EFFECT OF ARSENIC ON IMMUNITY, HAEMATOLOGICAL PARAMETERS AND ITS EXCRETION IN GROWING CROSSBRED CALVES

THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF

MASTER OF VETERINARY SCIENCE

IN

DAIRYING

(ANIMAL NUTRITION)

BY

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KARNAL – 132 001 (HARYANA), INDIA

2003

REGN. NO. 2080102

Dedicated to

My Parents

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ACKNOWLEDGEMENT

Words will fail to express my deepest sense of gratitude to my esteemed guide ***Dr. Veena Mani****, Senior Scientist, Dairy Cattle Nutrition Division, National Dairy Research Institute Karnal. I feel proud to be her student, without whose erudite and sagacious guidance, persistent help, calm and patient understanding, this work would not have been a success.*

My profound respect and sincere gratitude is extended to the members of advisory committee, Dr. Harjit Kaur, Principal Scientist, DCN Division, Dr. Aruna Chhabra, Principal Scientist, DCN Division, Dr. Bimlesh Mann, Senior Scientist, DC Division and Dr. V.K.Kansal, Principal Scientist, ABC Division for their concern, continuous advice and encouragement during the course of this study.

I am thankful to Dr K.K.Singhal, Head, DCN Division for providing necessary facilities during the course of this study.

I am thankful to all the staff of DCN Division for their help during this study.

I would like to convey my thanks to Barun, Neeraj and Nitin with whom I have spent so many colourful days of my stay at NDRI.

I gladly acknowledge the love and affection rendered by Tusar Bhai and Bhauja, Ashok Bhai and Bhauja, Bhabes Bhai, Nira Bhai and Bhauja, Rashmi didi and Satyaswarup.

Rhythmic beats of my heart evoke a sense of heartiest complements to the pillars of the loveliest triangle i.e,,Kallol Bhai,. Prajna Nani and Anjali Nani for their love, affection and helping hands.

My special appreciation goes to Shritapa nani and Binayak bhaina who deserves thanks for keeping my morals always high. I also extend my thanks to my near and dear ones.

Emotions are surpassing expression for the self-abnegation, sacrifice; my ***PARENTS*** *have made to make me see this horizon of science in a rain bowish coloured imagination.*

Place: Karnal
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Chaudhury Shripati Mishra
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CONTENTS

Chapter	Title	Page No.
1.	**INTRODUCTION**	**1-3**
2.	**REVIEW OF LITERATURE**	**4-17**
	2.1 Physical and chemical properties of arsenic	**4**
	2.2 Sources of arsenic contamination in the diet of animals	**5**
	2.2.1 Arsenic content in the environment	**8**
	2.2.2 Recommendations for level of arsenic	**8**
	2.3 Metabolism Of Arsenic In The Animal Body	**8**
	2.3.1 Tissue levels of Arsenic	**9**
	2.4 Arsenic toxicosis in animals	**10**
	2.4.1 Chronic toxicity	**10**
	2.4.2 Acute toxicity	**11**
	2.4.3 Synergistic or antagonistic effects of other elements with arsenic	**12**
	2.5 Effect of Arsenic on growth	**13**
	2.6 Effect of Arsenic on Immunity	**14**
	2.6.1 Effect of arsenic on oxidative stress	**15**
	2.7 Effect on certain haematological parameters	**16**

3.	**MATERIALS AND METHODS**	**18-31**
	3.1 Selection and housing of animals	**18**
	3.1.1 Details and distribution of experimental animals	**18**
	3.1.2 Composition of concentrate mixture	**19**
	3.1.3 Feeding Schedule	**19**
	3.2 Effect of arsenic administration on growth performance	**20**
	3.3 Effect of arsenic administration on Immunity status of animals	**20**
	3.3.1 Measurement of cell mediated Immunity by lymphocyte proliferation assay	**20**
	3.3.2 Zinc sulphate turbidity for estimation of total immunoglobulins in calves	**22**
	3.4 Analysis of hematological parameters	**23**
	3.4.1 Blood Collection	**23**
	3.4.2 Analysis of blood/ serum	**23**
	3.4.2.1 PCV and Hb	**24**
	3.4.2.2 TLC and DLC	**24**
	3.4.2.3 Arsenic content in blood	**24**
	3.5 Estimation of superoxide dismutase activity in blood	**27**
	3.6 Assay of Serum glutamate pyruvate transminase (Alanine transminase)	**28**
	3.7 To study the excretion pattern of arsenic	**29**
	3.7.1 Collection of feed, faeces and urine samples	**30**
	3.7.2 Aliquoting of faeces	**30**
	3.7.3 Aliquoting of urine	**30**
	3.7.4 Estimation of arsenic content in hair, feed, fodder, faeces and urine samples	**30**

4.	**RESULTS AND DISCUSSION**	**32-40**
	4.1 Growth Performance of Calves	**32**
	4.1.1 Changes in Body Weight	**32**
	4.2 Immunity of Calves	**33**
	4.2.1 Lymphocyte Proliferation Assay	**33**
	4.2.2 Total Immunoglobulin (Ig) Level in Serum	**34**
	4.3 Effect of Dietary Arsenic Administration on haematological Parameters	**34**
	4.3.1 Haemoglobin	**34**
	4.3.2 Packed Cell Volume (PCV) of Blood	**35**
	4.3.3 Total Leukocyte Count (TLC)	**35**
	4.3.4 Differential Leukocyte Count (DLC)	**36**
	4.3.5 Blood Arsenic Concentration	**36**
	4.4 Hair Arsenic Concentration	**37**
	4.5 Arsenic Balance	**37**
	4.6 Activity of Serum Glutamate Pyruvate Transminase (SGPT)/Alanine Transminase (ALT)	**39**
	4.7 Activity of Superoxide Dismutase	**39**
	4.8 Clinical Manifestation	**40**
5.	**SUMMARY AND CONCLUSIONS**	**41-41**
	5.1 Summary	**41**
	5.2 Conclusions	**42**
6.	**BIBLIOGRAPHY**	**I - VII**

LIST OF TABLES

Table No.	Title	After Page No.
2.1	Effect of arsenic administration on uminants	11
4.1	Effect of dietary arsenic supplementation on growth (kg body weight) of calves	33
4.2	Analysis of variance for growth (Mean sum of squares)	33
4.3	Effect of dietary arsenic supplementation on lymphocyte proliferation assay in calves	33
4.4	Analysis of variance for lymphocyte proliferation assay (Mean sum of squares)	33
4.5	Effect of dietary arsenic supplementation on total immunoglobulin level (mg/ml) in serum of calves	33
4.6	Effect of dietary arsenic supplementation on haemoglobin level (g/dl) in blood of calves	33
4.7	Analysis of Variance for blood parameters (Mean sum of squares)	35
4.8	Effect of dietary arsenic supplementation on packed cell volume (%) of calves	35
4.9	Effect of dietary arsenic supplementation on total leukocyte count (WBC/ml of blood) in calves	35
4.10	Effect of dietary arsenic supplementation on differential leukocyte count (%) of calves	37
4.11	Effect of dietary arsenic supplementation on blood arsenic level (ppb) of calves	37
4.12	Effect of dietary arsenic supplementation on hair arsenic level of calves (ppb)	37
4.13	Analysis of variance for hair arsenic level of calves (Mean sum of squares)	37
4.14	Arsenic balance in crossbred calves under metabolic trial	39
4.15	Effect of dietary arsenic supplementation on SGPT activity (units/ml of serum)	39
4.16	Analysis of variance for enzymatic activity (Mean sum of squares)	39
4.17	Effect of dietary arsenic supplementation on superoxide dismutase activity (unit/g Hb)	39

LIST OF FIGURES

Figure	Title	After PageNo:
1.	Standard Curve of Serum Ig Level (mg/ml)	23
2.	Standard Curve of Arsenic (ppb)	27
3.	Standard Curve of GPT (units/ml)	29

INTRODUCTION

Arsenic is known for its various uses since ancient times. The arsenical compounds were chiefly known for their medicinal as well as toxic properties. The Charaka-Sanhiti, the most valuable ancient text on medical science, recommended Ala (Orpiment) and Manashila (Realgar) for external and internal medication (Bagachi, 1969). Arsenic (As_sO_3) was also the preferred poison because of its high toxicity and easy availability.

With the passage of time, arsenic became known for its other beneficial purposes. In the present scenario, Arsenic is an element which has got wide applications in various industries such as insecticide, fungicide, wood preservative, glass, alloys, synthetic paint, electroplating and pharmaceuticals. In the modern world, the most important source of arsenic to the environment is the smelting activity of copper, lead, cobalt and gold ores. The other sources of arsenic which are diverse in nature include ground water, sewage sludge, insecticides, wood preservatives, vehicular and thermal plant emissions and even some common industrial processes in food processing. All these sources of arsenic contamination finally lead to the exposure of animals and human beings to toxic levels of the heavy metal affecting their health adversely.

In the Indian subcontinent, the ground water poses a major threat of exposing animals and human beings to toxic level of arsenic in some geographical locations. In six district of West Bengal, groundwater contained higher arsenic levels than the WHO recommended level of 0.01 mg/litre of drinking water (Dipankar *et.al.,* 1996). In Gohana block, Haryana, the ground water is reported to contain 20-27.5 mg arsenic/litre water at several locations (Kamra *et.al.,* 2002). The arsenic affected areas in Chhattisgarh are restricted to the Dongargarh-Kotri rift zone (Acharya, 2002). High arsenic levels in groundwater have also been reported in some areas in Bhojpur district of Bihar (Dainik Bhaskar, 2003) and number of deaths have been recorded due to arsenic poisoning. Information is available from Rajnandgaon district (Madhya Pradesh) where arsenic contamination of groundwater was found to be 880 ppb (Chakraborti *et al.,* 1995). Reports are also there from Chennai

(Ramesh and Ramanathan, 2002) indicating higher levels (50 to 146 μg/litre) of arsenic in groundwater.

Even soil in 40 locations of Punjab under sewage water irrigation contained higher arsenic level as compared to the sewage free soils (Arora *et.al* 1995). It is obvious that increased arsenic content in sewage water and soil will also reflect in the crops grown under such conditions. Exposure to higher than the recommended levels of arsenic in feed as well as drinking water has deleterious effects on various physiological functions in animals and human beings.

Arsenic is reported to cause depression in growth rate of monogastric animals. Significant depression in growth rate of rats was observed when 100 ppm As was given through drinking water (Glattre *et al.,* 1995). This depression was highly correlated with altered thyroid function. However, the implication of As administration through feed or drinking water in farm animals is meagerly reported.

Arsenic has been reported to be responsible for defective cell mediated immunity and decreased percentage of T-helper cells in the body (Yu *et.al*. 2002). Immunosuppressive action of arsenic has been responsible for decreased spleenic phagocytic activity of macrophages (Sengupta *et.al.,* 2002). Arsenic administration results in delayed type hypersensitivity, decrease in MCV of RBC and decrease in the weight of organs like thymus and spleen which are the active sites for antibody production (Schulz *et.al.,* 2002). Decreased immunity makes the animals susceptible to diseases making them unthrifty and uneconomic. There are also altered haematological parameters in case of chronic exposure to arsenic. Some reports suggest an increase in the fragility of erythrocyte membrane by chronic arsenic administration. Arsenic toxicity is also held responsible for reduced growth and infertility in animals. Carcinogenicity has been reported because of arsenic toxicity in animals. The accumulating nature of arsenic in

animal tissues as well as its secretion in milk may pose great threats to animals and human beings. As is also responsible for altered liver function by binding with the thiol group of liver enzymes and some carrier proteins (Jones, 1954). Thus, chronic arsenic exposure leads to various adverse effects on health, production and reproduction in animals.

Although literature is available on adverse effects of arsenic on monogastric animals as well as human beings but information relating to its effects on ruminants is scanty. However with the increasing environmental pollution load, it is important that the effect of feeding arsenic should also be assessed in ruminants as well. This is highly essential as it can affect the rumen microbes as well as the host animal.

Taking into consideration the above said effect of arsenic on animals, a research work was planned on growing crossbred calves with the following objectives: -

1. To study the effect of arsenic administration on growth, haematological parameters and immunity of crossbred male calves.

