Effect of Arsenic on Immunity, Oxidative Enzyme and Various Hematological Parameters in Cross Bred Calves

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ABSTRACT : An experiment was conducted on crossbred male calves to study the effect of arsenic (As) on immunity status and certain hematological parameters. Ten crossbred male calves of 3-4 months of age were distributed into two equal groups. Group I was kept as control, whereas, group II was supplemented daily with 50 ppm As (as As_sO_3) up to 90 days, in the diet. Calves of both groups were fed as per ICAR standards and their requirements were fulfilled by feeding concentrate mixture and green oats. All calves were kept under similar managemental conditions. Blood samples were collected at fortnightly intervals to estimate various haematological parameters and superoxide dismutase (SOD) enzyme activity. Serum Ig and serum glutamic pyruvate transaminase (SGPT) were also measured. Cell-mediated immune responses of the calves were monitored at 0, 45 and 90 of experimental feeding, through lymphocyte proliferation. No change in blood total leukocyte counts (TLC), differential leukocyte counts (DLC), packed cell volume (PCV), haemoglobin (Hb) and SGPT was observed with As supplementation. A decrease in SOD activity was noticed in group II calves. Stimulation index (SI) for lymphocyte proliferation decreased from 1.14 to 0.79 in group II calves during 90 days experimental feeding , whereas, there was no change in SI values in group I indicating significant decrease in immune response of As supplemented calves. Blood As concentration increased in group II calves with the decrease in immune response. Short term supplementation of As to growing calves suggested suppressive effects on cell-mediated immunity. However, long term experiments are required to demonstrate clearly the efects of this toxic metal in calves. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 4 : 497-501)***

Key Words : Arsenic, Calves, Hematology, Immunity, SOD, SGPT

INTRODUCTION

With rapid industrialization, technological advancement and unprecedental increase in population, pollutants such as heavy metals are increasing in the environment. Besides this, ground water also poses a major threat of exposing animals and human beings to toxic levels of arsenic in some geographic locations (Dipankar et al., 1996; Singh et al., 1998; Annon, 2003).

Now a days, major concern with regard to pollutants is towards subclinical exposure of these metals. Continuous exposure to heavy metals even at normal recommended or maximum tolerable levels also may have deleterious effect on various physiological functions of the living beings. Immune system and oxidative stress components are considered to be sensitive indicators of toxicity especially for environmental contaminants and pollutants (Chauhan and Mahipal 1994; Ahmad, 1995). Arsenic has been reported to induce conditions leading to defective cell mediated immunity in various cell systems (Yu et al., 2002) Decreased phagocytic index was observed in rats exposed to As as sodium arsenate (Sen gupta and Bishayi, 2002) and in chicks exposed to As through drinking water (Vodela et al., 1997). Excessive Arsenic is also known to affect the liver function of living organisms. Information relating to its effects on ruminants is meagerly reported, therefore, an attempt has been made to study the effect of As administration on immunity status, superoxide dismutase activity and various hematological parameters in growing crossbred calves.

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MATERIALS AND METHODS

Feeding and management of calves

Ten growing male crossbred calves (Holstein Friesian× Sahiwal) of about 3-4 months age were selected from the herd maintained at NDRI, Karnal and were randomly distributed into two groups of five each. Prior to the experimental feeding, the animals were dewormed with required doses of albendazol and butox.

All animals were offered concentrate mixture (CP 21%, TDN 70%) consisting of maize grain 33%, ground nut cake 33%, wheat bran 31% and mineral mixture 3%. Besides concentrate mixture, green oats were offered *ad libitum* to meet their nutrient requirements as stipulated by ICAR feeding standards (1998). Group II calves were daily supplemented with 50 ppm As (as As_sO_3) in their diet. To ensure complete consumption of As, the calculated amount of As_2O_3 for each treatment group animal was mixed with little quantity of concentrate mixture in the trough and was offered to the calves. Only after its consumption, the rest of concentrate mixture was offered to the calves. Weighed quantity of green oats were offered to the animals in the afternoon.

Collection of blood samples

Blood samples were drawn from jugular vein at fortnighty intervals in two sets of test tubes; one set with anticoagulant heparin and other without anticoagulant for examining TLC, DLC, Hb, PCV and SOD. Serum was harvested from the blood without anticoagulant and total immunoglobulin content (Ig) and serum glutamic pyruvate transaminase (SGPT) activity was estimated. Cellular immune response was estimated in blood samples collected at 0, 45 and 90 days of the experimental feeding.

