Indian Journal of Animal Sciences **78** (3): 317–321, March 2008

Change in minerals bioavailability in chronic arsenic toxicity with or without methionine and betaine supplementation in layer chicken

GOBINDA HALDER[1], SWAPNENDU MONDAL[2], BARUN ROY[3] and GAUTAM SAMANTA[4]

West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal 700 037 India

Received: 20 April 2007; Accepted: 7 August 2007

ABSTRACT

An experiment was conducted to find out effect of chronic toxicity of sodium arsenite with or without extra-supplementation of methionine and methionine-betaine combination on bioavailability of minerals in Rhode Island Red (RIR) layer chicken. RIR 16-week-old pullets (120) were randomly distributed in 4 experimental groups, each having 3 replicates with 10 birds (9 hens, 1 cock). The experimental groups were control (C)–provided basal diet to meet all the nutrients requirement as per BIS, treatment 1 (T_1)–birds were offered 5.5 ppm arsenic (As) through water; treatment 2 (T_2)–birds were offered 5.5 ppm As through water with 50g methionine through feed; and treatment 3 (T_3)–offered 5.5 ppm As through water and 25 g methionine + 25 g betaine through feed. The birds were maintained replicate wise in deep litter system of housing with a common system of management. Bioavailability of different minerals were significantly reduced in arsenic treated group (T_1) where no extra methionine or methionine + betaine combination were supplemented from external source. But in most cases bioavailability of minerals was enhanced in T_2 and T_3 groups in which methionine and methionine- betaine combination were supplemented respectively in their feed.

Key words: Arsenic, Layer chicken, Methionine, Methionine+betaine, Mineral bioavailability

Arsenic toxicity has got great importance particularly in India, due to its residual effect which may be associated with the public health hazards via contaminated animal products (Singh *et al.* 2005). Chronic ingestion of As through drinking water may produce toxicity in the birds, which not only reduce the bioavailability of minerals by increasing excretion rate of minerals, reducing the intake of dietary minerals as well as due to its residual concentration in animal tissue and excretion through animal products (egg, meat etc.) may cause threat to human health (NRC 2001). So far there is no available medicine for chronic arsenic toxicity; safe water, nutritious food (free from arsenic contamination), nutritional manipulation and physical exercise are the only preventive measures to fight chronic arsenic toxicity. 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 spots side by side, buccal mucus membraemelanosis, rough and dry skin, often with palpable nodules (Mazumder *et al.* 1998). To reduce the effect of arsenic toxicity in T_2 and T_3 group methionine or methionine-betaine is incorporated in their respective ration as methionine and betaine both increase the rate of detoxification of arsenic (Vaheter and Marafante 1987, Sardar 2005). Methylation of arsenic is the first defense of arsenic toxicity. To enhance the methylation activity thereby, to improve the bioavailability of minerals extra methionine or betaine is supplemented in feed as both are the active methyl donor and required for methylation process. Therefore, present study was envisaged to investigate the arsenic detoxification capability of methionine and betaine to increase bioavailability of minerals.

MATERIALS AND METHODS

Pullets (120), 16-week-old of strain Rhode Island Red were randomly distributed in 4 experimental groups, each having three 3 replicates with 10 pullets (9 hens and 1 cock) each. The experimental groups were control (C)–provided basal diet without methionine and betaine supplementation; treatment 1 (T_1)–birds were offered 5.5 ppm arsenic through water; treatment 2 (T_2)–birds were offered 5.5 ppm arsenic through water with 50 g methionine in feed; and treatment 3 (T_3)–offered 5.5 ppm As through water and 25 g methionine + 25 g betaine through feed. The feed and samples were analyzed for proximate components (AOAC 1995).

Present address: [1]Ph.D student, [3]Reader, [4]Professor, Department of Animal Nutrition.

[2]Veterinary Officer, Daspur II, Medinipur (P), Government of West Bengal, Medinipar, West Bengal.

