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Elimination of Arsenic Toxicity in Some Tissues and Organs by Supplementing Methionine and Methionine-Betaine in Laying Hens

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Abstract: An experiment was conducted to find out the effect of excess methionine and methionine-betaine supplementation on deposition of arsenic in different tissues, organs and eggs of hen and contribution of hen egg and meat to human health hazard along with the arsenic contaminated drinking water. One hundred twenty Rhode Island Red, 16 week-old, were allocated into four groups having three replicates with nine hens and one cock in each replicate. The experimental groups were C (control group fed with basal diet only), T_1 (fed control diet with 5.5 ppm arsenic through water), T_2 (fed control diet with 5.5 ppm arsenic through water + 50 g methionine per 100 kg of feed) and T_3 (fed same as T_2 but 50% of the excess methionine supplement was replaced with betaine). The birds were maintained in deep litter system of housing. Statistical analysis confirmed that the concentration of arsenic in different organs, tissues and eggs were varied significantly ($p<0.01$) due to dietary treatments. The long term intake of such arsenic contaminated hen eggs and meat by human being may act as some contributory factor to suffer from the serious health hazard. Statistical analysis confirmed that the concentration of arsenic (As) in various organs, tissues and eggs was significantly ($p<0.01$) reduced due to supplementation of methionine and methionine-betaine in T_2 and T_3 groups, respectively as significantly ($p<0.01$) larger amount of arsenic was voided through faeces in T_2 and T_3 groups than T_1 group where no excess methionine or betaine was added in diet. So it may be concluded that supplementation of either methionine or methionine-betaine combination may able to protect the chronic arsenic toxicity during exposure of As in laying hen.

Key words: Arsenic deposition, methionine, methionine+betaine, laying hen, health hazard

INTRODUCTION

Arsenic (As) is the word derived from the Greek word arsenicon means fearless. Toxic elements such as selenium, arsenic, cadmium, chromium, lead, molybdenum, fluorine etc. may adversely affect the health and performance of birds. Among important mineral toxicity faced by the birds, arsenic toxicity has got great importance, due to the residual effect of these minerals which may be associated with the public health hazards via contaminated animal products. Supplementation of methionine, protein and methyl donor in feed give protection against arsenic toxicity in animals (Buchet and Lauwerys, 1985; Vaheter and Marafante, 1987). At present, more than 130 million people (50 million from West Bengal and 80 million from Bangladesh) are at risk of arsenic contamination (Chowdhury *et al*., 2001) due to drinking of water contaminated with As which is above the recommended safe limit (0.01 mg As L^{-1}) (WHO, 1996). Chronic ingestion of As through drinking water may produce toxicity to the birds, thereby hamper the performance of bird. Degree of As

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toxicity depends on methylating capability of liver and kidney. For methylation of As methyl donor is needed (Vaheter and Marafante, 1987). Methionine and betaine both are active methyl donor. Methylation of arsenic is the first defense against arsenic toxicity. In this study, to enhance the methylation activity of liver and kidney methionine or betaine is supplemented in feed. Therefore, present study was done investigate the arsenic detoxification capability of methionine and betaine by estimation of As concentration in different organs and tissues.

MATERIALS AND METHODS

Experimental Design

One hundred and twenty Rhode Island Red (RIR) pullets aged 16 weeks were randomly distributed in four experimental groups, each having three replicates with 10 pullets (9 hens and 1 cock). The experimental groups were control (C)-provided basal diet, treatment 1 (T_1)-birds were offered 5.5 ppm arsenic (As) through water, treatment 2 (T_2)-birds were offered diet with excess 50 g methionine/100 kg of feed along 5.5 ppm As through water and treatment 3 (T_3)-offered diet with excess 25 g methionine and 25 g betaine/100 kg of feed along with 5.5 ppm As through.

