

CLINICAL STUDIES ON SELENOSIS IN DAIRY ANIMALS

by
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(L-92-V-277-M)

Thesis

*Submitted to the Punjab Agricultural University
in partial fulfilment of the requirements
for the degree of*

**MASTER OF VETERINARY SCIENCE
IN**

VETERINARY MEDICINE

(Minor : Veterinary Physiology)



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TO MY REVERED FATHER

who sacrificed his today for my better tomorrow without uttering a single word of dismay amidst heavy torrents of odds in his life.

TO MY REVEREND MOTHER

an apostle of sacrifices and pillar of my strength

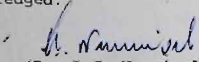
TO MY WIFE

for her encouragement, endurance and sympathetic understanding

CERTIFICATE I

This is to certify that this thesis entitled, "Clinical studies on selenosis in dairy animals" submitted for the degree of M.V.Sc. in the subject of Veterinary Medicine (Minor subject: Veterinary Physiology) of the Punjab Agricultural University, is a bonafide research work carried out by Pardeep Kumar Verma (L-92-V-277-M) under my supervision and that no part of this thesis has been submitted for any other degree.

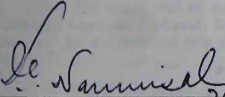
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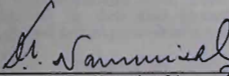

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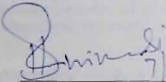
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CERTIFICATE II

This is to certify that the thesis entitled, "Clinical studies on selenosis in dairy animals" submitted by Pardeep Kumar Verma (L-92-V-277-M) to the Punjab Agricultural University in partial fulfilment of the requirement for the degree of M.V.Sc. in the subject of Veterinary Medicine (Minor subject: Veterinary Physiology) has been approved by the student's advisory committee, after an oral examination on the same, in collaboration with an External Examiner.


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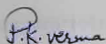
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ABSTRACT

The present investigation was undertaken in 30 clinical cases (20 crossbred cows and 10 buffaloes) in the selenium toxic belt comprising villages Barwa and Bagouran of Jalandhar district and Simbly of Hoshiarpur district of Punjab. Experimentally Se toxicity was induced in 18 buffalo calves divided in 3 groups of 6 each with sodium selenite @ 2.0 mg/kg body weight orally, daily for 14 doses.

Characteristic clinical manifestations in natural cases were abnormalities of horns/hoooves, chronic wounds on hind quarters, tail gangrene, emaciation and pronicity to secondary bacterial infections, anestrus and abortions in first trimester of pregnancy. These anomalies arose due to prolonged feeding of seloenosed fodder raised on seleniferous soils. Prevalence was more in adult animals (>3 years age) with severity of symptoms more in cattle than in buffaloes, irrespective of sex in both species. In experimental selenosis, clinical findings were anorexia, nasal exudation and terminally respiratory distress.

Comparative analysis reflected microcytic hypochromic anaemia in clinical selenosis and non-significant macrocytic normochromic anaemia in experimental selenosis. Blood glucose, plasma ALT/GPT and AST/GOT showed non-significant variations

while plasma urea nitrogen was significantly higher in selenosed animals. ALP in plasma and keratinized epithelium depicted a 2.5 and 1.64 fold increase respectively while RBC carbonic anhydrase (Zn-metallo enzyme) activity was 2 times lower in clinically selenosed animals. Significant reduction in total plasma proteins was due to a significant reduction in plasma globulin and fibrinogen levels 'S' amino acids in plasma and keratinized epithelium of clinical cases showed a significant reduction.

Plasma Se was markedly high in both clinical and experimental selenosis with significant reduction in whole blood iron and plasma Cu of clinical cases only. Hair and ruminal fluid of selenosed animals showed a 70 and 7 fold increase respectively over the healthy control. Comparative study of soil-water in seleniferous and non-seleniferous areas revealed 4 and 7 times higher Se level respectively with non-significant fluctuations in the S content. Gross and histopathological examination revealed mild degenerative changes in various internal organs. Therapeutic measures with sodium arsenate @ 10 ml/300 kg B.W. (750 mg/750 ml) plus ZnSO_4 @ 5g/day/animal in clinical cases led to visible improvement in symptoms. In experimental selenosis sodium arsenate + ZnSO_4 was found to be better than anthiomaline (5 ml deep i/m) + ZnSO_4 in alleviating symptoms and decreasing plasma Se levels.

P.K. verma

Signature of student

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Signature of Major Advisor

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LIST OF ABBREVIATIONS USED IN THE TEXT

@	: At the rate of
Ag	: Silver
A/G	: Albumin Globulin ratio
ALP	: Alkaline phosphatase
ALT	: Alanine amino transferase
As	: Arsenic
AST	: Aspartate amino transferase
BGL	: Blood glucose
BUN	: Blood urea nitrogen
BW	: Body weight
°C	: Degree celsius
Cd	: Cadmium
Cu	: Copper
CuSO ₄	: Copper sulphate
dl	: Decilitre
Fe	: Iron
Fig(s)	: Figure/s
fl	: Femtolitre
Hb	: Haemoglobin
Hg	: Mercury
i/m	: Intramuscular
I.U.	: International unit
MCH	: Mean corpuscular haemoglobin
MCHC	: Mean corpuscular haemoglobin concentration
MCV	: Mean corpuscular volume
ml	: Millilitre
mm ³	: Cubic millimeter
mol	: mole
μ	: micron
μg	: Microgram
μmol	: Micromole
PCV	: Packed cell volume
pg	: Picogram
S	: Sulphur
Sb	: Antimony
Se	: Selenium
SGOT	: Serum glutamic oxaloacetate transaminase
SGPT	: Serum glutamic pyruvate transaminase
SH	: Sulph-hydral group
SI	: Serum iron
TEC	: Total erythrocyte count
TLC	: Total leucocyte count
TPP	: Total plasma proteins
TSP	: Total serum proteins
WMD	: White muscle disease
Zn	: Zinc
ZnSO ₄	: Zinc sulphate
>	: Greater than
<	: Lesser than
%	: Per cent

CHAPTER I

INTRODUCTION

The soil is the source of various macro and micro-nutrients for plants, animals and human beings. The requirement and physiological role of the macro-nutrients has been well documented but a very scarce information is available with respect to few micro-nutrient elements, of which, selenium (Se) is one.

Selenium is a metalloid, which is very similar to sulphur in its chemical properties. It has an atomic number of 34 and atomic weight of 78.96. It lies between the metals tellurium and polonium and the non-metals oxygen and sulphur (Mcdowell, 1992).

Selenium occurs in a wide variety of rocks, lunar and volcanic materials, fossil fuels, soils, plant materials and waters. It enters soils primarily as a result of the weathering of Se-containing rocks, although volcanic activity, dusts (e.g., in the vicinity of the burning of coal), some phosphatic fertilizers and some waters can also be sources of selenium for soils (Mcdowell, 1992). Se is scarcely present in soil, water

and everywhere in the environment and is unevenly distributed (Mcdowell, 1992).

Selenium was named by the Swedish chemist J.J. Berzelius in 1818 after its discovery in the residues of sulphuric acid preparations from a mining operation. The oldest recorded case of disease syndrome known as alkali disease which resembles selenium poisoning was described by Polo Marco in 1295 from one of his journeys to Asia.

The early interest in Se was related to its properties as a toxic nutritional element. During the mid 1930s Se was first identified as the toxic element in some forages that caused animals to loose hairs, nails and hooves. The requirement of selenium in preventing nutritional muscular dystrophy in calves and lambs, reproductive disorders in cows, hepatosis dietetica in growing pigs, increasing susceptibility to mastitis in deficient cattle and various immunological aspects of farm animals and birds has been established. (Shamberger, 1983; Radostits *et al.*, 1994).

Although total areas of the world affected by Se deficiency are far greater than Se excess, yet in Punjab Se problem has been acting as a graveyard for animals in villages lying on the boundary line between districts Hoshiarpur and Jalandhar, owing to its toxicity (Gupta *et al.*, 1982; Dhillon and Dhillon, 1991b).

Se, as a trace element, is not an essential element for plant growth, but its level in fodder crops is very

important for animal health. Discovery of selenium as an essential element for animals (Schwartz and Foltz, 1957) has stimulated surveys on Se concentration of field crops in several countries.

Selenium (Se) levels of normal soils in the world fall in the range of 0.1 to 2.0 ppm (Swaine, 1955). Animals suffer from deficiency of Se in areas producing fodder crops containing less than 0.1 ppm Se and in the areas producing crops more than 4-5 ppm Se, animals suffer from toxicity of Se (National Research Council, 1983).

The biochemical role of selenium has been reported as a component of the enzyme glutathione peroxidase (Rotruck et al., 1973) which catalyzes the conversion of hydrogen peroxide to water and converts lipid peroxides to lipid alcohols. Thus, glutathione peroxidase alongwith superoxide dismutase, catalase and vitamin E protects the damage to cellular components by preventing the accumulation of peroxides in tissues.

Pockets of soil with relatively higher level of Se usually exist in more arid regions such as Western United States (Rosenfeld and Beath, 1964) but are also found in areas where rainfall is normal as in Punjab (Gupta et al., 1982). Selenium toxic pockets have been identified in Hoshiarpur and Jalandhar districts of Punjab and also has been reported in Haryana (Arora et al., 1975).

Fodder crops at selenium toxic sites in Punjab contained 4.5 to 159.5 ppm of Se (Dhillon and Dhillon, 1991b).

A number of animals feeding on fodders having more than 5 ppm of Se suffer from Se poisoning and exhibit typical Se toxicity symptoms like peeling off of horns, gradual detachment of hooves and falling of hair (Gupta et al., 1982). In addition to this, selenium toxicity provokes decreased productivity, anoestrus or delayed onset of oestrus, failure of conception, abortion and ultimately death of affected animals thus causing extensive economic losses (Dhillon et al., 1992). Chronic selenium toxicity also causes impairment of hepatic and renal functions (Randhawa et al., 1992).

The geochemistry of selenium parallels that of sulphur. The behaviour of two elements in living organisms could also be expected to be similar in some respects. In fact, biological interactions between selenium and sulphur have been shown to occur (Legget and Epstein, 1956; Ulrich and Shrift, 1968).

Except in isolated cases, selenium does not occur in water in sufficient amounts to produce selenium toxicity in plants or animals. It has been reported that water containing 20 ppb Se can be used for irrigation upto 20 years continuously without showing any toxic effects (Ayers and Westcot, 1976).

Farmers of Hoshiarpur and Jalandhar districts of Punjab are experiencing a colossal loss in animal wealth due to consumption of fodders containing toxic levels of Se. The land affected by high level of Se is on the increase. If such degradation of soil continues, the losses in terms of animals'

health as well as grain production will increase enormously.

As per available literature, there is scarcity of information regarding pathogenesis of selenium toxicity, alterations in animal system due to Se poisoning and scientific information in establishing inter-relationship of Se toxicosis in soil-plant-animal-human chain.

Thus, to study the patho-physiology of Se toxicosis and to work out the ameliorative measures, the studies were undertaken with following objectives.

OBJECTIVES OF INVESTIGATION:

1. To study the clinical symptoms, clinical pathology and post-mortem lesions of the affected animals.
2. To study the soil, water and the selenium level of the blood and ruminal fluid of affected animals.
3. To study the effect of supplementation of selenium at different doses in experimental calves.
4. To study the effect of preparations of antimony, arsenical and some other compounds for the treatment of clinical cases or experimental cases.
5. To study (qualitatively and quantitatively) the alterations in certain amino acids and enzymes in animals affected with selenosis.

CHAPTER II

REVIEW OF LITERATURE

The biological significance of selenium is reflected not only in its role in several agricultural disease syndromes but also in the numerous physiological systems it may influence and its essentiality in the mammalian diet. Various forms of toxicity both acute and chronic, have been attributed to the ingestion of this element. Two commonly observed chronic selenosis, "blind staggers" and "alkali disease", can be observed in agricultural animals feeding on plants containing moderate to high levels of selenium compounds. The third chronic selenosis is an experimental form usually induced by the presentation of inorganic salts. As is known in chemical toxicities, the extent of the toxic manifestations may be altered by the subject species, sex, age and chemical form of selenium and its route of administration. There are also various forms of deficiency disorders observed in animals fed on diets low in selenium.

In animals, blind staggers, ataxia, elevation in body temperature, laboured respiration, rapid but weak pulse, and

death from respiratory failure are the acute toxic symptoms. A subacute organic selenosis called alkali disease is caused by consuming plant material containing 25 to 50 ppm Se. The symptoms are loss of weight, irritability, degeneration and fibrosis of the heart, liver and kidneys and impaired reproductive capacity with foetal resorption. However, the literature on various aspects of selenosis in dairy animals is relatively meagre so incorporation of literature of other species was indispensable in the present venture which has been reviewed with respect to history, incidence, pathogenesis, symptoms, haematological and biochemical alterations, post-mortem lesions, treatment, selenium in blood/tissues, selenium in soil/fodder/hair and selenium content of water respectively.

2.1 HISTORY

Polo Marco (1295) in his travels in Western China (1295) near the borders of Turkestan and Tibet, was perhaps the first to describe a disease syndrome resulting from ingestion of seleniferous plants. He reported a poisonous plant that, if eaten by horses, caused hooves to drop off.

Simon (1560) described a disease in Columbia causing malformations in chicks and children and loss of hair and nails in human beings. It was referred to as selenium poisoning only after the discovery of selenium.

Berzelius (1817) discovered selenium (Se) from sulfur used in the Swedish sulfuric acid industry, from falun pyrites at Gripsholm, Sweden. These discoveries were first published in

1817 and later disclosed in a letter to Berthollet dated February 9, 1818. In fact, before Berzelius, a man by the name of Arnold de Villanova was the first to observe this element but failed to name it. During the beginning of 14th Century, he observed the deposition of some material along the walls of the container used for vaporization of sulfur, which he later named as Sulphur rubeum.

Beath and his co-workers (1917, 1921, 1932, 1934a, 1934b, 1935, 1936, 1937a, b, c, 1939a, 1939b, 1940, 1941, 1943 and 1947) conducted a most extensive programme which included a study of plants, animals and geological structures of Wyoming and other states and listed the Beath indicator plants which grow on seleniferous soils.

Shirlaw (1939) first of all reported the symptoms and lesions similar to alkali disease in buffaloes and to some extent in cattle in rice growing areas bordering the DEG rivulet in Pakistan and so this disease was termed as Degnala disease. However, the causative factor was unknown at that time.

Zingaro and Cooper (1974) pointed out that reddish deposits of selenium were first believed to be that of tellurium, but further examination showed that it was readily volatile and reducible. Since tellurium was named after the earth, selenium for its similarity to tellurium, was named after the moon.

Selenium toxicity was reported to be the main cause by Arora et al. (1975), for the development of this disease in buffalo on feeding rice straw.

According to Kalra et al. (1977), mycotoxin produced by Fusarium equiseti was considered to be the causative factor for degnala disease, although the toxin could not be detected in the experimentally inoculated rice straw with the above fungus.

2.2 INCIDENCE

Cadell and Cousins (1960) suggested direct relationship between urinary selenium concentrations and the prevalence of dental caries in American children. Although selenium has not been demonstrated to be essential in man, its suspected role in electron transport may suggest a possible modification of the calcified structures.

Bakshi et al. (1986) reported that while conducting comparative studies on urea enriched wheat/rice straw rations, symptoms as described for Degnala disease, by various workers, had developed in buffaloes fed rice straw. The intake of toxic levels of Se through rice straw, in the present study, than any other factor had resulted in producing the symptoms and the lesions typical of selenosis. It was, therefore, concluded that Degnala like symptoms on feeding rice straw to buffaloes can arise in the areas having soil environment interaction and agronomic practices favourable for the increased Se uptake by the rice plant.

Ghosh et al. (1993) reported the incidence of selenium toxicosis in grazing buffaloes in West Bengal. The pH of the soil was acidic. Both soil and forages analysis indicated toxic level of selenium. In this tropical region with unusual heavy rainfall there was a marked leaching of the said element from the Himalayan region to the sub-Himalayan belt for deposition. Cold weather or low temperature had a synergistic effect to produce the disease when the average atmospheric temperature was 12°C and humidity 66 to 82%.

2.3 PATHOGENESIS

Cousins and Cairney (1961) found that faecal excretion of selenium, even on low levels of intake, was high and it was suggested that reduction of dietary selenium to a non-available form occurred in rumen.

Rosenfeld (1962) reported that most selenium analogues were fully active as substrates for the enzymes of sulphur metabolism. Therefore, proteins were altered by substitution of selenomethionine for methionine. So sulphate treatment can be adopted to antagonise the effect of selenium toxicity at tissue level in animals.

In an evaluation of selenium as either a dietary essential or a carcinogenic risk, McConnell (1963) noted that administered selenium was retained by the animal for extended periods of time. Selenium passed the placental barrier as well as the mammary glands and into the milk proteins. In specific

cells, as the erythrocytes, selenium once incorporated remained there throughout the life span of the cell. Selenium got readily incorporated into rapidly growing and embryonic tissues. Selenium was incorporated into tissue proteins such as hemoproteins, enzymes and nucleoproteins. It was also present in the various intracellular fractions of liver.

Pan *et al.* (1964) observed in an experiment that a similar pathway was involved in the metabolism of both sulphur and selenium containing amino acids. The pattern of incorporation of label from the Se-alkyl labelled seleno-amino acids was found to be essentially similar to that of sulphur-amino acids.

Whanger (1970) found that in plant and animal metabolites selenium has been related intimately to sulphur-containing components, and the two elements may utilize the same metabolic pathways. Since no known biochemical pathway has been identified in non ruminant animals for reduction of inorganic sulphate and biosynthesis of sulfur amino acids, the possible biosynthesis of inorganic selenium into selenoamino acids has been questioned.

Ganther (1971) evaluated the role of SH groups in the non-enzymic^{at} incorporation of selenium into proteins and found that selenium was incorporated into the proteins at disulphide bridge to form seleno-trisulphide at low pH, whereas at pH 7.0 the reaction was accompanied by extensive liberation of elemental selenium.

Madan et al. (1973) concluded that there existed a biochemical interrelationship between selenium and sulphur in plants and microorganisms. Sulphur and selenium were interchangeable and excess of selenium replaced the sulphur in the proteins. Since sheep produced wool containing a very high level of sulphur, selenium in the feed may affect the quality and quantity of wool. Probably sulphur atoms were replaced by selenium atoms at the proper position in the molecule, without affecting the diameter of the fibre.

McConnel et al. (1974) said that generally selenium has been thought to be metabolized by incorporation into isologues of the sulphur amino acids and particularly into methionine. None of the selenium derived from orally administered selenite in chick serum proteins was either selenomethionine or selenocystine and suggested that the majority of the selenium was covalently bound between the sulphurs of half-cystine polypeptide residues and could be released by non-proteolytic means. If so, protein incorporation and release of selenium would not be dependent on protein synthesis or catabolism.

Donaldson (1976) suggested that Se inhibited fatty acid synthetase (FAS) by reversible bonding of Se to the SH groups (possibly at the active sites for acetyl-COA and/or malonyl-COA binding of FAS).

Ray and Ray (1976) pointed out that selenium was

capable of inhibiting certain enzyme systems and its toxic effect may be exerted by inhibiting the enzyme involved in cellular respiration. It could be concluded that oxygen uptake by the cell was reduced and this might be due to the sodium selenite which partially inhibited the enzyme system or there might be less ascorbic acid in the treated animals.

Underwood (1977) reported that absorbed Se was at first carried mainly in the plasma, from which it entered all tissues, including the bones, hair and leucocytes. Selenite (Se) had to undergo a chemical transformation by the erythrocytes in order to be wound by plasma proteins. Recently, it has been shown that Se that has been processed by the erythrocytes was taken up largely by β -lipoprotein and an unidentified fraction located electrophoretically between α_1 and α_2 -globulin fractions. The exact nature of the Se-binding of these tissues and the extent of its incorporation into selenoamino acids in tissue proteins was not fully determined yet.

According to Prasad et al. (1982) the toxic rice husk containing 6.23 ppm selenium, presented an altered picture of concentration of 'S' containing amino acids during analysis of this rice husk (rice-straw) using Beckman's amino acid analyser. This toxic rice-straw was estimated to contain 3.128 mM selenocystine and 10.522 mM selenomethionine per gm sample which were found to be far higher as compared to 2.098 mM selenocystine and 4.530 mM selenomethionine per gm in non-toxic

control rice straw. Normally a small amount of seleno-amino acids were synthesized by rumen microbes, but the increased dietary source of seleno amino acids altered the levels of 'S' containing amino acids in the animal body system and has been documented as cause of Degnala syndrome. Cystine in the toxic rice-straw samples was analysed to be 3.502 mM per gm while methionine was found only in traces and could not be detected in the sample probably due to Se : S antagonism or due to conversion of 'S' amino acids into seleno amino acids.

Sharma and Singh (1983) reported that still today, the precise way in which selenium at toxic levels interferes with tissue structure and functions was not clear. One of the reasons can be the replacement of sulph-hydral groups essential to the exudative process by selenium, leading to possible tissue injury and malfunction. They also reported, in an experiment on goat milk, that selenomethionine replaced part of the methionine in the casein fraction of milk.

Jubb et al. (1985) concluded that selenium toxicosis has been described as cause of claw lesions in young pigs but the mechanism by which selenium exerted this effect was unknown. Possibly its competition with sulphur might modify the structure of keratin.

Mensink et al. (1990) were of the view that severe hoof and claw abnormalities in livestock indicated that the excess selenium interfered with more than just the process of cornification. The degeneration of the podal epidermis may have

been due to a selenium-induced oxidation of mitochondrial NAD(P)H and a subsequent effect on mitochondrial calcium homeostasis.

Ghosh et al. (1993) studied pathogenesis of selenosis in West Bengal and reported that selenium reduced the sulphur and protein content of liver and this resulted in the anaemia. The low level of proteins and cobalt in the affected animals might had also some role in acute selenosis in some cases along with the excess intake of it from the plants grown in seleniferous soils.

Radostitis et al. (1994) observed that selenium analog was active for the enzymes of sulphur metabolism and thereby proteins were altered by substitution of selenomethionine for methionine. So sulphate treatment was beneficial to antagonise the effect of selenium toxicity at tissue level.

2.4 SYMPTOMS

Rosenfeld and Beath (1946) pointed out that selenium poisoning in sheep caused a gradual decrease in vitamin A and total proteins content of the blood. A more rapid decrease in the ascorbic acid and an increase in non -protein nitrogen was noted in all sheep dying from selenium poisoning. The heart and liver were affected by selenium feeding while the kidneys, lungs and spleen showed no changes in total proteins, sulphur and nitrogen content. Blood gradually became normal after selenium feeding was discontinued.

Rosenfeld and Beath (1947) found that about 75 per

cent of the malformed lambs died at birth and that 10 per cent died when 3-5 months of age. The lambs that lived showed deformities of the eyes and extremities and hypoplasia of the reproductive organs.

Glenn et al. (1964a) came up with a view that selenium treatment had no effect on reproduction in sheep. Body weights were unaffected until near the time clinical signs of toxicosis appeared. The disease was always acute and terminated fatally regardless the size of the toxic dose of selenium or length of treatment. Clinical signs were anorexia, weakness, apathy, loss of condition, respiratory distress, cyanosis and nasal exudation.

Harr (1973) reported that conditioned zinc deficiency caused poor wound healing and poor vascularisation.

Arora et al. (1975) observed that in Degnala disease caused by excessive selenium content in forage the apparent gross lesions exhibited by different animals usually involved body extremities. Animals showed multiple cutaneous and sub-cutaneous lesions on the skin of limbs distal to knee or hock joints, desquamation of epidermis in muzzle region, gangrenous tail and necrosed eartips, severe inflammation of limbs, deformed hooves resulting in lameness. Some cases exhibited sloughing off of hooves due to puss formation in coronet and pastern regions. Distinct loss of appetite, secondary infections leading to death by slow starvation and emaciation occurred only in later stages. Amongst recorded cases in

domestic animals buffalo seemed to be more susceptible to selenium toxicity than cattle.

Gupta et al. (1982) reported that most significant symptoms and lesions in chronic selenium toxicity were seen in hooves and horns which started with cracks, below the coronary bands, which deepen gradually followed by sloughing. Secondary bacterial infection in cracks lead to pus formation leading to off smell, difficulty in standing, staggering gait, lameness and recumbency. The covering of keratinized necrosed layer of horn sloughed off leaving the raw horn core. Animals finally became weak, depressed, emaciated and revealed hide-bound condition. Failure to conception and abortion in pregnant animals were frequent observations of this disease irrespective of species.

