and hematological parameters in arsenic toxicities in ducks. The experiment was designed and following methodology was adopted for performing the experiment.

One hundred and seventy five, Xinding day old male ducklings were purchased from Kishoregonj Poultry Farm and fed with Aftab broiler starter feeds, Bangladesh. At fifteen day old the ducklings were randomly divided into 5 equal groups (n = 35) and were marked as group  $T_0$ ,  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_4$ .

- T<sub>0</sub>: Ducklings were fed with recommended feed and drinking water *ad. lib*.
- T<sub>1</sub>: Ducklings were fed with recommended feed and drinking water treated with arsenic trioxide@ 100 mg L<sup>-1</sup>.
- T<sub>2</sub> : Ducklings were fed with recommended feed and drinking water treated with arsenic trioxide@ 100 mg L<sup>-1</sup> plus spirulina@ 30 mg L<sup>-1</sup>.
- $T_3$ : Ducklings were fed with recommended feed and drinking water treated with arsenic trioxide@ 100 mg  $L^{-1}$  plus spirulina@ 60 mg  $L^{-1}$ .
- $T_4$ : Ducklings were fed with recommended feed and drinking water treated with arsenic trioxide@ 100 mg  $L^{-1}$  plus spirulina@ 120 mg  $L^{-1}$ .

Arsenic trioxide and spirulina at different dose rate were fed to different groups of ducklings with drinking water daily for 90 days. The parameters were taken fortnightly at day 15, 30, 45, 60, 75, 90 and 105. Fortnightly five birds were sacrificed from each group for studying following parameters:

- Toxic signs and body weight.
- Hematological parameters i.e. TEC, Hb, ESR and PCV

**Toxic signs and body weight:** After feeding arsenic trioxide and spirulina all the control and treated birds were observed carefully for appearance of any toxic signs up to day 105. Body weight of the birds was measured at 15 days interval up to day 105 (15, 30, 45, 60, 75, 90 and 105).

Hematological parameters: Total Erythrocyte Count (TEC), Hemoglobin content (Hb), Erythrocyte Sedimentation Rate (ESR) and Packed Cell Volume (PCV) were studied. For determination of hematological parameters blood samples were collected from five birds from each group fortnightly up to day 105 by from jugular vein i.e. on day 15, 30, 45, 60, 75, 90 and 105. Around 1.5 mL blood was collected in the sterile glass test tubes containing citrated anticoagulant. The hematological parameters were determined as per method cited by Lamberg and Rothstein (1977).

**Statistical analysis:** The experimental data were designed in CRD and analyzed statistically using one way analysis of variance with the help of the MSTAT software and Duncan's Multiple Range Test (DMRT) were also done for ranging (Gomez and Gomez, 1984).

#### **RESULTS AND DISCUSSION**

The results of the studies on the effect of spirulina in reducing toxic signs, body weight and hematological parameters in arsenic induced toxicities in ducks following different doses are given below:

**Toxic signs:** All the control ducks were quiet normal without any toxic signs during the whole experimental period

Ducks of group  $T_1$  (only arsenic trioxide) were apparently normal up to 45 days of arsenic trioxide feeding. After 45 days of arsenic trioxide administration, sudden onset of depression, reduced feed intake, dullness and ruffled feathers were observed.

Ducks of group  $T_2$  (arsenic trioxide plus spirulina @ 30 mg  $L^{-1}$ ), group  $T_3$  (arsenic trioxide plus spirulina @ 60 mg  $L^{-1}$ ) and group  $T_4$  (arsenic trioxide plus spirulina @ 120 mg  $L^{-1}$ ) were apparently normal up to 45 days of arsenic trioxide feeding. After 45 days of arsenic trioxide administration the signs of depression, reduced feed intake, dullness and ruffled feathers were observed but was mild in nature.