Housing and Management

The experiment was conducted in the departmental layer shed, in deep litter system for six months of period. Three days prior to arrival of the pullets, the layer shed was thoroughly cleaned with soap water, fumigated by the use of potassium permanganate ($KMnO_4$) and formaldehyde solution (20 g $KMnO_4$ + 40 mL formaldehyde solution per 100 square feet). 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 sulfate (6 kg lime + 1 kg copper sulphate for 100 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* in clean water troughs every day, but prior to giving *ad lib* water to the treatment groups, As solution (1000 mg L^{-1}) was offered to the birds so that they could be able to drink the above solution. 1/20th of LD_{50} (Balatskii, 1989) value i.e., 5.5 ppm was selected in this study to produce chronic toxicity in layer. The water troughs 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. Diets of T_2 group were prepared by adding 50 g methionine per 100 kg of feed through replacing 50 g Lime Stone Powder (LSP) in control diets of respective group. Diets for T_3 group of grower or layer birds were prepared by adding 25 g methionine and 25 g betaine/100 kg feed by replacing 50 g LSP/100 kg of control diets.

Dietary Ingredients of Feeds

The dietary ingredients were in Table 1. Proximate principles were analyzed according to AOAC (1995).

Total Arsenic Estimation

Collection of Samples

At the time of metabolism trial, droppings voided daily by each replicate of birds during the preceding 24 h were collected randomly from all the portions and kept in clean container. Droppings so for collected were thoroughly mixed and representative sample for each replicate was stored in clean plastic zipper bags for further analysis.

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	Crude protein (%)$^{++}$	16.20	18.33
De-oiled 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	3.74	2.69	Total ash (%)$^{++}$	8.82	11.46
Vegetable oil	-	0.50	ME (Kcal kg^{-1})$^{+++}$	2572.00	2622.00
Lime stone powder	1.00	1.00	Calcium (%)++	1.85	3.25
Di calcium phosphate	1.00	1.00	Available phosphorus (%)$^{++}$	0.68	0.57
Oyster shell	2.00	6.00	Methionine^{+++}	0.46	0.48
Nicomix^{-}	0.025	0.025	Lysine^{+++}	0.90	1.00
Methionine	0.05	0.05	Methionine+ cystine^{+++}	0.62	0.66
Choline chloride	0.14	0.15			
Trace mineral mixture+	0.075	0.075			
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; $^{+++}$: Calculated value

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^{-1} concentration), sterilized and labelled tubes. The blood was centrifuged at 2500 rpm for 20 min. in order to separate plasma from red blood cells. Supernatants were collected in sterilized vials and stored in deep freeze at -20°C for subsequent analysis.

Care was taken to avoid contamination during sample collection. Different organs such as liver, kidney, lungs, spleen, heart, skin, proventriculus, gizzard, ovary, intestine and brain, various tissues like thigh and breast muscle, feather, claw, blood, fat and bone, faeces and eggs were collected to estimate total arsenic concentration. The feed and water offered to the birds also subjected to arsenic estimation.

Pre Treatment of the Samples

Before digestion, feather and claw samples were washed with triple distilled deionized water and finally washed with acetone as recommended by the International Atomic Energy Agency. Then it was dried in a hot air oven at 50-60°C temperature for 12 h. Care was taken to avoid contamination during egg collection and eggs were collected to estimate total arsenic concentration.

Procedure

Arsenic in water was generally analyzed without oxidative treatment. Biological samples to be analyzed for total arsenic were generally completely oxidized by digestion according to AOAC (1980). Out of the numerous methods employed for total arsenic estimation, here Gutzeit's or silver diethyldithiocarbamate complex (SDDC) method was adopted for estimation of total arsenic (Vasak and Sendivce, 1952; AOAC, 1995). As was estimated in whole blood as well as plasma by the method described above.

Statistical Analysis

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 test (Duncan, 1995) whenever any effect was found significant.

Table 2: Arsenic concentrations (ppm) in different organs of RIR hen

Groups	C	T_1	T_2	T_3
Liver	0.075±0.005^{c}	1.654±0.047^{a}	0.337±0.016^{b}	0.414±0.025^{b}
Kidney	0.061±0.005^{c}	0.666±0.058^{a}	0.231±0.010^{b}	0.305±0.009^{b}
Spleen	0.093±0.009^{b}	0.218±0.009^{a}	0.112±0.021^{b}	0.125±0.021^{b}
Ovary	0.014±0.001^{c}	0.348±0.014^{a}	0.080±0.008^{b}	0.097±0.007^{b}
Intestine	0.027±0.004^{c}	0.501±0.075^{a}	0.105±0.004^{c}	0.238±0.013^{b}
Bursa	0.018±0.001^{c}	0.247±0.016^{a}	0.060±0.004^{b}	0.074±0.003^{b}
Brain	0.004±0.001^{d}	0.019±0.001^{a}	0.007±0.000^{c}	0.011±0.001^{b}
Skin	0.097±0.006^{d}	1.818±0.039^{a}	0.266±0.028^{c}	0.427±0.030^{b}
Heart	0.050±0.004^{b}	0.697±0.029^{a}	0.080±0.010^{b}	0.092±0.003^{b}
Lung	0.044±0.007^{c}	1.061±0.087^{a}	0.074±0.003^{bc}	0.200±0.018^{b}
Proventriculus	0.049±0.013^{d}	0.782±0.009^{a}	0.221±0.005^{b}	0.105±0.003^{c}
Gizzard	0.053±0.002^{b}	0.406±0.026^{a}	0.067±0.004^{b}	0.074±0.002^{b}