James et al. (1982) reported in the article "Suspected phytogenic selenium poisoning in sheep" that acute selenium intoxication was characterized in cattle and horses by lack of appetite, refusal to drink, quiet stance, unsteady gait, increased pulse and laboured respiration. The animal may die in a matter of few hours. However, in sheep selenium poisoning was best characterized by an increased respiratory rate and sudden death.

Prasad et al. (1982) demonstrated that rice husk from seleniferous areas containing 6.23 ppm selenium was found to be highly toxic and symptoms of its toxicity could be induced in experimental animals. They fed this rice-husk to nine Murrah

buffalo calves, out of which 4 died and rest of 5 buffalo calves showing skin necrosis on legs below the knee or hock joints leading to desquamation of necrosed skin from legs and necrosis of ear tip or tail tip were diagnosed as cases of Degnala disease due to selenium toxicity, because, the selenium content in rice husk was found to be (6.23 ppm), which was far above the normal requirement of 0.1 ppm. In one animal severe gastroenteritis was observed a few days before death.

Bakshi et al. (1986) reported that with urea enriched wheat straw feeding, no symptoms or lesions were observed. In the urea enriched rice straw fed buffaloes, initial symptoms of swollen extremities followed by necrosed skin below the hock joints were observed within 30 days. The swelling of forelimbs was more aggravated with phimosis. The necrosis on tail and ear tips had also started. At a later stage the desquamation of the necrosed skin spots and skin around hooves had resulted in separation of hoof from the laminae resulting in the sloughing off of hoof in certain individuals.

The animals showed alopecia, staggering gaits and general debility. At the terminal stage the animal would lie prostrate with stretched limbs, gritting of teeth with grunt due to pain before death. The development of symptoms in the buffaloes fed on rice straw supplemented with concentrate mixture also followed the same trend till their death as described above. Some of the symptoms observed were common for the Se toxicity and Zn deficiency in the rations.

Dhillon *et al.* (1990) produced symptoms similar to Degnala disease by feeding selenourea to buffalo calves. It included oedema of fetlock region, tip of tail and ears. Patchy hair loss from fetlock was followed by skin and hoof cracks. Skin cracks took the shape of regular wounds. Hoof cracks were followed by ring formation and overgrowth (Table 1). However, these observed symptoms were not as severe as in natural cases. Necrosis of the muzzle and tip of the tongue was not observed in any animal.

Table 1. Chronic selenium toxicity symptoms as produced by feeding selenourea @ 0.15 mg/kg body weight

Symptoms	Period in days
*Oedema of fetlock region	5-11
*Hair loss	6-10
*Skin cracks	8-12
*Wounds	11-20
Hoof cracks	11-14
Tail oedema and necrosis	10-20
Ear tip necrosis	11-25
Hoof rings	12-20

* Symptoms 1-4 were observed in fetlock region (Dhillon *et al.* (1990).

In selenium toxicosis in grazing buffaloes of West Bengal, Ghosh *et al.* (1993) reported that clinically affected buffaloes showed general malaise, abdominal pain, reluctance to move with severe laminitis and anorexia because of the marked swelling of the tongue. There was oedema of fetlock region and other extremities of the body leading to ulceration. In all cases there was loss of hair, skin cracks, hoof cracks with ring formation just below the coronary band. Necrosis and oedema of the tail and ear tips was recorded. Animals passed voluminous semisolid to loose stool. Terminally animals became cachectic and skin became like parchment. Some animals were moribund and died after some days.

2.5 HAEMATOLOGICAL AND BIOCHEMICAL ALTERATIONS

Rosenfeld and Beath (1946) pointed out gradual decline in total proteins content of blood in sheep following addition of Se in diet.

Gerov and Cuskov (1964) reported that a dose of 10 mg of Se dioxide/kg body weight and 40 mg of sodium selenite/kg body weight was fatal for lambs and caused decline in Hb, TLC and TEC values.

Hirooka and Galambos (1966) confirmed the previously described association of injected inorganic ^{75}Se with lipid rich globulins of rat serum. The high degree of association of ^{75}Se with the α_2 and β globulins was observed not only in rats but also in humans. The affinity of selenium to the α_2 and β globulins seemed to be characteristic of mammals and was not

found in birds. A 17.5 fold increase of carrier SeO_4^{-2} resulted in a 5-fold decrease of the serum protein-bound ^{75}Se as determined by electrophoresis. This decrease was confined to the globulins only.

Dhillon *et al.* (1992b) observed significant decline in Hb, PCV and TEC values but no change in TLC values in cattle and buffaloes thriving on fodder raised on seleniferous soils for more than a year. An increase in MCV and decline in MCHC values was also observed, reflecting macrocytic and hypochromic anaemia.

Randhawa *et al.* (1992) observed a significant increase in the mean activity of alkaline phosphatase (271.44 ± 8.61 IU/L) in bovines suffering from chronic selenosis, as compared to that in healthy control (141.00 ± 8.83 IU/L). However, ALT (Alanine aminotransferases) and AST (Aspartate aminotransferase) activity of some animals showed non-significant variation. They also reported elevated blood urea nitrogen in Se toxicotic animals (24.85 ± 1.74 mg/dl) as compared to those of healthy control (4.66 ± 0.91 mg/dl) and decreased total plasma proteins in such animals i.e. 5.89 ± 0.30 mg/dl as compared to those of healthy control (7.00 ± 0.36 g/dl).

Ghosh *et al.* (1993) reported haematological and biochemical profiles of selenosed and control animals which have been presented in Table 2. All clinically affected buffaloes developed hypo-haemoglobinaemia, erythrocytopenia and low PCV which were suggestive of anaemia that possibly resulted

from the decreased level of proteins in the liver as the selenium reduced the sulphur and protein content of liver. The biochemical examination revealed hypoglycaemia, hypoproteinaemia, hypocobalosis and hyperselenosis. There was marked change in iron and copper concentration in blood of clinically affected animals in comparison to control animals, which possibly was due to interference in the utilization of iron resulting from hypoproteinaemia for formation of haemoglobin.

Table 2. Haematological and biochemical status of clinically affected and healthy buffaloes

Profiles	Clinically affected	Control
Hb (g/dl)	8.27 $\pm$ 0.83**	13.58 $\pm$ 0.46
PCV (%)	23.11 $\pm$ 1.05**	28.00 $\pm$ 0.77
TBC ($10^6/\text{mm}^3$)	5.37 $\pm$ 0.89**	6.96 $\pm$ 0.61
BGL (mg%)	46.92 $\pm$ 0.54**	57.32 $\pm$ 1.21
TSP (g%)	5.19 $\pm$ 1.08**	7.29 $\pm$ 0.67
SI (ppm)	3.89 $\pm$ 0.51**	4.01 $\pm$ 0.39
Se (ppm)	1.21 $\pm$ 0.03**	0.12 $\pm$ 0.02
SCu (ppm)	1.06 $\pm$ 0.08**	1.56 $\pm$ 0.11
SCo (ppm)	0.14 $\pm$ 0.005**	0.39 $\pm$ 0.020

**, $P < 0.01$

Ghosh et al. (1993)

2.6 POST-MORTEM LESIONS

Glenn et al. (1964b) studied post-mortem lesions of selenium toxicosis in 30 ewes fed subtoxic to toxic levels of sodium selenate for 1-5 months. Seventeen of the 30 ewes died of selenium toxicosis. The most severe and consistent pathological changes were found in the myocardium and lungs. Myocardial alterations were focal to diffuse degeneration, necrosis and early replacement fibrosis. Pulmonary changes, consisting of edema and interstitial haemorrhages, were typical of the degenerative processes which characterize passive congestion of the lungs resulting from left ventricular insufficiency. Atrophy of lymphoid centres in the spleen and lymph nodes was common. Degenerative changes were occasionally found in liver, kidneys and gastroenteritinal tract.

Van Vleet et al. (1974) studied post-mortem lesions in pigs induced with acute selenium toxicosis. Pathological alterations observed grossly and histologically included pulmonary edema, skeletal-myodegeneration, hepatic degeneration and necrosis, transudation into body cavities and widespread circulatory disturbances.

Prasad et al. (1982) induced typical Se toxicity symptoms by feeding rice husk to 9 Murrah buffalo calves. Out of these 4 animals died and 5 developed typical symptoms of Degnala syndrome. The autopsy lesions (gross and histopathological) observed in different animals were reported as congested liver and kidney, pale heart with jelly like

deposits, haemorrhagic gastroenteritis, necrotic foci on liver parenchyma, petechial haemorrhages on myocardium and dark red haemorrhagic kidney.

Hopper *et al.* (1985) studied histopathology in the lambs that died of selenium poisoning. At necropsy the gross lesions were consistent, with subcutaneous haemorrhages, marked pulmonary edema with hydrothorax and extensive destruction of renal cortices (similar to that found in clostridial enterotoxaemia) being detected. Other changes detected, but less consistently, were abomasitis, hepatic congestion and haemorrhages around the brain stem.

Histopathological examination revealed marked tubular degeneration in kidneys with, in some instances, tubular cast formation. Vacuolation of hepatocytes was noted irregularly.

Bakshi *et al.* (1986) studied gross and histopathological lesions in the animals (12) that died of selenosis due to feeding of selenosed rice straw and reported that animals in general showed acute gastroenteritis, ulcerative and hemorrhagic gastroenteritis, gangrenous syndrome, necrotic hepatitis and fibrous pericarditis.

According to Dhillon *et al.* (1990) histopathological examination of the tissues of animals in which selenosis was induced by feeding selenourea, revealed proliferation of fibrous tissue and infiltration with mononuclear cells, neutrophils and eosinophils. Occlusion of blood vessels due to thrombus formation and renal and hepatic degeneration was also

observed.

2.7 TREATMENT

Hurd-Karrer (1938) concluded on the basis of previous observations that inorganic sulphate will counteract selenate toxicity in plants and in microorganisms as measured by growth response. That selenate is in some cases a biological analogue of sulphate, has become apparent particularly in microbiological systems. For example, Escherichia coli under suitable circumstances can produce amino acids containing selenium in place of sulphur and selenate as well as molybdate and several other ions appeared to be an alternate substrate to sulphate for the yeast enzyme sulfurylase.

Moxon et al. (1940) recorded that p-bromobenzene administered to animals is detoxified by conjugation with tissue cystine and methionine and excreted in the urine as p-bromophenyl-mercapturic acid. Since selenium occurred in the body proteins of animals fed seleniferous diets, and since it was possible that it may replace sulphur in amino acids, the p-bromobenzene appeared to aid the organism in excreting the selenium by taking it from the proteins of the blood and tissues, possibly as selenium-cystine and selenium-methionine which were conjugated with the p-bromobenzene and excreted as the mercapturic acid. It has also given excellent results in the treatment of a human case of selenium poisoning.

Halverson et al. (1955) said, it was possible that linseed meal might be providing protection against selenosis

against seleniferous corn, by providing high level of protein available from linseed meal.

Wahlstrom et al. (1955) reported that excellent protection against up to 10 ppm of selenite was given by two arsenicals namely arsanilic acid and 3-nitro-4-hydroxyphenylarsonic acid at levels of 0.02 per cent and 0.005 per cent respectively. Partial protection, as evidenced by reduced mortality or increased growth, was shown at the lower levels used (arsanilic acid 0.01 per cent and 3-nitro-4-hydroxyphenylarsonic acid 0.0025 per cent). No toxic effects due to the arsenicals were observed at any time during either experiment.

Ganter and Baumann (1962a) showed that the elimination of volatile selenium can be greatly influenced by the type of diet fed. Arsenite was studied as a typical agent that minimises selenium toxicity; cadmium was included because it increases selenium toxicity and because both arsenite and cadmium have selective affinity for dithiols. Arsenite markedly increased the excretion of selenium into the gastrointestinal tract, this excretion amounting to 30 to 40% of the injected dose in most animals, compared with 5 to 15% in the absence of arsenite. Levels of selenium in the blood, liver and carcass were decreased by arsenite as were the amounts in the expired air, kidney levels were increased. Urinary excretion of selenium was not affected by arsenite.

Ganter and Baumann (1962a) also reported that arsenic

increased the excretion of selenium into gastrointestinal tract and its deposition in the kidney of rats, but decreased Se contents of blood, liver and carcass.

Hintz and Hogue (1964) reported that indirect evidence of competitive interactions involving selenium and sulphur in ruminants has come from field trials, whereby the incidence of white muscle disease (WMD) has been shown to increase with the addition of sulphate to the diet or likewise where the beneficial effect of selenite has been counteracted by added sulphate.

Arora et al. (1975) demonstrated that in all 23 out of 28 sick animals treated with Degcure mixture, responded to treatment and recovered completely. The other five sick animals did not respond because of their advanced stage and attack by secondary organisms resulting in pus formation in the affected tissues. The dose regimen followed with Degcure mixture was 30 g orally daily for 50-60 days. Inhibitory effect of sulphur to selenium uptake, by proteins at the disulphide bridge or by release of selenium from the proteins by replacement might have alleviated the toxicity symptoms because of close relationship between sulphur and selenium.

Jensen (1975) reported that high levels of dietary copper reduced the toxic effects of selenium in chicks apparently by causing the accumulation of a non-deleterious selenium compound in the liver.

Buck et al. (1976) reported that arsenicals were said

to decrease the toxicity of selenium salts by causing a shift from respiratory excretion to biliary excretion of selenium.

Fractionation studies conducted by Jenson and Chang (1976) showed that a protective factor in linseed meal was extracted by methanol and ethanol and was not destroyed by autoclaving. Concentrates of the factor were active at less than 1% of the dry matter of the diet. The results of these studies showed that linseed meal contained heat stable, organic, polar factor that modified selenium toxicity in the chicks. The factor in linseed meal could be interfering with adsorption of selenium or facilitating its elimination from the tissues.

Stowe and Brady (1978) reported that copper pretreatment reduced selenium toxicity in ponies. The mechanism of this protective effect had not been established. The astringent effect of Cu (CuSO_4) on the gastrointestinal mucosa may reduce Se absorption, however, Cu pretreated and non pretreated ponies had similar, highly elevated serum Se levels at 2 and 5 hours post Se administration. The rate of Se clearance from the serum was enhanced by Cu pretreatment.

Pope *et al.* (1979) reported from an experiment in sheep that urinary excretion of selenium accounted for between 12% with 0.05% sulphur and 22% with 0.24% sulphur respectively, of the dose after 7 days, with treatment differences being significant. Levels of radioactive ^{75}Se in blood were significantly higher in sheep fed the 0.05% S diet compared

with those fed the higher levels. The results showed that sulphur affected apparent selenium excretion and suggested that the metabolism of these two elements was intimately related.

While simultaneously feeding toxic rice straw containing 6.23 ppm selenium to buffalo calves, treatment was undertaken by Prasad *et al.* (1982) with "Degcure mixture"* evolved at National Dairy Research Institute, Karnal (India) in severely toxic animals. Degcure was recommended at the rate of 15-40 gm daily, orally, depending upon live-weight of animal and content of Se in diet until the recovery of animal. Animals recovered completely and Degcure prevented the necrotic spread on the ear pinna and other extremities.

Lowry and Baker (1989) found that supplementing the high selenite diet (15 mg/kg Se) with isoarsenous (14 mg/kg As) additions of As_2O_5 , As_2O_3 , phenylarsonic acid, phenylarsine oxide and roxarsone ameliorated the Se-induced growth depression : As_2O_5 almost totally restored growth rate; As_2O_3 , phenylarsonic acid and phenylarsine oxide gave intermediate responses; and roxarsone gave only a small ameliorative growth response. These findings suggested that both inorganic and organic arsenicals as well as cysteine ameliorated selenium toxicity by different mechanisms.

* Ingredients of Degcure mixture:

Magnesium sulphate	1 kg
Ferrous sulphate	166 gm
Copper sulphate	24 gm
Zinc sulphate	75 gm
Cobalt sulphate	15 gm

(evolved at NDRI, Karnal, in the Division of Dairy Cattle Nutrition and Physiology).

Fraser et al. (1991) suggested that stiffness in cattle and horses that may occur as part of chronic selenosis, may be relieved by the oral administration of compounds such as naphthalene and bromobenzene, which form mercapturic acids. The usual treatment could be, to give 40.5 g of naphthalene daily for 5 days and to repeat the dosage after a 5-day interval.

McDowell (1992) reported that high dietary protein content reduced toxicity of seleniferous diets through the endogenous sulfate yielded by the metabolism of the sulphur-amino acids in the excess protein. Linseed meal had a unique protective activity against chronic Se toxicosis, which was not associated with proteins. The cyanogenic glycosides, linustatin and neolinustatin were responsible for the protective action of linseed meal. Other dietary factors those could ameliorate Se toxicity were As, Hg, Ag, Cu and Cd so that tolerance to a particular dietary Se level would depend on the concurrent dietary levels of these elements in the diet.

Ghosh et al. (1993) treated 17 cases of selenium toxicosis in grazing buffaloes (Bubalus bubalis) with a mixture of compound sulphate of various elements such as magnesium, iron, copper, zinc and cobalt (i.e. Degcure) @ 15 to 40 g/day orally for 60 days. Linseed-cake @ 500 g - 2 kg/day and linseed oil @ 150-250 ml according to body weight were administered thrice orally in divided doses along with Degcure mixture. The 14 animals recovered from the disease, remaining 3 died owing to severity of the condition and secondary bacterial

infections. It proved very effective in alleviating the syndromes of toxicity.

Ghosh et al. (1993) also studied the efficacy of a peripheral vasodilator in grazing buffaloes suffering from selenium toxicosis. They administered complamina @ 4 ml/day (600 mg Xanthinol nicotinate) i/m, with a total of 5 such injections daily in 5 cases along with others. But it was reported to be little effective.

2.8 SELENIUM IN BLOOD/TISSUES

Glenn et al. (1964c) reported that the levels of selenium and distribution of selenium in the body tissues of adult sheep fed subtoxic to toxic quantities of sodium selenate daily for 1 to 5 months, varied with the tissue type and with the level and duration of selenium consumption. Selenium concentration was highest in the liver, followed by (in descending order) the kidneys, lungs, spleen, myocardium, skeletal muscles and brain.

McLaughlin and Cullen (1986) reported that concentration of Se in blood of horse grazing on seleniferous pastures ranged from 8.40 $\mu\text{mol/L}$ as compared to the normal value of 2.5 $\mu\text{mol/L}$.

In selenium toxicosis in pigs fed accumulating Astragalus plant species or sodium selenate, Baker et al. (1989) documented that urine and bile concentrations were increased by the greatest factor (40 to 100 times) with tissue concentrations of selenium increased by lesser factor (6 to 17

times).

Randhawa *et al.* (1992) inferred that mean Se content in blood of buffaloes suffering from chronic selenosis was 3.78 ± 2.4 $\mu\text{g/ml}$ which was 47 times higher than those of healthy animals.

2.9 SELENIUM IN SOIL/FODDER/HAIR

Arora *et al.* (1975) reported the range of selenium content in different fodder samples i.e. Berseem 2.2 to 6.7 ppm, paddy straw 1.7 to 4.5 ppm, lucerne 0.9 to 2.8 ppm, maize 4.6 ppm and oats fodder 2.0 ppm respectively. The safe level of Se in fodders is reported to be 0.5 ppm. Levels above 1 ppm might be toxic.

Dhillon *et al.* (1977) investigated selenium status of some common fodders and natural grasses growing in different climatic regions of Punjab. The effect of different doses of selenium on the growth of maize and its interaction with sulphur were also investigated. The ranges and averages of selenium content of plants in gramineae, leguminosae, chenopodiaceae and cruciferae were traces to 0.38 ppm and 0.15 ppm, 0.04 to 0.91 ppm and 0.21 ppm, 0.09 to 0.54 ppm and 0.23 ppm, and 0.11 to 1.50 ppm, respectively. The crucifers, in general, accumulated more selenium than the leguminous and cereal plants.

Gupta *et al.* (1982) analysed the samples of major fodder crops (wheat straw and berseem) from the affected land and also of hairs from the selenosed animals were analysed for

Dhillon and Dhillon (1991a) reported in a survey that tops of sugarcane (Sachharum officinarum) accumulated high levels of Se (7.9 to 67.5 mg/kg) in seleniferous areas, which was 6-10 times higher than those of non-seleniferous areas. They also reported a significant reduction of Se-content in tops and canes of Sachharum officinarum with the application of gypsum at the rate of 1 ton/hectare.

Dhillon and Dhillon (1991b) found that among the plant species primarily grown as fodder for animal consumption, the average Se content was highest in tops of Brassica campestris (119.92 ± 28.31 mg/kg) and lowest in Zea mays (6.53 ± 2.12 mg/kg). Among the cereal grains, the highest amount of Se was found in Triticum aestivum (33.09 ± 15.31 mg/kg) and lowest in oats (Oryza sativa) (7.53 ± 1.78 mg/kg).

Dhillon and Dhillon (1991b) reported that the average Se content of surface (0-15 cm) and subsurface (15-45 cm) layers of soil was 2.12 ± 1.13 and 1.16 ± 0.51 mg/kg respectively in seleniferous areas. The corresponding values of the two layers in the non-seleniferous areas were 0.42 ± 0.24 and 0.32 ± 0.27 mg/kg respectively which was about 5 times less than that of seleniferous soils.

Randhawa et al. (1992) pointed out that mean Se content in hairs samples of dairy animals suffering from chronic selenosis was 37.09 ± 18.5 μ g/g, which was 271 times higher than the selenium level in the hairs of healthy animals.

2.10 SELENIUM CONTENT OF WATER

Robberecht and Van Grieken (1980) reported that the selenium was predominantly present as selenate and total dissolved selenium ranged from 0.9 to 27 ppb in ground water from Israel.

Robberecht et al. (1983) reported that the selenium content of ground water in Belgium ranged from <0.05 to 1.33 ppb and selenate is predominant species. However, the snow and rain water collected in Antwerp, showed a selenium content of about 0.3 ppb.

In seleniferous areas of Punjab, Se content of underground water used for irrigation of crops ranged from 2.54 to 69.53 ppb (Dhillon and Dhillon, 1991b).

CHAPTER III

MATERIALS AND METHODS

3.1 PLACE OF WORK:

The research was carried out in the Department of Veterinary Medicine, College of Veterinary Science, Punjab Agricultural University, Ludhiana.

3.2 COLLABORATION WITH OTHER DEPARTMENTS:

For completing this task, help was sought of the Department of Veterinary Physiology and Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Punjab Agricultural University, Ludhiana.

3.3 TIME OF WORK:

The work was undertaken from March 1993 to February 1995.

3.4 ESTABLISHING CONTROL NORMS:

For comparing the results of clinically selenosed animals, control values were established in healthy and normal animals. For this purpose, healthy buffaloes (six) and cross bred cows/calves (six) were selected randomly from the Dairy Farm of Punjab Agricultural University, Ludhiana. These animals were maintained as normal control group. These animals were

clinically healthy, were not under the effect of any medication prior to and during the study and were dewormed twice at the interval of 15 days prior to the start of study. Throughout the study these animals were maintained under identical conditions of management and nutrition.

Sampling of control group was synchronized with sampling of clinical cases of selenium toxicity to have simultaneous assessment of altered parameters in clinical cases with respect to control group.

3.5 INVESTIGATION OF CLINICAL CASES

3.5.1 Epidemiology:

Study was conducted in the disease affected areas of villages Barwa and Bagouran of Jalandhar district and Simbly of Hoshiarpur district of Punjab. Prevalence of Se toxicity was taken into account on the basis of clinical manifestations and confirmed by analysis of plasma and hair samples for selenium content. Information regarding incidence of disease in various age groups, sex and species has also been elicited. An attempt has been made to understand the sequential clinical findings in affected animals of toxic belt.

3.5.2 Animals:

Thirty clinical cases (20 crossbred cows and 10 buffaloes) suffering from clinical selenosis were selected in disease prone areas of Hoshiarpur and Jalandhar districts (Punjab). Animals were selected by diagnosis based on the typical clinical manifestations of selenosis i.e. deformities

of horns, malformation, cracking, mutilation, slight elongation and sloughing off of hooves, shedding of hair, loss of body weight and skin elasticity, gangrene of tail and tips of ears, chronic traumatic injuries on quarters (especially hind quarters) and emaciation. These findings were confirmed by estimating Se level in plasma and hair samples.

3.5.3 Clinical pathology:

From the clinically selenosed animals blood was collected to study haematology, blood biochemicals, plasma enzyme activities, plasma sulphur amino acids, plasma or blood macro- and micro-elements including selenium. Ruminant fluid was collected to estimate the level of selenium.

Hair samples were analysed for level of selenium. Keratinized tissue from affected horn and hooves was collected to estimate level of certain enzymes. Sulphur amino acids were estimated from samples of keratinized epithelium i.e. tip of gangrenous tail and ear or other extremities.