In the present study, similar toxic signs were observed in ducks following arsenic trioxide feeding and arsenic trioxide and spirulina feeding in 3 different doses. Most of the signs observed in  $T_1$  (only arsenic trioxide) were also, observed in other three groups ( $T_2$ ,  $T_3$  and  $T_4$ ) but in

Table 1: Body weight (g) of control, arsenic induced and arsenic and spirulina treated ducks

	Day	Day	Day	Day	Day	Day	Day	(%) not
Treatment	15	30	45	60	75	90	105	gained
Control (T <sub>0</sub> )	230.40	605.40ª	798.80°	1001.20 <sup>a</sup>	1159.60°	1410.40ª	1529.60°	-
Arsenic trioxide@ 100mg L <sup>-1</sup> (T₁)	226.80	540.80⁵	713.60⁵	891.60b	1020.40b	1235.20b	1301.20b	14.93
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 30 mg L <sup>-1</sup> (T <sub>2</sub> )	228.20	555.20°	734.20°	918.40°	1056.80°	1278.40°	1357.40°	11.26
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 60 mg L <sup>-1</sup> (T <sub>3</sub> )	233.40	572.60d	755.40 <sup>d</sup>	945.60⁴	1090.20d	1321.60d	1407.80d	7.97
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 120 mg L <sup>-1</sup> (T <sub>4</sub> )	232.20	590.40°	780.60°	977.80°	1135.40°	1372.20°	1467.20°	4.08
SE	3.26	3.72	3.56	3.72	3.85	4.09	4.58	-

Figures in mean, SE

Table 2: TEC (million  $\mu L^{-1}$ ) of control, arsenic induced and arsenic and spirulina treated ducks

	Day	Day	Day	Day	Day	Day	Day	(%) not
Treatment	15	30	45	60	75	90	105	decreased
Control (T <sub>0</sub> )	2.05	2.23ª	2.39"	2.54	2.68ª	2.83ª	3.03*	-
Arsenic trioxide@ 100mg L <sup>-1</sup> (T <sub>1</sub> )	2.06	1.82°	1.74°	1.63⁴	1.53°	1.46°	1.36°	55.12
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 30 mg L <sup>-1</sup> (T <sub>2</sub> )	2.03	1.92⁴	1.89⁴	1.84°	1.81⁴	1.79⁴	1.77⁴	41.59
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 60 mg L <sup>-1</sup> (T <sub>3</sub> )	2.05	2.03°	2.05°	2.07⁵	2.11°	2.15°	2.21°	27.07
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 120 mg L <sup>-1</sup> (T <sub>4</sub> )	2.08	2.13⁵	2.22₺	2.32⁵	2.41⁵	2.54⁵	2.69⁵	11.23
SE	0.03	0.03	0.03	0.03	0.03	0.05	0.05	-

Figures indicate mean, SE

mild form indicating that spirulina has a protective role against skin lesions caused by arsenic trioxide. Toxic signs observed in T<sub>1</sub> (only arsenic trioxide) are in partial agreement with that of Islam et al. (2005) who reported that arsenic treated rats showed severe symptoms of excitement, restlessness, anorexia, ruffled hair coat and skin lesions in all parts of the body especially on tail region. Reduced feed intake and ruffled hair coat were also observed in arsenic treated rats by Alam (2004). Hsueh et al. (1995) showed that chronic exposure in human in the range of 0.01-0.04 mg/kg/day has been associated with skin cancer in Taiwan. Gonsebatt et al. (1997) reported the skin lesions in arsenic exposed individuals. The present findings are also, in agreement with Smith et al. (2000), Lasky et al. (2004) and Mitra et al. (2004). Lasky et al. (2004) also, stated that inorganic forms of arsenic are classified as carcinogens, with chronic exposure (10-40 µg day<sup>-1</sup>) associated with skin, respiratory and bladder cancers.

Effect on body weight: The body weight of ducks in each group was recorded at fifteen days interval following administration of arsenic trioxide and spirulina (Table 1). In a column figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance (p<0.01).

The body weight gain of chickens of control group ( $T_0$ ) at 105 days was highest (1529.6±11.52 g) but in arsenic treated group ( $T_1$ ) the body weight gain was lowest (1301.2±10.94 g) whereas in arsenic plus spirulina treated groups ( $T_2$ ,  $T_3$  and  $T_4$ ) body weight gains (1357.4±11.28, 1407.8±9.88 and 1467.2±6.83 g) were better than arsenic treated alone but less than control group.

Though the birds were in growing age however, the body weight of most of them was increased gradually but in case of the birds of group  $T_1$  the increasing trend of the body weight was lower significantly (p<0.01). The not

gained body weight was maximum (14.93%) in birds of only arsenic treated group  $T_1$  in comparison to control group  $(T_0)$ . On the other hands, other treated groups i.e. arsenic plus spirulina treated groups  $T_2$ ,  $T_3$  and  $T_4$  the reduction of body weight was 11.26, 7.97 and 4.08%, respectively which were lower than only arsenic treated group.

