

*Studies on Effect of Trace Mineral supplementation on
Haemato-biochemical profiles in Goat*

Thesis

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22.	Mean standard value of MCH
23.	Mean standard value of MCHC
24.	Mean standard value of Alkaline Phosphatase
25.	Mean standard value of Albumin
26.	Mean standard value of Total Protein

LIST OF ABBREVIATIONS

AAS	:	Atomic Absorption Spectrophotometer
<i>ad lib</i>	:	ad libitum
A/G ratio	:	Albumin/Globulin ratio
ALT	:	Alanine transaminase
AST	:	Aspartate transaminase
AOAC	:	Association of Official Analytical Chemists
Avg.	:	Average
b. wt.	:	Body weight
°C	:	Degree centigrade
Ca	:	Calcium
d	:	Day
CRD	:	Completely randomize Design
Cu	:	Copper
C	:	Cobalt
DM	:	Dry matter
ECIL	:	Electronic Corporation India Limited
Edn.	:	Edition
Fe	:	Iron
Fig.	:	Figure
FM	:	Fish meal
g	:	gram
GNP	:	Gross National Product
h	:	hour
ha	:	hectre
Hb	:	Haemoglobin
I	:	Iodine
K	:	Potassium
kg	:	Kilogram
l	:	litre
LW	:	Liveweight
meq	:	Milliequivalent
mg	:	milligram
Min. Mix	:	Mineral mixture
ml	:	mililiter
Mn	:	Manganese
Mo	:	Molybdenum
N	:	Nitrogen
Na	:	Sodium
NaCl	:	Sodium chloride
NH ₃ -N	:	Ammonia Nitrogen
NFE	:	Nitrogen free extract
NPN	:	Non protein nitrogen
OM	:	Organic matter
P	:	Phosphorous
P<0.01	:	Significant at 1% level
P<0.05	:	Significant at 5% level

pH	:	Potenz hydrogen
ppm	:	Parts per million
SRL	:	Strained rumen liquor
SE	:	Standard Error
TCA	:	Trichloroacetic acid
TVFA	:	Total Volatile Fatty acid
USA	:	United states of America
VFA	:	Volatile fatty acid
viz	:	Namely
Zn	:	Zinc
%	:	per cent

CERTIFICATE-I

This is to certify that the thesis entitled “**Studies on Effect of Trace Mineral supplementation on Haemato-biochemical profiles in Goat**” submitted in partial fulfilment of the requirements for the degree of **Master of Veterinary Science** of the Indira Gandhi Krishi Vishwa Vidyalaya, Raipur, is a record of the bonafide research work carried out by **Anjum Rani Lal** under my guidance and supervision. The Student’s Advisory Committee and the Director of Instructions have approved the subject of the thesis.

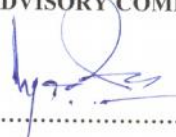
No part of the thesis has been submitted for any other degree of diploma or has been published/published part has been fully acknowledged. She has duly acknowledged all the assistance and help received during the course of the investigations.


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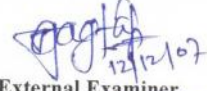
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CERTIFICATE-II

This is to certify that the thesis entitled "**Studies on Effect of Trace Mineral supplementation on Haemato-biochemical profiles in Goat**" submitted by **Anjum Rani Lall** to the Indira Gandhi Krishi Vishwa Vidyalaya, Raipur, in partial fulfillment of the requirements for the degree of **Master of Veterinary Science** in the Department of Veterinary Physiology has been approved by the Student's Advisory Committee after oral examination in collaboration with the external examiner.


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Head of the Department


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(D. G. JAGTAP)

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Director of Instructions

Date

CHAPTER-I

INTRODUCTION

India possesses the largest livestock wealth in the world but its contribution to Gross National Product in the country is very minimal, like wise is the situation of livestock economy in several other developing countries.

The goat is a very important livestock species in the developing countries, and known as poor man's cow in India. Nearly 94 per cent of the world population of 557 million goats exists in developing countries with 332 million in Asia, 174 million in Africa, 114 million in North Central America, 23 million in South America, 50 million in Europe, 1.9 million in Oceania and 6.4 million in the former USSR Republics (Devendra, 1992). In India the population of goat is about 10 million. All animals must receive the essential dietary nutrients such as energy, protein, minerals and vitamins in optimum amount in order to maintain health and good condition and to grow and reproduce to their full potential. Many mineral elements are essential for the growth and production of animals. Large number of livestock in many parts of the world consumes mineral deficient diets and suffers from mineral imbalances leading to nutritional disorders characterized by clinical symptoms often accompanied by mortality. Transient or sub clinical conditions occur that are difficult to diagnose with certainty and expressed merely as unthriftiness or unsatisfactory growth, production or fertility (Underwood, 1999). High concentrations of certain minerals in the diet may influence both the utilization and availability of other minerals or have toxic effects on animals.

In India, for the small and marginal farmers the crop production is the primary source of earning. Also in this context, livestock diets consist mainly of low quality crop residues. Competition for land is a major constraint for livestock feed production and deficiency have been noticed regionally (Hutagalung, 1978; Devendra, 1981) as well as an individual farms (Van Eys *et al.*, 1984).

The animal body contains different concentration of mineral elements in different tissues. Minerals are involved in the biological functions of the body and have important roles in nutrition their physiological make up such as maintenance, lactation growth production. Although the concentrations of trace elements in the body are lower than those of microelements, they must be maintained within narrow units for optimum performance of animals. The fusion of all body tissues can be affected and disordered when one or more essential minerals become deficient to various extents.

The availability absorption of minerals in the animal body can be affected by plant type and its stage of maturity, pasture fertilization animal difference and type of feed (Ammerman, *et al* 1989). Also the bioavailability is affected to a high degree by their interrelationships, which contribute to the complexity of mineral nutrition in animals (Miller 1979). Even it is also found out that the absorption of certain elements decreases at intakes as required for example Ca, Zn and Fe are absorbed progressively less as intake rises (Suttle 1983).

The concept of feeding trace minerals comes from the analysis of the status of feeds and fodder, which are feed to livestock. The feeds, fodder and soil were analyzed for finding the trace minerals status.

Bedi and Khan (1989) found that feed and fodder, which are cultivated on soil, are deficient in mineral profiles. Similar work was undertaken to assess the micro mineral status of feed and fodder in Dahod and Panchmahal district of Gujrat (Garg *et al.*, 2003). They concluded that the mineral was not upto the mark to meet the mineral requirement of livestock. Chew (2000) documented the essentiality of minerals for growth, reproduction, production and physiological functions of the body. Therefore, it is very important to meet the requirements in animals by external supplementation of mineral mixture.

In 2002 (Centre for Food and Nutrition Policy) the scientist come to a conclusion that mineral chelates and complexes may be considered to be added in animal diet.

The bioavailability is influenced by chemical form of minerals and physiological status to accurately assess the bioavailability more than one indicator status should be monitored (O'Dell and Sude, 1997).

The trace mineral bioavailability in ruminants viz. selenium, copper, zinc, manganese etc. has been well documented (Spears, 2003). According to him the absorption of selenium and copper is much lower in small ruminants than in non-ruminants. The low absorption of these minerals in ruminants is due to modification that occurs in the rumen environment.

The non-chelated minerals are generally found to be in appropriate for animal tissue whereas the chelated minerals are readily recognized and used by the body (Llewellyn, 2006).

Daily requirements of minerals may be greatly reduced by the addition of organic trace minerals elements to animal's diets.

Bioavailability is the amount of nutrient ingested that is absorbed and therefore available in the body for metabolic use for growth, maintenance of body tissue reproduction and other performance factors. No matter how high the nutrient levels of well formulated the product is, if it is not bioavailable, money and effort go waste.

In physiology mineral need of ruminant animals depend greatly on their physiological make up, age, health, nutritional status and function such as producing meat milk or developing of fetus. For example, dairy cow producing greater volume of milk have higher mineral requirements than dry cow or cows producing low quantity milk.

The bioavailability of minerals is reflected in terms of plasma concentration, hematological responses, which will indicate the quantum of minerals, made available to the body for the physiological functions.

It is known that bioavailability of chelated/organic minerals is higher than non-chelated or inorganic ones.

Keeping this in view the present study has been designed to assess the bioavailability of minerals, in goats with the following objectives:

1. To assess the bioavailability of inorganic and organic forms of trace minerals in goats under grazing condition.
2. To determine the effect of supplementing these minerals on rumen fermentation pattern in goats.
3. To study the effect of trace minerals on hematological and biochemical profile in goats.

CHAPTER-II

REVIEW OF LITERATURE

Adequate supply of nitrogen, energy and minerals in the diet of ruminants is essential for maintaining optimum ruminal activity. Like the macro elements status, the microelements also lacked any definite correlation between soil, pasture and blood plasma, it is suggested that analysis of soils and pastures are not reliable for assessing the mineral status of grazing goats in the plains, plateau or hills. Present study with the supplementation of minerals and concentrate have been met the requirement of goats adequately. A long-acting reticulo-rumen trace mineral bolus containing Cu, Se, and Co has been developed in the United Kingdom and has shown promise for helping alleviate trace mineral deficiencies (Buckley *et al.*, 1987, Hidioglou *et al.*, 1987; Givens *et al.*, 1988). As indiscriminate supplementation of minerals through mineral mixture supplements is neither economical nor practical, it will be useful to know the mineral status of feeds and fodder to ascertain the extent of deficiencies/excesses (Hinders, 1999: Garg *et al.*, 2002).