3.5.4 Post-mortem lesions:

Post-mortem lesions were studied in various internal organs of animals that died of experimental induction of selenium toxicity. Gross and histopathological changes were studied.

3.6 STUDIES ON EXPERIMENTAL SELENIUM TOXICITY

Three groups of buffalo calves were kept in all. Group I and II comprised of 6 buffalo calves each of 6-8 months of age, with average body weight of 80 kg. Group III consisted of

6 buffalo calves, each of 6-7 month of age with average body weight of 75 kg. Before the induction of selenium toxicity all the calves were dewormed with broad spectrum anthelmintic. The calves were kept on ad-lib feeding of green fodder depending upon their availability. Animals were subjected to identical nutritional and managerial conditions during the whole period of experimental study.

3.6.1 Experimental induction of selenium toxicity:

In all the three groups of animals, experimental selenium toxicity was induced with sodium selenite given orally @ 2.0 mg/kg body weight. First seven doses were given on alternate days, orally, followed by seven doses given daily. One animal each from Group I and Group II died after receiving 9 and 11 doses in all, respectively. Tissue samples of these animals were collected in 10% formaline solution for histopathological examination.

3.6.2 Gross and histopathological observations:

In the dead animals of Group I and II, gross lesions were studied. Various internal tissues of dead animals were evaluated histopathologically. The tissues were collected, as early as possible, after the death of the animals, in 10% neutral buffered formaline solution. For making paraffin sections, the tissue samples were processed by cedar wood oil method (Luna, 1968). By a rotatory microtome, paraffin sections of 5-7 μ were obtained on glass slides. For histopathological evaluation, Haematoxylin-Eosin stain (Luna, 1968) was used.

3.7 COLLECTION OF DIFFERENT SAMPLES

3.7.1 Blood samples:

From clinical cases blood samples were collected in two batches, one, before the undertaking of therapeutic measures and second, 40 or 50 days after the therapy. From experimental calves, blood samples were collected once prior to start of the experiment i.e. before the induction of selenium toxicity and thereafter the induction of selenium toxicity, subsequently at 4 days interval for 20 days (5 samplings). Treatment was instituted in Group I and Group II on 20th day, after which the samples were taken at 5 days of interval for 15 days.

No treatment was advocated to the Group 3rd animals but after 20 days of dosing with sodium selenite @ 2.0 mg/kg body weight samples were collected on same days as for treated animals to compare the fall in level of selenium in blood in treated and untreated animals. All the blood samples were collected in the 30 ml heparinized vials (0.1 mg/ml) aseptically by jugular veinipuncture. 5 ml of blood was also collected in a 5 ml vial containing sodium citrate @ 2-3 mg/ml of blood. These 5 ml blood samples were used for analysis of haematological indices and plasma from each of this blood sample was utilized for estimation of fibrinogen.

Blood glucose was estimated by GLUKOSTIX-GLUCOMETER,* at the site of collection of blood itself. The 30 ml blood samples were centrifuged at 3000 rpm for 15 minutes to separate plasma. Plasma was also separated from each of 5 ml blood sample collected in sodium citrate for estimation of fibrinogen. Plasma from all samples was then transferred to plastic vials (7 ml) and stored at -20°C for estimation of various biochemical, amino acid, mineral and enzymatic activities.

The RBCs left after removing plasma were diluted with equal volume of normal saline solution and centrifuged again. The clear solution was removed and the procedure was repeated 3 times in all, to remove the traces of plasma adhered to the red blood cells. Finally after draining the clear normal saline solution RBCs were transferred into 7 ml plastic vials and stored at -20°C for estimation of carbonic anhydrase.

3.7.2 Keratinized epithelium/tissue samples:

For estimation of certain enzymes, keratinized tissue was collected from horns/hooves. Keratinized tissue was collected in clinical cases. Before collection in clinical cases the horns/hooves were washed with water and scrappings of keratinized tissue (1 gm) were collected by scalpel blade or sickle, after complete drying of tissue. Such tissues were processed as early as possible.

*Glucostix-Miles India Ltd., AMES DIVISION, SAYAJIPURA, BARODA

For estimation of sulphur containing amino acids tip of gangrenous tail/ear/muzzle/horns/hooves were taken in a manner described above by scalpel blade.

3.7.3 Hair samples:

The hair were collected only from clinically affected animals. After collection of about 5g of hair samples, they were washed with tap water and then with double distilled water to remove any extraneous matter adhered to the hair. The hair samples were further dried in air and subsequently utilized for Se estimation.

3.7.4 Soil samples:

Soil samples from surface (0-15 cm depth) and plant samples were collected from the same affected field as well as from normal areas of Hoshiarpur/Jalandhar districts (Punjab). Soil samples collected during survey were air dried in shade, ground in wooden pestle and mortar, sieved through 60 mesh sieve and stored in cloth bags for analysis of minerals.

3.7.5 Plant/fodder samples:

Plant/fodder samples were also collected both from normal and disease prone areas of Hoshiarpur/Jalandhar districts (Punjab). In the laboratory these samples were washed successively with tap water, distilled water and double distilled water respectively. Then the samples were air dried

for some time in shade and then placed in an oven for drying at 55-60°C. After drying, the plant samples were ground in a *Willey-Grinding Mill and were stored for analysis of minerals.

3.7.6 Water samples:

In the present study the water samples were collected from the handpumps supplying water to the animals for drinking purpose or from tubewells where they are only source of water supply to animals. Water samples were collected in plastic bottles of about two litre capacity. Water was acidified with hydrochloric acid to prevent loss of selenium (Measures and Burton, 1980) and stored at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ till the estimation of selenium was completed. Two ml of toluene was also added to prevent fungal growth.

3.7.7 Ruminal fluid samples:

Five ml of ruminal fluid was collected from the left paralumbar fossa with the help of a hypodermic needle and syringe. It was acidified with 3-4 drops of concentrated HCl to prevent loss of selenium (Measures and Burton, 1980). 2 ml of toluene was also added to prevent fungal growth and samples were deep frozen at -20°C as soon as possible till the estimation of selenium. This ruminal fluid was collected in clinical cases only.

*Thomas Willey Mill, Model ED-5 supplied by Arthur H. Thomas Co., Phila, P.A., U.S.A.

3.8 BLOOD ANALYSIS

3.8.1 Haematological indices:

Haemoglobin (Hb):- It was estimated by Cyanmethaemoglobin method (Raymond and Wilkinson, 1963) using HB-meter*.

Haematocrit (PCV):- It was determined by using haematocrit tubes (Wintrobe, 1962).

Total leucocyte count (TLC) was done as per the method of Benjamin (1985)

Total erythrocyte count (TEC) was made by the method of Benjamin (1985).

Erythrocytic indices:

Mean corpuscular volume (MCV):- expressed the average volume of individual erythrocyte.

Mean corpuscular haemoglobin (MCH) - gives the amount of Hb by weight in an average erythrocyte.

Mean corpuscular haemoglobin concentration (MCHC) - gives the ratio of weight of Hb to the volume in which it is contained.

These erythrocytic indices were calculated according to the formulae given by Schalm et al. (1975) i.e.

$$\begin{aligned}
 1. \text{ Mean Corpuscular Volume (MCV)} &= \frac{\text{PCV (\%)} \times 10}{\text{Erythrocyte count (million/mm}^3\text{)}} \\
 2. \text{ Mean Corpuscular Hb (MCH)} &= \frac{\text{Hb (g/dl)} \times 10}{\text{Erythrocyte count (million/mm}^3\text{)}} \\
 3. \text{ Mean Corpuscular Hb Conc. (MCHC)} &= \frac{\text{Hb(g/dl)} \times 100}{\text{PCV (\%)}}
 \end{aligned}$$

* Erma-Hb-Meter, Japan (Model 303A).

3.8.2 Blood Biochemicals

Blood glucose was estimated by GLUCOSTIX-GLUCOMETER¹. Total plasma proteins were estimated by the Biuret method. Plasma albumin was estimated by the Biuret method and plasma globulin was determined by subtracting albumin from the total plasma proteins. Plasma urea nitrogen was determined by Dam method as described by Wootton (1964). These estimations were done using diagnostic reagents kits.² Fibrinogen was estimated by turbidity method as described by Parfentjev and Johnson (1953).

3.8.3 Plasma enzyme activities

Asparatate aminotransferase (AST) or Glutamic Oxaloacetate Transaminase (GOT) and Alanine aminotransferase (ALT) or Glutamic Pyruvate Transaminase (GPT) were estimated according to the method of Reitman and Frankel (1957) using diagnostic kits¹. Alkaline phosphatase (ALP) was estimated as per the method described by Wootton (1964) using diagnostic reagents kits².

3.8.4 RBCs enzyme activity

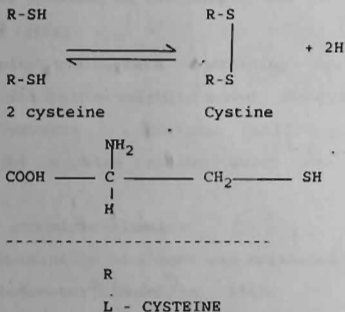
Carbonic anhydrase was determined from the RBCs after suitable dilution by the method described by Roughton and Booth (1946).

1 Glucostix Miles India Ltd., AMES DIVISION, SAYAJIPURA, BARODA.

2 Span Diagnostics Pvt. Ltd., 173-B, New Industrial Estate, Udhana - 394210 (Surat), India..ls2

3.8.5 Plasma sulphur amino acids

Sulphur amino acids methionine, cysteine and cystine were estimated. Experiments on cysteine have been grouped with those on cystine because cysteine is recognised as being present as such in the intact protein molecule but it is not ordinarily obtained as one of the products of protein hydrolysis unless precautions are taken against oxidation, since it is readily converted by oxidation of the -SH group into various sulfinic and sulfonic acids and into cystine. The relation between cysteine and cystine may be illustrated as follows:



The sulphur containing amino acids have been estimated colorimetrically after hydrolysis of the samples with 5.5N HCl at 110°C for 24 hours. Spectronic 20, Bausch and Lomb, Spectrophotometer¹ was used in the colorimetric estimations.

3.8.5.1 Hydrolysis of the sample

1ml of the plasma sample was hydrolysed with 10ml of 5.5N hydrochloric acid in the sealed tubes which were kept in an oven at 110°C for 24 hours. The total volume of the hydrolysate was made 50ml with the same acid and filtered. The clear filtrate was used for the determination of methionine and cysteine/cystine.

3.8.5.2 Estimation of methionine was done by the method described by Horn et al. (1946).

3.8.5.3 Estimation of cysteine/cystine

The cystine content of the sample was determined by the method of Leach (1966).

The protein hydrolysate containing cystine was buffered to pH 5.1 and sodium sulphite added. The cystine thus converted to S-cysteine - sulfonic acid, gave with phosphotungstic acid a blue colour which was measured colorimetrically.

3.8.6 Plasma macro- and micro-elements

Plasma concentration of copper was estimated by atomic absorption spectrophotometer² (Ludmilla, 1976).

For the analysis of plasma copper, 2 ml of each plasma sample was digested with triple acid mixture in the

1 Bausch and Lomb Spectrophotometer- Model Spectronic-20, Milton, Roy and Co. Rahway, New Jersey, U.S.A.

2 Atomic absorption Spectrophotometer (Model AA6 Varian Techtron Melbourne, Australia).

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ratio of 10:2:1 (concentrated Nitric acid : 70% perchloric acid:conc. sulphuric acid). The mixture was evaporated to a volume of 1-2 ml. The final volume was made upto 8ml with ultradistilled water and the samples were analysed with atomic absorption spectrophotometer.

Plasma level of Se was estimated by the method described by Cummins et al. (1964).

3.8.7 Whole Blood Iron

It was estimated by the method of Wong (1928).

3.9 KERATINIZED TISSUE ENZYME ACTIVITIES

For the estimation of alkaline phosphatase (ALP) and carbonic anhydrase in the keratinised tissue, each collected horn/hooves samples was well minced in a grinder with normal saline solution in a 1:6 or 1:9 or in other suitable dilution. The mixture solution was collected in a 50 ml flask, the mouth of which was covered with an aluminium foil paper. The flask was kept at room temperature for two days with occasional vigorous shaking. After this the material of each sample was transferred to a plastic container and was stored at -20°C temperature.

Before the analysis, the samples were thawed, material was filtered by a simple filter paper and filtrate was used for the estimation of activity of enzymes, alkaline phosphatase and carbonic anhydrase.

The enzyme alkaline phosphatase was estimated by the method described by Wootton (1964) using diagnostic reagents kits¹.

¹ Span Diagnostics Pvt. Ltd., 173-B, New Industrial Estate, Udhana - 394210 (Surat), India.

The enzyme carbonic anhydrase was estimated as per the method described by Roughton and Booth (1946).

3.10 KERATINIZED EPITHELIUM SULPHUR AMINO ACIDS

Each collected sample was homogenised/grinded to small pieces. First it was dried in shade and then in an oven at 70°C temperature.

3.10.1 Hydrolysis of the sample

250 mg of the sample was hydrolysed with 10ml of 5.5N hydrochloric acid in the sealed tubes which were kept in an oven at 110°C for 24 hours. The total volume of hydrolysate was made 50 ml and filtered. The clear filtrate was used for the determination of methionine, cysteine/cystine in a manner as described for plasma 'S' amino acids.

3.11 ANALYSIS OF HAIR SAMPLES

Selenium level in hair samples was estimated by the method of Cummins *et al.* (1964).

3.12 ANALYSIS OF SOIL SAMPLES AND PLANT SAMPLES

Soil and plant samples collected from normal and affected areas were subjected to same treatment as for plasma, for macro- and micro-elements analysis. 'Se' content was estimated by the method of Cummins *et al.* (1964).

Sulphur in soil and plant samples was determined by turbidometric method (Chesnin and Yein, 1950).

3.13 ANALYSIS OF WATER SAMPLES

One litre of each acidified water sample was taken in 500 ml beaker (in two lots) and evaporated to 50-60 ml on a hot

plate maintained at a temperature of 80-85°C. The concentrated solution was subjected to macro- and micro-elements analysis. Se in water samples was estimated by the method of Cummins et al. (1964).

For analysis of sulphur in water samples, 50 ml of each water sample was evaporated up to a volume of 5-10 ml and then sulphur was determined by turbidity method described by Chesnin and Yein (1950).

3.14 ANALYSIS OF RUMINAL FLUID SAMPLES

Selenium content of ruminal fluid samples was estimated by the method of Cummins et al. (1964).

3.15 THERAPEUTIC MEASURES

3.15.1 Clinical cases

Therapy was undertaken in 15 animals out of thirty animals examined for clinico-biochemical profile. The solution of sodium arsenate¹ was drenched at the following rate. 750 mg of sodium arsenate was dissolved in 750 ml of water and 10 ml out of this solution, containing 75 mg was drenched per adult animal unit (>300 kg BW), after further diluting it to 250 ml. This therapy continued for 5 days alternately followed by a gap of 3 days till blood samples were collected after treatment. Above therapy was supplemented with 5 g of ZnSO₄ (Zinc sulphate)² to be continued orally in a similar manner as

1 Sodium arsenate GR Hetal Chemicals, Bombay.

2 Zinc sulphate Pure, Romali, India

selenium arsenate i.e. 5 days on and 3 days off till sampling. Therapy regimen followed, was, to administer sodium arsenate in the morning and zinc sulphate in the evening.

Blood samples were collected once before treatment and thereafter on 40th or 50th day post-treatment and were analysed in the manner as described earlier.

3.15.2 Experimental cases

Group I: In this group the treatment of induced Se toxicity was undertaken with sodium arsenate solution and zinc sulphate powder.

Sodium arsenate solution was prepared by dissolving 750 mg of sodium arsenate in 750 ml water and was drenched @ 5 ml per experimental calf, further diluting it to 250 ml. It was given in the schedule of 5 days on and 3 days off, over a period of 15 days, starting from 20th day of experimental induction.

Zinc sulphate powder was given @ 2.5 g orally per experimental calf. It was dissolved in 250 ml of water and was drenched following a schedule as mentioned for the sodium arsenate in experimental cases. Blood samples were collected on the day 5th, 10th and 15th post treatment and were analysed as per standard procedures described earlier.

Group II: Treatment in this group was undertaken with ANTHIOMALINE* given @ 5 ml as deep i/m injection on alternate

* ANTHIOMALINE: 16% Antimony (Lithium antimony thiomalated)
May and Baker (India) Ltd. May Baker House, Bombay-25.

gluteal muscle on days 21st, 24th, 27th, 30th and 33rd of the experiment.

In addition to ANTHIOMALINE, $ZnSO_4$ was also given in the same dose regimen and schedule as for Group I experimental calves. Blood sampling was similarly done on day 5th, 10th and 15th post treatment and samples were analysed as per standard procedures described earlier.

Group III: No treatment was undertaken in this group. Blood sampling was done on day 5th, 10th and 15th after the last dose of sodium selenite given on day 20th, so as to determine the rate of fall of concentration of Se in blood plasma in untreated cases as compared to the treated cases of Group I and Group II experimental animals.

3.16 STATISTICAL ANALYSIS

The results were subjected to student's t-test and were tested at 1 per cent 5 and per cent level of significance (Snedecor and Cochran, 1968).

CHAPTER IV

RESULTS

4.1 EPIDEMIOLOGICAL SURVEY

It has been reported by Dhillon et al. (1992b) that entire Eastern border of Punjab along the Shivalik hills region is affected with selenium toxicosis. Keeping this fact in view, the present investigation was conducted in the area located between Garshankar-Balachaur road and upper Bari Bist Doab Canal which mainly comprised villages Barwa, Bagouran and a portion of Simbly village. These villages are situated along the boundary line between Hoshiarpur and Jalandhar districts of Punjab, which are located geographically at 30°58' to 31°12' North and 76°07' to 76°21' East of Punjab State of India (Fig.1). This area has been classified as hot and moderately dry zone in the sub-Himalayan Shivalik hills region.

Selenium (Se) as an aerosol and large particulate pollutant that results from such human activities as the combustion of fossil fuels, the smelting and refining of non-ferrous metals and manufacturing of glass and ceramics can be ruled out owing to the absence of any industrial unit in the

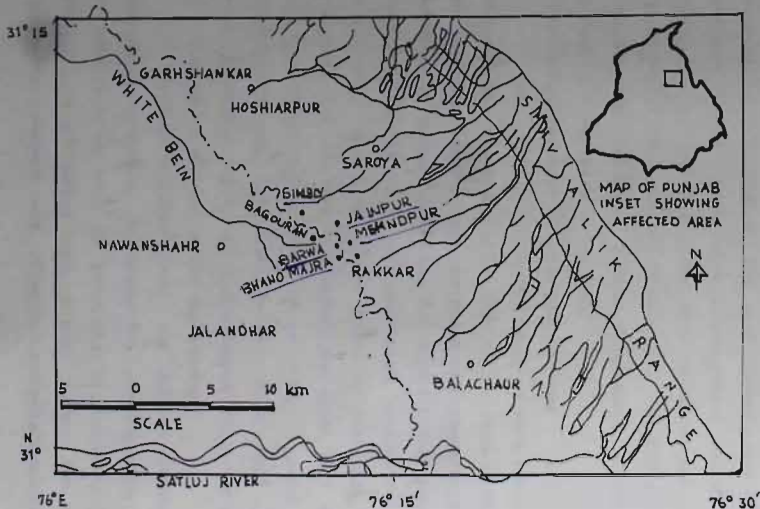


FIG.1 MAP SHOWING THE SELENIUM TOXIC SITES (•) IN JALANDHAR & HOSHIARPUR DISTRICTS OF PUNJAB, INDIA

(Lines from the Shivalik range indicate the path of seasonal rivulets)

(From Dhillon and Dhillon, 1990)

affected area. It seems that Se enters soils primarily as a result of the weathering of Se-containing rocks of Himalayan region. With usual heavy rainfall during monsoon season, there is a marked leaching of the said element from the Greater Himalayan region to Shivalik hills region and then to sub-Himalayan belt comprising the above said villages leading to deposition of this element in the soil. During rainy season, some seasonal channels, streams and canals can also act as sources of selenium (Se) for soil.

4.1.1.1 Incidence vis-a-vis clinical findings

Almost all the affected cases of selenium toxicity in Barwa village were dealt with. Most of the farmers have changed the source of fodder due to which the severity of visible symptoms has already waned out but chronicity of problem still shows its marks in the form of malformed, cracked and overgrown hooves (Figs. A and B). Fresh cases of selenosis were also encountered in the area surrounding the toxic belt and are on the increasing trend year after year. Animals depicted sloughing-off of hooves, switch of tail or tail gangrenes. Cases of chronic wounds on hind quarters (Figs.C,D and E) as well as on udder and teats were encountered.

In the village Simbly the animals showed typical symptoms of selenosis i.e. emaciation, deterioration in health and condition as well as proximity to secondary bacterial diseases like actinobacillosis (Fig.F). Animals had overgrown, mis-shapen, cracked and slightly elongated hooves, which

Fig.A. Malformed, overgrown and cracked hooves of a cow suffering from chronic selenosis

Fig.B. Hooves of buffalo showing progressive cracking and deep fissure formation in chronic selenosis



Fig.C. Emaciation, tail gangrene, chronic wounds on hind quarters and malformed hooves (left forelimb) in a cow as a result of chronic selenosis

Fig.D. Same cow with chronic wounds on hind quarters, hock joint and tail gangrene due to chronic selenium toxicity in a close view



Fig.E. A cow exhibiting tail constriction, malformed digits and cracking of hooves as a result of chronic selenosis

Fig.F. Hoof anomalies (hind limbs), weakness, emaciation and actinobacillosis in a cow suffering from chronic selenium toxicity.



Fig.G. Malformation, elongation and partial sloughing off of hooves in a cow due to chronic selenium toxicity

Fig.H. Superficial cracks turning to deep fissures in the horn corium of a buffalo as a result of chronic selenosis



Fig.I. Browning, wrinkling and partial sloughing off of nails
in human adults.

Fig.J. Browning, wrinkling and partial sloughing off of nails
in children.



years) dairy animals owing to chronicity of the disease and also severity and incidence of selenosis was encountered more in cattle than in buffaloes, irrespective of the sex in both species.

The native human inhabitants of the affected area showed visible clinical manifestations both in the children and adults. The visible symptoms included loss of hair, browning of hair and nails, sloughing and crackling of nails. Fresh nail growth showed wrinkling and brownish discolouration (Figs. I and J).

4.2 BLOOD/KERATINIZED TISSUE PROFILES

4.2.1 Haemogram:

Table 4 and Figs. 2 & 3 show the alterations in haematological indices of clinically selenosed dairy animals as compared to the haematological indices of the normal healthy dairy animals.

In dairy animals suffering from chronic selenium (Se) toxicity, the mean values of various haematological indices i.e. Hb, PCV, TEC, TLC, MCV, MCH and MCHC were observed as 8.12 ± 0.11 g/dl, 29.90 ± 0.44 per cent, $5.84 \pm 0.07 \times 10^6/\text{mm}^3$, $5.80 \pm 0.10 \times 10^3/\text{mm}^3$, 51.46 ± 1.02 fl, 13.95 ± 0.25 pg and 27.45 ± 0.54 per cent respectively. On the other hand, the same indices, in normal healthy dairy animals were observed to be 10.35 ± 0.26 g/dl, 37.00 ± 1.28 per cent, $6.09 \pm 0.26 \times 10^6/\text{mm}^3$, $7.67 \pm 0.67 \times 10^3/\text{mm}^3$, 61.85 ± 3.14 fl, 17.34 ± 0.84 pg and 28.33 ± 1.21 per cent respectively.

Table 4. Haematological indices of dairy animals suffering from chronic selenium toxicity as compared with normal healthy dairy animals

Parameter	Mean $\pm$ S.E.	
	Normal healthy animals (n=12)	Selenosed animals (n=30)
Hb (g/dl)	10.35 $\pm$ 0.26	8.12 $\pm$ 0.11**
PCV (%)	37.00 $\pm$ 1.28	29.90 $\pm$ 0.44**
TBC ($\times 10^6/\text{mm}^3$)	6.09 $\pm$ 0.26	5.84 $\pm$ 0.07
TLC ($\times 10^3/\text{mm}^3$)	7.67 $\pm$ 0.67	5.80 $\pm$ 0.10**
MCV (fl)	61.85 $\pm$ 3.14	51.46 $\pm$ 1.02**
MCH (pg)	17.34 $\pm$ 0.84	13.95 $\pm$ 0.25**
MCHC (%)	28.33 $\pm$ 1.21	27.45 $\pm$ 0.54

** significant at 1% level of significance ($P < 0.01$)

n = no. of animals examined.

