STUDIES ON SELENIUM STATUS IN DAIRY ANIMALS OF PUNJAB

Thesis

Submitted to the Guru Angad Dev Veterinary and Animal Sciences University in partial fulfillment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE
in
VETERINARY MEDICINE
(Minor Subject: Veterinary Public Health and Epidemiology)

By

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(L-2013-V-29-M)

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LUDHIANA – 141 004

2015

CERTIFICATE - I

This is to certify that the thesis entitled **"Study on Selenium status in dairy animals of Punjab"** submitted for the degree of **M.V.Sc.**, in the subject of **Veterinary Medicine** (Minor subject: **Veterinary Public Health and Epidemiology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Darleen Kaur Grewal (L-2013-V-29-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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This is to certify that the thesis entitled "**Study on Selenium status in dairy animals of Punjab**" submitted by **Darleen Kaur Grewal (L-2013-V-29-M)** to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfillment of the requirements for the degree of **Master of Veterinary Science** in the subject of **Veterinary Medicine** (Minor subject: **Veterinary Public Health and Epidemiology**) 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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ACKNOWLEDGEMENT

First of all, I bow my head to ***The ALMIGHTY*** *whom I must acknowledge for making me competent enough to takeover and complete this research work. Next to be acknowledged are my parents and my sister without whose inspiration I wouldn't have completed this journey.*

It was a privilege of completing this research work under the amicable guidance of ***Dr SNS Randhawa,*** *(Director of Research). I express my sincere gratitude to him for his valuable ideas and introspective guidance with constructive criticism and altruistic help throughout the course. His patience and support helped me overcome many crisis situations and I thank him for giving me an opportunity to learn and complete this work under his care.*

I extend my respect, heartiest gratitude to worthy members of my advisory committee, ***Dr. S.K. Uppal*** *(Dean PGS Nominee), Professor, Veterinary Medicine,* ***Dr. C.S. Randhawa****, Professor, Veterinary Medicine and* ***Dr.Randhir Singh****, Assistant Professor, Veterinary Public Health and Epidemiology,* ***Dr Dhanwinder Singh****, Senior Soil Chemist Department of Soil Sciences, PAU for their advice, supervision, esteemed guidance and persistent encouragement. Their help, encouragement, assistance, cooperation and concerns throughout the duration of this work which directed me to the right path in difficult situations.*

I express my heartfelt thanks ***Dr Shashi Nayyar*** *Senior Biochemist and Head, Department of Veterinary Physiology and Biochemistry, for providing required facilities and constant encouragement throughout the research programme. Her soft hearted nature and readily helpful suggestions always made things lighter.*

I owe deep sense of respect to ***Dr Amarjeet Singh*** *Professor and Head, Animal Disease Research Centre, for his enduring support, ever willing help in providing the facility and scheduling all the sampling tours for the research work even in the most crucial and tight schedule. I would like to thank* ***Dr Vishal Mahajan*** *Assistant Professor and* ***Dr G.K Philia*** *Senior Scientist for providing their time and guidance during the survey.*

It is my pleasure to record deep sense of gratitude and sincere thanks to other faculty members of my department ***Dr. B.K, Bansal****, Sr. Scientist-cum-Head, Veterinary Medicine,* ***Dr. P.S. Dhaliwal, Dr. C.S. Randhawa, Dr. S.S. Randhawa, Dr.Sushma Chhabra, Dr.Shukriti Sharma, Dr. D.K, Gupta, Dr. Sikh Tejinder Singh, Dr.Neetu Saini*** *and* ***Dr. Sujata Turkar*** *for their encouragement, assistance and cooperation during my research.*

I owe my sincere gratitude to ***Dr. C S Mukhopadhaya*** *Assistant Professor SABT for his motivation, and inspiring guidance during the brain wrecking Statistical work.*

Timely help and cooperation extended by technical and non teaching staff of the department of veterinary medicine is highly acknowledged with special thanks to ***Mr. Baljinder Singh ,Mr. Kewal, Mrs. Anita, Mr. Ganga, Mr. Chote Lal, Mr. Bhrampal and Mr Vasu ;****Lab Technicians* ***Mr. Yogesh*** *for their valuable help. My heartfelt gratitude to* ***Mr Kirpal Singh and Mr Dhanvinder Singh*** *(Department of Soil Sciences, PAU) for their help and valuable suggestions which was provident guidance.*

I wish to offer my deepest thanks to ***Dr Prateek Sharma****. Who has been a solid anchor on whom I rely again and again. Words cannot express how grateful I am to have him as my best friend and for his presence in all the good and bad times. I imagine if I would have been able to ever complete this work without his cooperation, continuous motivation, care, and moral support.*

I will always cherish the uninterrupted cooperation and the funfilled days with my dear friends ***Dr(s).Navjot Kaur Pannu, Satinderpal Singh Saini and Ankit Narang.*** *Who have been bearing the brunt of my frustrations and sharing in the happiness and their highly lively company during my investigation. I could always ask them for help, advice, opinions and ungrudging assistance on lab related issues.*

A sense of heartfelt gratitude is felt towards my classmates for their co-operation and motivating company Dr(s) ***Manasa, Divya, Razia, Sukhmeen, Shabnam, Neha, Ankush, Amandeep, Adarsh, Manjot.***

I humbly acknowledge the unconditional and unlimited love of my mother ***Mrs. Jagdeep Kaur Grewal*** *&for being the sculptors of my beautiful life and whose everlasting efforts, dedication, selfless sacrifices, silent prayers, blessings and encouragement enabled me to reach this horizon.*

The everlasting love, affection, and constant support of my grandmother ***Harvinder Kaur*** *and loving sister* ***Miss Kirat Grewal*** *who have been my support pillars and who have beard with my rough schedules and moods and constantly adjusted to anything that I asked for. My maternal grandfather* ***S.Amrik Singh*** *who has always seen and shown me my potentials and encouraged me all along.*

It would be the moment of proud and great joy for my father ***Dalbir Singh Grewal*** *who I believe has being bestowing his blessing on me from heaven and is looking upon us; celebrations would be incomplete without him but the proud look of his face and his lessons of life has kept me going in the worst situations of these past years. He taught me,* ***he that ventures fails not a****nd made me able to face the world.*

Though only my name appears on the cover, a great many people have contributed to its production. I owe my gratitude to all those people who have made this thesis possible and because of whom my postgraduate experience has been one that I will cherish forever.

As an amateur investigator, it is a matter of great pride and pleasure to present this thesis, which is the climax of my dedication, devotion and ardor to my field of interest. At last, I apologize for the faux pass of the persons who extended help in one way or other and deserve thanks directly or indirectly.

Place: Ludhiana

Date: _____ *(Darleen Kaur Grewal)*

Title of the Thesis	:	STUDY ON SELENIUM STATUS IN DAIRY ANIMALS OF PUNJAB
Name of the student	:	Darleen Kaur Grewal
Admission No.	:	*L-2013-V-29-M*
Major Subject	:	*Veterinary Medicine*
Minor Subject	:	*Veterinary Public Health and Epidemiology*
Name and Designation of Major Advisor	:	Dr. SNS Randhawa Director of Research Veterinary Medicine
Degree to be Awarded	:	M.V.Sc.
Year of award of Degree	:	2015
Total Pages of Thesis	:	87 + APPENDICES + VITA
Name of University	:	Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004 (Punjab), India

ABSTRACT

The study was planned to determine the present status of selenium in animal- plant system in Punjab with special reference to selenosis. A total of 112 crossbred cows and 187 buffaloes from 22 districts of Punjab were sampled randomly. Mean Se levels in 20 districts on basis of plasma, hair, water and fodder analysis were within normal range and significantly high in two districts viz Nawansheher and Hoshiarpur. Clinical manifestations of chronic selenosis were poor health, unthriftiness, emaciation, overgrown hooves with visible horizontal cracks leading to shedding in severe cases, associated with cracking of horns followed by avulsion of horn corium. Alopecia with generalized or patchy alopecia of trunk and tail switch hair loss, inappetance and decreased productivity along with various forms of reproductive problems were also observed associated with lameness, reluctance to move in severe cases along with arching of back and difficulty to get up. Haematological profile showed non-significant alterations in mean values of Hb, PCV, TLC and TEC in selenotic animals and thus had no diagnostic and prognostic significance. Plasma mineral analysis reflects inverse relationship of Se with As and B where as positive relationship with Pb, Ni and Cr. Therapeutic trial with oral administration of 'Degcure mixture' (pentasulphate) given@ 30 g/day /animal for 45 days resulted in increase in appetite, with sloughing of the hooves and horns in severely affected cases. Lesions of hooves and horns started showing regeneration but at a very slow rate and lameness was markedly improved with increase in milk production.

Keywords: Selenosis, Pentasulphates, Seleniferous, non- seleniferous.

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Signature of Major Advisor *Signature of the Student*

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LIST OF ABBREVIATIONS

°	:	Degree
%	:	Per cent
±	:	Plus-minus
<	:	Less than
>	:	More than
ALP	:	Alkaline phosphatase
ALT	:	Alanine amino transferase
AST	:	Aspartate transaminase
Cr		Chromium
GGT	:	Gamma glutamyl transferase
DLC	:	Differential leukocyte count
TLC	:	Total leucocyte count
et al	:	and others
EDTA		Ethylene diamine tetra-acetic acid
g/dl	:	Gram per deciliter
g/l	:	Gram per litre
Hb	:	Haemoglobin
IU	:	International unit
IU/kg	:	International unit per kilogram
mean±SE	:	Mean plus-minus Standard error
Mg	:	Magnesium
Mg	:	Milli gram
mmol/l	:	Millimole per litre
Na	:	Sodium
ng/ml	:	Nanogram per millilitre
nmol/l	:	Nanomole per litre
PCV	:	Packed cell volume
µL		Micro litre
TEC	:	Total erythrocyte count
G		Gram
u/l	:	Units/litre
ppm	:	Parts per million
ppb	:	Parts per billion

mg/L	:	Milligram per litre
mg/kg	:	Milligram per kilogram
Ha	:	Hectare
DM	:	Dry matter
MCHC	:	Mean corpuscular hemoglobin concentration
MCV	:	Mean corpuscular volume
MCH	:	Mean corpuscular hemoglobin
Se	:	Selenium
Co	:	Cobalt
Cu	:	Copper
Fe	:	Iron
Zn	:	Zinc
P	:	Phosphorus
K	:	Potassium
Mn	:	Manganese
Cd	:	Cadmium
S	:	Sulphur
Al	:	Aluminium
mg/dL	:	Miligram per decilitre
IU/L	:	International units per litre
ESR	:	Erythrocyte sedimentation rate
g/dl	:	Gram per deciliter
H_2O_2	:	Hydrogen per oxide
ROS	:	Reactive oxygen species
GPx	:	Glutathione peroxidase
SOD	:	Superoxide dismutase
Pi	:	Inorganic phosphorous
SUN	:	Serum urea nitrogen
TSP	:	Total serum proteins
μg/g	:	microgram per gram
μg/L	:	microgram per litre

CHAPTER I

INTRODUCTION

Mineral imbalances in soil and forages have been long held responsible for low production and reproduction in livestock in the tropical countries. This is because the livestock is largely dependent upon forages which in turn depend on the concentration of minerals in soils. There appears to be a definitive role of mineral deficient soils to cause deficient level in ration (McDowell and Conrad 1990 and Rajora and Panchuri 1993).

Soil, plant and management factors can influence the amount of mineral in the feedstuff (Sharma and Joshi 2002). The concentrations of minerals within the soil have a direct bearing on plant concentrations which in turn influence the mineral concentration of animals; however, diagnosis is most commonly confirmed by blood analysis. Soil analysis is less informative because assimilation of trace elements remains complex. The trace elements content of roughages is not always representative of the trace element levels in blood because presence of antagonist may affect the absorption from feed and water e.g. high level of manganese, molybdenum, iron, calcium or goitrogenic substances (Puls 1994). But logically insufficient contents of trace elements in feed stuff will definitely depress respective blood levels.

Raw materials, from which the soils are derived, are so varied and the soils formation process is so different from one climatic region to the other that average soil composition has little significance (Krauskopf 1972). Allaway (1968) reported that plants growing on same soil under different environment condition showed marked differences in plant mineral uptake. Concentrations of minerals in plants are influenced by soil genesis, fertilizer practices and stage of maturity.

Selenium (Se) is an essential metalloid trace element, naturally occurring and required in small amounts, as a part of selenoproteins and selenoenzymes. The name is derived from Selene—goddess of the moon. Selenium was discovered as an essential nutrient in 1957. The discovery of Se in glutathione peroxidase enzyme was the key to understanding its importance in nutrition and health. The primary pathway of exposure to Se is food followed by water and air (Barceloux 1999). There is very narrow margin of safety between the toxic and deficient doses of Se in animals and

humans. Selenium behaves antagonistically with Asand S in human beings and animals inhibiting the uptake and functions of these elements.

Selenium is found in soils in the form of elemental Se, such as selenate salts and ferric selenite or in its organic form. Selenite ($SeO_3^{2}-$) and selenate forms (SeO_4^{2-}) are common in most soils. These anionic forms are highly soluble, mobile, bio-available and potentially toxic. Organic forms come mainly from the decomposition of plants that accumulate Se. The Se in soil varies with soil type and texture, organic matter content and with rainfall.

Selenium concentrations in plants, grains and vegetables are related to Se levels in the surrounding soils. Incorporation and redistribution of Se by the roots occurs rapidly, but is dependent on the species and physiological conditions of the plant. In most cases, 85 percent of selenate and 70 percent of selenite are found in the aerial tissues. The normal content of Se in forages ranges from 0.1 to 0.5 ppm. The risk of livestock poisoning becomes high beyond 5 ppm. There are seleniferous plants, Se accumulating plants and others plants with an average content of Se. Seleniferous plants are characterized by a high content of Se. Selenium is also found in water. It originates from atmospheric deposits or soil drainage and sub-soils which are naturally rich in Se. The concentration in water varies from a few to several 100 mg/L. In most cases it does not exceed 10 mg/L (Mehdi *et al* 2013).

The atmosphere plays an important role in the bio-geochemical cycling of Se. It influences the transport and transformation. The presence of Se is linked to natural activities such as soil erosion, volcanism and forest fires. It is also related to human activities like burning fossil fuels and incineration of garbage, tires and papers. Burning coal and oil are the primary sources of emissions of Se compounds in the air.

Selenium deficiency is well known in animals and humans. In animals, Se deficiency is fairly common without supplementary feeding, especially with forages that are grown on neutral or acidic soils. Manifestations of Se deficiency differ in the young and the adult animals.

Selenium forms a vital constituent of the biologically important enzyme glutathione peroxidase (GPx). This enzyme reduces peroxides in cells, thus, preventing oxidative injury to cells. Because of this vital role, deficiency of Se in dairy animals may result in a wide variety of clinical manifestations. Nutritional

muscular dystrophy in calves and lambs; reproductive disorders in cows; as well as increased susceptibility to mastitis in cattle are most commonly seen due to its deficiency (Blood and Radostits 1989). Selenium can be converted in the organism into various metabolites and also play a role in cancer prevention. Many methods can be used to prevent deficiencies, such as the use of enriched Se mineral salts, application of fertilizer with Se, incorporation of Se in drinking water, injections, implants and Se bolus. Deficiency affects blood levels of IgG and T cell function, and this determines a higher prevalence and severity of Se deficiency diseases in animal populations (Arthur *et al* 2000). However, in India till date no clinical or subclinical deficiency of Se has been recorded.

Selenium (Se) toxicity also referred as selenosis is a serious threat when an excess of it is found in soils. Chronic toxicity studies have indicated that diets containing 5 mg/kg or more of Se result in chronic toxicity in laboratory animals.

Chronic selenosis has been reported in India in winter season with the symptoms of hair loss, horizontal cracks on hooves and horns, leading to elongation and sloughing of hooves, lameness and recumbency in severe cases (Gupta *et al* 1982). The toxicity of Se depends on its chemical form and invariably the organic forms are more toxic than the inorganic forms. Arid environments with alkaline soils, in times of drought or where less irrigation water is available predisposes to high soil Se levels and thus, greater uptake of Se by plants. Such conditions exist in Rajasthan and southern parts of the Haryana states of India where above normal soil Se levels have been recorded (Yadav *et al* 2005).In dairy animals decline in production and reproduction, alopecia especially involving tip of the tail leading to gangrene followed by sloughing has been recorded (Dhillon *et al* 1990). These signs were associated with anoestrous and delayed onset of oestrous, failure of conception and abortions and ultimately death of affected animals and thus causing extensive economic losses (Gupta *et al* 1982; Dhillon and Dhillon 1991; Ahuja 1993). Chronic selenosis also caused impairment of hepatic and renal functions (Blood and Radostits 1989; Randhawa *et al* 1992). However, since 1981 onwards pockets of seleniferous soils have been identified in north eastern parts of Punjab, specifically Nawansheher district and a few adjoining areas of Hoshiarpur district. Dhillon and Dhillon (1991)

and subsequently along with Randhawa *et al* (1992) and Singh (1999) observed chronic Se poisoning of plants and animals in seleniferous regions of Punjab.

However, earlier no systematic study had been undertaken to establish Se status in dairy animals, subsoil water and plants in other 20 districts of Punjab. As per the availability of scientific literature, scanty information exists on interrelationship of Se toxicosis in animal- plant system.

Thus, keeping in view the importance of Se in dairy animals and limited knowledge about the present scenario on Se toxicity and its deficiency in animal-plant system in Punjab. The present study was undertaken with the following objectives:

1. To study the Se status in dairy animals of Punjab.

2. To determine Se status, haematological and biochemical alterations in dairy animals manifesting alopecia from switch of the tail, necrosis of the tip of the tail and other associated signs (if any).

3. To study the role of pentasulphates on Se status of dairy animals.

CHAPTER II

REVIEW OF LITERATURE

The plant is the great intermediary by which certain elements of the rocks, after their conversion into soil, are assimilated and made available for the vital processes of animals and human beings.

Selenium is not essential for the growth of plants and is not found in their chemical structure and composition. Plants growing on Se rich soils accumulate it in their leaves and other soft parts. When dairy animals feed on such vegetation than excess of Se in their blood leads to Se toxicity, also referred as selenosis and poisoning causing loss of hair, horns and hooves. In some parts of the world Se is very deficient but in others it is more than the permissible level. Selenium is very toxic in certain parts of Punjab and very highly concentrated in soil especially the soil of Hoshiarpur and Nawansheher district (Anonymous 2015a).

Selenium is rapidly and efficiently absorbed from naturally toxic or near toxic seleniferous diets and also from soluble salts of the element added to the normal diet. Selenium is absorbed mainly from duodenum (Wright and Bell 1966) and after absorption is carried mainly in plasma (Buescher *et al* 1960) where it is associated with plasma protein and enters all tissues, bone, hair and red blood cells (Cousins and Cairney 1961, McConnell and Levy 1962).

Thermal plants are great pollution causing agents and source of atmospheric pollution in Punjab, especially in Ropar and Bhatinda districts. In addition to that some soils also contain excess selenium, released from natural degradation of seleniferous rocks. The soils containing 5 mg/kg Se or above are said to be toxic or seleniferous soils. Selenium is present in soils and in water in insoluble form. The soils having very low concentration of selenium as low as 0.10mg/kg could produce vegetation containing toxic levels of selenium.

Selenium exists in different compounds forms in the soil and nature. Its compound formation depends on the pH value of the soils. The high pH soils contain selenate form of Se while low pH soils contain biselenite compounds. The most vegetative crops that are prone to its high concentration are wheat, sugarcane, potatoes, carrot, turnips and tomatoes.

Selenium has dual nature, in some conditions it is every essential and in others it is harmful and toxic. It is essential in low concentration to animal life. On the other hand, animals thriving on rich Se vegetations show varied kinds of symptoms like development of cracks on hooves, peeling of the horns, loss of hair from the body parts, loss of tail and its degeneration, detachment of hooves etc. In animals it is deposited in blood, hooves and hair causing their speedy loss (Shamberger 1983).

Literature has been reviewed to understand the subject matter with regard to epidemiology, symptomatology and biochemical analysis of blood, mineral status in plasma, hair, plant and water as well as therapeutic measures initiated in Se toxicity in dairy animals.

2.1 EPIDEMIOLOGY

Ammerman and Miller (1975) stated that the early interest in Se was related primarily to its toxicity but since 1957 the element has been recognized as a dietary essential. The dietary requirement for Se by most species is about 0.1 ppm. Deficiencies of Se in cattle and sheep have been confirmed under natural grazing conditions in many countries of the world. The dietary requirements for Se and its metabolism are influenced by many nutrient interrelationships, including its interactions with sulphur, lipids, vitamin E, proteins, amino acids and several microelements.

Gupta *et al* **(**1982) recorded chronic Se toxicity in buffaloes, cattle and goats on certain dairy farms in Hoshiarpur district of Punjab.

In India, Se poisoning in cattle may arise within 10-42 days of feeding rice straw, leucern or barseem with Se levels 0.50-6.7 mg/kg DM and the problem soils have Se levels of 1.0-10.5 mg/kg (Arora 1975). Thus, the toxic levels of Se in feed and soils in India are lower than the usual toxic levels in Ireland. International standards for Se requirement in cattle are usually given as 0.10-0.18 mg/kg DM.

Dhillon and Dhillon (1991) identified pockets of seleniferous soils in north eastern parts of Punjab, India, by examining the Se content of soils, irrigation water, plants and animal tissues. Toxic sites ranging from 4 - 16 ha were sporadically distributed in the study area and occupied more than 100 ha. In the seleniferous areas, the selenium content of surface (2.12 ± 1.13 mg/kg) and subsurface (1.16±0.51mg/kg) layers of soils was 4–5 times higher than that of non-seleniferous areas. It was

proposed that the deposition of seleniferous materials transported by seasonal rivulets from higher reaches of the Shivalik hills and use of underground water for frequently irrigating crops like lowland rice lead to the development of seleniferous pockets in the area.

Gerloff (1992) monitored responses of dairy herds in a veterinary practice specializing in nutritional consultation and stated that a reference range of 70 to 100 ng of Se/ml of serum was an acceptable target concentration. This range can be attained most often by providing > 6 mg of supplemental Se per animal per day, but several factors affect the serum Se responses of different cows to specific Se intakes. These factors may include forage types and sources, ruminal environment, supplemental fat, dietary calcium, trace metals, and genetics. The major benefits, observed experimentally, of maintaining optimal Se intakes include minimizing the incidence of mastitis and preventing calf losses associated with myopathy and (or)respiratory disease.

Dhillon *et al* (1991) observed chronic Se poisoning of plants and animals, which has been reported in north-western India, with the affected region being traditionally a maize wheat growing area.

Seko *et al* (1996) reported that, in areas with high Se content in soil and plants, the incidence of Se toxicity in animals and humans was higher.

Several hundred deaths have been reported in sheep from acute/subacute selenium intoxication following grazing of seleniferous plants growing on reclaimed phosphate mines in south-eastern Idaho (Fessler *et al*, 2003). Natural Se toxicosis was reported from seven states of the USA. Over supplementation with Se was reported as a cause of toxicosis in 15 states (Edmondson *et al*, 1993). Chronic selenosis has often been reported in India in winter season with the symptoms of hair loss, cracks on skin, hooves and horns, leading to elongation and sloughing of hooves, lameness, ataxia and recumbency (Gupta *et al*, 1982).

Khan *et al* (2006) evaluated the trace elemental nutritive values of soils and forages collected from south-western part of the province of Punjab. Seasonal effects were found in all soil micro-minerals except Zn, while forage Fe, Zn, and Se were affected by seasonal changes. Concentrations of some trace minerals varied greatly among seasons and sampling periods. All soil mineral levels except Co and Se were

sufficiently high to meet the requirements of plants for normal growth and soil Co and Se levels were severely deficient and considered inadequate for plant growth. Soil Fe, Zn, Co, and Se levels were higher, and Cu and Mn lower during winter than those during summer. Although forage micro-minerals were within the range required by the ruminants, they were not sufficiently high to prevent the predisposition to various diseases caused by nutrient deficiency. Consequently, grazing animals at that location needed continued mineral supplementation of these elements with a mixture of high bio-availability rather than of high micro-mineral contents to support optimum ruminant productivity.

