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TOXICOPATHOLOGICAL EFFECTS OF ORAL SODIUM ARSENITE ON PRODUCTION OF LAYERS

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ZAN conceived and designed the study and reviewed the manuscript. BRT conducted experiments, MT, IHL and AAM analyzed the data and drafted the manuscript.

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ABSTRACT

Arsenic is a highly toxic metal found in surface as well ground water in many areas of South Asia and can affect humans, animals as well as commercial poultry industry. The present study aimed to assess toxico-pathological effects of arsenic on egg production, quality and health of layers. Seventy-five Hy-Line W-36 layers were divided into four treatment groups (B, C, D, E) which were daily given 1mg, 5mg, 10mg, and 20 mg/kg/bw respectively of sodium arsenite in drinking water for three weeks while one group (A) served as control. The groups were examined weekly for determination of body weight. Further, egg production, weight and quality parameters like albumin, yolk, shell weight, thickness and Haugh unit were assessed. Deleterious effects of sodium arsenite were found to be dose and time dependent. There was a significant decrease in egg production, egg weight, albumin weight, yolk weight, shell weight, shell thickness and Haugh unit. There was decreased feed intake, increased water intake and reduced body weight. In conclusion, the sodium arsenite in drinking water produces adverse effects on egg production and quality of layers in time and dose dependent manner.

1. INTRODUCTION

The poultry industry is rapidly growing all over the world and is considered as largest sector of agriculture in the world (Memon, 2012). Fresh eggs are one of the most nutritious foods and used in many other food products (Sharkawy & Ahmed, 2002, Ieggli et al., 2011). However, arsenic contaminated drinking water can have deleterious effects on layer health and production. Moreover, toxic chemical contaminants, including arsenic, in eggs and poultry products via feed and drinking water are of serious public health concern (Sharaf et al., 2013).

Arsenic (As) is the 20th most toxic metalloid element in the earth's crust (Cancès et al., 2008). Arsenic contamination of river and ground water is a serious concern in South Asia.

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In Pakistan, maximum level of Arsenic up to 100 µg/L in ground water is reported in the central and southern parts, which is well above maximum acceptable limit set by WHO which is 10 µg/L (Islam et al., 2009).

The groundwater is used as drinking water in poultry farming in Pakistan, therefore Arsenic toxicity can affect both health and production of poultry especially in areas with high arsenic concentration (Jabeen et al., 2012). The toxicity of arsenic depends upon animal species along with dose and duration of exposure (Halder et al., 2009). Arsenic contaminated food or drinking water can have an impact not only on human health but also may affect wild animals, birds, poultry, and livestock. Current research was designed to measure the effect of various concentrations of sodium arsenite in drinking water on production and health of layers.

2. MATERIALS AND METHODS

Experimental design, birds, and management

Seventy-five Hy-Line W-36 layers 40 weeks of age, were obtained from the local commercial farmers. Before the experiment, all birds were provided adaptation period of one week. The layers were randomly divided into four treatment groups and one control group. The treatment groups were given sodium arsenite (Sigma-Aldrich, USA) in drinking water daily at the rate of 1 mg, 5 mg, 10 mg, and 20 mg/ Kg/body weight for three weeks. The control was given untreated drinking water. All birds were kept under hygienic and well-ventilated conditions under 16 hours of light and 8 hours of darkness regimen. Commercial layer feed and water were given ad libitum.

Egg quality parameters

The Digital Egg Tester (DET6000, NABEL Co., Ltd. Japan) was used for the analysis of freshly laid eggs daily. Eggshell thickness, eggshell weights, weight of albumin, yolk weight, were measured.

Yolk index

The yolk index was used as an egg quality indicator in the 1930s. Sharp and Powell (1930) described a method for calculating a value that they dubbed the "yolk index." After removing the yolk from the albumen, the process required a five-minute wait for the yolk to expand before measuring its height and breadth in two directions at right angles (Sharp and Powell 1930). It was calculated by dividing the yolk height by the yolk diameter of the egg broken onto a flat surface. The height and diameter were measured by a vernier caliper.