Analytical techniques

Blood TLC and DLC were counted as per the method described by Jain (1986). Packed cell volume was determined using Wintrobe tubes which were filled with freshly collected blood and centrifuged at 3,000 rpm. The volume of packed cells was noted (Schalm, 1965). Hb concentrations in blood samples were determined by acid haematin method (Oser, 1979) in which blood is diluted with dilute HCl to split hemoglobin into heme and globin. Heme was further converted to acid hematin (brown coloured compound) and compared with the standard colour in a hemometer. The method of Marklund and Marklund (1974) was followed for the measurement of SOD activity. For the estimation of SOD, red blood cell lysate was prepared by centrifuging 2 ml of freshly drawn blood at 5,000 rpm for 15 minutes in a refrigerated centrifuge and washing thrice with cold normal saline solution. The reaction mixture contained different concentrations of appropriately diluted blood lysate from 0.2 to 2.0 ml which were made 3 ml with tris buffer (50 Mm; pH 8.2) and 0.2 ml (2 mM) pyrogallol. A standard was prepared without addition of sample. The rate of auto-oxidation of pyrogallol was taken from the increase in absorbance at 420 nm against reference cuvette containing 3.0 ml tris buffer with the help of Cary-1 Varian (Australia) double beam UV- visible spectophotometer. The increase in absorbance was 0.02 min^{-1} in the absence of SOD. A unit of enzyme was defined as amount of enzyme that inhibited the reaction by 50%. SGPT activity was estimated by using diagnostic reagent kit supplied by Span-Diagnostic Ltd, India. Total Ig in serum was estimated by precipitation method of McEvan and Fisher (1970). Test reagent was prepared by 4.1 ml of 5% zinc sulphate and volume was made to one liter. For turbidity development, 0.1 ml plasma was mixed with 12 ml test reagent and optical density was measured at 460 nm after keeping for one hour. Standards (4-40 mg/ml) were prepared in foetal calf serum and were processed similar to samples. Cell mediated immune response was calculated as stimulation index (SI) during blastogenic response of lymphocytes cell culture on stimulation with mitogen. The incorporation of ^{3}H- thymidine in lymphocyte DNA during proliferation was measured (Larson, 1979). For lymphocyte proliferation assay, total and differential leukocyte were counted in blood samples to calculate number of lymphoytes. Blood samples were diluted with culture media (consisting of 1 vial RPMI, 2.20 g sodium bicarbonate, 2.38 g HEPES, 0.11 g sodium pyruvate, 0.58 g glutamine, 0.06 g pencillin and 0.1 g streptomycin) containing 5% foetal calf serum to give 1×10^{6} lymphocytes/ml. To each well, 100 µl of diluted blood was added, followed by addition of 100 µl mitogen (20 µg concanavalin A) or cell media. Culture plates were incubated in sterile atmosphere containing 5% CO_2 for 72 h at 37°C. ^{3}H-thymidine (0.5 µci) in 10 µl media was added to each microculture well 18h before terminating incubation to estimate DNA synthesis during blastogenesis. Thereafter cultures were harvested by adding 50 µl 30% TCA to each well. Contents were filtered through glass fibre filter paper and washed with saline and methanol. Filter papers were transferred to scintillation vials and were dried in oven. Then, 10 ml scintillation fliud was added to each vial and activity was counted in automatic Tricarb scintillation counter (Model, 1,600 CA, Packard, USA). Blastogenic activity was expressed as stimulation index (SI), which was obtained by dividing mean CPM in culture with mitogen by mean CPM in culture without mitogen.

Blood arsenic concentration was measured in digested samples by flourometric method as described by Pal et al. (1996). Five ml blood samples were digested in closed tubes as recommended by AOAC (1995) and the volume was made to 20 ml with double distilled water. The digested sample (5 ml) was evaporated to dryness in water bath. Then, complexation with catechol solution and Na_2 EDTA was done. The sample was dried and extracted with toluene via the formation of ion-associate with acridine orange (ACO) followed by regeneration of ACO by H_2SO_4. (As (v)-catechol-ACO) in toluene gave bright yellow flourescence in alkaline medium which was monitored at 480/530 nm using Cary-1 Varian (Australia) double beam UV- visible spectophotometer. Standard solutions of As (V) were prepared in the range of 4-400 ppb.

The data obtained was analysed statistically as Randomized block design for sources of variation between two treatments, seven periods and five replicates (Snedecor and Cochran, 1980). All the results were interpreted for interaction also.

RESULTS AND DISCUSSION

When As (as As_2O_3) was supplemented at 50 ppm level for a period of 90 days in the diet of crossbred calves, no clinical sign of toxicity was observed. Periodic changes in PCV, Hb, TLC and DLC are presented in Table 1. The values of these parameters were within the normal range (Swenson and Reece, 1996). The statistical analysis of the data indicated no significant difference due to treatment.