Normal blood Se levels are 0.10 to 0.20 ppm. Marginal levels are 0.05 to 0.08 ppm. Symptoms of frank deficiency, such as white muscle disease, are to be expected when blood Se levels fall below 0.05 ppm. Cattle should be supplemented when blood Se levels are below 0.08 ppm. Responses of deficient cattle to Se supplementation may include increased weight gain, reduced scours, improved feed efficiency, fewer retained placenta and improved immune response. Long-term whole blood Se levels of 2.0 ppm have not been excessive but due to possible antagonisms with other trace minerals, whole blood Se levels are ideally not above 0.40 ppm.

2.2 CLINICAL SYMPTOMS OF CHRONIC SELENOSIS

Selenosis occurs as an acute (blind staggers) or chronic disease (alkali disease) affecting herbivores. The intensity of clinical symptoms associated with selenosis varies with form of Se available in the soil-plant system for animals, nature of diet, duration of excess to dietary sources. Animals require 0.05- 0.1 mg/kg Se in diet to prevent diseases related to its deficiency but suffer from Se toxicity if intake increases to 2-5 mg/kg (Gissel-Nielsen *et al* 1984).

Typical symptoms of Se poisoning in plants i.e snow-white or papery-white chlorosis with pink colouration at lower side of the leaves and sheath of wheat were, at first, observed by Hurd-Karrer (1934).

Prasad *et al* (1982) induced Se toxicity by feeding rice husk to nine buffalo calves. Five out of nine showed characteristic symptoms of “Degnala disease” i.e. necrosis of the skin of the legs below the knee and hock joints, ear tips and tip of the tail. The clinical cases of Se toxicity were treated with oral administration of

"Degcure mixture". The recommended dosage was varying from 15-40 gm orally until recovery, depending upon the live weight of the animal and the level of Se in the diet.

James *et al* (1991) recorded that Se in certain soils might be taken up by plants in amounts sufficient to make forage toxic to animals. Intoxication of livestock by seleniferous plants has been classified as acute or chronic. Selenosis has been described in two forms viz. alkali disease and blind staggers. Alkali disease also referred as chronic selenosis results from ingestion of plants containing 5-40 ppm Se in inorganic or organic forms. Alkali disease causes loss of hair, lameness due to cracks and sloughing of hooves, weight loss and probably reduces reproductive efficiency. Blind staggers referred as acute selenosis is said to result from the consumption of Se indicator plants. Blind staggers causes animals to wander, walk in circles, associated with difficulty in swallowing; In addition, it may also cause blindness.

Yaeger *et al* (1998) studied the effect of subclinical Se toxicosis on pregnant beef cattle. The relationship between abortion and subclinical Se toxicosis was evaluated in the dams associated with subclinical Se toxicosis on the bovine immune system. Cows were fed diets containing 0.25 (control), 6.0, and 12.0 ppm Se. Although Se toxicosis has been earlier reported to cause abortions, however, the above study failed to reproduce abortions. Calf born had myocardial lesions consistent with those described for selenosis and had hepatic selenium levels of 9.68 ppm (wet weight). Elevated dietary Se resulted in the depression of several leukocyte function parameters in cows. A statistically significant depression in forced antibody response was identified in both Se supplemented groups. The above findings indicated that even in the absence of clinical alkali disease, elevated Se levels might adversely affect both pregnancy outcome and the bovine immune system.

Kaur *et al* (2005) observed effects of oral administration of sodium selenite on clinical and haematological parameters of crossbred cows. Toxic manifestations of sub-acute Se toxicity included anorexia, salivation, redness of the eyes, swelling of the joints, wound formation in the pastern area, reluctance to move, diarrhoea, stiffness of the neck, laboured breathing, and subnormal body temperature, and recumbency during the terminal stages. In chronic selenosis, the main symptoms observed were rough hair coat, alopecia, swelling of the coronet, enlargement of the

hooves, interdigital lesions and gangrene of the tip of the tail. Both forms of selenosis significantly affected Hb, PCV, TEC and TLC. However, MCV and MCH were significantly altered in chronic toxicity only.

Tiwari *et al* (2006) reported Se poisoning based upon a variety of clinical signs including weight loss, poor growth rates, lameness, defective hoof growth, horizontal ridges or cracks in the hoof wall, hair loss, infertility and deaths especially when there was presence of high Se content in animal feeds or overdosing injectable selenium products. A garlicky odour on the animals' breath was evident.

Kaur *et al* (2005) also studied the effect of sodium selenite induced subacute and chronic toxicity in cross-bred cow calves. Subacute and chronic selenosis was induced by oral administration of sodium selenite at dose rate of 2.5 mg/kg for 21 days and 0.25 mg/kg for 16 weeks, respectively. Toxic manifestations in subacute selenosis included anorexia, salivation, redness of eyes, swelling of joints, wound formation in the pastern area, reluctance to move, diarrhoea, stiffness of neck, laboured breathing and subnormal body temperature and recumbency in terminal stages. In chronic selenosis, main symptoms observed were rough hair coat, alopecia, swelling of coronet, enlargement of the hooves, interdigital lesions and gangrene at the tip of tail.

2.3 SELENIUM DEFICIENCY

Selenium is necessary for growth and fertility in animals, neutrophil and lymphocyte function, and antibody production. Clinical syndromes of Se deficiency include white muscle disease (nutritional muscular dystrophy) in ruminants (Ammerman and Miller 1975; Swecker 1997). Clinical signs of Se deficiency in animals include reduced appetite, growth, production, and reproductive fertility, a general unthriftiness and muscular weakness. Retained placenta has been reported in selenium deficient cows, while 'mulberry heart' disease is noted in pigs. Selenium deficiency in animals is very common and widespread around the globe (Fordyce 2005).

Carter *et al* (1968) studied Se content of forage and hay crops in different sections of the Pacific Northwest The primary criterion was to delineate areas where forage and hay crops generally contain insufficient Se to meet requirements of lambs and calves, and thus prevent white muscle disease (WMD) and other Se responsive

diseases. The minimal requirements may vary from 0.03 to 0.10 ppm Se in the diet, depending upon the diet level of vitamin E and possibly other substances. The authors reported WMD was common when forages and hay contain less than 0.10 ppm Se and the incidence was greater at lower Se levels.

Khan *et al* (2006) evaluated the trace elemental nutritive values of soil and forages collected from south western part of the province of Punjab, Pakistan. Seasonal effects were found in all soil micro minerals except Zn, while forage Fe, Zn, and Se were affected by seasonal changes. All soil mineral levels except Co and Se were sufficiently high to meet the requirements of plants for normal growth. In contrast, soil Co and Se levels were severely deficient considered inadequate for plant growth. Soil Fe, Zn, Co, and Se levels were higher, and Cu and Mn lower. Forage Zn levels during summer were at marginal deficient levels, and in contrast, all other forage micro-minerals was within the required range for ruminants. Although forage micro minerals were within the range required by the ruminants, they were not sufficiently high to prevent the predisposition to various diseases caused by nutrient deficiency.

Ahmed *et al* (2009) determine the Se concentrations in soil and forage at a livestock farm in district Sargodha, Punjab, Pakistan. Soil and forage samples exhibited very low levels of Se which were far below the critical levels for plant growth and animal requirements for various metabolic processes. Therefore, high incidence of deficiency was found particularly in some months of the samplings. These findings suggested the need of application of Se containing fertilizers for pastures or supplementation with mixture containing Se for animals being reared therein.

2.4 HAEMATOLOGY

Dhillon *et al* (1992) reported significant decline in Hb, PCV and TEC 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 macrocyctic and hypochromic anaemia.

Randhawa *et al* (1992) recorded significant increase in mean blood urea nitrogen in Se toxicotic animals (24.85 ± 1.74mg/dl) as compared to those of healthy control (4.66 ± 0.91mg/dl) and decreased total plasma proteins in Se toxicotic animals

(5.89 ± 0.30 mg/dl) as compared to those of healthy (7.00 ± 0.36mg/dl). Also observed was 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 ± 8.83 IU/L). However, non-significant alterations in the activity of alanine amino transferase (ALT) and aspartate amino transferase (AST) had been observed in the same animals.

Ghosh *et al* (1993) recorded decrease in Hb (8.27 ± 0.83 g/dl), PCV (23.11 ± 1.05 %) and TEC values (5.37 ± 0.89 x 10^6/ cu mm) in clinical cases of chronic selenosis in buffaloes as compared to the respective mean values of (13.58 ± 0.46 g/dl), (28 ± 0.77 %) and (6.96 ± 0.61 x 10^6/ cu mm), recorded in healthy control animals.

Ahuja (1993) observed significantly decline in mean values of Hb and non-significant decline in PCV and TEC along with an increase in mean value of MCV and decrease in mean value of MCHC in selenotic dairy animals as compared to those records in healthy dairy animals.

Radostits *et al* (1994) suggested that the moderate degree of anaemia could occur in chronic selenosis and a depression of Hb level to 7g/dl was indicator of selenosis.

Verma (1995) found significant reduction in mean value of Hb, PCV and TLC in clinical cases of selenosis in livestock.

Feldman *et al* (2000) detected that Hb, PCV and TEC values in cattle varied from 8.0-15.0 g/dl, 26-46 percent, 5-10 x 10^6/ cu mm, respectively.

Singh (2000) indicated that mean Hb, PCV and TEC crossbred cattle were 9.4g/dl, 27.84 percent and 5.69 x 10^6/cu mm, respectively

Randhawa *et al* (2002) induced copper deficiency in male buffalo calves by adding molybdenum (30 ppm wet basis) to their diet. Copper status was monitored from the liver Cu concentration and a level below 30 ppm (DM basis) was considered as deficient. Haemoglobin, haematocrit, total and differential leucocyte numbers were determined and the results revealed that mean total leukocyte count was unaffected, whereas a significant fall in neutrophil count coincided with the fall in mean hepatic copper level to 23.9±2.69 ppm.

Tiwari *et al* (2006) observed significant of clinical blood haemoglobin, packed cell volume, total erythrocyte count and total leukocyte count in induced cases of subacute and chronic selenosis. However, mean corpuscular volume and mean corpuscular haemoglobin were significantly altered in chronic toxicity only. There was no significant effect of selenosis on erythrocyte sedimentation rate and mean corpuscular haemoglobin concentration.

2.5 BIOCHEMISTRY

Ahuja (1993) observed significant increase in mean plasma ALP and plasma urea nitrogen and significant decrease in total plasma proteins in selenotic bovines. Non-significant fluctuations in blood glucose levels and non-significant elevation in mean plasma AST and ALT activity in selenotic bovines was also recorded.

Randhawa *et al* (1992) reported significant increase in mean plasma alkaline phosphatase activity (271.44 ± 8.81 IU/L), blood urea nitrogen (24.85 ± 1.74 mg/dl) and plasma creatinine (1.47 ± 0.18 mg/dl) along with significant decrease in total plasma proteins (5.89 ± 0.30 g/dl) in selenotic bovines as compared to the respective mean value of 141.00 ± 8.83 IU/L, 4.66 ± 0.91 mg/dl and 0.62 ± 0.36 mg/dl and 7.00 ± 0.36 g/dl in healthy controls . However, non-significant alternations in the mean plasma albumin, ALT and AST activities had been observed in the same animals.

Potter *et al* (1939) pointed out that increase or decrease in blood glucose depends on nutritional status of the animal which might not the direct of selenium toxicity.

Stevens *et al* (1985) detected significantly higher mean serum AST activity in cattle raised on the Se variable and Se-toxic areas than in the Se-deficient areas.

Verma (1995) concluded that chronic selenosis lead to hyperproteinaemia which was due to hyperglobulinaemia and decrease in fibrinogen. Globulin being prime biochemical for immunity, the animals became susceptible to secondary bacterial infections e.g. actinobacillosis.

Fraser *et al* (1997) stated that chronic selenosis lead to increase in plasma alkaline phosphatase, ALT and AST activity.

2.5.1 Glutathione Peroxidase Activity

Reactive oxygen species (ROS), such as peroxides, hydrogen peroxide and hydroxyl radicals, are produced during aerobic metabolism. If ROS are not removed in a timely manner by an antioxidant system, mammalian cells may encounter oxidative stress that causes destruction of macromolecules and abnormal function. Glutathione peroxidase along with SOD and catalase are considered the main antioxidant enzymes in mammals. Cellular GPx (glutathione: H2O2 oxidoreductase, EC.1.11.1, GPx1) was the first identified selenoproteins and is the most abundant form of body Se.

Flohe *et al* (1973) reported that the GPx functions in the cellular oxidation-reduction reactions to protect the cell membrane from oxidative damage caused by free radicals.

Oh *et al* (1974) stated that GSH is metalloenzyme containing four atoms of Se per molecule of enzyme and because of high concentration of Se in GSH there is a direct correlation between the amount of erythrocyte GSH activity and Se concentration.

Hoffman *et al* (1978), in their study, found that the initial glutathione peroxidase activity in heifers increased from 14.6 ±0.9 to 21.6 ± 1.6 u/mg Hb six weeks after supplementation with vitamin E and Se. Further, it was reported that the maximum GPx response to treatment was achieved between four to six weeks post treatment.

Whole blood has a Se concentration that is approximately three times higher than that in serum (Scholz and Hutchinson, 1979) and often is better for Se determination because any hemolysis of the erythrocytes will cause serum to have a false high value for Se (Maas *et al*, 1992).

Scholz *et al* (1979) indicated that the erythrocytes of cattle contain only Se-dependent GPx activity; therefore, blood or erythrocyte Se concentration and blood or erythrocyte GPx activity are excellent indicators of long-term Se status in cattle.

Stevens *et al* (1985) stated that erythrocytic GPx activity might be useful in determining the diagnosis of chronic Se toxicosis.

Counotte and Hartman (1989) demonstrated that there was a highly significant correlation between Se content in whole blood and the concentration of the Se containing enzyme glutathione peroxidase in red blood cells.

Osama *et al* (1992) reported that the blood GPx in cows increased with vitamin E and Se injections and was significantly higher in treatment group as compared to the control group.

Miller *et al* (1993) reported that the reduction in Se intake, at a time when there was excessive exposure to stimulators of ROS production resulted in a relative deficiency of GPx activity and development of oxidative stress.

Hogan *et al* (1993) observed that GPx maintained the integrity of membranes and thus reduced the oxidative stress which protected cellular membrane and lipid containing organelles from peroxidative damage by destruction of endogenous peroxides.

Finch and Turner (1996) based upon the data on 210 Nigerian Sahel goats, reported that the erythrocyte glutathione (GSH) concentration were ranging from 2.4 – 175.2 with mean value of 46.5 ± 36.4 mg/100ml RBC or 0.1- 7.3 (mean 1.9 ± 1.4)mg/gHb. Majority of the goat population (74.8%) had low or deficient erythrocyte GSH concentrations (≤60 mg/100ml RBC). Sixteen animals with very low erythrocyte GSH values of 2.4 to 9.8 (6.9 ±2.9 units) mg/100ml RBC were not anaemic and the anaemic goats with PCV of 17.0 ± 0.9 percent had GSH concentrations of 3.5 – 97.5 (45.0 ± 42.8 units) mg/100ml RBC.

Arthur (2000) reported that GSH is located in the cystol and utilizes the reducing potential of glutathione to reduce H_2O_2 and other organic peroxides to water.

Shrivastava and Kadu (2005) in their study on effect of reduced glutathione treatment on selenosis, blood Se concentration and GPx activity, concluded that the blood Se had good correlation with toxic signs during subcutaneous selenosis and 2.5 and 3.5g/ml could be considered as the threshold level for appearance of signs and mortality in buffalo species. The GSH given i.v. could be used as a therapeutic measure during subcutaneous selenosis as it reduced the blood Se concentrations, arrested the toxic signs, thus prevented mortality and lowered GPx activity.

Philip and Rogers (2001) stated that GPx levels in whole blood closely reflected blood Se status and blood GPx and Se levels in bovine kidneys and liver had positive linear relationships.

2.5.2 Lipid Peroxidation

Donkoh (1989) reported that high ambient temperature caused impaired antioxidant status which was characterized by elevated lipid peroxidation in serum.

Altan *et al* (2003) observed that heat stress increased lipid peroxidation which was associated with production of large number of free radicals which were capable of initiating peroxidation of polyunsaturated fatty acids.

Castillo *et al* (2003) documented that detection of free radical damage and the protection against it had become very important in the studies related to ruminant production/reproduction as the level of lipid peroxidation and antioxidant status provided complementary information about the metabolic status of the animal rather than metabolic parameters alone.

Simsek *et al* (2006) inferred that lipid peroxidation was an indicator of oxidative stress in cells and tissues. Lipid peroxides derived from polyunsaturated fatty acids were unstable and decomposed to form a series of compounds like malondialdehyde (MDA). The estimation of MDA was widely used as an indicator of lipid peroxidation

Yarovan (2008) observed that free radicals oxidation was activated in animals under various types of stresses and lipid peroxidation products accumulated in various organs.

2.6 PLASMA MINERAL CONCENTRATIONS

Stevens *et al* (1985) found that Se serum concentrations ranged from 0.021 to 0.789 microgram/ml in cattle, whereas 0.05 to 0.40 microgram/ml was the reported range for adequate serum Se concentrations in cattle. It was observed that some herds had adequate serum Se concentrations while other herds had less than adequate serum Se concentrations (less than 0.05 microgram/ml). Therefore, more cattle were at risk of developing Se-deficiency disease. Serum Se concentrations (up to 0.789 microgram/ml) correlated with GPx enzyme activity. It was inferred that mean serum Se concentration of 0.789 microgram/ml was approximately 6.2 times higher than

previously reported in dairy cattle. Therefore, it was considered that RBC glutathione peroxidase activity might be useful in determining the diagnosis of chronic Se toxicosis.

Levander (1986) recorded that concentrations of Se in whole blood were responsive to Se intake.

Maas *et al* (1992) analysed Se concentration of paired blood and serum samples from 344 cattle using Inductively Coupled Argon Plasma Emission Spectroscopy using hydride generation (ICP). The correlation coefficients, by simple linear regression of serum Se on blood Se, was 0.88 ($r^2 = 0.77$).

Christodoulopoulos *et al* (2003) studied Se concentration in blood and hair of Holstein dairy cows. Although, the feeding regime in these farms was similar, the Se content of feeds was variable. The Se content of concentrate feeds was 0.104±0.086 mg/kg on dry matter (DM) and on basis of silage was 0.025±0.018 mg/kg. A significantly positive correlation was found between the mean Se concentration in black hair and the mean Se concentration in blood (r^2=0.610, p<0.001), the mean Se concentration in white hair and the mean Se concentration in blood (r^2=0.770, p<0.001) and the mean Se concentration in white hair and the mean Se concentration in black hair (r^2=0.921, p<0.001). The mean Se concentration in white hair was significantly lower than that in black hair (p<0.001).

2.7 HAIR MINERAL CONCENTRATIONS

Kursa and Kroupová (1975) assessed Se content in cattle hair in areas with incidence of nutrition-induced muscular dystrophy and found the lowest mean Se values in the fur dry matter, with repeated findings of mere 0.09 ppm Se. About 60 to 100 per cent of samples from those farms showed selenium values below the 0.25 ppm level. The fur of heifers which recovered from nutritional muscle dystrophy and were treated contained 0.29±0.11 ppm Se. In other breeds, 0.30 ± 0.07 to 3.33 ± 0.16 ppm Se was found in the fur of cows and young cattle. The initial field assay of Se content in cattle fur indicated a relation of low Se values to the frequency of clinical forms of nutritional muscle dystrophy in domestic ruminants.

Combs *et al* (1982) examined that mineral content of hair was affected by season, breed, hair colour within and between breeds, sire, age and body location. Seasonal effects might be due to stage of growth of hair and to changes caused by

perspiration, surface contamination and diet. Breed and sire effects on mineral content of hair complicated prediction of nutritional status based on hair analyses because, in many commercial cattle, neither breed nor sire was known. Hair from young animals might be lower in Zn, Mn and Fe, but is higher in Na, Ca, Cu and K than that from older animals. It was also observed that pigmented hairs apparently were higher in Ca, Mg, K and Na than white hair, but trace mineral concentrations were similar in hair of different colours. The effect of body location on mineral content of hair might be due to differences in surface contamination, differences in hair growth cycles and differences in texture of the hair. Concentrations of Ca, P and Cu in hair were not affected by dietary intake of these minerals. Zn and Se contents of hair might reflect dietary intake. Information on other required minerals is lacking. The Pb, As and possibly Cd levels in hair may be related to dietary or environmental exposure

Gupta *et al* (1982) recorded higher levels of Se in hairs of livestock affected with chronic selenosis. The increase in hair Se content was in the range of 7.50 -16.32 ppm.

Frank *et al* (1986) found 20 per cent decline in liver Cu concentration in cattle supplemented with 0.58 mg Se/Kg in the control diet when fed over seven to eight months period in both the groups.

Combs (1987) stated endogenous minerals that were incorporated into hair by several routes. Most attention on hair mineral incorporation has focused on element uptake within the hair follicle. Minerals incorporated within the follicle are presumably chemically or physically associated with cortical cells of the hair shaft and reflect mineral status at the time that the hair filament was synthesized. Mineral deposition in hair does not cease when the follicle is not producing a hair fibre. For several elements significant correlations exist between mineral concentrations in hair and mineral intake. It has been further reported that dietary intake of Ca, P and Fe were also known to affect uptake of other elements in hair. Hair mineral analyses might be useful, however, when combined with other indicators of mineral status to provide a more precise assessment of mineral status in livestock.

Dhillon *et al* (1990) stated that mean Se content in hair samples was ranging from 18.7 ± 5.9 to 38.6 ± 17.7 ppm in dairy animals raised on seleniferous areas as

compared to mean hair Se value of 0.06 ± 0.03 ppm recorded in dairy animals from non- seleniferous area in certain villages of Punjab.

Dhillon and Dhillon (1991) recorded that the average Se content of hair in animals fed with fodder containing high levels of Se was 28.21 ± 14.99 µg/g as compared to the 0.057 ±0.031µg/g in healthy control animals, thus, reflecting roughly 500 times high Se content in hair of affected animals.

Randhawa *et al* (1992) inferred that mean Se content in blood of buffaloes suffering from chronic selenosis was 3.78 ± 2.4 µg/ml, which was 47 times higher than those of healthy animals. Verifying, similarly, mean Se content in hair samples of dairy animals suffering from chronic selenosis was 37.09 ± 18.5 µg/g, which was 271 times higher than that of healthy animals.

Ahuja (1993) reported Se content of 14.30 ± 1.31 µg/ml in hair samples of selenotic animals as compared to mean Se content of 0.24 ± 0.05 µg/ml recorded in hair of healthy animals

2.8 WATER AND FODDER MINERAL CONCENTRATIONS

Elemental concentration of different forages is mostly affected by soil characteristics including pH, fertilization practices, drainage system, plant species forage stage of maturity and, various types of interactions among different mineral elements. When animals depend exclusively on forage plants to fulfil their fodder requirements it is necessary to identify various attributes that may change forage composition and to measure strategy program to improve livestock productivity and performance (Khan *et al* 2012).

The availability of minerals in the soil depends upon the effective concentration in soil solution, which is influenced by pH, moisture, organic matter, leaching, and presence of other elements and microbial activity of soil.

Se, As and S are great pollutants of soil. The poisoning of these trace elements causes many undesired effects on animals and humans. The high concentrations of these trace elements in blood causes toxic effects that are antagonistic to the cell formation and growth of body. Their deficiency especially of Se can lead to many health related issues and problems. Selenium is a metalloid with its chemical properties somewhat similar to Sulphur Metalloid is an element that has some characteristics of a metal and some of a non-metal (Anonymous 2015b).

Watts (2006) conducted research on trace elements and stated that Se is antagonist to Cu, Zn, Fe, Mn, S, Hg, As, Cd, Pb, T, Sn, F, Ag, Vitamin B_2, C, A, K but has synergistic effect with Ca, Na, K, Cu, Fe, Mn, Co and Vitamin B_1, B_6, A,C, D and E.