2.1 Trace Mineral Status in ruminants:

The animal body contains different concentrations of mineral elements in different tissues. Minerals are involved in the biological functions of the body and have important roles in various physiological functions, lactation, growth and production. Although the concentrations of trace elements in the body are lower than those of macro elements, they must be maintained within narrow limits for optimum performance of animals. The function of all body tissues can be affected and disordered when one or more essential minerals become deficient to various extents.

The availability and absorption of minerals in the animal body can be affected by plant type and its stage of maturity, pasture fertilization, animal differences and type of feed (eg. pasture grass, hay, silage or concentrate) (Ammerman, 1989). Their bioavailability is affected to a high degree by their interrelationships (Fig 2). Absorption of certain elements decreases as intake increases above requirement. For instance Ca, Zn and Fe are absorbed progressively less as intake rises (Fig 3).

Table 1 Recommended dietary intake of minerals for kids^a and does^b and critical levels of minerals in bone, plasma and liver.

Element	Kids ^a	Does ^b	Tissue	Critical level ^c
Ca (%)	0.30-0.52*	0.43	Bone (fat free)	24.5 (%)
			Plasma	8 mg/100 ml
P (%)	0.25-0.40	0.28	Bone (fat free)	11.5 (%)
			Plasma	4.5 mg/100 ml
Mg (%)	0.10	2.20	Plasma	1-2 mg/100 ml
Na (%)	0.10	0.18	-	-
Fe (ppm)	30	50	Liver	180 ppm
Mn (ppm)	40	40	Liver	8 ppm
Cu (ppm)	8	10	Liver	25-75 ppm
			Plasma	0.65 µg/ml
Zn (ppm)	30	40	Plasma	0.6-0.8 µg/ml

* Requirement below, which a deficiency occurs (NRC goat, 1981).

* Recommendation for the (NRC goat, 1981).

2.2 Macro elements:

2.2.1. Calcium (Ca):

Calcium (Ca) is the most abundant element found in the animal body. About 99% of the Ca is found in the skeleton and teeth. The remainder is present in the body fluids and soft tissues. It is required for normal blood clotting, rhythmic heart action and neuromuscular excitability. Calcium can also act as an activator or stabilizer of enzymes. Calcium is mainly absorbed in the small intestine. Its solubility and absorption is favored under acid conditions. Its absorption is decreased with low vitamin D, due to lower 1,25-D hormone production in the body, age, Phosphorus (P) and Calcium (Ca) intake (Ammerman and Goodrich, 1983).

The Calcium requirement for maintenance of lactating Beetal goats in mid lactation were 0.54 g Ca/kg metabolic W and 1.16 g Ca in diet/g Ca secreted in milk (Singh and Mudgal, 1987). According to NRC (1989) availability of Ca in most feeds is about 45%, while ARC (1980) gives a figure of 68%. Calcium availability however, depends not only on sufficient dietary supply but also on the chemical form in which it occurs in the diet and on the P content.

The Ca requirements of animals depend primarily on the daily milk yield. McDowell *et al.*, (1991) observed that serum Ca concentration in goats were above critical levels in seasonal dermatitis in affected and unaffected farms. Bhattacharya *et al.*, (1994) observed that mean serum Ca concentration of non-descript goats of Assam at pre-pubertal, pubertal and post-pubertal stages were 11.05 ± 0.19 , 9.91 ± 0.44 and 9.90 ± 0.30 mg/100 ml respectively, while, Prabha *et al.*, (2000) reported that mean serum Ca level were 13.65, 11.80, 12.83, 12.23,

13.35 mg/dl in pre parturient crossbred cow, calves at birth, calves at 1 month, calves at 4-5 months and calves at 10-11 months respectively. Lall and Prasad in (1990) conducted a study to find out the effect of certain Ca and P supplements on rumen metabolites and blood Ca and P levels. They observed that blood Ca and P level were similar in all the treatment i.e. in control and treated. Plumlee *et al.*, 1958 documented that different chemical forms of Ca in supplement alter the normal metabolic profile of rumen. They also showed that different source of Ca and P fed had no significant difference ($P>0.05$) in the feed intake. It was concluded that there was no significant difference in the respective groups thus indicating that cellulolytic organisms are not affected by different source of Ca and P supplements.

Bryant *et al.*, 1959 indicated that different forms of supplemental P did not have any effect on cellulolytic activity of rumen bacteria. However, results of other study conducted by Fisher, (1978) are contradictory which showed that Monosodium phosphate as source of phosphorus resulted in higher production of propionate in rumen as compared to other source of phosphorus such as mono calcium phosphate etc. They concluded that no difference in Ca and P concentration was found suggesting that even at lower dietary Ca and P supply in control the homeostatic mechanisms were adequate to maintain serum Ca and P level.

2.2.2 Phosphorus (P):

About 80% of the P of body is found in the skeleton and teeth. It has an important role in phospholipid formation and therefore fatty acid transport amino acid and protein formation. Phosphorus is mainly absorbed in the small intestine

in which a carrier-dependent absorption mechanism is present (Scott *et al.*, 1984). Rumen bacteria take up part of the consumed P in ruminants. Generally there is very little evidence of variation in absorption of P between different types of feed. The amounts of phytate P in ruminant feeds have little effect on the availability of P due to the ability of microbial phytases in the rumen to degrade phytates (Suttle, 1983).

Durand *et al.* (1989) reported that impaired rumen microbial activity resulted in the negative effect of P deficiency on voluntary feed intake and decreased rate of growth. Salivary P secretion is generally higher than dietary P when animals receive sufficient amounts of dietary P (Cohen, 1980). This salivary P provides a substantial amount of P for microbes in the rumen. In a P deficient diet, particularly one with a high Ca content, the low salivary P secretion into the rumen can substantially reduce ruminal inorganic P levels. A value lower than 20-28 mg rumen inorganic P in sheep has been shown to depress rumen microbial activity (Durand *et al.*, 1989).

Changes in blood P is the first known response to a deficiency of P, which leads to a withdrawal of this element from the bone (Underwood 1981). After a few weeks or months on a P deficient diet the blood plasma P concentrations fall to 2-3 mg/100 ml. The critical level of P deficiency determined by blood plasma analysis has been suggested to be < 4.5-mg/100 ml (McDowell, 1985). Niekerk *et al.* (1990) estimated that blood P level was 3.50, 2.41, 2.15 m mol/lit in kid, 12 month old and adult Angora goat. Growth requirements of pregnant and lactating swiss-type goats were at least 3.0 g P/kg ration DM/ day (Anke *et al.*, 1987). Pre- and postpartum growth, conception rate, feed consumption and milk yield were

reduced at 2.0 g/kg DM, but abortion rate, mortality and milk protein contents increased with no effects on milk fat. Nursing Granadina goat kids from birth to 30 day of age required 1.31 g P/kg (Sanz-sampelayo *et al.*, 1987). Maintenance requirements for growing goats were 27 mg P/kg BW, 12 mg adult females and 19.2 mg for castrated males (Kessler, 1991).

2.3 Trace Elements:

2.3.1 Zinc (Zn):

Zinc has long been known to be essential for animals. Zinc is a component of many metallo enzymes such as super oxide dismutase, carbonic-anhydrase, alcohol dehydrogenase, carboxypeptidase, alkaline phosphatase and RNA polymerase, which affect the metabolism of carbohydrate, protein, fed and nucleic acids. Zinc deficiency alters the synthesis of prostaglandins, which may affect luteal function (Underwood, 1981). Zinc and Copper are antagonistic to each other. Zinc requirements of beef and dairy cattle are estimated to be between 20 and 40 ppm, depending on age and growth rate (NRC, 1989).

Chhabra and Arora (1985) showed that 15 and 65 mg Zn resulted in some metabolic disorders. Ramana *et al.*, (2000) estimated the mineral content of blood plasma from Southern transition zone of Karnataka and reported that the levels of Zn (0.66 ppm) was low in the blood plasma of animals in this zone. While studying plasma trace mineral concentration Das *et al.*, (2003) observed that plasma Zn was highest in calves and lowest in lactating animals.

Organic chelators of Zn can increase or decrease the bioavailability of Zn. phytate is an organic form insoluble complex with Zn and greatly diminishes the availability of Zn for absorption of monogastric and pre ruminant animals.

However, a rumen microbe metabolizes Zn absorption in ruminant animals (Reece, 2004).

However, the dietary factors that affect Zn bioavailability in ruminants are not clearly defined. Phytate can be degraded by microbial phytase in the rumen. High dietary Ca reduces serum Zn concentration in ruminants (Perry, *et al.*, 1968 and Pond, 1986).

Studies in lambs indicate that Zn sulphate and Zn oxide are similar in bioavailability (Kegley *et al.*, 1992; Sandoval *et al.*, 1997). Also research suggested that supplementing certain organic forms of Zn can improved animal production responses compared with those supplemented with only inorganic Zn. (Spears, J. W. 1996).

High tissue concentration of Zn also seen in calves and lambs fed with Zn proteinate relatively to the Zn sulphate (Wright *et al.*, 2001; Cao *et al.*, 2000).

On the other hand supplemented Mo did not affect the plasma Zn level. A lower ceruloplasmin and erythrocyte superoxide desmutase activity associated with supplemental Mo. Ward and Spear (1999) indicated the inhibitory effect of Mo on Cu bioavailability.