2. To observe the excretion pattern of arsenic in crossbred calves.

REVIEW OF LITERATURE

Pollution is a global problem posing a threat of incalculable dimension for human health and quality of the environment. The industrialization, technological advancement and population explosion have been the root causes for environmental pollution in the last few decades. The ever-increasing need for industrial goods and demand for higher agricultural production have further aggravated the problem of environmental pollution. The above activities have led to the use of high chemical fertilizer application, mining activity and other physical processes, which contribute vast amounts of toxic metals such as arsenic, lead, mercury and cadmium to the natural ecosystem.

In the current study on the effect of arsenic on immunity, hematological parameters and its excretion from body, a comprehensive review of literature has been presented under the following subtitles.

2.1 Physical and chemical properties of arsenic

2.2 Sources of arsenic contamination in the diet of animals

2.3 Metabolism Of Arsenic In The Animal Body

2.4 Arsenic toxicosis in animals

2.5 Effect of Arsenic on growth

2.6 Effect of As on Immunity

2.7 Effect on certain haematological parameters

2.1 Physical and chemical properties of arsenic

Arsenic compounds are widely distributed in nature in various forms. It is considered as a heavy metal due to its specific gravity higher than 5 (specific gravity = 5.73). Its atomic number and atomic weight are 18

and 74.91, respectively. Arsenic occurs in nature in two forms i.e. trivalent and pentavalent. Its compounds may also occur in organic or inorganic form. The inorganic compounds of arsenic are more toxic than the organic ones. The common inorganic compounds of arsenic are arsenic trioxide, arsenites and arsenates while the major organic compounds of arsenic are arsanilic acid and dimethyl arsinic acid.

The most common compound of arsenic in use is the arsenic trioxide (As_2O_3). It occurs as an amorphous or crystalline, tasteless, odorless, white powder, sparingly soluble in water. With alkalis, it forms different arsenites. The arsenites are basic ingredients in the preparation of colouring pigments. The arsenates are commonly used in fungicides and wood preservatives. The arsanilic acid is known as a growth promoter and is added to animal feed at low levels.

2.2 Sources of arsenic contamination in the diet of animals

The sources of arsenic are diverse in nature. But the major contributors are mentioned below: -

Ores are the most abundant source of arsenic in nature. The quantity of arsenic associated with lead and copper ores ranges from traces to 2-3%, whereas gold ores contain upto 11% (Nriagu, 1994). Usually, during the smelting activity, arsenic is released to the environment. Some of the most commonly occurring arsenic bearing ores are Arsennargentite (Ag_3As), Chloannthite ($(Ni,Co)As_3$), Domeykite (Cu_3As), Loellingite ($FeAs_2$), Niccolite (NiAs), Sperrylite ($PtAs_2$), Orpiment (As_2S_3), Realgar (AsS), Adamite ($Zn_2As\ O_4OH$) and Enargite ($Cu_3As\ S_4$) (Nriagu and Azcue, 1990).

Arsenic contamination of ground water in several geographical regions around the world has been reported in the last few decades. Arsenic in groundwater above the WHO maximum permissible limit of 0.05 mg/litre is found in six districts of West Bengal, India (Dipankar *et.al* , 1996) and Smith 2000). However, Dhar *et al.* (1997) reported toxic levels of As in 27 districts of West Bengal. A few districts of M.P. are reported to have arsenic content of ground water upto 880 ppb (Charkraborti *et al.,* 1995). Groundwater in

Gohana block of Haryana is contaminated with arsenic in the range of 20.0 – 27.5 mg/l (Kamra *et al*, 2002). A recent report has indicated human beings in some villages of Bihar suffering from arsenic toxicity (Dainik Bhaskar, 2003).

The sewage water from industries and households contain high amounts of heavy metals. When this untreated sewage water is used for irrigation, there is a sharp increase in the content of toxic elements in the soil. In 40 sewage irrigated vegetable farming locations of Punjab, the soil samples were found to contain potentially toxic levels of As, Cr and Pb (Arora and Brar, 1995).

Supplementation of chemical fertilizers at high levels to crops has been a common trend in the Indian cultivation practices. Monoammonium phosphate application causes higher soil arsenic uptake by plants with a phosphorus-enhanced solid-phase arsenic release mechanism and phosphate fertilizer additions to soils containing lead arsenate pesticide residues can increase arsenic solubility (Creger and Peryea, 1994). On the contrary, supplemental magnesium reduces arsenic uptake in plants by 40-50% and decreases arsenic content by about 30% in bulbs or grains (Kiss *et.al,* 1992).

The reported arsenic content of groups of coals range from 3 to 45 mg/kg (Wedepohl, 1969). Despite various losses, approximately 2.5g arsenic is released to the atmosphere for every ton of coal consumed (Bertine and Goldberg, 1971). The smelting or refining activities to extract minerals in purified form also contribute vast amounts of arsenic to the environment. Lead smelters are especially great contributors of arsenic. A lead smelter is responsible for annual average deposition of 0.016 to 453g of As/ha within a range of 0.5-40 km from the smelter (Pilgrim and Hughes, 1994).

Sodium and potassium arsenite (Na_3AsO_3 and K_3AsO_3) and thioarsenites (derivatives of the hypothetical orthothio-arsenious acid, H_3AsS_3) are extensively used as weed-killers and as an insecticide for grains. Sodium arsenite solutions used as weed-killers may contain 14-40% of arsenic as As_2O_3. Sodium arsenate (Na_2HAsO_4) is sometimes used as the

poison on fly-papers and lead and calcium arsenates are extensively used as sprays for fruit trees. Arsenic in the form of monosodium methylarsonate (MSMA), disodium methylarsonate (DSMA), dimethylarsinic acid and arsenic acid is employed widely in the agriculture fields which is a common source of arsenic poisoning for the livestock grazing in these pastures or cultivated lands.

Despite immense controversy, arsenic acid is still in use in the preparation of wood preservative salts (Warner and Solomon, 1990) while sodium arsenite solution is used for debarking trees. Ingestion of ash from the burned wooden posts treated with arsenic containing preservative CCA (copper-chrome arsenate) can be lethal for cows (Hullinger *et.al.,* 1998).

Arsenic is an essential constituent of many organic and inorganic colouring agents such as King's yellow (As_2S_3), mineral blue or copper potassium arsenate, Scheele's green ($CuHAsO_3$) and Paris or emerald green [$Cu(AsO_2)_2\ Cu(C_2H_3O_2)_2$]. The Paris green is also used as an insecticide, an anthelmentic and a slug bait. Thus effluents from dyeing industry may be a source of As contamination.

Medicinal inorganic preparations of arsenic like fowler's solution and Donovan's solutions have been in wide use. Organic arsenical compounds (acetarsol, neoarsphenamine (salvarsan) and sulpharsphenamine (sulpharsen)) are employed for treating histomoniasis (blackhead), leishmaniasis, trypanosomiasis, amoebiasis and sleeping sickness. Arsenical dips (sodium and potassium arsenite and thioarsenites) for sheep and cattle are usually combined with sulphur and contain about 20% of soluble arsenic and 3% of insoluble arsenious sulphide. In the diluted form of use, the soluble arsenic concentration is commonly 0.25-0.5%, so any negligence in usage may increase the chances of toxicity in the animals.

Arsenic is used in making alloys with lead and copper. Arsenic is added in traces to lead-antimony grid alloys used in acid batteries and at a level of 0.02-0.5% to copper alloys to raise the recrystallisation temperature and corrosion resistance (Carapella, 1978). Highly pure arsenic metal

(99.99%) is used in the electronics industry in the form of gallium and indium arsenide, to form semi conductor compounds. Arsenical copper alloys are used extensively in locomotive fireboxes, condenser tubes, heat exchanger and distillation tubes. Arsenic is also in use as a decolorizing agent in glass and enamel industry. Therefore, the discharge of these industrial effluents may increase As content in that particular area.

2.2.1 Arsenic content in the environment

The soil arsenic content usually varies between 1.5 to 2 ppm. Most feeds contain less than 0.5 ppm arsenic and rarely exceed 1 ppm on fresh basis (Underwood, 1977). Arsenic is not readily absorbed from the soil by plant even when the arsenic level in soil is enhanced, but surface contamination is the key factor that causes high arsenic contamination above 0.5 ppm level (Underwood, 1973).

The arsenic concentration in seawater ranges from 2 to 5 ppb (Schroeder and Balassa, 1966). The arsenic content of commercial fish meals used in livestock production ranged from 2.6 to 9.1 ppm (air dry basis) (Lunde, 1968).

2.2.2 Recommendations for level of arsenic

The maximum allowable concentration of arsenic in drinking water as per WHO is 0.05 mg/litre (WHO, 1991) whereas it is 0.10 mg/litre as recommended by FAO (Pescod, 1992). For cattle, the maximal tolerable dietary arsenic level is 50 mg/kg and 100 mg/kg for inorganic and organic forms, respectively per kg of diet (NRC, 2001). Sheep and cattle do not find arsenic unpalatable and may even develop a taste for it (Clarke and Clarke, 1975).

2.3 Metabolism of arsenic in the animal body

The rate of absorption of inorganic arsenicals in the body depends on their solubility. Sodium arsenite is readily soluble and rapidly absorbed,

hence it is highly toxic. But arsenic trioxide, sparingly soluble in water and slowly absorbed, is largely excreted unchanged in the faeces.

Around 80% of the arsenic absorbed is stored in the body. It is most widely distributed in the tissues including the liver, abdominal viscera, bone, skin and particularly hair and nails. Arsenic has a tendency to accumulate in the liver. After storage in the liver, it is slowly released to other tissues. But in the case of long continued exposure, the major sites of arsenic accumulation are bone, skin and keratinised tissue such as hair and hoof. Arsenic continues to be present at high levels in these tissues long after its levels have come down to traces in urine and faeces (Grollman and Slaughter, 1947). Once deposited, arsenic is irremovable from keratinised tissues like hair.

Arsenic is excreted in the urine, faeces, sweat and milk. Urine and hair are the most useful specimens for chemical analysis even at low dietary levels of arsenic (Neiger and Osweiler, 1992). Arsenic is excreted in urine within 5-6 hrs of administration and can exist even in traces upto 8 days in the horse (Louw, 1952) and 14 days in cattle (Maas, 1947).

2.3.1 Tissue levels of Arsenic

Arsenic levels are higher in skin, hair, liver, kidney and spleen than in other tissues of animals exposed to arsenic. In cattle poisoned under field conditions, liver As levels were 3.5 to 60.4 ppm (dry weight) (Reagor, 1973). Cattle that had consumed 1.25 ppm dietary arsenic for 3 weeks had muscle arsenic levels of 0.2 ppm (dry weight) and all tissue concentrations were 1 ppm arsenic or less (Peoples, 1964). Cows consuming 18 ppm arsenic as dried manure of poultry that had received 3-nitro-4-hydroxyphenyl arsonic acid had no arsenic residue in milk after 5 days on the diet (Calvert and Smith, 1972). In the final report of ICAR ad-hoc project, Singh *et al.* (2000) reported blood level of As in the range of 0.087 – 0.779 ppm; while egg contained 0.0 to 1.604 ppm, milk contained 0.0 to 0.2 ppm and goat meat contained upto 2.943 ppm As in these two villages.

Few studies conducted on effects of administration of different chemical forms of As through different routes in ruminants are summarized in the Table 2.1.

2.4 Arsenic toxicosis in animals

The arsenicals differ widely in their toxicity basing on their form, absorption into the cells, rate of oxidation, rate of elimination, route of exposure, age of animals, nutritional status, duration of exposure and presence of any synergistic or antagonistic element in the feed.

Trivalent arsenicals, which specially block lipoate dependent enzymes, are generally more toxic than pentavalent arsenicals (Frost, 1967). Trivalent arsenical compounds combine with thiol group (SH) of lipoic acid moiety of lipothiamide pyrophosphate, an essential coenzyme in oxidative decarboxylation of pyruvic acid and α-ketoglutaric acid. Arsenic also appears to exert toxic action by attachment to sulphydryl groups of protein. The attachment is loose enough so that compounds with sulphydryl groups with greater affinity for arsenic can withdraw the tissue arsenic for urinary excretion.