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

Ingredient	Grower ration	Layer ration	Chemical composition,% DM basis	Grower ration	Layer ration
Maize	42.00	48.68	Crude protein (%) ++	16.20	18.33
Deoiled rice bran	36.75	16.87	Crude fibre (%)++	6.92	4.99
Soybean meal	10.32	17.51	Ether extract (%)++	1.51	2.55
Til-cake	2.87	5.42	Nitrogen free extract (%)++	65.95	62.82
Fish meal	3.77	2.74	Total ash (%)++	8.82	11.46
Vegetable oil	-	0.50	ME (Kcal/kg) (calculated)	2572	2622
Lime stone powder	1.00	1.00	Cacium% ++	1.85	3.25
Di-calcium phosphate	1.00	1.00	Available Phosphorus%++	0.68	0.57
Oyster shell	2.00	6.00	Zinc (mg/kg)++	40.65	35.74
Nicomix ¯	0.025	0.025	Manganese (mg/kg)++	47.98	29.03
Methionine	-	-	Methionine (calculated)	0.3	0.4
Choline chloride	0.160	0.15	Lysine (calculated)	0.7	0.82
Trace mineral mixture+	0.075	0.075			
Common salt	0.03	0.03			

¯ Each gram of nicomix contained vitamin A–40,000 IU, vitamin D_3–6,000 IU, vitamin B_1–3.2 mg, vitamin B_2–20 mg, vitamin B_6–6.4 mg, vitamin B_{12}–82 mcg, niacin–48 mg, calcium pantothenate–32 mg, vitamin K- 4 mg, vitamin E–32 mg and folic acid–3.2 mg.
+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. ++Estimated value.

Housing, management and experimental diets

The experiment was conducted in the departmental layer shed, in deep litter system. Three days prior to arrival of the pullets, the layer shed was thoroughly cleaned with soap water, disinfected by the use of $KMnO_4$ solution, and fumigated with the formaldehyde solution. The feeding and watering troughs were properly cleaned and disinfected. The deep litter was prepared with sun-dried sawdust, rice husk and chopped rice-straw. Lime and copper sulphate (6 kg and 1 kg copper sulphate for 1000 square feet) were added to the litter for disinfection. Birds were exposed to 18 h light and 6 h darkness throughout the period of the experiment. All the birds of control group were offered water *ad lib.* by clean water troughs 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 above solution. The water troughs and feeder were cleaned every day. The birds were fed as per the feeding standards for layer chicken (BIS 1992). As per the objective of the study, the control diet (Table 1) of respective ration as such used for T_1 group. Diet of T_2 group were prepared by adding 50 g methionine/100kg of feed as in control diet of respective group. Diet for T_3 group birds were prepared by adding 25 g methionine/ 100kg of feed + 25 g betaine/100kg of feed.

Blood collection

The collection of blood from the birds was done at the end of 26th week of the trial. The birds were bled to collect sufficient amount of blood in heparinised (200 IU/ml concentration) proper sterilized and labelled tubes. The blood was centrifuged at 2500 rpm for 20 min to separate plasma from red blood cells. Supernatants were collected in sterilized vials and stored in deep freeze at–20 °C for subsequent analysis.

Estimation of plasma minerals

Plasma calcium and plasma trace minerals (Zn, Mn, Fe and Cu) were estimated as per method Sandel (1950) and as modified by Arenza *et al.* (1977) using atomic absorption spectrophotometer (AAS). Plasma (1ml) was diluted with 40 ml and 10 ml of triple distilled water respectively for Ca and trace minerals estimation and was transferred to separate sterilized plastic vial, labelled properly and kept for subsequent analysis with the help of AAS by using specific lamp and standard. Plasma phosphorus was estimated by the method as described by Gowenlock *et al.* (2002) in Varley's Practical Clinical Biochemistry (2002) with the help of U V visible spectrophotometer.

Collection of tissues and bones

Two birds were sacrificed from each replicate at the end (26th week) of the trial. Liver, thigh muscle, breast muscle, kidney and femur were collected and preserved in closely packed plastic zipper bags well labelled and kept in–20°C for further analysis.

Mineral estimation of bone

Bones were boiled for approximately 10 min in distilled water, cleaned of all soft tissue, dried for 12 h at 105 °C, and extracted in a Soxhlet apparatus with petroleum ether for 48 h before drying. After drying in hot air oven it was properly weighed and ashed. It was subject to acid digestion by 50% HCl. After boiling with 50% HCl and cooling, it was left to precipitate for 1h. Then repeated washing with deionised triple distilled water, the solution was filtered. The final volume was made up to 50 ml and the sample was preserved in pre-labelled plastic vials till the estimation by AAS.

Estimation of liver, kidney and muscle minerals

Liver, kidney and muscle were also digested by use of tri-acid (concentrated nitric acid, concentrated sulphuric acid and concentrated perchloric acid at the ratio of 9:2:1). 1 g of the dried sample (dried in hot air over at 100 ± 2°C) of liver, kidney and muscle were digested with 20 ml of tri- acid mixture in hot plate at 180°–200°C. Then the solution was filtered through Whatman filter paper no. 42 and volume was made up to 50 ml. This aliquot was then used to determine the concentration of trace mineral with the help of AAS.