Similar alphabets at superscript denote homogenous means due to Duncan's test at 1% level of significance, Values are shown in Mean±SE

Table 3: Arsenic concentrations (ppm) in different tissues of RIR hen

Groups	C	T_1	T_2	T_3
Thigh muscle	0.047±0.001^{c}	0.492±0.036^{a}	0.083±0.008^{c}	0.183±0.037^{b}
Breast muscle	0.019±0.001^{c}	0.228±0.012^{a}	0.035±0.003^{c}	0.076±0.012^{b}
Feather	0.238 0.012^{c}	2.553±0.112^{a}	0.488±0.010^{b}	0.331±0.013^{bc}
Claw	0.203±0.004^{c}	3.293±0.190^{a}	0.389±0.006^{b}	0.682±0.010^{bc}
Blood	0.034±0.006^{c}	0.109±0.014^{a}	0.057±0.011^{bc}	0.078±0.006^{ab}
Plasma	0.017±0.003^{c}	0.053±0.007^{a}	0.029±0.005^{bc}	0.040±0.003^{ab}
Blood cell	0.051±0.009^{c}	0.167±0.021^{a}	0.088±0.016^{bc}	0.121±0.009^{ab}
Fat	0.008±0.002^{b}	0.045±0.005^{a}	0.014±0.001^{b}	0.010±0.001^{b}
Meat	0.046±0.003^{d}	0.391±0.014^{a}	0.107±0.031^{c}	0.165±0.007^{b}
Bone	0.090±0.004^{b}	1.193±0.092^{a}	0.165±0.005^{b}	0.217±0.025^{b}

Similar alphabets at superscript denote homogenous means due to Duncan's test at 1% level of significance, Values are shown in Mean±SE

RESULTS AND DISCUSSION

Deposition of Arsenic in Different Organs and Tissues

Statistical analysis revealed that arsenic concentration of organs showed significant variation ($p<0.01$) among different groups. The arsenic concentration in T_1 group was highest in skin followed by liver, lung, proventriculus, heart, kidney, intestine, ovary, gizzard, bursa, spleen and brain (Table 2).

Statistical analysis revealed that arsenic concentration of tissues (Table 3) varied significantly ($p<0.01$) among the various groups but As concentration in plasma differed significantly ($p<0.05$) at 5% level. The highest concentration was found in claw, followed by feather, meat, bone, thigh muscle and breast muscle. It was observed that among edible parts highest concentration of arsenic was found in skin and liver, which was also observed by Proudfoot *et al.* (1991). Numerous studies revealed that skin, hair and tissues high in squamous epithelium have a strong tendency to accumulate and maintain higher levels of total arsenic (Hulinger *et al.*, 1998). This is apparently a function of the binding of inorganic arsenic to sulfhydryl rich keratin in these tissues. Auto radiographic studies have also revealed a tendency of arsenic to accumulate in epidydimis of testis and lens of eye of mice (Lindgren *et al.*, 1982). In birds arsenic was eliminated through droppings and eggs.

It was found that thigh muscle contained higher values of arsenic than that of breast muscle possibly may be due to the more blood circulation to thigh muscle which caused more deposition of arsenic in that part of the body. Proudfoot *et al.* (1991) also found a significant differences between thigh and breast muscle and reported that 60% or more lower As accumulation in breast muscle than thigh muscle may be due to the higher activity of later one.