Browne (1938) stated plants possess a very; evident faculty of assimilating certain elements, as Ca, K, Mg, P, and S, in much greater quantities than their abundance in the soil might lead us to suppose, and of more or less effectively rejecting other elements such as Al, the second most abundant mineral constituent of soils.

Mayland *et al* (1986) found synergistic relationship between Se and Pb and also indicated that Se in feed increased the absorption of Pb and subsequent toxicity in mature sheep.

Reith (1965) and Flemming (1973) listed several factors influencing the mineral uptake by crop and pastures. As the soil pH increases availability of soil Mo and Se increases, however, availability of Cu, Co, Fe and Mn decreases (McDowell 1993). Poor soil aeration and drainage increases the availability and uptake of Cu, Co, Mn and Fe by the plants (Grace and Clark 1991). As the plant matures mineral concentration decreases due to dilution process and translocation of minerals to the root system.

Increased concentration of Se in ground water is mainly observed in the Nawansheher district. The concentration of selenium is observed to be more than 0.01 mg/L in samples collected from various depths in Nawansheher district. Groundwater in few other districts has also been found to be contaminated with Se. In Fatehgarh Sahib district, 3 of the 93 samples were found to be contaminated by Se. In Hoshiarpur district, 1.12 percent of the 178 samples; in Ludhiana district 6 percent of the 83 samples and in Ropar 0.8 percent of the 234 samples were found to be Se contaminated. The problem of Se contamination was observed many years ago as well, but no worthwhile measures for reduction of Se concentration to permissible limits by rain water harvesting has been implemented for removal of Se from ground water.

Dhillon (2001) reported that maximal tolerable levels of Se in livestock's feed varied from 2-5 ppm.

Spears (2003) evaluated trace mineral bioavailability in ruminants and stated that absorption of Se and Cu is much lower in ruminants than in non-ruminants. The low absorption of these minerals in ruminants is due to modifications that occur in the rumen environment. Se bioavailability is reduced by high dietary S and the presence of cyanogenetic glycosides in certain legumes. Feeding organic selenium from selenomethionine or selenized yeast results in much higher tissue and milk selenium concentrations than are obtained with selenite. Authors also stated high dietary Mb in combination with moderate to high dietary S resulted in formation of thiomolybdates in the rumen. Thiomolybdates greatly reduced Cu absorption, and certain thiomolybdate species could be absorbed and interfere systemically with Cu metabolism. Independent of Mb, high dietary S reduces Cu absorption perhaps via formation of copper sulfide. High dietary iron also reduced Cu bioavailability. Mn is very poorly absorbed in ruminants, and research suggested that high dietary Ca and P might reduce Mn absorption.

Yadav *et al* (2005) used ICP-AES technique and stated Se status in soils of agricultural lands of northern districts of India. The drier lands, where lesser rains were received or where less irrigation water was available in Rajasthan and southern parts of the Haryana states, had above normal soil selenium levels. These soils were also found to be alkaline. Punjab, Himachal Pradesh and northern parts of the Haryana states had normal levels of Se in their soils, except with slightly lower Se levels in a few areas that were affected by floods along the river Yamuna. In the Nawansheher–Hoshiarpur region of Punjab, more than 1000 hectares of agricultural land has been significantly affected by high levels of selenium (Se). The Se concentrations in soil and crops such as wheat grains, wheat husk, rice, maize and mustard products were found to be ranging from 2.7 to 6.5 mg/kg and 13 to 670 mg/kg, respectively, indicating significantly high Se in these crop products.

Enjalbert *et al* (2006) estimated plasma Cu and Zn and erythrocyte GPx from cattle herds to evaluate the relationship between trace-element status and production, reproduction and health in cows and their calves. Inadequate Cu status was not associated with adult disorders but was an important risk factor for poor calf performance or health. Se deficient status was associated with most studied disorders in cows and both deficient and marginal herd status were strongly associated with

poor health of calves, particularly with increased risks of myopathy and infectious diseases. Zinc insufficiency was strongly associated with low milk production and impaired locomotion in dairy herds, and was also associated with diarrhoea and poor growth in calves. Because a low-adequate status increased the risk of many disorders in adults and calves, and classified herds as deficient and marginal when the lower terciles of plasma zinc concentration are below 12 and between 12 and 14 μmol/L respectively.

Report of the high level expert group on water logging in Punjab in 2013 stated high concentration of As (more than 50 µg/L) has been reported from Muktsar, Bhatinda, Mansa and Sangrur Districts. Jain further recoded that As concentration exceeding the limit of 0.01mg/L was encountered at a few places in the districts of Amritsar, Gurdaspur, Hoshiarpur, Kapurthala and Ropar.

Kojouri *et al* (2009) concluded that Zn and Se have a determinant role in immune status and the response of animal's immunity system to dermatophytosis as serum concentration of Se and Zn in cattle with dermatophytosis were found to be significantly lower ($P \leq .05$).

Shukla *et al* (2010) analysed soil, feeds, fodder samples as well as blood serum of animals (cattle and buffaloes) in four villages of district of Uttarakhand for different macro and micro mineral contents to establish the mineral correlation among soil, plants and animals. The macro and micro mineral contents in soils were found to be higher than their respective critical levels except Ca. Average daily macro and micro mineral intake per animal through different feed ingredients were found optimum except Ca and Cu which were deficient with an average value of 11.4 g and 48.16 ppm, respectively. The soil and plants ($r=-0.07$) and soil and animals ($r=-0.34$) showed non-significant negative correlations while, significant ($P<0.05$) negative correlation ($r=0.08$) between plants and animals for Ca was observed. The correlation for P, Mg, Fe, Cu, Co, Mn and Zn was observed between soil-plants, plants-animals as well as soil-animals. The total mineral intake showed non-significant positive correlations for P, Zn and Cu while negative correlations for Ca, Mg, Fe and Mn.

Kumaresan *et al* (2010) studied the soil–plant–animal continuum in subtropical hilly areas. Soil, fodder, and blood serum samples from dairy cattle were collected from eight districts of Mizoram, a hilly state in India. The samples were

digested using diacid mixture (HNO_3:$HClO_4$; 10:4) and analysed for macro (Ca, P, Mg, Na, and K) and micro (Cu, Co, Mn, Fe, and Zn) mineral concentrations. The macro and micro mineral concentrations varied among the different districts. The correlation values between fodder and cattle were significant for all the minerals studied except for P and K. The correlation value between fodder and cattle was highly significant ($P < 0.01$) for Ca (0.878), Mg (0.88), Cu (0.885), and Zn (0.928). However, such correlations were not observed between the mineral levels in cattle and mineral levels in soil except for Ca (0.782).

Bajaj *et al* (2011) found hazardous concentrations of Se in soil and groundwater in North-West India and stated that prevailing intensive irrigation practices in Punjab with Se enriched groundwater might be the cause of Se accumulation in soils. Toxic concentrations of Se (45–341 g/L) were present in groundwater (76m deep) of Jainpur and Barwa villages in Punjab. Selenium enrichments were also found in top soil layers (0–15 cm) of Jainpur (2.3–11.6 mg/kg) and Barwa (3.1mg/kg). Mineralogical analyses confirmed silicates and phyllosilicates as main components of these soils, also reflected by the high content of SiO_2 (40–62 wt.%), Al_2O_3 (9–21 wt.%) and K_2O (2.2–3.2 wt.%). Sequential extraction revealed >50% Se bioavailability in Jainpur soils. Detailed biogeochemical studies of Se in sediments or groundwater of Punjab was not available so far; it was concluded that intensive investigations needs to be started for better understanding of the problem of Se toxicity.

Pasha *et al* (2012) conducted a study to assess the macro-minerals status of buffaloes on the basis of blood plasma, feedstuffs, soil and water analysis in Districts Hafizabad and Sheikhupura rice zone of Punjab province in Pakistan. Ca status in lactating buffaloes was found to be significantly deficient. Plasma Na and K levels were also affected by season ($P \leq 0.05$). Lower plasma Na and sufficient K values were found in all physiological stages of buffaloes. Sodium levels in water, soil and feedstuffs were lower than required. Higher levels of K were found in roughages, soil and water. Higher levels of Mg were found in soil. It was concluded that macro-mineral levels were significantly different ($P \leq 0.05$) in blood plasma of different physiological stages of buffaloes in those areas of Punjab and that animals needed minerals supplementation for health and economic benefits.

Sun *et al* (2014) stated that As and Se were unusual metalloids as they both induce and cure cancer. They both cause carcinogenesis, pathology, cytotoxicity, and genotoxicity in humans, with reactive oxygen species playing an important role. They hypothesize that there were two types of interactions between As and Se. At low concentration, Se could decrease As toxicity via excretion of As–Se compound, but at high concentration, excessive Se could enhance As toxicity by reacting with S–adenosylmethionine and glutathione.

Tomza-Marciniak *et al* (2011a) analysed concentrations of Cd, Pb, Fe, Zn, Cu, Cr, Ni, Al and Ar in cattle on inductively coupled plasma atomic emission spectrometry. Authors inferred that Pb was significantly correlated with Cd, Zn, Fe, Cu and Ni. A significant positive correlation between the concentration of Cd and Zn, Cu and Ni concentrations was observed.

Tomza-Marciniak*et al* (2011b) further evaluated the concentration of Se and selected heavy metals and their possible relationship in serum healthy lactating cows. Cd, Pb, Cu and Zn concentrations were determined by inductively coupled plasma-atomic emission spectrometry. The content of Se, Zn, and Cu was 0.083 ± 0.026, 0.629 ± 0.413, and 0.152 ± 0.042 μg/mL respectively. The presence of the Cd and Pb was found with mean concentration of 0.0009 ± 0.0008 and 0.018 ± 0.016 μg/mL, respectively. Analysis of correlations between Se and toxic metals showed a negative and significant ($P < 0.05$) relationship between Se concentration and Pb and Cd concentration in the serum, with correlation coefficients of $r = -0.595$ and $r = -0.618$, respectively. For Cu and Zn, this relationship was also negative but not significant ($r = -0.255$ and $r = -0.203$). Overall the study demonstrated that the levels of toxic metals decreased as serum selenium concentrations increased.

Zwolak and Zaporowska (2012) human studies have demonstrated that Se may reduce As accumulation in the organism and protect against As-related skin lesions. Selenium was found to antagonise the pro-oxidant and genotoxic effects of As in rodents and cell cultures. Also, studies on Se effects against oxidative stress induced by Cd in various animal tissues produced promising results. Reports suggested that Se protection against toxicity of As and Cd was mediated via sequestration of these elements into biologically inert conjugates. Selenium-dependent antioxidant enzymes probably play a secondary role in As and Cd detoxification.

Fordyce (2012) said Se has one of the narrowest ranges between dietary deficiency (<40μg/day) and toxic levels (>400μg/day) (WHO 1996), which makes it necessary to carefully control intakes by humans and other animals, hence, the importance of understanding the relationships between environmental exposure and health. Geology exerts a fundamental control on the concentrations of Se in the soils on which we grow the crops and animals that form the human food chain. The Se status of populations, animals, and crops varies markedly around the world as a result of different geological conditions. Because diet is the most important source of selenium in humans, understanding the biogeochemical controls on the distribution and mobility of environmental Se is key to the assessment of Se-related health risks.

Laven and Nortje (2013) determined Cu and Se status of dairy cattle, by assessing liver Cu and serum Se concentrations. The Se concentration in serum, the standard deviation of each batch varied from 0.5-147 nmol/L, and for Cu concentration in liver, the standard deviation varied from 173-829 μmol/kg fresh weight.

Ademi *et al* 2015 assessed Se status of 160 cows in whole blood in some of the Western Balkan countries using inductively coupled plasma mass spectrometry. The results showed 45.6 percent of cows had inadequate (≤100 ng/mL) level of whole blood-Se concentration. The whole blood-Se concentration was significantly higher in Se supplemented cows than in those without any Se supplementation. Therefore, they suggested Se supplementation to the animal feed or Se-biofortified feed used to ensure adequate level of Se in cows.

2.9 TREATMENT

Arora *et al* (1975) reported that 23 out of 28 cattle and buffaloes suffering from Se toxicity were recovered completely after giving “Degcure mixture” @30gm per day for about 55 days. The remaining five animals did not respond due to their advance stage and secondary complications.

Deore *et al* (2002) inferred that although there were no specific treatments to correct Se toxicities in animals, recognition of seleniferous plants, proper land management and selective grazing might help to prevent selenosis. Animals having a blood Se level >1.5 μg/ml were indicative of impending Se toxicosis and such animals should receive corrective measures to alleviate Se toxicity. Administration of

reduced glutathione (GSH) intravenously at 5 mg/kg of body weight reportedly arrested the toxic signs, prevented mortality and lowered glutathione peroxidase (GPx) activity.

Fessler *et al* (2003) recorded that feeding high protein diets and well balanced mineral mixture that contained S and Cu could reduce Se toxicity.

CHAPTER III

MATEIRIALS AND METHODS

3.1 PLACE AND TIME OF WORK

The research was carried out in the Department of Veterinary Medicine, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The work was undertaken from July 2014 to July 2015.

3.2 BASELINE SURVEY OF MINERAL STATUS IN DAIRY ANIMALS OF PUNJAB

3.2.1 Selection of Animals

Dairy animals from 22 villages representing all districts of Punjab state, were selected using survey tool box as per National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Hebbal, Bengaluru, Karnataka. Twelve to twenty dairy animals from each village were selected from two different regions representing east and west part of the village to see any variability. Blood, hair, fodder and water samples were collected from randomly selected dairy animals and farms. Dairy animals were examined for any signs of clinical or sub-clinical deficiency or toxicity of Se. Information on health status of the animals, reproductive status, plane of nutrition, mineral supplementation was obtained by physical examination as well as per a comprehensive questionnaire.

3.2.2 Collection of Samples

Samples were collected from 112 crossbred cows and 187 buffaloes from 22 districts of Punjab. One village from each of twenty districts as per the list was visited and twelve to twenty apparently healthy adult dairy animals were sampled from each village randomly. However, in Nawansheher district six villages were sampled and in Hoshiarpur district three villages were selected for sampling (Table 1).

3.2.2.1 Blood samples

Whole blood was collected aseptically from each crossbred cows and buffalo selected for sampling. About 20 ml whole blood was collected by jugular venipuncture in to stoppered mineral free heparinised glass vials and was centrifuged at 3000 rpm for 30 minutes to separate plasma which was subsequently stored in acid washed glass vials in deep freezer for estimation of various minerals. The remaining

RBC pellet obtained after centrifugation was used to prepare hemolysate for estimation of various enzymes activities viz. Glutathione peroxidase activity and Lipid peroxidase activity.

Table 1: List of the districts of the dairy animals sampled during the survey

Districts	Villages Visited	Cattles	Buffaloes
Amritsar	2	5	8
Barnala	2	2	10
Bathinda	1	2	10
Faridkot	1	6	6
Fatehgarh Sahib	1	3	9
Fazilka	1	4	8
Ferozepur	1	2	10
Gurdaspur	1	3	9
Jalandhar	1	7	5
Kapurthala	1	7	5
Ludhiana	1	4	14
Mansa	1	4	15
Moga	1	8	12
Mohali	1	7	5
Muktsar	1	6	7
Pathankot	1	6	6
Patiala	1	6	8
Ropar	1	5	7
Sangrur	1	8	4
Tarn Taran	1	5	5
Nawansheher	6	5	9
Hoshiarpur	3	7	8
Total	31	112	187

Five ml of blood was collected in EDTA coated vials (Vaku- 8 HMD Pvt. Ltd) and used for haematological studies viz. Haemoglobin (Hb), packed cell volume (PCV), total erythrocytic count (TEC), total leukocyte count (TLC) and platelet count. Similarly, five ml of blood was collected in clot activating vials and centrifuged at 3000 rpm for 30 minutes to separate serum for estimation of various biochemical parameters viz. ALT, AST, GGT, BUN, creatinine and total proteins.

3.2.2.2 Hair samples

Hair samples (1-2 gm) were collected from the same animal from tail region and washed with tap water and detergent to remove dirt, dust and dung. The samples were rinsed twice with double distilled water followed by dipping in acetone, dried at 50°C and stored at room temperature for subsequent mineral analysis.

3.2.2.3 Fodder and water samples

Fodder samples currently being fed to the animals were collected and brought to laboratory. They were first washed with tap water and then with double distilled water. The samples were dried at room temperature for some time and then placed in oven for drying at 55 to 66° C. After drying the fodder samples were grinded and stored for analysis of macro and micro minerals.

Water which was provided to animals for drinking was taken in plastic bottles and brought for analysis of macro and micro-minerals.

3.3 BLOOD ANALYSIS

3.3.1 Haematological Parameters

Blood samples collected in EDTA coated vials (Vaku Pvt. Ltd), were used for determination of following parameters.

a) Haemoglobin (Hb; g/dl)
b) Packed cell volume (PCV; %)
c) White blood cells(WBC; $x10^3/\mu l$)
d) Total erythrocyte count (TEC; $\times 10^6$)
e) Platelet count ($\times 10^3$)

The above parameters were determined using fully Automatic Laser Based Haematology Analyser (ADVIA ® 2120 Haematology system, Siemens Healthcare Diagnostics Inc., USA).

f) Differential leukocyte count (DLC;%) was performed manually under oil immersion of light microscope in blood smears stained by Wright Giemsa stain or Leishman stain (Jain 1986).

3.3.2 Blood Biochemical Analysis

For biochemical analysis, blood samples collected in Clot activating (serum vials) (Vaku) were analysed on VITROS 350/250/250AT Chemistry systems (Ortho-Clinical Diagnostics, Johnson and Johnson Company) kits were used for estimation of following parameters.

a) AST
b) ALT
c) GGT
d) SUN
e) Creatinine
f) TSP

3.3.3 Preparation of hemolysate

After the plasma was separated from the sediment (erythrocytic pellet) after removal of the buffy coat, the sediment was washed thrice with normal saline solution (0.9% NaCl) to prepare hemolysate. Hemolysate was prepared by adding distilled water slowly upto the initial marked level with constant stirring. Aliquots for estimation of LPO were immediately separated and the rest was stored in deep freeze till the activity of various enzymes was estimated.

3.3.4 Glutathione Peroxidase Activity (GPx)

The GPx activity was measured by method of Hafeman *et al* (1974). This assay is based on the principle that GPx catalyses the reaction between H_2O_2 and reduced glutathione (GSH) to form oxidized glutathione (GSSG) and water. The rate of oxidation of GSH by H_2O_2 is used as a measure of GPx activity.

3.3.4.1 Procedure

Hemolysate was diluted 1:200 times with distilled water. Take 0.2 ml of reduced glutathione, 0.2 ml of buffer and 0.1 ml of sodium azide in test, control and blank tubes. Add 0.1 ml of hemolysate and 0.2 ml distilled water to test tubes. Add 0.1 ml of hemolysate and 0.2 ml of distilled water to test and control tubes. In the blank, add only 0.3 ml of distilled water. After 5 minutes pre-incubation. After 5 min

pre-incubation, add 0.2 ml pre-warmed H_2O_2 (at 37˚ C) to test and blank except control (to which 0.2 ml of distilled water was added). After 3 min interval, add 4 ml of metaphosphoric acid precipitation solution to all the tubes and centrifuged. To 2ml of filtrate pipetted from all the tubes, add 2 ml of 0.4 M disodium hydrogen phosphate solution and 0.1 ml of DNTB reagent. Mix thoroughly and optical density was read at 420 nm.

$$\text{GPx activity (units/mg Hb)} = \frac{10\ (\log C - \log T)}{X} \times 200$$

Where, X is Hb in mg/ 0.1 ml

3.3.5 Estimation of Lipid Peroxidation

Lipid peroxidation was assayed by method of Placer *et al* (1966). The method is based on the principle that the reaction of malonyldialdehyde (MDA), an end product of lipid peroxidation with thiobarbituric acid (TBA) yields a pink coloured trimethine complex which is measured at 548 nm.

3.3.5.1 Procedure

Test and control (volume 1.5 ml) were run without H_2O_2. They were prepared as follows:

Test and Control without H_2O_2: 0.1 ml of hemolysate + 1.4 ml of buffer.

- Incubated all the test solutions at 37˚C for 30 minutes but control solutions were not incubated.
- Added 1.5 ml of TBA reagent to all the tubes.
- Heated the test solutions in boiling water bath for 10 minutes using marbles as condenser.
- Cooled the test solutions.
- Control solutions were not heated and cooled.
- Added 3 ml of pyridine/ n- butanol (3/1, V/V) and 0.1 ml of 1N NaOH to all solutions. Mixed thoroughly.
- Read optical density of test and control against a water blank at 548 nm.

$$\text{Lipid peroxidation nmol MDA produced g/Hb} = \frac{(OD_T - OD_C)}{X} \times 1000$$

Where, X = Hb in mg/0.1 ml

3.4 MINERAL ANALYSIS

3.4.1 Plasma Samples

Plasma samples of 2 ml each for minerals analysis were taken and digested overnight in 10 ml di-acid mixture of distilled concentrated nitric acid AR (3 parts) and Perchloric acid AR (1 Part), boiled at 110°C on open hot plate until reduced to 1 ml colourless digestates. Digestates, diluted to 10 ml with double glass distilled water, were measured for the concentrations of various macro and micro elements viz. Na, K, Ca, Mg, P, S, Cu, Mn, Fe, Zn, Co, B, Se, Al, Ni, Cd, Cr, As and Pb using Inductively Coupled Argon Plasma Atomic Emission Spectroscopy (ICP-AES).

3.4.2 Hair Samples

One gm of hair sample was digested in 13 ml of one cycle distilled concentrated nitric acid AR (5 parts) and 2 ml of perchloric acid AR (1 Part), boiled at 110°C on open hot plate until reduced to 1 ml. The digested extract was diluted with double glass distilled water to make 25 ml of volume and filtered using Whatmann filter paper No.1 and then analysed for various macro and micro minerals.

3.4.3 Fodder Samples

One gm of grinded fodder sample was digested with 10 ml of distilled concentrated nitric acid AR (3 parts) and perchloric acid AR (1 Part). Digested extracts was diluted to 25 ml of volume and filtered using Whatmann filter paper No. 42, then analysed for various micro and macro minerals.

All digested samples were analysed on Inductively Coupled Argon Plasma Atomic Emission Spectroscopy (ICP-AES).

3.5 THERAPEUTICS MEASURES

Seventeen clinical cases showing signs of chronic Se toxicity were identified following comprehensive survey conducted in villages of Nawansheher district viz. Jainpur, Barwa, Rakkar Dhahan (Saroya block) and Barwa, as well as villages of Hoshiarpur district viz. Najjarpur, Simbli, Dhamai (Garhshankar block). Dairy animals showing symptoms of hoof elongation/overgrowth and cracks, horn cracks, patchy/ generalized loss of hair from the body (alopecia) and tail, along with other signs like lameness, repeat breeding followed by shedding of dew claws, hooves and

horns etc. were identified after complete physical examination and history related to onset of signs was also taken.

Blood samples from 17 clinically identified cases showing signs of chronic Selenosis were collected before undertaking therapeutic measures (0 day) and thereafter on 45^{th} day post treatment. Hair samples (17), fodder samples fed to the animals (16), water samples (15) from farmers' house and field were also collected for analysis of minerals.

Blood (7), hair(11), water(4) and fodder(11) samples from villages of Nawansheher districts viz. Rurki khurd (Balachaur), Majara and Karawar were also collected and analysed for Se to assess the present mineral status in these areas of the district, animals from these areas did not exhibit any sign of Selenosis.

