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## **Chronic Arsenic Toxicity with and Without Excess Supplementation of Methionine on the Performance and Metabolizability of Nutrients in Layer Chicken**

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**Abstract:** An experiment was conducted to find out the effect of arsenic with or without excess supplementation of methionine and methionine-betaine combination on performance of layer chicken. Sixteen week old pullets (n = 120) of Rhode Island Red (RIR) were randomly assigned into four dietary treatments replicated three times with 10 birds/replicate. The groups were control (C)-provided basal diet to meet all the nutrients requirement, T<sub>1</sub>-birds were offered basal diet and 5.5 ppm arsenic (As) through water, T<sub>2</sub>-birds were offered control diet with 50 g methionine per 100 kg of feed and 5.5 ppm As through water and in T<sub>3</sub>- 25 g methionine + 25 g betaine per 100 kg of control feed and 5.5 ppm As through water were provided. The birds were maintained replicate wise in deep litter system of housing with a common system of management. Final body weight and body weight gain was significantly (p<0.01) varied among the groups. Reduced feed intake was noticed in arsenic treated groups. Egg production and egg weight were significantly (p<0.01) decreased in arsenic treated groups than control. The first laying age were differed significantly (p<0.01) among the experimental groups.

**Key words:** Layer chicken, methionine, arsenic, weight gain, egg production, laying age

### **INTRODUCTION**

Toxic elements such as selenium, arsenic, cadmium, chromium, lead, molybdenum, fluorine etc., may adversely affect the health and production performances of birds. Among important nutritional disorders faced by the birds, mineral toxicity or poisoning has got great importance, due to the residual effect of toxic minerals which may be associated with the public health hazards via contaminated animal products. Arsenic (As) is known for a long time and the word is derived from the Greek word arsenicon means fearless. Among toxicities produced by different elements, arsenic toxicity in concern with human health is now a burning problem throughout the world, especially in India. Protein, methionine, cysteine, choline, selenium, manganese and vitamins give protection against arsenic toxicity in animals (Vaheter and Marafante, 1987; Currey *et al.*, 1992; NRC, 1999; Sardar, 2005). At present various parts of world specially in India are suffering from As toxicity due to drinking of water contaminated with As which is above the recommended safe limit [0.01 mg As L<sup>-1</sup>] (WHO, 1996). Chronic ingestion of As contaminated feed and drinking water may produce toxicity to the animals, which not only reduce productivity of animals but also its residual concentration in animal tissue and excretion through animal products (egg, meat etc.) may threat to human health (NRC, 1999). Continuous consumption of contaminated eggs and meat along with drinking water may produce following skin lesions in human being: Darkening of the skin, spotted pigmentation, white and black

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spots side by side, buccal mucus membraemelanosis, rough and dry skin, often with palpable nodules. Methylation of arsenic is the first defense against arsenic toxicity. Excess methionine and methionine with betaine were added as a methyl donor in diets to facilitate As metabolism for As detoxification.