2.4.1 Chronic toxicity

Chronic toxicity of arsenic in animals has mostly been reported in geographical locations where ground water is contaminated with arsenic.

Abnormalities detected in cattle in the villages of Nadia district of West Bengal where the groundwater arsenic level is well above the WHO recommended arsenic levels, are skin diseases of various types-skin eruption, black/ white spot in skin in neck, leg and other parts of the body, fall of hair from skin, abortion, anoestrus, anorexia, watery secretions from eye, poor health, weakness, diarrhoea, constipation and dark coloured urination (Singh *et al.,* 1998).

Abnormalities detected in goats include abortion, skin problems, white and black spot on body, sometimes diarrhoea with blood, stunted growth, weakness, anorexia, dark and cloudy urine (Singh *et al.,* 1998).

When the growth promoter, arsanillic acid, was offered to swine at the level of ten times the normal recommended level (100 ppm in feed) for 20 days, severe posterior paresis or quadriplegia was observed in several animals by day 15 (Ledet *et al.,* 1973). The tissue arsenic levels increased with time and the peripheral nerve tissues showed an affinity for arsenic.

2.4.2 Acute toxicity

Arsenic poisoning is commonly an acute, clinical syndrome and mostly accidental in nature. In most of the cases, death occurs prior to detection of the cause and proper treatment.

The signs of the inorganic arsenic toxicosis vary with the quantity and method of administration. The usual signs displayed by cattle that have been dipped in solutions containing excessive arsenic include colic pain, vomiting, diarrhoea, marked depression and dermatitis usually due to increased capillary permeability and cellular necrosis. The time until onset and severity are governed by the amount of arsenic absorbed through the skin (Kinsley, 1929).

Signs of acute ingestion toxicosis are similar. However, skin lesions are rarely present. The lesions at necropsy of acute cases include gastroenteritis, glomerular nephritis and dermatitis. Acute signs in horse include cerebral involvement and signs of intense pain with head banging (Lillie, 1970).

Animals may survive a high single oral dose that can be toxic with a short period of repeated exposures, although it has been suggested that tolerance of low oral doses may be increased by repeated oral doses (Clarke and Clarke, 1975). Following a single oral dose, almost all of the administered arsenic is excreted within a few days. Ruminants that survive arsenic intoxication by a single dose should be withheld from market for 2

Table 2.1 Effect Of Arsenic Administration On Ruminants

No. of animals	Age/ Weight	Quantity of elements	Source	Duration	Route	Effect	Reference
Cattle-2	400kg	1.17 mg/kg 2.34 mg/kg 4.68 mg/kg	Lead arsenate	126 days	Diet	No adverse effect --------do------- --------do-------	Marshall *et.al.*1963
Cattle-1	Mature	343 mg 1.367 g	As_2O_3	3 days	Drench	No adverse effect Increased milk arsenic	Fitch *et.al.* 1939
Cattle-4	Mature	40mg /head/day	Dried poultry Manure	18 days	Diet	No adverse effect	Calvert and Smith, 1972
Cattle-2	Mature	1.6 mg/kg 1.6 mg/kg 3.2 mg/kg 3.2 mg/kg	Arsonic acid Arsanillic acid Arsonic acid Arsanillic acid	3 days	Capsule	No adverse effect --------do------- --------do------- --------do-------	Calvert and Smith, 1972
Sheep-3	45kg	5.7 ppm 11.7 ppm 17.1 ppm 5.7 ppm 11.7 ppm 17.1 ppm 5.7 ppm 11.7 ppm 17.1 ppm	Potassium arsenite Arsanillic acid Arsonic acid	34 days	Diet	No adverse effect --------do------- --------do------- --------do------- --------do------- --------do------- --------do------- --------do------- --------do-------	Bucy *et.al.*, 1954
Sheep-2	45kg	17.1 ppm 34.2 ppm 68.4 ppm 17.1 ppm 34.2 ppm 68.4 ppm 17.1 ppm 34.2 ppm	Arsonic acid Arsanillic acid Potassium arsenite	63 days	Diet	No adverse effect --------do------- --------do------- --------do------- --------do------- --------do------- --------do------- --------do-------	Bucy *et.al.*, 1954
Sheep-1	35kg 30kg 28kg 30kg 32kg 25kg 34kg 30kg 28kg 29kg 30kg 32kg	142.4 ppm 284.8 ppm 570 ppm 1139 ppm 142.4 ppm 284 ppm 570 ppm 1139 ppm 142 ppm 284 ppm 570 ppm 1139 ppm	Arsonic acid Arsanillic acid Potassium arsenite	56 days	Diet	No adverse effect --------do------- --------do------- --------do------- --------do------- --------do------- Convulsion by 56th day, loss of wt Convulsion by 28th day No adverse effect --------do------- Wt. loss, ↓ feed intake Wt. loss, ↓ feed intake	Bucy *et.al.*, 1955

weeks and for 6 weeks following multiple doses (Selby *et al.,* 1974). Swine and poultry that have received arsenic as a growth promotant must be held 5 days following withdrawal before slaughter for market (AAFCO, 1978).

Most non-ruminants are more susceptible to intoxication than are ruminants or horses. Arsanillic acid is the least toxic of the common arsenicals. The fatal dose for horse and cows are 300 grains (approximately 40 mg/kg body weight) arsenic per day as contrasted with 4-8 grains (approximately 6-12 mg/kg body weight) for sheep. Horse and cattle can ingest 20-30 grains (2.66 to 4 mg/kg body weight) arsenic daily continuously with no apparent ill effect (Reives, 1925).

The oral LD_{50} of arsanillic acid and 3-nitro-4-hydroxy phenyl arsonic acid in rats is about 800 and 100 mg of arsenic per kg of body weight respectively (Frost, 1953).

2.4.3 Synergistic or antagonistic effects of other elements with arsenic

Presence of some other elements or compounds in feed or water may interfere with the availability of arsenic and its metabolism in the body.

A study involving sheep in which the grazing animals were exposed to arsenic through the pasture, it was found that when feeds/grass contained arsenic>700 mg/kg dry matter, arsenic-selenium antagonism was evident (Yanchev *et al.,* 1992). In spite of 22% higher selenium intake of sheep, the selenium content of wool decreased by 17%. The Se level was as low as 98mg/kg wool whereas the normal value is 250mg/kg wool. This indicates antagonistic relationship of arsenic and selenium.

Meso-2, 3-dimercaptosuccinic acid (DMSA) and sodium 2,3-dimercaptopropane-1-sulfonate (DMPS) chelate with arsenicals making them unavailable to the body (Domingo, 1994). These two chelating agents have been effectively used therapeutically for metal induced developmental toxicity. The level of chelation may be affected by the form of arsenical in diet. The chelated compounds fail to affect the physiological processes like

their unchelated or free counterparts. This prevents the undesired toxic effects of the arsenicals. The dose rates of these chelating agents for counteracting the toxicity of different arsenicals are highly variable.

High fluorine level in feed or water is responsible for dental and skeletal flourosis in animals as well as human beings. But arsenic and fluorine in combination reduced the body weight of the rats significantly presenting a synergistic effect between arsenic and fluorine. (Li et.al, 1996).

Synergistic effect has also been reported between arsenic and copper. Rats exposed to dietary arsenite administration revealed that in comparison to controls with copper levels of about 10 μg Cu/g net weight of the renal cortex, dietary administration of arsenite upto 60 mg As/kg of diet for 3 weeks, increased cortical levels to 65 μg Cu/g net weight (Hunder et.al 1999).

2.5 Effect of Arsenic on growth

Arsenic content in food and drinking water supplied to animals has been found to adversely affect the growth of the animal significantly.

Drinking water containing low (0.8 ppm arsenic) or high (8.6 ppm arsenic) concentration of contaminant when offered to broiler breeders for ten weeks, a linear relationship indicated between increasing concentration of contaminant in drinking water and decreasing body weight of hens (Vodela *et al.,* 1997). The low concentration of contaminant significantly decreased egg production and egg weight and increased percentage of embryonic mortality. Although water consumption significantly decreased, the feed consumption was not affected at any level.

A study conducted on the toxic effects of arsenic compounds on cell division using Chinese hamster V79 cells revealed that inorganic arsenic (arsenite and arsenate) inhibited cell growth at very low concentrations whereas monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsine oxide (TMAO), which are methylated metabolites of inorganic arsenic were less cytotoxic than the inorganic arsenic compounds (Eguchi *et al.,* 1997). The cytotoxicity of the three methylated metabolites decreased as

the number of methyl groups increased. Arsenobetaine (AsBe) and arsenocholine (AsC) did not show any cytotoxicity. The methylated metabolites MMA, DMA and TMAO, induced mitotic arrest. Tetraploidy production was observed in cell exposed to DMA or TMAO. Arsenite, arsenate, AsBe and AsC did not induce mitotic arrest or tetraploidy.

Arsenic also imparts its deleterious effect on growth by altering the thyroid hormone tri-iodothyronine (T_3) and thryoxine (T_4). Seventy-eight wistar rats pretreated with arsenate (arsenic 100 mg/litre of drinking water) for four weeks showed the tendency of arsenic to accumulate in the thyroid tissue (Glattre *et al.,* 1995). Post mortem examinations showed that the thyroid tissue of rats pretreated with arsenic exhibited obvious toxic changes in comparison to minor or no changes in the control group. The T_4/T_3 ratio was found to be negatively correlated with arsenic ($P<0.012$). This altered thyroid hormone metabolism may affect the growth adversely.

2.6 Effect of As on Immunity

Arsenic has been found to induce apoptosis in various cell systems. This condition leads to a defective cell-mediated immunity and decreased percentage of T cell and T helper cell subpopulations in peripheral mononuclear cells thus adversely affecting the T cell survival and function in mononuclear cells (Yu *et al.*, 2002). An arsenic concentration higher than 1 µM induces release of tumor necrosis factor α from the mononuclear cells and causes cytotoxic effect on T cells.

Spleenic macrophages are the essential sites for trapping and concentrating foreign substances carried in the blood and for synthesis and release of antibodies to the circulation. In vivo exposure of rodents to sodium arsenite (0.5 mg/Kg body weight) decreases the phagocytic activity for ingestion and digestion of exogenous antigens such as whole microorganisms, as evident from the phagocytic index, 11444.55 ± 62.86 (in control) to 5555.5 ± 1571.33 in arsenic treated rates (Sengupta and Bishayi, 2002). Decreased phagocytic index indicates higher susceptibility of animals to diseases.

Effect of inorganic arsenicals on DNA synthesis in unsensitised human blood lymphocytes is biphasic. These chemicals at very low concentrations enhance blast formation and DNA synthesis. Maximum stimulating effect is observed at the concentration of 1 x 10^{-5}M to 2 x 10^{-6}M for sodium arsenite exposure whereas for sodium arsenate, the concentration varies form 1 x 10^{-5} M to 1 x 10^{-6}M (Meng and Meng, 2000). The longer the exposure of the lymphocytes to arsenicals, the lower the concentrations of arsenicals at which the maximum stimulating effect is found. It is observed that the stimulating effect of trivalent arsenic (sodium arsenite) is stronger than pentavalent arsenic (sodium arsenate). By inhibiting the DNA synthesis of lymphocytes, arsenic affects the cellular immunity adversely.

Supplementation of arsenic through drinking water has been found to suppress the natural, humoral and cell mediated immune response in broiler chicks (Vodela *et al.,* 1997). A deficiency of dietary vitamins and minerals causes increased sensitivity to the adverse effects of arsenic contaminated drinking water.

2.6.1 Effect of arsenic on oxidative stress

Oxidative stress is a major ill effect of high tissue arsenic level. In male wistar rats, mitochondrial dysfunction has been found to be responsible for oxidative stress related diseases. Supplementation of arsenic in drinking water resulted in binding of arsenic to thiol or SS-group in enzyme and proteins and releasing oxidant species during the redox cycling and metabolic activation process e.g. by cytochrome – P450 in liver (Cobo and Castineira, 1997).