2.3.2 Copper (Cu):

The presence of Cu in animal tissues was recognized at the beginning of the nineteenth century (Underwood, 1966). Copper is involved in hemoglobin production, in the central nervous system, in the functioning of enzyme systems, bone metabolism and heart function. It is interrelated with Mo, S, Zn, and Fe, which can reduce its absorption in the body (Humphries *et al.*, 1985). Anemia is a common expression of Cu deficiency in all species where the deficiency is severe or prolonged (Underwood, 1981).

Factors such as Cu by passing the rumen, its interrelationships with other trace elements of the diet such as S, Mo and Fe, or high body Cu requirement, can affect the Cu absorption. The causes of Cu deficiencies have been categorized by Ward (1977) in four groups viz, high levels of Mo in feeds (more than 20 ppm), low Cu but significant amounts of Mo, deficient Cu (< 5 ppm) and normal Cu and low Mo, with high levels of soluble protein which increases the amounts of sulphide produced in the rumen, thus resulting in unavailable Cu sulphide. Cu deficiency is the most commonly limiting mineral to grazing livestock throughout the world (McDowell *et al.*, 1984).

The mean plasma Cu concentration of calves, heifers and milch cattle were 0.69, 0.89 and 0.97 g/ml respectively (Das *et al.* 2002).

A significant decline of copper was found in the experiment conducted on goat fed with different level of Mo. An increased level of Mo intake produced a gradient fall in plasma Cu concentration (Tola *et al.*, 2002). They also found that apparent absorbability of Zn with a concomitant decline in Cu absorption corroborated the reports of Oestreicher and Cousins (1985) and Solomons (1986). They reported Cu and Zn share common pathway of absorption in the intestine and hence the removal of one element from the site of absorption would lead to the enhancement in the absorption of the other.

In the above experiment the plasma Cu concentration was similar in treated groups but as the end of the mean concentration declined by gradually from control to treated this suggesting an antagonist effect of Mo orientation absorption of Cu due to formation of Cu thiomolybdates complex. Jerry and Spears (2003) documented that thiomolybdates greatly reduced copper absorption

and interfere systemically with copper metabolism. Dietary iron also reduces copper bioavailability.

Thiomolybdates associated with solid rumen digesta (bacteria, protozoa and undigested feed particles) form insoluble complexes with copper that do not release copper even under acidic conditions, Allen *et al.*, (1987).

A number of studies evaluated various organic forms of copper. In cattle fed diets that were high in molybdenum, copper proteinate was more bioavailable than cupric sulfate in some studies, but not in others (Kincaid, *et al.*, 1986; Ward, *et al.*, 1996 and Wittenberg, *et al.*, 1990).

2.3.3. Cobalt (Co):

Study was conducted with dairy cattle fed diets supplemented with cobalt and the level of the cobalt in liver and blood was examined. The cobalt concentration of liver taken on day 60 was not affected by dietary cobalt but was higher in young cows. Cobalt supplement in the starter diet did not affect cobalt in serum or liver of young calves. In conclusion supplemental dietary cobalt did not affect secretion of cobalt in milk, tissue or sub-cellular distribution of cobalt within the liver. (Kincaid, *et al.*, 2003).

Cobalt is an essential element in case of ruminants. Since microbes in the rumen, to synthesize vitamin B₁₂, utilize it. The identity of cobalt essentiality was established in 1935 for ruminant. Cobalt is scarcely absorbed from the digestive tract and only 3 percent is converted into vitamin B₁₂ in the rumen of ruminants and its 3 percent component is absorbed. Cobalt supplementation may be necessary in ruminant where the soils and herbage grown over it, is deficient. Cobalt is a requirement for only ruminants, which is required indirectly for rumen

microbes to synthesize vitamin B₁₂ Cobalt requirement for of sheep 0.1 mg/kg BM. (Tiffany *et al.*, 2006).

2.3.4 Iron (Fe):

Iron has important biochemical functions in the animal body. It is a component of hemoglobin, myoglobin, cytochrome and the enzymes catalase and peroxidase. It is absorbed in all the sections of the small intestine but mostly in the duodenum. Thomas (1970) has summarized concepts on Fe absorption as follows; (1) absorption is more efficient when body stores are low, (2) the amount absorbed is usually a small portion of that ingested, (3) many dietary factors influence the amount absorbed, (4) absorption occurs directly into blood, with limited amounts into the lymphatic system, (5) the quantity absorbed from the duodenal parts exceeds that from other portions of the tract and (6) orally administered ferrous (Fe²⁺) salts are more effective for hemoglobin regeneration than ferric (Fe³⁺).

Iron is dietary essential for goats and influence the efficiency of production. ARC (1980) suggested 500 mg/kg DM as the maximum tolerance for ruminants. Requirements for iron by goats can be met by 40 mg/kg DM (Wilkinson and Stark, 1987). Grazing animals may receive substantial amounts of Fe by soil ingestion, which could negatively affect the absorption of Cu or other elements (Probowo, 1989).

High dietary Iron did not affect Copper status in young preruminant calves, which suggests that a functional rumen is needed for Iron to interfere with Copper metabolism.

Tola, *et al.*, (2002) showed that increased dietary Mo reduces the plasma Fe concentration. Sharma *et al.*, (1995) documented that Fe deposition occurred as hemosiderin in various tissues in goats supplemented with Mo.

2.3.5 Manganese (Mn):

Manganese (Mn) is an essential trace element for animals. It is a cofactor for many enzymes involved in carbohydrate metabolism. It is needed for bone structure, reproduction and functioning of the central nervous system. The liver, kidney and pancreas are the organs most sensitive to Mn deficiency (Hidiroglou, 1970).

Anke (1966) reported that 5.1 mg Mn/kg DM was adequate for goats during pregnancy to maintain normal birth weight in their offspring. However, 6 mg Mn/kg DM induced tarsal bone deformities in some does and their kids (Groppe and Anke, 1971). Levels of Mn that sustain growth are not sufficient to maintain normal skeletal development (ARC, 1980). Mn level of 40 mg/kg DM has been suggested as fully adequate for goats (Wilkinson and Stark, 1987). Das *et al.*, (1997b) reported that serum Mn level was higher in goats from red laterite soil zone compared with those of new alluvial soil zone.

Mn is very poorly absorbed in ruminants and limited research suggests that dietary Ca and P may reduce Mn bioavailability. Relative bioavailability of Mn from Mn methionine was 120% of that present in the sulfate form.

2.3.6 Molybdenum (Mo):

Molybdenum (Mo), an integral part of various enzymes systems like xanthine oxidase and aldehyde oxidase which catalyze basic metabolic reactions in the nitrogen. Sulfur and carbon cycles of body has obtained more importance in

animal nutrition because of its inhibitory role on the bioavailability of other trace elements particularly coppers (Kisher *et al.*, 1997).

Thiomolybdates associated with solid rumen digesta (bacteria, protozoa and undigested feed particles) form insoluble complexes with copper that do not release copper even under acidic conditions (Allen *et al.*, 1987).

When ruminal sulfide concentrations are low, molybdenum may have little effect on copper bioavailability. In sheep, fed diets that contained only 1.0 g of sulphur/kg of diet, increasing the dietary molybdenum from 0.5 to 4.5 mg of molybdenum/kg of diet did not affect copper bioavailability (Suttle, N.F. 1991).

2.3.7 Iodine (I):

Feeding high proportions of glucosinolate rapeseed meal in concentrate feeds depress the metabolism of thyroid gland in ruminants (Hill 1991) leading to reduced performances such as low milk production and infertility. In India for example, prevalence of iodine deficiency disorders is quite widespread, especially in-Himalayan belt of Northern India (Pandav and Anand, 1997; Bedi 1997).

Iodine is necessary for synthesis of the thyroid hormones, which regulate energy metabolism and basal metabolic rates (Reece, 2004).

Lubin, *et al.*, (1979) have reported greater digestibility of organic nutrient upon supplementation with iodine. On the contrary, Bedi *et al.*, (2000) have failed to record any positive influence of extra iodine on the nutrient utilization by Barbari goats. Likewise provision of additional iodine was also ineffective in boosting the nutrient digestibility in pigs fed with a rapeseed meal diet (Schone *et al.*, 1986).

Supplementation of iodine did not have any effect on the intake and retention of Ca and P by the goats. This is in agreement with findings of Khan *et al.*, (2002). Present observations are however, in contradiction to the findings of Koval'skii *et al.*, (1972), who reported that iodine supplementation resulted increased retention of Ca and P. The physiological state of the animals had no effect on metabolism of Ca and P.

2.4 Chelated and Non-Chelated minerals and Action of Chelated and Non-Chelated minerals:

It is found that in natural food, essential vitamins and minerals needed to function healthily and efficiently are not found in free state. Difference between chelated or renatured state and non-chelated or free state determine the ways the body deals with material in the two states. The chelated makes them more recognizable to the body increasing their absorption and availability and conveys a degree of protection to that mineral; while preventing chemical interaction from occurring. Eric, Llewellyn (2006).

Reports from various workers throughout the world confirm that metal chelates of amino acids and peptides enhance the bioavailability of the trace elements. (Paik, 2001) thereby leading to the improvement of growth and general health status when they are otherwise unavailable in sufficient amounts to meet animal's need. (Dutta, C., Mondal, M.K. and Biswas, R.P. 2006).

