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# Arsenic(III) Toxicological Impacts on *Gallus gallus domesticus* Early Days Brooding and its Bio-Accumulation Studies in Blood Samples by DPASV

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Unchecked release of industrial effluents containing arsenic in the fresh water is resulting in environmental pollution of water resources. Actual exposure of this water contamination was checked by measuring the As(III) bio-accumulation in blood samples of broiler. Eco-physiological impacts of this bio-accumulation were also captured like body weight, feed consumption, feed conversion, mortality rate and core internal body temperature. Analysis of As(III) bio-accumulation revealed that the effects on the health conditions of birds due to As(III) are extremely adverse since it results in decreased body weight by 22 %, decreased feed consumption by 18 %, decreased feed conversion by 0.015 g/g, higher mortality rate by 7.38 % and lowered core internal body temperature by 7.8 % within first 14 days of brooding period. Overall, there was bio-accumulation of 25.8 µg/L of As(III) in blood samples of *Gallus* sp after 14 days of brooding period.

**Keywords: As(III), Broiler, Bio-accumulation, Voltammetry, Mortality.**

## INTRODUCTION

Arsenic has been under man's usage as medicine since prehistoric times. Man has also tried to use it as warfare agents like in the form of Adamsite. Later on toxicity issue arose when in 1900, 6000 people suffered from arsenic poisoning due to contaminated beer in England [1]. Arsenic contaminated water has played a catastrophic role in many countries including Pakistan [2]. Arsenic is most problematic among the metalloids and oxy anion forming elements as it has greater mobility range under both oxidizing and reducing conditions. Natural water mainly contains inorganic form as oxyanions of trivalent arsenite [As(III)] or pentavalent arsenate [As(V)] [3].

Natural and anthropogenic sources are the two mainstream source of arsenic leaching. Natural sources include earth crust in the form minerals of arsenic like arsenopyrite [4]. Burning of fossil fuels [5] and smelting of ores are some other established natural sources [3]. Anthropogenic sources have contributed a lot in arsenic pollution as compared with natural sources. Arsenic has been employed as insecticide's component in form of lead arsenate, copper acetoarsenite, mono sodium methane arsonate (MSMA) and arsenic acid for cotton production [6]. Similarly arsenic acid, 3-nitro-4-hydroxy phenyl arsenic acid were used as feed additives under food additives law of 1958 [7]. Arsenic acid is extremely used as cotton desiccant for many years. Wolman salts, osmosalts, zinc and chromium arsenate are used as wood preservative [8]. The first wood preservative was fluorchrome arsenic phenol (FCAP), which was used in 1918 in USA. Sodium arsenite has been widely applied as weed killer in about 1890. Before the advent of antibiotics, arsenic compounds were used as medicine. Donovan's solution (arsenic iodide) and valagin's solution (arsenic trichloride) were recommended for treatment of rheumatism, arthritis, asthama, malaria and diabetes [9,10]. Today the use of arsenic compounds in treatment of cancer is under consideration [11-12]. Now a day's arsenic compounds have found application in glass industry and semiconductor industry. Moreover it has also found application in catalyst formation and alloy production [13,14].

Arsenic pollution is a major public concern now in Pakistan [15] where elevated levels of arsenic were found in major fresh water reservoirs of the country. According to National Standards for Drinking Water Quality [16] the maximum permissible limit for arsenic in drinking water is 50 µg/L while it is 10 µg/L according to EPA and WHO [17,18]. Fresh water contains arsenic in the range of 1-10 µg/L and this concentration reached up to level of 100-5000 µg/L in regions of sulfide mineralization and mining [19]. Major water reservoirs of Pakistan have found to be arsenic contaminated like Tarbela (620 µg/L), Chashma (750 µg/L) and Lloyd (620 µg/L). Similarly ground water

sample from different cities of Pakistan showed concentration of 80 μg/L [20].