The data were subjected to statistical analysis for drawing inferences as per the standard method given by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Plasma, liver, kidney, muscle and bone minerals are presented in Table 2.

Bioavailability of plasma minerals

Calcium and phosphorus (mg/dl): The levels of plasma calcium (Ca) and phosphorus (P) varied significantly (P<0.01) among the experimental groups due to dietary treatments. This finding was in disagreement with the result of Hoffman *et al.* (1992). They observed slightly higher value in arsenic treatment group than the value found in control group. The least level of both Ca and P was found in T_1 group. This level increased in T_2 and T_3 groups and comparable to the control group. As plasma calcium level was reduced in arsenic intoxicated group (T_1), plasma phosphorus level should be increased. But it was not found in present study. The decrease of plasma P concentration due to chronic arsenic toxicosis in layer chicken might break possibility of reciprocal relationship with plasma Ca indicating impairment of normal level of plasma Ca: P ratio caused the P and Ca deprivation adversely, but improved due to addition of methionine and betaine. Arsenic mediated plasma P deprivation might be due to the formation of unabsorable Ca phytate, which might also reduce the Ca availability as observed in the present investigation. Laying hens normally have higher and more variable Serum Ca values than non-laying chicken (9–12 mg/dl) due to the presence of Ca-lipophosphoprotein, viellogenin, a precursor of egg yolk proteins (Hurwitz 1996)

Copper and iron (mg/ml): Significant variation (P<0.01) in plasma Cu was noticed among the groups showing the least level in T_1 group. But no significant (P>0.05) difference was found among control, T_2 and T_3 groups. So, Arsenic intoxicated group (T_1) showed marked depletion of plasma Cu level. This finding is in agreement with the finding of Uthus (2001) who reported that high dietary arsenic caused a marked deprivation of copper in rats. The mechanism for the apparent exacerbation of copper deficiency by arsenic is not known. Statistical analysis confirmed that T_1 group showed significantly lower level of plasma iron (P<0.01) than other experimental groups which might be due to toxic effects of arsenic that can be overcome by addition of methionine or betaine in the feed as higher bioavailability of Fe in plasma was found in T_2 and T_3 group.

Table 2. Effect of chronic arsenicosis with or without extra supplementation of methionine and methionine-betaine combination on mineral concentrations of plasma, liver, kidney, muscle and bones

Parameter	Control	T_1	T_2	T_3	SEM	P Value
Plasma mineral concentration						
Ca mg/dl	23.57^a	17.77^c	21.25^b	20.68^b	0.18	**
P mg/dl	9.75^a	7.68^b	9.67^a	9.68^a	0.10	**
Cu µg/dl	0.46^a	0.26^c	0.42^a	0.36^b	0.01	**
Zn µg/dl	2.79^a	2.21^b	3.04^a	2.99^a	0.05	*
Mn µg/dl	4.82^a	4.05^b	4.68^a	4.54^a	0.04	*
Fe µg/dl	3.46^a	2.16^c	3.41^a	2.87^b	0.03	**
Liver mineral concentrations						
Ca g%	0.03	0.03	0.03	0.024	0.00	NS
P g%	0.01	0.01	0.01	0.01	0.00	NS
Cu mg/kgDM	6.25^a	5.05^b	6.34^a	6.26^a	0.09	*
Zn mg/kgDM	36.13^a	29.59^b	35.60^a	34.33^a	0.31	*
Mn mg/kgDM	7.35^a	6.02b	6.87^a	6.72^a	0.08	**
Fe mg/kgDM	417.55^a	354.87^b	406.51^a	396.45^a	1.84	**
Kidney mineral concentrations						
Ca g%	0.06	0.06	0.06	0.06	0.00	NS
P g%	0.03	0.03	0.03	0.030	0.00	NS
Cu mg/kgDM	12.51^c	19.24^a	13.97^{bc}	15.38^b	0.17	**
Zn mg/kgDM	25.20^a	21.65^b	24.33^a	23.83^{ab}	0.34	*
Mn mg/kgDM	23.91^a	20.58^b	23.34^a	23.00^a	0.25	**
Fe mg/kgDM	235.50^a	208.25^b	237.95^a	231.92^a	1.42	**
Muscle mineral concentrations						
Ca g%	0.07	0.06	0.07	0.06	0.00	NS
P g%	0.03	0.03	0.03	0.03	0.00	NS
Cu mg/kgDM	5.32	5.70	5.25	5.11	0.06	NS
Zn mg/kgDM	42.71^a	35.96^b	42.08^a	40.58^a	0.67	*
Mn mg/kgDM	3.53^a	3.00^b	3.44^a	3.36^a	0.05	*
Fe mg/kgDM	122.97^a	108.59^b	120.53^a	117.12^a	1.28	*
Bone mineral concentration						
Ca%	28.28^a	24.14^b	27.70^a	27.19^a	0.19	**
P%	13.14^a	11.38^b	12.85^a	12.59^a	0.17	*
Cu mg/kgDM	10.80^a	8.81^c	10.10^b	9.95^b	0.09	**
Zn mg/kgDM	116.71^a	98.85^b	114.50^a	110.99^a	1.81	*
Mn mg/kgDM	6.83^a	5.89^b	6.63^b	6.51^b	0.09	*
Fe mg/kgDM	52.71^a	42.65^c	50.98^{ab}	47.42^b	0.65	**