Thus, it inferred that mean values of haematological indices in clinically selenosed animals such as Hb, PCV, TLC, MCV and MCH were significantly lower than the comparable mean values of these indices in normal healthy dairy animals at 1% level of significance ($P < 0.01$), while the mean values of other indices such as TEC and MCHC depicted non-significant fluctuations as compared to their counterparts of normal healthy dairy animals.

The same haematological indices were observed in clinically selenosed animals before and after the undertaking of therapeutic measures and have been shown in Table 5 and Figs.2 & 3). It showed that before undertaking therapeutic measures in animals suffering from chronic Se toxicity the mean values of indices such as Hb, PCV, TEC, TLC, MCV, MCH and MCHC were 8.06 ± 0.15 g/dl, 29.60 ± 0.56 per cent, $5.85 \pm 0.14 \times 10^6/\text{mm}^3$, $5.73 \pm 0.16 \times 10^3/\text{mm}^3$, 50.78 ± 1.72 fl, 13.82 ± 0.38 pg and 27.44 ± 0.79 per cent respectively. The present treatment could not help significant but a non-significant variations in the above mentioned haematological indices which were respectively observed to be 8.80 ± 0.47 g/dl, 31.06 ± 1.08 per cent, $5.88 \pm 0.11 \times 10^6/\text{mm}^3$, $5.98 \pm 0.23 \times 10^3/\text{mm}^3$, 52.96 ± 1.98 fl, 15.01 ± 0.85 pg and 28.74 ± 1.76 per cent.

4.2.2 Blood/plasma biochemical profiles

Mean blood/plasma biochemical profiles such as plasma urea nitrogen, total plasma proteins and blood glucose, both

Table 5. Haematological indices of clinically selenosed dairy animals before and after undertaking the therapeutic measures

Mean $\pm$ S.E.

Parameter	Before treatment (n=15)	After treatment (n=15)
Hb (g/dl)	8.06 $\pm$ 0.15	8.80 $\pm$ 0.47
PCV (%)	29.60 $\pm$ 0.56	31.06 $\pm$ 1.08
TEC ($\times 10^6/\text{mm}^3$)	5.85 $\pm$ 0.14	5.88 $\pm$ 0.11
TLC ($\times 10^3/\text{mm}^3$)	5.73 $\pm$ 0.16	5.98 $\pm$ 0.23
MCV (fl)	50.78 $\pm$ 1.72	52.96 $\pm$ 1.98
MCH (pg)	13.82 $\pm$ 0.38	15.01 $\pm$ 0.85
MCHC (g/dl)	27.44 $\pm$ 0.79	28.74 $\pm$ 1.76

n = no. of animals examined.

Fig. 2 Showing haematological indices in normal healthy, selenosed dairy animals before and after treatment

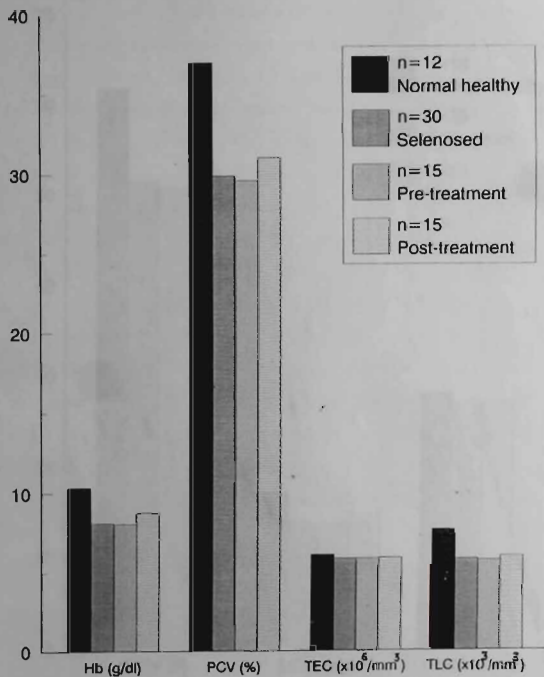


Fig.2 Showing haematological indices in normal healthy, selenosed dairy animals before and after treatment

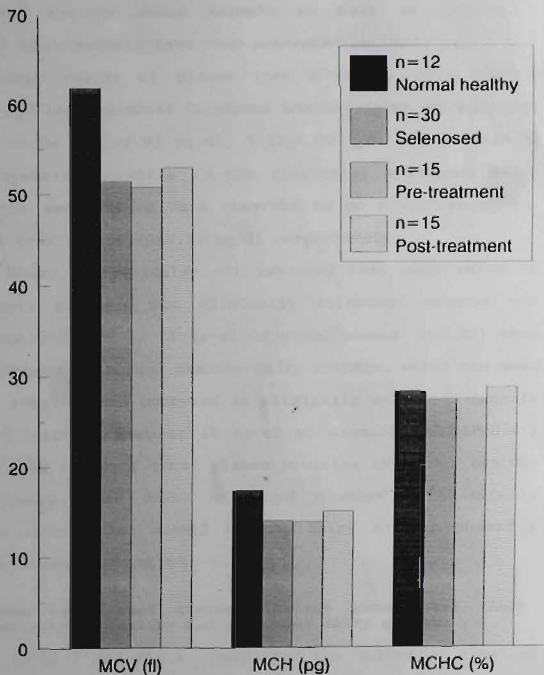


Fig.3 Showing erythrocytic indices in normal healthy, selenosed dairy animals before and after treatment

for normal healthy dairy animals as well as clinically selenosed dairy animals have been presented in Table 6.

Mean values of plasma urea nitrogen, total plasma proteins and blood glucose in normal healthy dairy animals were observed to be 5.02 ± 0.08 mg/dl, 7.32 ± 0.07 g/dl and 57.58 ± 0.75 mg/dl respectively, while in the clinically selenosed dairy animals the same values were observed to be 8.93 ± 0.10 mg/dl, 5.47 ± 0.05 g/dl and 56.40 ± 0.37 mg/dl respectively.

Thus, statistically, it revealed that mean value of plasma urea nitrogen for clinically selenosed animals was significantly higher at 1% level of significance ($P < 0.01$) than its counterpart in normal healthy dairy animals, while the mean value of total plasma proteins in clinically selenosed animals was significantly lower at 1% level of significance ($P < 0.01$) than the mean value of total plasma proteins in normal healthy dairy animals. Mean value of blood glucose in clinically selenosed animals and normal healthy dairy animals showed a non-significant fluctuation.

4.2.3 Plasma/keratinized tissue alkaline phosphatase (ALP) activity in healthy and selenosed dairy animals

Table 7 and Fig.4 depicted the status of Alkaline phosphatase (ALP) enzyme in plasma and keratinized tissue.

Mean value of ALP in plasma of normal healthy dairy animals was 58.09 ± 3.49 units/l, whereas the mean value of ALP in clinically selenosed animals was 145.41 ± 3.86 units/l. It indicated that the mean plasma ALP activity in clinically

Table 6. Blood/plasma biochemical alterations in dairy animals suffering from chronic selenium toxicity as compared with normal healthy dairy animals

Mean $\pm$ S.E.

Parameter	Normal healthy animals (n=12)	Selenosed animals (n=30)
Plasma urea nitrogen (mg/dl)	5.02 $\pm$ 0.08	8.93 $\pm$ 0.10**
Total plasma proteins (g/dl)	7.32 $\pm$ 0.07	5.47 $\pm$ 0.05**
Blood glucose (mg/dl)	57.58 $\pm$ 0.75	56.40 $\pm$ 0.37

** significant at 1% level of significance (P<0.01)

n = no. of animals examined.

Table 7. Plasma/keratinized tissue alkaline phosphatase activity in normal healthy and selenosed dairy animals

Mean $\pm$ S.E.

Plasma alkaline phosphatase	Normal healthy animals (n=12)	Selenosed animals (n=30)
ALP (units/l)	58.09 $\pm$ 3.49	145.41 $\pm$ 3.86**
Keratinized tissue alkaline phosphatase	Normal healthy animals (n=10)	Selenosed animals (n=15)
ALP (units/l)	146.90 $\pm$ 4.37	241.90 $\pm$ 8.48**

** significant at 1% level of significance (P<0.01)

n = no. of animals examined.

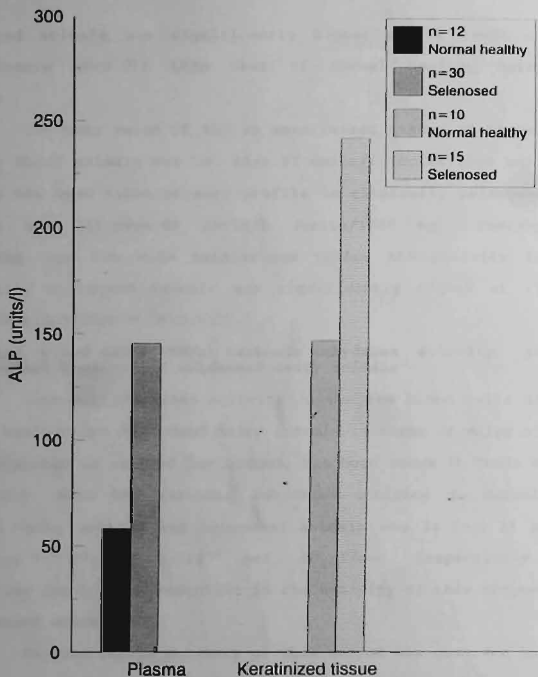


Fig.4 Showing ALP activity in plasma and keratinized tissue in normal healthy and selenosed dairy animals

selenosed animals was significantly higher at 1% level of significance ($P < 0.01$) than that of normal healthy dairy animals.

The mean value of ALP in keratinized tissue of normal healthy dairy animals was 146.90 ± 4.37 units/l (Units/1000 mg), whereas the mean value of same profile in clinically selenosed animals was 241.90 ± 8.48 units/l (units/1000 mg), thereby revealing that the mean keratinized tissue ALP activity in clinically selenosed animals was significantly higher at 1% level of significance ($P < 0.01$).

4.2.4 Red blood cells (RBCs) carbonic anhydrase activity in normal healthy and selenosed dairy animals

Carbonic anhydrase activity in the red blood cells of normal healthy and selenosed dairy animals in terms of moles of CO_2/l absorbed or evolved per second, has been shown in Table 8 and Fig.5. Mean RBC carbonic anhydrase activity in normal healthy dairy animals and selenosed animals was $38.69 \pm 3.93 \times 10^{-3}$ and $17.97 \pm 1.46 \times 10^{-3}$ mol. $\text{CO}_2/\text{l}/\text{sec}$. respectively, reflecting significant reduction in the activity of this enzyme in diseased animals.

No qualitative activity of this enzyme was observed in the keratinized epithelium of either normal healthy or selenosed dairy animals.

4.2.5 Other plasma enzyme activity

Mean plasma enzyme activities for AST/GOT and ALT/GPT in normal healthy dairy animals and animals suffering from

Table 8. Red blood cells carbonic anhydrase activity in normal healthy and selenosed dairy animals

Parameter	Mean $\pm$ S.E.	
	Normal healthy animals (n=12)	Selenosed animals (n=17)
Carbonic anhydrase (mol.CO ₂ /l/sec) $\times 10^{-3}$	38.69 $\pm$ 3.93	17.97 $\pm$ 1.46**

** significant at 1% level of significance (P<0.01)

n = no. of animals examined.

Table 9. Other plasma enzyme activities in normal healthy and selenosed dairy animals

Parameter	Mean $\pm$ S.E.	
	Normal healthy animals (n=12)	Selenosed animals (n=30)
AST/GOT (units/l)	69.19 $\pm$ 3.19	72.84 $\pm$ 2.00
ALT/GPT (units/l)	12.05 $\pm$ 2.78	16.12 $\pm$ 1.44

n = no. of animals examined.

Fig 5 Showing red blood cells carbonic anhydrase activity in normal healthy and selenosed dairy animals

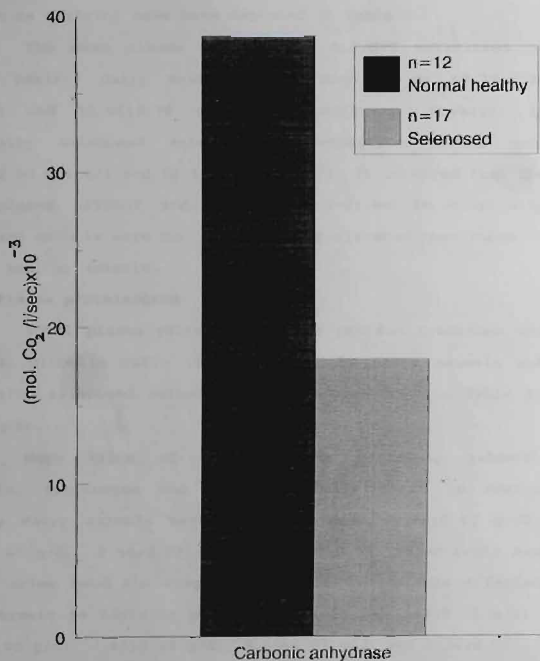


Fig.5 Showing red blood cells carbonic anhydrase activity in normal healthy and selenosed dairy animals

chronic Se toxicity have been depicted in Table 9.

The mean plasma AST/GOT and ALT/GPT activities in normal healthy dairy animals were found to be 69.19 ± 3.19 units/l and 12.05 ± 2.78 units/l respectively. However, in clinically selenosed animals the respective values were 72.84 ± 2.00 units/l and 16.12 ± 1.44 units/l. It inferred that the mean plasma AST/GOT and ALT/GPT activities in clinically selenosed animals were non-significantly elevated than those of normal healthy animals.

4.2.6 Plasma proteinogram

Total plasma proteins, various protein fractions and albumin: globulin ratio in normal healthy dairy animals and clinically selenosed animals have been presented in Table 10 and Fig.6).

Mean value of total plasma proteins, albumin, globulin, fibrinogen and albumin:globulin ratio in normal healthy dairy animals were 7.32 ± 0.07 g/dl, 2.95 ± 0.12 g/dl, 4.36 ± 0.05 g/dl, 0.50 ± 0.02 g/dl and 0.67 ± 0.03 respectively and on the other hand the respective values for animals affected with chronic Se toxicity were analysed to be 5.47 ± 0.05 g/dl, 2.99 ± 0.06 g/dl, 2.47 ± 0.08 g/dl, 0.20 ± 0.02 g/dl and 1.26 ± 0.06 .

This presentation revealed that in clinically selenosed animals there was significant decrease at 1% level of significance ($P < 0.01$) in mean values of total plasma proteins, globulin and fibrinogen than the same values in the normal healthy dairy animals. But in the clinically selenosed animals

Table 10. Proteinogram alterations in plasma of dairy animals suffering from chronic selenium toxicity as compared with normal healthy dairy animals

Parameter	Mean $\pm$ S.E.	
	Normal healthy animals (n=12)	Selenosed animals (n=30)
Total plasma proteins (g/dl)	7.32 $\pm$ 0.07	5.47 $\pm$ 0.05**
Albumin (g/dl)	2.95 $\pm$ 0.12	2.99 $\pm$ 0.06
Globulin (g/dl)	4.36 $\pm$ 0.05	2.47 $\pm$ 0.08**
Fibrinogen (g/dl)	0.50 $\pm$ 0.02	0.20 $\pm$ 0.02**
Albumin:Globulin (A/G)	0.67 $\pm$ 0.03	1.26 $\pm$ 0.06**

** significant at 1% level of significance (P<0.01)

n = no. of animals examined.

Table 11. Proteinogram alterations in plasma of clinically selenosed dairy animals before and after undertaking the therapeutic measures

Parameter	Mean $\pm$ S.E.	
	Before treatment (n=15)	After treatment (n=15)
Total plasma proteins (g/dl)	5.43 $\pm$ 0.07	6.00 $\pm$ 0.11**
Albumin (g/dl)	3.08 $\pm$ 0.06	3.06 $\pm$ 0.05
Globulin (g/dl)	2.35 $\pm$ 0.11	2.94 $\pm$ 0.15**
Fibrinogen (g/dl)	0.23 $\pm$ 0.02	0.28 $\pm$ 0.03
Albumin:Globulin (A/G)	1.36 $\pm$ 0.09	1.08 $\pm$ 0.07**

** significant at 1% level of significance ($P < 0.01$)

n = no. of animals examined.

Fig.6 Showing proteinogram alterations in normal healthy, selenosed dairy animals before and after treatment

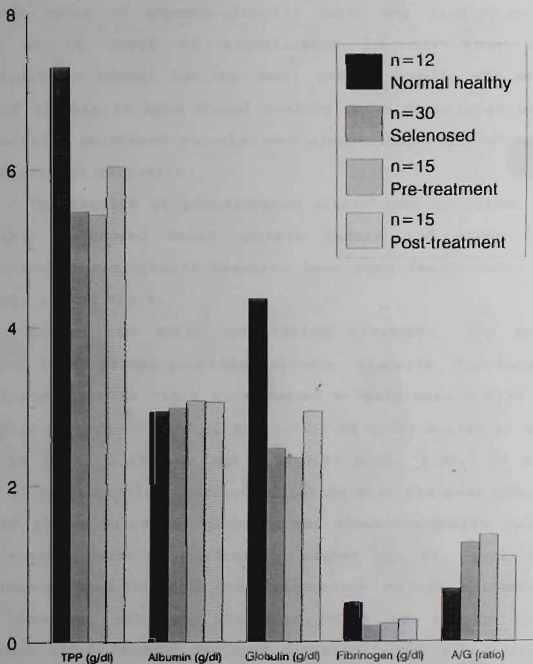


Fig.6 Showing proteinogram alterations in normal healthy, selenosed dairy animals before and after treatment

the mean value of albumin:globulin ratio was significantly higher at 1% level of significance ($P<0.01$) than its counterpart in normal healthy dairy animals, while the mean value of albumin in both normal healthy dairy animals as well as clinically selenosed animals, was almost comparable without any significant variation.

The results of proteinogram alterations in plasma of clinically selenosed dairy animals before and after the institution of therapeutic measures have been demonstrated in the Table 11 and Fig.6.

Before and after undertaking treatment, the mean values of total plasma proteins, albumin, globulin, fibrinogen and albumin:globulin ratio in diseased animals were 5.43 ± 0.07 and 6.00 ± 0.11 g/dl; 3.08 ± 0.06 and 3.06 ± 0.05 g/dl; 2.35 ± 0.11 and 2.94 ± 0.15 g/dl; 0.23 ± 0.02 and 0.28 ± 0.03 g/dl; 1.36 ± 0.09 and 1.08 ± 0.07 respectively, thereby indicating that the mean values of total plasma proteins, globulin and albumin:globulin ratio after therapy were significantly higher at 1% level of significance ($P<0.01$) than the respective values estimated before therapy, whereas, the mean value of albumin and fibrinogen after therapy depicted non-significant alterations than their respective counterparts recorded before therapy.

4.2.7 Plasma sulphur containing amino acids

Table 12 and Fig.7 show the level of sulphur containing amino acids in plasma of normal healthy dairy animals and clinically selenosed animals.

Table 12. Concentration of sulphur containing amino acids in plasma of dairy animals suffering from chronic selenium toxicity as compared with normal healthy dairy animals

Parameter	Mean $\pm$ S.E.	
	Normal healthy animals (n=12)	Selenosed animals (n=30)
Cysteine (cystine) (mg/ml)	3.40 $\pm$ 0.06	2.76 $\pm$ 0.02**
Methionine (mg/ml)	0.99 $\pm$ 0.01	0.71 $\pm$ 0.01**

** significant at 1% level of significance ($P < 0.01$)

n = no. of animals examined.

Table 13. Concentration of sulphur containing amino acids in plasma of clinically selenosed dairy animals before and after undertaking the therapeutic measures

Parameter	Mean $\pm$ S.E.	
	Before treatment (n=15)	After treatment (n=15)
Cysteine (cystine) (mg/ml)	2.79 $\pm$ 0.02	2.97 $\pm$ 0.02**
Methionine (mg/ml)	0.71 $\pm$ 0.005	0.79 $\pm$ 0.03**

** significant at 1% level of significance ($P < 0.01$)

n = no. of animals examined.

In animals suffering from chronic selenium toxicity the mean values of cysteine (cystine) and methionine were respectively 2.76 ± 0.02 mg/ml and 0.71 ± 0.01 mg/ml, whereas these values in normal healthy dairy animals were observed to be 3.40 ± 0.06 mg/ml and 0.99 ± 0.01 mg/ml respectively. Thus, the concentrations of cysteine/cystine and methionine in diseased animals were significantly reduced at 1% level of significance ($P < 0.01$) than those of normal healthy dairy animals.

Mean concentrations of sulphur containing amino acids in plasma of clinically selenosed dairy animals before and after undertaking therapeutic measures have been depicted in Table 13 and Fig.7.

Mean values of cysteine/cystine and methionine in affected animals before and after undertaking therapy were recorded as 2.79 ± 0.02 and 2.97 ± 0.02 mg/ml; 0.71 ± 0.005 and 0.79 ± 0.03 mg/ml respectively, thus revealing a significant improvement at 1% level of significance ($P < 0.01$) in the mean values of cysteine/cystine and methionine in plasma after the institution of treatment than before the treatment.

4.2.8 Keratinized tissue/epithelium sulphur containing amino acids

Concentrations of sulphur containing amino acids in keratinized epithelium of normal healthy dairy animals and clinically selenosed animals have been shown in Table 14 and Fig.7.

Mean concentrations of cysteine/cystine and methionine

Table 14. Concentration of sulphur containing amino acids in keratinized epithelium/tissues of animals suffering from chronic selenium toxicity as compared with normal healthy dairy animals

Parameter	Mean $\pm$ S.E.	
	Normal healthy animals (n=12)	Selenosed animals (n=30)
Cysteine (cystine) (mg/100 mg)	5.65 $\pm$ 0.45	3.24 $\pm$ 0.16**
Methionine (mg/100 mg)	0.25 $\pm$ 0.06	0.11 $\pm$ 0.02**

** significant at 1% level of significance ($P < 0.01$)

n = no. of animals examined.

Table 15. Concentration of sulphur containing amino acids in keratinized epithelium/tissues of clinically selenosed dairy animals before and after undertaking the therapeutic measures

Parameter	Mean $\pm$ S.E.	
	Before treatment (n=15)	After treatment (n=15)
Cysteine (cystine) (mg/100 mg)	3.46 $\pm$ 0.28	3.79 $\pm$ 0.30
Methionine (mg/100 mg)	0.14 $\pm$ 0.03	0.16 $\pm$ 0.04

n = no. of animals examined.

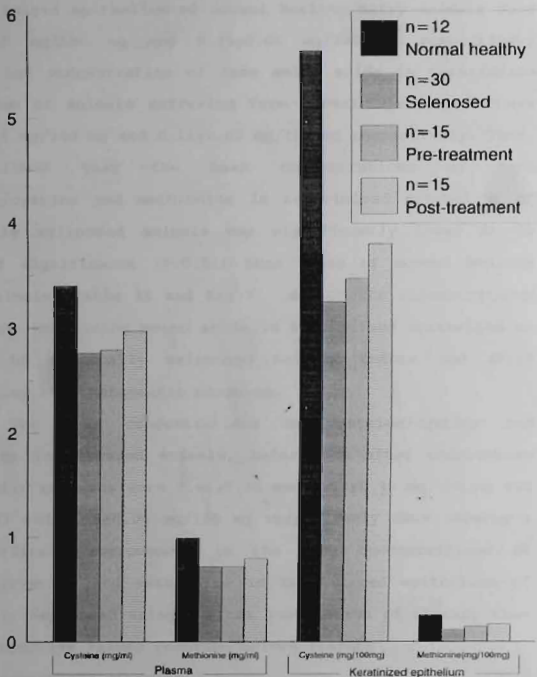


Fig.7 Conc. of (S) amino acids in plasma and keratinized epithelium in normal healthy, selenosed dairy animals before and after therapy

in keratinized epithelium of normal healthy dairy animals were 5.65 ± 0.45 mg/100 mg and 0.25 ± 0.06 mg/100 mg respectively whereas the concentration of same amino acids in keratinized epithelium of animals suffering from chronic Se toxicity are 3.24 ± 0.16 mg/100 mg and 0.11 ± 0.02 mg/100 mg respectively. Thus, it followed that the mean concentrations of both cysteine/cystine and methionine in keratinized epithelium of clinically selenosed animals was significantly lower at 1% level of significance ($P < 0.01$) than those of normal healthy dairy animals. Table 15 and Fig.7 depict the concentrations of sulphur containing amino acids in keratinized epithelium or tissues of clinically selenosed animals before and after undertaking the therapeutic measures.