Inorganic arsenic is more readily absorbed than organic arsenic. Dermal absorption of trivalent form is rapid than pentavalent form. The absorption site of arsenic is gastrointestinal tract or respiratory tract. After absorption 93-99 % of arsenic binds with the globin of hemoglobin in red blood cells and distributed within whole body in 24 h. It persists in hair, nail and skin [21]. A number of analytical techniques are available for arsenic detection. Atomic absorption spectrometry of four types Flame atomic absorption spectrometry, Graphite furnace atomic absorption spectrometry, atomic fluorescence spectrometry and ICP is used for arsenic detection [22]. Once spectrophotometric methods for metal detection were very popular but recently they had become obsolete because they are time consuming and do not ensure sufficient low detection limit [23]. In this study, electrochemical method was used for the detection of bio-accumulation of arsenic in blood samples of bird. A reported method of voltammetric detection of As(III) with *Porphyridium cruentum* based modified carbon paste electrode was applied for the determination of the As(III) in blood samples. This method was used because of its accuracy of detection to as low as 2.5 μg/L [24].

Freshly hatched one day old chicks were brooded under controlled conditions of 32 °C with 30-50 % relative humidity (RH) in the litter at placement [25]. Body weight (g), feed consumption (g), feed conversion (g/g), mortality rate (numbers) and core internal body temperature (°C) were used as the determining factors in the analysis of As(III) bio-accumulation resulting from polluted water of unchecked release of industrial effluents in fresh waters. Decreased body weight, decreased feed consumption, slow feed conversion, high mortality rate and lowered core internal body temperature were found correlated with the elevated levels of As(III) in the blood samples of selected bird. Broiler was purposefully selected for this analysis because of its high consumption of this bird in the human population. Annual consumption of this bird's meat is 12 kg per person [26].

## EXPERIMENTAL

Ground water was taken from catchment areas of river Ravi from different places in Punjab. Arsenic(III) level was calculated in these samples and 0.08 mg/L concentration was detected. In literature effects of arsenic contamination in drinking water have been studied at 8.6 mg/L [27] so by standard addition method the concentration of sample was increased up to 10 mg/L.

Mineral water was purchased from the market with mineral contents (mg/L) in following concentrations; calcium (40-70), magnesium (4-15), sodium (7-30), potassium (0.02-0.10), fluoride (0.2-0.05), chloride (77-150) and sulphate (12-50).

**Birds:** *Gallus gallus domesticus*, broiler chicks were purchased from a hatchery. The day old chick birds were purchased with equal distribution of male and female density. A total number of 200 chicks were purchase and these were of exact same weight of 37 ± 2 (g). These were then separated in two flocks on proportionate basis. Flock 1 and Flock 2 (each containing hundred chicks) were formulated. Flock 1 and Flock 2 were given mineral and ground water respectively for drinking during brooding period of 14 days.

**Brooding temperature (BT) and relative humidity (RH):** *Gallus gallus domesticus*, the day old freshly hatches chicks were brooded under controlled conditions of 32 °C [35]. Two 100 Watt bulb hung over demand pans at the end of the line for each flock were used as light source. First day weight, seven day weight and fourteen day weight was used as tool to check uniformity of the health conditions in each flock. 30-50 % relative humidity (RH) in the litter at placement was maintained. PS-500-020 Digital Thermo-hygrometer by OMEGA was used for the measurement of the relative humidity.

**Chicks core internal body temperature (CIBT) and weights measurements:** The chick's core internal body temperature (CIBT) measurement in birds was done by inserting a probe into a cloaca to determine CIBT of the bird. PS-TC-100A Digital Compact probe Thermometer by OMEGA was used for the measurement of the CIBT. ACB600H Precision Compact Balance of resolution of 0.01 g by sheen was used for the measurement of weights.

**Arsenic measurement in blood samples:** Differential pulse anodic stripping voltammetric (DPASV) studies were carried out with a potentiostat/galvanostat (Reference 600 TM, Gamry, Germany) instrument equipped with Gamry Framework and Echem Analyst software by a reported method [24]. Stock solution of As(III) of concentration of 1000 ± 2 mg/L solution was taken from Merck. This stock solution was diluted from 2.5 to 200 μg/L and it was used as standard for the voltammetric studies of As(III) bio-accumulation in the blood samples of the birds. The mineral oil (Light, White; Sigma-Aldrich, USA) was used as binder in biologically modified carbon paste electrode, *Porphyridium cruentum* was used as biomass of particle size of 250 μm, 0.1 M $HNO_3$ was used as stripping medium, preconcentration or accumulation time of 8 min was given to every sample at deposition potential of -0.8 V, pH of the solution was maintained at 6 and the composition of the electrode was 5:65:30 (w/w %) of biomass:carbon powder: binder [24].

