



## Effect of vitamin E supplementation on growth, nutrient utilization, mineral balance and immune status of arsenic exposed goats

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### ABSTRACT

This experiment aimed to investigate ameliorative efficacy of vitamin E in the goat (*Capra hircus*) kids exposed to arsenic (As). Twenty-one Black Bengal male goat kids (4–6 months age,  $6.71 \pm 0.49$  kg body weight) were divided into seven blocks of three animals in each after arranging them in an ascending order on the basis of their body weight and then one animal from each block was randomly allotted to three groups. The kids were fed either basal diet as such (control, T1), or supplemented with 60 mg As/kg diet (T2) and 60 mg As/kg diet + 250 IU vitamin E/kg diet (T3) for 180 days including a 6 days metabolic trial. Daily feed intake and fortnightly body weight were recorded throughout the experimental period. To assess humoral immune response, animals were inoculated with *Pasteurella multocida* inactivated antigen after 135 days of experimental feeding and blood samples collected on day 0, 7, 14, 21, 28 and 35 post-inoculation. For assessing cell mediated immune response, kids were exposed to phytohaemagglutinin-p antigen after 170 days of experimental feeding and skin fold thickness was measured up to 96 h of exposure. Growth rate, feed conversion efficiency and intake and digestibility of different nutrients viz. dry matter, crude protein, ether extract, acid detergent fibre and neutral detergent fibre did not vary ( $P > 0.05$ ) among the three groups. However, absorption as well as retention of As were higher ( $P < 0.001$ ) in both the As exposed groups (T2, T3) as compared to control group. Addition of As also adversely affected the retention of zinc ( $P = 0.024$ ), cell mediated immunity ( $P = 0.014$ ) and humoral immune responses ( $P = 0.024$ ), which were largely ameliorated by vitamin E supplementation.

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**Abbreviations:** ADF, acid detergent fibre; ADG, average daily gain; AOAC, AOAC International; As, Arsenic; BW, body weight; CMI, cell mediated immunity; CP, crude protein; DM, dry matter; DTH, delayed type hypersensitivity; EE, ether extract; ELISA, enzyme linked immunosorbent assay; NDF, neutral detergent fibre; OM, organic matter; SEM, standard error of mean.

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## 1. Introduction

Arsenic (As), one of the most toxic elements, is rapidly emerging as a serious environmental pollutant. Groundwater in more than 30 countries has been reported to contain As at levels which are much higher than its permissible/safe limits (Datta et al., 2010; Bundschuh et al., 2012; Rodríguez-Lado et al., 2013) leading to its increased levels in feeds and fodders (Bergqvist and Greger, 2012; Ghosh et al., 2013) thereby increasing its level in blood and body tissues (Datta et al., 2010; Rajaganapathy et al., 2011; Mohanta et al., 2013) of the animals. Ruminants with chronic exposure of As suffer with anorexia along with digestive disturbances, liver and kidney dysfunctions (Gupta, 2007; Goyer and Clarkson, 2008). These adverse effects of As are due to accelerated production of free radicals in the body leading to oxidative stress (Jomova et al., 2011; Naujokas et al., 2013). Vitamin E is a potent anti-oxidant and protects body tissues against oxidative damage by reducing free radicals produced by toxic elements such as lead and cadmium (Flora, 2011).

Toxic minerals alter normal nutrient metabolism, particularly that of other minerals (Cui and Okayasu, 2008; Suttle, 2010). Apparent digestibility of dry matter (DM), crude protein (CP) and calcium (Ca) was significantly reduced in 100 and 150 mg/kg fluorine-added groups (Tao et al., 2005). However, information on the effect of arsenic on utilization of nutrients is scanty (Gonzalez et al., 1995; Mishra et al., 2004). Therefore, this study was conducted to test the efficacy of vitamin E as an ameliorative agent on growth rate, nutrient utilization and immune status of kids exposed to As.

## 2. Materials and methods

### 2.1. Animals, feeding and management

Twenty-one Black Bengal male goat (*Capra hircus*) kids (4–6 months,  $6.71 \pm 0.49$  kg body weight) were divided into three groups of seven animals each on the basis of their body weight (BW). To correct for variation in the body weight in the three groups, initially all the animals were arranged in the ascending order of their body weights and divided into seven blocks of size three each. Then, one animal from each block was randomly placed in either group T1, T2 or T3.

The experiment was conducted after approval of Institutional Ethics Committee and the Committee for the Purpose of Control and Supervision of Experiments on Animals in India. Kids were maintained under similar managerial conditions in a well ventilated shed with cemented floors and individual feeders, except during the metabolism trial period, when they were kept in metabolic cages. The animals were treated for both ecto and endo-parasites before the start of the experiment, and subsequently at regular intervals and vaccinated for common animal diseases at the start of the study.

Kids were offered a common basal diet comprising of concentrate mixture (450 g/kg wheat bran, 270 g/kg ground maize grain, 250 g/kg deoiled soybean meal, 20 g/kg mineral supplement and 10 g/kg common salt), 100 g wheat straw and about 250–300 g green maize (*Zea mays*) fodder daily to meet their nutrient requirements (NRC, 2007) for 50 g daily live weight gain. Experimental feeding was similar in all the groups except for addition of 60 mg arsenic (as aqueous solution of sodium arsenite)/kg in the diet of T2 and T3 animals. Kids in group T3 were additionally supplemented with 250 IU vitamin E/kg diet. All the kids were fed the same basal diet for 28 days before starting the experimental feeding which was continued for 180 days, during which body weight of the animals were recorded at fortnightly interval. The amount of concentrate mixture required for each animal was revised at 15-day intervals, based on their BW. The amount of As and vitamin E supplemented was also revised at fortnightly intervals, depending upon the DM intake of the individual kids. Clean and fresh drinking water was offered *ad libitum* at 10.00 and 15.00 h daily.

### 2.2. Metabolism trial

After 105 days of experimental feeding, a metabolic trial of six days duration was conducted involving quantification of feed offered andorts along with faeces and urine voided by kids during the preceding 24 h to determine nutrient digestibility and balances of nitrogen (N) and different minerals i.e. Ca, Phosphorus (P), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), selenium (Se) and arsenic (As) on four kids randomly selected from each group, kept in metabolic cages with facilities for separate collection of faeces and urine. The kids were adapted for 5 days to the cages before actual sampling was done. Representative samples of feed, orts, faeces and urine were brought to the laboratory in plastic containers to avoid metal contamination. The DM content was determined daily by drying a suitable aliquot of the feed, orts and faecal samples at  $80 \pm 5^\circ\text{C}$  in a hot air oven. The dried samples of feeds and faeces of each animal for 6 days were pooled, ground in a Willey mill to pass through 1 mm sieve and stored in airtight plastic containers for further analysis. For N determination in the faeces, an appropriate aliquot of the wet faeces was weighed, mixed with 10 mL of 1: 5 sulphuric acid and pooled into a previously weighed air tight container. A suitable aliquot of urine sample of each animal was transferred daily into Kjeldahl flask containing 50 mL concentrated sulphuric acid for N determination and another aliquot was pipetted into a plastic container daily for each animal and kept under refrigeration for analysis of minerals.

### 2.3. Analytical procedures

Feeds, orts and faeces samples were analyzed using standard procedures of AOAC (2005): DM (934.1) after drying at  $95\text{--}100^\circ\text{C}$  for 24 h; N (2001.11 and 984.13) by a Kjeldahl method after acid hydrolysis; ether extract (EE; 920.39) after

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