Table 1. Effect of As supplementation on hematological parameters of calves

Days	Hb (%)	PCV (%)	TLC/ml	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)	Monocytes (%)
Group I								
0	8.7±0.3	25.4±2.4	8,621±1,171	50.6±11.9	32.6±1.1	9.2±0.8	0.2±0.2	7.2±0.8
15	8.2±0.3	33.8±0.9	10,910±717	56.4±2.4	32.0±2.3	7.4±0.9	0.2±0.2	3.8±0.6
30	8.1±0.1	34.0±1.6	11,680±1,932	55.4±2.8	32.6±1.9	7.6±0.9	0.4±0.2	4.4±1.1
45	8.0±0.1	32.6±0.9	11,510±1,647	52.4±3.1	32.4±2.2	10.4±0.9	0.2±0.2	4.8±0.8
60	8.0±0.2	30.0±0.9	11,178±1,471	55.2±2.1	29.0±1.1	8.6±0.7	0.2±0.2	7.0±1.1
75	8.0±0.2	30.8±0.8	12,700±867	54.6±2.2	30.2±2.6	8.2±0.8	0.6±0.4	6.4±1.2
90	8.0±0.2	35.0±1.3	11,596±1,170	49.0±1.8	33.6±1.1	8.6±0.5	0.6±0.2	8.0±1.0
Group II								
0	8.5±0.9	29.0±2.2	11,670±2,106	50.6±3.4	33.0±2.3	9.4±0.8	0.4±0.2	6.4±0.7
15	7.8±0.4	34.2±2.5	11,210±795	57.8±2.0	31.6±2.1	6.4±1.1	0.4±0.2	4.2±1.1
30	8.3±0.2	35.4±1.8	11,530±1,514	55.6±2.5	31.6±2.4	8.6±1.2	0.4±0.2	4.2±0.7
45	8.5±0.4	33.0±1.4	12,410±830	52.0±3.1	32.8±2.1	12.0±1.9	0.2±0.2	3.0±1.0
60	8.5±0.1	31.4±0.9	11,966±1,091	55.2±2.8	29.6±2.4	8.0±0.6	0.4±0.2	6.8±0.8
75	8.2±0.1	31.6±1.0	14,390±2,626	55.2±2.3	28.8±0.9	8.6±0.7	0.6±0.2	6.8±0.9
90	7.7±0.2	33.6±1.5	11,920±1,509	49.8±1.7	33.2±1.9	8.2±1.0	0.2±0.2	6.6±0.6
Significance test								
Group I vs. group II	NS	NS	NS	NS	NS	NS	NS	NS

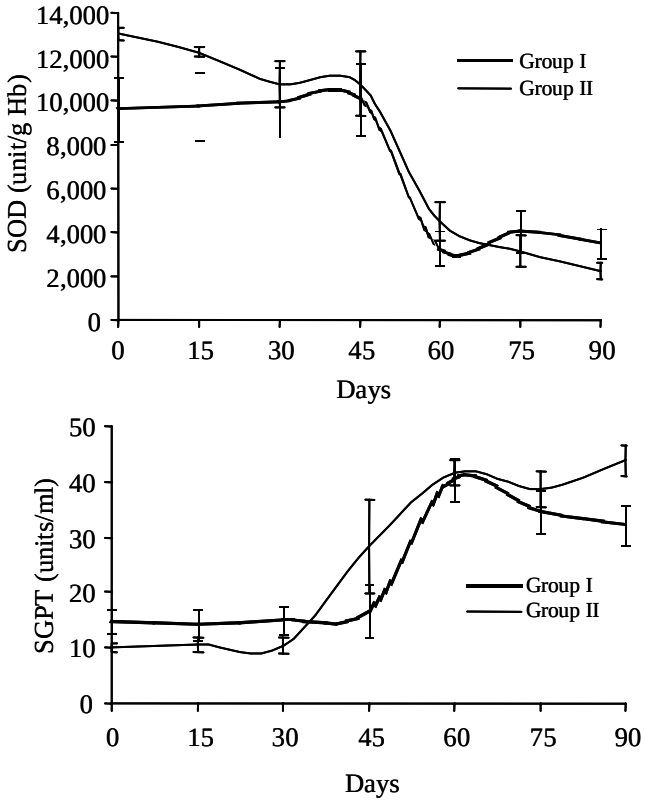

Figure 1. Effect of As supplementation on SOD and SGPT enzymes in calves.

Satake et al. (1997) reported that chronic toxicity of As resulted in distruction of RBC and bone marrow cells leading to anemia, but in this study, no such observation was recorded. Scultz et al. (2002) also reported reduced mean corpuscular volume (MCV) in red blood cells of rats given inorganic As at the level 2-6 mg/kg body weight.