In the present study, arsenic reduced the increasing trend of body weight in ducks. Similar to present findings, Islam *et al.* (2001) reported that arsenic significantly (p<0.01) reduced the body weight in experimentally induced arsenic toxicosis in mice. Purohit (2005) also observed a reduction in the body weight of acephate treated chickens in India.

Likewise, Mahaffey *et al.* (1981) studied concurrent exposure to lead, cadmium and arsenic and effects on toxicity and tissue metal concentrations in the rat. Cd and As reduced weight gain even when differences in food intake were taken into account and administration of both Cd and As depressed weight gain more than did either metal alone. In accordance to the present findings significantly (p<0.01) reduced body weight was also, observed in arsenic induced rats by Alam, (2004) and Islam *et al.* (2005). Sharma *et al.* (2007) reported that decreased body weight was observed in arsenic treated group of Swiss albino mice.

#### Hematological parameter

**Total Erythrocyte Count (TEC):** Daily administration of arsenic trioxide alone and in combination with spirulina in different doses in drinking water for 90 days on TEC in ducks is presented in Table 2.

In a column figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance (p<0.01).

The TEC values were decreased significantly (p<0.01) (55.12%) in arsenic trioxide fed group ( $T_1$ ) in relation to control group ( $T_0$ ). The reduction of TEC values in other

Table 3: Hb (g%) of control, arsenic induced and arsenic and spirulina treated ducks

	Day	Day	Day	Day	Day	Day	Day	Decreased
Treatment	15	30	45	60	75	90	105	(%)
Control (T <sub>0</sub> )	8.75	8.92ª	9.13ª	9.33°	9.55³	9.76ª	9.97ª	-
Arsenic trioxide@ 100mg L <sup>-1</sup> (T₁)	8.77	7.02°	6.69°	6.29°	5.87⁴	5.46°	5.03°	49.55
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 30 mg L <sup>-1</sup> (T <sub>2</sub> )	8.73	7.49⁴	7.28d	7.04⁴	6.89⁴	6.66⁴	6.31⁴	36.72
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 60 mg L <sup>-1</sup> (T <sub>3</sub> )	8.78	7.99°	7.88°	7.79°	7.91°	7.94°	8.04°	19.36
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 120 mg L <sup>-1</sup> (T <sub>4</sub> )	8.74	8.51⁵	8.72⁵	8.79⁵	8.97⁵	9.23⁵	9.41⁵	5.62
SE	0.03	0.03	0.04	0.04	0.05	0.04	0.05	-

Figures indicate mean, SE

Table 4: ESR (mm/in 1st h) of control, arsenic induced and arsenic and spirulina treated ducks

	Day	Day	Day	Day	Day	Day	Day	Increased
Treatment	15	30	45	60	75	90	105	(%)
Control (T <sub>o</sub> )	1.90	2.20	2.40⁵	2.70⁵	3.10°	3.40⁴	3.70⁴	-
Arsenic trioxide@ 100mg L <sup>-1</sup> (T₁)	1.80	3.90	4.80°	5.90°	7.20ª	8.20°	9.40ª	60.64
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 30 mg L <sup>-1</sup> (T <sub>2</sub> )	1.70	3.30	4.20ab	5.10 <sup>a</sup>	5.90⁴	6.90ab	7.70ªb	51.95
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 60 mg L <sup>-1</sup> (T <sub>3</sub> )	2.00	2.90	3.80ab	4.30ab	4.70₺	5.40™	6.10₺	39.35
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 120 mg L <sup>-1</sup> (T <sub>4</sub> )	1.60	2.50	2.80⁵	3.00⁵	3.70°	4.10⁰	4.40⁰	15.91
SE	0.22	0.51	0.59	0.66	0.66	0.63	0.72	-

Figures indicate mean, SE

Table 5: PCV (%) of control, arsenic induced and arsenic and spirulina treated ducks