The mean concentrations of cysteine/cystine and methionine in diseased animals, before and after undertaking therapeutic measures were 3.46 ± 0.28 and 3.79 ± 0.30 mg/100 mg and 0.14 ± 0.03 and 0.16 ± 0.04 mg/100 mg respectively thus showing a non-significant improvement in the mean concentrations of cysteine/cystine and methionine in keratinized epithelium of clinically selenosed animals after institution of therapy than their respective values recorded before therapy.

4.2.9 Plasma/blood minerals

The concentration of minerals in the plasma or blood of normal healthy dairy animals and clinically selenosed animals have been presented in Table 16 and Figs.8 & 9.

In normal healthy dairy animals the mean

Table 16. Plasma/blood minerals in normal healthy and selenosed dairy animals

Parameter	Mean $\pm$ S.E.	
	Normal healthy animals (n=12)	Selenosed animals (n=30)
Plasma selenium ($\mu\text{g/ml}$)	0.20 $\pm$ 0.01	0.85 $\pm$ 0.04**
Whole blood iron (mg/100 ml)	35.88 $\pm$ 0.88	28.10 $\pm$ 0.40**
Plasma copper ($\mu\text{g/ml}$)	1.62 $\pm$ 0.06	1.02 $\pm$ 0.04**

** significant at 1% level of significance ($P < 0.01$)

n = no. of animals examined.

Table 17. Plasma/blood minerals in clinically selenosed dairy animals before and after undertaking the therapeutic measures

Parameter	Mean $\pm$ S.E.	
	Before treatment (n=15)	After treatment (n=15)
Plasma selenium ($\mu\text{g/ml}$)	0.98 $\pm$ 0.06	0.88 $\pm$ 0.03
Whole blood iron (mg/100 ml)	27.95 $\pm$ 0.52	30.53 $\pm$ 1.63
Plasma copper ($\mu\text{g/ml}$)	1.12 $\pm$ 0.04	1.20 $\pm$ 0.04

n = no. of animals examined.

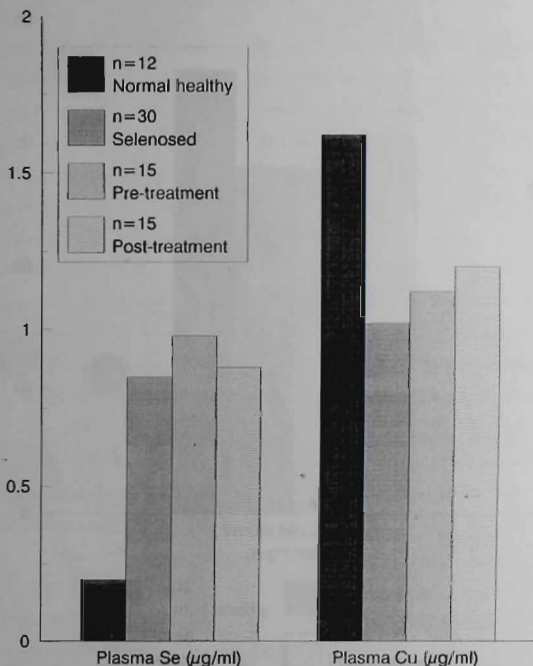


Fig.8 Showing plasma Se and Cu in normal healthy, selenosed dairy animals before and after treatment

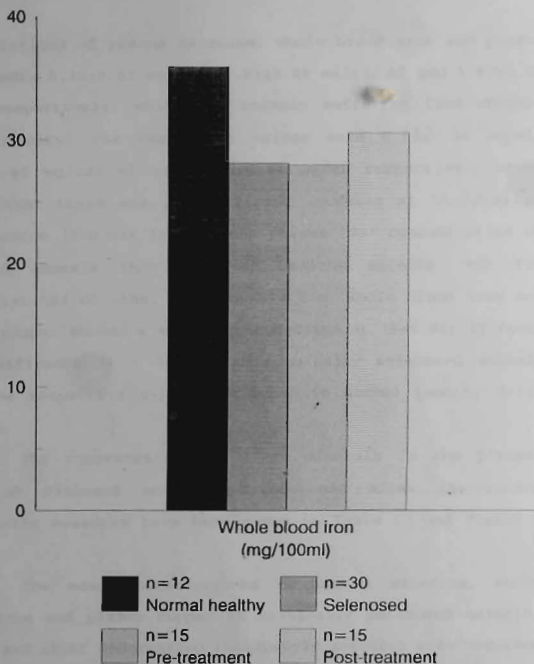


Fig.9 Showing whole blood iron in normal healthy, selenosed dairy animals before and after treatment

concentrations of plasma selenium, whole blood iron and plasma copper were 0.20 ± 0.01 $\mu\text{g/ml}$, 35.88 ± 0.88 mg/100 ml and 1.62 ± 0.06 $\mu\text{g/ml}$ respectively, while, in animals suffering from chronic 'Se' toxicity, the respective values were 0.85 ± 0.04 $\mu\text{g/ml}$, 28.10 ± 0.40 mg/100 ml and 1.02 ± 0.04 $\mu\text{g/ml}$ respectively which showed that there was a significant increase at 1% level of significance ($P < 0.01$) in the mean plasma 'Se' concentration of diseased animals than that of healthy animals. But the concentrations of other two minerals i.e. whole blood iron and plasma copper showed a significant decline at ($P < 0.01$) 1% level of significance ($P < 0.01$) in clinically selenosed animals than the respective values estimated in normal healthy dairy animals.

The concentration of these minerals in the plasma/blood of diseased animals, before and after instituting therapeutic measures have been shown in Table 17 and Figs.8 & 9.

The mean concentrations of plasma selenium, whole blood iron and plasma copper in clinically selenosed animals, before and after undertaking therapeutic measures were recorded as 0.98 ± 0.06 and 0.88 ± 0.03 $\mu\text{g/ml}$; 27.95 ± 0.52 and 30.53 ± 1.63 mg/100 ml ; 1.12 ± 0.04 and 1.20 ± 0.04 $\mu\text{g/ml}$ respectively which implied that the mean plasma selenium, whole blood iron and plasma copper concentrations after therapy have improved or changed to add comfort to the diseased animals but only non-significantly than their respective counterparts before

Table 18. Selenium content of the hair of normal healthy and selenosed dairy animals

Parameter	Mean $\pm$ S.E.	
	Normal healthy animals (n=12)	Selenosed animals (n=30)
Selenium in hair ($\mu\text{g/g}$)	0.22 $\pm$ 0.01	15.39 $\pm$ 0.79**

** significant at 1% level of significance ($P < 0.01$)

n = no. of animals examined.

Table 19. Selenium content of hair in clinically selenosed dairy animals before and after undertaking the therapeutic measures

Parameter	Mean $\pm$ S.E.	
	Before treatment (n=15)	After treatment (n=15)
Selenium in hair ($\mu\text{g/g}$)	15.77 $\pm$ 1.27	14.17 $\pm$ 1.66

n = no. of animals examined.

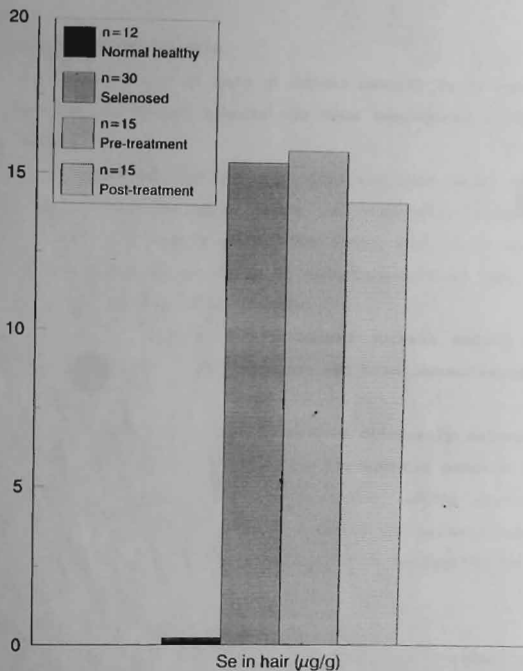


Fig.10 Showing selenium content of hair in normal healthy, selenosed dairy animals before and after treatment

therapy.

4.3 SELENIUM CONTENT OF HAIR

The Se content of hair of normal healthy dairy animals and clinically selenosed animals has been documented in Table 18 and Fig.10.

In normal healthy dairy animals the mean level of Se in hair was 0.22 ± 0.01 $\mu\text{g/g}$, while in clinically selenosed animals it was 15.39 ± 0.79 $\mu\text{g/g}$. The later was found to be significantly higher at 1% level of significance than the same value in normal healthy dairy animals.

Se content of hair of diseased animals before and after undertaking therapeutic measures has been demonstrated in Table 19 and Fig.10.

The mean 'Se' content of hair in clinically selenosed animals, before and after undertaking therapeutic measure was 15.77 ± 1.27 and 14.17 ± 1.66 $\mu\text{g/g}$ respectively, which depicted that mean 'Se' content of hair of affected animals after therapy was non-significantly lower than the respective value estimated before therapy.

4.4 SOIL-WATER MINERAL ANALYSIS

The results showing selenium (Se) and sulphur (S) content of soil and water both in non-seleniferous and seleniferous areas of Punjab have been listed in Table 20 and Fig.11.

The mean concentration and range of Se in the soil of non-seleniferous and seleniferous areas of Punjab was 0.28 ± 0.03

Table 20. Total selenium and sulphur content of soil and water samples of seleniferous and non-seleniferous areas of Punjab

Mean $\pm$ S.E.

S. No.	Type of sample	Parameter	Area	
			Non-seleniferous (n=15)	Seleniferous (n=25)
1	Soil	Total selenium (ppm)	0.28 $\pm$ 0.03 (0.08-0.55)	1.17 $\pm$ 0.19** (0.23-4.55)
		Total sulphur (ppm)	135.60 $\pm$ 12.35 (74.00-225.00)	161.80 $\pm$ 8.01 (96.00-250)
2	Water	Total selenium (ppm)	1.64 $\pm$ 0.29 (0.25-4.25)	12.80 $\pm$ 3.57** (0.65-69.50)
		Total sulphur (ppm)	1.34 $\pm$ 0.25 (0.48-3.80)	1.21 $\pm$ 0.17 (0.30-3.90)

** Significant at 1% level of significance ($P < 0.01$)

n = no. of samples analysed

Values in parenthesis () indicate the range.

and 0.08-0.55 ppm; 1.17 ± 0.19 and 0.23-4.55 ppm, respectively, thus, revealing that the concentration of Se was significantly higher at 1% level of significance ($P < 0.01$) in the soil of seleniferous areas than that of non-seleniferous areas of Punjab.

The mean S level of soil and range of S level in the soil of non-seleniferous and seleniferous areas of Punjab was 135.60 ± 12.35 and 74 to 225 ppm; 161.80 ± 8.01 and 96 to 250 ppm respectively, thereby indicating, a non-significant higher level of S in the soil of seleniferous areas than that of non-seleniferous areas of Punjab.

The mean Se and range of Se in water of non-seleniferous and seleniferous areas of Punjab was respectively 1.64 ± 0.29 and 0.25 to 4.25 ppb; 12.80 ± 3.57 and 0.65 to 69.50 ppb, thus, reflecting a significantly higher level of Se at 1% level of significance ($P < 0.01$) in water samples of seleniferous areas as compared to those of non-seleniferous areas. The average sulphur (S) level and its range in water samples of non-seleniferous areas and seleniferous areas of Punjab was 1.34 ± 0.25 and 0.48 to 3.80 ppm; 1.21 ± 0.17 and 0.30 to 3.90 ppm respectively which presented a non-significantly lower level of S in water samples of seleniferous areas than those of non-seleniferous areas of Punjab.

4.5 PLANT AND RUMINAL FLUID MINERAL ANALYSIS

Table 21 and Figs. 11 & 12 illustrate the comparison of Se and S level of various fodder crops and ruminal fluid of

Table 21. Selenium and sulphur content of various crops and ruminal fluid of animals in seleniferous and non-seleniferous areas of Punjab

Mean $\pm$ S.E.

S. No.	Type of sample	Parameter	No. of samples	Area	
				Non-seleniferous	Seleniferous
1.	Sugarcane leaves (tops)	Selenium (ppm)	5	1.95 $\pm$ 0.30 (1.00-2.71)	36.66 $\pm$ 11.08** (10.50-67.50)
	(<u>Sachhbraum officinarum</u>)	Sulphur (%)	5	0.85 $\pm$ 0.17 (0.48-1.43)	0.70 $\pm$ 0.19 (0.23-1.33)
2.	Wheat (<u>Triticum aestivum</u>)	Selenium (ppm)	5	1.58 $\pm$ 0.38 (1.10-3.10)	11.14 $\pm$ 4.22* (2.60-25.90)
		Sulphur (%)	5	0.70 $\pm$ 0.10 (0.41-1.06)	0.49 $\pm$ 0.07 (0.28-0.69)
3.	Mustard (Sarson) (<u>Brassica campestris</u>)	Selenium (ppm)	5	1.20 $\pm$ 0.13 (0.76-1.50)	14.82 $\pm$ 5.86* (3.50-36.40)
		Sulphur (%)	5	1.13 $\pm$ 0.24 (0.64-2.00)	0.98 $\pm$ 0.27 (0.41-1.90)
4.	Maize (<u>Zea mays</u>)	Selenium (ppm)	5	0.97 $\pm$ 0.19 (0.40-1.52)	15.48 $\pm$ 4.79** (5.64-30.83)
		Sulphur (%)	5	0.74 $\pm$ 0.07 (0.53-0.97)	0.61 $\pm$ 0.06 (0.40-0.74)
5.	Barseem (<u>Trifolium alexandrinum</u>)	Selenium (ppm)	5	0.87 $\pm$ 0.15 (0.40-1.24)	5.11 $\pm$ 0.24** (4.41-5.74)
		Sulphur (%)	5	0.68 $\pm$ 0.04 (0.60-0.84)	0.58 $\pm$ 0.05 (0.40-0.72)

Table 21 contd...

S. No.	Type of sample	Parameter	No. of samples	Area	
				Non-seleniferous	Seleniferous
6.	Ruminal fluid	Selenium (ppm)	10	0.66±0.13 (0.08-1.33)	5.04±0.93** (1.25-9.55)
		Sulphur (%)	10	1.05±0.18 (0.50-2.20)	0.92±0.08 (0.58-1.33)

* Significant at 5% level of significance ($P < 0.05$)

** Significant at 1% level of significance ($P < 0.01$)

Values in parenthesis () indicate the range.

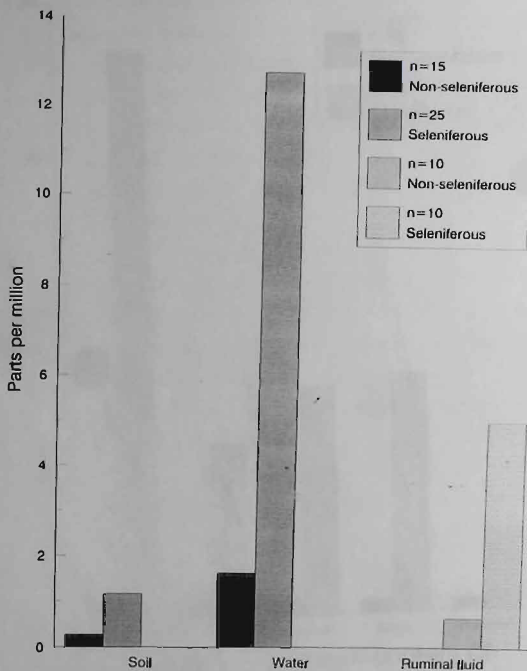


Fig.11 Showing selenium content of soil, water and ruminal fluid from non-seleniferous and seleniferous areas of Punjab

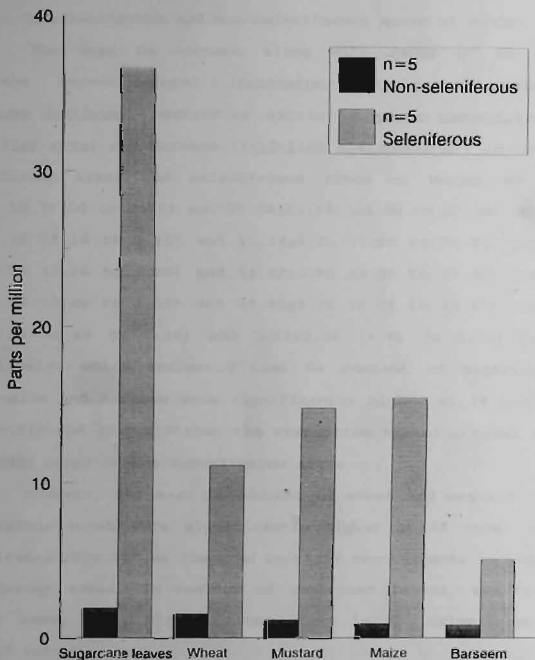


Fig.12 Showing selenium content of various crops from non-seleniferous and seleniferous areas of Punjab

animals in seleniferous and non-seleniferous areas of Punjab.

The mean Se content along with range of Se of sugarcane leaves (tops) (Sachharum officinarum), wheat (Triticum aestivum), mustard or sarson (Brassica campestris), maize (Zea mays) and Berseem (Trifolium alexandrinum) in non-seleniferous areas and seleniferous areas of Punjab were, 1.95 ± 0.30 (1.00 to 2.71) and 36.66 ± 11.08 (10.50 to 67.50) ppm; 1.58 ± 0.38 (1.10 to 3.10) and 11.14 ± 4.22 (2.60 to 25.90) ppm; 1.20 ± 0.13 (0.76 to 1.50) and 14.82 ± 5.86 (3.50 to 36.40) ppm; 0.97 ± 0.19 (0.40 to 1.52) and 15.48 ± 4.79 (5.64 to 30.83) ppm; 0.87 ± 0.15 (0.40 to 1.24) and 5.11 ± 0.24 (4.41 to 5.74) ppm respectively, which reflected that Se content of sugarcane tops, maize and berseem were significantly higher at 1% level of significance ($P < 0.01$) than the respective values arrived at for fodder crops of non-seleniferous areas.

However, the mean Se content of wheat and mustard in seleniferous areas were significantly higher at 5% level of significance ($P < 0.05$) as compared to their counterparts in non-seleniferous areas. Se content of sugarcane leaves, was the highest among the different crops grown in the seleniferous areas of Punjab.

The mean sulphur(S) content (and range of S) of sugarcane (tops), wheat, mustard, maize and berseem in non-seleniferous and seleniferous areas of Punjab were 0.85 ± 0.17 (0.48 to 1.43) and 0.70 ± 0.19 (0.23 to 1.33) per cent; 0.70 ± 0.10 (0.41 to 1.06) and 0.49 ± 0.07 (0.28 to 0.69) per cent) 1.13 ± 0.24

(0.64 to 2.00) and 0.98 ± 0.27 (0.41 to 1.90) per cent; 0.74 ± 0.07 (0.53 to 0.97) and 0.61 ± 0.06 (0.40 to 0.74) per cent; 0.68 ± 0.04 (0.60 to 0.84) and 0.58 ± 0.05 (0.40 to 0.72) per cent respectively, thus, furnishing that the S content of all the fodder crops mentioned above, in the seleniferous areas of Punjab, were non-significantly lower than the S content of these fodder crops in the non-seleniferous areas of Punjab.

The average content of Se (with range of Se) in the ruminal fluid samples of animals in non-seleniferous areas and seleniferous areas of Punjab were 0.66 ± 0.13 (0.08 to 1.33) and 5.04 ± 0.93 (1.25 to 9.55) ppm respectively which illustrated that Se content of ruminal fluid of animals in seleniferous areas of Punjab was significantly higher at 1% level of significance ($P < 0.01$) as compared to the respective value in the animals of non-seleniferous area. The mean S content in the ruminal fluid samples of animals from non-seleniferous areas was 1.05 ± 0.18 per cent with a range of 0.50 to 2.20 per cent which was comparable with the S content in the ruminal fluid samples of animals from seleniferous areas with a mean value of 0.92 ± 0.08 per cent and with range of 0.58 to 1.33 per cent. Thus, it depicted that the mean S content in the ruminal fluid of animals, from seleniferous areas, was non-significantly lower than that of non-seleniferous areas of Punjab.

4.6 STUDIES ON EXPERIMENTAL INDUCTION OF SELENOSIS IN BUFFALO CALVES

4.6.1 Clinical manifestations

In all, 18 buffalo calves were divided into 3 groups, each of 6 calves. All the calves were healthy, alert, active and had normal appetite and body norms before induction of selenosis. During induction of selenosis they were subjected to identical conditions of management and feeding. All the 3 groups of buffalo calves received first seven sodium selenite doses at the rate of 2.0 mg/kg B.W. on alternate days from day 0 to day 13, so appetite remained near normal till day 10 of induction. Thereafter the feed intake declined slowly till day 15 and animals were partially to nearly completely anorectic between day 16 and day 20 of induction of selenosis.

One buffalo calf from group I died on day 15 and another buffalo calf from group II died on day 17 of induction. No death occurred in group III buffalo calves. After becoming partially off-feed the animals became weak and depressed. Few buffalo calves developed constipation while some developed diarrhoea. Signs of respiratory distress were observed in 10 out of 18 buffalo calves on day 16 which included dyspnea, nasal exudation and excessive salivation. The buffalo calf of group II that died on 17th day, also developed cyanosis that appeared only during terminal stages of illness.

In general, the clinical signs of experimentally induced selenosis in buffalo calves were weakness, anorexia,

loss of condition, apathy, nasal exudation, excessive salivation, respiratory distress, constipation or diarrhoea and terminally cyanosis followed by death.

4.6.2 Blood profiles

4.6.2.1 Haemogram

The effect of experimentally induced Se toxicity and its treatment with sodium arsenate + zinc sulphate combination on haematological indices in group I buffalo calves have been detailed in Table 22.

In group I experimentally toxic animals, the average values of Hb and PCV at day zero of experiment were 9.85 ± 0.59 g/dl and 34.08 ± 1.98 per cent respectively. Thereafter, Hb was progressively decreased upto day 12 (8.80 ± 0.69 g/dl) and then showed an abrupt rise at day 16 (9.44 ± 0.72 g/dl) followed by a fall at day 20 (8.64 ± 0.82 g/dl), all non-significantly. From 20th day onwards Hb showed a non-significant rising trend till the end of experiment (9.16 ± 0.77 g/dl) which remained comparable to the value at day zero of the experiment. PCV decreased throughout non-significantly till day 16 (27.80 ± 3.85 per cent) with a non significant and marked increase at day 20 (30.80 ± 2.47 per cent) and then depicted non-significant variation after undertaking therapeutic measures till day 35 (31.60 ± 2.15 per cent) which remained non-significantly lower than the value at the start of induction (34.08 ± 1.98 per cent).

The mean TEC and TLC at the start of experiment were $6.30 \pm 0.33 \times 10^6/\text{mm}^3$ and $8.30 \pm 0.65 \times 10^3/\text{mm}^3$ respectively, which

Table 22. Effect of experimentally induced selenosis and its treatment with sodium arsenate and zinc sulphate combination, on haematological indices in group I buffalo calves
Mean $\pm$ S.E.

Parameter	Day of sampling								
	0	4	8	12	16	20 ^x	25	30	35
Hb (g/dl)	9.85 ± 0.59	9.60 ± 0.61	9.41 ± 0.59	8.80 ± 0.69	9.44 ± 0.72	8.64 ± 0.82	8.72 ± 0.82	8.92 ± 0.78	9.16 ± 0.77
PCV (%)	34.08 ± 1.98	33.33 ± 2.06	30.16 ± 2.00	29.16 ± 2.19	27.80 ± 3.85	30.80 ± 2.47	31.60 ± 2.29	30.60 ± 2.15	31.60 ± 2.15
TEC ($\times 10^6/\text{mm}^3$)	6.30 ± 0.33	6.12 ± 0.32	5.79 ± 0.42	5.50 ± 0.46	5.83 ± 0.41	5.28 ± 0.51	5.35 ± 0.50	5.36 ± 0.48	5.31 ± 0.49
TLC ($\times 10^3/\text{mm}^3$)	8.30 ± 0.65	8.08 ± 0.71	7.92 ± 0.75	7.79 ± 0.77	7.85 ± 0.78	7.91 ± 0.77	8.02 ± 0.75	8.10 ± 0.74	8.03 ± 0.72
MCV (fl)	54.70 ± 3.82	55.21 ± 4.51	53.47 ± 4.86	54.83 ± 5.74	49.25 ± 8.38	61.04 ± 8.00	61.68 ± 7.88	59.00 ± 6.58	61.51 ± 6.41
MCH (pg)	15.78 ± 1.02	15.78 ± 0.94	16.55 ± 1.16	16.29 ± 1.15	16.37 ± 1.21	16.79 ± 1.65	16.69 ± 1.66	17.04 ± 1.73	17.69 ± 1.72
MCHC (%)	29.39 ± 2.53	29.32 ± 2.64	31.77 ± 2.82	31.00 ± 3.60	35.94 ± 4.90	28.65 ± 3.63	27.95 ± 3.06	29.44 ± 2.84	29.18 ± 2.41

^x Induction ceased and therapeutic measures undertaken on day 20 of experiment.

reflected a non-significant decrease throughout the period of induction of selenosis. After undertaking therapeutic measures at day 20, these values furnished a non-significant change throughout the period of therapy. Mean TEC and TLC values at the end of therapy were respectively $5.31 \pm 0.49 \times 10^6/\text{mm}^3$ and $8.03 \pm 0.72 \times 10^3/\text{mm}^3$ respectively which were non-significantly higher than the respective values on day 20 (day of undertaking therapeutic measures) and were non-significantly lower than the respective values at day zero of experiment.