Different alphabets at superscript differ significantly at P<0.05 or P<0.01, **P<0.01; *P<0.05.

Zinc and manganese (mg/ml): Plasma zinc (Zn) and manganese (Mn) level in T_1 group showed significantly (P<0.05) lower value than control, T_2 and T_3 groups. The highest plasma Zn level was observed in T_2 group, whereas the highest plasma Mn was observed in control group followed by T_2, T_3 and T_1 group. Chronic ingestion of arsenic to birds of T_1 group caused significant depletion of plasma Zn and Mn level. But no significant decrease in plasma Zn

and Mn level was observed between T_2 and T_3 groups, as methionine and betaine increased methylation procedure needed for arsenic detoxification.

Bioavailability of liver minerals

Calcium, phosphorus (g%), copper, iron, zinc and manganese (mg/kg DM): Ca and P level in liver was not differed significantly among different experimental groups. The lowest Ca value was observed in T_3 group followed by T_1, T_2 and Control groups, but non-significantly the lowest P value was found in T_1 group followed by T_3, T_2 and control.

Statistical analysis confirmed that T_1 group showed significantly lower Cu level ($P<0.01$) and Fe level ($P<0.05$) in liver than any other experimental groups. But Cu and Fe level obtained in T_2 and T_3 groups showed almost similar value, which is comparable to control. Descending trend of copper level obtained in different groups in the following manner $T_2 > T_3 >$ Control $> T_1$.

Statistical analysis revealed that T_1 group showed the lowest Zn level in liver followed by T_3, T_2 and control groups. The Zn level of liver was 36.13, 29.59, 35.60 and 34.33 mg/kg DM in C, T_1, T_2 and T_3 groups, respectively. Above results revealed that arsenic caused significantly ($P<0.05$) marked depletion of liver zinc than either control group or T_2 or T_3 group. The level of manganese in liver also differed significantly ($P<0.01$) among different groups due to dietary treatments.

Bioavailability of kidney minerals

Calcium, phosphorus (g%), copper, iron, zinc and manganese (mg/kg DM): Both Ca and P levels in kidney remained similar ($P>0.05$) amongst treatment groups. Statistical analysis revealed that kidney Cu level varied significantly ($P<0.01$) among the groups. The highest copper level was found in T_1 (19.24 mg/kg DM), followed by T_3 (15.38 mg/kg DM), T_2 (13.97 mg/kg DM) and C (12.51 mg/kg DM) groups. This result was corroborated with the findings of Uthus (2001), who reported that high dietary arsenic caused a marked accumulation of copper in the kidney of rats. The mechanism for the apparent exacerbation of copper deficiency by high arsenic level is yet to be known. From statistical analysis it was found that T_2 group showed the lowest iron level amongst all experimental groups. Significant ($P<0.01$) variation was observed in Fe level among the experimental groups due to dietary treatments. The statistical analysis confirmed that T_1 group showed significantly ($P<0.05$) lower Zn level followed by T_3, T_2 and control groups. The mechanism for the apparent exacerbation of Zn in kidney due to As toxicity is yet to be known. Kidney Mn concentration differed significantly ($P<0.01$) among the different groups showing the same trend as Zn concentration in kidney.