## MATERIALS AND METHODS

The experiment was conducted at Departmental poultry shed, Department of Animal Nutrition, Kolkata, during the period of January 2004-July 2004. One hundred and twenty Rhode Island Red (RIR) pullets of 16 weeks age were randomly distributed into four experimental groups, each consisting of three replicates ( $n = 10$  i.e., 9 hens and 1 cock per replicate). The experimental groups were C (control group fed with basal diet only), T<sub>1</sub> (fed control diet (Table 1) with 5.5 ppm as through water), T<sub>2</sub> (fed control diet with 5.5 ppm As through water + 50 g methionine per 100 kg of feed) and T<sub>3</sub> (fed same as T<sub>2</sub> but 50% of the excess methionine supplement was replaced with betaine). As per the objective of the study, the control diet of respective ration as such used for T<sub>1</sub> group. In diet of T<sub>2</sub> 50 g Lime Stone Powder (LSP) was replaced by 50 g methionine in 100 kg of control diets. Similarly diets for T<sub>3</sub> birds were prepared where 25 g betaine was used in stead of 50% of excess methionine. The experiment was conducted in the departmental layer shed, in deep litter system. The deep litter was prepared with sun-dried sawdust, rice husk and chopped rice straw. Lime and copper sulfate were added to the litter as disinfectants. Three days prior to arrival of the pullets, the layer shed and floor space were thoroughly cleaned with soap water, disinfected with potassium permanganate and fumigated with the formaldehyde solution. The feeding and watering troughs were properly cleaned and disinfected. Birds were exposed to 18 h light and 6 h darkness throughout the period of the experiment. All the birds were fed accurately weighed feeds twice a day and the residue left over was amounted next morning to determine feed intake in 24 h. All the birds of the control group were offered water *ad lib* every day, but prior to giving *ad lib* water to the treatment groups, As solution was offered to the birds so that they could be able to drink the whole As solution. The water troughs and feeders were cleaned every day. The birds were fed as per the Indian Feeding Standards for layer chicken (BIS, 1992). Daily egg production were recorded. Age at onset of lay for each bird was also recorded. Egg weight was recorded daily. A digestibility trial was carried out at the terminal part of the experiment for 5 days to estimate metabolizability of Dry Matter (DM), Organic Matter (OM),

Table 1: Ingredient and nutrient composition of control diet for grower and layer ration (parts by weight)

Ingredients	Grower ration	Layer ration	Chemical composition		
			(% DM basis)		
			Grower ration	Layer ration	
Maize	42.00	48.68	C P (%) ++	16.20	18.33
Deoiled rice bran	36.75	16.87	C F (%) ++	6.92	4.99
Soyabean meal	10.32	17.51	E E (%) ++	1.51	2.55
Til cake	2.87	5.42	N F E (%) ++	65.95	62.82
Fish meal	3.74	2.69	Total ash (%) ++	8.82	11.46
Vegetable oil	-	0.50	ME (Kcal kg <sup>-1</sup> ) (calculated)	2572.00	2622.00
Lime stone powder	1.00	1.00	Ca% ++	1.85	3.25
Di calcium phosphate	1.00	1.00	Available P% ++	0.68	0.57
Oyster shell	2.00	6.00	Zinc (mg kg <sup>-1</sup> ) ++	40.65	35.74
Nicomix <sup>-</sup>	0.025	0.025	Manganese (mg kg <sup>-1</sup> ) ++	47.98	29.03
Methionine	0.05	0.05	Methionine	0.46	0.48
Choline chloride	0.14	0.15	Lysine	0.90	1.00
Trace mineral mixture <sup>+</sup>	0.075	0.075	Methionine+ cystine	0.62	0.66
Salt	0.03	0.03			

<sup>-</sup> Each gram of Nicomix contained. Vitamin A-40,000 IU, Vitamin D<sub>3</sub>-6,000 IU, Vitamin B<sub>1</sub>-3.2 mg, Vitamin B<sub>2</sub>-20 mg, Vitamin B<sub>6</sub>-6.4 mg, Vitamin B<sub>12</sub>-82 mcg, Niacin-48 mg, Calcium pantothenate-32 mg, Vitamin K-4 mg, Vitamin E-32 mg and Folic acid-3.2 mg. <sup>+</sup> for Grower ration: Ferrous sulphate 45 g, Zinc sulphate 12.50 g, Manganese sulphate 13.65 g, Copper sulphate 3.60 g, Potassium iodide 0.15 g and Sodium selenite 0.20 g; for layer ration: Ferrous sulphate 37.50 g, Zinc sulphate 18.55 g, Manganese sulphate 15 g, Copper sulphate 3.60 g, Potassium iodide 0.15 g and Sodium selenite 0.20 g. <sup>++</sup> Estimated value

Ether Extract (EE), Crude Protein (CP), Nitrogen Free Extract (NFE) as well as major and trace minerals. The feed and fecal samples were analyzed for proximate components (AOAC, 1995). The calcium in the feeds and droppings was estimated with the help of Atomic Absorption Spectrophotometer (Perkin Elmer, Analyst 100) as stated by Trudeau and Freier (1967). The phosphorus in the feeds and droppings was estimated with the help of UV visible Spectrophotometer as per the method followed by AOAC (1975). Trace minerals (Zn, Mn, Cu and Fe) estimation from feeds and droppings were done by wet oxidation as per the method of AOAC (1995) and analyzed with the help of Atomic Absorption Spectrophotometer.