Blood samples taken from chicks were kept at room temperature for 3 h and then at 4 °C for overnight. Each blood sample was centrifuged for 10 min at 2000 rpm. Serum was removed and stored in sterile plastic tubs until used for As(III) measurements [28].

## RESULTS AND DISCUSSION

Body weight of the chicks is directly related to the drinking water quality. Water is an essential nutrient that impacts virtually all physiological functions. In present study, it was observed that water consumption was decreased in chicks of Flock 2 because of the high concentration of the As(III) in water. According to Viola *et al.* [29], a 40 % water restriction decreases the feed intake (from 542 to 338 g), body weight (from 471 to 295 g) and FCR (from 1.28 to 1.37) at 14 days. One major sign of overt toxicosis in chicken is growth inhibition and consequently decreased body weight [27,29]. Flock 1 had greater body weight than Flock 2; an obviously accepted result. After brooding period of 14 days, a maximum decrease of 22 % was

observed (Figs. 1 and 2). Similar findings have been reported in literature depicting role of heavy metals on decreasing body weights of chicken [30,31].

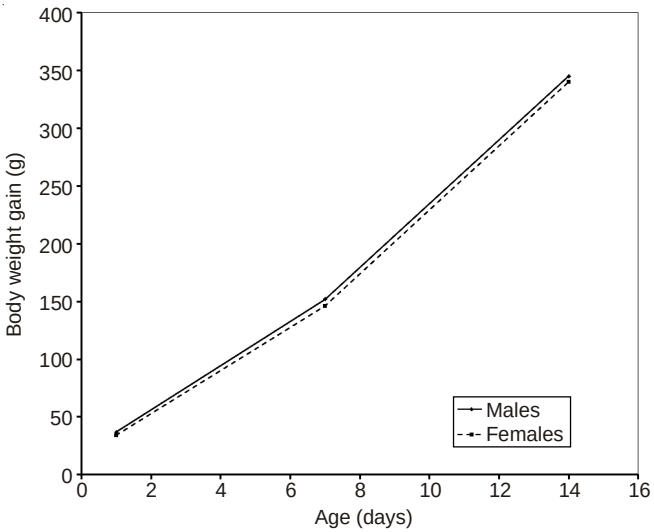


Fig. 1. Weight gain (g) in chicks of Flock 1 drinking mineral water

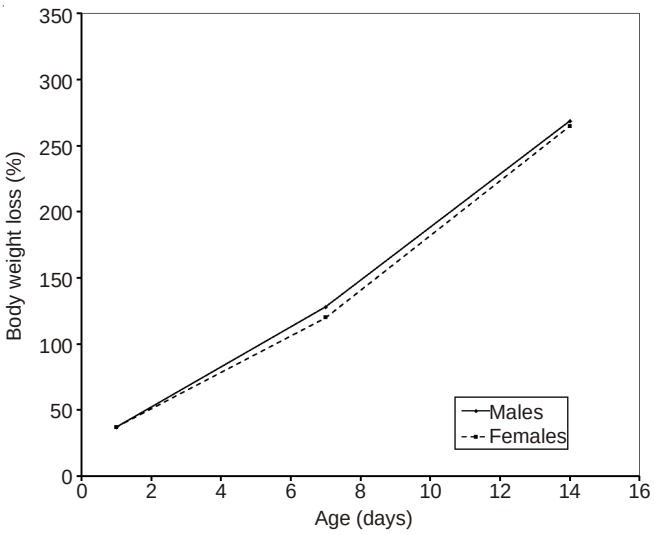


Fig. 2. Weight loss (g) in chicks of Flock 2 drinking As(III) contaminated water

**Feed consumption (g):** In this study there was reduction in feed consumption in Flock 2. A maximum reduction in feed consumption was of 18 % after 14 days brooding period (Figs. 3 and 4). It has been reported in literature that addition of heavy metals to the feed intake of broilers, even at the low dosing rate decreases feed intake, suppress humoral and cell-mediated immune response [32].

**Feed conversion (g/g):** Feed conversion was calculated by dividing body weight to feed intake. As we have discussed above that high level of As(III) in drinking water decreases the body weight and feed consumption in chicks of Flock 2 so it is quite obvious that feed conversion was not be equal to Flock 1 that received mineral water as drinking water (Figs. 5 and 6).