The yolk index was calculated by the following formula:

YI=YH/YD

YI: Yolk index

YH: Yolk height

YD: Yolk diameter

Haugh's unit

The Haugh's unit is a metric for measuring the grade of egg protein based on the height of the egg white (albumin). This is the logarithm of the inner thick albumin adjusted for egg weight (Haugh, 1937). The Haugh's unit was calculated by initially weighing of egg on electric weighing machine, then the egg was broken onto a flat surface and a micrometer was used to determine the height of albumin.

The Haugh's unit was calculated by the following formula:

$$HU = 100 * \log(h - 1.7w^{0.37} + 7.6)$$

HU: Haugh unit

h: observed height of the albumin in millimeters

w: weight of egg in grams

Clinical signs, morbidity and mortality

All layers were observed daily for development of clinical signs and mortality. Weekly body weight along with feed and water intake were recorded during the experiment.

Statistical analysis

All data are displayed as mean ± Stdev. Differences between groups were analyzed by using analysis of variance (ANOVA) to indicate statistically significant differences. Data were analyzed on GraphPad Prism 9 software.

3. RESULTS AND DISCUSSION

Layer production parameters

The results on bodyweight, feed intake and water intake are shown in **Table 1.** Bodyweight and feed intake in all treatment groups significantly ($p<0.05$) decreased in a time and dose-dependent manner as compared to control. There was a significant ($p<0.05$) decrease in body weight in dose-dependent manner all groups in the 2nd, 3rd, and 4th week of the experiment. The egg production, Haugh unit and yolk index significantly declined with time and was dose dependent as compared to control **(Table 2**). Water intake was also significantly ($p<0.05$) increased in all treated groups in time and dose-dependent manner as compared to control (**Table 1)**.

Egg quality parameters

The analysis of variance showed that egg weight did not significantly change in group A during three weeks of the experiment, while other groups (B-E) were significantly different from each other and control. Egg weight significantly ($p<0.05$) declined in treated groups (B-E) in time and dose-dependent manner as compared to control group(A) as shown in **Table 3**.

This study showed that albumin and yolk weights were significantly ($P < 0.05$) different among treated groups (B-E) as shown in **Table 3**. The weight of albumin, and yolk, in groups treated with Sodium arsenite, were lower than the control group (A) (**Table 3)**. The shell weight and thickness were significantly decreased in all treated groups as compared with control (**Table 4)**. Moreover, all observed egg quality traits were significantly different among groups ($P < 0.05$). Time and dose-dependent significant ($p<0.05$) decrease were seen in groups B -E as compared to the control group for both exterior and interior characteristics of the egg. Albumin, yolk and shell weight, shell thickness, Haugh unit of the control group (A) was higher than treated groups (A-E) with doses and times interval shown in (**Tables 2-4)**. Significant ($p<0.05$) dose-dependent decrease in egg production was noted in all treatment groups during the 2nd and 3rd, week of the experiment (**Table 2)**.

Clinical findings

Clinical signs such as depression, dullness, paralysis of legs in few birds, pale and anemic comb and wattles were observed in treated groups. Clinical signs were more pronounced with higher doses. Dose-dependent mortality rates were observed in groups E, D, C and B, i.e., 50, 30, 10 and 5 %, respectively while there was no mortality in control group. Arsenic is considered as a king of toxins and its exposure is considered as one of the major environmental health risks in many regions of the world (Silbergeld et al., 2008). It has been reported that severity of Arsenic toxicity is dose dependent and acute cases are result of high arsenic intake (Flora & Tripathi, 1998). Clinically, all organs are affected by arsenic, but mainly it affects the central nervous system, hematopoietic system, liver and kidneys (Hughes, 2002).