Data obtained in the present study was analyzed with the help of software SPSS 10.0 Package (SPSS, 1997). Levels of significance were calculated by Duncan (1995) Test whenever any effect was found significant.

## RESULTS AND DISCUSSION

### Live Weight Changes

Live weight of the birds at the end of the experiment varied ( $p < 0.05$ ) due to dietary treatments (Table 2). A significantly lower body weight was recorded in the  $T_1$  group receiving 5.5 ppm As. Supplementation of excess methionine alleviated this depression in the  $T_2$  group as it was revealed from the significantly higher body weight in the  $T_2$  dietary group. However, partial replacement of excess methionine with betaine was found not to be very much effective since the body weight of the  $T_3$  group of birds was significantly lower than that of  $T_2$  group. It is worth mentioning that the excess methionine supplementation in the  $T_2$  group not only alleviated the As induced depression in the body weight but also promoted body weight as it was revealed from the significantly higher body weight in the said dietary group relative to the control group. As induced growth retardation has been reported earlier (Wharton *et al.*, 1957; Vodela *et al.*, 1997; Abdo *et al.*, 1989; Kerr *et al.*, 1963) which is presumably caused by an arsenic mediated Zn deficiency leading to biochemical and metabolic changes related with amino acid utilization, protein synthesis and nucleic acid biosynthesis (Chesters, 1997; Sardar, 2005). But the result of Aguilar *et al.* (1997) and Holeman and Stibilij (1997) not corroborated with the result of present study. Thompson *et al.* (1991) reported that an excess arsenic may cause direct toxic effects and secondary deficiency of other trace elements like Zn, copper (Cu) and Mn to precipitate indirect toxicity and influences the feed utilization efficiency leading to poor growth. DNA synthesis as well cell replications may simultaneously be inhibited by Zn deficiency (Baker and Duncan, 1983) which may be precipitated by As toxicity as DNA synthesis requires methylation process. As in diet may affect DNA synthesis by reducing methylation of DNA through a decreased availability of methyl groups which are utilized by As itself resulting less DNA synthesis leading to reduce cell replication and finally poor growth. Arsenic mediated Mn deficiency might also lead to reduce growth (Zhu *et al.*, 1998). Mn is essential for some of the enzymes related

Table 2: Effect of arsenic toxicity with or without excess supplementation of methionine and methionine and betaine combination on the performance of RIR layer chicken

Parameters	C	$T_1$	$T_2$	$T_3$	Sig.
Initial body weight (g)	1504.17±4.29	1500.26±0.91	1500.31±1.36	1500.51±0.94	$p > 0.05$
Final body weight (g)	2001.05±1.44 <sup>b</sup>	1798.80±5.28 <sup>d</sup>	2098.73±2.16 <sup>e</sup>	1950.72±2.78 <sup>c</sup>	$p < 0.01$
Weight gain (g)	496.89±3.39 <sup>b</sup>	298.54±6.13 <sup>d</sup>	598.42±3.16 <sup>e</sup>	450.21±3.12 <sup>c</sup>	$p < 0.01$
DM consumed/bird (g)	18022.53±232.13	16942.69±175.90	17769.42±219.06	17747.48±343.83	$p > 0.05$
Egg production (%)	79.56±0.61 <sup>a</sup>	68.02±1.37 <sup>c</sup>	75.82±1.44 <sup>b</sup>	72.53±0.79 <sup>b</sup>	$p < 0.01$
Total number of eggs	1102.70±8.49 <sup>a</sup>	942.76±19.04 <sup>c</sup>	1050.65±19.79 <sup>b</sup>	1005.31±10.94 <sup>b</sup>	$p < 0.01$
Average egg weight (g)	55.32±0.94 <sup>a</sup>	49.97±0.51 <sup>b</sup>	55.20±0.26 <sup>a</sup>	54.32±0.24 <sup>a</sup>	$p < 0.01$
DM consumption $\text{kg}^{-1}$ of eggs	2660.48±53.49 <sup>a</sup>	3240.24±79.81 <sup>a</sup>	2764.38±56.99 <sup>bc</sup>	2927.11±15.79 <sup>b</sup>	$p < 0.01$
First laying age (days)	134.00±0.58 <sup>a</sup>	144.33±1.20 <sup>a</sup>	136.00±1.15 <sup>b</sup>	136.33±0.67 <sup>b</sup>	$p < 0.05$