Among the different compounds of blood, plasma showed lowest values, whereas blood cells contained highest value of arsenic and its intermediate value was found in whole blood. This observation indicated that blood cell has a tendency to accumulate more arsenic. The present finding

supported the report of WHO (1981), where it was shown that the whole blood contained arsenic level two times than plasma level and red cells contained three times of more arsenic than the plasma. Inorganic arsenic is rapidly cleared from the blood in most animals showing lower concentration in blood during chronic exposure as observed in the present study (Yamauchi and Yamamura, 1985). Less deposition of total arsenic in RBC may be another factor of lower concentration of total As in blood of birds. It was found that brain contained lower concentration of arsenic may be due to blood brain barrier which could not prevent the entry of arsenic completely but made its rate of entry slower (Lindgren *et al*., 1982).

Feather, claw and skin showed to contain higher values of arsenic than that of other tissues may be because of these body parts contained more protein, rich in sulfhydryl group and due to higher affinity of arsenic to sulfhydryl group, more amount of arsenic could be deposited in those parts of the body (Vaheter and Marafante, 1983).

Arsenic concentration (mg kg^{-1}) in layer meat was found to be 0.046, 0.391, 0.107 and 0.165 in C, T_1, T_2 and T_3 groups, respectively. Though the value was lower than Indian maximum permissible limit (MFPO-Amended in 1992) but in long term consumption of such contaminated layer meat may play a significant contributory role along with the contaminated drinking water to cause ailments of human health.

Arsenic Concentration in Egg and its Different Components

Table 4 has been represented the arsenic contents in different components of hen egg. The results indicated that yolk contained highest total arsenic concentration followed by whole egg, albumen and eggshell. The values between whole egg and albumen were not statistically different. All the components of egg from intoxicated birds contained higher value ($p<0.01$) than control bird. The ratio of arsenic content in yolk and albumen was found to be almost 3:1. This finding was in agreement with the finding of Holeman and Stibilij (1997). Increased total arsenic residues in egg with non-supplemented treatments during present investigation resembled the observation of Chiou *et al*. (1997). Total arsenic residues in egg yolk of arsenic treated treatment of non-supplemented group exceeded the permissible limit of 500 μg kg^{-1} of the Food and Drug Administration (FDA). Therefore, eggs produced from layer exposed to arsenic (sodium arsenite) may create the consumer safety problem via contaminated eggs (Chiou *et al*., 1997). Similar to present observation Anke *et al*. (1982) and Hoffman *et al*. (1992) showed that incorporation of arsenic in egg was extremely low and increased with time.

Total arsenic residues in liver of layer chicken exceeded the Canadian National Standard (Chiou *et al*., 1997), whereas total arsenic residues in pooled meat of arsenic treated non-supplemented treatments of layer chicken did not exceed the permissible limit of 0.5 ppm of FDA, therefore, long term consumption of spent layer meat may create a consumer safety problem.

As same type of feed ingredients were used for feed formulation and water source for all experimental birds was same, so no variation was found in arsenic concentration of feed and water of different groups (Table 4). But arsenic concentration in faeces of different groups varied

Table 4: Concentration of As in egg and in different parts, feed, water and faeces of hen

Groups	C	T_1	T_2	T_3
Egg (ppm)	0.025±0.001^{bc}	0.377±0.043^{a}	0.071±0.003^{bc}	0.103±0.009^{b}
Yolk (ppm)	0.037±0.003^{b}	0.550±0.069^{a}	0.106±0.006^{b}	0.150±0.016^{b}
Albumen (ppm)	0.012±0.001^{bc}	0.180±0.020^{a}	0.036±0.002^{bc}	0.051±0.005^{b}
Egg shell (ppm)	0.008±0.001^{b}	0.085±0.007^{a}	0.012±0.002^{b}	0.015±0.002^{b}
Feed (mg kg^{-1})	0.097±0.006	0.097±0.006	0.097±0.006	0.097±0.006
Water (mg kg^{-1})	0.013±0.001	0.013±0.001	0.013±0.001	0.013±0.001
Faeces (mg kg^{-1})	0.040±0.020^{d}	2.260±0.020^{c}	5.250±0.030^{a}	4.181±0.030^{b}

Similar alphabets at superscript denote homogenous means due to Duncan's test at 1% level of significance, Values are shown in Mean±SE

significantly ($p<0.01$) due to dietary treatments. The highest concentration (mg kg^{-1}) in faeces was found in T_2 group followed by T_3, T_1 and C group, respectively. The total faecal excretion provides a useful index of arsenic exposure. Pattern of faecal total arsenic excretion in the present investigation supported the findings of Chiou *et al*. (1997). In excess methionine and methionine -betaine combination supplemented groups a lesser accumulation of arsenic was found than non supplemented group which suggest that methionine or methionine and betaine promote detoxification process of arsenic.