3.6 TREATMENT

Treatment included Degcure mixture prepared as per formulation of Arora *et al* (1975) consisting of five sulphates (1 kg magnesium sulphate, 166 g ferrous sulphate, 24 g copper sulphate, 75 g zinc sulphate and 1.5 g cobalt sulphate). The treatment was given orally at a dose rate of 30g/day/animal for 45 days. Blood samples were collected before and after the completion of treatment. For estimation of Se along with other macro and micro minerals

3.7 STATISCAL ANALYSIS

Mean, Standard error of mean and range of various parameters were estimated. Statistical differences were analysed by test of significance (one way analysis of variance) wherein, differences were considered to be significant at 5 percent level ($P \leq 0.05$) between different districts and paired t test were performed using Systat and SPSS for windows.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 EPIDEMIOLOGICAL SURVEY

The primary objective of this study was to identify and determine the status of selenium in dairy animals of Punjab, to develop/formulate clinico-epidemiological data base with respect to all districts of Punjab and also to develop a better understanding of plant-animal relationship in respect to mineral deficiency and toxicity.

Study was conducted to understand the present status of Se in dairy animals and its importance, interaction with other minerals in different areas representing different possible soils type along with the assessment of adequacy of mineral mixtures fed to the dairy animals in different parts of Punjab state. Study was also aimed to assess the occurrence of chronic/acute selenium toxicity in animals of Nawansheher and Hoshiarpur districts with known toxic soils and fodders in these areas.

Seasonal green fodder and straw constituted the basic diet of the majority of the dairy animals under study. Maize and sorghum were given prominently during summer season and barseem and oats during winter season along with wheat straw being fed in both the seasons.

It was observed that 78.49 percent of dairy animals were on medium plane of nutrition (*ad lib* green fodder + small amount of straw), 18.54 percent were on high plane of nutrition (*ad lib* green fodder + concentrate) whereas 3.07 percent were on low plane of nutrition. Concentrate was mostly fed to animals on dairy farms. Mineral mixture supplementation was recorded in 39.93 % of animals in the study @ 40 -50 g daily (Table 2).

All the adult animals selected were examined for any overt clinical abnormality, if any, on the day of sampling. These animals were bright, alert, healthy and not under any type of medication prior to this. In general, body condition of the animals varied from fair to good. Health record and history of any problem or signs during previous years particularly suggestive of any mineral deficiency or toxicity were enquired along with type and source of diet, mineral supplementation and current reproductive status etc.

Table 2: District wise grading of plane of nutrition and mineral mixture feeding status of dairy animals of Punjab

S. No	Districts	Plane of Nutrition			Mineral Mixture	
		Low	Medium	High	Yes	No
1	Amritsar (N=13)	1	10	2	6	7
2	Barnala (N=12)	-	10	2	6	6
3	Bathinda (N=12)	-	10	2	6	6
4	Faridkot (N=12)	-	09	3	6	6
5	Fatehgarh Sahib (N=12)	-	10	2	6	6
6	Fazilka (N=12)	-	12	-	6	6
7	Ferozepur (N=12)	-	12	-	6	6
8	Gurdaspur (N=12)	-	08	4	8	4
9	Jalandhar (N=12)	-	12	-	5	7
10	Kapurthala (N=12)	-	6	6	5	7
11	Ludhiana (N=18)	-	14	4	7	11
12	Mansa (N=19)	4	15	-	4	15
13	Moga (N=20)	-	12	8	4	16
14	Mohali (N=12)	1	10	1	4	8
15	Muktsar (N=12)	-	11	1	4	8
16	Pathankot (N=13)	-	12	1	4	9
17	Patiala (N=14)	1	11	2	4	10
18	Ropar (N=12)	-	9	3	4	8
19	Sangrur (N=12)	-	3	9	10	2
20	Tarn Taran (N=10)	-	10	-	4	6
21	Nawansheher (N=14)	2	12	-	4	10
22	Hoshiarpur (N=16)	-	12	4	8	8
	Percentage (%)	3.07%	78.49%	18.54%	39.93%	60.07%

4.2 HAEMATO-BIOCHEMICAL ANALYSIS

The haematological values of dairy animals from 22 districts of Punjab have been presented in Table 3. The overall mean values of Hb, PCV, TEC, TLC and Platelets in dairy animals of Punjab were 10.41 ±0.57 g/dl, 31.73 ±0.39 %, 6.36±0.07 x 10^6/cu mm, 17.03±1.13 x 10^3/ cu mm and 242.14 ±6.59 x 10^3/ cu mm, respectively. Mean DLC recorded was 46.39±0.72 percent neutrophils, 52.46±0.71 percent lymphocytes and 1.18±0.11 percent eosinophils.

Species wise mean values of various haematological parameters (Hb, PCV, TEC, TLC, DLC and Platelets count) and biochemical parameters (Lipid peroxidase and Glutathione peroxides activity) in dairy cattle and buffaloes have been given in Table 4. Mean values of Hb, PCV, TEC, TLC and Platelets in cattle were found to be 10±0.021 g/dl, 29.19±0.62 percent, 6.05±0.13x 10^6/ cu mm, 13.86±1.87 x 10^3/ cu mm and 288.10±10.39 x 10^3/cu mm, respectively. Mean DLC recorded was 44.53±1.19 percent neutrophils, 54.15±1.18 percent lymphocytes and 1.34±0.18 percent eosinophils.

As per literature wide variations exist in normal values of these parameters which could be ascribed to physiological factors, season, temperature, quality of diet, animal excitement, time of sampling and laboratory methods for analysis. (Feldman *et al* 2000). The mean values recorded in the present study were well within the normal range. Singh (2000) indicated that mean Hb, PCV and TEC values in crossbred cattle were 9.4g/dl, 27.84 percent and 5.69 x 10^6/cu mm, respectively and those were comparable to the present study.

Randhawa (1993) reported comparatively higher mean values of Hb, PCV and TEC (12.06 g/dl, 36.09 percent and 7.65 x 10^6/ cu mm, respectively). Despite various factors affecting the haematological indices, Feldman *et al* (2000) detected that Hb, PCV and TEC varied from 8.0-15.0 g/dl, 26-46 percent, 5-10 x 10^6/ cu mm, respectively with respective mean values of 11 g/dl, 35 percent and 7 x 10^6/ cu mm. Therefore, it could be inferred that cattle as compared to buffaloes were having values in lower normal range.

Mean values of Hb, PCV, TEC, TLC and Platelets in buffaloes were 11.49±0.16 g/dl, 33.19±0.47 percent, 6.54±0.09 x 10^6/ cu mm. 18.85±1.41 10^3/ cu mm and 215.70±7.88 x10^3/ cu mm, respectively. Mean DLC recorded was 47.37±0.90 percent neutrophils, 51.78±0.89 percent lymphocytes and 1.09±0.13 percent eosinophils which were within the normal range and have been presented in table 4.

Table 3: Haematological values of cattle and buffaloes from all districts of Punjab (Mean±SE)

S.No	Districts	WBC ($x10^3$/cu mm)	TEC ($x10^6$/ cu mm)	Hb (g/dl)	PCV (%)	Platelets ($x10^3$/cu mm)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)
1	Amritsar (N=13)	34.38^{b} ±9.39	6.022^{abc} ±0.303	10.269^{ab} ±0.496	30.431^{abc} ±1.433	227.69^{bcdefg} ±28.436	62.76^{a} ±3.05	37.92^{ef} ±2.93	0.38^{bc} ±0.21
2	Barnala (N=12)	54.57^{a} ±8.873	7.102^{ab} ±0.482	9.733^{b} ±0.378	35.625^{ab} ±2.378	345.75^{a} ±30.107	46.08^{cdef} ±2.61	53.00^{abcd} ±2.65	0.83^{bc} ±0.386
3	Bathinda (N=12)	49.93^{a} ±10.394	6.411^{abc} ±0.412	11.325^{ab} ±0.556	33.450^{abc} ±1.591	172.83^{fg} ±16.36	52.50^{bc} ±3.28	46.66^{cde} ±3.34	1.16^{bc} ±0.45
4	Faridkot (N=12)	10.00^{c} ±1.04	6.558^{abc} ±0.619	11.333^{ab} ±1.342	32.250^{abc} ±3.443	212.83^{cdefg} ±37.28	48.33^{bcde} ±2.62	51.83^{abcd} ±2.34	0.66^{bc} ±0.37
5	Fatehgarh Sahib (N=12)	10.83^{c} ±0.74	6.176^{abc} ±0.326	11.050^{ab} ±0.377	31.308^{abc} ±1.108	212.66^{cdefg} ±18.67	45.83^{cdef} ±2.76	54.00^{abc} ±2.70	0.33^{c} ±0.22
6	Fazilka (N=12)	9.41^{c} ±0.64	6.195^{abc} ±0.418	9.667^{b} ±0.359	29.125^{c} ±1.338	289.33^{abcde} ±26.46	40.66^{def} ±2.34	59.33^{a} ±2.20	0.66^{bc} ±0.28
7	Ferozepur (N=12)	9.61^{c} ±0.49	5.672^{c} ±0.289	9.600^{b} ±0.569	29.242^{c} ±1.859	259.75^{abcdef} ±26.98	42.50^{cdef} ±1.69	57.67^{ab} ±1.57	0.66^{bc} ±0.37
8	Gurdaspur (N=12)	10.41^{c} ±0.97	6.858^{abc} ±0.369	12.375^{a} ±0.808	36.400^{a} ±2.471	189.91^{fg} ±24.71	44.00^{cdef} ±1.74	54.00^{abc} ±1.82	2.00^{b} ±0.65
9	Jalandhar (N=12)	11.83^{c} ±0.752	5.989^{abc} ±0.185	11.050^{ab} ±0.507	28.492^{c} ±1.112	260.91^{abcdef} ±36.09	40.16^{ef} ±1.914	56.50^{abc} ±1.79	3.50^{a} ±0.74
10	Kapurthala (N=12)	12.00^{c} ±1.40	5.842^{bc} ±0.747	10.408^{ab} ±0.511	30.733^{abc} ±1.710	296.83^{abcd} ±46.15	41.33^{def} ±2.86	57.83^{ab} ±2.76	0.83^{bc} ±0.45
11	Ludhiana (N=18)	9.16^{c} ±0.55	5.950^{abc} ±0.201	10.328^{ab} ±0.418	30.544^{abc} ±1.307	182.05^{fg} ±14.56	43.11^{cdef} ±2.07	56.00^{abc} ±2.06	0.88^{bc} ±0.33
12	Mansa (N=19)	12.434^{c} ±0.750	7.092^{ab} ±0.315	12.237^{a} ±0.704	34.600^{abc} ±1.877	201.84^{defg} ±17.68	56.42^{ab} ±2.60	43.38^{def} ±2.578	0.63^{bc} ±0.26

S.No	Districts	WBC ($x10^3$/cu mm)	TEC ($x10^6$/ cu mm)	Hb (g/dl)	PCV (%)	Platelets ($x10^3$/cu mm)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)
13	Moga (N=20)	11.088^{c} ±0.563	6.539^{abc} ±0.204	10.970^{ab} ±0.473	32.015^{abc} ±1.195	191.15^{fg} ±19.63	42.50^{cdef} ±2.64	57.50^{ab} ±2.89	1.00^{bc} ±0.30
14	Mohali (N=12)	13.021^{c} ±0.749	6.542^{abc} ±0.289	11.200^{ab} ±0.589	32.725^{abc} ±1.787	256.58^{abdcef} ±28.12	45.33^{cdef} ±4.47	49.00^{bcd} ±4.63	4.00^{a} ±0.92
15	Muktsar (N=12)	12.175^{c} ±0.870	6.108^{abc} ±0.269	11.400^{ab} ±0.491	32.258^{abc} ±2.266	308.25^{abc} ±32.74	52.50^{bc} ±3.08	46.83^{cde} ±3.12	1.00^{bc} ±0.46
16	Pathankot (N=13)	12.159^{c} ±1.446	7.176^{a} ±0.644	11.485^{ab} ±0.983	32.900^{abc} ±2.817	296.76^{abcd} ±30.66	40.39^{def} ±2.94	58.15^{ab} ±2.88	1.53^{bc} ±0.51
17	Patiala (N=14)	12.616^{c} ±2.559	6.710^{abc} ±0.294	11.029^{ab} ±0.596	32.514^{abc} ±1.524	152.85^{g} ±31.62	41.14^{def} ±3.60	56.14^{abc} ±3.45	2.00^{b} ±0.75
18	Ropar (N=12)	13.021^{c} ±0.749	6.542^{abc} ±0.289	11.200^{ab} ±0.589	32.725^{abc} ±1.787	256.58^{abcdef} ±28.12	37.50^{f} ±3.99	60.66^{a} ±3.83	1.83^{bc} ±0.75
19	Sangrur (N=12)	31.481^{b} ±9.271	6.201^{abc} ±0.317	10.417^{ab} ±0.570	29.842^{bc} ±1.651	198.66^{efg} ±30.28	47.66^{bcdef} ±3.60	52.00^{abcd} ±3.66	0.50^{bc} ±0.35
20	Tarn Taran (N=10)	33.346^{b} ±419	6.083^{abc} ±0.373	10.480^{ab} ±0.593	31.100^{abc} ±1.728	253.10^{abcdef} ±33.02	62.10^{a} ±3.77	37.20^{f} ±3.83	0.50^{bc} ±0.26
21	Nawansheher (N=14)	10.418^{c} ±0.699	5.918^{abc} ±0.260	11.057^{ab} ±0.561	29.336^{bc} ±1.398	323.28^{ab} ±40.54	50.42^{bcd} ±3.06	48.28^{bcd} ±3.32	1.28^{bc} ±0.49
22	Hoshiarpur (N=16)	7.956^{c} ±0.37	6.124^{abc} ±0.21	11.531^{ab} ±0.49	29.944^{bc} ±1.03	304.18^{abc} ±22.64	38.93^{ef} ±2.81	60.50^{a} ±2.86	0.50^{bc} ±0.27
	Total (N=299)	17.03 ±1.13	6.36 ±0.07	10.94 ±0.13	31.73 ±0.39	242.14 ±6.59	46.39 ±0.72	52.64 ±0.71	1.18 ±0.11

Mean values bearing different superscripts in a row differ significantly ($P \leq 0.05$)

The mean values of Hb, PCV and TEC in the present study were comparable with the respective mean values of 11.29 g/dl, 34.83 percent, 6.79 x 10^6/ cu mm as reported in adult buffaloes by Randhawa *et al* (1993). Similarly, Singh (1999) also recorded a mean Hb, PCV and TEC of 10.67 g/dl, 31.88 percent and 6.35 x 10^6/ cu mm, respectively.

The mean values of Hb, PCV, TLC, TEC and DLC showed non-significant variations between species (cattle and buffaloes) with no definite pattern, whereas, there was significantly higher platelets count in cattle as compared to buffaloes.

Table 4: Haematological and biochemical profile of dairy animals in Punjab (Mean±SE)

Parameters	**Cattle(N=112)**	**Buffalo(N=187)**
Hb (g/dl)	10.00 ± 0.213	11.49 ± 0.161
PCV (%)	29.19 ± 0.62	33.19±0.47
TEC(x 10^6/cu mm)	6.05 ± 0.13	6.54 ± 0.09
TLC(x10^3/cu mm)	13.86 ± 1.87	18.85 ± 1.41
Neutrophils(%)	44.53 ± 1.19	47.37 ± 0.90
Lymphocytes(%)	54.15 ± 1.18	51.78 ± 0.89
Eosinophils(%)	1.34 ± 0.18	1.09 ±0.13
Platelets(10^3/cu mm)	288.10 ± 10.39*	215.70 ± 7.88
GPx (Units/mg Hb)	138.25 ± 21.88	81.93±16.59
LPO (nmol/g Hb)	699.43± 83.13	615.94±63.05

N=Number of dairy animals

* Significant (P≤0.05)

Table 5: Plasma, hair, fodder and water selenium concentrations in dairy animals of all the districts of Punjab (Mean±SE)

Districts	Plasma (µg/ml) (N=286)	Hair(µg/g) (N=286)	Fodder (mg/kg) (N=96)	Water(µg/L) (N=85)
Amritsar	0.087±0.00^{c}	1.79±0.19^{c}	0.34±0.06^{a}	1.7±0.9^{a}
Barnala	0.147±0.080^{bc}	2.62±0.21^{c}	0.29±0.03^{a}	32.2±2.7^{a}
Bathinda	0.044±0.011^{c}	0.99±0.18^{c}	0.33±0.05^{a}	3.7±2.0^{a}
Faridkot	0.050±0.004^{c}	2.61±0.65^{c}	0.48±0.14^{a}	0.7±0.3^{a}
Fatehgarh Sahib	0.047±0.005^{c}	2.83±0.46^{c}	0.56±0.75^{a}	11.3±0.9^{a}
Fazilka	0.008±0.002^{c}	1.45±0.07^{c}	1.62±0.15^{a}	0.3±0.3^{a}
Ferozepur	0.019±0.006^{c}	1.91±0.21^{c}	0.53±0.12^{a}	4.3±2.4^{a}
Gurdaspur	0.088±0.009^{c}	1.55±0.28^{c}	0.50±0.14^{a}	10.0±1.1^{a}
Jalandhar	0.077±0.008^{c}	1.64±0.21^{c}	0.46±0.12^{a}	12.0±0.1^{a}
Kapurthala	0.185±0.016^{bc}	5.63±0.58^{b}	3.73±0.59^{a}	24.8±8.1^{a}
Ludhiana	0.085±0.005^{c}	0.78±0.12^{c}	0.39±0.05^{a}	1.3±1.3^{a}
Mansa	0.065±0.004^{c}	1.49±0.21^{c}	2.23±0.98^{a}	1.0±0.6^{a}
Moga	0.050±0.004^{c}	2.30±0.23^{c}	0.57±0.23^{a}	3.0±1.2^{a}
Mohali	0.050±0.006^{c}	1.17±0.40^{c}	0.24±0.23^{a}	5.5±3.5^{a}
Muktsar	0.052±0.003^{c}	0.68±0.14^{c}	0.87±0.42^{a}	4.7±3.7^{a}
Pathankot	0.062±0.006^{c}	2.06±0.27^{c}	0.60±0.19^{a}	7.3±2.6^{a}
Patiala	0.036±0.009^{c}	2.03±0.14^{c}	0.07±0.10^{c}	14.8±0.6^{a}
Ropar	0.067±0.005^{c}	1.98±0.30^{c}	1.71±0.98^{a}	6.0±1.2^{a}
Sangrur	0.072±0.005^{c}	1.68±0.19^{c}	0.21±0.08^{a}	7.0±3.0^{a}
Tarn Taran	0.044±0.009^{c}	1.43±0.22^{c}	0.70±0.25^{a}	16.7±6.4^{a}
Nawansheher	0.601±0.103^{a}	37.00±5.42^{a}	15.77±8.82^{a}	101.64±35.58^{a}
Hoshiarpur	0.607 +0.150^{a}	24.01±6.36^{a}	6.67±1.14^{a}	27.87±11.65^{a}

Mean values bearing different superscripts in a row differ significantly (P≤0.05)

The mean concentration of Se in selenotic fodder fed to the animals and grown on seleniferous soils in Nawansheher district was 15.77 ± 8.82 mg/kg, whereas, Se content of fodders grown on non-seleniferous soils of the area was 1.74 ± 0.38 mg/kg.

The mean concentration of Se in selenotic fodder grown on seleniferous soils from affected areas of Hoshiarpur district and fed to the animals included in the present study was 6.67 ± 1.41mg/kg, whereas, mean concentration of Se in fodders grown on non-seleniferous soils of the area was 1.72 ± 0.36mg/kg.

4.2.1 Selenium status in dairy animals of Punjab

4.2.1.1 Plasma

The mean Se values recorded in plasma of dairy animals of 22 districts of Punjab are given in Table 5.

The mean plasma Se values of dairy animals from each of the twenty two districts of Punjab were: 0.087±0.00 µg/ml (Amritsar), 0.147±0.080 µg/ml (Barnala), 0.044±0.011 µg/ml (Bathinda), 0.050±0.004 µg/ml (Faridkot), 0.047±0.005 µg/ml (Fatehgarh Sahib), 0.008±0.002 µg/ml (Fazilka), 0.019±0.006 µg/ml (Ferozepur), 0.088±0.009 µg/ml (Gurdaspur), 0.077±0.008 µg/ml (Jalandhar), 0.185±0.016 µg/ml (Kapurthala), 0.085±0.005 µg/ml (Ludhiana), 0.065±0.004 µg/ml (Mansa), 0.050±0.004 µg/ml (Moga), 0.050±0.006 µg/ml (Mohali), 0.052±0.003 µg/ml (Muktsar), 0.062±0.006 µg/ml (Pathankot), 0.036±0.009 µg/ml (Patiala), 0.067±0.005 µg/ml (Ropar), 0.072±0.005 µg/ml (Sangrur), 0.044±0.009 µg/ml (Tarn Taran) 0.601±0.103 µg/ml (Nawansheher) and 0.607±0.15 µg/ml (Hoshiarpur).

The mean Se contents in plasma of dairy animals from 22 districts of Punjab ranged from 0.008±0.002 to 0.607±0.15 µg/ml. The minimal mean plasma Se value recorded (0.008±0.002 µg/ml) was from Fazilka district whereas, the maximal mean value recorded 0.607±0.150 µg/ml was from Hoshiarpur followed by 0.601±0.103 µg/ml from Nawansheher district of Punjab, respectively. Mean plasma Se values were within normal range in twenty districts of Punjab. However, they were significantly higher in Nawansheher and Hoshiarpur districts. Therefore, animals from Nawansheher and Hoshiarpur districts having high Se contents in plasma were designated as selenotic animals.

Comprehensive analysis and surveillance studies done among the twenty non seleniferous districts of Punjab revealed that mean plasma Se levels in dairy animals

of Kapurthala and Barnala district were in comparatively higher normal range and those of Ferozepur and Patiala in lower range. It was also revealed that mean Se status of the dairy animals of Kapurthala district, on the basis of plasma Se content was higher.

Epidemiological studies conducted on dairy animals of Punjab revealed the present status of Se in different parts of the state. Animals require 0.05- 0.1 mg/kg Se in diet to prevent diseases related to its deficiency but suffer from Se toxicity if intake increases to 2-5 mg/kg (Gissel-Nielsen *et al* 1984). Plasma Se levels of 0.10 -0.20 µg/ml have been considered normally within safe range in dairy animals; levels below 0.02 µg/ml are deficient and above 0.2 µg/ml are considered toxic.

The mean plasma Se value recorded in healthy dairy animals was 0.122µg/ml, whereas, in animals suffering from chronic selenosis, the mean plasma level recorded was 0.317µg/ml (Stevens *et al,* 1985).

4.2.1.2 Hair

The mean Se values recorded in hair samples of animals of 22 districts of Punjab are given in the Table 5.

The mean hair Se values of dairy animals from 22 districts of Punjab were: 1.79±0.19 µg/g (Amritsar), 2.62±0.21 µg/g (Barnala), 0.99±0.18 µg/ml(Bathinda), 2.61±0.65 µg/g (Faridkot), 2.83±0.46 µg/g (Fatehgarh Sahib), 1.45±0.07 µg/g (Fazilka), 1.91±0.21 µg/g (Ferozepur) 1.55±0.28 µg/g (Gurdaspur), 1.64±0.21 µg/g (Jalandhar), 5.63±0.58 µg/g (Kapurthala), 0.78±0.12 µg/g(Ludhiana), 1.49±0.21 µg/g (Mansa), 2.30±0.23 µg/g (Moga), 1.17±0.40 µg/g (Mohali), 0.68±0.14 µg/g (Muktsar), 2.06±0.27 µg/g (Pathankot),2.03±0.14 µg/g (Patiala), 1.98±0.30 µg/g (Ropar), 1.68±0.19 µg/g (Sangrur) , 1.43±0.22 µg/g (Tarn Taran) 37.00±5.42µg/g (Nawansheher) and 24.01±6.36 µg/g (Hoshiarpur).

The mean Se content in hair samples of animals from 22 districts of Punjab ranged from 0.68±0.14 to 37.00±5.42 µg/g. The minimal mean hair Se value (0.68±0.14µg/g) was recorded from Muktsar district, whereas, the maximal mean value (37.00±5.42 µg/g) was recorded from animals of villages of Nawansheher district of Punjab. Mean hair Se values were within normal range in twenty non-seleniferous districts of Punjab; however, they were significantly higher in

Nawansheher and Hoshiarpur districts. Therefore, animals from Nawansheher and Hoshiarpur districts having significantly high Se content in hair were referred as selenotic animals.