Similar alphabets at superscript denote homogenous means due to Duncan's test at 5% level of significance

to protein metabolism, e.g., glutamine synthetase, arginase etc. Deficient level of Mn in the body could have impaired protein metabolism which results in lower nitrogen retention and lower body weight as found in the present investigation (Ye *et al.*, 1991).

As induced growth retardation might be due to less feed consumption, less nitrogen retention and less organic matter retention, which might be altered by biochemical and metabolic changes related to amino acid utilization, protein synthesis and nucleic acid biosynthesis. The weight gain in T<sub>2</sub> group was more than control and other treatment groups (T<sub>1</sub> and T<sub>3</sub>) probably methionine itself also induced growth rate. Methionine also detoxified the arsenic by promoting methylation procedure (Vaheter and Marafante, 1987).

#### **Dry Matter Intake (DMI)**

DMI was not affected by dietary treatments significantly during the experimental period. However, the cumulative feed intake per bird up to the termination of trial was numerically lower in T<sub>1</sub> group but feed intake of T<sub>2</sub> and T<sub>3</sub> group was closer to C group. This might be due to the fact that induction of arsenic toxicity produced stresses which might be revived with excess supplementation of methionine. Reduced feed intake under investigation probably due to arsenic induced stress causing deficiency of trace minerals specially Zn and Cu by unknown mechanism (Thompson, 1993) resulting biochemical changes related to the sense of taste as described by Henkin (1977) in rats. Arsenic mediated injury in mouth might be also another cause of reduction of feed intake. Pathological changes, like erosion and ulceration of surrounding oral epithelium and break down in the integrity of epithelium in mouth was found in T<sub>1</sub> group which may be an important factor in the impairment of appetite (Gentle *et al.*, 1981). Reduced feed intake also observed by Savabieasfahani *et al.* (1998) in rats, Chen and Chiou (2001) in ducks; Prikhod'ko (1998) in chickens by inducing arsenic toxicity.

#### **Laying Performance**

The percentage of egg production (hen house basis) with egg weight varied significantly ( $p < 0.01$ ) among the control and treated groups showing reduced egg production percent in T<sub>1</sub> group. Total number of eggs produced in 22 weeks (from 21st week of age to 42nd week of age) were showed significant ( $p < 0.01$ ) variation among the control and treatment groups. From the result of present study it was clear that induction of chronic arsenic toxicity reduced percent egg production and total egg production significantly. But excess supplementation of methionine and partial replacement of methionine by betaine increased percent egg production as well as total egg production by reducing toxic effects of arsenic on egg production in layer. Chiou *et al.* (1996) and Vodela *et al.* (1997) observed significant decrease in egg production, egg weight and total egg number among the arsenic treated and control groups. But Holeman and Stibilij (1997) found no significant effects on egg production in hen fed a diet containing As up to 30 mg kg<sup>-1</sup> for 19 days. Arsenic induced reduction of egg production in layer chicken might be owing to impairment of Ca metabolism (Taylor *et al.*, 1961). Decreased total egg production in T<sub>1</sub> group might be due to reduced DM intake, inefficient utilization of macro and micro nutrients and pathological changes in reproductive organs which might be revived by extra-methionine and betaine supplementation. From the results it was clear that arsenic intoxicated group (T<sub>1</sub>) showed significantly lower value than control (C), extra-methionine supplementation group (T<sub>2</sub>) and extra-methionine-betaine supplementation group. Exposure of dietary concentration of 400 ppm of arsenic for 4 weeks in mallard ducks (Stanley *et al.*, 1994) and (0.36 mg kg<sup>-1</sup> body weight) for 8 weeks in chicken (Donoghue *et al.*, 1994) resulted in decreased egg weight. Arsenic induced decrease in egg weight might be due to the decrease of estrogen which plays a role in controlling egg weight via changes in fat metabolism (Wiebe and Barr, 1988). Statistical analysis revealed that for 1 kg egg production the highest dry matter was required for T<sub>1</sub> group followed by T<sub>3</sub>, T<sub>2</sub> and C group. Treated groups consumed more dry matter for per kg egg production due to noticeable decrease in egg