The results of blood SOD and SGPT activity measured at fortnightly interval are presented on Figure 1. At 0 day, SOD activity (unit/g Hb) was 9,603 and 13,035 in group I and II respectively. The data obtained at periodic intervals revealed that SOD activity decreased gradually in group II, even though the initial values were higher in this group. Though there was a sharp decline in SOD activity in both the groups on 60th day and thereafter, but, the decline was more noticeable in group II. The decreased activity may be correleted with the increased enviornmental stress as there was sharp increase in enviornmental temperature Perusal of the data indicated that though the effect was non-significant due to treatment, but it had an indication of increasing oxidative stress in treatment group. As supplementation both as trivalent or pentavalent form altered SOD activity (Zaman et al., 1995; Cobo and Castineira, 1997). Chaudhuri et al. (1999) also reported decreased SOD activity in brains of rats fed 0.05 to 3.0 ppm As in drinking water, but no such studies are reported in ruminants.

Mean SGPT activity (units/ml) was found to be 23.97 and 26.20 in group I and II (Figure 1). The differences between the two groups were non-significant though significant variation existed between periods. Serum glutamic pyruvate transaminase is an enzyme present in cytoplasm of hepatic cells and any sort of liver damage will increase SGPT concentration. Arsenate has been documented to disturb pyruvate metabolism as pyruvate cycle is catalysed by SGPT. But in the present study, no adverse effect was recorded indicating that probably dose of As and/or period of its supplementation were not so critical to disturb the enzyme function.

The results of lymphocyte proliferation assay indicated stimulation index values of 1.45 and 1.14 at 0 day in group

Table 2. Changes in serum immunoglobulin and blood As concentration in calves

Days	Serum immunoglobulin (mg/ml)		Blood arsenic (ppm)	
	Group I	Group II	Group I	Group II
0	36.34±1.69	39.96±1.18	156.22±2.89	112.10±20.79
15	35.52±1.90	40.08±0.82	187.60±11.76	181.18±19.48
30	35.18±2.00	39.64±1.28	165.84±10.14	196.98±19.79
45	35.50±1.92	39.84±1.02	166.66±11.51	194.94±18.60
60	35.80±1.81	39.42±1.11	157.48±14.34	211.14±15.39
75	36.16±1.70	39.94±1.10	155.48±7.67	214.58±14.81
90	36.06±1.73	40.38±0.94	147.92±10.93	273.26±18.26
Significance	NS		CD treatment: 13.42; CD period: 25.11. CD (treatment×period): 35.05.	

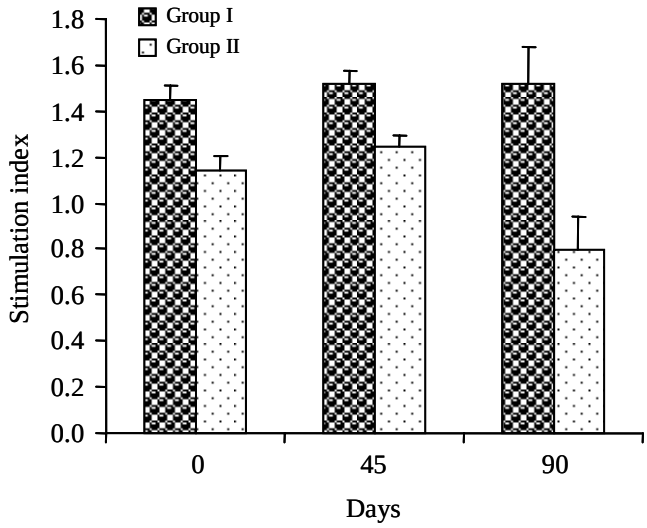

Figure 2. Effect of As supplementation on lymphocyte proliferation in calves.

I and II respectively. The values were almost maintainted in control group,throughtout experimental period of 90 days, but in the treatment group the values decreased to 0.794. The period of feeding also significantly reduced the stimulation index (Figure 2).

The periodic changes in total serum immunoglobulin (mg/ml) are given in Table 2, indicating no statistical difference due to treatment. The average Ig concentration was 35.79 and 39.89 in groups I and II, respectively. At the start of the experiment, the animals in group II had higher status with regard to total Ig content which persisted till the end of the experiment. Vodela et al. (1997) found suppresed natural, humoral and cell mediated immune response in hens given As through drinking water. No reports illustrating the effect of As supplementation on Ig level in ruminants are available.

The periodic changes in blood As concentration are presented in Table 2. Blood As concentration at the end of 90 days experimental feeding averaged 162.45 and 197.14 ppb in groups I and II, respectively, the differences being significant ($p<0.05$). Singh et al. (2001) reported blood As concentrations of 0.525 mg/L in cattle of Ghentugachi and Gontra villages of Nadia district, West Bengal. This area is highly polluted with As and higher blood As concentration was suggested to be due to the fact that 95-99% of absorbed As binds to the globulin of Hb in erythrocytes (Satake, 1997).

It was concluded that As @ 50 ppm in the diet had tendency to suppress the immune function of crossbred calves. Long term studies are required to clearly demonstrate the adverse effects of As on growth and antioxidative enzyme activity in calves.

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