	Day	Day	Day	Day	Day	Day	Day	Decreased
Treatment	15	30	45	60	75	90	105	(%)
Control (T <sub>0</sub> )	28.90	29.28	29.90ª	31.20°	32.40	33.30°	34.40°	-
Arsenic trioxide@ 100mg L <sup>-1</sup> (T <sub>1</sub> )	29.10	27.12	26.30⁵	24.90⁵	22.70d	20.1 <sup>d</sup>	17.30⁴	49.71
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 30 mg L <sup>-1</sup> (T <sub>2</sub> )	29.20	27.70	27.40ab	26.60ab	25.30⁰	23.60°	21.90°	36.34
Arsenic trioxide@ 100mg L <sup>-1</sup> plus spirulina@ 60 mg L <sup>-1</sup> (T <sub>3</sub> )	28.80	28.20	28.50ab	28.30ab	28.20₺⁰	27.60°	27.10 <sup>b</sup>	21.23
Arsenic trioxide@ 100 mg L <sup>-1</sup> plus spirulina@ 120 mg L <sup>-1</sup> (T <sub>4</sub> )	29.10	28.90	29.30°	29.90°	31.10 <sup>ab</sup>	31.90°	32.60°	5.24
SE	0.59	0.77	0.79	0.81	0.82	0.81	0.82	-

Figures indicate mean, SE

three groups  $(T_2, T_3)$  and  $T_4$  i.e. combined administration of arsenic and spirulina were 41.59, 27.07 and 11.23%, respectively which were less than arsenic treated groups.

Hemoglobin (Hb): Administration of arsenic trioxide alone and in combination with spirulina in different doses in drinking water on Hb level in ducks is presented in Table 3.

In a column figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance (p<0.01).

Similar to TEC, the Hb values were also decreased significantly (p<0.01) to the extent of 49.55% in arsenic treated group (T<sub>1</sub>). However, in arsenic plus spirulina treated group (T<sub>2</sub>) Hb value was reduced upto 36.72%. But in other two groups (T<sub>3</sub> and T<sub>4</sub>), the Hb values were reduced upto 19.36 and 5.62%, respectively.

Erythrocyte Sedimentation Rate (ESR): ESR of ducks following daily administration of arsenic trioxide alone and in combination with spirulina in different doses in drinking water for 90 days were observed and is presented in Table 4.

In a column figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance (p<0.01).

The ESR values were increased (60.64%) significantly (p<0.01) in arsenic trioxide treated group  $(T_1)$  in comparison to control group (Tn) but the increasing percentage of ESR values in other three groups (T2, T3 and T<sub>4</sub>) i.e. combined administration of arsenic and spirulina were 51.93, 39.35 and 15.91%, respectively which were less than arsenic treated groups.

Packed Cell Volume (PCV): Daily administration of arsenic trioxide alone and in combination with spirulina in different doses in drinking water for 90 days on PCV in ducks is presented in Table 5.

In a column figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance (p<0.01).

Similar to TEC and Hb, the PCV values in ducks were also decreased significantly (p<0.01) to the extent of 49.71% in arsenic treated group (T<sub>1</sub>) in relation to control group  $(T_0)$ . However, in other three groups i.e.  $T_2$ ,  $T_3$  and T<sub>4</sub>the PCV values were decreased to the extent of 36.34, 21.23 and 5.24%, respectively.

Similar to present findings, reduction of hematological parameters by arsenic has been reported by many authors. Islam et al. (2005) found that TEC, Hb and PCV values were significantly (p<0.01) reduced in arsenic treated rats but ESR value was significantly (p<0.01) increased. Alam, (2004) observed significantly (p<0.01) decreased TEC, Hb and PCV and significantly (p<0.01) increased ESR in arsenic treated rats. Ahmed (2004) also reported a significant (p<0.01) reducing in TEC, Hb and PCV values and significant (p<0.01) increased values of ESR was observed in arsenic treated rabbit.

In contrary, Mahaffey et al. (1981) observed the

increased numbers of circulating RBCs in rats. However, they found that hemoglobin and hematocrit values were reduced in arsenic toxicities in rats as observed in the present study.

The cause of change in hematological values might be due to the toxic effect of arsenic on haematotopoeitic system which is responsible for such alterations in hematological parameters. However, Islam *et al.* (2005) assumed that toxic effects of arsenic trioxide on bone marrow may be responsible for erythrocytopenia.

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