Another mechanism of oxidative stress by arsenite, arsenate and the herbicide monosodium methyl arsenate (MSMA) mediated by generation of reactive oxygen intermediates which cause lipid peroxidation and cellular damage (Schlenk *et al.,* 1997). Here, metallothioneins (MTs) are induced by oxidative stress and act as scavengers of reactive oxygen intermediates. Even the responses to arsenate exposure showed a trend towards a dose dependent induction of MT and MT mRNA.

The oxidative stress by trivalent arsenic (As^{3+}, $NaAsO_2$) and pentavalent arsenic (As^{5+}, Na_2HAsO_4) in drinking water studied in an insect model (Musca domestica) after an exposure of 48 hours revealed alterations of the antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione transferase (GST) and glutathione reductase (GR) and increase in lipid peroxidation and protein oxidation. SOD (1.3 fold), GST (1.6 fold) and GR (1.5 fold) were induced by As^{3+} . Lipid peroxidation and protein oxidation which represent stronger indices of oxidative stress were elevated upto 2.9 fold (Zaman et.al. 1995). This evidence favours the pro-oxidant nature of arsenic.

A study on oxidative stress showed that arsenic as a pro-oxidant causes lipid peroxidation, protein and enzyme oxidation and GSH depletion in addition to DNA oxidation and formation of DNA adducts (Ahmad, 1995). A wasting syndrome is observed under sub-acute stress whereas in acute toxicity, vital physiological processes impaired are haemolymph melanization and diuresis.

2.7 Effect on certain haematological parameters

Arsenic has a tendency to bind to the erythrocyte membrane via sulphydryl group which is indicated by the decrease of the sulphydryl level of membrane proteins (Zhang *et al.,* 2000). The binding to the membrane induces changes in the fluidity of the membrane lipids and in the negative charge density in the outer surface of the membrane affecting the transport-system across it.

In wistar rats chronic inorganic arsenic administration reduced the Mean Corpuscular Volume (MCV) of red cells affecting the overall health status (Schulz *et al.,* 2002).

In wistar rats, arsenic (V) administration at the level of 5 μg/ 10 gm of food for 10 weeks increased plasma cholesterol level from 47.27 mg/ dl to 96.83 mg/dl (Aguilar *et al.,* 1997*)*. The sodium arsenite administration (20 mg/kg body weight) resulted in increased serum glucose level and serum aspartate aminotransferase activity but significant decrease in activity of

alkaline phosphatase and lactate dehydrogenase was observed. Thus, the arsenic affects various physiological processes by numerous complex mechanisms.

MATERIALS AND METHODS

The material used and experimental techniques followed during the investigation to study the effect of arsenic administration on growth, haematological parameters and immunity of crossbred male calves are presented in this chapter.

3.1 Selection and housing of animals

Ten growing male crossbred calves of 3-4 months of age were selected randomly from the herd maintained at NDRI, Karnal. All the calves were housed in well-ventilated separate pens. Each pen had a separate manger with high partition walls on both sides so that an animal could not have access to other animals' diet. The pens as well as the animals were washed thoroughly everyday. The experimental sheds were maintained in a very hygienic condition. Prior to the beginning of the experiment, the animals were dewormed with required dose of Albendazole and Butox.

3.1.1 Details and distribution of experimental animals

Treatments	**Calf No.**	**Date of Birth**	**Initial Body Wt. (kg)**	**Avg. Body Wt. (kg)**
T_1 (control)	6620	24.9.2002	70	76.5
	6621	26.9.2002	99.5	
	6623	1.10.2002	79	
	6633	24.10.2002	55	
	6634	31.10.2002	79	

T_2 (50 ppm As)	6617	15.9.2002	96.5	
	6622	30.9.2002	48	
	6624	8.10.2002	69	
	6632	21.10.2002	79.5	72.4
	6638	07.11.20002	69	

3.1.2 Composition of concentrate mixture (Supplied by Godrej Feed)

Ingredients

Ground nut cake	33.0 %
Maize grain	33.0 %
Wheat bran	31.0 %
Mineral mixture	2.0 %
Common salt	1.0 %

Nutrient requirement for calves with body weight gain of 550gm/day

Live weight (kg)	DM (kg)	DCP (g)	TDN (kg)	Ca (g)	P (g)
70	2.10	259	1.39	8	5
80	2.33	282	1.53	9	6
100	2.78	328	1.80	12	9
120	3.23	373	2.07	15	11
140	3.67	419	2.34	17	12

(Indian Council of Agricultural Research Feeding Standards, 1985)

3.1.3 Feeding Schedule

All the animals were offered their daily quota of concentrate mixture (containing 21% CP and 70% TDN) and green oat fodder to meet the nutrient requirements as stipulated by ICAR feeding standards (1985). The animals in two groups were offered two different dietary treatments as follows.

T_1: Control diet (concentrate mixture + green oat fodder)

T_2: Control diet + 50 ppm As as As_2O_3 in concentrate mixture.

Weighed concentrate mixture was offered at 9:30 a.m. The pre-weighed required quantity of arsenic trioxide was added to a small quantity of concentrate mixture in a plastic tub and offered to the animals. After the animal consumed this small amount, then the rest amount of concentrate mixture was given. This was done to ensure that the animal consumed the As everyday at the calculated level.

Green oat fodder in weighed quantity was offered to calves at 2:00 p.m.. Clean drinking water was offered twice a day at 9:30 a.m. and 3:00 p.m.

3.2 To study the effect of arsenic administration on growth performance

The body weights of the calves were recorded at weekly interval.

3.3 Effect of arsenic administration on Immunity status of animals

3.3.1 Measurement of cell mediated Immunity by lymphocyte proliferation assay

Cellular immune response was estimated in blood samples collected from calves on 0 day, 45 day and 90 day of the study. The treatment response was calculated as stimulation index (SI) during the blastogenic response of lymphocyte cell culture on stimulation with mitogen. The incorporation of ^{3}H-thymidine in lymphocyte DNA during proliferation was measured (Larson, 1979).

Chemicals

RPMI 1640, fetal calf serum (FCS), concanavalin A, penicillin, streptomycin, ^{3}H thymidine (specific activity 6.7 μci/mM), HEPES buffer, sodium pyruvate, glutamine, sodium bicarbonate, sodium chloride, methanol, 2,5-diphenyloxazole (PPO), 1,4-bis(5-phenyloxazoyl) benzene (POPOP), scintillation grade toluene.

Preparation of reagents

Media

One litre of cell culture media consisted of 1 vial RPMI, 2.20 g sodium bicarbonate, 2.38 g HEPES, 0.11g sodium pyruvate, 0.58 g glutamine, 0.06g penicillin and 0.1 g streptomycin. All components were mixed with gentle stirring and filtered through 0.22 μ filter paper under negative pressure. The filtered media was aseptically dispersed in bottles with minimum air space. At the time of use, FCS @ 5% was added to the media.

Mitogen

20 μg concanavalin A was prepared in media containing 5% FCS.

Procedure

Total and differential leukocytes were counted in blood samples to calculate the number of lymphocytes. The blood samples were diluted with cell culture media containing 5.0% FCS to give $1x10^6$ lymphocytes/ml. Culturing was done in microplates. To each well, 100 μl of diluted blood was added followed by addition of mitogen (100 μl) (20μg/ml) or media. The culture plates were incubated in sterile atmosphere at 5% CO_2 for 72 hours at 37°C, 0.5 μci of ^{3}H-thymidine in 10 μl media was added to each microculture well 18 hours before terminating incubation of cell cultures to estimate DNA synthesis during blastogenesis.

Culture harvest of lymphocytes

Cultures were harvested by adding 50 μl of 31% TCA to each well. This forms 5% of total volume in the well. The entire well contents were mixed and transferred to glass filter. Four washings were given to wells with normal saline. Thereafter, filters were washed with absolute methanol. Filters were transferred to scintillation vials and were allowed to dry overnight at 70°C in an oven. Ten ml of scintillation fluid was added to each vial. All the samples were counted in an automated scintillation counter. Blastogenic response was calculated as stimulation index.

$$SI = \frac{\text{Mean cpm in culture with mitogen}}{\text{Mean cpm in culture without mitogen}}$$

3.3.2 Zinc sulphate turbidity test for estimation of total immunoglobulins in calves

Chemicals

5% Zinc sulphate solution (Sigma chemical), fetal calf serum (Sigma Chemical), rabbit gamma globulin (Sigma Chemical)

Procedure

Zinc sulphate turbidity test as described by McEwan (1970) was modified for the estimation of total immunoglobulin in the serum samples.

4.1 ml of 5% zinc sulphate solution was diluted to 1000 ml with freshly boiled and cooled distilled water. This working solution contained 205 mg of $ZnSO_4$ per litre. For each serum sample to be tested, three tubes were taken. The first two constituted the unknown sample tubes whereas the third tube served as control or blank. 12 ml of $ZnSO_4$ solution was pipetted in unknown sample tubes. In the blank tube 12 ml of distilled water was pipetted. Then 0.1 ml of serum to be tested was added in each tube. The tubes were properly shaken and kept for 1 hour for the turbidity to develop. The turbidity developed in each tube was read in a spectrophotometer at a wavelength of 460 nm. Before taking the reading, null adjustment was made against the zinc sulphate solution. Just prior to noting the optical density all the tubes were shaken properly to make uniformly turbid solution. Blank reading was subtracted from the average unknown readings to arrive at the corrected optical density (OD) for each individual serum sample. Simultaneously, a series of standards ranging from 4 to 40 mg/ml FCS (fetal calf serum) were processed in an identical manner as unknown samples described above. The gamma globulin concentrations were calculated from the standard curve.

Preparation of standard curve

300 mg of rabbit gamma globulin was dissolved in 7.5 ml of fetal calf serum. A suitable quantity of this stock solution was diluted further with FCS for preparation of standard solutions with the concentrations 0,4,8,12,16,20,24,28,32,36 and 40 mg/ml. 12.0 ml of $ZnSO_4$ was added to 0.1 ml of each standard in duplicate. The contents were shaken properly and incubated at room temperature for 1 hour along with unknown sample tubes. The standard curve was prepared by plotting OD against concentration (Figure 1).

3.4 Analysis of hematological parameters

3.4.1 Blood Collection

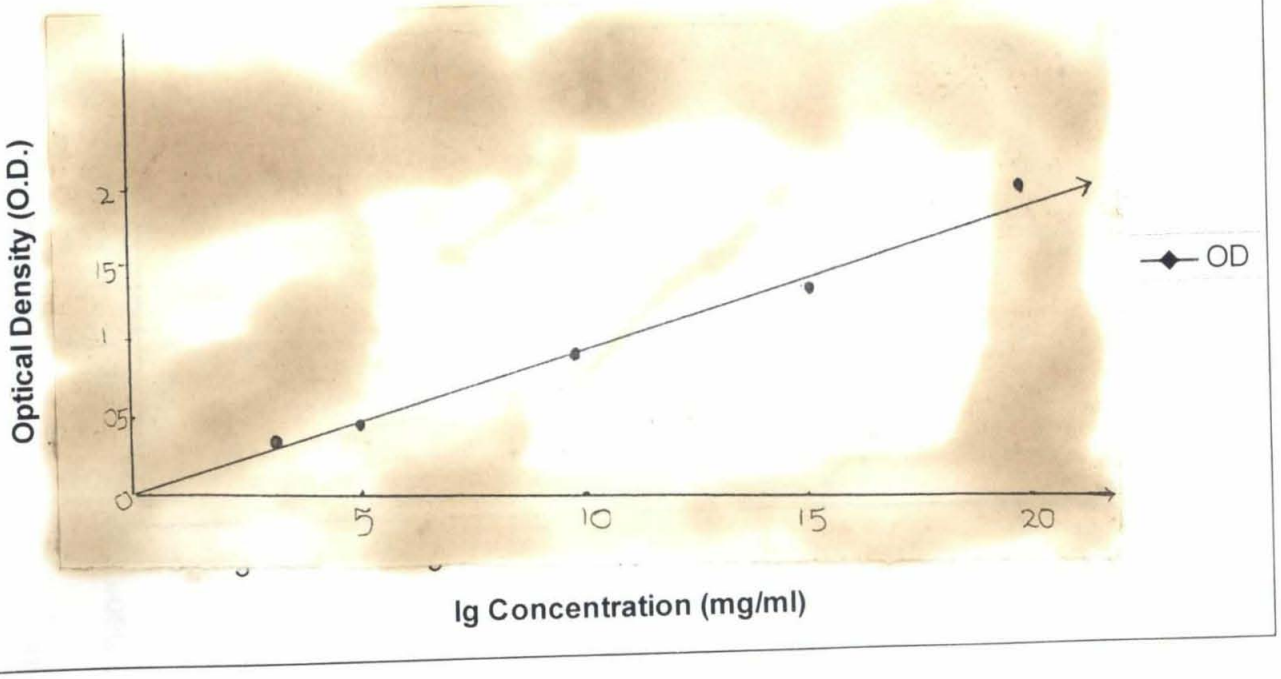
Blood samples (approximately 15 ml) from the calves were collected on 0 day (prior to start of the experiment) and thereafter at fortnightly intervals to determine blood arsenic, haemoglobin, packed cell volume (PCV), total leukocyte count (TLC), differential leukocyte count (DLC), serum glutamate pyruvate transaminase and super oxide dismutase activity.