Comprehensive analysis and surveillance studies among the animals from twenty non seleniferous done revealed that Se levels in hair samples of animals in Kapurthala were comparatively higher and that of Muktsar, Bathinda and Ludhiana were in lower range. It was also observed Se status of the dairy animals of Kapurthala district, on basis of hair Se content was higher.

Selenium content of in hair is considered as an important index of Se toxicity in dairy animals. If hair contains more than 3.00 µg/g of Se, the animal may be suffering from some degree of Se poisoning (Dhillon *et al*, 1996). Dhillon *et al* (1990) stated that mean hair Se value recorded in healthy dairy animals was (0.06±0.03 µg/g) whereas, in animals raised on seleniferous areas, the mean hair Se levels recorded was 18.7±5.9 to 38.6±17.7 µg/g. (Ahuja 1993) reported mean Se content of 0.24±0.05 µg/g in hair samples of healthy animals as compared to mean Se content of 14.30±1.31 µg/g in hair samples from animals induced Se toxicity.

4.2.1.3 Fodder

The mean Se value recorded in fodder samples in Punjab is given in the Table 5.

The mean Se values in fodder samples fed to the dairy animals from 22 districts of Punjab were 0.34±0.06 mg/kg (Amritsar), 0.29±0.03 mg/kg (Barnala), 0.33±0.05 mg/kg (Bathinda), 0.48±0.14 mg/kg (Faridkot), 0.56±0.75 mg/kg (Fatehgarh sahib), 1.62±0.15 mg/kg (Fazilka), 0.53±0.12 mg/kg (Ferozepur), 0.50±0.14mg/kg (Gurdaspur),0.46±0.12 mg/kg (Jalandhar), 3.73±0.59 mg/kg (Kapurthala), 0.39±0.05 mg/kg (Ludhiana), 2.23±0.98 mg/kg (Mansa), 0.57±0.23 mg/kg (Moga),0.24±0.23 mg/kg (Mohali),0.87±0.42 mg/kg (Muktsar), 0.60±0.19 mg/kg (Pathankot),0.07±0.10 mg/kg (Patiala),1.71±0.98 mg/kg (Ropar), 0.21±0.08 mg/kg (Sangrur), 0.70±0.25 mg/kg (Tarn Taran), 15.77±8.82 mg/kg (Nawansheher) and 6.67±1.14 mg/kg (Hoshiarpur).

Mean Se content in fodder (seasonal green fodders and wheat straw) fed to animals in 22 districts of Punjab ranged from 0.07 ± 0.10 to 15.77 ± 8.82 mg/kg. The minimum mean fodder Se concentration recorded (0.07 ± 0.10 mg/kg) was from

Patiala district, whereas, the maximum mean value recorded (15.77±8.82 mg/kg) was from villages of Nawansheher district of Punjab. Mean fodder Se contents were within normal range in twenty districts of Punjab; however they were significantly higher in Nawansheher and Hoshiarpur districts. The dairy animals from villages of Nawansheher and Hoshiarpur districts were fed fodders grown on seleniferous soils of the area. Thus, plasma and hair samples of such animals as well as fodder samples have significantly higher Se contents as compared to fodders from other districts of Punjab. Thus, affected areas/villages of Nawansheher and Hoshiarpur districts were referred as seleniferous.

Mean Se concentrations in fodders grown on non seleniferous soils of Nawansheher district (village Barwa) and Hoshiarpur districts (Village Dhamai) was 1.74±0.038 mg/kg and 1.72±0.36mg/kg, respectively.

When samples were considered on the basis of villages, the average Se content in fodder samples from Nawansheher district were in following order: Jainpur> Rakkar Dhahan> Barwa and that from Hoshiarpur district was in following order: Simbli > Najjarpur > Dhamai.

Comprehensive analysis and surveillance of all the districts done revealed Se levels in fodder samples from Kapurthala were comparatively higher and those of Patiala and Fatehgarh Sahib were lower. It was also observed that status of Se in plasma, water and fodder samples of Kapurthala district was non-significantly higher among the 20 non seleniferous districts, whereas, hair Se was significantly higher.

Selenium is not an essential element for plant growth but its concentration in fodder and grain crops is important for animals and human health. For proper nutrition of domestic animals, it is desirable to keep Se concentration in daily diet between 0.1 and 1.0 mg/kg. If the animals are exposed to fodder/roughage/straw containing more than 2-5 mg/kg Se, it may result in toxic effects (Gissel-Nielson *et al* 1984).

4.2.1.4 Ground water

The mean Se contents of water samples from of each of the 22 districts of Punjab is given in the table 5.

The mean Se concentration in water samples from villages of 22 districts of Punjab were: 1.7±0.9 µg/L (Amritsar), 32.2±2.7 µg/L (Barnala), 3.7±2.0 µg/L (Bathinda),0.7±0.3 µg/L (Faridkot), 11.3±0.9 µg/L (Fatehgarh Sahib),0.3±0.3 µg/L (Fazilka),4.3±2.4 µg/L (Ferozepur),10.0±1.1µg/L (Gurdaspur), 12.0±0.1 µg/L (Jalandhar), 24.8±8.1 µg/L (Kapurthala), 1.3±1.3 µg/L (Ludhiana), 1.0±0.6 µg/L (Mansa), 3.0±1.2 µg/L (Moga), 5.5±3.5 µg/L (Mohali), 4.7±3.7 µg/L (Muktsar), 7.3±2.6 µg/L (Pathankot), 14.8±0.6 µg/L (Patiala), 6.0±1.2 µg/L (Ropar), 7.0±3.0 µg/L (Sangrur), 16.7±6.4 µg/L (Tarn Taran), 101.64±35.58 µg/L (Nawansheher) and27.87±11.65µg/L (Hoshiarpur).

The mean Se contents recorded in water samples from 22 districts of Punjab ranged from 0.3±0.03 to 101.64±35.58 µg/L. The minimum mean water Se content (0.3±0.03 µg/L) recorded among 20 non-seleniferous regions was from Fazilka district, whereas, the maximum mean contents recorded (101.64±35.58 µg/L) was from villages of Nawansheher district of Punjab. Mean Se water contents were within normal range in 20 non- seleniferous districts of Punjab, however, they were significantly higher in other two districts Nawansheher and Hoshiarpur. Therefore, underground water samples tested from selected villages of Nawansheher and Hoshiarpur districts was regarded as seleniferous.

In the survey undertaken, samples of underground water were collected from houses being used for drinking purposes and also from farmer's fields used for irrigation purposes from Nawansheher and Hoshiarpur districts of Punjab. Selenium content of water samples from Nawansheher district ranged from 8.90 to 371.10 µg/L whereas in Hoshiarpur district ranged from 1.50 to 85.50µg/L. It was inferred that the Mean Se content was highest in water samples from Nawansheher district followed by Hoshiarpur.

Eight out of 12 water samples collected from Nawansheher district, had significantly higher mean Se concentration (146.10±46.23µg/L) whereas four samples showed mean Se concentrations within normal limits (12.72 ± 1.35µg/L). When water samples were considered on the basis of the selected villages, the average Se content was in the following order: Jainpur> Rakkar Dhahan> Barwa. It was further observed that the water samples collected from farmer's fields of Nawansheher district, which was used for irrigation purposes and where fodder fed to the animals was grown,

showed significantly higher mean Se content (162.48 ± 49.9 µg/L) as compared to water samples collected from the houses, used for drinking purposes with mean Se concentration of 16.46 ± 3.87 µg/L.

Three out of eight samples collected from Hoshiarpur district, had significantly higher mean Se concentration (65.00 ± 11.50µg/L) whereas other 5 samples showed mean Se concentrations within normal limits (5.60 ± 3.18 µg/L). When water samples were considered on the basis of selected villages in Hoshiarpur district, the average Se content was in the following order: Simbli > Najjarpur >Dhamai. Samples collected from farmer's fields of Hoshiarpur district, used for irrigation of crops, showed significantly higher Se contents as compared to the samples collected from the houses, used for drinking purposes. However, subsoil water samples collected from houses of Simbli and Dhamai villages used for drinking purposes, also had Se concentrations above the critical limits.

Results of the comprehensive analysis and surveillance of all the districts revealed that mean water Se levels of villages selected in Kapurthala and Barnala districts were significantly in higher range, whereas, those of Fazilka and Faridkot districts were in lower range, thereby, indicating that mean subsoil water Se levels of the dairy animals of Kapurthala and Barnala districts were highest among the twenty non – seleniferous districts.

Underground water constitutes the only major source of water available for irrigation and drinking purposes in Punjab. Water samples in the present study were collected from tube wells and being used by animals for drinking purpose as well as ground water from farmer's fields used for irrigation purpose, where fodder fed to the animals was grown.

Selenium content in ground water is an important criteria for determining its suitability for different purposes. The maximum permissible level of Se in water used for drinking purposes is 10µg/L and that for water used for irrigation of crops is 20µg/L (NAS-NAE, 1973).

4.3 SELENIFEROUS AND NON-SELENIFEROUS

Based on the report of results obtained, regarding Se status from 22 districts of Punjab; Nawansheher and Hoshiarpur districts, on the basis of plasma, hair, fodder and water samples had significantly higher Se contents and were referred as

seleniferous. However, in other 20 districts viz. Amritsar, Barnala, Bathinda, Faridkot, Fatehgarh Sahib, Fazilka, Ferozepur, Gurdaspur, Jalandhar, Kapurthala, Ludhiana, Mansa, Moga, Mohali, Muktsar, Pathankot, Patiala, Ropar, Sangrur and Tarn Taran, mean Se contents were within apparently normal range and were referred as non-seleniferous.

The haematological indices of apparently healthy dairy animals, referred as non selenotic from 20 districts of Punjab and clinically affected animals referred as selenotic from two known seleniferous districts, have been presented in table 6.

Table 6: Haematological and biochemical profile of non-selenotic and selenotic dairy animals (Mean±SE)

Parameters	Non-Selenotic animals (N=263)	Selenotic animals (N=23)
Hb (g/dl)	10.90±0.14	11.31±0.42
PCV (%)	31.96±0.41	29.66±1.22
TEC (x 10^6/cu mm)	6.40±0.08	6.03±0.25
TLC(x10^3/cu mm)	11.39±1.42	9.10±3.52
Neutrophils(%)	46.57±0.76	44.30±2.26
Lymphocytes(%)	52.40±0.76	54.80±2.24
Eosinophils(%)	1.22±0.12	0.86±0.34
Platelets(x 10^3/cu mm)	234.04±6.81	313.10±20.16*
GPx (Units/mg Hb)	100.06±14.05	123.94±41.60
LPO (nmol/g Hb)	606.80±52.58	993.91±155.69*

N=Number of dairy animals

* Significant at 5% level (P≤0.05)

The mean values of Hb, PCV, TEC, TLC and Platelets in selenotic dairy animals were 11.31±0.42 g/dl, 29.66±1.22 per cent, 6.03± 0.25 x 10^6/cu mm, 9.10±3.52x 10^3/cu mm and 313.10±20.16 x 10^3/ cu mm, respectively. Similarly, mean DLC recorded were 44.30 ± 2.26 percent neutrophils, 54.80 ± 2.24 percent lymphocytes, 0.86 ± 0.34 percent eosinophils. However, in healthy animals the respective mean values of Hb, PCV, TEC, TLC and Platelets were 10.90±0.14 g/dl, 31.96±1.0.41 percent, 6.40± 0.08 x 10^6/cu mm, 11.39±1.42x 10^3/ cu mm and

233.04±6.81 x 10^3/cu mm, whereas, mean DLC recorded were 46.57 ± 0.76 percent neutrophils, 52.40±0.76 percent lymphocytes and 1.22 ± 0.12 percent eosinophils. Thereby, indicating that, mean Hb, PCV, TEC and TLC in selenotic animals were within the normal range and therefore, cannot be used in diagnosis and assessment of prognosis of chronic selenosis. However, mean value of Platelets was significantly higher in affected animals as compared to healthy animals which needs further investigations. The mean values of other parameters viz. Hb, PCV, TEC and DLC in selenotic animals revealed no significant variations as compared to those of healthy animals.

Gradual decrease in PCV values was recorded during induction of selenosis by Singh (1999). Similarly, Ahuja (1993) indicated that mean PCV value was 29.06 percent in selenotic animals as compared to 31.50 percent recorded in healthy animals, which was comparable as the present study.

Erythrocytic GPx sensitivity has been linked with Se deficiency and toxicity reflective of plasma Se status. The mean GPx activity in healthy and diseased animals was 100.06±14.05 and 123.94±41.60 units/mg Hb, respectively thereby, indicating no significant difference between the two groups in the present study.

Mean erythrocytic lipid peroxidation in affected animals with significantly higher Se content in plasma as well as its activity in healthy dairy animals have been given in table 6. Significant increase in lipid peroxidation activity was seen in diseased animals (993.91±155.69 nmol/g Hb) as compared to healthy animals (606.80±52.58 nmol/g Hb). The results revealed ultimately cellular destruction in the form of lipid peroxidation.

4.3.1 Plasma minerals concentrations

The mean concentrations of minerals in the plasma of healthy dairy animals from 20 districts of Punjab and selenotic animals from two known seleniferous districts are depicted in table 7.

Mean plasma Se concentration in non selenotic animals and selenotic animals was 0.06 ± 0.01 and 0.604 ± 0.003 µg/ml respectively, thereby, indicating a significant ($P \leq 0.05$) 10 fold increase in plasma Se concentration in selenotic animals from villages of Nawansheher and Hoshiarpur district as compared to healthy non selenotic animals of non-seleniferous districts.

Ahuja (1993) reported significant higher mean value of plasma Se of 0.90 ± 0.10 µg/ml in clinical cases of chronic selenosis in bovines in selenotic areas of Punjab. However, Stevens *et al* (1985) recorded values of Plasma Se (0.317 µg/ml) and (0.275 µg/ml), respectively in cattle from Se toxic areas and cattle supplemented with toxic dose of Se (10 ppm) and were lower as compared to the present study.

Singh (1999) also observed mean plasma Se concentration of 0.40 ± 0.05 µg/ml in buffalo calves fed fodders from areas with high soil Se.

Table 7: Plasma minerals concentrations in non selenotic and selenotic dairy animals (Mean±SE)

Parameters	Plasma mineral concentrations	
	Non Selenotic animals (N=263)	Selenotic animals (N=23)
Selenium (µg/ml)	0.06±0.01	0.604±0.003*
Arsenic(µg/ml)	0.061±0.007	0.040±0.021
Sulphur (µg/ml)	729.94±13.03	757.95±38.60
Boron(µg/ml)	4.20±0.44	0.96±1.3*
Calcium (mg/dl)	9.78±0.23	9.85±0.68
Cadmium(µg/ml)	0.006±0.001	0.003±0.004
Cobalt(µg/ml)	0.004±0.00	0.007±0.001*
Chromium (µg/ml)	0.055±0.008	0.117±0.024*
Copper (µg/dl)	58.60±0.50	61.10±4.40
Iron (µg/ml)	7.338±0.767	9.476±0.2.266
Magnesium (mg/dl)	2.68±0.06	2.51±0.17
Manganese (µg/dl)	10.50±0.90	11.40±2.80
Nickel (µg/ml)	0.068±0.008	0.135±0.023*
Phosphorus (mg/dl)	8.74±0.19	8.78±0.57
Lead (µg/ml)	0.18±0.02	0.45±0.07*
Zinc(µg/dl)	168.20±11.40	145.00±33.60

N=Number of dairy animals

* Significant (P≤0.05)

Fodder and water were the primary sources of this toxicity in selenotic dairy animals. Plants and plant products constituent the primary source of Se for livestock and thus Se concentration in fodders is critical to animal health. Thus, intake of Se enriched diet/ fodder from the Se toxic soils might have led to rise in plasma Se levels in the present study. Selenium is rapidly and efficiently absorbed from naturally toxic or near toxic seleniferous diets and also from soluble salts of the element added to the normal diet. Selenium is absorbed mainly from duodenum (Wright and Bell 1966) and after absorption is carried mainly in plasma (Buescher *et al* 1960) where it is associated with plasma protein and enters all tissues, bone, hair and red blood cells (Cousins and Cairney 1961; McConnell and Levy 1962).

The mean plasma As and S concentrations in healthy animals were 0.061 ± 0.007 and 729.94±13.03 µg/ml, respectively and in selenotic animals 0.040± 0.021 and 757.95±38.60 µg/ml, respectively in selenotic animals showing non-significant difference between the two groups (Table 7).

The mean plasma concentrations of Cr, Ni and Pb in apparently healthy non selenotic animals were 0.055± 0.008, 0.068± 0.008 and 0.18±0.02 µg/ml, respectively. However in selenotic animals from seleniferous areas the respective values were 0.0117 ± 0.024, 0.135± 0.023 and 0.45± 0.07 µg/ml. The results revealed significant increase in mean plasma Cr, Ni and Pb concentrations in diseased animals as compared to healthy animals. Over two fold increase in mean plasma Pb levels recorded in the present study was comparable to the findings of Mayland *et al* (1986) who also showed synergistic relationship between Se and Pb and also indicated that Se in feed increased the absorption of Pb and subsequent toxicity in mature sheep.

Significant decreased in mean plasma B concentration was recorded in selenotic animals with mean value of 0.96±1.30 µg/ml as compared to mean value of 4.20 ± 0.44 µg/ml recorded in non-selenotic animals.

There was non–significant variation in the mean plasma values of Cd, Cu, Co, Fe, Zn, Mn, Mg, Ca and P between selenotic and non- selenotic animals.

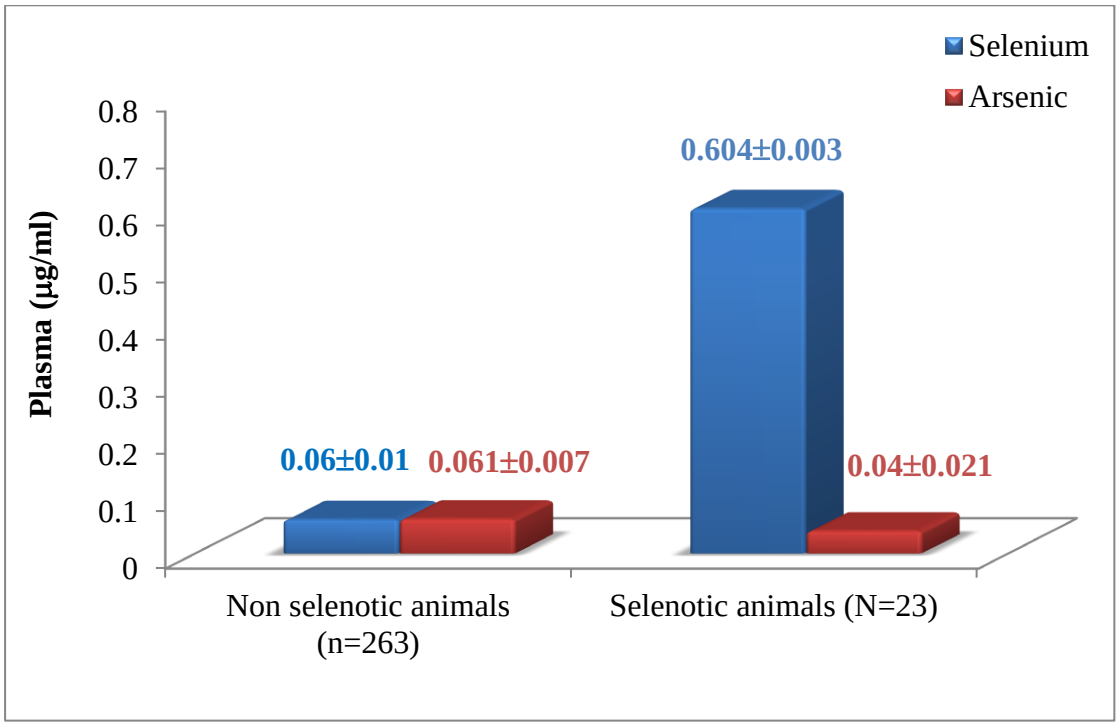

Fig. 1: Plasma Selenium and Arsenic concentrations in non-selenotic and selenotic animals (Mean±SE)

4.3.2 Hair minerals concentrations

Mean minerals concentrations in hair samples of non selenotic animals from 20 non seleniferous districts and selenotic animals from two known seleniferous districts of Punjab have been presented in table 8.

Mean Se concentrations in hair samples of healthy animals from 20 non seleniferous districts was fifteen times higher in selenotic animals from seleniferous districts as compared to healthy animals.

Significantly higher Se content of hair samples in affected animals could be ascribed to excessive accumulation of Se in animal's tissues and systems including hair, due to prolonged feeding of Se rich diets.

Table 8: Hair minerals concentrations in non-selenotic and selenotic dairy animals (Mean±SE)

Parameter(µg/g)	Non Selenotic (N=263)	Selenotic (N=23)
Selenium	1.90±0.46	30.50±6.50*
Arsenic	2.57±0.32	2.51±0.89
Sulphur	39928.31±918.73	40302.50±2504.04
Boron	19.52±0.73	16.92±2.00
Calcium	9564.52±675.74	13825.20±1841.60*
Cadmium	0.147±0.007	0.087±0.018*
Cobalt	0.55±0.04	0.39±0.12
Chromium	4.14±0.54	2.92±1.47
Copper	7.82±0.02	7.77±0.56
Iron	324.41±37.81	442.66±103.06
Magnesium	4889.75±333.50	9517.40±907.80*
Manganese	70.67±4.85	95.00±13.23
Nickel	2.26±0.16	3.18±0.46
Phosphorus	442.90±17.30	492.8±47.2
Lead	2.18±0.20	1.14±0.54
Zinc	86.48±2.07	89.37±5.65

N=Number of dairy animals

* Significant at 5% level (P≤0.05)

Rosenfeld and Beath (1964) reported that animal with high Se content contained more than 10 µg/g of Se in hair.

Dhillon *et al* (1990) recorded that mean Se in hair samples ranged from 18.7±5.9 to 38.6 ±17.7 µg/g in animals from seleniferous areas as compared to mean Se value of 0.06±0.03 µg/g recorded in hair samples of dairy animals from non-seleniferous areas of Punjab.

Randhawa *et al* (1992) reported mean hair Se content of 37.09±18.50 µg/g in dairy animals suffering from chronic Se toxicity.

Similarly, Ahuja (1993) observed mean Se content of14.30±1.31 µg/g in hair samples of selenotic animals as compared to mean Se content of 0.24±0.05µg/g recorded in hair of healthy animals. However, values in affected animals were

significantly lower than the mean values recorded in the present study. The variations could be ascribed to the Se status of the diet and duration of their access to the affected animals.

Singh (1999) recorded hair selenium content of 11.485±0.240 µg/g in animals experimentally fed with fodder high in selenium concentrations.

The mean concentrations of As and S from hair samples of healthy animals were 2.57±0.32 µg/g and 39928.31± 918.73 µg/g, respectively whereas, in selenotic animals respective values were 2.51±0.89µg/g and 40302.50±2504.04 µg/g. The result revealed that was no significant difference observed in hair As and S values between selenotic and non selenotic animals.

Mean value of hair Ca was (9564.52±675.74µg/g) in selenotic animals as compared to healthy animals (13825.20±1841.60 µg/g), similarly, hair Mg concentration increased from 4889.75 ±33.30 µg/g to 9517.40± 907.80 µg/g.

Mean hair Cd concentration in animals suffering from selenosis was 0.087± 0.018 µg/g whereas in healthy animals the concentration was 0.147±0.007µg/g. The result reflected that significant decreased in mean concentration was observed in selenotic animals as compared to healthy animals.