In the present investigation, considerable reduction of tissue accumulation of total arsenic and increment of faecal excretion of arsenic in layer chicken due to supplementation of either methionine or methionine-betaine combination was observed. Poor nutritional status may indicate an increased susceptibility of As toxicity leading to reduced methylation of inorganic As and therefore, increased tissue retention of As and vice versa (Hopenhayn-Rich *et al*., 1996). The greater retention of total arsenic in tissue is a consequence of its reactivity and binding with tissue constituents, most notable to sulfhydryl group (Vahter and Marafante, 1983) of a variety of essential enzymes and proteins leading to tissue damage may be due to impairment of energy metabolism (Szinicz and Forth, 1988). Reduced tissue accumulation and increased excretion of arsenic due to methionine supplementation in the present investigation was in agreement with the observation of Vahter and Marafante (1983) who examined the effect of low dietary intake of methionine, choline or protein on excretion of methylated metabolites in rabbits in which total arsenic excretion in urine was significantly decreased compared to control of all diet groups. The diet low in methyl group donors resulted 2-3 times increased tissue accumulation of total As.

A large size of human and animal population of West Bengal is exposed to the killer agent, arsenic, through contaminated drinking water. Animals particularly poultry is directly exposed to this agent. Thereby, long term intake of some poultry product, like hen eggs and layer meat by human being may act as some contributory factor to suffer from the serious health hazard. The mean arsenic concentration of different edible parts of layer meats and different component of eggs were presented in Table 2-4. Though the estimated concentration did not exceed the Indian maximum permissible limit (MFPO, 1992) but in long term consumption of such contaminated layer meat and eggs may play a contributory role along with the contaminated drinking water to affect human health adversely.

The WHO recommended value of arsenic in drinking water of 10 μg L^{-1} and the maximum permissible limit of 50 μg L^{-1} are based on water consumption of 2 L per day. West Bengal are in a tropical region where the average water consumption of an adult is about 4 L day^{-1} and those who work in fields consume a much higher quantity of water. Thereby, the recommended value and maximum permissible limit of arsenic in drinking water for villages of West Bengal should be at least half of the value recommended by the WHO in order to keep the total consumption within the safe limit. Again it is reported by WHO (1981) that 1.0 mg of inorganic arsenic per day may give rise to skin lesions within a few years and according the National Research Council (NRC) 500 μg of arsenic per day results in the risk of 13 out of every 100 persons developing cancer in their lifetime (Smith *et al*., 1999). If WHO (1981) and NRC postulations are applied to people drinking arsenic contaminated water in West Bengal, then large number of people are at risk for cancer. This arsenic burden on people of West Bengal may further elevated the risk of suffering due to consumption of contaminated poultry meat and eggs.

From the results of the total amount of arsenic consumed both through poultry products (meat and egg) and drinking water, the body burden of arsenic became higher of safe limit. In group T_1, whole egg contain arsenic 0.377 ppm, meat contain 0.391 ppm. So, it is obvious that layer products (meat and eggs) have a significant contribution of arsenic to produce clinical manifestation in human being of affected area.

People must understand that so far there is no available medicine for chronic arsenic toxicity; safe water, nutritious food (free from arsenic contamination), vitamins and physical exercise are the only preventive measures to fight against 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 membrane melanosis, Keratosis was a late feature of arsenical dermatitis, Rough and dry skin, often with palpable nodules. So, people should be aware of consumption of As contaminated eggs and meat. So it can be concluded that besides contaminated drinking water the contribution of different poultry products in the affected area may also cause human health hazard. Such exposure of arsenic in a very minute level for a long time may cause chronic arsenicosis of the individuals. With this, it can be concluded that the total effects of arsenic i.e., from drinking water and sources of poultry products highly dangerous for human health.

CONCLUSION

From the present findings it may be concluded that supplementation of either methionine or methionine-betaine combination may able to protect the chronic arsenic toxicity during exposure of As in layer chicken.

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