Blood was collected in sterilized 20 ml vacutainer tubes with 16 gauge needle. Heparin was used as an anticoagulant at the rate of 20 IU/ml of blood. Heparin was reconstituted in 0.9% normal saline solution.

Just after blood collection, the samples were kept in sealed flask with ice packing. For serum collection, 5 ml blood was separately collected without anticoagulant. It was kept undisturbed in slanting position for 2-3 hours at room temperature and then centrifuged at 2500 rpm for 30 minutes to separate out clear serum. Then, the serum was drawn by using a syringe and was transferred to 2 ml micro centrifuge tube and stored at –20°C till further use.

3.4.2 Analysis of blood/ serum

Fig.1 **Standard Curve of Serum Ig Level (mg/ml)**



3.4.2.1 PCV and Hb

Packed cell volume and haemoglobin concentration in blood samples were determined by Wintrobe (Schalm, 1965) and acid hematin method (Oser, 1979) respectively.

3.4.2.2 TLC and DLC

Total leukocyte count (TLC) and differential leukocyte count (DLC) were performed in the blood samples as per the method described by Jain (1986).

3.4.2.3 Arsenic content in blood

5ml of blood sample was taken with 10ml of tri-acid mixture and wet digested on slow heat until it became transparent. The contents were made upto a volume of 20ml with double distilled water. Then, the procedure for total arsenic estimation as described below was followed.

Estimation of arsenic content by fluorometric method

Arsenic was estimated by fluorometric method Pal *et al.,* (1996).

Apparatus Cary 1 Varian UV-visible double beam spectrophotometer

Parameters		**Advanced Parameters**	
Photometric mode	% Trans	Factor	1.0
Abscissa mode	NM	Calibration conc.	1.0
Ordinate (Y)	0.0/100.00	Source change (nm)	310.0
Abscissa (x)	480.0/530.0	Beam mode	Single
SBW (nm)	4.0	Gain	200
Signal Avg. Time (Sec)	0.5	Beam interchange	Normal

Data Interval	1.0	Cycle time (mm)	1.0
Scan Rate (nm/min)	120.0	Cycle count	10.0
Lamps on	Vis	Peak threshold	10.0
Baseline correct	Off	Peak type	Min/max
Autoscale	Yes	Wt correction	No
Autoscore	No	Volume correction	No

Chemicals

As (III)(As_2O_3) (S.D. Fine Chem.)

As (V)(H_3AsO_4) (Merck Chem.)

Catechol AR (S.D. Fine Chem.)

Analytical reagent grade acridine orange (S.D. Fine Chem.)

Analytical reagent grade Na_2EDTA (S.D. Fine Chem.)

Preparation of Standard solution

(i) As (III)(As_2O_3) solution: 1.320g of As_2O_3 was dissolved in minimum volume of 20% NaOH in 1 litre volume flask. It was acidified with HCl (1+1) and diluted to volume with water.

(ii) As (V) (H_3AsO_4) solution: Proper dilution in distilled water can be made in the range of 2-20 μg As/ml for the standard solution.

Preparation of Reagents

1. Acridine Orange Solution (ACO) – 5×10^{-3} M. dm^3

1.85 gm of ACO was dissolved in 1 litre of distilled water to make 5×10^{-3} M/lt solution.

2. Catechol AR Solution – 10^{-2}M/lt or 10^{-2}M/dm^3

1.1011 gm of catechol AR was dissolved in 1 litre of distilled water to make 10^{-2} M/lt solution.

3. Na_2 EDTA solution – 2 x 10^{-2} M/lt or 2 x 10^{-2}M/dm^3

7.4448 gm of Na_2 EDTA was dissolved in 1 litre of distilled water to make 10^{-2} M/lt solution.

Procedure

For total arsenic estimation as described for water sample to convert all the arsenic into arsenic (V), 5ml of the water sample was placed in a beaker and 2-3 drops of conc. HNO_3 was added and evaporated to dryness on a water bath. Traces of nitric acid were neutralized with slight excess of ammonia solution and the sample was again evaporated to dryness on a water bath.

Catechol solutions (5 ml each) mixed with 1 ml Na_2 EDTA solution were taken in a set of 25 ml beakers. Variable amount of As(v) (0.2 to 20 μg) from the above treated samples were added to the beakers and evaporated to dryness on a water bath for complexation with catechol. The dry residue was transferred quantitatively into 10 ml volumetric flasks and volumes were made up. The resulting concentrations ranged from 0.02-2.0 μg/ml. Then 1 ml portion of each of these solutions were mixed separately with 3ml distilled water, 50 μl ACO and 50 μl glacial acetic acid and extracted with 3 ml toluene. 2ml portions form each of the toluene extracts were mixed with 1 ml distilled water and 0.25 ml of conc. H_2SO_4 and were vigorously shaken for 2-3 minutes in a stoppered glass vial. After the water layer had settled down, 0.8 ml of the layer was taken out with a micropipette and placed in a curette. To this was added 1 ml distilled water and 0.35 ml liquor ammonia. After thoroughly mixing the solutions, fluorescence spectra were recorded using …ex = 480 nm, sl 5/5. Fluorescence peak appears at 530 nm. It was observed that the final concentrations of arsenic (v) were in the range of 4-400 ppb.

The standard curve was drawn with the help of these working standards, it was linear (Figure 2).

3.5 Estimation of superoxide dismutase activity in blood sample

The activity of the enzyme superoxide dismutase (EC 1.15.1.1.) was assayed by the method described by Marklund and Marklund (1974). The reaction mixture contained different concentrations of appropriately diluted blood lysate preparation ranging from 0.2 to 2.0 ml which were made upto 3 ml by tris-HCl buffer (50 mM, pH 8.2) containing 1mM diethylene triamine pentaacetic acid and 0.2 ml of 2mM pyrogallol. A standard was prepared without addition of sample. The rate of autooxidation of pyrogallol was taken from the increase in absorbance at 420 nm against a reference cuvette containing 3.0 ml tris-HCl buffer (50mM, pH 8.2) with the help of Cary 1 Varian double beam UV-visible spectrophotometer. The increase in absorbance was $0.02min^{-1}$ in the absence of superoxide dismutase. The inhibition of pyrogallol autooxidation is brought about by superoxide dismutase, which was employed for the determination of enzyme activity. A unit of enzyme was defined as the amount of enzyme that inhibits the reaction by 50 percent.

Preparation of solutions

a. Pyrogallol (2mM)

25.2 mg of pyrogallol was dissolved in 100 ml of 10 mM HCl.

b. Tris buffer (50mM)

605 mg was dissolved in 100 ml of distilled water. 39 mg of diethylene triamine pentaacetic acid was added to 100 ml of the buffer. The pH was adjusted to 8.2 using HCl.

Preparation of blood lysate

2 ml of freshly collected blood with anticoagulant was centrifuged at 5000 rpm for 15 minutes using a refrigerated centrifuge. The RBC was

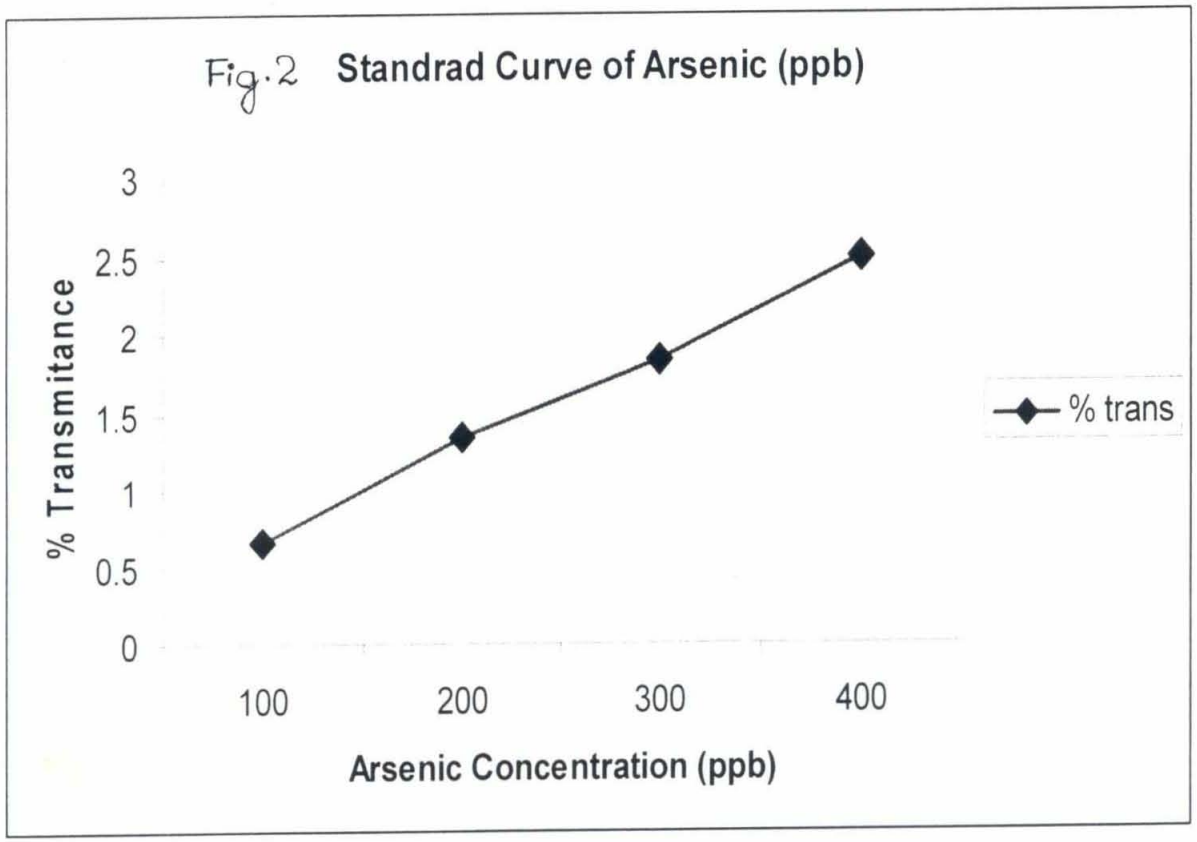


Fig.2 Standrad Curve of Arsenic (ppb)
% Transmitance
3
2.5
2
1.5
1
0.5
0
100
200
300
400
Arsenic Concentration (ppb)
% trans

washed with cold normal saline solution (0.9% NaCl Soln) for 3 times. RBC lysate was prepared by adding 1 ml distilled water and mixed for 30 seconds using vortex mixer. Then, for assay of SOD, dilutions were made with tris buffer.

3.6 Assay of Serum glutamate pyruvate transminase (Alanine transminase)

(Reitman and Frankel, 1957)

This enzyme study was performed at 15 days interval.

Apparatus Cary 1 Varian UV-visible spectrophotometer.

Chemicals

Reagent 1: Buffered alanine - α-KG substrate, pH 7.4

Reagent 2: DNPH colour reagent

Reagent 3: Sodium hydroxide solution, 0.4N

Reagent 4: Working pyruvate standard, 2mM

Supplied in a diagnostic reagent kit by Span Diagnostics Ltd.