Mean MCV, MCH and MCHC values of this group, before induction of experimental selenosis were 54.70 ± 3.82 fl, 15.78 ± 1.02 pg and 29.39 ± 2.53 per cent respectively. Among these values MCV fluctuated both ways (increased or decreased) non-significantly till day 16 (49.25 ± 8.38 fl) of induction of selenium toxicity and then rose markedly but non-significantly at day 20 (61.04 ± 8.00 fl) of induction as compared to the value at day zero of the experiment. Undertaking therapeutic measures at day 20, could not help MCV, throughout the period of therapy, than remaining comparable to the value at day 20.

MCH showed a gradual but non-significant increase throughout period of induction of selenosis (till day 20) and even after undertaking the therapeutic measures from day 20 to day 35 (17.69 ± 1.72 pg). The value at day 35 was non-significantly higher than the value at day 0 of experiment (15.78 ± 1.02 pg).

MCHC followed a zig-zag path during the period of

induction of Se toxicity, with a marked and non-significantly higher value at day 16 (35.94 ± 4.90 per cent) as compared to the respective counterpart at day zero (29.39 ± 2.53 per cent). Undertaking therapeutic measures from day 20 to day 35, non-significantly stabilized MCHC and kept it almost comparable to the values, both, at day zero and day 20 of the experimental induction of selenosis.

Table 23 illustrate the haematological indices of group II buffalo calves under the effect of experimentally induced Se toxicity and its treatment with anthiomaline and zinc sulphate combination. The trend followed that to start with, at day zero, the Hb and PCV were 9.65 ± 0.73 g/dl and 35.41 ± 2.33 per cent respectively. Hb decreased non-significantly till day 20 (8.56 ± 0.80 g/dl) except a slight rise during day 12 to day 16 i.e. from 8.76 ± 0.73 g/dl to 9.10 ± 0.70 g/dl respectively. Then during the period of therapy it remained comparable to the values in the period of treatment. Final value of Hb at day 35 (8.76 ± 0.82 g/dl) remained non-significantly lower than the respective value in group I buffalo calves, after undertaking therapeutic measures, thus showing a non-significantly lower degree of improvement in Hb of group II calves as compared to group I calves.

PCV followed a non-significant declining trend till day 16 (28.5 ± 3.22 per cent) of induction of Se toxicosis followed by a non-significant rise at day 20 (31.4 ± 2.47 per cent) which remained almost constant throughout and till the

Table 23. Effect of experimentally induced selenosis and its treatment with anthiomaline and zinc sulphate combination, on haematological indices in group II buffalo calves
Mean $\pm$ S.E.

Parameter	Day of sampling								
	0	4	8	12	16	20 ^x	25	30	35
Hb (g/dl)	9.65 ± 0.73	9.36 ± 0.74	9.16 ± 0.70	8.76 ± 0.73	9.10 ± 0.70	8.56 ± 0.80	8.68 ± 0.80	8.76 ± 0.82	8.76 ± 0.82
PCV (%)	35.41 ± 2.33	34.50 ± 2.47	31.50 ± 2.18	30.16 ± 2.28	28.50 ± 3.22	31.40 ± 2.47	31.80 ± 2.78	31.40 ± 2.41	31.40 ± 2.63
TEC ($\times 10^6/\text{mm}^3$)	6.18 ± 0.28	6.04 ± 0.29	5.77 ± 0.37	5.53 ± 0.42	5.70 ± 0.40	5.17 ± 0.52	5.20 ± 0.51	5.21 ± 0.51	5.18 ± 0.53
TLC ($\times 10^3/\text{mm}^3$)	8.62 ± 0.59	8.22 ± 0.64	7.92 ± 0.73	7.75 ± 0.77	7.88 ± 0.79	8.16 ± 0.77	8.23 ± 0.72	8.24 ± 0.72	8.21 ± 0.79
MCV (fl)	57.73 ± 4.09	57.78 ± 4.99	55.54 ± 4.61	56.09 ± 5.45	51.22 ± 6.39	63.78 ± 8.59	64.02 ± 8.61	63.14 ± 8.35	63.67 ± 8.88
MCH (pg)	15.71 ± 1.15	15.55 ± 1.06	16.04 ± 1.13	16.07 ± 1.15	16.11 ± 1.03	17.07 ± 1.80	17.23 ± 1.86	17.33 ± 1.86	17.46 ± 1.85
MCHC (%)	27.93 ± 3.10	27.99 ± 3.47	29.83 ± 3.40	30.09 ± 4.00	33.74 ± 4.56	27.89 ± 3.64	27.84 ± 3.24	28.36 ± 3.29	28.65 ± 3.85

^x Induction ceased and therapeutic measures undertaken on day 20 of experiment.

end of therapeutic measures (31.4 ± 2.63 per cent). The trend that PCV followed in both group I and group II was apparently similar.

Mean TEC and TLC of this group at the beginning of induction were $6.18 \pm 0.28 \times 10^6/\text{mm}^3$ and $8.62 \pm 0.59 \times 10^3/\text{mm}^3$ respectively, both of which decreased non-significantly till last day of induction with respective values at day 20 as $5.17 \pm 0.52 \times 10^6/\text{mm}^3$ and $8.16 \pm 0.77 \times 10^3/\text{mm}^3$. Non-significant uneven increase was shown by both these parameters between day 12 and day 16 i.e. TEC from $5.53 \pm 0.42 \times 10^6/\text{mm}^3$ to $5.70 \pm 0.40 \times 10^6/\text{mm}^3$ and TLC from $7.75 \pm 0.77 \times 10^3/\text{mm}^3$ to $7.88 \pm 0.79 \times 10^3/\text{mm}^3$ respectively. From day 20 to day 35, the mean values of TEC and TLC remained comparable to the values throughout period of undertaking therapeutic measures. The value of TEC at the end of treatment ($5.18 \pm 0.53 \times 10^6/\text{mm}^3$) in group II calves was non-significantly lower than the respective value in group I calves ($5.31 \pm 0.49 \times 10^6/\text{mm}^3$).

Mean MCV, MCH and MCHC before induction of selenosis were 57.73 ± 4.09 fl, 15.71 ± 1.15 pg and 27.93 ± 3.10 per cent respectively. MCV remained almost same till day 4 of induction then followed a wavy curve upto the mean value of 63.78 ± 8.59 fl at day 20, thereafter, undertaking the therapeutic measures, kept the MCV almost similar to the value at day 20 with the final value at day 35 of experiment reaching upto 63.67 ± 8.88 fl.

MCH followed gradual rising slope throughout the

period of induction, being 17.07 ± 1.80 pg at day 20. Further during the period of therapy it maintained its rising trend, thus reading 17.46 ± 1.85 pg on the last day of experiment. This furnished that the rise in the MCH in group II buffalo calves was non-significantly lower than the respective value at concluding day of experiment in group I calves (17.69 ± 1.72 pg).

MCHC remained almost unchanged till day 8 (29.83 ± 3.40 per cent) of induction followed by slight increase from day 8 to day 16 (33.74 ± 4.56 per cent). The values remained comparable to each other throughout the period of treatment in group II animals but were non-significantly lower than the respective values throughout the period of treatment in group I animals. The MCHC at day 35 in group II and group I animals was respectively 28.65 ± 3.85 per cent and 29.18 ± 2.41 per cent.

Table 24 represents the haematological indices of group III buffalo calves under the influence of experimentally induced selenium toxicity followed by no therapeutic measures. Mean Hb and PCV at day zero were respectively 9.48 ± 0.71 g/dl and 36.58 ± 2.02 per cent respectively. Both these parameters followed the same trend as in other two groups throughout the period of induction with the final values at day 20 as 8.63 ± 0.84 g/dl and 32.50 ± 2.02 per cent respectively. With no therapeutic measures beyond day 20, Hb non-significantly improved to 8.70 ± 0.82 g/dl at day 35, thus reflecting that improvement in Hb brought about in group I buffalo calves (with sodium arsenate and zinc sulphate combination) at day 35 was

Table 24. Effect of experimentally induced selenosis followed by no therapy, on haematological indices in group III buffalo calves

Mean $\pm$ S.E.

Parameter	Day of sampling								
	0	4	8	12	16	20 ^x	25	30	35
Hb (g/dl)	9.48 ± 0.71	9.28 ± 0.69	9.01 ± 0.73	8.70 ± 0.84	8.98 ± 0.82	8.63 ± 0.84	8.66 ± 0.85	8.75 ± 0.85	8.70 ± 0.82
PCV (%)	36.58 ± 2.02	35.33 ± 2.28	33.16 ± 2.31	32.16 ± 2.56	30.16 ± 3.03	32.50 ± 2.02	32.50 ± 2.26	33.16 ± 2.37	32.80 ± 2.48
TEC($\times 10^6/\text{mm}^3$)	6.06 ± 0.26	5.98 ± 0.27	5.76 ± 0.32	5.42 ± 0.37	5.70 ± 0.31	5.26 ± 0.40	5.33 ± 0.46	5.36 ± 0.43	5.32 ± 0.45
TLC($\times 10^3/\text{mm}^3$)	8.80 ± 0.56	8.60 ± 0.53	8.23 ± 0.65	7.95 ± 0.69	8.11 ± 0.66	8.27 ± 0.63	8.21 ± 0.62	8.26 ± 0.62	8.28 ± 0.61
MCV (fl)	60.75 ± 3.84	59.52 ± 4.45	58.43 ± 4.98	60.81 ± 6.04	53.61 ± 5.71	64.24 ± 7.46	63.73 ± 7.68	64.11 ± 7.48	64.69 ± 8.56
MCH (pg)	15.68 ± 1.04	15.52 ± 0.93	15.73 ± 1.09	16.11 ± 1.15	15.75 ± 1.11	16.86 ± 1.91	16.85 ± 1.98	16.87 ± 2.02	17.00 ± 2.02
MCHC (%)	26.29 ± 2.52	26.78 ± 2.75	27.70 ± 2.91	27.65 ± 3.31	31.11 ± 4.18	26.90 ± 3.07	27.20 ± 3.45	27.01 ± 3.54	26.99 ± 3.10

^x Induction ceased and no therapeutic measures undertaken beyond day 20 of experiment.

better (9.16 ± 0.77 g/dl) than the respective value in group II buffalo calves (8.76 ± 0.82 g/dl) treated with anthiomaline and zinc sulphate combination.

PCV under the effect of no treatment remained similar (at day 20) with the value at day 35 as 32.80 ± 2.48 per cent.

Mean TEC and TLC started before induction with $6.06 \pm 0.26 \times 10^6/\text{mm}^3$ and $8.80 \pm 0.56 \times 10^3/\text{mm}^3$ respectively and followed the same declining trend till day 20 as in other two groups. Undertaking therapeutic measures, kept these parameters comparable to their respective values at day 20. Mean MCV, MCH and MCHC starting respectively from 60.75 ± 3.84 fl, 15.68 ± 1.04 pg and 26.29 ± 2.52 per cent showed same tendency as in other two groups throughout the induction period till day 20. The final value of MCV at day 35 (64.69 ± 8.56 fl) was comparable to the value at day 20 (64.24 ± 7.46 fl). Mean MCH values maintained their rising trend from day 20 (16.86 ± 1.91 pg) to day 35 (17.00 ± 2.02 pg) at a much lower rate than the respective values of MCH in other two treatment groups during the same period. MCHC with value of 26.90 ± 3.07 per cent at day 20 remained almost constant during the period of treatment, reaching 26.99 ± 3.10 per cent at day 35.

The haematological indices of group III buffalo calves, in general, provide the ground values to evaluate the comparative efficacy of the drugs i.e. sodium arsenate + zinc sulphate in group I and anthiomaline + zinc sulphate in group II, experimentally toxic (selenosed) buffalo calves,

respectively.

4.6.2.2 Plasma/blood minerals

Variations in the level of plasma/blood minerals in group I buffalo calves, under the effect of experimentally induced selenosis and its treatment with sodium arsenate plus zinc sulphate combination, have been documented in Table 25 and Fig.13.

Mean plasma selenium value before induction of selenosis, at day zero, was 0.21 ± 0.02 $\mu\text{g/ml}$, which increased significantly at 1% level of significance ($P < 0.01$) on day 4, 8, 12, 16 and 20 to 0.31 ± 0.02 , 0.44 ± 0.02 , 0.54 ± 0.02 , 0.70 ± 0.04 and 0.84 ± 0.02 $\mu\text{g/ml}$ respectively. Undertaking therapeutic measures at day 20 reduced the mean plasma selenium successively to become non-significant from day 30 (0.28 ± 0.06 $\mu\text{g/ml}$) onwards.

Whole blood iron reduced non-significantly from 34.17 ± 2.03 $\text{mg}/100$ ml at day zero to 29.96 ± 2.86 $\text{mg}/100$ ml at day 20 of experiment. Undertaking therapeutic measures couldn't help whole blood iron than to remain comparable to the value at day zero, with final value reading at 31.81 ± 2.66 $\text{mg}/100$ ml at day 35.

The decrease exhibited by plasma copper from 1.98 ± 0.34 $\mu\text{g/ml}$ at day zero to 1.77 ± 0.43 $\mu\text{g/ml}$ at day 20, was non-significant; consequently the slight increase following the therapeutic measures i.e. from 1.77 ± 0.43 $\mu\text{g/ml}$ on day 20 to 1.81 ± 0.38 $\mu\text{g/ml}$ on day 35, was also non-significant.

Table 26 and Fig.13 exhibit the effect of

Table 25. Effect of experimentally induced selenosis and its treatment with sodium arsenate and zinc sulphate combination, on plasma/blood minerals in group I buffalo calves
Mean $\pm$ S.E.

Parameter	Day of sampling								
	0	4	8	12	16	20 ^x	25	30	35
Plasma selenium ($\mu\text{g/ml}$)	0.21 ± 0.02	0.31** ± 0.02	0.44** ± 0.02	0.54** ± 0.02	0.70** ± 0.04	0.84** ± 0.02	0.58** ± 0.03	0.28 ± 0.06	0.23 ± 0.05
Whole blood iron (mg/100 ml)	34.17 ± 2.03	33.30 ± 2.11	32.66 ± 2.05	30.52 ± 2.40	32.75 ± 2.51	29.96 ± 2.86	30.27 ± 2.86	30.95 ± 2.74	31.81 ± 2.66
Plasma copper ($\mu\text{g/ml}$)	1.98 ± 0.34	2.00 ± 0.35	1.82 ± 0.36	1.91 ± 0.35	1.66 ± 0.44	1.77 ± 0.43	1.79 ± 0.42	1.71 ± 0.43	1.81 ± 0.38

** Significant at 1% level of significance ($P < 0.01$)

^x Induction ceased and therapeutic measures undertaken on day 20 of experiment.

experimentally induced selenosis and its treatment with anthiomaline and zinc sulphate combination on plasma/blood minerals in group II buffalo calves.

At day zero, the mean plasma selenium was 0.20 ± 0.01 $\mu\text{g/ml}$ and it increased significantly at 1% level of significance ($P < 0.01$) to 0.30 ± 0.01 , 0.42 ± 0.03 , 0.54 ± 0.03 , 0.68 ± 0.04 and 0.82 ± 0.03 $\mu\text{g/ml}$ on day 4, 8, 12, 16 and 20 of induction of selenosis. Therapeutic measures undertaken from day 20 to day 35 reduced the plasma selenium which became non-significant only on day 35 of experiment. Till day 30, the plasma selenium, though decreased than its ultimate preceding values at day 25 and day 20, remained significantly higher at 1% level of significance ($P < 0.01$) than the value at day zero of experiment.

Comparison of mean plasma selenium values of group I and group II buffalo calves reflect that, following treatment, the mean plasma Se decreased more rapidly to become non-significant at day 30 (0.28 ± 0.06 $\mu\text{g/ml}$) in group I, than the respective values on respective days in group II where it became non-significant only at day 35 (0.28 ± 0.07 $\mu\text{g/ml}$).

Whole blood iron averaged 33.49 ± 2.55 $\text{mg}/100$ ml at day zero and remained comparable to the values estimated upto induction of selenosis and after undertaking therapeutic measures except a marginal non-significant decrease at day 20 (29.68 ± 2.81 $\text{mg}/100$ ml) of experiment.

Plasma copper fluctuated non-significantly both during

Table 26. Effect of experimentally induced selenosis and its treatment with anthiomaline and zinc sulphate combination, on plasma/blood minerals in group II buffalo calves
Mean $\pm$ S.E.

Parameter	Day of sampling								
	0	4	8	12	16	20 ^x	25	30	35
Plasma selenium (μ g/ml)	0.20 ± 0.01	0.30** ± 0.01	0.42** ± 0.03	0.54** ± 0.03	0.68** ± 0.04	0.82** ± 0.03	0.71** ± 0.05	0.56** ± 0.06	0.28 ± 0.07
Whole blood iron (mg/100 ml)	33.49 ± 2.55	32.50 ± 2.58	31.81 ± 2.45	30.41 ± 2.55	31.57 ± 2.44	29.68 ± 2.81	30.09 ± 2.81	30.37 ± 2.87	30.38 ± 2.88
Plasma copper (μ g/ml)	1.94 ± 0.34	1.91 ± 0.34	1.74 ± 0.35	1.81 ± 0.34	1.56 ± 0.34	1.76 ± 0.39	1.79 ± 0.38	1.84 ± 0.38	1.75 ± 0.31

** Significant at 1% level of significance ($P < 0.01$)

^x Induction ceased and therapeutic measures undertaken on day 20 of experiment.

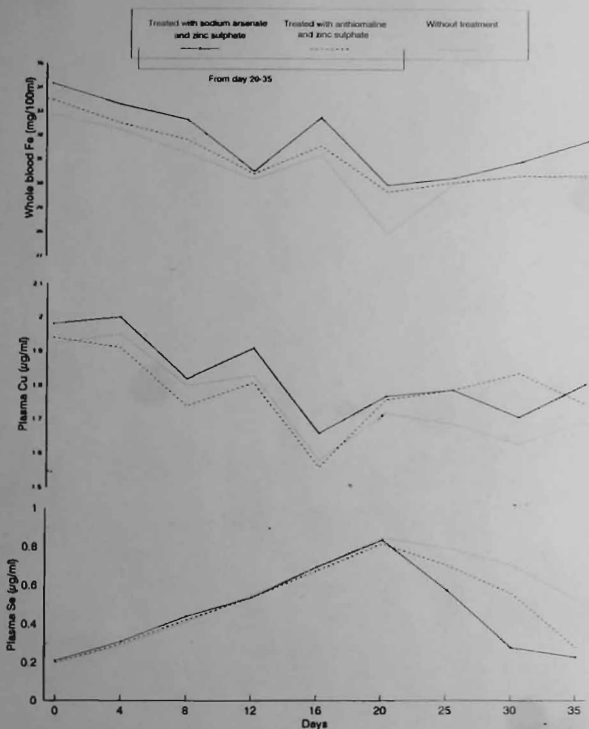


Fig.13 Showing changes in plasma Se, Cu and whole blood Fe in different experimental groups of buffalo-calves.

induction of selenosis as well as during period of therapy, starting with 1.94 ± 0.34 $\mu\text{g/ml}$ at day zero and finishing at 1.75 ± 0.31 $\mu\text{g/ml}$ at day 35 respectively.

Alterations in plasma/blood minerals of group III buffalo calves, under the effect of experimentally induced selenosis followed by no therapy, have been documented in Table 27 and Fig.13. Mean plasma/blood mineral values of this table provided a ground to compare the therapeutic efficacy of sodium arsenate plus zinc sulphate combination in group I with that of anthiomaline plus zinc sulphate combination in group II, respectively for restoring these plasma/blood minerals to normalcy.

Mean plasma selenium once normal at 0.20 ± 0.01 $\mu\text{g/ml}$ concentration on day zero of experiment increased significantly at 1% level of significance ($P < 0.01$) to 0.29 ± 0.02 , 0.41 ± 0.01 , 0.55 ± 0.02 , 0.70 ± 0.01 and 0.85 ± 0.02 $\mu\text{g/ml}$ on day 4, 8, 12, 16 and 20 of induction of selenosis respectively. Thereafter, following no therapy, from day 20 onwards, it reduced, but never became non-significant, showing the concentration of 0.53 ± 0.05 $\mu\text{g/ml}$, even 15 days after cessation of induction i.e. on day 35.

Whole blood iron with mean value 32.88 ± 2.46 mg/100 ml at day zero, varied non-significantly and remained comparable to the values upto induction of selenosis and thereafter following no therapy.

Mean plasma copper read 1.92 ± 0.30 $\mu\text{g/ml}$ at day zero

Table 27. Effect of experimentally induced selenosis followed by no therapy on plasma/blood minerals in group III buffalo calves

Mean $\pm$ S.E.

Parameter	Day of sampling								
	0	4	8	12	16	20 ^x	25	30	35
Plasma selenium ($\mu\text{g/ml}$)	0.20 ± 0.01	0.29** ± 0.02	0.41** ± 0.01	0.55** ± 0.02	0.70** ± 0.01	0.85** ± 0.02	0.80** ± 0.03	0.71** ± 0.04	0.53** ± 0.05
Whole blood iron (mg/100 ml)	32.88 ± 2.46	32.23 ± 2.41	31.26 ± 2.55	30.19 ± 2.92	31.20 ± 2.83	27.94 ± 2.93	30.06 ± 2.95	30.35 ± 2.95	30.30 ± 2.84
Plasma copper ($\mu\text{g/ml}$)	1.92 ± 0.30	1.95 ± 0.31	1.80 ± 0.32	1.83 ± 0.32	1.58 ± 0.35	1.72 ± 0.31	1.69 ± 0.29	1.63 ± 0.30	1.70 ± 0.30

** Significant at 1% level of significance ($P < 0.01$)

^x Induction ceased and no therapeutic measures undertaken beyond day 20 of experiment.

and followed a wavy path to 1.70 ± 0.30 $\mu\text{g/ml}$ concentration at day 35 of experiment, reflecting no significant change throughout its path of fluctuation, both during period of induction as well as period of no therapy.

4.7 POST-MORTEM LESIONS

Post-mortem lesions were studied in two buffalo calves, one from group I and other from group II that died on day 15 and day 17 of induction of selenosis respectively.

4.7.1 Gross-pathological alterations

Heart: Gross lesions in heart were irregularly shaped. Indistinct pale focal areas were found throughout the thick muscular wall of the left ventricle and interventricular septum in group I buffalo calf. Subepicardial and subendocardial haemorrhages were found only in group II buffalo calf.

Lungs: Pulmonary edema was grossly visible in the interlobular areas throughout all lobes in both calves. Trachea and bronchi contained white to cream-coloured froth. Focal to large diffused haemorrhages in the lung tissue was a common finding in both calves.

Spleen: The spleen appeared grossly normal.

Liver: It was pallor with small white necrotic areas. Liver was also slightly enlarged.

Kidneys: No significant gross change was observed in the kidneys except necrotic foci in the cortical tissue and some haemorrhagic foci with generalized hyperaemia of kidney.

Gastrointestinal tract: The mucosa of the anterior ventral sac

and anterior pillar of the rumen was hypraemic. Mild catarrhal gastroenteritis was observed.

Skeletal muscle: No gross lesion was observed in the skeletal muscle.

All the other tissues appeared grossly normal.

4.7.2 Histopathological alterations:

Heart: The entire intracellular mass became more acidophilic and distinctly granular thus depicting hyaline degeneration of mild intensity in cardiac muscle fibres and purkinje fibres. Haemorrhages inbetween the cardiac muscle fibres were also noticed.