The influence of form of supplemented Zn (Organic vs Inorganic) on sheep. Zn status has not been well documented. They found in their study that liver copper concentrations were higher for organic minerals than the inorganic source of copper. (Hatfield, *et al.* 2001). Eckert *et al.*, (1999) reported that the

liver copper concentration tend to decrease with higher amounts of copper were fed from organic or proteinate source, but increase when greater amount of copper were fed from sulphate. Nockels *et al.*, (1993) also reported no difference between organic and inorganic forms of copper on fecal output.

The supplementation of chelated minerals, will increase absorption and utilization of minerals, therefore in an animals digestive system organic trace minerals that are bound to an organic ligand such as protein, amino acid and carbohydrates becomes more biologically available than inorganic traces minerals (Parks and Harmston, 1994).

2.5 Hematological parameters:

Effect of supplementation of Cu on hematological profiles i.e. whole blood Hemoglobin (g/dl), Packed Cell Volume (PCV), Total erythrocyte Count (TEC) and Total Leukocyte Count (TLC) etc. throughout the experimental period were similar in all the treatment groups. The dose of some trace minerals (mainly Cu) did not affect the concentration of Hb, PCV, TEC and TLC throughout the experimental period. (Dutta, C. *et al.* 2006).

In contradiction to the above findings Tola, *et al.* (2003) documented that Hb concentration decrease as the Mo concentration in diet increases. Similarly like Hb under influence of graded doses of Mo, TEC declined. The values of TLC did not differ much.

2.6 Biochemical parameters:

The supplementation of trace minerals premix resulted in similar ($P>0.05$) growth rates and significantly ($P<0.01$) more dry matter intake than control. The blood biochemical profile viz. total protein albumin, globulin, urea, creatinine,

aspartate, amino transferase and alkaline phosphatase were similar ($P>0.05$) in both groups. It was concluded that trace elements in salt premix did not improve the biochemical profile (Rao *et al.*, 2004).

Hatfield, P.G. *et al.*, (2001) found that serum alkaline phosphate activity did not differ between control and supplemented ewes. Alkaline phosphatase activity was greater in Zn than Zn-Cu supplements ewes. In addition, alkaline phosphatase activity tended to be greater in sulphate than complex supplemented ewes.

The blood alkaline phosphatase activity has been used as an indicator of animals Zn status. Wan, *et al.* (1993) and Kraus, *et al.*, (1997) reported that the plasma alkaline phosphatase activity was higher in Zn adequate than Zn deficient rats. In the above study serum alkaline phosphatase activity did not differ between the two groups i.e. supplemented and control ewes.

In pigs serum alkaline phosphatase activity was not affected by source of Zn either as Zn sulphate or as chelated zinc (Swinekl, *et al.*, 1996).

2.7 Ruminal Parameters:

Minerals supplementation may be necessary to stimulate rumen digestion of fibrous crop byproducts (Leng and Preston, 1983) However, free green forages, will generally provide adequate levels of soluble mineral, for maximum microbial activity. (Durand and Dewashima, 1980)

A study was conducted to investigate the effect of trace minerals supplementation on live weight gain, blood biochemical profile, etc. The supplementation of trace mineral premix resulted in growth rates and significantly ($P 0.01$) more dry matter intake (DMI) than control. The total volatile fatty acid

concentration and individual fatty acids were also similar in both groups. It was concluded that added trace minerals elements in the both premix did not improve the live weight gain, rumen fermentation, nutrient digestibility; blood biochemical profile and composition of muscle and tissues, except for the liver copper content indicating that the trace mineral content of basal ration was adequate. (Rao, *et al.*, 2004).

Bang (1990). Reported that the elevation of abomasal pH reduced the absorption of copper.

After supplementation of certain Ca and P the rumen pH in different treatments were found similar and treatments are not influenced. The ammonia nitrogen in strained rumen liquor was statistically similar in all the treated groups. As comparison to control it was significant lower ($P < 0.05$) in treated groups with rock phosphates. In general there was no significant difference ($P > 0.05$) in respective groups with regard to total volatile fatty acids, thus indicating that cellulolytic organism are not affected by different source of Ca and P supplements. The study also revealed that different forms of supplemented phosphorus did not have any effect on cellulolytic activity of rumen bacteria (Lall, and Prasad, 1990).

The majority of long chain fatty acid, methane, ammonia and pH were not greatly affected by Cobalt supplementation. (Tiffany, *et al.*, 2006).

2.7.1 pH:

A study revealed that rumen pH is affected by the level of protein intake. (Briggs, 1967)

2.7.2 Total Volatile Fatty Acid (TVFA):

The amino acid produced, either incorporated into bacterial cells or further deaminated to form ammonia, CO₂ and VFA (Hungate, 1960).

2.7.3 Ammonia Nitrogen:

Ammonia nitrogen depends on the amount of mineral granules consumed by the goats. (Leng, 1989; Mehra, *et al.*, 1991; Sahoo, *et al.*, 1992; Tiwari, *et al.*, 1990, Niral, *et al.*, 1999).

2.7.4 Total nitrogen:

It was suggested that the total nitrogen concentration of rumen increased in response to increase in nitrogen intake in sheep (Hume, *et al.*, 1970). Further, Leng (1984) found similar result in his experiment performed on goat.

2.7.5 Total Bacterial Count:

A report of significant increase in total bacterial count (TBC) existed as result of long term urea feeding in cattle (Ogra, 1980).

2.7.6 Total protozoal count (TPC):

According to Mathur *et al.*, (1991); Ogra, (1980) there was significant increase in total protozoal count after supplementation of urea rich products.

2.7.7 Bacterial Biomass:

Availability of carbon skeleton form VFA favors the growth of bacteria. (Hungate, 1966).

CHAPTER-III

MATERIALS AND METHODS

Experiment was conducted in the Department of Veterinary Physiology and Animal Nutrition, College of Veterinary Science and A.H., Anjora, Durg.

3.1 Selections, Housing and Management of Experimental Animals:

Twenty-one graded non-descript adult goats of about three years of age were procured from Department of Animal Nutrition, College of Veterinary Science & A.H. for the present study. The animal weighed from 20-25 kg and of either sex was selected. They are dewormed with the calculated dose of panacur (5 mg/kg b.w.). These animals were ear tagged with identification numbers. The animals were kept in the goat shed having cemented floor for conducting the experiment. Strict disinfectant, hygiene and sanitary conditions were provided in the experimental pens. *Ad libitum* clean and wholesome drinking water was provided to the animals twice daily.

3.2 Treatment and Feeding schedule:

The selected goats were divided into three groups and the treatment was given for twenty-one days. The animals were allowed to graze freely. The concentrated mixture was stall-fed @ of 150gm/goat/day for 21 days. The animals were supplied with *ad lib* drinking water during the course of the study. The details of the treatment of mineral mixtures, the chelated or organic and non-chelated or inorganic given as per the Table No. 2

Table 2. Details of the three groups and the treatments given

S. No	Groups	No. of animals	Treatments
1.	Group-I	7	Control
2.	Group-II	7	Non-chelated trace mineral @ 5g/day (inorganic form)
3.	Group-III	7	Chelated trace mineral @ 5g/day (organic form)

3.3 Feeding pattern:

The experiment trail was conducted for 21 days having 7 goats in a group. The three groups, Group I, which kept as a control were as Group II was given non-chelated trace mineral and Group III received chelated trace mineral mixture @ the rate of 5g/day/goat for 21 days in morning at 7 am before feeding and watering throughout the experiment. At the end of 21st day all the animals were kept together and collection of blood/serum was done on 22nd, 23rd, and 24th day for haemato-biochemical and trace mineral analysis. The strained rumen liquor was collected from the animals for the study of various ruminal parameters through stomach tube.

3.4 Collection of blood/serum and rumen liquor:

Rumen liquor samples were collected from individual animals at the end of trail before feeding and watering with the help of especially designed stomach tube. The tube was inserted through mouth along with esophagus to rumen. About 100ml of rumen liquor was collected and strained through 4 layers muslin cloth into conical flask and processed for the determination of pH, total volatile fatty acids, ammonia, nitrogen, total nitrogen, total bacterial count, total protozoal count and bacterial biomass as per the procedure outline by Pathak *et al.* (1996).

3.5 Blood and serum samples for hematological and biochemical estimation:

The blood samples were collected from jugular vein of each goat in an sterile glass tube while containing anticoagulant EDTA (Ethylene di-amine-tetra acetate @ 1 ml solution for hematological studies (Jain 1996).

For biochemical analysis blood samples without anticoagulant was taken in test tube and the test tube was kept in slanting position for 8-10 hours and serum was collected in a separate vial and stored at -20⁰C deep freezer. One set was used for the determination of calcium, phosphorus, alkaline phosphatase, total protein, albumin and another set for estimation of all the minerals i.e. macro, micro and trace minerals as per the procedure of AAS.