Procedure

Standard Curve preparation

Tube No:	1	2	3	4	5
Enzyme activity (units/ml)	0	28	57	097	150
Reagent 1(ml)	0.250	0.225	0.2	0.175	0.15
Reagent 4(ml)	-	0.025	0.05	0.075	0.1
Distilled water (ml)	0.05	0.05	0.05	0.05	0.05
Reagent 2(ml)	0.25	0.25	0.25	0.25	0.25
Mixed well and allowed to stand at room temperature for 20 minutes					
Reagent 3 (ml)	2.5	2.5	2.5	2.5	2.5

The solutions in the tubes were mixed well and allowed to stand at room temperature for 10 minutes. The OD was measured against distilled water at 505 nm. A standard graph was plotted by taking enzyme activity on x-axis and OD on y-axis.

TEST

Reagent 1	0.25ml
Incubated at 37°C for 5 minutes.	
Serum sample	0.05 ml
Mixed well and incubated at 37°C for 30 minutes.	
Regent 2	0.25 ml
Mixed well and allowed to stand at 37°C for 20 minutes.	
Regent 3	2.5 ml

Mixed well and allowed to stand at 37°C for 10 minutes. The OD was recorded against distilled water at 505 nm.

Calculation

The OD of test was marked on the Y-axis of the standard curve and extrapolated to the corresponding enzyme activity on X-axis (Figure 3).

3.7 To study the excretion pattern of arsenic

The excretion pattern of arsenic was studied by conducting a metabolic trial on the experimental calves. A metabolic trial of 6 days duration was conducted on all the calves after 2 months of feeding arsenic trioxide supplemented diet. The calves were duly harnessed in individual metabolic stalls and given two days adaptation period before the actual sampling of

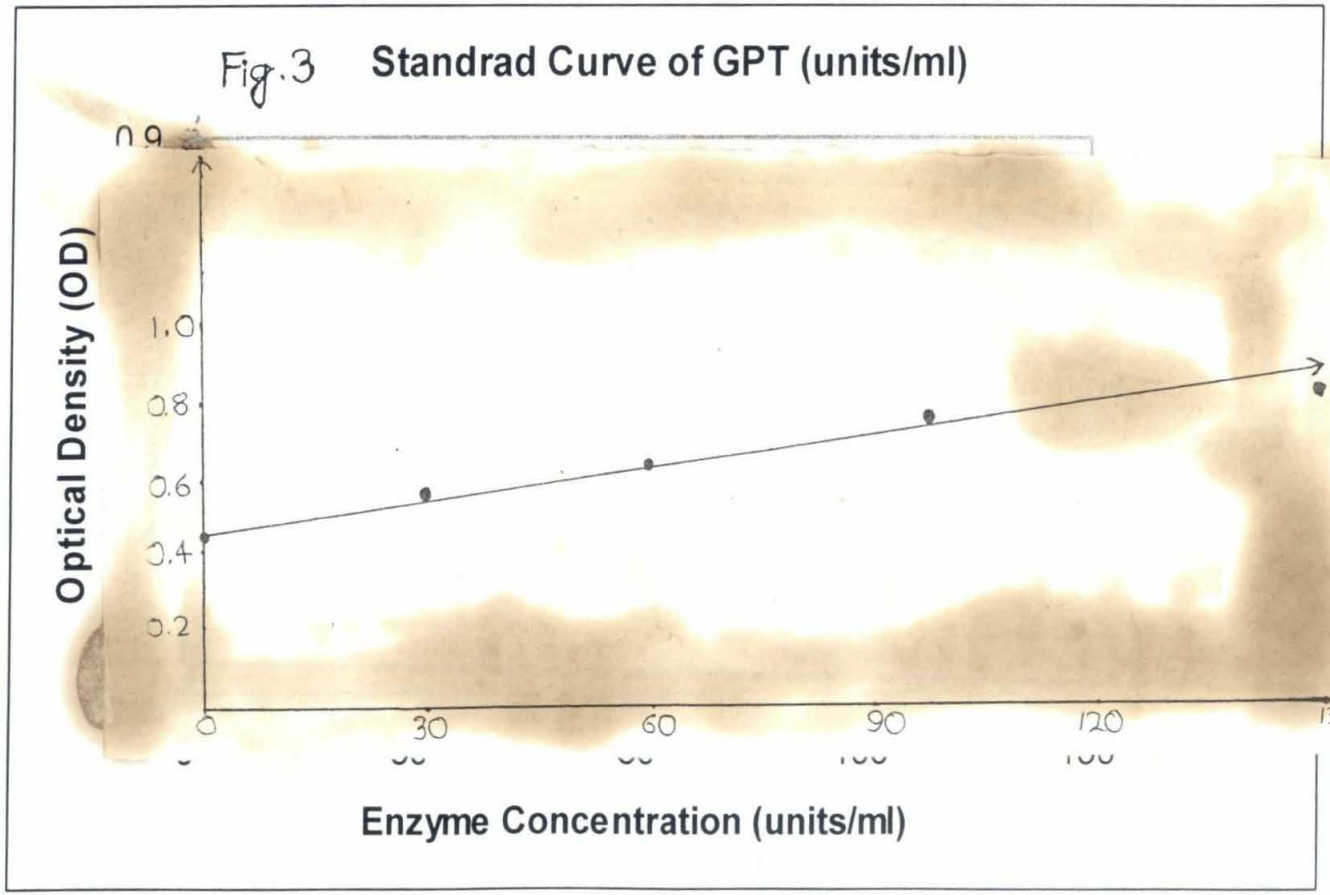


Fig.3 Standrad Curve of GPT (units/ml)
Optical Density (OD)
0.9
1.0
0.8
0.6
0.4
0.2
0
30
60
90
120
150
Enzyme Concentration (units/ml)

feeds, faeces and urine. The body weights of the calves were recorded before and after the metabolic trial.

3.7.1 Collection of feed, faeces and urine samples

Samples of offered and left over feed were collected in the morning for dry matter, proximate principles and arsenic estimation. Water samples were also collected for determination of arsenic content. The quantities of urine and faeces voided by the calves (24-hour duration) were recorded every morning. Representative aliquots of faeces and urine were taken separately after thorough mixing in dry plastic bags and plastic bottles, respectively.

3.7.2 Aliquoting of faeces

For determination of As content, a suitable aliquot (1/100) of faeces was dried daily in hot air oven at 90°C. The dried samples were pooled after 8 days for individual calf. At the end of the metabolic trial, the dried pooled samples were ground in a laboratory Wiley mill.

3.7.3 Aliquoting of urine

For arsenic estimation, an aliquot (1/250) was collected daily for each calf in clean glass bottle. These aliquots were pooled over the trial period of six days. A known amount (20 ml) of concentrated sulphuric acid was added in the bottles for preservation.

3.7.4 Estimation of arsenic content in hair, feed, fodder, faeces and urine samples

Hair sample was collected from all the calves on 10^{th} day and 90^{th} day for arsenic estimation. Prior to hair sample collection, the calves were given a thorough bath. Hair was collected with sharp razor with close proximity to the skin. Then the samples were kept in plastic packets. After overnight drying in oven, 1 gm of each sample was wet digested with 10 ml of tri-acid mixture (Conc. HNO_3: Conc. H_2SO_4: 70% Perchloric acid::3:2:1) and the final volume was made upto 20ml with double distilled water.

1 gm of dried and finely ground feed and fodder samples were subjected to wet digestion and the final volumes were made upto 20ml with double distilled water.

1 gm of dried and ground pooled faeces samples and 10 ml of pooled urine samples from metabolic trial were wet digested and the final volumes were made upto 20 ml with double distilled water.

After the above treatments, the earlier described procedure for total arsenic (both As (V) and As (III)) estimation was followed by taking 5 ml of digest.

RESULTS AND DISCUSSION

The effect of arsenic administration on growth, immunity, haematological parameters and excretion pattern was studied in growing Karan Fries calves of about 4 months age. The results obtained are presented and discussed in this chapter-

4.1 Growth Performance of Calves

The growth performance of male KF calves was studied for a period of 90 days.

4.1.1 Changes in Body Weight

The changes in body weight at weekly intervals in the two experimental groups are given in Table 4.1 and the statistical analysis in Table 4.2. The average body weight of the animals at the beginning of experiment was 76.50 $\pm$ 7.23 and 71.70 $\pm$ 8.45 kg in groups T_1 and T_2 respectively. The corresponding body weights at the end of the experiment were 107.00 $\pm$ 8.19 and 102.80 $\pm$ 7.85 kg respectively. It was evident from the results (Table 4.2) that the body weights were not affected significantly due to the treatment. The calves in T_1 and T_2 gained 30.5 to 31.1 kg during 90 days, thus depicting slower average growth rate.

The results showed that the supplementation of arsenic in the diet, at 50 ppm level did not cause any adverse effect on body weight gain in the calves under study. However, no literature is available on As effects on growth in ruminants, though the adverse effects have been observed in monogastric animals by Vodela *et.al.* (1997). They offered drinking water containing 0.8 ppm to 8.6 ppm arsenic to broiler breeders for 10 weeks and observed an inverse relationship between increasing concentration in drinking water and body weights of hens.

4.2 Immunity of Calves

4.2.1 Lymphocyte Proliferation Assay

Lymphocytes are responsible for providing immunity against foreign pathogens because of their phagocytic activity. The degree of phagocytic activity in response to mitogen is the measure of the level of immunity. Higher phagocytic activity by the lymphocytes naturally provides higher immunity to the animal. The extent of DNA synthesis of the lymphocytes in the presence of mitogen is the basis of SI (Stimulation index) in lymphocyte proliferation assay.

In the present study, the stimulation index at the end of 90 days was found to be 1.495± 0.05 and 0.794 ± 0.15 in case of T_1 and T_2 respectively (Table 4.3). The statistical analysis of the data (Table 4.4) revealed that though the SI value at 0 day was higher in group $T_{1,}$ it remained unaltered for 90 days. However, there was significant decrease (upto 30 %) in SI value in group T_2. The differences between the treatments were found to be significant ($P<0.05$).

The above result suggests the immuno-suppressive nature of arsenic. The decreased stimulation index in the treatment group shows the altered immunity status of the calves due to dietary arsenic administration. Sengupta and Bishayi (2002) also observed decreased phagocytic activity for ingestion and digestion of exogenous antigens such as whole microorganisms and higher susceptibility to diseases in rats by sodium arsenite administration. Similarly, Meng and Meng (2000) observed that the inorganic arsenicals by inhibiting DNA synthesis of lymphocytes, affected the cellular immunity adversely. The reduced cellular immunity renders the animals more susceptible to disease conditions because of decreased lymphocyte proliferation.