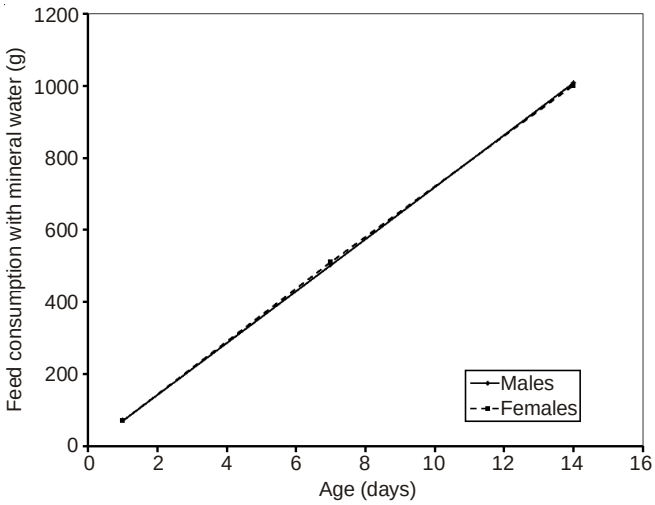


Fig. 3. Effect of mineral water on feed consumption (g) of chicks in Flock 1

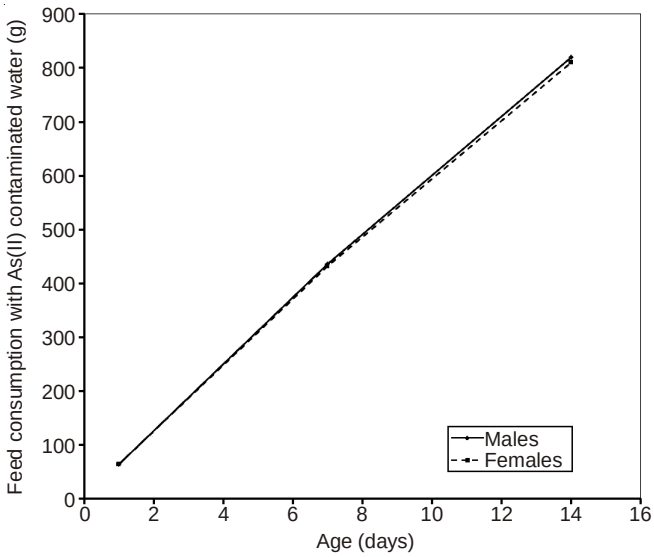


Fig. 4. Effect of As(III) contaminated water on feed consumption (g) of chicks in Flock 2

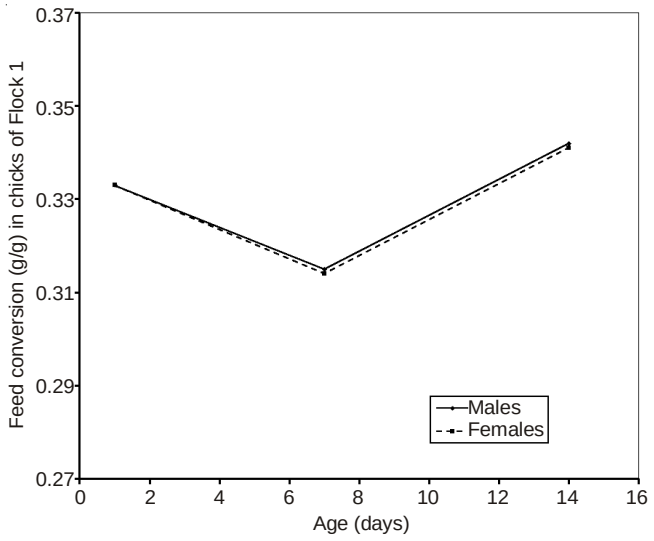


Fig. 5. Trend of feed conversion (g/g) in chicks of Flock 1

**Mortality rate (No):** The mortality rate (7.38 %) was on the higher side in chicks of Flock 2 as compared with chicks of Flock 1 (Figs. 7 and 8). In similar studies mortality rate was