Lungs: Microscopic examination revealed pulmonary congestion and edema. Transudate was located in some of the alveoli as well as in the interlobular, perivascular, peribronchial and subplural tissue (Fig. K). Haemorrhages in the interlobular tissues were occasionally encountered. Leukocyte and mononuclear macrophage infiltration was limited or absent in most lung sections.

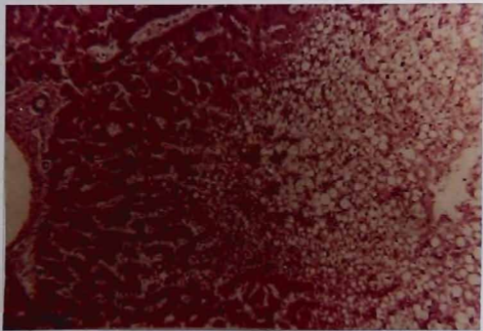
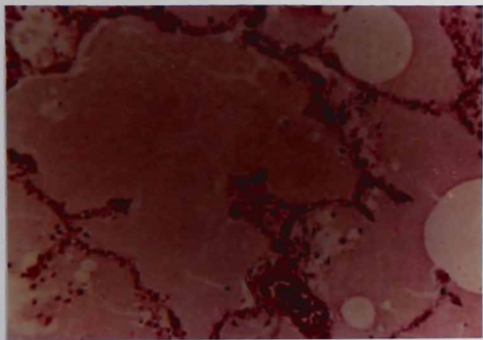
Spleen: The spleen sections appeared near normal.

Liver: Congestion and dilatation of sinusoids was common. Liver section revealed mild to moderate necrotic changes and fatty metamorphosis at some places along with venous congestion and thrombus formation in artery (Fig. L).

Kidneys: Congestion of the cortex as well as the medulla was seen in many sections. Kidney section depicted edema and mild necrotic changes in the tubule and glomeruli. There were red

Fig.K. Section of lung of a buffalo calf, showing congestion of alveolar capillaries and alveoli filled with edematous fluid. H&E X 280

Fig.L. Photomicrograph of liver of a buffalo calf showing fatty change (vacuolation), haemorrhages (chronic venous congestion), atrophy of hepatocytes and distension of sinusoids around the centrilobular zone (right) while the hepatocytes at periphery of lobule (left) appear normal. H&E X 140



cells indicating haemorrhages and a proteinaceous fluid in the subcapsular space of the glomerulus.

Gastrointestinal tract:

Rumen: No characteristic histopathological alteration was observed.

Omasum: Pathology of omasal leaves microscopically appeared near normal.

Abomasum: Gastric glands showed normal pattern of parietal and chief cells with no histopathologic distortion.

Intestine: the intestinal wall i.e. mucosa, submucosa, muscularis mucosa were almost normal.

CHAPTER V

DISCUSSION

Selenium is a paradox because the essentiality of Se ends at the level of 0.1 ppm while toxicity is likely to be at the level of 1 ppm of the diet. Selenium absorption, retention, distribution within the body, toxicity and the amounts, forms and routes of excretion vary with the chemical forms and amounts of the element ingested and the dietary levels of several other elements such as arsenic and mercury. On a molar basis Se salts have been reported the most toxic among the essential minerals. Depending on the intake of As, tocopherols and proteins, 3 to 10 ppm Se in the diet has been reported to cause chronic intoxication in animals.

Selenium compounds have been reported as the most toxic of the group VI elements. Selenium toxicity or selenosis, might be caused by both organic and inorganic forms of Se. A subacute organic selenosis or chronic selenosis called Alkali disease might occur by consuming plant material containing more than 5 ppm Se in diet, over a period of years, with abnormal hoof development in cattle, more pronounced as the age of

animals advanced (Sharma and Singh, 1983).

5.1 DISCUSSION ON CLINICALLY SELENOSED DAIRY ANIMALS BEFORE AND AFTER UNDERTAKING THERAPEUTIC MEASURES

5.1.1 Epidemiological survey

The scientific information regarding the epidemiological survey of selenosis is meagre in the available literature, because of poor surveillance system and non availability of diagnostic facilities. Se toxicity or selenosis has been reported from different parts of India viz. Haryana (Arora et al., 1975), Punjab (Gupta et al., 1982; Bakshi et al., 1986; Dhillon and Dhillon, 1991b) and West Bengal (Ghosh et al., 1993).

Present epidemiological survey was undertaken in the foothills of Shivalik range of Himalayas comprising villages Barwa, Bagouran and a portion of Simbly village. These areas had been already demarcated as selenium toxic by Dhillon et al. (1992b). Increased occurrence of selenosis in these villages could be ascribed to the entry of selenium element in soil and water primarily as a result of the weathering of Se-containing rocks of Himalayan region. Seasonal rivulets and streams during monsoon rains led to marked leaching of the said element from greater Himalayan region to Shivalik hills region and then to sub-Himalayan belt comprising the above said villages leading to deposition of this element in the soil. A similar explanation was given by Ghosh et al. (1993) for selenium toxicosis in grazing buffaloes in the district of Jalpaiguri.

the sub-Himalayan areas of West Bengal, situated in the northern part of the State. Lack of any industrial pollution activity in the study area further confirmed the above hypothesis.

5.1.2 Incidence vis-a-vis clinical findings

Severity of the symptoms had waned out in some animals as farmers had been arranging green forages from the non-seleniferous areas i.e. distant from the Shivalik hill belts. Due to the effect of depletion of elemental Se from mountaneous rocks by monsoon rains and its deposition in the vicinity of Shivalik hills, the emergence of fresh cases of selenosis were found to be increasing. Animals in the affected area were raised on the green fodders and cereal straws grown on seleniferous soils and their consumption for a prolonged period led to the development of clinical manifestations of Se toxicity. Animals in the study area were subjected to similar managerial practices.

Rumen micro-organisms were thought to reduce Se to unavailable forms so the increased faecal excretion was owing to the poor gastrointestinal absorption of Se (Sharma and Singh, 1983; McDowell, 1992). This supported the fact that it could take very long time for Se to absorb upto toxic concentrations in blood to exhibit symptoms of selenosis, indicating the chronic nature of disease.

Cracking and sloughing off of the hooves could be ascribed to the disturbed production of keratin or decreased

production or decreased strength of keratin. Since the keratin consisted mainly of sulphur-containing amino acids cystine/cysteine and methionine (Oser, 1965). Selenium-sulphur antagonism in organisms had been well documented. Selenium and sulphur are inter-changeable and excess of selenium replaced the sulphur as a part of sulphur containing a.a. in the proteins (Madan et al., 1973). The hooves, horns and keratinized epithelium abnormalities recorded in present investigation could be due to the fact that majority of the selenium was covalently bound between sulphurs of half-cystine polypeptide residues (thus resulting in distortion of keratin) and could be released by non-proteolytic means (Jenkins, 1968). This showed that protein incorporation and release of selenium would not be dependent on protein synthesis or catabolism but there existed a competitive antagonism between Se and sulphur. Jubb et al. (1985) said that competition of Se with sulphur might modify the structures of keratin in claws of young pigs. Generally, selenium had been thought to be metabolized by incorporation into isologues of the sulphur containing amino acids particularly into methionine (McConnel et al., 1974). Jenkins (1968) further showed that none of the selenium derived from orally administered selenite in chick serum proteins was either seleno-methionine or seleno-cystine which were normally synthesized in the body system as part of glutathione peroxidase that protected cellular and sub-cellular membranes from oxidative damage; further confirming the competitive

antagonism in synthetic activity between the selenium and sulphur.

Keratinized epithelial defects on extremities could be explained on the basis of above facts but chronic wounds on hind quarters, udder, teats and other extremities required further documentation and investigation. However, Bakshi *et al.* (1986) reported that some of the symptoms observed were common for the Se toxicity and Zn deficiency in the rations and also present study revealed the decreased activity of Zn metallo enzyme carbonic anhydrase in selenosed animals as compared to normal healthy dairy animals; hence it followed that excess of Se in diet conditioned the secondary Zn deficiency in body system which lead to poor wound healing (Harr, 1973). Another explanation sought for the poor wound healing at extremities could be the poor vascularisation of tissues caused by Cu, Zn and Se imbalances i.e. excess of Se and decrease of Cu and Zn (Harr., 1973) which was consistent with the present venture. One of the reasons could be the replacement of sulph-hydral groups essential to the oxidative process, by selenium, leading to possible tissue injury and malfunction (Sharma and Singh, 1983).

Clinical findings of emaciation, deterioration in general condition of health were also earlier reported by Arora *et al.* (1975); Dhillon *et al.* (1992b); Ghosh *et al.* (1993) and many others. These symptoms could be due to depressed nutrient digestibility due to high Se intake which had been reported to

depress the rumen microbial activity, leading to impaired digestion along with interference in absorption (Tekchandani and Arora, 1978; Hansard, 1983).

Selenosed or emaciated animals became susceptible to secondary bacterial diseases like actinobacillosis owing to decreased immunity in emaciated state of health. Moreover, it had been shown recently that Se that had been processed by the erythrocytes was taken up largely by β -lipoprotein and an unidentified fraction located electrophoretically between α_1 and α_2 -globulin fractions, thus reducing globulin portion of plasma proteins, which was a prime biochemical to provide immunity in the form of antibodies (Sharma and Singh, 1983). Marked swelling of tongue during selenosis in buffaloes had been reported by Ghosh et al. (1993) which supported the finding of actinobacillosis in present investigation.

Typical keratinization rings, cracks proceeding to fissures in horns followed by sloughing off of horn corniums in buffaloes were consistent with the findings of Gupta et al. (1982) and well supported by other published works of Arora et al. (1975); Bakshi et al. (1986) and Ghosh et al. (1993).

Anorexia within few days of feeding sugar cane tops could be due to ability of sugar cane tops to accumulate highest concentration of Se than other forage crops due to its perennial nature, a finding consistent with present survey. As already explained, higher level of Se in diet depressed ruminal microbial activity and disturbed normal digestion. Continued

feeding of diet containing more than 5 ppm of Se lead to digestive disturbances, partial to complete anorexia and finally emaciation.

Animals in advanced stages of selenosis were emaciated due to the fact that overgrown hooves and laminitis restricted their movement from their resting place to manger (Bakshi et al., 1986; Ghosh et al., 1993). Immense pain in hooves during walking and even standing left them to rest on knees for hours together which was consistent with the findings of Gupta et al. (1982).

Pronicity of forelimbs or hind limbs to such defects could not be hypothesised but abnormalities of hooves were encountered more in hind limbs than in forelimbs probably due to the fact that more weight bearing nature of hind limbs hooves made them to crack earlier than hooves of forelimbs. However, both the fore and hind limbs were equally prone to hoof abnormalities and little higher hind limbs pronicity could not be accepted as a major symptom.

The gynaecological disorders such as infertility, anoestrous, delayed onset of oestrous, failure of conception and abortion in first trimester of pregnancy were in agreement with the findings of Gupta et al. (1982); Dhillon and Dhillon (1990); and Dhillon et al. (1992b). Selenium might effect reproduction primarily or secondarily. Primarily Se could exert its deleterious effect by interfering with the cellular oxidative process during embryonic development. Secondarily,

the chronic selenosis in the form of emaciation, impaired digestion and absorption, might lead to secondary or conditioned deficiency of essential minerals like Cu and Zn (a finding consistent with this work) which were required for normal oestrous cyclicity.

Pallor mucosae and anaemia in clinical cases of selenosis was also observed by Ghosh et al. (1993) who reported that all clinically affected buffaloes developed hypo-haemoglobinaemia, erythrocytopenia and low PCV which were suggestive of anaemia, possibly resulted from the decreased level of proteins in the liver as the selenium reduced sulphur and protein content of liver. These findings were in accordance with the report of Radostitis et al. (1994).

The lesser incidence of selenosis in calves could be due to the feeding of calves solely on milk and since very little amount of Se was excreted in milk (McConnell, 1948) and milk was poor source of selenium (Underwood, 1977). Generally this disease might take about 6 months or more for its development in a new born or freshly purchased animals to exhibit the typical symptoms of selenosis (Gupta et al., 1982). Both the sexes were equally prone to the severity of symptoms which was in accordance with the findings of Gupta et al. (1982) and Bakshi et al. (1986).

Arora et al. (1975) and Ghosh et al. (1993) reported higher incidence and severity of selenosis in buffaloes while Gupta et al. (1982) reported equal susceptibility of cattle and

buffaloes to occurrence and severity of symptoms. In the present investigation the higher incidence and severity of disease in cattle could be ascribed to the more population of cross-bred cows in the affected area and due to the known susceptibility of cross-breds to the vagaries of climatic conditions prevailing in Punjab or owing to their poor tolerance to Se excess.

The therapeutic measures were undertaken with sodium arsenate and zinc sulphate combination. The adoption of this therapy revealed visible improvement in the symptoms of selenosis. The chronic wounds on the hind quarters and the tail constrictions started healing probably due to decrease in selenium toxicity by causing a shift from respiratory excretion to biliary excretion of Se through feces or urine.

Zinc sulphate was included in the treatment regimen as sulphate helped to antagonise deleterious effects of Se due to competitive antagonism between the two elements. The basis of using sulphate mixture was that there were evidences wherein it has been shown that selenium analogues were active for the enzymes of sulphur metabolism and thereby proteins were altered by substitution of seleno-methionine for methionine (Rosenfeld, 1962). Rats and sheep given selenium, excreted substantially more selenium into urine when sulfate was present in diet. Urinary excretion of selenium increased significantly by increasing % S in diet. Although the increased urinary excretion of selenium was accompanied by a decreased excretion

into the gastrointestinal tract, there were small but consistent decreases in the retention of Se in the blood, liver, kidneys and carcass. The results showed that sulphur (sulphate) affected apparent selenium excretion and suggested that the metabolism of 'S' and Se was intimately related (Ganter and Baumann, 1962b; Halverson et al., 1962 and Pope et al., 1979).

Severity of symptoms involving horns and hooves of animals ceased to deteriorate further and appetite of animals improved remarkably reflecting positive response of the above therapeutic regimen in spite of feeding Se enriched forages to the affected animals.

However, complete remedial measures include removal of source of selenium by changing the source of forage crops and continuous therapy with above mentioned agents for at least 6 months, intermittently, because adoption of therapeutic measures for just 40 and 50 days in present study reflected only a non-significant decline in plasma Se content and a non-significant improvement in other plasma/blood biochemicals, after the therapy.

5.2 BLOOD/KERATINIZED TISSUE PROFILES

5.2.1 Haemogram:

The overall mean of haematological and erythrocytic indices depicted in Table 4 and Figs. 2 & 3 were comparable to the findings of Jain (1986) in cattle. However, these normal indices may vary depending upon age, sex, breed, species,

climatic conditions, management and nutrition, time of sampling, sampling error, effect of storage, reproductive status and method employed for analysis (Swenson, 1970). Significant decline in Hb and PCV were in accordance with the findings of Ghosh et al. (1993). Baker et al. (1989) reported higher PCV in selenosed pigs than controls pigs. Ahmed et al. (1988) reported increase in Hb, PCV and TEC values in goats intoxicated with sodium selenite. These differences could be ascribed to rate of selenium treatment, duration of selenium intoxication (acute or chronic), mode of administration, individual animal susceptibility as well as chemical form of Se salts (Glenn et al., 1964a).

Occurrence of anaemia in present investigation was in accordance with the findings of Ghosh et al. (1993) who reported that all clinically affected buffaloes developed hypo-haemoglobinaemia, erythrocytopenia and low PCV which were suggestive of anaemia. All these findings were consistent with the present work. Mean TLC of selenosed animals was significantly lower than the normal healthy dairy animals indicating lowered resistance and immunity. The decrease in the mean TEC value was not significant which reflected that the chronicity of problem might have inhibited the depression on bone-marrow that might have existed in the earlier stages of selenosis leading to release of immature erythrocytes in peripheral circulation thus exhibiting macrocytic anaemia as immature erythrocytes were larger in size than normal ones

(Schalm et al., 1975). Body compensatory mechanisms seemed to overcome the depression of bone-marrow in this chronic disease.

There was a significant decrease in mean MCV and mean MCH values of selenosed animals than in normal animals. The average decrease in MCV, MCH together with mean decrease in Hb and PCV resulted in development of microcytic hypochromic anaemia which inferred that although the number of erythrocytes produced in selenosed animals were almost similar to that of normal healthy dairy animals but in the former the erythrocytes were smaller in size and had less haemoglobin than normal.

Occurrence of microcytic hypochromic anaemia could be well explained keeping in view the well documented Se vs Cu interaction (Hill, 1974; Jensen, 1975; Stowe and Brady, 1978; Stowe, 1980 and Fehrs et al., 1981) that caused plasma copper to decrease. Plasma mineral profile (Table 16 and Figs.8 & 9) indicated a significant decrease in plasma copper level of selenosed animals as compared to normal healthy animals which was well corroborated by the findings of Ghosh et al. (1993). Further Frankel et al. (1970) reported that in animals a copper deficient diet led to the development of hypochromic microcytic anaemia, since copper was apparently essential for normal erythropoieses in animals. The decline in whole blood iron in selenosed animals corroborated the hypochromic anaemia in present investigation.

Undertaking therapeutic measures increased all the haematological and erythrocytic indices but non-significantly,

owing to increased biliary excretion of Se by sodium arsenate and sulphur replacement of selenium as affected by zinc sulphate.

5.2.2 Blood/plasma biochemical profiles

Mean value of plasma urea nitrogen of normal healthy dairy animals in present study (Table 6). was in close approximation with the mean BUN value recorded by Randhawa *et al.* (1992) in normal healthy dairy animals. Mean values of total plasma proteins and blood glucose in normal healthy dairy animals were supported by the findings of Radostitis *et al.* (1994) in bovines and dairy cattle.

Blood urea nitrogen (BUN) is the end product of the body metabolism that undergoes excretion via kidneys. Excessive accumulation of Se in kidney lead to Se induced nephrotoxicity (Maag *et al.*, 1960; Rosenfeld and Beath, 1964; Radostitis *et al.*, 1994). Plasma urea nitrogen in selenosed animals was significantly higher (Table 6) which was in accordance with the findings of Ahmed *et al.* (1988) and Randhawa *et al.* (1992) in cattle and buffaloes. Kidney damage due to Se- accumulation might lead to significant increase in plasma urea nitrogen in selenosed dairy animals. However, Kaneko (1980) reported an increased hepatic urea production during decreased glucose utilization as a cause of elevated plasma urea nitrogen in selenosed dairy animals.

Present investigation revealed a significant decrease in total plasma proteins in selenosed animals (Table 6 and

Fig.6) which simulated the findings on TSP reported by Ghosh et al. (1993) who reported that selenium reduced the sulphur and hence the protein content of liver thus leading to anaemia. The decreased plasma proteins in selenosed dairy animals indicated a chronic hepatic insufficiency (Kaneko, 1980). Rosenfeld (1962) reported that selenium analogues were fully active as substrates for the enzymes of sulphur metabolism, therefore, proteins were altered by substitution of selenomethionine for methionine. Decrease in different types of proteins or various fractions of proteins has been discussed under the heading plasma proteinogram of present discussion.

High level of Se in diet (as in present investigation) depressed ruminal microbial activity and disturbed normal digestion and absorption leading to partial to complete anorexia (Tekchandani and Arora, 1978; Hansard, 1983). Depending upon these findings the decrease in blood glucose of selenosed buffaloes depicted by Ghosh et al. (1993) was well supported, but in the present investigation, it revealed almost same or slight non-significant decrease in blood glucose of selenosed animals. This could be ascribed to the adaptability of selenosed animals to selenosed fodder or due to increased compensatory glycogenolysis and/or decreased peripheral utilization of glucose (Kaneko, 1980). In chronic selenosis, nutritional status of animal governed the rise or fall in blood glucose and liver glycogen (Potter et al., 1939).

5.2.3 Plasma/keratinized tissue alkaline phosphatase (ALP) activity in healthy and selenosed dairy animals

The present investigation revealed a significant increase in plasma ALP activity (Table 7 and Fig.4) which was well supported by the findings of Randhawa *et al.* (1992). The ALP activity has been reported to increase in plasma due to hepatobiliary damage or chronic hepatic insufficiency or with obstructive jaundice (Frankel *et al.*, 1970). However, Kaplan and Righetti (1970) reported increased plasma ALP activity in bone, small intestine and kidney damage as well. Thus it is inferred that about 2.5 fold increase in plasma ALP activity was due to release of ALP from either bone, liver, kidney and other organs owing to cumulative toxic effect of Se.

The present study also depicted a significant rise in ALP activity of keratinized tissue of horns or hooves in selenosed dairy animals (Table 7 and Fig.4). The ALP activity of keratinized tissue of horns/hooves of normal healthy dairy animals was higher than its counterpart in plasma as the origin of the ALP was believed to be the osteoblastic cells of bone, which being reservoir of the ALP had higher concentration of ALP (Frankel *et al.*, 1970). Horns and hooves being keratinized extensions of bone (osteogenic tissue) were expected to contain the same higher ALP activity as in bone as compared to plasma.

Since the site of function of ALP is outside the osteoblastic cells in the local interstitial fluid, some phosphatase activity was found in the circulating plasma.

Apparently there had been a balance between uptake of enzyme by plasma and the excretion and destruction since the plasma activity remained fairly constant (Frankel et al., 1970).

Disturbance in this balance, due to the increased plasma ALP activity than its normal constant level, owing to its release from various internal organs, under the cumulative toxic effect of Se, might have triggered the 1.64 fold increased synthesis of ALP in osteoblastic cells of bone and hence 1.64 fold increase in ALP activity in keratinized tissues of selenosed animals over that of normal healthy dairy animals, was justified.

5.2.4 Red blood cells carbonic anhydrase activity in normal healthy and selenosed dairy animals

The significant reduction in the activity of carbonic anhydrase in RBCs of selenosed animals (Table 8 and Fig.5) as compared to normal healthy dairy animals reflected that the enzyme activity might have decreased due to the conditioned Zn deficiency caused by excess of selenium, since carbonic anhydrase has been reported as a Zn- metallo enzyme i.e. containing 33% Zn (McDowell, 1992).

Absence of qualitative activity of this enzyme in keratinized epithelium of normal healthy and selenosed animals could be attributed to the specificity of carbonic anhydrase to the RBCs only (Roughton and Booth, 1946) and absence of this enzyme in keratinized epithelium.

5.2.5 Other plasma enzyme activities

The present investigation exhibited the slight non-significant increase in AST/GOT and ALT/GPT activities respectively in diseased animals (Table 9) as compared to normal healthy animals. Rise in plasma AST/GOT and ALT/GPT levels indicated the cellular damage (Drotman and Lawhorn, 1978) and altered permeability of plasma membrane (Ramazzotto and Carlin, 1978). These biochemicals could not be ascribed to indicate specific damage to any one organ in ruminants (Boyd, 1962).

A non-significant increase in AST/GOT and ALT/GPT might indicate a gradual damage to various internal organs namely liver, kidney, spleen and muscles, thus indicating the chronicity of the disease, which was hypothesised in the present study. Herigstad and Whitehair (1974) observed a marked rise in AST/GOT and ALT/GPT values after injecting sodium selenite intramuscularly which might be due to direct and severe muscular damage.

5.2.6 Plasma proteinogram

A significant decrease in the total plasma proteins in selenosed animals as compared to normal healthy dairy animals in the present study has already been explained based on various published works. Constancy in the mean albumin values between normal healthy and selenosed dairy animals (Table 10 and Fig.6) simulated with the findings of Baker et al. (1989)

who reported that albumin concentration was either unchanged or significantly decreased from prefeeding values in groups fed selenium. According to Sharma and Singh (1983) Se underwent a chemical transformation by the erythrocytes in order to be bound by plasma proteins and was taken up largely by β -lipoproteins and an unidentified fraction located electrophoretically between α_1 and α_2 -globulin fractions thus reducing globulin, a finding consistent with the significant decrease in globulin of selenosed animals in present study (Table 10 and Fig.6). Hirooka and Galambos (1966) also confirmed the decrease in globulin fraction of proteins by demonstrating high degree of association of ^{75}Se with lipid rich α_2 and β globulins, which was characteristic of mammals. The decrease in total plasma proteins concentration might be due to decreased synthesis of albumin, fibrinogen and most of the globulins (Kaneko, 1980). Changes in blood due to excessive selenium intake included decreased fibrinogen levels (Fraser *et al.*, 1991) which corroborated the significant decline in plasma fibrinogen level of selenosed animals in the present study (Table 10 and Fig.6).

Albumin globulin ratio depicted a corresponding significant alteration.