3.5.1 Parameter studied:

3.5.1.1 Hematological study:

The hematological parameters including Total Erythrocyte Count, Total Leukocyte Count, Hb Estimation, Packed Cell Volume, MCV, MCH and MCHC were analyzed as per procedure outline in Veterinary Hematology by Jain (1996)

3.5.1.2 Biochemical study:

The blood samples from the treated goats were collected in a vial with Z serum separator clout activator to estimate biochemical parameters. These parameters were estimated by standard procedures by using semi-automated analyzer (Logotech Tech.-168 model) by using biochemical kits (Bayer Autopk biochemistry kits- Baroda, Span Diagnostics Ltd. – Sachin, Lab-care Diagnostics Pvt. Ltd.- Sarigam) and methodology recommended by manufacturer. The parameters are as follows:

- a) Total protein

- b) Albumin
- c) Alkaline phosphatase
- d) Serum calcium
- e) Inorganic phosphorus

3.6 Rumen fermentation pattern:

3.6.1 pH of strained rumen liquor:

After collection of strained rumen liquor (SRL) it was processed for determination of pH by means of pH meter (NIG 333)

3.6.2 Total volatile fatty acids:

One ml of centrifuged rumen liquor was taken in Markham's distillation apparatus. To this 1 ml of oxalic acid (5%) added and 1 ml of potassium oxalate (10%) buffer was added. Thereafter 100 ml of the steam distillate was collected in a marked conical flask. An aliquot of this was taken and titrated against standard 0.01 N NaOH using phenolphthalein as an indicator to find out the concentration of TVFA in rumen liquor (Bennett and Reid, 1957). TVFA (meq/100ml SRL) = Volume (ml) of 0.01 N NaOH used.

3.6.3 Ammonia- Nitrogen:

Ammonia- Nitrogen was determined in the strained rumen liquor following the method of micro-kjeldahl (AOAC, 1995). Equal volume of SRL and 40 % NaOH was distilled and titrated against 0.01 N standard H₂SO₄. It was calculated by the formula: -

$$\text{NH}_3\text{-N (mg/100ml SRL)} = \frac{\text{Volume of standard acid used (ml)}}{\text{Volume of standard acid used (ml)}} \times 100$$

Volume of sample taken (g)

3.6.4 Total Nitrogen:

Total nitrogen content in pooled SRL was determined by conventional Micro- Kjeldahl method (AOAC 1995). 5 ml of SRL was digested with 10ml of concentrated sulphuric acid (AR grade) and distilled in Micro- Kjeldahl distillation apparatus. The distillate was collected in a conical flask having 10 ml of 2% boric acid mixed indicator. About 50 ml of distillate was collected and titrated against 0.01 N standard H₂SO₄. Total nitrogen was calculated as:

$$\text{Total N (mg/100ml SRL)} = \frac{\text{Volume of standard boric acid used (ml)}}{\text{Volume of sample taken for digestion (g)}} \times 100$$

3.6.5 Total Bacterial and Protozoal counts:

The rumen liquor was collected through a stomach tube and Buckner's flask, which in turn was connected to a suction pump and filtered through 4 layers of muslin cloth. Samples of SRL were drawn from each kid just before feeding and watering. The total bacterial and protozoal counts were done in SRL.

3.6.5.1 Total bacterial count:

The live bacterial count was done by the methods as compiled in the laboratory manual (Pathak *et al.*, 1996).

Five milliliters of SRL was diluted to 25ml with 10% formal saline. One ml of this was again diluted to 50 times with normal saline. To a 1.0 sq. cm marked area of slide 0.01ml of this fluid was spread. The smear was fixed and stained with Gram's stain. The bacteria were counted in 30 microscopic fields by

using 10 ocular and oil immersion object. The bacterial concentration was determined as:

Area of each field = r^2 , where = radius of the microscopic field.

Number of fields/100 sq.mm = $100/r^2 = F$

Total number of fields counted = $30(X)$

Average number of bacteria/field = N

Volume of SRL used = 0.00002ml

Hence, the number of bacteria in 1ml of SRL = $F \times N \times 10^5/2$

(Note: using stage micrometer, the diameter of the field in various magnifications were measured).

3.6.5.2 Total protozoal count:

Protozoal counts were done under stage laboratory microscope at low power as per the method described in the laboratory manual (Pathak *et al.*, 1996).

Five milliliter of SRL was diluted to 25ml with 10% formal saline. Eosin stain (2%) was added to the diluted fluid at the rate of 1 drop per ml. It was then kept for 5-10 min. The haemocytometer was then charged with this diluted fluid and 8 WBC chambers were counted for the protozoa numbers. The protozoa numbers were calculated as:

Dilution ratio = 1 in 5

Average number of protozoa = Total number/8 = X

i.e., 1 Sq.mm contains = X protozoa

Depth of chamber = 0.1mm

Therefore, 1 cm contains = $X \times 10$ protozoa

Therefore, 1 ml contains = $X \times 10 \times 1000$

i.e., 1 ml of diluted SRL contains = $X \times 10 \times 1000$ protozoa

$$\begin{aligned} \text{Therefore, 1 ml of undiluted SRL, contains} &= X \times 10 \times 1000 \times 5 \\ &= X \times 10^4 \times 5 \\ &= X \times 0.5 \times 10^5/\text{ml} \end{aligned}$$

3.6.7 Bacterial biomass:

20 ml of SRL was centrifuged at 200xg for 2 minutes to remove coarse feed particles. The supernatant was centrifuged at 1,500xg for 15 min to separate protozoal pellets. The pellets were given washings with normal saline. The contents were again centrifuged at 1,500xg for 15 minutes. The protozoal pellets were treated once with 10% TCA. Thereafter it was dried and weighed to know the mass of protozoal pellet. The bacterial biomass was determined as above by centrifuging the protozoal free rumen liquor at 15,000xg for 15 minutes. The remaining process is similar to that of protozoal biomass as complied in laboratory manual (Pathak *et al.*, 1996).

3.7 Estimation of trace minerals:

Minerals were then estimated by using an atomic absorption spectrophotometer (ECIL-Elements AAS 4141).

3.8 Statistical Analysis:

Data were statistically analyzed as following statistical analysis.

1. Mean range and standard error from each parameter for all the groups.
2. Analysis of variance for the comparison of the three groups for each parameter using Completely Randomized Design (CRD).
3. t-Test for the testing of significance of difference between mean values for the parameter found to be significant in analysis of variance.

Twenty-one animals of three years of age were randomly divided in to three groups (I, II, III) of 7 animals in each following completely randomized design (CRD) (Snedecor and Cochran 1976)

CHAPTER-IV

RESULT AND DISCUSSION

4.1 Mineral analysis:

The mineral content of blood serum is directly proportional to the bioavailability of macro and microelements across the gastro intestinal tract, which depends mainly on the pH and presence of other minerals at the site of absorption. It is also influenced by the concentration of fatty acid in the gastro intestinal tract.

4.1.1 Micro Minerals:

4.1.1.1 Zinc (Zn):

Data pertaining to the concentration of zinc have been given in Table No. 3 and Fig. 1. It ranged from 2.52 to 3.67 in groups II, I and III respectively. Although there was no significant difference amongst groups III, where goats were supplemented with chelated zinc. There are different reports on the concentration of Zn in the grazing ruminants Ramana *et al.*, (2000). Reported that the values were lower in animals while Das *et al.*, (2003) reported the higher values. Although they depend upon the area where animals are actually allowed for grazing which was repeated by the soil status of zinc.

Organic chelator Zn could increase as decrease the bioavailability of zinc. Phytate is an organic matter, which forms insoluble complex with zinc and greatly diminishes the availability of Zn for absorption in the pre-ruminant. The results of the present study investigate no significant difference amongst groups irrespective of the inorganic or organic form of dietary supplementation in goats.

Fig. 1 Mean standard value of Zinc

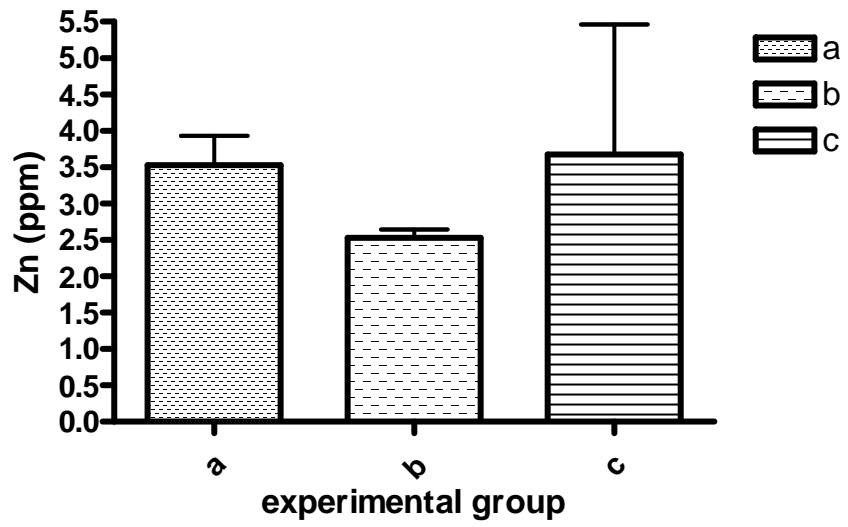
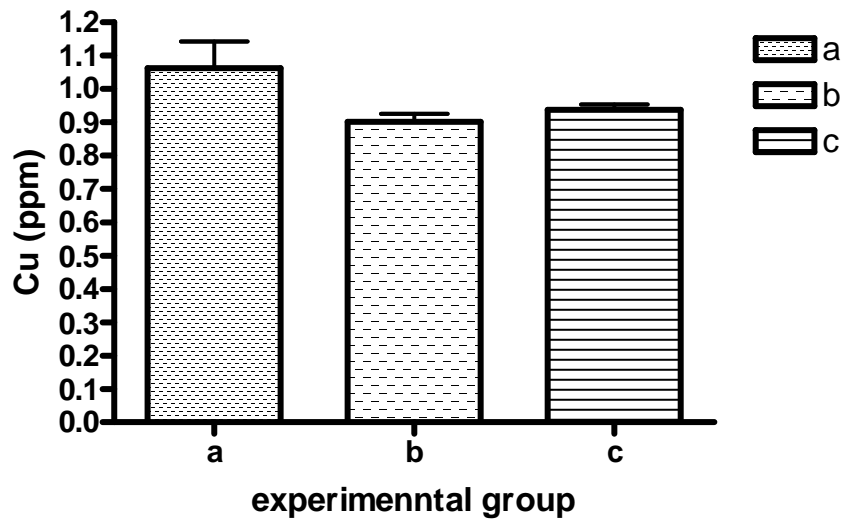