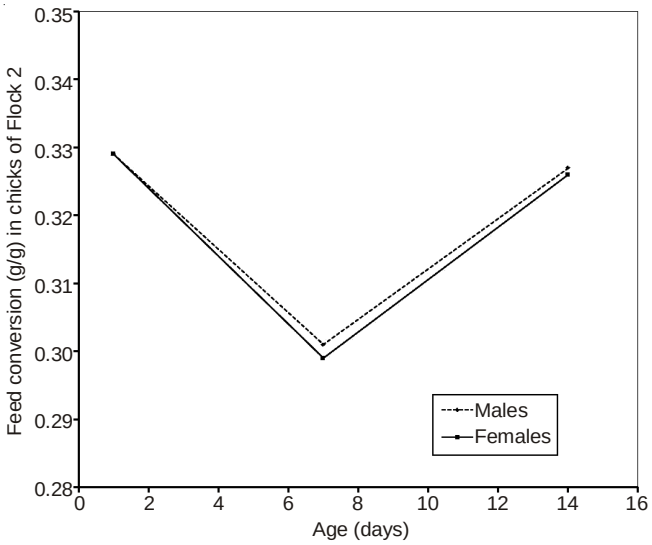


Fig. 6. Trend of feed conversion (g/g) in chicks of Flock 2

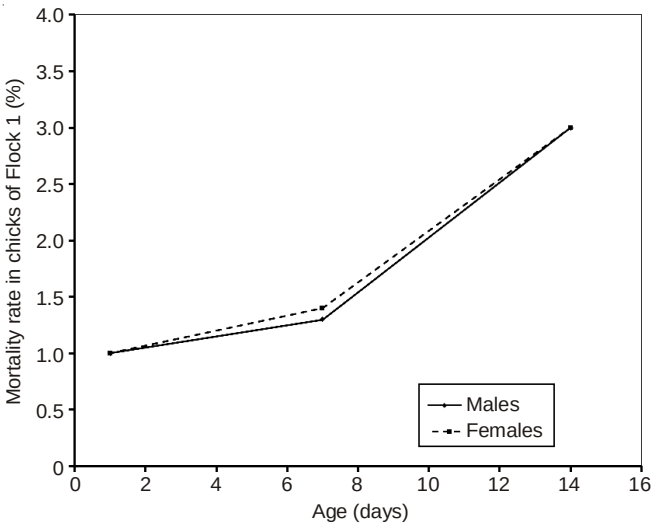


Fig. 7. Effect of mineral water on mortality rate (%) of chicks in Flock 1

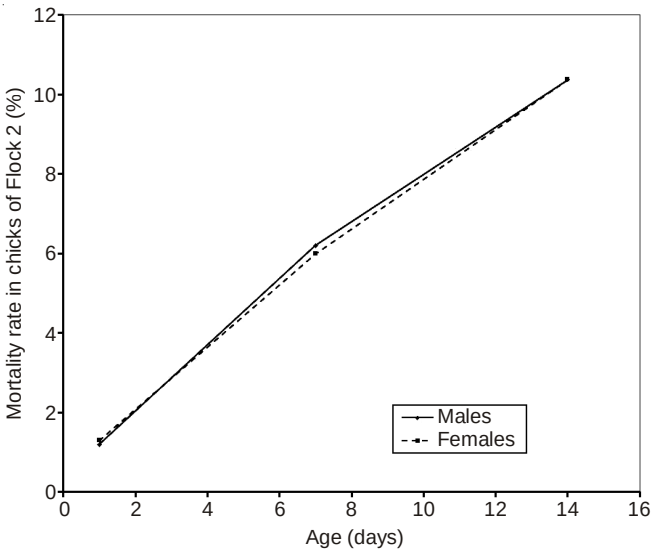


Fig. 8. Effect of As(III) contaminated water on mortality rate (%) of chicks in Flock 2

found on the higher sides when brooding chicks were given heavy metals contaminated water [32].

**Core body internal temperature (CBIT):** One day old chick's average core body temperature is found at 41.8 ± 0.18 °C. Body temperature found to have a direct relationship with feed intake [33]. As As(III) contaminated drinking water has suppressed feed intake so it was obvious that body temperature in chicks of Flock 2 was low. There was a decrease of 7.8 % in body temperature in chicks of Flock 2 (Fig. 10). The literature reported that body temperature of chicks increase on a regular basis during 3 weeks of brooding period. A similar behaviour was observed in chicks of Flock 1 (Fig. 9).