Bioavailability of minerals in muscle

Calcium, phosphorus (g%), copper, iron, zinc and manganese (mg/kg DM): Both muscle Ca and P level did not vary significant among the experimental groups. It indicates that arsenic have little effect on muscle Ca and P level. But the lowest Ca and P value was found in T_1 group. Statistical analysis also revealed that there was no significant variation regarding muscle Cu level among the experimental groups. In this study, the highest copper level was found in T_1 group followed by Control, T_2 and T_3 groups. The significant ($P<0.05$) reduction in muscle Zn, Mn and Fe levels were observed in T_1 group than T_3, T_2 and control groups. From the above result it was clear that administration of arsenic reduced the bioavailability of Zn, Mn and Fe in muscle which was increased due to supplementation of methionine and betaine.

Bone calcium, phosphorus (g%), copper, iron, zinc and manganese (mg/kg DM): Both bone calcium ($P<0.01$) and phosphorus ($P<0.05$) showed significant variation among the experimental groups. The lowest value was observed in T_1 group than T_2, T_3 and control groups. Statistical analysis revealed that T_1 group showed significantly ($P<0.01$) lower level of bone copper than any other experiment groups. The highest level of bone copper was seen in control group followed by T_2, T_3 and T_1 groups. Significant difference also found between T_1 and T_2 or T_3 group. But no significant difference was found between T_2 and T_3. Statistical analysis revealed that the bone iron level was differed significantly ($P<0.01$) among the different groups showing the lowest value in T_1 than T_3, T_2 and control. Methionine or betaine supplementation did not improve bone iron level as compared to control value. The bone Zn and Mn level in T_1 group was significantly ($P<0.05$) lower value than other experimental groups. The highest value was in control group, followed by T_2, T_3 and T_1 groups. Supplementation of methionone or methionine- betaine combination elevated the level of Zn which was comparable to the level of control. Supplementation of methionone or methionine- betaine combination did not improve the level of Mn and remained similar to diet T_1.

It may be concluded that chronic toxicity of sodium arsenite reduce bioavailability of Ca, P, Cu, Fe, Zn and Mn in various parts of body of RIR layer chicken. This reduced level of minerals can be improved with supplementation of either methionine or methionine betaine combination in layer chicken. However, methionine supplemented group showed better bioavailability of minerals than methionine-betaine combination. Therefore, supplementation of methionine would be desirable to counteract the toxicity caused by high arsenic in diet.

REFERENCES

AOAC. 1995. *Official Methods of Analysis.* 16th edn. Association of Official Analytical Chemists. Washington DC.

Arenza J S, Holbi D S, Singh B and Verman P N. 1977. Status of some macro-and micro-minerals in neonatal buffalo calves and

their mothers. *Indian Journal Dairy Science* **30**: 255–57.

BIS. 1992. Bureau of Indian Standard. *Nutrient Requirements for Poultry*. IS: 13574:1992.

Gowenlock A H, McMurray J R and Mclauchlan D M. 2002. *Varley's Practical Clinical Biochemistry*. 6th edn. CBS Publishers and Distributors, New Delhi, India.

Hurwitz S. 1996. Homeostatic control of plasma calcium concentration. *Critical Review of Biochemistry and Molecular Biology* **31**: 41–100.

Mazumder D N G, Haque R, Ghosh N, De B K, Santra A, Chakraborty D and Smith A H. 1998. Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India. *International Journal of Epidemiology* **27**: 871–77.

NRC. 2001. *Arsenic in Drinking Water*. Board of Environmental Studies and Toxicology, Subcommittee on Arsenic in Drinking Water, National Academy Press, Washington, D.C.

Sardar P. 2005. 'Chronic arsenicosis in chicken and common carp (*Cyprinus carpio* L.) and its remedial measures through nutritional manipulation.' Ph. D. thesis, West Bengal University of Animal and Fishery Sciences, Kolkata, India.

Sandel E E. 1950. *Colorimetric Determination of Traces of Metals*. Interscience Publishers, INC, New York.

Singh R P, Saha R P and Misra R K. 2005. *Arsenic profile in livestock, livestock feed and livestock product in West Bnegal*. National Dairy Research Institute, Eastern Regional Station, Nadia, West Bengal, India.

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 6th edn. Oxford and IBH Publications, New Delhi.

Uthus E O. 2001. High dietary arsenic exacerbates copper deprivation in rats. *Journal of Trace-Elements in Experimental Medicine* **14**(1): 43–55.

Vaheter M and Marafante E. 1987. Effect of low dietary intake of methionine, choline or proteins on the biotransformation of arsenite in the rabbit. *Toxicological Letter* **37**: 41–46.