Fig. 2 Mean standard value of Copper



4.1.1.2 Copper (Cu):

Data of serum copper in the different groups have been presented in Table No. 3 and Fig. 2. It varied from 0.91 to 1.062 ppm. There was no significant difference ($P < 0.01$) amongst groups. Although the values were slightly higher in control groups when no dietary supplemented was given to goats. Since anemia is a common expression of Cu deficiency in all species were the deficiency is severe or prolonged. (Underwood, 1981) no such condition could be noticed in goats in the present study indicating a normal serum Cu level. Although Cu deficiency is the most commonly limiting mineral to grazing livestock through the World (Mc Dowell, *et al.*, 1984) which could be attributed to the soil Cu status which might have affected its level in the forage and animal system. Cu and Zn share a common pathway of absorption in the nutritive and hence the removal of one element from the site of absorption would lead to the enhancement in the absorption of the other (Solomons 1956).

4.1.1.3 Cobalt (Co):

Data pertaining to cobalt levels in the serum have been given in Table No. 3 and Fig. 3. It was higher in chelated mineral supplemented group III followed by group II and lowest in control group I. There was a significant ($P < 0.05$) difference amongst groups. It ranged from 0.386 ppm to 1.076 ppm being lowest in I and highest in III. The values were in accordance to the earlier findings (Kincard *et al.*, 2003). They reported that cobalt supplementation in the starter diet did not effect cobalt in serum or liver. However, in grazing animal cobalt supplementation affected its levels in serum and other vital organs.

Fig. 3 Mean standard value of Cobalt

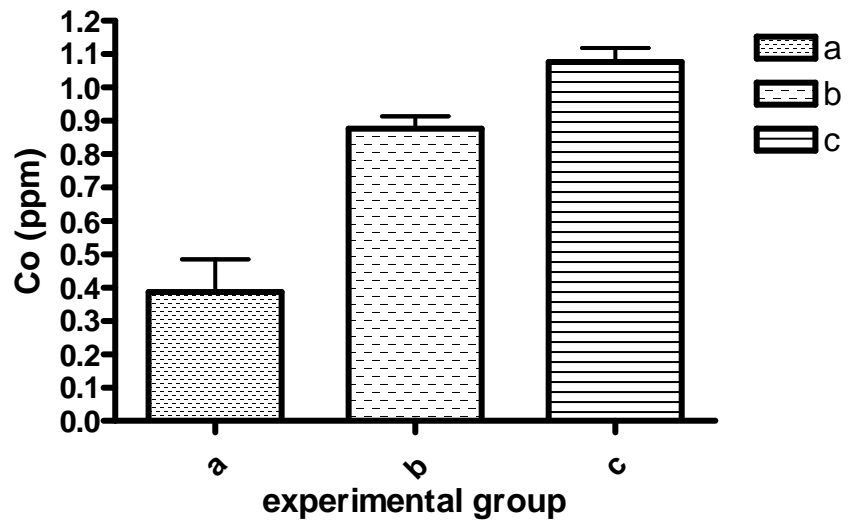


Fig. 4 Mean standard value of Iron

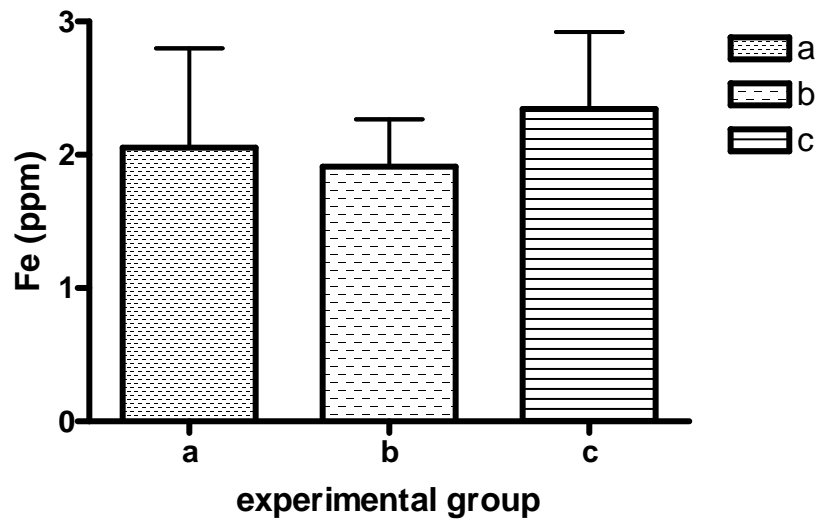


Table 3: Mean values and standard error of trace minerals parameters in three groups in goats

S.No.	Parameters	Group I	Group II	Group III
01.	Zinc	3.52±0.039	2.52±0.11	3.67±1.79
02.	Copper	1.062 ±0.08	0.901 ±0.02	0.937 ±0.01
03.	Cobalt	0.386±0.09 ^c	0.876±0.03 ^b	1.076±0.04 ^a
04.	Iron	2.053±0.74	1.825±0.33	2.343±0.58
05.	Manganese	0.505±0.04	0.432±0.01	0.489±0.07
06.	Molybdenum	15.54±0.46 ^{ab}	12.75±0.81 ^{ab}	9.048±1.20 ^c
07.	Iodine	4.05±0.13	3.97±0.06	3.91±0.15

Mean values in each row carrying common superscript do not differ (P<0.05) significantly

4.1.1.4 Iron (Fe):

The concentrations of serum iron in goats in the different groups have been given in Table No. 3 and Fig. 4. There was no significant difference amongst groups. It indicated that soil plant-animal system was well adjusted and concentrated with the iron content. The soil status of Chhattisgarh is very rich in the iron, which could supply the adequate concentration to the plant forages and to the biological system of the domestic animals maintained on such forages. However, the physiological system of animals allow only the require intake of this mineral which is well evident and documented in the farm animals. There are number of factors which affect the iron transport across the gastro-intestinal tract. The major factor is the low Fe storage of body. For the synthesis and regeneration of hemoglobin forms (Fe^{2+}) salts are more effective than the ferric (Fe^{3+}). The maximum tolerance of Fe by the ruminants is suggested to 500 ppm with a requirement of 40 ppm in goat. The results of the present study fulfill the physiological needs of goats though its level is greatly influenced by the dietary Cu (Probowo, 1989).

4.1.1.5 Manganese (Mn):

The concentration of Mn in goats under different groups has been presented in Table No. 3 and Fig. 5. No significant difference was obtained amongst groups though it was slightly higher in control group I as compared to groups II and III where goats were supplemented with inorganic and chelated form of minerals. This was probably due to the associative affect of diet. Similar trends were observed by the earlier worker (Anke, 1966). In contrary, Dass *et al.*, (1997) reported a higher serum Mn level in goats maintained on forage from red

Fig. 5 Mean standard value of Maganese

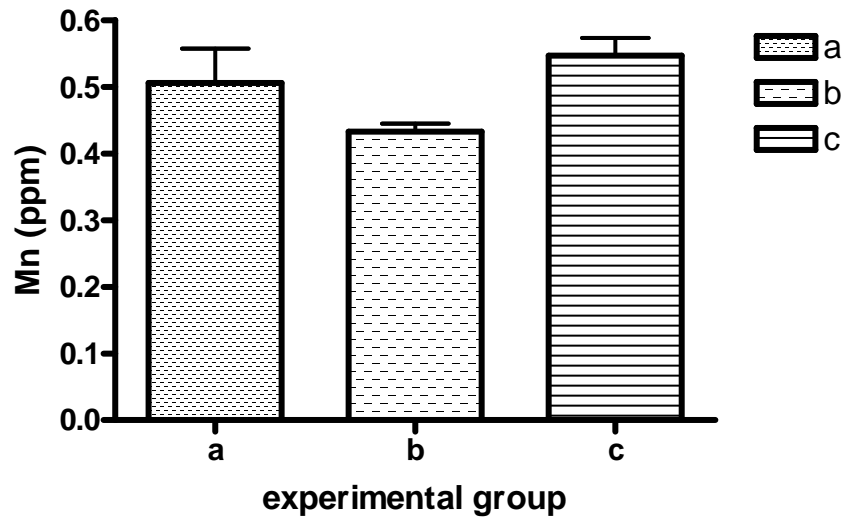
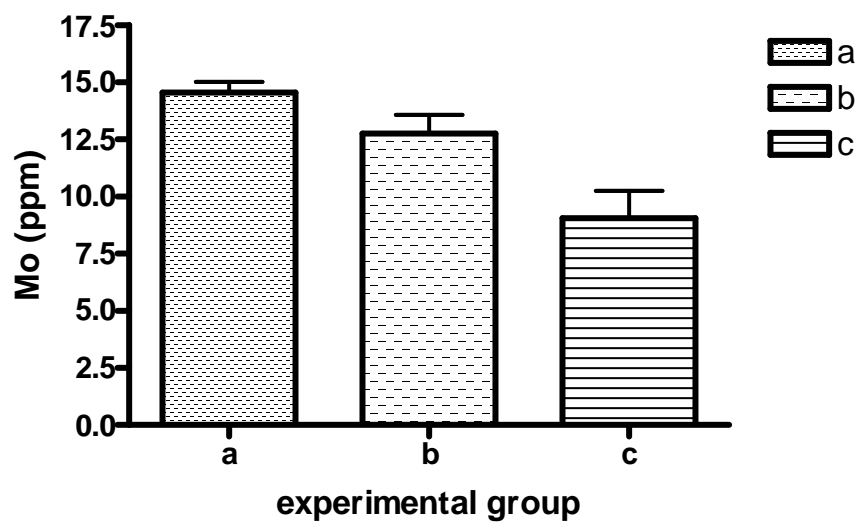


Fig. 6 Mean standard value of Molybdenum



laterite soil as compared to those from new alluvial soil. It indicated that soil texture affect the availability of Mn in the forage which in turn influence its level in the grazing ruminants.

