



# Transfer of arsenic from poultry feed to poultry litter: A mass balance study

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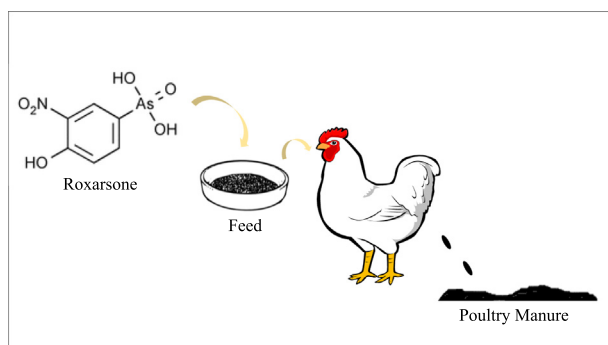
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## HIGHLIGHTS

- First study that describes mass balance of arsenic intake and excretion by chickens
- Two strains Ross and Cobb exhibited similar response to roxarsone-amended feed.
- Arsenic did not retain in chickens during their growth on arsenic containing feed.
- Poultry litter enriched in arsenic warrants investigations if used in crop production.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Roxarsone (rox), an arsenic (As) containing organic compound, is a common feed additive used in poultry production. To determine if As present in rox is excreted into the poultry litter without any retention in chicken meat for safe human consumption, the transference of As from the feed to poultry excreta was assessed using two commercial chicken strains fed with and without dietary rox. The results revealed that both the strains had similar behaviour in growth (chicken weight; 2.17–2.25 kg), feed consumption (282–300 kg pen<sup>-1</sup> initially containing 102 chicken) and poultry litter production (73–81 kg pen<sup>-1</sup>) during the growth phase of 35 days. Our mass balance calculations showed that chickens ingested 2669–2730 mg As with the feed and excreted out 2362–2896 mg As in poultry litter during the growth period of 28 days when As containing feed was used, yielding As recovery between 86 and 108%. Though our complementary studies show that residual arsenic species in rox-fed chicken meat may have relevance to human exposure, insignificant retention of total As in the chicken meat substantiates our mass balance results. The results are important in evaluating the fate of feed additive used in poultry production and its potential environmental implications if As containing poultry litter is applied to soil for crop production.

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## 1. Introduction

Arsenic (As) is a toxic element in the environment and a known carcinogen that consistently ranks first on the Agency for Toxic Substances and Disease Registry (ASTDR) Substance Priority List (<https://www.atsdr.cdc.gov/STP>).

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[atsdr.cdc.gov/spl/index.html](https://atsdr.cdc.gov/spl/index.html)). Sources of As contamination in the environment includes its elevated contents in some earth crust minerals, industrial activities (mining and smelting) and arsenical compounds used for different purposes (ATSDR, 2007). Drinking water and ingestion of contaminated food are primary As exposure pathways to biological receptors (humans and animals) that pose a significant threat to public health (Naujokas et al., 2013).

Roxarsone (rox; 3-nitro-4-hydroxyphenylarsonic acid), an organic compound containing As, was added routinely to the poultry feed for parasitic disease prevention, growth promotion, enhanced feed utilization and improved meat pigmentation for >60 years (Nachman et al., 2013; Fisher et al., 2015) until its use was discontinued in Europe (European Commission, 1999), USA (FDA, 2013), and Canada (<https://www.theglobeandmail.com/life/health-and-fitness/sales-halted-after-arsenic-found-in-chicken-drug/article591962/>). When the United States Food and Drug Administration (FDA) approved rox in 1944 as a feed additive (Nachman et al., 2013), it was believed that the non-toxic organic As present in rox would not change into toxic inorganic As inside the chicken body; rather organic As would be excreted unchanged into the poultry manure leaving chicken meat safe for human consumption (Schmidt, 2012). However, higher than tolerable amounts of total As (>2.0 mg kg<sup>-1</sup>) established by FDA before 1963 (FDA, 1963) were found in the livers of rox-fed chickens compared to the ones fed without rox (FDA, 2011).

Subsequent studies focused on the determination of different species of As in different chicken body parts as the toxicity of As is highly dependent on its chemical species (Moe et al., 2016). Higher concentrations of inorganic As were found in the conventional compared to the anti-biotic free conventional chicken meat samples in the US (Nachman et al., 2013). Similarly, inorganic forms of As was also detected in the feather meal products from six US states (Nachman et al., 2012). Yao et al. (2016) revealed that rox in chicken diet was transformed into its metabolites (different As species) in chicken manures which subsequently increased concentrations of these As species in rice plants (grain, straw and hull) when grown in soil amended with the chicken manures. However, no study to date has reported the mass balance of As from poultry feed to poultry litter. In our study, we conducted an experiment growing two strains of chicken on rox-supplemented feed or feed devoid of rox. Different arsenic species were analyzed in chicken liver (Peng et al., 2014; Peng et al., 2017), chicken breast (Liu et al., 2016) and chicken litter (Yang et al., 2016) samples taken from this study. In the current manuscript, we report mass balance that determines the amount of total As taken in by chickens with feed consumption and the amount of total As excreted in poultry litter to assess the possible retention of As inside the chicken body.