Table 4.1 Effect of dietary arsenic supplementation on growth (kg body weight) of calves

Interval	T_1	T_2
1	76.50±7.23	71.70±8.45
2	76.30±7.78	74.30±8.73
3	78.10±7.63	75.30±8.55
4	79.40±7.40	76.80±8.12
5	80.60±6.96	77.10±7.92
6	84.40±7.58	78.60±7.60
7	89.60±6.47	83.80±8.03
8	92.60±6.75	86.30±8.17
9	94.80±7.44	87.00±8.22
10	96.40±7.29	89.40±8.26
11	97.80±7.61	90.80±8.03
12	101.40±7.85	95.70±8.35
13	103.90±8.08	98.80±8.16
14	107.00±8.19	102.80±7.85
Mean	89.91±2.80	84.88±2.60

Table 4.2 Analysis of variance for growth (Mean sum of squares)

Source of Variation	Degree of Freedom	Growth
Treatments(T)	1	885.0286
Periods(P)	13	1040.131
T×P	13	8.043945
Error	108	294.1006

Table- 4.3 Effect of dietary arsenic supplementation on lymphocyte proliferation assay in calves

Day	T_1	T_2
0	1.448 ± 0.06	1.138 ± 0.07
45	1.518 ± 0.06	1.246 ± 0.05
90	1.52 ± 0.16	0.794 ± 0.15
Mean	1.495^{a} ± 0.056	1.059^{b} ± 0.074

Table 4.4 Analysis of variance for lymphocyte proliferation assay (Mean sum of squares)

Source of Variation	Degree of Freedom	LPA
Treatment (T)	1	1.42572
Periods (P)	2	0.13066
T×P	2	0.15633
Error	24	0.05102

Table-4.5 Effect of dietary arsenic supplementation on total immunoglobulin level (mg/ml) in serum of calves

Day	T_1	T_2
0	36.34 ± 1.69	39.96 ± 1.18
15	35.52 ± 1.90	40.08 ± 0.82
30	35.18 ± 2.00	39.64 ± 1.28
45	35.50 ± 1.92	39.84 ± 1.02
60	35.80 ± 1.81	39.42 ± 1.11
75	36.16 ± 1.70	39.94 ± 1.10
90	36.06 ± 1.73	40.38 ± 0.94
Mean	35.79^{b} ± 0.15	39.89^{a} ± 0.10

Table-4.6 Effect of dietary arsenic supplementation on haemoglobin level (g/dl) in blood of calves

Day	T_1	T_2
0	8.68 ± 0.31	8.56 ± 0.93
15	8.18 ± 0.29	7.80 ± 0.44
30	8.12 ± 0.13	8.30 ± 0.23
45	8.02 ± 0.14	8.52 ± 0.387
60	7.98 ± 0.20	8.48 ± 0.13
75	8.00 ± 0.17	8.24 ± 0.07
90	7.98 ± 0.17	7.74 ± 0.18
Mean	8.48 ± 0.09	8.23 ± 0.12

4.2.2 Total Immunoglobulin (Ig) Level in Serum

Periodic changes in total Ig level are given in Table 4.5. The average value of total Ig level in serum was found to be 35.79± 0.15 and 39.89± 0.10 in T_1 and T_2 groups respectively (Table 4.5).

Immunoglobulins, which are protein in nature, comprise the basic defence mechanism of the body against antigens. The immunity provided by different immunoglobulins at various stages involves diversified complex mechanisms. The concentration of total immunoglobulins in the serum is an indicator of the immunity status of the animals.

Critical analysis of data indicated that total Ig level remained unaltered during the 90 day experimental period. The values were found to be significantly higher in T_2 even at the onset of the experiment. On the contrary to the above finding, Vodela *et. al.,* (1997) found suppressed natural, humoral and cell mediated immune response through arsenic administration in drinking water in hen.

4.3 Effect of Dietary Arsenic Administration on Haematological Parameters

4.3.1 Haemoglobin

The haemoglobin level in blood determines the oxygen carrying capacity of blood. Hb value below normal range significantly reduces oxygen carrying capacity and the anaemic condition may lead to anorexia, depression and stunted growth.

The periodic changes in Hb content are presented in Table 4.6 and the results of statistical analysis in Table 4.7. At the start of the experiment, the Hb content in blood was 8.68± 0.31 and 8.56± 0.93 gm/dl in T_1 and T_2 groups respectively and it was found to be unaltered due to administration of arsenic

as evident from the observed values even upto 90 days. The average Hb content of blood during the study was 8.48 ± 0.09 and 8.23 ± 0.12 gm/dl in T_1 and T_2 groups respectively, revealing no statistical difference due to treatment. However, the values observed in both the groups were slightly lower than the normal values i.e. 11-12 g/dl of blood (Swenson and Reece, 1996). On the contrary, Satake *et.al.,* (1997) postulated that chronic toxicity of As resulted in destruction of red blood cells and bone marrow cells, thus leading to anaemia, but in this study no such observations were recorded.

4.3.2 Packed Cell Volume (PCV) of Blood

The mean PCV (%) of blood was found to be 31.71± 1.26 and 32.60± 0.80 in T_1 and T_2 groups respectively (Table 4.8). The normal value of PCV in bovines lies in the range of 27-30%(Swenson and Reece, 1996). The statistical analysis of data (Table 4.7) indicated no significant effect due to treatment, thereby suggesting that dietary As administration at 50 ppm level for 90 days did not have adverse effect on PCV value of blood. But, Schulz *et.al.,* (2002) reported reduced Mean Corpuscular Volume (MCV) of red cells in wistar rats when subjected to chronic inorganic arsenic ($NaAsO_2$) administration at the level of 2-6 mg/kg body wt. However, no studies are available on ruminants.

4.3.3 Total Leukocyte Count (TLC)

The leukocytes include both granulocytes and agranulocytes which are responsible for providing immunity to the body. Decreased total leukocyte count may affect the health of the animal because of altered phagocytic activity. During the study, the mean total leukocyte count per ml of blood was found to be 11156.43± 532.7 and 12156.57± 580.50 in T_1 and T_2 groups respectively (Table 4.9). The values were found to be nearly in the normal range of 7000-10000 (Swenson and Reece, 1996). The differences between treatments were found to be non significant (Table 4.7).

Table 4.7 Analysis of Variance for blood parameters (Mean sum of squares)

Source of Variation	Degree of freedom	Parameters								
		Hb	**PCV**	**TLC**	**Lymphocyte**	**Neutrophil**	**Eosinophil**	**Basophil**	**Monocyte**	**Blood As Level**
Treatment (T)	1	0.16466	13.7285	17505000	2.4142	1.1517	0.5142	0.0142	4.1285	21785.2000
Periods (P)	6	0.57824	72.5619	10785288	81.1619	26.0952	17.5619	0.1619	23.9000	5171.2750
T×P	6	0.30856	5.5619	3091134	0.9142	1.4571	2.1142	0.1142	1.7952	7135.6290
Error	52	0.50865	11.1983	11702274	26.2593	13.5170	4.9065	0.3071	4.5917	787.9007

Table 4.8 Effect of dietary arsenic supplementation on packed cell volume (%) of calves

Day	T_1	T_2
0	25.40 ± 2.42	29.00 ± 2.23
15	33.80 ± 0.96	34.20 ± 2.47
30	34.40 ± 1.60	35.40 ± 1.80
45	32.60 ± 0.92	33.00 ± 1.41
60	30.00 ± 0.89	31.40 ± 0.92
75	30.80 ± 0.86	31.60 ± 1.02
90	35.00 ± 1.30	33.60 ± 1.50
Mean	31.71 ± 1.26	32.60 ± 0.80

Table-4.9 Effect of dietary arsenic supplementation on total leukocyte count (WBC/ml of blood) in calves

Day	T_1	T_2
0	8521 ± 1171.28	11670 ± 2106.51
15	10910 ± 717.53	11210 ± 795.52
30	11680 ± 1932.27	11530 ± 1514.89
45	11510 ± 1647.53	12410 ± 803.03
60	11178 ± 1471.00	11966 ± 1091.38
75	12700 ± 867.04	14390 ± 2626.85
90	11596 ± 1170.92	11920 ± 1509.44
Mean	11156.43 ± 532.7	12156.57 ± 580.50

4.3.4 Differential Leukocyte Count (DLC)

Under differential leukocyte count, all the granulocytes and agranulocytes were studied at fortnightly interval (Table 4.10).

During the study, the mean lymphocyte content (%) of blood was found to be 53.37± 1.04 and 53.74± 1.11 in groups T_1 and T_2 respectively. The differences due to the treatments were found to be non significant (Table 4.7).

The neutrophil, eosinophil, basophil and monocyte (%) of blood were found to be 31.77± 0.60 and 31.51± 0.65; 8.57± 0.38 and 8.74± 0.64; 0.34± 0.07 and 0.37± 0.05; 5.94± 0.60 and 5.46± 0.60; in T_1 and T_2 groups respectively. The differences between treatments were found to be non significant (Table 4.7).

The normal values of lymphocyte, neutrophil, eosinophil, basophil and monocyte in case of bovines are 60-65, 25-30, 2-5, 1 and 5 respectively (Swenson and Reece, 1996).

4.3.5 Blood Arsenic Concentration

The periodic changes in blood As concentration are given in Table 4.11. The initial blood As concentration was observed to be 156.22 ± 2.89 and 112.10± 20.79 ppb in groups T_1 and T_2 respectively. The average blood arsenic concentration (ppb) at the end of the experiment was found to be 162.45± 4.84 and 197.14± 18.11 ppb in groups T_1 and T_2 respectively (Table 4.11). The differences between the treatments were found to be significant at 5 % level (Table 4.7). The blood arsenic concentration (ppb) during the period of study remained unchanged in control group, however, the blood arsenic level gradually increased during the experimental period in T_2 and at the end of the experiment of 90 days, it increased 2.4 folds. Singh *et.al.,*(2001) reported that average level of As in blood (mg/l) ranged from 0.0 to 0.525 in Ghentugachi and from 0.087 to 0.779 in cattle of Gontra village of Nadia district which are reported as As affected region in West Bengal.

Higher blood arsenic concentration might be due to the fact that 95-99% of absorbed binds to globin of Hb in erythrocytes (Satake *et.al.,* 1997).

4.4 Hair Arsenic Concentration

The As concentration of hair was estimated at day 10 and day 90 of feeding. The results are presented in Table 4.12. The statistical analysis is given Table 4.13. It was observed that the concentration remained more or less the same even after 90 days in the control group, however, the values increased 2.5 to 3 times in the As supplemented group T_2 revealing highly significant difference due to treatment. Once deposited in keratinised tissues like hair or nail, As is irremovable from these tissues (Satake *et.al.,* 1997).This accumulating nature of As in hair can be used as an indictor of arsenic status.

It is evident from the literature that arsenic has a tendency to accumulate in hair, bone and other body tissues (Satake *et.al.,* 1997, Grollman and Slaughter,1947, Neiger and Oswell,1992). The gradual accumulation of As in these tissues results in high levels after a long duration of exposure. As level particularly in hair can be used as a measure of As toxicity in the animals.

4.5 Arsenic Balance

A metabolic trial of 6 days duration was carried out on the experimental calves. The pooled urine and faeces samples were subjected to arsenic estimation by fluorimetric method.

The arsenic level was also estimated in the concentrate, green oat fodder and water offered to the animals during study. The concentrate mixture, green oat fodder and water samples were found to contain 1.2 ppm, 0.705 ppm and 30.7 ppb As respectively. The total arsenic intake was calculated by considering the arsenic intake through concentrate mixture, green oat fodder, water and arsenic supplementation in the form of As_2O_3 (in the T_2 group). The total excretion of arsenic was calculated by taking into consideration the amount of arsenic excreted through urine and faeces.