4.1.1.6 Molybdenum (Mo):

The data pertaining to the concentrate of Mo in goats under different dietary groups have been presented in Table No. 3 and Fig. 6. It varied from 9.048 ppm to 15.54 ppm. There was significant ($P < 0.05$) difference amongst group. However, no difference could be obtained between control group and the group II where goats were supplemented with inorganic form of Mo. The difference could be established only with group III where chelated form of minerals were supplemented. The values were lowest in group III followed by group II and I. Since the requirement of Mo is very low in small ruminants the chelated form of mineral might have counteracted its absorption and allowed the normal physiological concentration in the ruminants. As it is well evident that a strong correlation is established between the Mo, Cu and S, the chelated minerals containing amino acid which might have formed the required amount of Mo in the blood across the gastro-intestinal tract and maintain an adequate Mo level in the serum to perform the metabolic reaction for the exertion of unwanted non protein nitrogenous compound through kidney tubules.

When ruminal sulfide concentrations are low, molybdenum may have little effect on Cu availability (Allen et al., 1987). In sheep, fed diets that contained only 1 g of sulphur per kg DM, increasing the dietary Mo from 0.5 to 4.5 mg of Mo/kg DM did not affect Cu availability (Suttle, 1991).

4.1.1.7 Iodine (I):

The data pertaining regarding iodine shows that there is no significant difference in the values of iodine Table No. 2 and Fig. 7.

4.1.2 Macro Minerals:

4.1.2.1 Calcium (Ca):

The data pertaining to the serum Ca in goats have been presented in Table No. 4 and Fig. 8. There was significant difference amongst groups in the level of serum Ca. It was highest in the group where chelated form of minerals was supplemented in the diet of goat 10.04 mg/100 ml in III group followed by group I 9.22 mg/100 ml and lowest group II although there was no significant difference between group I and II. Ca is mainly absorbed in small intestine. Its solubility and absorption is favored under acidic conditions or the pH near neutrality, which was observed in the present study. However, its absorption is decrease with low vitamin D, due to lower 1, 25-D hormone production in the body (Ammerman and Goodrich, 1983).

Bhattacharya et al., (1994) observed the mean serum Ca concentration of non descript goats at pre pubertal, pubertal and post-pubertal stages, which varied from 9.90 to 11.05-mg/100 ml. These finding are in accordance to the present study. These values were significantly higher in other species like cattle and buffalo Prabha *et al.*, (2000). Lall and Prasad (1990) reported that different chemical forms of Ca and P did not influence the normal metabolic profile, however, Plumlee *et al.*, (1958) gave an opposite opinion. Fisher (1978) concluded from his studies that no significant difference in the Ca and P concentration was found suggesting that even at lower Ca and P supply in control

Fig. 7 Mean standard value of Iodine

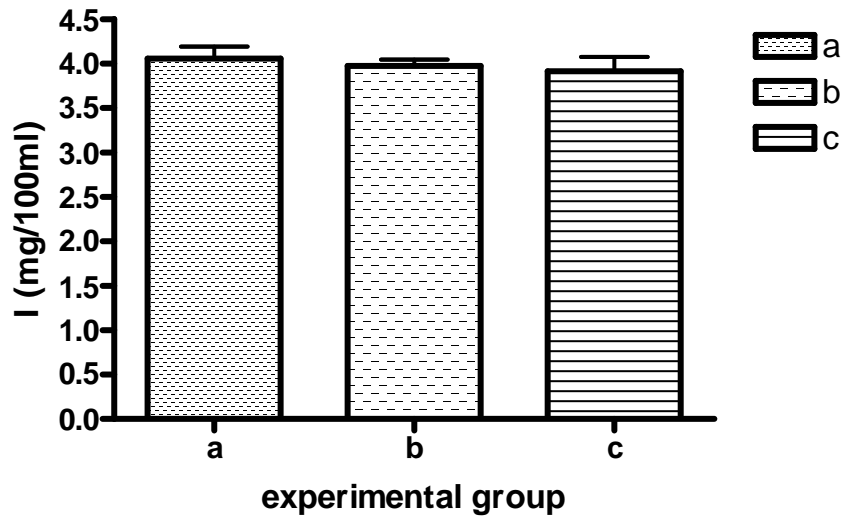
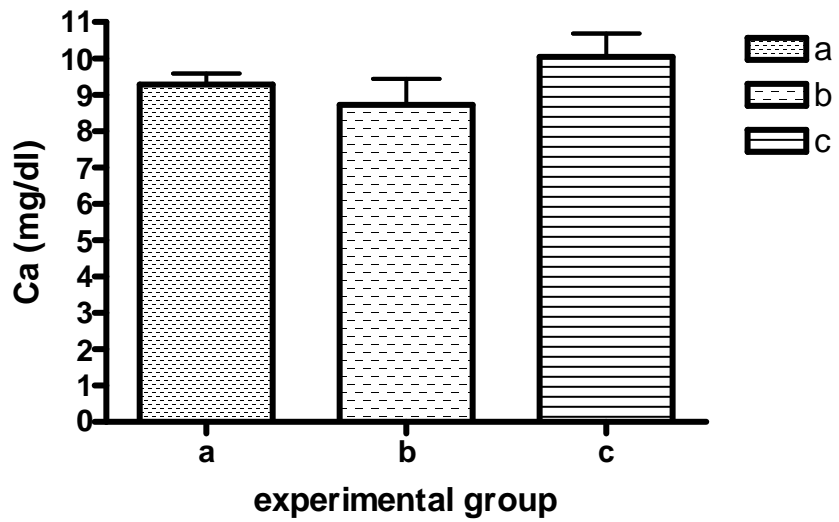


Fig. 8 Mean standard value of Calcium



the homeostatic mechanisms were adequate to maintain serum Ca and P level in the body.

4.1.2.2 Phosphorus (P):

The levels of P in goats of different groups have been given in Table No. 4 and Fig. 9. It was significantly ($P < 0.05$) higher (8.61 mg/100 ml) in-group II and lowest (6.12 mg/100 ml) in groups III indicating that dietary supplemented of P did not influenced much the serum P due to a constant physiological homeostatic mechanism in the body. The absorption of P is mainly, through a carrier dependent mechanism (Scott *et al.*, 1984). Changes in blood P is the first known response to a deficiency of P, which leads to a withdrawal of this element from the bone (Underwood, 1981). The findings are in accordance to the result of the present study. The serum blood P is normal as against 2-3mg per 100 ml during P deficiency (McDowell, 1985).

Table 4: Mean values and standard error of macro minerals parameters in three groups in goats

S.No.	Parameters	Group I	Group II	Group III
01.	Ca	9.22±0.29	8.72±0.71	10.04±0.63
02	P	7.62±0.27 ^b	8.61±0.18 ^a	6.12±0.39 ^c

Mean values in each row carrying common superscript do not differ ($P < 0.05$) significantly

4.2 Ruminal Parameters:

Adequate supply of nitrogen, energy and minerals in the diet of the small ruminant is essential for maintaining optimum ruminal microbial activity. Deficiency of nitrogen and minerals in diet not only depresses the voluntary feed intake but also causes high heat production without adequate formation of ATP