Arsenic utilization in poultry industry and its subsequent transport to different components of biosphere may have far-reaching consequences related to human health and the environment. Though rox containing poultry feed has been discontinued in Europe and North America, many other countries continue to use phenylarsenicals in the poultry industry (Nachman et al., 2012; Yao et al., 2016).

## 2. Methodology

### 2.1. Poultry feed and chicken growth experiment

Effect of two types of feed (rox containing feed: rox, and feed without rox: control) was studied on two commercial strains of poultry (Cobb 500 and Ross 308) grown in an environmentally controlled barn as described previously by Liu et al. (2016). Briefly, in the barn, age related temperature and ventilation protocols were followed as per the Strain Management Guides of Cobb (Cobb-Vantress, 2008) and Ross (Aviagen, 2009). The lighting program followed a photoperiod of 23 h for the first three days, and then 20 h' light and 4 h' dark from fourth day to the end of the experiment (35 days). In total, there were

16 pens with a treatment combination of two types of feed, two poultry strains and four replications. In each pen (169 cm × 420 cm), 102 chicks (one-day old, mixed sex, previously weighed) were reared. New soft-wood shavings of known weight were used as the bedding material in each pen that yielded a depth of 7.5 cm above the pen floor. For the rox treatment, 3-NITRO® (Alpharma Canada Corporation) containing 20% rox was added to the feed at 250 g ton<sup>-1</sup>. Because rox contains 28.48% As, the As content was 14.24 mg kg<sup>-1</sup> of the feed prepared for the rox treatment. The feed used for the control treatment had all the same ingredients except rox. All chickens received diet according to standard Poultry Research Centre diet composition (Table 1) - starter diet (3068 kcal kg<sup>-1</sup>; 23% crude protein) for two weeks, grower diet (3152 kcal kg<sup>-1</sup>; 20% crude protein) from two to four weeks, and finisher diet (3196 kcal kg<sup>-1</sup>; 19% crude protein) from four to five weeks of age. Rox was added to the starter and grower diets only. Chickens had ad libitum access to both feed and water (<1 µg As L<sup>-1</sup>).

### 2.2. Poultry litter sampling and analysis

Samples of poultry litter (excreta mixed bedding material) were collected from each pen on 14, 28 and 35th days using a steel core of 10 cm diameter. Fifteen fresh samples were collected randomly up to the full depth of litter, and then total amount of litter in each pen was estimated by extrapolating the measurements to the whole pen area. These 15 litter samples, on each respective sampling day, were pooled together and a representative sample (a quarter of the composite sample; ~0.5 kg) was put into a double Ziplock® polyethylene bag, sealed and frozen at -20 °C until chemical analysis was performed. The remaining sample was put back into the pen and mixed well with the litter. The total litter amount was also determined at 35th day (clean out day) by both estimating through sampling method employed for day 14, 28 and 35, and physically weighing all the litter collected from each pen. Total litter weight measured at 35th day was higher than the weight estimated through sampling method. Therefore, a factor of 1.4 was used, which represented an average ratio of measured to estimated poultry litter weight from 16 pens, to calculate the weight of poultry litter produced

**Table 1**  
Composition of different growth stage poultry feeds used in the experiment to feed chickens for 35 days.

Ingredient (%)	Control			Rox		
	Starter <sup>a</sup>	Grower <sup>b</sup>	Finisher <sup>c</sup>	Starter	Grower	Finisher
Corn, yellow grain	18	18	15	18	18	15
Fat, vegetable	3.8	3.4	4.1	3.8	3.4	4.1
Fish meal, menhaden	3.0	5.0	3.5	3.0	5.0	3.5
Soybean meal	26.9	16.2	15.1	26.9	16.2	15.1
Wheat, hard grain	43	53	58	43	53	58
Calcium carbonate	1.5	1.0	1.0	1.5	1.0	1.0
Dicalcium phosphate	1.5	1.0	1.1	1.5	1.0	1.1
Sodium chloride	0.43	0.34	0.36	0.43	0.34	0.36
L-Lysine	0.23	0.15	0.15	0.23	0.15	0.15
DL-Methionine	0.23	0.10	0.09	0.23	0.10	0.09
L-Threonine	0.05	0.10	0.03	0.05	0.10	0.03
Broiler vitamin premix (0.5% inclusion)	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride premix (0.5% inclusion)	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin E 5000 IU kg <sup>-1</sup>	0.30	0.30	0.30	0.30	0.30	0.30
Generic enzyme (0.5% inclusion)	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostat (Amprol)	0.05	0.05	0.05	0.05	0.05	0.05
Growth promoter (Roxarsone)	0.000	0.000	0.000	0.005	0.005	0.000

<sup>a</sup> 0–14 days.

<sup>b</sup> 15–28 days.

<sup>c</sup> 29–35 days.

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