Table 3: Chronic arsenicosis with and without excess supplementation of methionine and methionine and betaine combination on nutrient intake ( $\text{g bird}^{-1} \text{ day}^{-1}$ ) and metabolizability (%)

Parameters	C	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	Sig.
DM intake	104.05±1.28 <sup>a</sup>	93.68±0.74 <sup>c</sup>	101.05±1.26 <sup>ab</sup>	98.27±0.57 <sup>b</sup>	p<0.01
DM metabolizability	64.46±0.31	62.66±0.99	63.70±0.33	64.06±0.48	p>0.05
OM intake	92.13±1.13	87.90±0.41	89.47±1.12	90.77±1.67	p>0.05
OM metabolizability	69.65±0.25 <sup>a</sup>	63.26±0.68 <sup>c</sup>	66.64±0.69 <sup>b</sup>	65.51±0.51 <sup>b</sup>	p<0.01
CP intake	18.97±0.23 <sup>a</sup>	17.08±0.14 <sup>c</sup>	18.42±0.23 <sup>ab</sup>	17.91±0.10 <sup>b</sup>	p<0.01
CP metabolizability	56.94±0.53 <sup>a</sup>	53.14±0.39 <sup>b</sup>	55.66±0.55 <sup>a</sup>	55.70±0.63 <sup>a</sup>	p<0.01
EE intake	2.60±0.03	2.52±0.02	2.55±0.02	2.62±0.03	p>0.05
EE metabolizability	68.08±0.39 <sup>a</sup>	62.92±0.40 <sup>c</sup>	67.14±0.41 <sup>ab</sup>	66.10±0.59 <sup>b</sup>	p<0.01
CF intake	5.19±0.06	5.00±0.10	5.04±0.06	5.12±0.09	p>0.05
CF metabolizability	15.43±1.04	16.82±0.41	15.92±0.07	16.29±0.29	p>0.05
NFE intake	65.30±0.80	63.31±0.57	63.46±0.81	65.11±1.69	p>0.05
NFE metabolizability	82.98±0.21 <sup>a</sup>	76.95±0.31 <sup>d</sup>	82.02±0.04 <sup>b</sup>	80.54±0.31 <sup>c</sup>	p<0.01

Similar alphabets at superscript denote homogenous means due to Duncan's test at 5% level of significance

production and egg weight. Delayed first laying age (days) value was observed in T<sub>1</sub> group followed by T<sub>3</sub>, T<sub>2</sub> and C group. Arsenic toxicated groups (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>) showed delayed laying age. Supplementation reduced the time of onset of egg production with better result in methionine supplemented treatment than methionine-betaine supplementation, indicated that supplementation of these nutrients during chronic inorganic arsenic toxicity might be able to counter act the arsenic mediated adverse effect on reproductive system probably due to direct local effect of arsenic or indirectly through reduction of body trace minerals and impairment of Ca metabolism (Taylor *et al.*, 1961; Thompson *et al.*, 1991).