Undertaking therapeutic measures with sodium arsenate and zinc sulphate significantly improved total plasma proteins and plasma globulin of clinically selenosed animals (Table 11, and Fig.6). This was attributed to the sulphate component of

therapeutic regimen as Ghosh *et al.* (1993) observed that decreased level of proteins in the liver was due to the fact that selenium reduced sulphur and protein content of liver. The basis of sulphate treatment to antagonise the effect of selenium toxicity at tissue level, was the fact that selenium analogue was active for the enzymes of sulphur metabolism and thereby proteins were altered (Rosenfeld, 1962).

Corresponding significant improvement in A/G ratio was an obvious feature.

5.2.7 Plasma sulphur containing amino acids

Mean values of cysteine/cystine and methionine in normal healthy dairy animals was in accordance with the values referred by Oser (1965) as percentage of various fractions of serum proteins. These findings also simulated with the values of sulphur containing amino acids as reported by Fasman (1976) in human adult and infant plasma.

The significant decrease in the levels of sulphur containing amino acids in the selenosed animals as reported in this study (Table 12 and Fig.7) was expected owing to the well documented selenium-sulphur antagonism in organisms. Various aspects of mechanisms whereby selenium and sulphur act as competitive antagonists of each other has been well described under the heading, 'Incidence vis-a-vis clinical findings' of the present discussion.

The significant improvement in the mean levels of 'S' containing amino acids in plasma of diseased animals after

undertaking therapeutic measures (Table 13 and Fig.7) could be ascribed to the two way competitive antagonistic interaction of sulphate (as part of zinc sulphate) with selenium.

(i) Sulphur and selenium were shown to be interchangeable and excess of S replaced selenium (Madan *et al.*, 1973) which was covalently bound between sulphurs of half cystine polypeptide residues in proteins and could be released from proteins by non-proteolytic means (Jenkins, 1968).

(ii) The metabolism of 'S' and 'Se' was reported to be intimately related (Ganter and Baumann, 1962b and Pope *et al.*, 1979). Urinary excretion of Se increased significantly by increasing percentage of 'S' in the diet. Sulphate decreased the gastrointestinal excretion or uptake of Se but on the other hand decreased retention of Se in blood, liver, kidneys and carcass. These two hypotheses supported well, the increase in mean level of plasma 'S' amino acids and decrease in Se level in blood of selenosed animals after undertaking therapeutic trials with sodium arsenate + zinc sulphate combination.

5.2.8 Keratinized tissue/epithelium sulphur containing amino acids

According to Oser (1965), keratins as a class are characterized by their extreme insolubility in the usual protein solvents, their lack of digestibility and their high sulphur content, most of which is in the form of cystine that varies from 4-8% with negligible amount of methionine, which were just the same in normal healthy dairy animals of present

study (Table 14 and Fig.7).

Keratin molecule was considered to consist of closely packed polypeptide chains which are held together by the disulfide bond of cystine, the strength and resistance to solvents and enzymes being associated with close packing of these chains (Oser, 1965). Existence of excess of selenium in diet and competitive antagonistic interaction with S made Se to bound covalently between sulphurs of half cystine polypeptide residues, thus weakening the strength of keratin and making it susceptible to cracking, elongation or malformation.

The significant decrease in keratinized epithelium 'S' containing amino acids in selenosed animals (Table 14 and Fig. 7) could be explained on the basis of discussion under the heading plasma sulphur containing amino acids.

The non-significant slight rise in keratinized tissue S containing amino acids after therapy (Table 15 and Fig.7) could be ascribed to the fact that excessive accumulation of Se in cutaneous system appendages including keratin, might require long duration remedial measures. The Se was shown to be first processed in erythrocytes, then taken up by plasma proteins, further deposited in leucocytes, bones and cutaneous system appendages like hair and keratinized tissue (Sharma and Singh, 1983).

5.29 Plasma/blood minerals:

Mean plasma Se level in normal healthy dairy animals (Table 16 and Fig.8) was in accordance with the findings of

Radostitis *et al.* (1994). The 4.25 fold increase in plasma Se level of selenosed animals could be ascribed to the feeding of forage and water containing higher content of selenium, a finding which was supported by the analysis of forages and water samples from affected areas in present investigation. Similar significant rise in Se level of blood of cattle and buffaloes suffering from chronic Se toxicity was reported by Randhawa *et al.* (1992) and Ghosh *et al.* (1993)

Most (80%) of Se after ingestion has been reduced to unavailable forms by ruminal microbes resulting in increased faecal excretion of orally administered selenium (McDowell, 1992). Absorbed selenium, however small it may be, has to undergo a chemical transformation by the erythrocytes in order to be bound by plasma proteins specifically by β -lipoproteins and between α_1 and α_2 -globulin fractions (Sharma and Singh, 1983) which supported the present work due to decrease in globulins in this study. From plasma, it enters all tissues like liver, kidney, hair, muscles, bones and leucocytes and was accumulated excessively due to repeated and continued administration (Rosenfeld and Beath, 1964).

In the light of above information the significant rise in the level of plasma Se in selenosed animals (Table 16 and Fig.8) correlated with the continued long duration ingestion of Se rich forages and water for years together which justified the chronic nature of the disease in question.

Non-significant decline in mean plasma Se level of

affected animals after undertaking therapeutic measures (Table 17 and Fig.8) could be ascribed to the interaction of Se with dual forces i.e. sodium arsenate and zinc sulphate.

On one hand selenium has a strong tendency to make complex with heavy metals, of which arsenic (As) has been one (McDowell, 1992). Therefore, Se metabolism was influenced by dietary sodium arsenate leading to excessive mobilization from the sites of accumulation in various internal organs to plasma. Arsenicals as such are said to decrease the toxicity of selenium salts by causing a shift from respiratory excretion to biliary excretion of Se (Hatch *et al.*, 1979).

On the other hand metabolism of sulphate as a component of zinc sulphate was reported to be intimately related to Se. Sulphate in diet decreased the gastrointestinal excretion of Se, decreased retention of Se in blood, liver, kidneys and whole body and increased urinary excretion of Se significantly (Ganter and Baumann, 1962b) which might be so in the present case.

The significant decrease in whole blood iron of selenosed animals (Table 16 and Fig.9) could be due to the setting in of microcytic hypochromic anaemia which could be further due to the decrease in proteins and sulphur content of liver (Ghosh *et al.*, 1993). Ghosh *et al.* (1993) reported that this decrease in iron content was possibly due to interference in the utilization of iron resulting from hypoproteinaemia for formation of haemoglobin.

Slight improvement in the whole blood iron following therapy (Table 17 and Fig.9) could be due to increased excretion of Se to significant rise in total plasma proteins brought about by sodium arsenate + zinc sulphate.

Due to the well documented interaction between selenium and copper (Hill, 1974; Jensen, 1975; Stowe and Brady, 1978; Stowe, 1980 and Fehrs *et al.*, 1981) the significant fall in the plasma copper level of selenosed animals was expected (Table 16 and Fig.8) which has been in accordance with the findings of Ghosh *et al.* (1993). No typical symptoms of hypocuprosis were observed since only the levels below 0.19 $\mu\text{g/ml}$ of plasma represent functional deficiency or hypocuprosis and moreover levels of copper in blood may remain normal for long periods after hepatic copper levels commence to fall and early signs of copper deficiency appear. Normal average level of copper in bovine plasma has been reported to be 2 $\mu\text{g/ml}$ and levels between 0.19 $\mu\text{g/ml}$ to 0.57 $\mu\text{g/ml}$ represent the marginal deficiency (Radostitis *et al.*, 1994).

Slight improvement in Cu status of diseased animals after therapy (Table 17 and Fig.8) could be attributed to the elimination of Se effected by sodium arsenate and zinc sulphate.

5.3 SELENIUM CONTENT OF HAIR

Mineral analysis of hair might provide information on the nutritional status of animals at the time of its formation

(Werner and Anke, 1960). Significant increase in the selenium content of hair in selenosed animals in the present study (Table 18 and Fig.10) was well supported by the findings of Gupta *et al.* (1982) and Randhawa *et al.* (1992). A 70-fold rise in selenium content of hair in selenosed animals (Table 18 and Fig.10) could be ascribed to the repeated or episodal deposition of Se in leukocytes, bones and cutaneous system appendages like hair and keratinized tissue (Sharma and Singh, 1983) owing to the continued long duration ingestion of Se enriched forage and water. Selenium content of hair can be evaluated as a more reliable diagnostic index of extent of chronic Se toxicity in dairy animals (Radostitis *et al.*, 1994).

Persistent increase in Se content of hair was maintained even after therapeutic measures (Table 19 and Fig. 10) which calls for long duration remedial measures to eliminate excessively deposited selenium in cutaneous system appendages like hair and keratinized epithelium.

5.4 SOIL, WATER, PLANT AND RUMINAL FLUID MINERAL ANALYSIS

The soil of seleniferous areas have been renewed every year by the alluvium deposited by the rivers of Indus system. To be precise the White Bein - a seasonal rivulet from higher ranges of Shivalik hills has been reported to be responsible for leaching of finer particles from higher reaches to the affected area, over a period of many years. Thus the low lying areas of past times later became filled up with finer deposits containing Se brought along with the run off water.

The analysis of soil and water samples reflected a significant rise in Se level in seleniferous areas as compared to non-seleniferous areas (Table 20 and Fig.11) which was in agreement with the findings of Dhillon and Dhillon (1990, 1991b) and could be explained in the light of above described fact.

Significantly high level of Se in different fodder samples (Table 21 and Fig.12) could be ascribed to high level of Se content in soil and water and this was in accordance with the findings reported by Gupta *et al.* (1982); Dhillon and Dhillon (1991a, 1991b) and Dhillon *et al.* (1992a). Dhillon and Dhillon (1990) reported that underground water, the only source of irrigation to crops, contained about 9 times more Se content than that of non-seleniferous areas. Farmers traditionally followed corn-wheat cropping sequence. Owing to availability of plentiful water for irrigation, farmers adopted the practice of growing rice in rotation with wheat. Se content of wheat grown in rotation with corn for even 15 years on same soil and irrigation water increased but remained below the toxic levels but Se toxicity symptoms appeared in wheat wherever rice-wheat rotation was adopted for the last 5 years. The difference in Se accumulating capacity of wheat upto toxic levels when grown after rice or corn seemed to be existed in the differences in the water requirements of the two cropping systems. The rice-wheat rotation required 3 times more irrigation with water than

corn wheat sequence (Dhillon and Dhillon, 1990). Thus, it inferred that the 'Se' accumulating capacity of the wheat or various other crops, when grown on the same seleniferous soils, deposited by the seasonal channels, aggravated remarkably, depending upon the requirement of underground irrigation water, thus contributing to the increased Se toxicity in the area.

Significantly high level of Se in ruminal fluid of selenosed animals was corresponding to the high level of Se in forages and water of seleniferous area.

Wherever the Se was high, sulphur should reduce due to the interaction between the two elements. But this has not been uniformly followed in the present case probably due to the presence of other mineral elements in soil and use of phosphate fertilizers that might have resulted in multi-elemental interaction.

Dhillon and Dhillon (1990) reported that alkaline pH permitted more selenium to be absorbed by plants, whereas, acidic soils were not known to produce toxic seleniferous vegetation. However, Ghosh *et al.* (1993) reported selenium toxicosis in grazing buffaloes, when the pH of the soil was acidic.

5.5 STUDIES ON EXPERIMENTAL INDUCTION OF SELENOSIS IN BUFFALO CALVES

5.5.1 Clinical manifestations

Occurrence of various clinical signs in experimental cases could be explained on the basis of discussion under the

same heading in clinical cases, however, the differential features of excessive salivation, nasal exudation, constipation and diarrhoea need to be discussed. Excessive salivation could be due to stimulation of salivary glands when sodium selenite was given orally. Volatile selenium compounds formed during metabolism of selenium were reported to be excreted by lungs causing damage to lungs (Glenn *et al.*, 1964a) which was supported by post-mortem lesions of lungs in present study. Supervening of damaged lungs with secondary bacterial infection might have resulted in nasal exudation. Constipation could be ascribed to decreased feed and water intake. Diarrhoea in some cases might have resulted from necrotic changes observed in intestinal mucosa (Glenn *et al.*, 1964b).

5.5.2 Blood profiles

5.5.2.1 Haemogram:

The observations of haematological and erythrocytic indices of all the 3 groups upto the period of induction of selenosis (day 20) showed non-significant variations (Tables 22, 23 and 24) similar to the trend observed in the clinical cases except for MCV. Mean MCV values in all 3 groups till day 20 increased gradually, but non-significantly. The non-significant average decrease in Hb, PCV, TEC and non-significant increase in MCV followed by constancy in MCHC reflected macrocytic normochromic anaemia. Macrocytic anaemia could be attributed to compensatory increase in erythropoietic activity due to depression in Hb and proteins, resulting in

release of immature erythrocytes in peripheral circulation (Schalm *et al.*, 1975). Moreover, levels of copper in blood may remain normal for long periods after liver copper levels commence to fall (Radostits *et al.*, 1994) and microcytic anaemia appears. Normochromic anaemia in present investigation was supported by non-significant variation in whole blood iron as depicted in the plasma/blood mineral analysis.

Haematological and erythrocytic indices of group III (Table 24) provide base values to compare the therapeutic efficacy of sodium arsenate plus zinc sulphate in group I and anthiomaline plus zinc sulphate in group II respectively on the basis of improvement in the various indices following therapy.

5.5.2.2 Plasma/blood minerals:

Pattern of variation of minerals in all 3 groups of buffalo calves (Tables 25, 26 and Fig.13) was observed to be same as in the clinical cases. Gradual and significant increase in plasma Se level during experiment could be explained on the basis that daily oral administration of sodium selenite led to its regular absorption from intestine and hence exhibited an increasing accumulatory effect (Wright and Bell, 1966).

Whole blood iron and plasma copper exhibited non-significant slight decrease till induction of selenosis in all 3 groups of calves (Tables 25, 26 & 27 and Fig.13) which supported the occurrence of macrocytic and normochromic anaemia in the present work.

Generally, selenium had a strong tendency to form

complex with heavy metals. Therefore, selenium metabolism was influenced by the dietary levels of several such elements and radicals (Sharma and Singh, 1983). Comparison of the various parameters under inspection in group I and group II calves after undertaking therapeutic measures with group III calves (without therapeutic evaluation). Table 27 and Fig.13 revealed a better improvement in various parameters under the effect of sodium arsenate plus zinc sulphate combination than under the effect of anthiomaline plus zinc sulphate combination. This could be due to better complexing of Se with arsenate than with antimony (Sb). Uncomplexed Se continued to show its deleterious or accumulation defects. This further proved the specific antidotal properties of arsenate against selenium poisoning.

The rapid fall in plasma Se level in the experimental cases than the fall in level of plasma Se in the clinical cases, after undertaking therapeutic measures, was well supported by the finding of Sharma and Singh (1983) who reported that the elimination of organic Se was slow, while selenite was eliminated rapidly.

5.6 POST-MORTEM LESIONS

Post-mortem findings of selenosis similar to the present investigation were also reported by various other scientists namely Rosenfeld and Beath (1946) in cattle; Glenn et al. (1964b) in sheep; Herigstad et al. (1973) in young swine; Van Vleet et al. (1974) in baby pigs; Prasad et al.

(1982) in clinical cases in buffalo calves; Casteel et al. (1985) in swine; Dhillon et al. (1990) in experimental buffalo calves; Ghosh et al. (1993) in grazing buffaloes. Severity of the lesions (Gross + histopathological) varied with dose of sodium selenite, the latter being irritant to internal organs. Mild necrotic changes in various internal organs could be ascribed to low dose rate of sodium selenite and short duration of induction. Lesions appeared owing to the slow and gradual accumulation of Se in the various internal organs due to repeated dosing of the animals with sodium selenite.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The present study was conducted in the selenium toxic belt comprising villages Barwa and Bagouran of Jalandhar district and Simbly of Hoshiarpur district of Punjab. For comparing the results of present study a control group comprising of 12 normal healthy dairy animals was evaluated clinico-biochemically to establish control norms. In all, 30 clinical cases (20 cross-bred cows and 10 buffaloes) were selected for clinico-biochemical evaluation on the basis of diagnosis based on deformities of horns; cracking, slight elongation, malformation and sloughing-off of hooves, shedding of hairs, loss of skin elasticity, gangrene of tail and ears.

Blood, keratinized epithelium and hair samples were collected from affected animals, once before the treatment and then 40 or 50 days after undertaking therapeutic measures to study clinical pathology and clinico-biochemical alterations, if any. Soil, water, forage and ruminal fluid samples both from seleniferous and non-seleniferous areas, were also evaluated for Se and S status.

Eighteen experimental calves divided in 3 groups of 6 each were managed for inducing selenium toxicity with sodium selenite @ 2.0 mg/kg body weight orally. First 7 doses were given on alternate days and later 7 doses were given daily i.e. till day 20 of experiment. One animal each from group I and group II died after consuming 9 and 11 doses in all, respectively. Tissue samples from dead animals were collected for histopathological examination.

Therapeutic measures in 15 selected clinical cases included drenching of sodium arsenate and zinc sulphate in the morning and evening respectively. 750 mg of sodium arsenate was dissolved in 750 ml of water and 10 ml out of this solution containing 75 mg was drenched per adult animal (>300 kg body weight), after further diluting it to 250 ml. This continued for 5 days, alternatively followed by a gap of 3 days till the blood samples were collected post treatment. 5g zinc sulphate was given orally on a similar schedule as sodium arsenate. In experimental cases, group I animals were treated in a similar manner as clinical cases with sodium arsenate @ 5 ml per calf and ZnSO_4 @ 2.5 g per calf orally from day 20 onwards till day 35. In group II animals, in place of sodium arsenate anthiomaline was administered as deep i/m injections on alternate gluteal muscles on days 21, 24, 27, 30 and 33 of experiment. Zinc sulphate continued as in group I animals. No treatment was constituted in group III so as to compare the rate of fall of concentration of Se in blood plasma in

untreated cases as compared to the treated cases of group I and group II.

Blood samples in clinical cases were analysed for various haematological and erythrocytic indices. Biochemical analysis of plasma included, proteinogram evaluation, plasma urea nitrogen and blood glucose. Enzyme analysis included estimation of AST, ALT, ALP in plasma and that of ALP and carbonic anhydrase in the keratinized epithelium. 'S' containing amino acids cystiene/cystine and methionine were estimated both in plasma and keratinized epithelial tissues. Mineral analysis of plasma included Se, Fe and Cu estimation. Hair and ruminal fluid samples were evaluated for Se only while soil, water and forage were estimated for both Se and S content. In experimental study blood was analysed for haematological and erythrocytic indices and for mineral analysis.

The source of selenium in this study could be the marked leaching of Se during monsoon rains from greater Himalayan region to Shivalik hills region and then to sub-Himalayan belt comprising the above said villages leading to periodic and prolonged deposition of this element in the soil.

Symptoms observed in chronic selenosis were malformed, cracked, overgrown hooves, sloughing-off of hooves, switch of tail or tail gangrene, chronic wounds on hind quarters, deterioration in general health and condition leading to emaciation and pronicity to secondary bacterial infections like

actinobacillosis, anaestrous and abortion in first trimester of pregnancy. Typical keratinization rings with cracks proceeding to fissures in horns was characteristic of buffaloes. Clinical signs in experimentally induced selenosis were anorexia, nasal, exudation, excessive salivation, constipation or diarrhoea, terminally respiratory distress and cyanosis.

Incidence was more in adult animals (>3 years age) than in young calves and severity of symptoms was more in cattle than in buffaloes irrespective of the sex in both species.

Haemogram of clinical cases depicted significant reduction in Hb, PCV, TLC, MCV and MCH reflecting microcytic hypochromic anaemia while non-significant fluctuations in experimental selenosis, in various indices revealed macrocytic normochronic anaemia.

Biochemical analysis revealed non-significant fluctuations in blood glucose in clinical selenosis, while plasma urea nitrogen and total plasma proteins in clinical cases were decreased significantly than in normal healthy dairy animals.

ALP in plasma showed 2.5 fold increase and in keratinized epithelium 1.64 fold increase over the values in normal healthy dairy animals. RBC carbonic anhydrase, a Zn-metallo enzyme decreased significantly in clinically selenosed animals while plasma ALT/GPT and AST/GOT exhibited the slight non-significant increase. No activity of carbonic anhydrase was

recorded in keratinized epithelium.

Plasma proteinogram alterations revealed that the significant decrease in total plasma proteins in clinically selenosed animals was due to significant reduction in plasma globulin and fibrinogen levels. Analysis of 'S' containing amino acids in plasma and keratinized epithelium reflected significant reduction in cysteine/cystine and methionine content in selenosed animals.

Plasma/blood mineral analysis exhibited a significant increase in plasma Se level in both clinical and experimental selenosis, significant reduction in whole blood iron and plasma copper in clinical cases, while non-significant fluctuation in whole blood iron and plasma copper of experimental study.

Clinically selenosed animals exhibited a 70 fold increase in selenium content of hair than normal healthy dairy animals, which remained persistently higher even after therapy.

Soil, water, plant and ruminal fluid samples from seleniferous areas reflected a significant rise in Se content and non-significant fluctuations in 'S' content as compared to the respective samples from non-seleniferous areas. Gross and histopathological study revealed mild degenerative changes in heart, lungs, spleen, liver, kidneys and gastrointestinal tract.

Following therapeutic measures, severity of symptoms involving horns and hooves of clinical cases ceased to deteriorate further and appetite of animals improved remarkably

reflecting positive response to the therapeutic regimen inspite of feeding Se enriched forages to the affected animals. Experimental cases improved remarkably and almost completely.

Following conclusions were deduced from present study.

CONCLUSIONS:

1. Owing to the chronic nature of disease, the typical chronic selenosis symptoms were exhibited by adult animals as compared to young calves, irrespective of sex, with severity of symptoms much aggravated in cross-breds than in buffaloes.

2. Clinical manifestations in native humans of investigated area revealed keratinization defects and browing of nails, hairs and shedding-off of hairs, while principle complaints in animals being keratinization defects in hooves and horns, gangrene and necrosis of extremities, particularly switch of tail, lameness, emaciation leading to death.

3. The keratinization defects in horns and hooves of selenosed animals occurred owing to the reduction in 'S' containing amino acids due to Se and S antagonism leading to possible replacement of 'S' by Se in 'S' amino acids thus weakening the strength of hooves and horns and making them prone to cracking and malformations.

4. (a) Chronic selenosis resulted in hypc-haemoglobinaemia, low PCV, MCV, MCH and hypoproteinemia which were suggestive of microcytic hypochromic anaemia.

(b) A significant decline in plasma Cu and whole blood Fe of selenosed animals was suggestive of low MCV.

5. Chronic selenosis lead to hypoproteinaemia which in turn was due to hypoglobulinaemia and decrease in fibrinogen, globulin being prime biochemical for immunity, the animal became susceptible to secondary bacterial infections e.g. actinobacillosis, in the present study.

6. Decrease in red blood cells carbonic anhydrase activity gave indirect evidence of Zn-deficiency since this enzyme is a Zn-metallo enzyme, the Zn-deficiency being exhibited in the present study in the form of poor healing of chronic wounds or poor vascularisation of the peripheral tissues, on the hind quarters.

7. Marked increase in Se content of hair suggested that it was a better indicator of the chronicity of the disease than the levels of Se in the blood.

8. Providing sulphate in the form of zinc-sulphate in the ration of selenosed animals resulted in significant improvement in 'S' containing amino acids of plasma, significant improvement in total plasma proteins with non-significant improvement in the 'S' containing amino acids of keratinized epithelium.

9. Relatively acute selenium toxicity symptoms were observed by oral feeding of sodium selenite @ 2.0 mg/kg body weight in buffalo calves leading to non-significant decrease in Hb, PCV, TEC and non-significant increase in MCV and MCH reflecting macrocytic normochromic anaemia in acute selenium

toxicity.

10. The results of therapeutic measures in experimental acute selenosis indicated that sodium arsenate plus zinc-sulphate combination was better as compared to anthiomaline plus zinc-sulphate in alleviating symptoms of acute selenium toxicity.

11. On the basis of plasma Se level partial recovery of chronic selenosis cases in the field conditions and complete recovery of animals fed sodium selenite experimentally, following therapeutic measures, concluded that inorganic form of Se was rapidly eliminated from body than organic form of Se, as in field conditions of present study.

12. The deleterious effects of selenium in field conditions depend upon rate of ingestion of selenium enriched fodder, duration of ingestion of such fodder and individual animals' susceptibility.

13. Quantity of inorganic selenium (sodium selenite) ingested govern the severity of degenerative changes in different internal organs of experimental animals.

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