Our results show that sodium arsenite in drinking water leads to a dose and time dependent significant reduction in feed consumption and body weight in layer birds. Moreover, a significant increase in water consumption was also observed. Similar observations have been reported in broilers after administration of high doses of arsenic in water (Mashkoor et al., 2013,Vodela et al., 1997). Another study has reported weight loss, decreased feed consumption and increased thirst after administering birds with arsenite (Sharaf et al., 2013). Decrease in body weight may be due to an increase in oxidative instability, resulting in a low dietary intake, lack of adequate nutrition, and reduced metabolism of exposed birds (Ghaffar et al., 2017). Our findings are in agreement with other studies in birds, rats, mice, and rabbits (Khan et al., 2013).

This study found a significant decrease in egg quality parameters such as Haugh Unit and thickness of eggshell after sodium arsenite administration to layers. Similar results have also been previously reported in chicken (Vodela et al., 1997). This may be due to decrease in estrogen receptors as reported by Garcia-Morales et al., 1994, who indicated that arsenic leads to a decline of about 58% estrogen receptors in human breast cancer cell line. Additionally, Whitehead et al., 1993 have also observed that egg weight is controlled by estrogen which changes fat metabolism and protein synthesis in the oviduct. Our study has also reported decrease in Haugh unit significantly in layers due to sodium arsenite toxicity. Haugh unit is a measurement of the quality of albumin in fresh eggs (Silversides, 1994) and it is easily affected by heavy metal toxicity. Besides, eggshell thickness was also found significantly decreased which probably occurs due to metal toxicity leading to suppression of calcium metabolism (Edens & Garlich, 1983).

4. CONCLUSION

The present study shows that the sodium arsenite in drinking water affects egg production, egg quality and health of layers as reflected by increased morbidity, higher mortality and reduced body weight in dose and time dependent manner.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1 Body weight, feed intake and water intake with varying concentrations of sodium arsenite in drinking water

Weeks	Group	Body Weight (gm) /bird	Feed intake (gm)/bird	Water intake (gm)/bird
1st week	**A**	1400±0.25[ab]	1105±13[a]	3315±38[i]
	B	1412.5±25[a]	1090.5±1[ab]	3369.3±14[h]
	C	1387.5±25[abc]	1057±5.8[bc]	3371.8±15[gh]
	D	1375±50[acb]	1047.3±3.5[c]	3389.3±3.5[fg]
	E	1375±50[abc]	990.5±1[d]	3471.5±3[e]
2nd week	**A**	1387.3±26[abc]	1105±13[a]	3315±38[i]
	B	1354.8±47[abc]	958.8±3.8[de]	3401.5±3.8[f]
	C	1320.8±38[bc]	938.5±1.2[e]	3469.5±4.7[e]
	D	1215.5±10[d]	825.5±4[g]	3496.5±11[d]
	E	1159.5±44[de]	764.5±1.2[h]	3609.3±5.1[c]
3rd week	**A**	1387.3±17[abc]	1105.8±9.8[a]	3317±29[i]
	B	1312.5±26[c]	900.5±1.3[f]	36.93±5.1[c]
	C	1230.5±29[d]	869.8±1.7[f]	3758±6.3[b]
	D	1132.5±27[e]	789±1.8[h]	3767±5.4[b]
	E	1012.4±15[f]	650.5±1.3[i]	3815.5±3.8[a]

Table 2 Egg production, Haugh unit and yolk index with varying concentrations of sodium arsenite in drinking water