Table 4.10 Effect of dietary arsenic supplementation on differential leukocyte count (%) of calves

	Lymphocyte		Neutrophil		Eosinophil		Basophil		Monocytes	
Day	**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**	**T_1**	**T_2**
0	50.60 ±1.91	50.60 ±3.37	32.60 ±1.16	33.00 ±2.28	09.20 ±0.80	09.40 ±0.81	0.20 ±0.20	0.40 ±0.24	07.20 ±0.86	06.60 ±0.67
15	56.40 ±2.37	57.80 ±2.05	32.00 ±2.34	31.60 ±2.13	7.40 ±0.92	6.40 ±1.12	0.20 ±0.20	0.40 ±0.24	3.80 ±0.66	4.20 ±1.15
30	55.40 ±2.83	55.60 ±2.54	32.60 ±1.91	31.60 ±2.36	7.60 ±0.92	8.60 ±1.20	0.40 ±0.24	0.40 ±0.24	4.40 ±1.07	4.20 ±0.66
45	52.40 ±3.07	52.00 ±3.11	32.40 ±2.18	32.80 ±2.13	10.40 ±0.87	12.00 ±1.87	0.20 ±0.20	0.20 ±0.20	4.80 ±0.80	3.00 ±1.04
60	55.20 ±2.17	55.20 ±2.85	29.00 ±1.14	29.60 ±2.37	8.60 ±0.67	8.00 ±0.63	0.20 ±0.20	0.40 ±0.24	7.00 ±1.09	6.80 ±0.86
75	54.60 ±2.20	55.20 ±1.31	30.20 ±2.59	28.80 ±0.96	8.20 ±0.86	8.60 ±0.67	0.60 ±0.40	0.60 ±0.24	6.40 ±1.24	6.80 ±0.96
90	49.00 ±1.81	49.80 ±1.74	33.60 ±1.16	33.20 ±1.93	8.60 ±0.50	8.20 ±1.01	0.60 ±0.24	0.20 ±0.20	8.00 ±1.00	6.60 ±0.60
Mean	53.37 ±1.04	53.74 ±1.11	31.77 ±0.60	31.51 ±0.65	8.57 ±0.38	8.74 ±0.64	0.34 ±0.07	0.37 ±0.05	5.94 ±0.60	5.46 ±0.60

Table-4.11 Effect of dietary arsenic supplementation on blood arsenic level (ppb) of calves

Day	T_1	T_2
0	156.22 ± 2.89	112.10 ± 20.79
15	187.60 ± 11.76	181.18 ± 19.48
30	165.84 ± 10.14	196.98 ± 19.79
45	166.66 ± 11.51	194.94 ± 18.60
60	157.48 ± 14.34	211.14 ± 15.39
75	155.48 ± 7.67	214.58 ± 14.81
90	147.92 ± 10.93	273.26 ± 18.26
Mean	162.45^{b} ± 4.84	197.74^{a} ± 18.11

Table-4.12 Effect of dietary arsenic supplementation on hair arsenic level of calves (ppb)

Day	T_1	T_2
10	691.6 ± 44.62	941.52 ± 99.98
90	705.28 ± 32.04	2506.7 ± 267.56
Mean	698.44^{b} ± 66.32	1724.11^{a} ± 326.00

Table 4.13 Analysis of variance for hair arsenic level of calves(Mean sum of squares)

Source of Variation	Degree of Freedom	Hair As level
Treatment (T)	1	5259995
Periods (P)	1	3115999
T×P	1	3008940
Error	16	105755.2

The arsenic content in the faeces (ppm) was found to be 1.39± 0.12 and 1.85± 0.17 in the T_1 and T_2 groups respectively. The arsenic level (mg/litre) in the urine was found to be 0.1661± 0.01 and 0.2133± 0.01 in the T_1 and T_2 groups respectively. However, Singh *et.al.,*(2001) reported quite high As content of 21.65mg/kg DM in dung and 2.18 mg/l in urine of cows in the As affected villages of Nadia district of West Bengal. The results could not be compared as it was a field study in which the animals were continuously exposed to As.

The results obtained from the metabolic trial revealed that the retention of arsenic was 34.17% and 97.00% in the T_1 and T_2 groups respectively (Table 4.14) thereby indicating the accumulating nature of the element. 65.83% and 3.00% of total arsenic intake was excreted in the urine and faeces in the T_1 and T_2 groups respectively.

It was also confirmed and correlated with increased As content in blood and hair as observed in the study. From the critical perusal of the data on % retention of As, the results are in accordance with the observation by Satake *et. al.,* (1997) which states that most of the ingested (95% of trivalent As and 80% of sea-food As) or inhaled As is absorbed through the gastro-intestinal or the respiratory tract and after absorption 95-99% of the As binds to globin of the haemoglobin in the erythrocytes and then it gets transported to other body tissues and gets deposited in bone, hair, nail and skin.

Urine and hair are reported to be the most useful indicators of As exposure even at very low doses (Neiger and Osweller, 1992). As in foods is well absorbed and rapidly eliminated mainly through urine. Underwood (1973) reported that As of organic compounds disappeared rapidly from tissues of monogastric animals and was mostly excreted through faeces. But the findings of this study are not in accordance with the above findings as in this case, percentage excretion through urine or faeces was not altered due to inorganic arsenic as As_2O_3 supplementation in the diet. This resulted in higher concentration of As in blood as well as in hair, thus suggesting its tendency of deposition in these tissues.

4.6 Activity of Serum Glutamate Pyruvate Transminase (SGPT)/Alanine Transminase (ALT)

The periodic changes in SGPT activity are presented in Table 4.15. The values in calves are within the normal range 23.7± 17.3 units/ml (Kaneko and Comelius, 1971). The SGPT activity in serum (units/ml of serum) was found to be 23.97 ± 4.26 and 26.20 ± 5.89 in the groups T_1 and T_2 respectively (Table 4.15). The differences between the treatments were found to be non-significant (Table 4.16).

SGPT is an enzyme present in the cytoplasm of the hepatic cells. Any sort of hepatic tissue injury leads to the release of this enzyme to the blood circulation and increase in the concentration of this enzyme in blood. SGPT catalyses the following reaction:

α - Ketoglutarate + L- Alanine Z L-Glutamate + Pyruvate

Literature shows that pyruvate metabolism is disturbed due to arsenate. So, it was hypothesized that As may affect the liver enzyme function thereby, altering SGPT activity. But in this study, no such observation was recorded suggesting that probably the dose level and / or period was not so critical to alter the enzyme function.

4.7 Activity of Superoxide Dismutase

Superoxide dismutase is an anti-oxidative enzyme indicating the oxidative stress of the animals. Oxidative stress adversely affects the immunity of the animals.

The results of blood SOD activity measured at fortnightly intervals and its statistical analysis are presented in Table 4.17 and Table 4.16 respectively. At 0 day, SOD activity (Unit/gm Hb) was 9603± 1471 and 13035± 256 indicating higher values in T_2 group. A thorough analysis of data obtained at periodic intervals revealed that SOD activity decreased gradually in T_2. Although, there was sharp decline in activity in both the groups on 60^{th}

Table 4.14 Arsenic balance in crossbred calves under metabolic trial

Animal No.	As intake through Conc. (mg)	As intake through Roughage (mg)	As intake through Water (mg)	As Supplemented (mg)	Total As Intake (mg)	As voided through faeces (mg)	As voided through urine (mg)	Total As excreted (mg)	% As retained	Avg. % As retained	% As excreted	Avg. % As excreted
1	1.638	2.044	0.388	0	3.797	1.780	1.253	3.033	20.13		79.87	
2	1.638	2.129	0.326	0	3.820	1.697	0.683	2.380	37.7		62.30	
3	1.638	2.284	0.465	0	4.114	1.577	1.093	2.670	35.1	34.17	64.90	65.83
4	1.638	2.594	0.318	0	4.277	1.781	0.624	2.405	43.77		56.23	
5	1.638	2.178	0.442	0	3.985	1.639	0.986	2.625	34.13		65.87	
6	1.638	2.263	0.550	116	120.17	1.752	1.834	3.586	97.02		2.98	
7	1.638	1.692	0.288	61	64.34	1.139	0.704	1.843	97.14		2.86	
8	1.638	2.157	0.365	86	89.88	1.872	0.839	2.711	96.99	97.00	3.01	3.00
9	1.638	2.178	0.383	96	99.92	1.945	1.113	3.058	96.94		3.06	
10	1.638	1.910	0.406	87	90.68	1.743	1.080	2.823	96.89		3.11	

Table-4.15 Effect of dietary arsenic supplementation on SGPT activity (units/ml of serum)

Day	T_1	T_2
0	14.80 ± 2.17	10.00 ± 0.77
15	14.20 ± 2.78	10.60 ± 1.36
30	15.00 ± 2.58	10.40 ± 1.40
45	16.60 ± 4.78	28.40 ± 8.50
60	40.40 ± 3.82	41.60 ± 2.29
75	34.60 ± 3.89	38.60 ± 3.28
90	32.20 ± 3.52	43.80 ± 2.78
Mean	23.97 ± 4.26	26.20 ± 5.89

Table 4.16 Analysis of variance for enzymatic activity (Mean sum of squares)

Source of Variation	Degree of Freedom	Enzymes	
		SGPT (U/ml)	SOD (U/gm Hb)
Treatment (T)	1	86.91428	1.540344E+07
Periods (P)	6	1724.281	1.545527E+04
T×P	6	130.6810	7033338.0
Error	52	67.14341	4732356.0

Table-4. 17 Effect of dietary arsenic supplementation on superoxide dismutase activity (unit/g Hb)

Day	T_1	T_2
0	9603.65 ±1471.42	13035.15 ±256.07
15	9741.36 ±1584.56	12209.87 ±229.71
30	9915.90 ±1590.19	10771.72 ±1070.77
45	10046.13±1621.96	10777.36 ±1449.55
60	3272.08 ±766.83	4507.02 ±895.12
75	4047.63 ±977.43	3142.24 ±702.76
90	3505.84 ±676.43	2256.56 ±360.60

day and onwards, the decline was more noticeable in group T_2 from the onset of the experiment. This decreased activity may be co-related with the increased environmental stress as there was sharp increase in environmental temperature. Critical perusal of data indicated that this effect was non-significant due to the treatment. But it had an indication of increasing oxidative stress of treatment group animals as differences were significant ($P<0.10$). Probably the picture would have been more transparent if the study period could have been longer. Cobo and Castineira (1997) reported that supplementation of arsenic in drinking water caused oxidative stress by binding of arsenic to thiol or SS-group in enzyme and proteins and releasing oxidant species during the redox cycling and metabolic activation process e.g., by cytochrome – P450 in liver. The oxidative stress by trivalent arsenic (As^{3+}, $NaAsO_2$) and pentavalent arsenic (As^{5+}, Na_2HAsO_4) in drinking water also revealed alterations of the antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione transferase (GST) and glutathione reductase (GR) (Zaman *et.al.,* 1995). Chaudhuri *et.al.,* (1999) reported decreased superoxide activity in brain of rats fed 0.05 to 3.0 ppm As in drinking water.

4.8 Clinical Manifestation

At 50 ppm level of As supplementation (as As_2O_3), no sign of clinical manifestation of toxicity was observed during the period of study.

SUMMARY AND CONCLUSIONS

Environmental pollution has been a major cause of various health hazards in the recent times because of unexpectedly high rate of urbanization, population growth and industrialization. The extent of its effects on different physiological processes is dependent on the genetic, nutritional and environmental factors. Since, times immemorial, arsenic is considered as a toxic element and during the past few decades, environmentalists have stressed a lot on ever-increasing level of arsenic in the environment from various sources, so it has become the need of the hour to probe into the effects of arsenic on various physiological functions in human beings as well as animals. In the present investigation, the effect of arsenic on growth, immunity, haematological parameters and its excretion in crossbred calves has been studied. The salient findings of the study are summarized as follows:

5.1 Summary

Feeds, fodders, blood and hair samples were subjected to wet digestion and estimation of arsenic was carried out by the fluorometric method.

The growing KF calves were supplemented with 50 ppm As/ kg DM of feed for a duration of 90 days.

Dietary arsenic administration had no adverse effect on the growth of the animals.

The arsenic supplementation resulted in decreased phagocytic activity of the lymphocytes in the experimental group, but the total immunoglobulin level was not affected due to the treatment. Reduced phagocytic activity confirms the immunosuppressive nature of arsenic.

There was also no adverse effect of dietary arsenic supplementation on haematological parameters such as haemoglobin level, packed cell volume, total leukocyte count and differential leukocyte count. No alteration in these haematological parameters was observed during the 90 day study with the given dose of dietary arsenic supplementation.

Arsenic administration did not affect the liver enzyme SGPT activity at the given dose rate, but the anti oxidative enzyme SOD function was adversely affected by arsenic feeding.

A metabolic trial was conducted on the experimental animals to study the excretion pattern of arsenic.

The intake of arsenic through different sources such as concentrate mixture, fodder, water and supplementation were recorded. The amount of arsenic excreted was calculated by considering As excretion in the urine and the faeces. The amount of arsenic retained in the control and the experimental groups were found to be 34.17% and 97% respectively. The high level of arsenic retention was reflected in the increase of blood arsenic level (30%) and hair arsenic level (2.5-3 folds) in the experimental group.

CONCLUSIONS

- Arsenic administration did not have any adverse effect on growth of the crossbred calves.
- The haematological parameters such as Hb, PCV, TLC and DLC were not affected by arsenic feeding.
- Arsenic supplementation resulted in immunosuppression due to reduced phagocytic activity of lymphocytes.
- Arsenic administration did not have any adverse effect on activity of liver enzyme SGPT.
- Arsenic supplementation caused oxidative stress, which was evident from decreased SOD activity.
- The arsenic retention was much higher in the experimental group as compared to the control group due to its deposition in hair and blood.

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