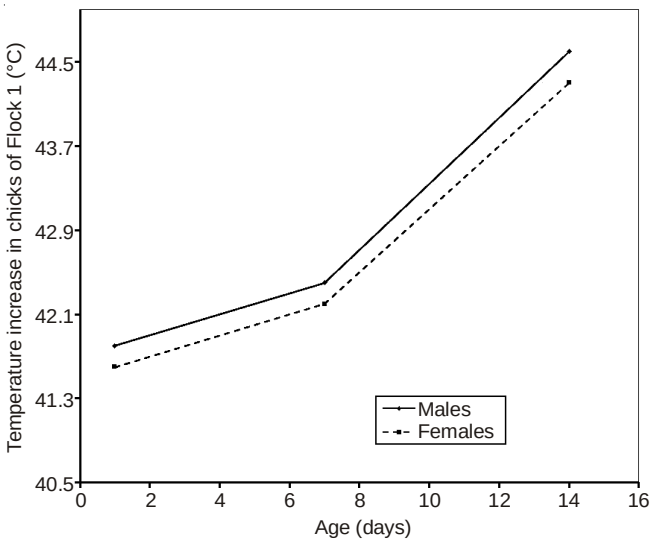


Fig. 9. Increase in core internal body temperature (CIBT) in chicks of Flock 1 drinking mineral water

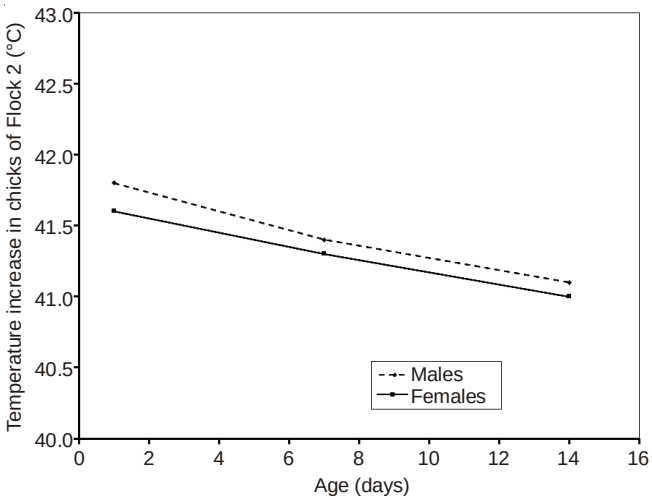


Fig.10. Increase in core internal body temperature (CIBT) in chicks of Flock 2 drinking As(III) contaminated water

**Arsenic(III) bio-accumulation in blood samples:** The literature reported that arsenic has a great potency of bio-accumulation [34,35]. This present study calculated bio-accumulation of As(III) by a reported method [24]. Results showed that factors indicating good brooding of chicks are disturbed in Flock 2 that was drinking water containing arsenic. As(III) was not detected after 1st day of brooding and it was less than 2.5 µg/L, on 7th day of brooding it was 14.38 µg/L and on 14th day of brooding it was found to be present in 25.8 µg/L. There was no distinction of bio-accumulation on the basis of gender found in this study (Tables 1 and 2).

TABLE-1
TOTAL AMOUNT (µg/L) OF As(III) BIO ACCUMULATED IN BLOOD SAMPLE OF CHICKS OF FLOCK 1

| Age (days) | Male (µg/L) | Female (µg/L) |
|---|---|---|
| 1 | ND | ND |
| 7 | ND | ND |
| 14 | ND | ND |
| Total 14 days bio-accumulation | ND* | ND* |

*Not detectable

TABLE-2
TOTAL AMOUNT (µg/L) OF As(III) BIO-ACCUMULATED IN BLOOD SAMPLE OF CHICKS OF FLOCK 2

| Age (days) | Male (µg/L) | Female (µg/L) |
|---|---|---|
| 1 | ≤ 2.5 | ≤ 2.5 |
| 7 | 14.4 ± 0.4 | 14.4 ± 0.4 |
| 14 | 25.8 ± 0.4 | 25.8 ± 0.4 |
| Total 14 days bio-accumulation | 25.8 | 25.8 |

## Conclusion

Arsenic contaminated drinking water during the 14 days brooding period impacts body weight, feed consumption, feed conversion, mortality and core internal body temperature indicated adversely. Similarly there was a bio-accumulation of 25.8 µg/L in the blood samples after 14 days of brooding. Overall, this study suggests that unchecked release of industrial effluents caused water contamination in catchment areas that was found to be a source of bio-accumulation in chicken.

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