Weeks	Group	Egg Production/bird	Haught Unit	Yolk Index
1st week	**A**	6.750^{a}±0.5^{a}	75.64±1.07^{a}	35.17±0.81^{a}
	B	6.0±0.81^{ab}	64.23±1.97^{b}	31.39±1.15^{b}
	C	5.25±0.95^{abcd}	61.74±1.30^{bc}	29.41±0.94^{bc}
	D	5.0±0.81^{abcde}	62.02±2.40^{bc}	27.23±1.28^{cd}
	E	4±1.41^{bcdef}	60.87±0.76^{bcd}	25.87±1.03^{de}
2nd week	**A**	6.5±1^{a}	74.56±1.84^{a}	35.37±0.44^{a}
	B	5.75±0.95^{abc}	60.19±4.94^{bcd}	29.28±1.07^{bc}
	C	4.50±1.29^{abcde}	58.35±3.05^{bcde}	27.39 ±1.7^{cd}
	D	4±0.81^{abcdef}	49.62±4.47^{fghi}	25.51±1.88^{de}
	E	3.75±1.25^{bcdef}	44.18±3.13^{hi}	23.5±1.29^{ef}
3rd week	**A**	6.5±0.5^{a}	74.81±1.38^{a}	35.01±1.14^{a}
	B	5.0±0.81^{abcde}	54.90±4.16^{cdef}	24.23±1.25^{ef}
	C	3.5±0.57^{cdef}	53.51±2.28^{defg}	21.90±0.61^{fg}
	D	3.75±0.95^{bcdef}	46.41±4.88^{ghi}	15.71±1.16^{i}
	E	1.75±0.95^{fg}	36.35±0.12^{jk}	13.05±0.1^{ij}

Table.3 Egg weight, Albumin and yolk weight with varying concentrations of sodium arsenite in drinking water

Weeks	Groups	Egg weight (gm)	Albumin weight (gm)	Yolk weight (gm)
1st Week	**A**	61.8±0.95^{a}	37.1 ±0.78^{a}	18.6±0.5^{a}
	B	52.6±1.7^{b}	31.5±1.37^{b}	15.7±0.2^{b}
	C	50.1±1.29^{bc}	30.1±0.94^{bc}	15.1±0.1^{bc}
	D	48.6±1.7^{cd}	29.4±0.75^{cd}	14.6±0.2^{cd}
	E	49.4±1.7^{cd}	29.6±1.34^{c}	14.8±0.4^{bc}
2nd Week	**A**	61.7±0.95^{a}	37.1 ±0.75^{a}	18.5±0.4^{a}
	B	50.5±0.82^{bc}	30.1±0.49^{bc}	15.1±0.4^{bc}
	C	46.2±1.82^{de}	27.7±1.17^{de}	13.8±0.5^{de}
	D	38.5±1.7^{f}	23.6±0.71^{f}	11.5±0.6^{f}
	E	33.6±1.29^{g}	20.1±0.84^{g}	10.1±0.2^{g}
3rd Week	**A**	61.2±0.81^{a}	36.6 ±1.7^{a}	18.3±0.4^{a}
	B	49.3±1.7^{cd}	29.6±1.7^{cd}	14.8±0.1^{bc}
	C	44.4±1.91^{e}	26.6±1.5^{e}	13.3±0.2^{e}
	D	30.8±1.7^{g}	18.5±1.29^{g}	9.3±0.2^{g}
	E	22±1.29^{h}	13.6±1.29^{h}	6.8±0.08^{h}

Table 4 Shell weight and thickness with varying concentrations of sodium arsenite in drinking water

Weeks	Groups	Shell weight (gm)	Shell thickness (cm)
1st Week	A	8.17±0.13[a]	0.54[a]±0.01[a]
	B	6.99±0.40[c]	0.44±0.01[c]
	C	6.62±0.19[c]	0.41±0.01[cd]
	D	5.63±0.53[d]	0.28±0.01[fg]
	E	4.27±0.43[f]	0.22[hi]±0.02[hi]
2nd Week	A	8.24±0.22[a]	0.55±0.01[a]
	B	6.93±0.08[c]	0.33±0.01[ef]
	C	5.33±0.28[de]	0.25±0.01[gh]
	D	4.76±0.51[ef]	0.22±0.01[hi]
	E	3.26±0.20[gh]	0.18±0.01[i]
3rd Week	A	7.99±0.40[ab]	0.55±0.02[a]
	B	5.68±0.43[d]	0.32±0.01[ef]
	C	4.40±0.27[f]	0.25±0.01[gh]
	D	3.98±0.40[fg]	0.18±0.01[i]
	E	2.52[h]±0.41	0.10[j]±0.01