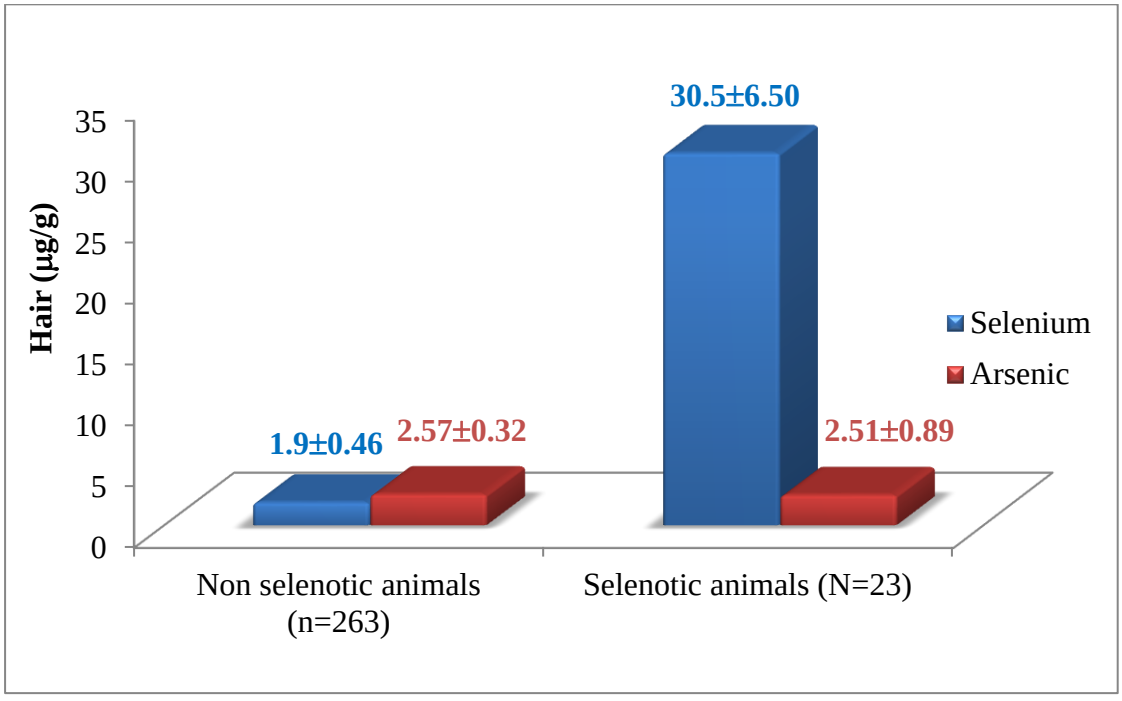

Fig. 2: Hair Selenium and Arsenic concentrations in non-selenotic and selenotic animals (Mean±SE)

4.3.3 Fodder minerals concentrations

Requirements of minerals, in the dairy animals are highly dependent on the levels of their productivity. The minerals are drained in milk and required for other physiological activity. Thus, an adequate intake through feed by the dairy animals is an important criterion in meeting the mineral requirement. With the introduction of multicut forage varieties, the soil is getting depleted in mineral elements and as a result the concentrations of minerals are decreasing in fodder crops and on the other hand, accumulating especially in soils, with excessive toxic concentrations of certain minerals.

Mean concentrations of macro and micro minerals in fodder crops and wheat straw from seleniferous and non-seleniferous areas have been presented in the table 9.

Mean concentration of Se of fodder crop grown in non-seleniferous and seleniferous areas was 0.89 ± 0.27 and 11.22 ± 4.55 mg/kg, respectively thereby, indicating that the mean value was 13 times higher in fodder crops grown in seleniferous areas of Nawansheher and Hoshiarpur districts as compared to that of non-seleniferous areas.

Significantly, higher content of Se in fodder crops of seleniferous areas has been reported by Dhillon *et al* (1991) which was ascribed to high level of Se in soils of those areas.

In the present study, the mean S content of fodder crops of in non seleniferous and seleniferous areas were 2040.12 ± 188.42 and 1251.36 ± 122.91 mg/kg respectively, which was significantly lower in fodder crops grown in seleniferous areas as compared to non seleniferous areas.

The average As concentration of fodder crops grown in non seleniferous and seleniferous areas was 1.69 ± 0.49 and 0.39 ± 0.32 mg/kg respectively. The result reflected that mean As concentration was significantly lower in fodder crops grown in seleniferous areas as compared to non seleniferous areas.

Table 9: Fodder minerals concentrations from non-seleniferous and seleniferous areas (Mean±SE)

Parameter (mg/kg)	Non Seleniferous(N=74)	Seleniferous(N=22)
Selenium	0.89±0.27	11.22±4.55*
Arsenic	1.69 ±0.49	0.39 0.32*
Sulphur	2048.12 ±188.42	1251.36 ±122.91*
Boron	8.99±1.47	7.46±0.96
Calcium	339.81±30.31	230.78±19.77*
Cadmium	0.128±0.014	0.077±0.009*
Chromium	6.93±0.77	5.18±0.50
Copper	1167.30±92.10	1645.70±60.10*
Iron	571.48±84.70	194.38±52.25*
Magnesium	163.16±15.88	57.29±10.30*
Manganese	3117.70±427.90	2233.10±279.10
Nickel	3.57±0.35	1.80±0.23*
Phosphorus	198.05±21.12	125.20±13.77*
Lead	2.55±0.50	4.19±0.328
Zinc	2805.90±273.60	1479.60±178.50*

N=Number of dairy animals

* Significant at 5% level (P≤0.05)

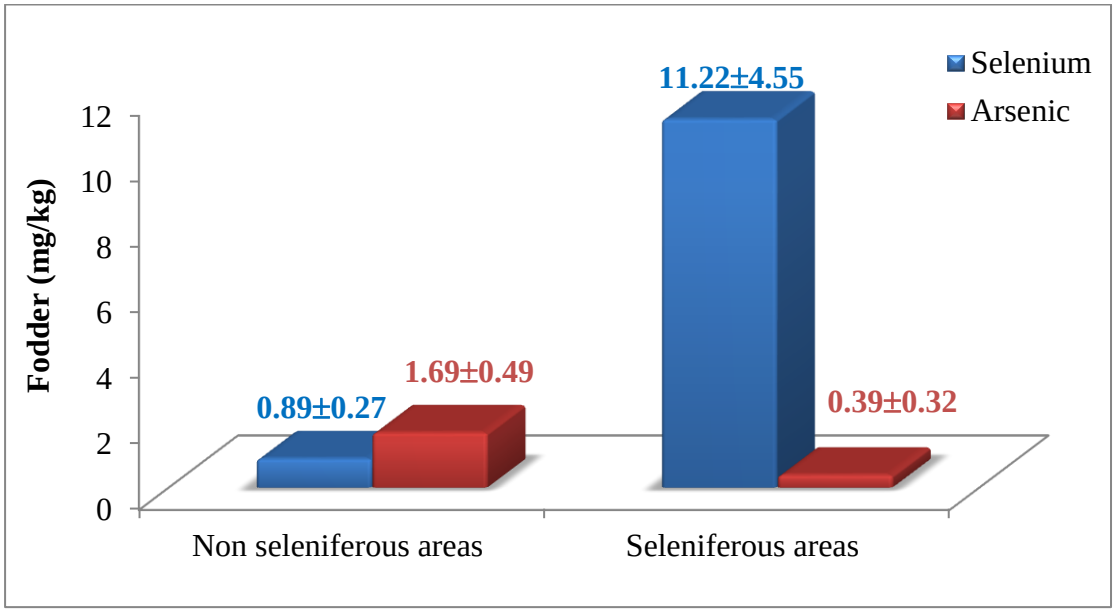

Fig. 3: Fodder Selenium and Arsenic concentrations in seleniferous and non seleniferous areas (Mean±SE)

4.3.4 Water minerals concentrations

The mean values of Se and other minerals concentrations in water samples from seleniferous and non seleniferous areas have been presented in the table 10.

The mean value of Se content in water samples from non seleniferous and seleniferous areas was 9.43 ± 6.10 and 64.75 ± 36.88 µg/L, respectively thereby, indicating significantly 7 times higher Se concentration in water samples from seleniferous areas as compared to that from non seleniferous areas.

Similarly, average S content of water in non seleniferous and seleniferous areas was 30852.12 ± 5032.52 and 2511.11 ± 901.80 µg/L, respectively which was significantly lower (over 10 times) in water from seleniferous areas as compared to non seleniferous areas.

The mean water Pb content in non seleniferous and seleniferous areas was 16.07 ± 2.17 and 49.53 ± 4.26 mg/L, respectively, which was significantly higher in water samples that from seleniferous areas as compared to non seleniferous areas.

The average As content in water from non seleniferous and seleniferous areas was 5.88 ± 1.12 and 2.02 ± 2.00 mg/L, respectively.

Table10: Water minerals concentrations from seleniferous and non seleniferous areas (Mean±SE)

Parameter	Non Seleniferous (N=64)	Seleniferous (N=25)
Selenium	9.43±6.10	64.75±36.88
Arsenic	5.88±1.12	2.02±2.00
Sulphur	30852.12± 5032.45	2336.31±338.62*
Boron	354.85±73.80	72.84±13.10
Calcium	42652.10±864.50	23351.10±1546.44
Chromium	16.81±2.54	26.59±3.11*
Iron	14.34±9.77	2.75±15.12
Manganese	16.40±12.7	36.41±21.15
Phosphorus	21.28±11.71	15.31±19.54
Lead	16.07±2.17	49.53±4.26*
Zinc	32.29±12.10	38.05±22.48

N=Number of dairy animals
* Significant at 5% level (P≤0.05)

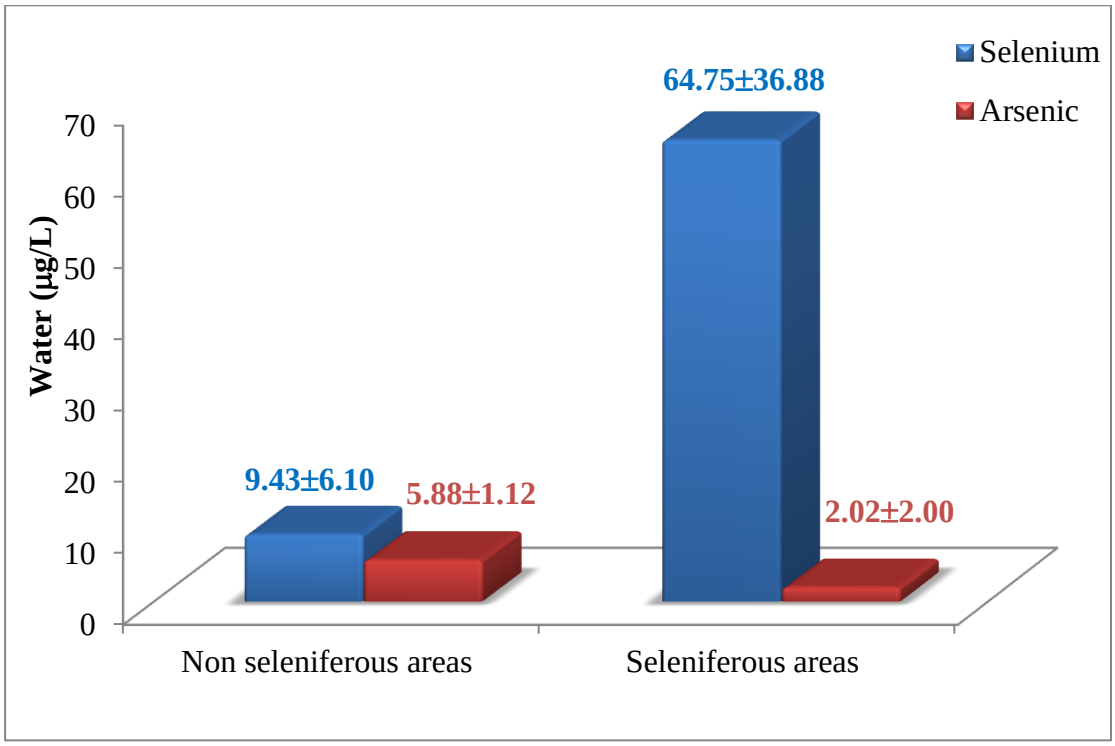

Fig. 4: Water Selenium and Arsenic concentrations in seleiferous and non-seleniferous areas (Mean±SE)

On the basis of comprehensive analysis of plasma, hair, fodder and water Se of 20 non selenotic districts, it was confirmed that mean Se levels were within normal range. Dairy animals of these districts served as healthy control as none of the animals revealed signs associated with deficiencies of Se.

4.4 CLINICAL MANIFESTATION OF SELENOTIC DAIRY ANIMALS AND ROLE OF PENTASULPHATES IN THEIR TREATMENT

4.4.1 Clinical Manifestation

Chronic Selenium toxicity referred as selenosis earlier in certain villages of Nawansheher and Hoshiarpur districts of Punjab Dhillon and Dhillon (1991). Significantly high Se concentration in ground water of Nawansheher district and also in certain belts of soils of Jainpur and Barwa villages of Nawansheher districts had been reported by Dhillon (1992), Gupta *et al* (1982) and Bajaj *et al* (2011). Crops growing on these Se enriched soils have significantly high Se contents. Se toxicity occurred in a localized pattern; this variation being produced through leaching or transportation of previously formed seleniferous deposits. Animals being fed fodder crops growing on soils having high Se content intend to suffer from selenosis.

A comprehensive baseline survey was conducted in various blocks and villages of Nawansheher and Hoshiarpur districts as depicted in the table 11. It was observed that dairy animals in studied areas were raised on green fodders and cereal straws grown on seleniferous soils and their ingestion for prolong period lead to the development of clinical manifestation of Se toxicity. During the survey animals showing clinical signs of selenosis in the form of slow but progressive emaciation, poor health (Fig. 10), unthrifty appearance, overgrown hooves with visible horizontal cracks (Figs. 5, 7 and 8), associated with cracking and even loss of horn core followed by avulsion of horn corium, alopecia with generalized/ patchy loss of hair from the body, loss of hair from the switch of the tail (Fig.6). Inappetance and decreased productivity along with poor reproductive health. overgrowing, soft and brittle hooves and hair loss was also seen. Similar findings were reported in dairy animals suffering from chronic selenosis by Dhillon *et al* (1990). Kaur *et al* (2005) observed manifestations of chronic selenosis, as rough hair coat, alopecia, swelling of the coronet, enlargement of the hooves, interdigital lesions and gangrene of the tip of the tail. Similarly, Tiwari *et al* (2006) also recorded selenium poisoning based upon weight loss, poor growth rates, lameness, defective hoof growth, horizontal ridges or cracks in the hoof wall, hair loss and infertility. In the present study complaints of anoestrus, abortions and delayed onset of estrus were also recorded in dairy animals which are comparable to the findings of Gupta *et al* (1982), Dhillon and Dhillon (1991) and Dhillon *et al* (1992).

Abnormality of hooves and horn could be due to inefficient keratin production due to Se antagonism with thio-amino acid, which is important constituent of keratin.

The appearance of above symptoms could be ascribed to prolonged feeding of Se enriched forages and straw grown on seleniferous soil and related to impaired digestion associated with interference in absorption. Signs of lameness were also observed there was reluctance to move in severe cases along with arching of back and difficulty to get up.

In dairy animals decline in production and reproduction, alopecia especially involving tip of the tail leading to gangrene followed by sloughing has been earlier

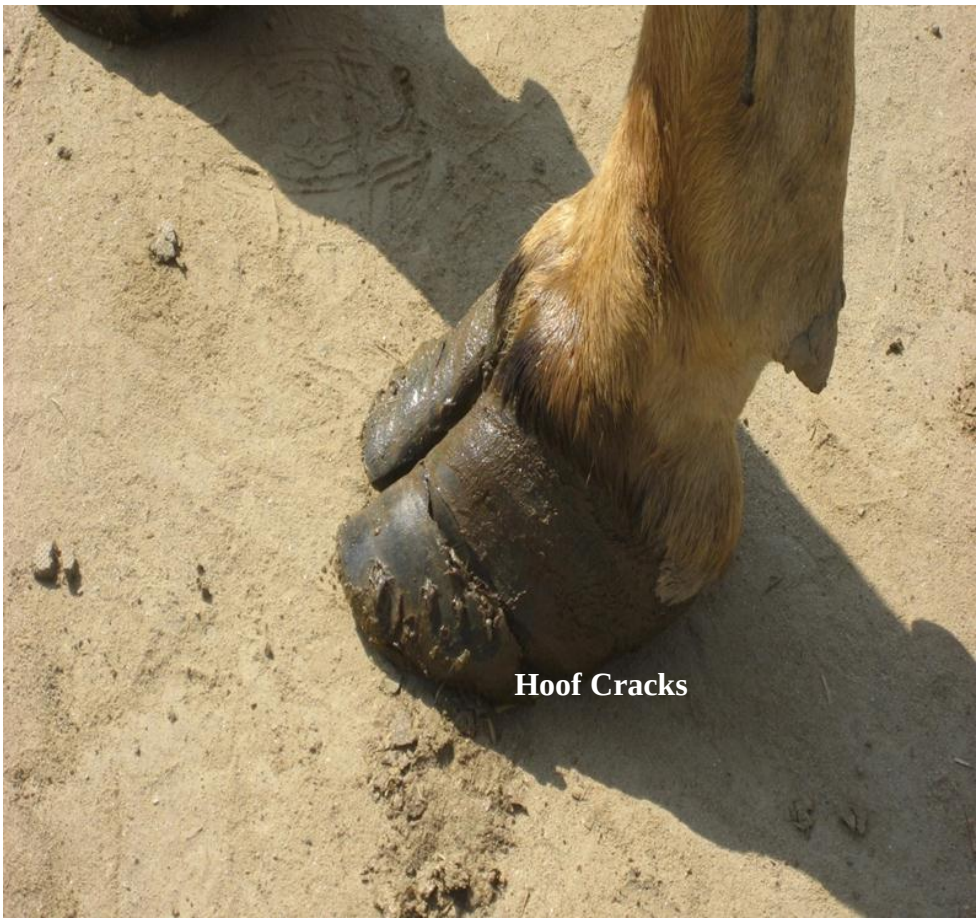

Fig. 5: Animal showing clinical signs of deep hoof cracks in chronic selenosis

Fig. 6: Selenotic animal with alopecia of tail

Fig. 7: Overgrowth of hoof in adult buffalo

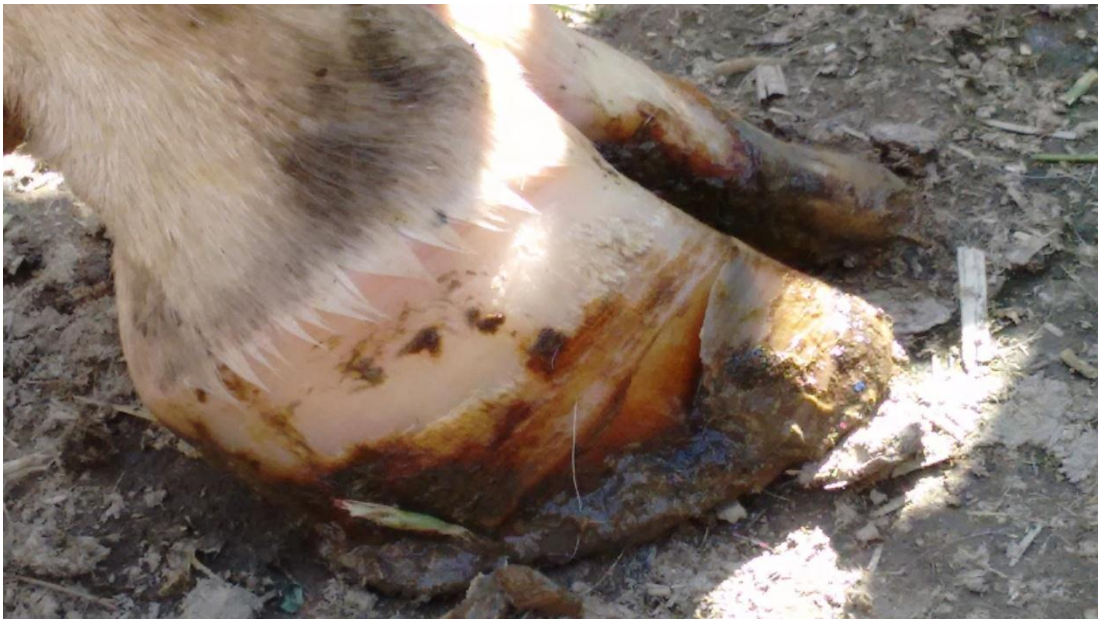

Fig. 8: Horizontal cracks in a hoof of crossbred cow

Fig. 9: Selenotic buffalo showing alopecia on head region with cracks on horns

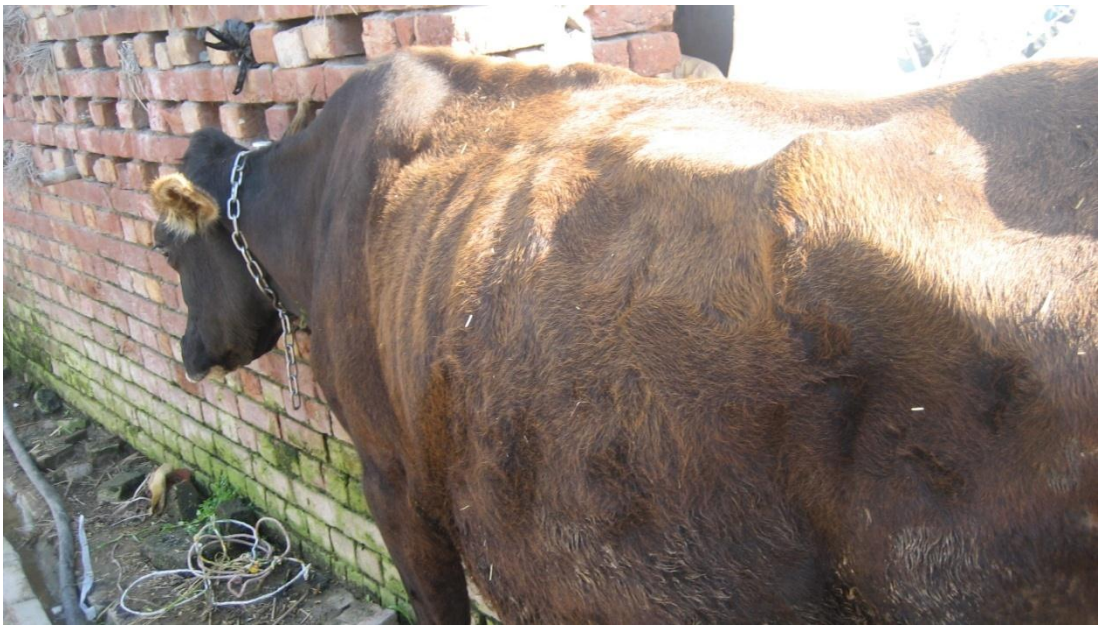

Fig. 10: Crossbred cow showing emaciation, loss of body condition with achromotrichia and rough starry coat

Fig. 11: Buffalo showing avulsion of left horn and alopecia of neck region

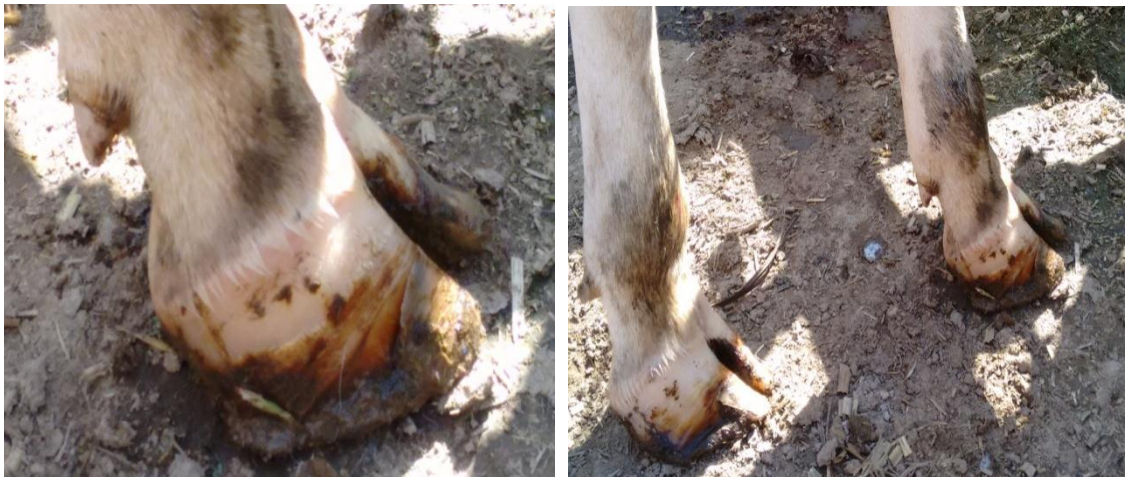

Fig. 12: Cattle with cracked hooves with coronet inflammation

recorded (Dhillon *et al* 1990). The signs were associated with anoestrous and delayed onset of oestrous, failure of conception and abortions (Gupta *et al* 1982; Dhillon and Dhillon 1991; Ahuja 1993). Chronic selenosis also caused impairment of hepatic and renal functions (Blood and Radostits, 1989; Randhawa *et al* 1992). The changes and clinical signs observed in chronic selenosis in dairy animals of Punjab were more severe due to the feeding of selenium rich diets resulting in death of affected animals within 4-5 months and thus caused extensive economic losses. However, over the period of time recommendations by Dhillon and Dhillon (2009) were adopted by the farmers like use of gypsum to soils which reduce the absorption of Se in plants, use of organic manure also reducing Se toxicity, changing the cropping sequence with recommendation of crops with less uptake of Se and increasing the depth of water (deep boring) in the affected areas as well as avoiding the feeding of crops grown on seleniferous soils markedly reduced the uptake of Se by crops raised on seleniferous soils, It was considered that adoption of these recommendations might have led to decreased incidence of clinical cases and severity of signs thus reducing the effect of chronic selenosis on animal system.