#### Effect on Nutrients Intake and Metabolizability

Dry matter and crude protein intake ( $\text{g/bird/day}$ ) (Table 3) varied significantly ( $p<0.01$ ) between the control (C) and other experimental groups. The present finding was in close agreement with the observation of Chiou *et al.* (1996). But different observation was obtained by Vodela *et al.* (1997). OM, EE, CF and NFE intake were not varied due to dietary treatments. But there was significant ( $p<0.01$ ) variation in metabolizability of CP, OM, EE, NFE among the dietary treatments. The lowest retention was found in T<sub>1</sub> group of birds without excess supplementation. It might be due to fact that arsenic caused impairment of appetite or due to pathological changes in mouth like erosion and ulceration of surrounding oral epithelium. CP retention was significantly ( $p<0.01$ ) higher in control and T<sub>3</sub> groups when compared to T<sub>2</sub> and T<sub>1</sub> groups. Arsenic intoxicated groups (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>) voided more amount of CP and could not be used for cell synthesis. Reduction of intake of OM, CP, EE, CF, NFE and TA and its actual metabolizability in layer might be due to As induced reduction of DMI. Sodium arenites might exert its adverse effect through deficiency of trace minerals particularly Zn and Mn. Yi *et al.* (1996) reported that Zn deficiency caused lower DMI in broiler chicken. Reduced protein intake and retention reflected on reduced growth. Zn is necessary for cell cycle. This might be one of the reasons for reduced N-retention and growth as observed in the present investigation.

From the above discussion it was clear that arsenic caused reduction in nutrient intake along with reduction in retention of nutrients. This finding is corroborated with the findings of Thompson *et al.* (1991). This might be due to direct toxic effects or indirect toxic effects which caused secondary deficiencies of nutrients by reducing the nutrient utilization efficiency with showing some neurological disorders, poor growth, poor appetite etc.

#### Inorganic Matter Intake and Metabolizability

T<sub>1</sub> group showed lower ( $p<0.01$ ) intake of calcium (Ca), phosphorus (P), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) than other experimental groups. Metabolizability of Ca, P, Fe, Cu, Mn and Zn (Table 4) varied significantly due to dietary treatments showing least metabolizability in T<sub>1</sub> group. But supplementation of excess methionine improved minerals intake as well as its

Table 4: Chronic arsenicosis with and without excess supplementation of methionine and methionine and betaine combination on inorganic matter intake (g or mg bird<sup>-1</sup> day<sup>-1</sup>) and metabolizability (%)

Parameters	C	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	Sig.
Ca Intake (g)	3.49±0.04 <sup>a</sup>	3.14±0.02 <sup>c</sup>	3.39±0.04 <sup>ab</sup>	3.29±0.02 <sup>b</sup>	p<0.01
Ca metabolizability	46.94±0.65 <sup>a</sup>	40.21±1.12 <sup>b</sup>	46.15±0.52 <sup>a</sup>	46.67±0.52 <sup>a</sup>	p<0.05
P intake (g)	1.00±0.01 <sup>a</sup>	0.90±0.01 <sup>c</sup>	0.97±0.01 <sup>ab</sup>	0.94±0.01 <sup>b</sup>	p<0.01
P metabolizability	45.92±0.66 <sup>a</sup>	42.58±0.73 <sup>b</sup>	45.33±0.75 <sup>a</sup>	43.62±0.81 <sup>ab</sup>	p<0.05
Fe mg	10.96±0.13 <sup>a</sup>	9.90±0.12 <sup>b</sup>	10.90±0.20 <sup>a</sup>	10.79±0.20 <sup>a</sup>	p<0.01
Fe metabolizability	57.67±0.52 <sup>a</sup>	47.62±3.20 <sup>b</sup>	55.86±0.91 <sup>a</sup>	55.51±1.58 <sup>a</sup>	p<0.05
Cu intake (mg)	1.70±0.02 <sup>a</sup>	1.53±0.01 <sup>b</sup>	1.65±0.02 <sup>ab</sup>	1.61±0.01 <sup>b</sup>	p<0.01
Cu metabolizability	53.47±0.66 <sup>a</sup>	47.72±0.47 <sup>b</sup>	51.81±0.80 <sup>b</sup>	51.64±1.34 <sup>b</sup>	p<0.01
Mn intake (mg)	8.74±0.11 <sup>a</sup>	7.87±0.06 <sup>c</sup>	8.49±0.11 <sup>ab</sup>	8.25±0.05 <sup>b</sup>	p<0.01
Mn metabolizability	26.73±0.21 <sup>a</sup>	16.96±0.23 <sup>b</sup>	19.78±0.23 <sup>b</sup>	17.52±0.12 <sup>b</sup>	p<0.01
Zn intake (mg)	11.52±0.14 <sup>a</sup>	10.37±0.08 <sup>c</sup>	11.19±0.14 <sup>ab</sup>	10.88±0.06 <sup>b</sup>	p<0.01
Zn metabolizability	30.04±0.37 <sup>a</sup>	23.25±0.32 <sup>c</sup>	28.69±0.23 <sup>bc</sup>	28.05±0.57 <sup>b</sup>	p<0.01

Similar alphabets at superscript denote homogenous means due to Duncan's test at 5% level of significance

metabolizability. Reduction of Ca retention might show impairment of such physiological function as nerve conduction, contraction and relaxation and bone mineralization leading to leg weakness (Whitehead, 1995). Important of Ca metabolism in laying hen might reduce egg production and egg quality as confirmed in the present study, because in poultry, Ca performs in unique function of protecting the egg through the deposition of an egg shell during passage down the oviduct. Reduced retention of P might be due to the fact that of reduction of Ca increased opportunities for Ca to unabsorbed phytate in the gut or reduction of Ca retention might be accompanied by excretion of P with increasing renal 1  $\alpha$ -hydroxylase and plasma 1, 25 (OH)<sub>2</sub> D<sub>3</sub> (Littledike and Goff, 1987). Deprivation of P manifested the loss of appetite subnormal growth and weight loss as observed in the present investigation. It is accompanied by reduction of egg production, reduced feed intake as confirmed in the present study, but it is much likely to occur than Ca deficiency, because of the smaller requirements for P. Reduction of Fe availability in non supplemented group might be due to the fact sodium arsenite formed as unabsorbable complex with Fe and excreted through faces (Banerjee *et al.*, 2000). Fe deprivation might cause impairment of haemoglobin formation leading anaemia, loss of appetite, poor growth, lethargy as confirmed in the present investigation and it might also cause the disturbance of energy metabolism via glycolysis. Uthus (2001) reported that high dietary arsenic (50  $\mu$ g As g<sup>-1</sup>) exacerbated the Cu deficiency in rats but the mechanism of Cu deficiency by high dietary As was unknown. Since Fe promotes free radical generation and catalase protects tissue from H<sub>2</sub>O<sub>2</sub> and hydroperoxide damage, this is potentially a two edged pathogenic sword (Golden and Ramdath, 1987). Thus Cu deficiency might aggravate free radical formation and reduction of catalase activity resulting increased tissue damage leading to poor growth and overall performance as observed in the present investigation. As mediated Zn deprivation might cause loss of appetite leading to reduced growth (Droke *et al.*, 1993). Mn deficiency lowered Mn-SOD activity in the heart and lung and increased the per-oxidative cell damage might lead to reduced feed intake and growth as found in the present investigation. As mediated Mn deficiency might be one of the causes of reduced egg production in the present investigation. The arsenic intoxicated group (T<sub>1</sub>) showed less utilization of both macro and micro minerals than other groups. This might be due to direct or indirect toxic effect of arsenic, also due to pathological changes in different organs which is accompanied with biochemical and haematological changes, physiological disorder. But other treatment groups utilized minerals in better way than T<sub>1</sub> group. Because excess supplementation of methionine or methionine-betaine involved in methylation procedure to detoxify sodium arsenite. Correlation between trace mineral retention and intake was in close agreement with the reports of Chesters (1997), Yi *et al.* (1996) and Thompson (1993).

## CONCLUSIONS

It might be concluded that due to chronic arsenic toxicity, less amounts of dry matter was utilized for body growth, body function, metabolic function and heat production leading to reduce performance in non supplemented treatments and vice versa in control and supplemented treatments of birds indicated role of methionine and betaine in mitigation of chronic arsenic toxicity.

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