Thirty dairy animals diagnosed on basis of clinical symptoms, were treated with oral administration of Degcure mixture given @ 30 g/day /animal for 45 days. Degcure mixture consisted of five sulphates viz. 1 kg magnesium sulphate, 166 g ferrous sulphate, 24 g copper sulphate, 75 g zinc sulphate and 1.5 g cobalt sulphate. Ganther (1971) evaluated the role of SH group in the non enzymic incorporation into protein and found that Se was incorporated into the protein at disulphide bridge to form seleno- trisulfide at low pH, whereas, at pH 7, the reaction was accompanied by liberation of element Se. Sulphates lead to indirect excretion of Se in urine and faeces and therefore important in elimination of chronic selenosis (Levander and Morris 1970).

Follow up of the treatment was undertaken in 17 out of 30 Cases. Three animals were reported dead due to other causes; five were sold by the owner; five showed reluctance to eat the prescribed pentasulphates mixture and thus, did not complete the treatment. Blood and hair samples were collected again from the animals for evaluating the efficacy of pentasulphates in treatment of chronic selenosis in dairy animals.

Table 11: Seleniferous areas identified with clinical cases of selenosis in dairy animals

District	Block	Village
Hoshiarpur	Garhshankar	Simbli, Najjarpur, Dhamai
Nawansheher	Saroya	Jainpur, Mehindpur, Rakkar Dhahan
	Nawansheher	Barwa
	Balachaur	Rurki khurd, Phirni Majra, Karawar

4.4.2 Haematology

The haematological indices of diseased animals before and after undertaking therapeutic measures have been presented in table 12.

Mean values of haematological indices in selenotic dairy animals before undertaking therapeutic measures were (pre-treatment; 0 day) were 11.32±0.55 g/dl (Hb), 29.71±1.33 percent (PCV), 5.91±0.23 x 10^6 cells/cu mm (TEC), 9.11±0.66 x10^3cells/cu mm (TLC), 47.52±2.93 percent (neutrophils), 51.17±3.12 percent (lymphocytes), 0.94±0.42 percent (eosinophils) and 341.88±34.23 x10^3/cu mm (platelets) respectively.

After undertaking therapeutic measures for 45 days respective post treatment mean values were 11.06±0.75g/dl (Hb), 31.40±1.42 percent (PCV), 6.46±0.25 x10^6cells/cu mm (TEC), 15.80±4.11 x10^3cells/cu mm (TLC), 41.88±3.25 percent (neutrophils), 56.82±3.44 (lymphocytes), 1.29±0.51percent (eosinophils) and 226.82±27.96 x 10^3/cu mm(platelets)respectively. Thus, comparative studies of haematological indices (Hb, PCV, TLC and DLC) before and after instituting therapeutic measures revealed non-significant variations, however, mean TEC count showed significant increase and mean platelet count decreased significantly after 45 days of therapeutic trial. The dairy animals of non-seleniferous areas served as healthy controls.

Ahuja (1993) reported lower mean values of 8.96 ± 0.94 g/dl (Hb), 31.37 ± 2.50 percent (PCV), 4.69 ± 0.38 x 10^6/ cu mm (TEC) and 5.89 ± 0.37 x 10^3 / cu mm (TLC) in diseased animals. Dhillon *et al* (1992), Ghosh *et al* (1993) also observed significant decline in mean Hb, PCV and TEC but no change in TLC values in cattle and buffaloes thriving on fodder raised on seleniferous soils for more than a year. However, in the present study mean Hb, TEC and TLC values were higher non-significantly.

Table 12: Haematological indices of dairy animals (cattle and buffalo N=17) before and after therapeutic trial (Mean ± SE)

Parameter	Non-Selenotic animals	Pre-Treatment	Post-Treatment
Hb (g/dl)	10.90±0.14	11.32±0.55	11.06±0.75
PCV (%)	31.96±0.41	29.71±1.33	31.40±1.42
TEC (x 10^3 / cu mm)	6.40±0.08	5.91±0.23	6.46±0.25
TLC (x10^3 / cu mm)	17.93±1.19	9.11±0.66	15.80±4.11
Neutrophils (%)	46.57±0.76	47.52±2.93	41.88±3.25
Lymphocyte (%)	52.40±0.76	51.17±3.12	56.82±3.44
Eosinophil (%)	1.22±0.12	0.94±0.42	1.29±0.51
Platelet (x10^3 / cu mm)	234.04±6.81	341.88±34.23	226.82±27.96

* Significant at 5 percent level (P≤0.05)

4.4.3 Blood/Serum Biochemical Profile:

The results of blood/serum biochemical analysis of diseased animals before and after undertaking therapeutic measures have been presented in table 13.

The pre-treatment mean values of serum AST, ALT, Plasma Urea Nitrogen, Creatinine, TP and GGT in diseased animals were 101.58±11.45 U/L, 177.58±32.07 U/L, 15.82±1.66 mg/dl, 1.84±0.17 mg/dl, 9.27±0.43 g/dl and 35.52±3.51 U/L respectively.

Table 13: Biochemical profile of dairy animals before and after therapeutic trial (Mean±SE)

Enzymes	Healthy Control group (n=10)	Pre-Treatment	Post-Treatment
GPx (U/mg Hb)	110.09±19.28	108.24 ± 21.68	1182.45 ± 121.95
LPO (nmol/gHb)	657.68±75.59	1051.86 ± 256.16	480.07 ± 94.99*
AST (U/L)	182±22.05	101.58 ± 11.45	95.23 ± 9.96
ALT (U/L)	144±42.50	177.58 ± 32.07	214.23 ± 17.33
BUN (mg/dl)	20.18±1.55	15.82 ± 1.66	23.412±2.633
Creatinine (mg/dl)	1.6±0.32	1.84 ± 0.17	1.72 ± 0.09
GGT (U/L)	30.45±2.90	35.52 ± 3.51	35.41 ± 3.41
Total Protein (g/dl)	8.2±1.24	9.27 ± 0.43	10.29 ± 1.02

* Significant at 5 percent level (P≤0.05)

After undertaking therapeutic measures respective post-treatment values were 95.23±9.96 U/L, 214.23±17.33 U/L, 23.41± 2.63 mg/dl, 1.72± 0.09 mg/dl and 10.29 ± 1.02 g/dl and 35.41±3.41 mg/dl respectively.

The results revealed that blood biochemical profile was unaffected by treatment.

Randhawa *et al* (1992) reported comparable Plasma Urea Nitrogen levels in cattle and buffaloes, but significantly decreased levels of TPP (5.89 ± 0.30 mg/dl).

Ahuja (1993) observed significant increase in mean plasma ALP, Plasma Urea Nitrogen and significant decrease in TPP in selenotic bovines. However, non-significant fluctuations were recorded in mean plasma AST and ALT activities in selenotic bovines.

Fraser *et al* (1997) indicated that chronic selenosis caused to increase in ALP, ALT and AST activities.

The mean value of GPx activity (Glutathione peroxidase), before and after therapeutic measures was 108.24±21.68 and 1182.45±121.95 U/mg Hb, respectively thereby, indicating significantly higher GPx activity ($P<0.001$) after undertaking therapeutic measures. The significant increase in erythrocytic glutathione peroxidase activity could be due to the mobilisation of Se and due to neutralization effect of Se by pentasulphates.

The mean value of LPO (Lipid peroxidase) before and after therapeutic measures was 1051.86±256.16 and 480.07± 94.99 nmol/ g Hb, respectively, thereby, indicating significant decrease in LPO activity after undertaking therapeutic measures. The decrease in LPO activity could be due to the effect of pentasulphates.

4.4.4 Plasma minerals concentrations

The mean concentrations of minerals in plasma of the selenotic animals before and after instituting therapeutic measures have been presented in table 14.

The mean concentrations of plasma Se, As and S in selenotic animals pre-treatment were 0.64±0.10 µg/ml, 0.03±0.006 µg/ml, 764.14±35.92 µg/ml and post-treatment were0.78±0.13 µg/ml, 0.06±0.01 µg/ml, 801.80± 42.05 µg/ ml respectively, thereby, indicating no significant differences in the respective mean values after undertaking therapeutic trial. There was significant increase in plasma mineral concentrations of diseased animals as compared to the non-selenotic animals

from 20 districts of Punjab and these animals also served as healthy control group in the present study.

Table 14: Plasma minerals concentrations of selenotic dairy animals before and after treatment (Mean±SE)

Parameters	Non Selenotic animals (N=263)	Pre-treatment	Post-treatment
Selenium (µg/ml)	0.06±0.01	0.64±0.11	0.78±0.13
Arsenic (µg/ml)	0.061±0.007	0.03±0.01	0.06±0.03
Sulphur (µg/ml)	729.94±13.03	764.14±35.92	801.80±42.05
Boron (µg/ml)	4.20±0.44	0.99±0.11	0.95±0.08
Calcium (mg/dl)	9.78±0.23	9.50±0.54	9.86±0.56
Cobalt (µg/ml)	0.004±0.00	0.008±0.002	0.01±0.003
Chromium (µg/ml)	0.055±0.008	0.16±0.11	0.07±0.01
Copper (µg/dl)	58.60±0.50	58.43±5.41	60.71±4.10
Iron (µg/ml)	7.338±0.767	13.05±5.28	15.16±5.91
Magnesium (mg/dl)	2.68±0.06	2.49±0.14	2.42±0.18
Manganese (µg/dl)	10.50±0.90	13.25±3.10	12.58±3.66
Nickel (µg/ml)	0.068±0.008	0.16±0.09	0.10±0.01
Phosphorus (mg/dl)	8.74±0.19	7.70±0.46	8.43±0.58
Lead (µg/ml)	0.18±0.02	0.32±0.08	0.80±0.02*
Zinc (µg/dl)	168.20±11.40	152.25±16.44	129.50±12.55

* Significant at 5 percent level ($P \leq 0.05$)

In the present study mild increase in mean plasma Se content has been observed in dairy animals after treatment with pentasulphates. Intake of Se enriched diet/ fodder from the Se toxic soils might have led to rise in plasma Se levels in the present study. Selenium is rapidly and efficiently absorbed from naturally toxic or near toxic seleniferous diets and also from soluble salts of the element added to the normal diet. Selenium is absorbed mainly from duodenum (Wright and Bell 1966) and after absorption, is carried mainly in plasma (Buescher *et al* 1960), where it is associated with plasma proteins and enters all tissues, bone, hair and red blood cells (Cousins and Cairney 1961, McConnell and Levi 1962).

The principle source of Se to affected dairy animals was dietary constituents - green forages and wheat straw. Feeding these forages grown on seleniferous soils for

prolonged period throughout the year might have accounted for the appearance of the clinical signs. In general, occurrence was not related to age, sex and species. It appears that mobilization of Se from various tissues especially liver, hair, hooves and horns might have led to high plasma Se even after treatment.

Arsenic is a specific antidote of Se toxicity. It has been reported that toxic effects of Se in animals can be counteracted by Arsenic compounds. Rising plasma Se concentrations increased the excretion of As through urinary and hepatic system, due to competitive antagonism between As and Se.

Although the interaction between Se and S is well known but only nonsignificant increase in S concentration was observed during post treatment with pentasulphates. This could be due to mobilization of S from liver which masked the effect of Se on plasma S concentration. The mean value of plasma Lead (Pb), before and after therapeutic measures, was 0.33±0.09and 0.81±0.02 µg/g, respectively, thereby, indicating significant higher Pb values ($P<0.001$) after undertaking therapeutic measures. The 3 fold increase could be due to the synergistic relationship between Se and Pb. Also, comparable to the findings of Mayland *et al* (1986), it was found that high levels of Se in feed might have increased absorption of lead.

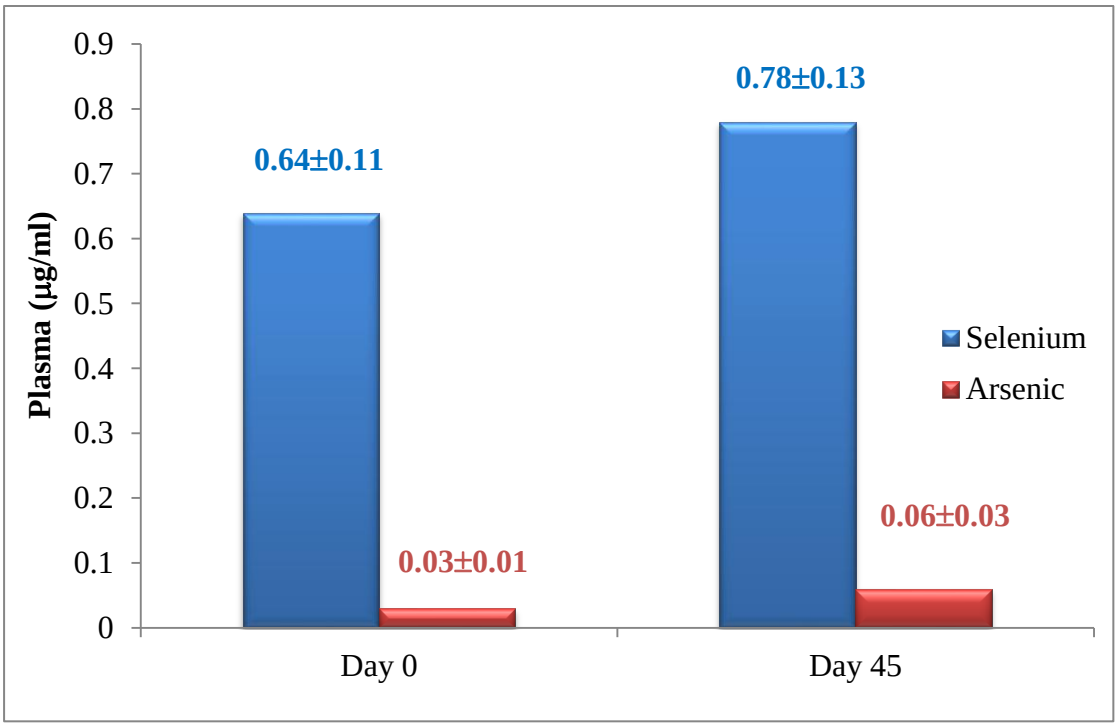

Fig. 13: Plasma Selenium and Arsenic concentrations before and after treatment in selenotic animals (Mean±SE)

4.4.5 Hair minerals concentrations

Concentration of minerals in the hair of the selenotic animals, before and after instituting therapeutic measures, have been presented in table 15

The mean concentrations of hair Se, As and S in selenotic animals pre-treatment were 34.07± 5.02, 2.32± 0.50 and 52106.06±4428.80 µg/g, respectively. 45 days of after treatment (post-treatment) were 24.36± 3.27, 1.63± 0.27, 29443.93 ± 2370.42 µg/g, respectively. The results reflected non-significant decrease in mean Se content of hair post-treatment whereas, mean S decreased significantly after 45th days of therapeutic trial. Although there was significant increase in hair mineral concentrations of diseased animals as compared to the non-selenotic animals from 20 districts of Punjab and these animals also served as healthy control group in the present study.

Table 15: Concentration of minerals in hair samples of selenotic dairy animals before and after treatment (Mean±SE)

Minerals(µg/g)	Non Selenotic (N=263)	Pre-treatment	Post-treatment
Selenium	1.90±0.46	34.07±5.020*	24.36±3.27
Arsenic	2.57±0.32	2.32±0.51	1.63±0.27
Sulphur	39928.31±918.73	52106.06±4428.80*	29443.93±2370.42
Boron	19.52±0.73	19.07±2.06*	12.38±2.69
Calcium	9564.52±675.74	15722.235±1870.44*	5928.55±957.33
Cadmium	0.147±0.007	0.09±0.02	0.20±0.01*
Cobalt	0.55±0.04	0.32±0.03*	0.24±0.04
Chromium	4.14±0.54	3.07±0.16*	1.22±0.22
Copper	7.82±0.02	10.23±0.92*	8.07±0.64
Iron	324.41±37.81	369.70±40.20*	137.49±23.34
Magnesium	4889.75±333.50	11319.37±2058.57*	2896.98±575.30
Manganese	70.67±4.85	94.27±15.68*	24.82±4.69
Nickel	2.26±0.16	3.12±0.63*	0.86±0.072
Phosphorous	442.90±17.30	583.43±52.40*	296.51±47.64
Lead	2.18±0.20	1.29±0.30	1.94±0.11
Zinc	86.48±2.07	102.34±8.71*	72.17±5.84

* Significant at 5 percent level (P≤0.05)

. Mineral status of hair provides information of nutritional status of the animal. Decrease in mean Se and S concentrations are observed and could be ascribed to excessive accumulation of Se the cutaneous system especially hair, due to prolonged feeding of Se rich diets following action of pentasulphates there was mobilisation of S and Se these minerals from the keratin tissues into the plasma and subsequent elimination from the animal's system. It was concluded that, mineral analysis of hair might be considered as important diagnostic index of chronic Selenosis in dairy animals.

The mean concentration of Pb in hair samples showed non-significant increase from pretreatment value of 1.290 ± 0.30 to 1.94 ± 0.11 µg/g recorded after undergoing therapeutic measures, respectively.

The mean concentration of B, Ca , Co, Cr, Cu, Fe, Mg, Mn, Ni, P and Zn in hair samples before undertaking treatment in selenotic animals were 19.07 ± 2.06, 15722.23 ± 1870.44, 0.32 ± 0.03, 3.07 ± 0.16, 10.23 ± 0.92, 369.70 ± 40.20, 11319.37 ± 2058.57, 94.27 ± 15.68, 3.12 ± 0.63, 583.43 ± 52.40 and 102.34 ± 8.71 µg/g and 12.38 ± 2.69, 5928.55 ±957.33, 0.24 ± 0.04, 1.22 ± 0.22, 8.07 ± 0.64, 137.49 ±23.34, 2896.98 ± 575.30, 24.82 ± 4.69, 0.86 ± 0.07, 296.51± 47.64 and 72.17 ± 5.84µg/g, respectively thereby, indicating significant decrease in mineral concentrations in diseased animals after therapeutic measures.

The mean concentrations of Cd in hair samples post-treatment increased significantly to 0.20 ± 0.01 from 0.09 ± 0.02 µg/g in selenotic animals.

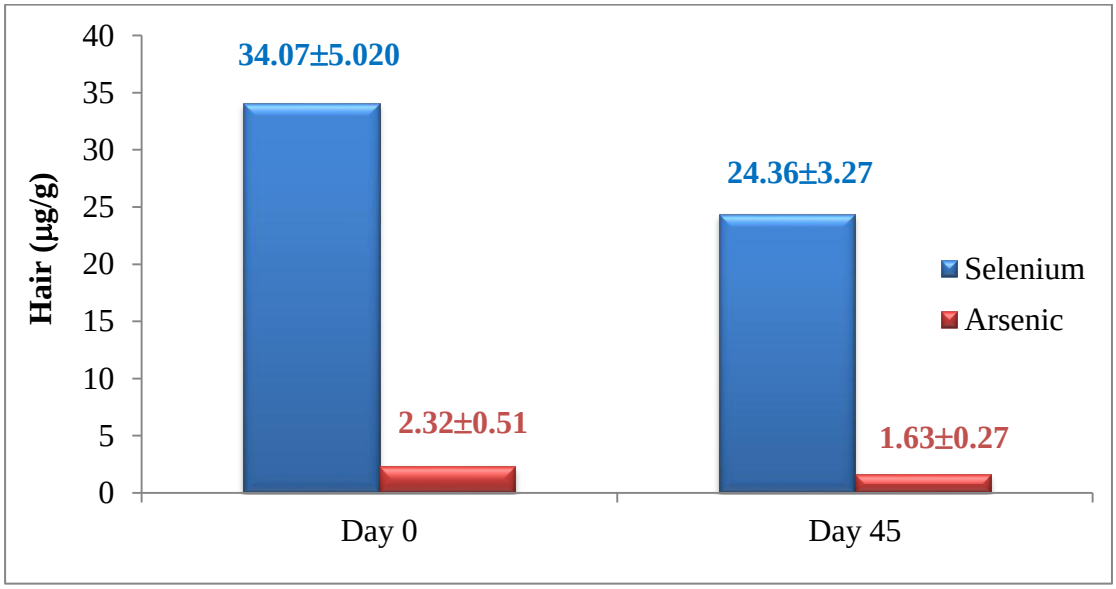

Fig. 14: Hair Selenium and Arsenic concentrations before and after treatment in selenotic animals (Mean±SE)

4.4.7 Water minerals concentrations

The mean concentrations of Se and other minerals in water samples from seleniferous areas, which were used for drinking purposes by the diseased animals is given in table 16.

The mean Se concentration in water from seleniferous areas, which was used for drinking purposes by the diseased animals and also used for irrigation of crops was 86.43±29.53 µg/L.

The mean S content in water samples from seleniferous areas which were being used for drinking purposes by the diseased animals was 2336.31 ± 338.62 µg/L.

Table 16: Concentrations of minerals in water samples from seleniferous areas (Mean±SE)

Minerals (µg/L)	Water mineral concentrations (N=15)
Selenium	86.43±29.53
Arsenic	2.02 ±0.28
Sulphur	2336.31±338.62
Boron	71.85 ±5.20
Calcium	23350.84 ±4090.21
Chromium	26.00 ±2.57
Copper	1.68 ±0.58
Iron	1.73 ±0.27
Magnesium	18791.65 ±885.39
Manganese	36.42 ±34.01
Nickel	1.07 ±0.10
Phosphorous	15.31 ±1.69
Lead	48.53 ±6.00
Zinc	38.05 ±10.92

In the present investigation total number of 15 water samples from seleniferous areas of Nawansheher and adjoining areas of Hoshiarpur districts were collected and analysed for Se. Nine of the samples were from farmer's house where the animals were kept and the same water was used by animals for drinking purpose. Six water samples were from the fields were the fodder crops fed to the animals were

grown. The mean Se content in water samples collected from houses was 23.00 ± 6.76 µg/L. Water samples from Six household out of the nine were having Se content in normal range whereas, two samples from Hoshiarpur district villages Dhamai and Simbli had Se content higher than the critical level.

Six samples out of the 15 were collected from farmers' fields of Nawansheher district (villages Rakkar Dhahan, Jainpur and Barwa), where fodder fed to the animals was grown. All sub-soil water samples of the area had Se concentration higher than the critical levels with mean Se concentration being 181.58±54.56 µg/L.

4.4.8 Fodder minerals concentrations

The mean concentration of Se and other minerals in fodder crops and wheat straw from seleniferous areas, which were being fed to the diseased animals, are given in the table 17.

Table 17: Concentrations of minerals in fodder samples from seleniferous areas (Mean±SE)

Mineral(mg/kg)	Fodder concentrations (N= 16)
Selenium	13.95±7.19
Arsenic	0.39±0.05
Sulphur	1251.36±122.91
Boron	7.46±1.05
Calcium	2307.82±196.51
Cadmium	0.077±0.003
Chromium	5.18±0.44
Copper	16.45±0.56
Iron	194.38±16.18
Magnesium	572.97±24.01
Manganese	22.33±2.53
Nickel	1.80±0.16
Phosphorous	1252.03±132.83
Lead	4.19±0.38
Zinc	14.79±1.46

The mean Se content in fodder crops and wheat straw from seleniferous areas, which were being fed to the diseased animals was 13.95 ± 7.19 mg/kg.

The mean S content in fodder crops and wheat straw from seleniferous areas, which were being fed to the diseased animals was 1251.364±120.521mg/kg.

Ahuja (1993) reported fodder crops grown on seleniferous soils had mean Se content of 21.70 ± 4.53 mg/kg. The average Sulphur content in fodder grown on seleniferous areas was 0.57 ± 0.06 percent.

23 dietary samples (green fodder and wheat straw) were collected. Sixteen fodder samples being fed to the animals collected at zero day had mean Se content of 13.95 ± 7.19 mg/kg. Due to prolonged feeding of fodder and straw with high Se content grown on seleniferous soils of the area dairy animals developed signs of chronic selenosis and had significantly high plasma and hair Se content. On comprehensive survey and interaction with dairy farmer's it was discovered that a few owners have discontinued the feeding of the seleniferous fodder and instead purchased fodder being grown away from the periphery of affected villages. The mean Se concentration of 7 samples of such non seleniferous fodder was 1.83 ± 0.30 mg/kg.

During the survey plasma, hair, water and fodder samples were also collected from Rurki khurd, Phirni Majra and Karawar villages of Balachaur block. Animals did not exhibit any signs of Se toxicity in these villages and samples were analysed to study the present status of Se in the areas. Mean Se plasma, hair, water and fodder concentrations recorded were 0.095 ± 0.005 µg/ml, 3.15 ± 1.08 µg/g, 4.30 ± 1.18µg/L and 0.64 ± 0.10 mg/kg respectively. Thereby, indicating that mean Se concentrations in plasma, hair, water and fodder samples of Balachaur block of Nawansheher were well within the normal range and thus, the areas surveyed in the present study had no incidence of selenosis prevailing in the animals.

4.4.9 Selenium toxicity symptoms in different crops in seleniferous areas

During the present study, classical symptoms of Se toxicity were observed in different crops growing on seleniferous soils of Nawansheher village Mehindpur in the district. Wheat crop at the farmer's field showed white-pinkish colouration of the tip with restricted growth (Fig. 15). On analysis, Se concentration of wheat crop was exceptionally high in wheat crops (254.4 mg/kg) and of sugarcane tops was (57.3

mg/kg). The mean Se concentration of water used for irrigation in field was 60.8 µg/L. Thus indicating that wheat and sugarcane crops when grown on soils and irrigated with water with high Se concentrations absorb Se in exceedingly high amounts. Changes in colour of the leaves of the crop was observed, thus making them unfit for consumption for humans and animals therefore it is also recommended that ground water in affected areas should not be used for drinking purposes.

The pinkish red colour indicated that Se has accumulated in elemental form the above portion of the crop showed varying degrees of toxicity symptoms.

Typical symptoms of Se poisoning in plants i.e snow-white or papery-white chlorosis with pink colouration at lower side of the leaves and sheath of wheat were, at first, observed by Hurd-Karrer (1934). Similar observations were recorded first time on wheat growing under field conditions on naturally occurring seleniferous soils of Punjab by Dhillon and Dhillon (1991) which might contain Se ranging from 100 to 450 mg/kg. Similar findings were seen during the present study in crops growing in field of village Mehindpur of Nawansheher.

4.5 Health status after treatment with pentasulphates

On questioning the farmer's about the health status of the animals after the treatment it was reported that in some of the animals there was improvement in body condition. There was increase in appetite, sloughing of the hooves and horns were seen, lesions of hooves and horns started showing regression slowly.

Fig. 15: Symptoms of selenium poisoning as white chlorosis of wheat crop – Snow white or papery white colouration of leaves

CHAPTER V

SUMMARY

Mineral imbalances in soil and forages have been long held responsible for low production and reproduction in livestock in the tropical countries. This is because the livestock is largely dependent upon forages which in turn depend on the concentration of minerals in soils. Selenium (Se) is an essential metalloid trace element, naturally occurring and required in small amounts as a part of selenoproteins and selenoenzymes. It has a very narrow margin of safety between the toxic and deficient doses in animals and humans.

Selenium concentrations in plants are related to selenium levels in the surrounding soils. Incorporation and redistribution of selenium by the roots occurs rapidly, but is dependent on the species and physiological conditions of the plant. The normal content of selenium in forages ranges from 0.1 to 0.5 ppm. The risk of livestock poisoning becomes high beyond 5 ppm. Seleniferous plants are characterized by a high content of selenium.

Selenium deficiencies are well known in animals and humans. In animals, selenium deficiency is fairly common without supplementary feeding, especially with forages that are grown on neutral or acidic soils. Deficiency of Se in dairy animals may result in a wide variety of clinical signs. Selenium (Se) toxicity also referred as selenosis is a serious threat when an excess of it is found in soils. Chronic toxicity studies have indicated that diets containing 5 mg/kg or more of Se result in chronic toxicity. Chronic selenosis has been reported in India in winter season, specifically Nawansheher district and a few adjoining areas of Hoshiarpur district with the symptoms of hair loss, horizontal cracks on hooves and horns, leading to elongation and sloughing of hooves, lameness and recumbency in severe cases (Gupta *et al* 1982).

Selenium forms a vital constituent of the biologically important enzyme glutathione peroxidase (GPx). Therefore, the present investigation envisaged the present scenario on Se toxicity and deficiency in animal- plant system in Punjab

Dairy animals from 22 villages, representing all districts of Punjab state, were selected using survey tool box. Information on health status of the animals,

reproductive status, plane of nutrition and mineral supplementation was obtained by physical examination as well as per a comprehensive questionnaire. Samples were collected from 112 crossbred cows and 187 buffalos from 22 districts of Punjab. One village from each district as per the list was visited and twelve to twenty apparently healthy adult dairy animals were sampled from each village randomly. Whereas in Nawansheher district six villages were sampled and in Hoshiarpur district three villages were selected for sampling.

Whole blood was collected by jugular venipuncture from each crossbred cows and buffaloes selected for sampling. 20 ml whole blood was collected by jugular venipuncture. Hair samples from the switch of tail of same animal from which blood sample was taken, drinking water samples and fodder fed was also collected, digested and then the concentration of various macro and micro elements viz. Na, K, Ca, Mg, S, Cu, Mn, Fe, Zn, Co, B, Se, Al, Ni, Cd, Cr, As, P and Pb was analysed using Inductively Coupled Argon Plasma Atomic Emission Spectroscopy (ICP-AES).

The Mean Se content in plasma of dairy animals from 22 districts of Punjab viz. Amritsar, Barnala, Bathinda, Faridkot, Fatehgarh Sahib, Fazilka, Ferozepur, Gurdaspur, Jalandhar, Ludhiana, Mansa, Moga, Mohali, Muktsar, Pathankot, Patiala, Ropar, Sangrur, Tarn Taran, Kapurthala, Nawansheher and Hoshiarpur ranged from 0.008 ± 0.002 to 0.607 ± 0.150 µg/ml. Lowest plasma Se value recorded (0.008 ± 0.002 µg/ml) was from Fazilka district and the High value recorded (0.601±0.103 µg/ml and 0.607 ± 0.150 µg/ml) was from Nawansheher and Hoshiarpur district of Punjab, respectively. Mean plasma Se values were within normal range in twenty districts of Punjab. However, they were significantly higher in Nawansheher and Hoshiarpur districts. Therefore, animals from Nawansheher and Hoshiarpur Districts having high Se content in plasma were designated as selenotic animals.

Similarly, mean Se content in hair samples of animals from 22 districts of Punjab ranged from 0.68 ± 0.14 to 37.00 ± 5.42 µg/g. Lowest hair Se concentrations (0.68 ± v0.14µg/g) was recorded from Muktsar district and highest concentration (37.00 ± 5.42 and 24.01 ± 6.36 µg/g) was recorded from animals of villages of Nawansheher and Hoshiarpur districts, respectively. Mean hair Se values were within normal range in twenty non-seleniferous districts of Punjab. However, they were significantly higher in Nawansheher and Hoshiarpur districts. Therefore, animals

from Nawansheher and Hoshiarpur Districts having significantly high Se content in hair were referred as selenotic animals.

Mean Se content in fodder (seasonal green fodders and wheat straw) fed to animals in 22 districts of Punjab ranged from 0.07±0.10 to 15.77± 8.82 mg/kg. The minimum mean fodder Se concentration recorded (0.07±0.10 mg/kg) was from Patiala district, whereas, the maximum mean value recorded (15.77±8.82 mg/kg) was from villages of Nawansheher district of Punjab. Mean fodder Se contents were within normal range in twenty districts of Punjab, however they were significantly higher in Nawansheher and Hoshiarpur districts. The animals from villages of Nawansheher and Hoshiarpur districts were fed fodder grown on seleniferous soils of the area. Thus, plasma and hair samples of animals and fodder samples had significantly high Se content as compared to fodders from other districts of Punjab. Thus, areas/villages of Nawansheher and Hoshiarpur districts were referred as Seleniferous.

Comprehensive analysis and surveillance studies among the animals from twenty non seleniferous districts revealed that Se levels in hair samples of animals in Kapurthala (5.63±0.58 µg/g) were comparatively higher and fodder Se concentrations (3.73±0.59 mg/kg) were also comparatively higher. Therefore, it was also observed that status of Se in plasma, hair, water and fodder of Kapurthala district was higher among the 20 non seleniferous districts.

Similarly, mean Se contents recorded in water samples from 22 districts of Punjab ranged from 0.3±0.03 to 101.64±35.58 µg/L. The minimum mean water Se content (0.3±0.03 µg/L) recorded among 20 non-seleniferous regions was from Fazilka district, whereas the maximum mean contents recorded (101.64±35.58 µg/L) was from villages of Nawansheher district of Punjab. Mean Se water content were within normal range in 20 districts of Punjab, however they were significantly higher in Nawansheher and Hoshiarpur districts. Therefore, underground water tested from Nawansheher and Hoshiarpur districts was regarded as seleniferous.

The mean values of Hb, PCV, TEC, TLC and Platelets in selenotic dairy animals were 11.31 ± 0.42 g/dl, 29.66 ± 1.22 per cent, 6.03 ± 0.25 x 10^6/cu mm, 9.10 ± 3.52x 10^3/cu mm and 313.10±20.16 x 10^3/ cu mm, respectively. Mean DLC recorded were 44.30 ± 2.26 percent neutrophils, 54.80 ± 2.24 percent lymphocytes,

0.86 ± 0.34 percent eosinophils. However in healthy animals the mean values of Hb, PCV, TEC, TLC and Platelets were 10.90 ± 0.14 g/dl, 31.96 ± 1.0.41 percent, 6.40 ± 0.08 x 10^6/cu mm, 11.39±1.42x 10^3/ cu mm and 233.04 ± 6.81 x 10^3/cu mm, respectively. Mean DLC recorded were 46.57 ± 0.76 percent neutrophils, 52.40±0.76 percent lymphocytes, 1.22 ± 0.12 percent eosinophils. Thereby, indicating that the mean value of TLC in selenotic animals was significantly ($P \leq 0.05$) lower as compared to the mean TLC in healthy animals. However, mean value of Platelets was significantly higher in affected animals as compared to healthy animals. The mean values of other parameters viz. Hb, PCV, TEC and DLC revealed no significant variations as compared to those of healthy animals.

A comprehensive baseline survey was conducted in various blocks and villages of Nawansheher and Hoshiarpur districts. It was observed that dairy animals in studied areas were raised on green fodders and cereal straws grown on seleniferous soils and their ingestion for prolong period lead to the development of clinical manifestation of Se toxicity. During the survey animals showing clinical signs of selenosis in the form of slow but progressive emaciation, poor health, unthrifty appearance, elongated hooves with visible horizontal cracks, associated with cracking and even loss of horn core followed by sloughing, alopecia with generalized/ patchy loss of hair from the body, loss of hair from the switch of the tail. Inappetance and decreased productivity along with various forms of reproductive problems were also observed.

The appearance of above symptoms could be ascribed to prolonged feeding of Se enriched forages and straw grown on seleniferous soil and related to impaired digestion associated with interference in absorption. Signs of lameness were also observed there was reluctance to move in severe cases along with arching of back and difficulty to get up. Abnormality of hooves and horn could be due to inefficient keratin production due to Se antagonism with thio-amino acid, which is important constituent of keratin.

Thirty dairy animals diagnosed on basis of clinical symptoms, were treated with oral administration of Degcure mixture given @ 30 g/day /animal for 45 days. Degcure mixture consisted of five sulphates viz. 1 kg magnesium sulphate, 166 g ferrous sulphate, 24 g copper sulphate, 75 g zinc sulphate and 1.5 g cobalt sulphate.

Blood and Hair samples were collected again from the animals for evaluating the efficacy of pentasulphates in treatment of chronic selenosis in dairy animals.

Mean values of haematological indices in selenotic dairy animals before undertaking therapeutic measures were (pre-treatment; 0 day) were 11.32 ± 0.55 g/dl (Hb), 29.71 ± 1.33 percent (PCV), 5.91 ± 0.23 x 10^6 cells/cu mm (TEC), 9.11 ± 0.66 x10^3cells/cu mm (TLC), 47.52 ± 2.93 percent (neutrophils), 51.17 ± 3.12 percent (lymphocytes), 0.94 ± 0.42 percent (eosinophils) and 341.88 ± 34.23 x10^3/cu mm (platelets), respectively. After undertaking therapeutic measures for 45 days respective post treatment mean values were 11.06 ± 0.75g/dl (Hb), 31.40 ± 1.42 percent (PCV), 6.46 ± 0.25 x10^6cells/cu mm (TEC), 15.80 ± 4.11 x10^3cells/cu mm (TLC), 41.88 ± 3.25 percent (neutrophils), 56.82 ± 3.44 (lymphocytes), 1.29 ± 0.51 percent (eosinophils) and 226.82 ± 27.96 x 10^3/cu mm (platelets), respectively. Thus, comparative studies of haematological indices (Hb, PCV, TLC and DLC) before and after instituting therapeutic measures revealed non-significant variations, however, mean TEC count showed significant increase and mean platelet count decreased significantly after 45 days of therapeutic trial.

The pre-treatment mean values of serum AST, ALT, BUN, Creatinine, TP and GGT in diseased animals were 101.58 ± 11.45 U/L, 177.58 ± 32.07 U/L, 15.82 ± 1.66 mg/dl, 1.84 ± 0.17 mg/dl, 9.27 ± 0.43 g/dl and 35.52 ± 3.51 U/L, respectively. After undertaking therapeutic measures respective post-treatment values were 95.23 ± 9.96 U/L, 214.23 ± 17.33 U/L, 23.41 ± 2.63 mg/dl, 1.72 ± 0.09 mg/dl, and 10.29 ± 1.02 g/dl and 35.41 ± 3.41 mg/dl, respectively.

The results revealed that blood biochemical profile before and after instituting therapeutic measures reflected non-significant variations. The mean value of GPx activity (Glutathione peroxidase), before and after therapeutic measures was 108.24 ± 21.68 and 1182.45 ± 121.95 U/mg Hb, respectively, thereby, indicating significantly higher GPx activity ($P<0.001$) after undertaking therapeutic measures. The significant increase in erythrocytic glutathione peroxidase activity could be due to the mobilisation of Se and due to neutralization effect of Se by pentasulphates.

The mean value of LPO (Lipid peroxidase) before and after therapeutic measures was 1051.86 ± 256.16 and 480.07 ± 94.99 nmol/g Hb, respectively thereby,

indicating significant decrease in LPO activity after undertaking therapeutic measures. The decrease in LPO activity could be due to the effect of pentasulphates.

The mean concentrations of plasma Se in selenotic animals pre-treatment was 0.64 ± 0.10 µg/ml and post-treatment was 0.78 ± 0.13 µg/ml, thereby, indicating no significant differences in the respective mean values after undertaking therapeutic trial. The mild increase in mean plasma Se content has been observed in dairy animals after treatment with pentasulphates. Intake of Se enriched diet/fodder from the Se toxic soils might have led to rise in plasma Se levels in the present study. Selenium is rapidly and efficiently absorbed from naturally toxic or near toxic seleniferous diets and also from soluble salts of the element added to the normal diet. Selenium is absorbed mainly from duodenum (Wright and Bell 1966) and after absorption, is carried mainly in plasma (Buescher *et al* 1960), where it is associated with plasma proteins and enters all tissues, bone, hair and red blood cells (Cousins and Cairney 1961; McConnell and Levi 1962).

The mean concentrations of hair Se in selenotic animals pre-treatment was 34.07±5.02 µg/g and post-treatment was 24.36 ± 3.27 µg/g. The results reflected non-significant decrease in mean Se content of hair post-treatment after 45th day of therapeutic trial. Mineral status of hair provides information of nutritional status of the animal. Decrease in mean Se concentrations are observed and could be ascribed to excessive accumulation of Se the cutaneous system especially hair, due to prolonged feeding of Se rich diets following action of pentasulphates there was mobilisation of S and Se these minerals from the keratin tissues into the plasma and subsequent elimination from the animal's system. It was concluded that, mineral analysis of hair might be considered as important diagnostic index of chronic Selenosis in dairy animals.

The mean Se concentration in water from seleniferous areas, which was used for drinking purposes by the diseased animals and also used for irrigation of crops was 86.43±29.53 µg/L and the mean Se content in fodder crops and wheat straw from seleniferous areas, which were being fed to the diseased animals was 13.95 ± 7.19 mg/kg.

Due to prolonged feeding of fodder and straw with high Se content grown on seleniferous soils of the area dairy animals developed signs of chronic selenosis and had significantly high plasma and hair Se content. On comprehensive survey and interaction with dairy farmer's it was discovered that a few owners have discontinued the feeding of the seleniferous fodder and instead purchased fodder being grown away from the periphery of affected villages.

Mean Se concentrations in plasma, hair, water and fodder samples of Balachaur block of Nawansheher were well within the normal range and thus, the areas surveyed in the present study had no incidence of selenosis prevailing in the animals.

After the treatment it was reported that in some of the animals there was improvement in body condition. There was increase in appetite and sloughing of the hooves and horns were seen, lesions of hooves and horns started showing regeneration but at a very slow rate.

CONCLUSIONS

1. Se status in non seleniferous 19 districts viz. Amritsar, Barnala, Bathinda, Faridkot, Fatehgarh sahib, Fazilka, Ferozepur, Gurdaspur, Jalandhar, Ludhiana, Mansa, Moga, Mohali, Muktsar, Pathankot, Patiala, Ropar, Sangrur and Tarn Taran is within normal range on basis of plasma, hair, water and fodder analysis. However, Se status in non seleniferous in Kapurthala district, on basis of hair analysis, is significantly higher. However, none of the dairy animals from 20 non seleniferous districts showed signs associated with deficiencies of Se.
2. In seleniferous regions of Nawansheher and Hoshiarpur district, on basis of plasma, hair, water and fodder analysis Se concentration is significantly higher.
3. Common clinical manifestations of chronic selenosis were rough starry coat, horizontal cracks followed by elongation of hooves and horns associated with lameness, decline in production and reproduction.
4. Emaciation, loss of hair from tip of tail followed by sloughing of tail are also observed in severe chronic selenosis.

5. Mean Hb, PCV, TEC and TLC in selenotic animals were within the normal range and therefore cannot be used in diagnosis and assessment of prognosis of chronic selenosis
6. Hair selenium is 15 times and plasma selenium 10times higher in selenotic animals. Thus, hair Se is better indicator of chronic selenosis as compared to plasma Se concentration.
7. Plasma mineral analysis reflects inverse relationship of Se with As and B where as positive relationship with Pb, Ni and Cr.
8. Oral feeding of pentasulphate mixture for 45 days @ 30 gm daily results in moderate and gradual improvement in appetite but only mild clinical improvement in lameness and reproduction in severely affected animals.
9. On basis of results of therapeutic trial it is recommended that treatment needs to be continued for a prolonged period associated with oral feeding of sodium arsenate @60 mg daily for 10 days followed by gap of 10 days then repeating as per clinical improvement.

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ANNEXURE I

QUESTIONNAIRE PERFORMA

1. Name Sample No._____ Date_____

Address_____

_____

District_____

Block_____

Village_____

2. Subject Particulars

Species:

Age:

Breed:

Identification mark:

Lactation no.:

Milk yield per day**:**

Type of feeding: Stall/Grazing

Last calving date/month:

3. Plan of nutrition: Low (Green fodder + more of straw)

Medium (Ad Lib green fodder + small amount of straw)

High (Ad Lib green fodder + concentrate)

4. Mineral supplementation: Yes / No

Make of mineral mixture

5. Type of soil: _____

6. Type of fodder/forages grown: Winter: Barseem/Oats/Any other

Summer: Maize/Sorghum/Guinea grass/Napier/Bajra

Any other

7. Health problems reported:

- MM colour
- Hair loss
-Hoof and Horn abnormality
- Coat colour
- Reduced milk yield Yes / No

8. Reproductive status:

-Infertility (Repeat breeder/Sub oestrus/Anoestrus)

-Lactation No: Heifer/Lactation No:

-Date of calving:

-Parturition: Normal or abnormal

-Dystocia

-Calf mortality

-Abortion: Yes/No

If yes- every time /once

-Retention of placenta: Yes/no

Ifyes- every time/once

-Sporadic /Storm: Calving- Normal/Assisted

Uterine/Vaginal prolapse

Alopecia (hair loss)

Lameness- Yes/No (if yes signs of lameness)

10.Amount of fodder/Concentrate:(please tick)

Green fodder	1	2	3	4
Wheat straw	1	2	3	4
Concentrate (kg)	1	2	3	4

11. Owner/Individual affected with any signs:

- Hair loss
- Blackening of the nails
- Any other

ANNEXURE II

LIST OF VILLAGES VISITED DURING SURVEY

District	Village
Amritsar	Ghonewahla Mashiwahla
Barnala	Handiya Khudi Khurd
Bathinda	Nangla
Faridkot	Panjgrain
Fatehgarh sahib	Lalon
Fazilka	Mahatam nagar
Ferozepur	Jalalabad
Gurdaspur	Hargobindpura
Jalandhar	Atta
Kapurthala	Mothawala
Ludhiana	Malo Daud
Mansa	Nangal Khurd
Moga	Takhanwad
Mohali	Gharuan
Muktsar	Giddarwaha
Pathankot	Sangher
Patiala	Faridpur
Ropar	Chandesar
Sangrur	Phalloud kalan
Tarn Taran	Algon
Nawansheher	Jainpur Barwa Rakhar Dhahan
Hoshiarpur	Najjarpur Simbli Dhamai Rurki Khurd Karawar

VITA

Name of the student	:	Darleen Kaur Grewal
Father's name	:	S. Dalbir Singh Grewal
Mother’s name	:	*Jagdeep Kaur Grewal*
Nationality	:	*Indian*
Date of birth	:	27.02.1990
Permanent home address	:	230, Model House, Ludhiana-141002 Punjab

EDUCATIONAL QUALIFICATIONS

Bachelor's degree	:	*B.V.Sc. & A.H.*
University	:	ACVM, RAJUVAS, Bikaner
Year of Award	:	*2012*
OCPA	:	*7.35/10.00*
Master's degree	:	M.V.Sc.
OCPA	:	7.80/10.00