Fig. 9 Mean standard value of Phosphorous

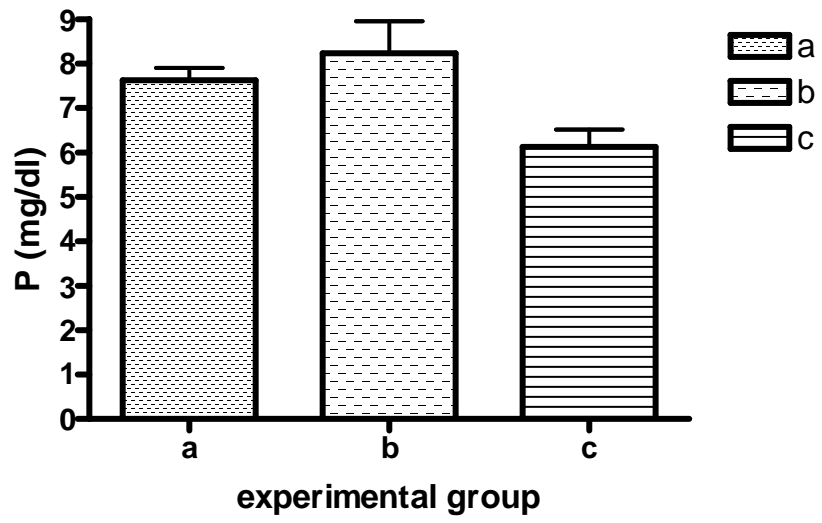
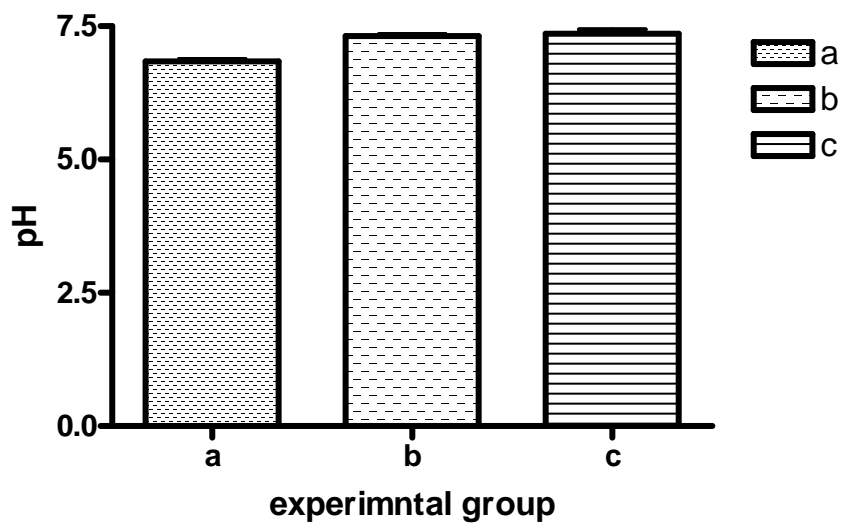


Fig. 10 Mean standard value of pH



due to energy uncoupling. Consistence and continuous supply of the catalytic amount of trace element has been shown to be necessary for maintaining optimum microbial activity for improved digestion of fibrous diet. In the present study attempts were made to improve the rumen fermentation activity for optimum nutrient utilization by providing inorganic and organic form of minerals. It is well evident that pH, volatile fatty acid, total protozoal count, ammonia nitrogen and total nitrogen together contribute for maintaining the favorable rumen environment for improved nutrient utilization and availability of nutrient to the animals. The comparative picture of rumen fermentation pattern has been dealt below:

4.2.1 pH:

The pH in rumen of goat under different group is presented in Table 5 and Fig. 10. The pH of rumen liquor ranged from 6.88 to 7.12 in-group I to III, being lowest in group I. It is higher in-group II as compared to others groups. No significant difference could be obtained. The supplementation of organic minerals to the diet of grazing goats in group III resulted in a normal buffered rumen environment, which indicated that the normal growth and health, of goats. In contrary, Briggs (1967) reported that higher protein diet could also influence the pH of rumen. In the present study it was noticed that in spite of inorganic or organic forms of mineral supplementation in-group II and III the pH was not much affected. Lall and Prasad (1990) reported that supplementation of Ca and P in the diet maintained pH at par in all the groups. In contrary to this, Bang (1990) pointed out that elevation of abomosal pH due to supplementation of extrogeneous mineral reduces the absorption of Copper.

4.2.2 Total Volatile Fatty Acid (TVFA):

TVFA concentrations were determined in all the animals maintained on grazing pasture supplemented with or without chelated and inorganic minerals. The data pertaining to TVFA (meq/100ml/SRL) concentration has been given in Table No. 5 Fig. 11. The mean values of concentration ranged from 2.01 to 7.08, from group I and III. The values were significantly ($P < 0.05$) higher in-group III as compared to I and II. Durand and Dewashima (1980) reported that there was no significant difference amongst groups in regards to volatile fatty acid concentration due to supplementation of minerals in the diet. However, Hungate (1966) showed that higher concentration of volatile fatty acid in the rumen increases the availability of carbon required for the bacterial growth. The increased volatile fatty acid in groups III was probably due to the involvement of minerals specially Ca and P and trace elements in energy metabolism, where it acts as a cofactor for activating the enzyme system responsible for the VFA production. The higher VFA in group III spares energy in the form of ATP for the improved productivity of animals. In this way supplementation of chelated minerals reduces the requirement of energy to the animals.

4.2.3 Ammonia Nitrogen (NH₃-N):

The ammonia nitrogen concentration was significantly affected amongst the groups in Table No. 5 and Fig. 12. It was significantly ($P < 0.05$) higher in-group II and as compared to group I. It ranged from 7.4 to 9.94 and the value was higher (9.94) in group III and lowest (7.40 ml/100ml) in-group I. This may be due to the fact that mineral matters being alkaline in nature might have increase the rumen pH as evident from the present result, which favors the degradation of feed

Fig. 11 Mean standard value of TVFA

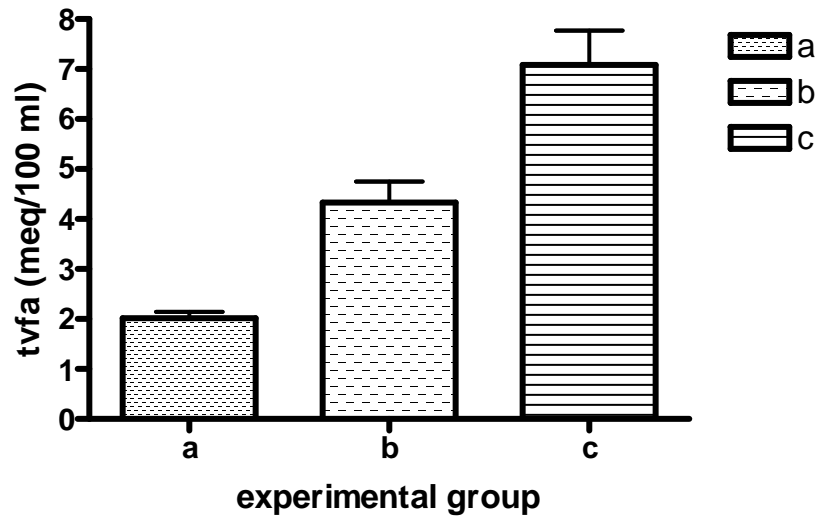
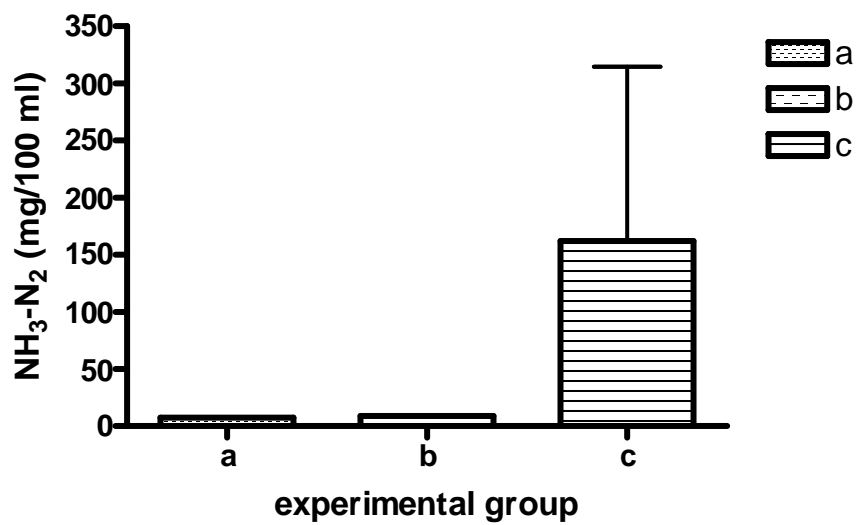


Fig. 12 Mean standard value of Ammonia nitrogen



protein by the process of proteolysis. Lall and Prasad (1990) reported that supplementation of minerals did not affect much the ammonia nitrogen concentration in rumen. However, the values were slightly higher in groups where minerals were supplemented as compared to the control group.

Table 5 Mean values and standard error of ruminal parameters in three groups in goats

S.No.	Parameters	Group I	Group II	Group III
01.	TVFA	2.01 ±0.12 ^c	4.33 ±0.42 ^b	7.08 ±0.68 ^a
02	pH	6.88±0.05	7.12±0.07	6.95±0.04
03.	NH ₃ -N ₂	7.40±0.20 ^c	8.77±0.35 ^{ab}	9.94±0.31 ^{ab}
04.	Total Nitro.	1.85±0.26 ^c	5.00±0.37 ^{ab}	4.51±0.57 ^{ab}
05.	TBC	7.58±0.12 ^{bc}	8.06±0.21 ^{bc}	12.06±0.29 ^a
06.	TPC	3.4±0.01 ^c	3.80±0.291 ^{ab}	3.77±0.208 ^{ab}
07.	B. Biomass	0.0427±0.00139 ^{ab}	0.0486±0.00178 ^{ab}	0.0508±0.00261 ^a

Mean values in each row carrying common superscript do not differ (P<0.05) significantly

4.2.4 Total Nitrogen (TN₂):

Data pertaining to total Nitrogen concentration in rumen of goats has been presented in Table No. 5 and Fig. 13. There was no significant difference between group II and III where diet was supplemented with inorganic or organic minerals respectively. However, it differs slightly (P<0.05) with group I. where animals were getting green grass and 150 gm of concentration mix/need/day. The values of total nitrogen concentration were higher in-group II 5.00 (ml/100ml) followed by group III (4.51 ml/100ml) and 1.85 (ml/100ml) in group I. This was a very

Fig. 13 Mean standard value of Total nitrogen

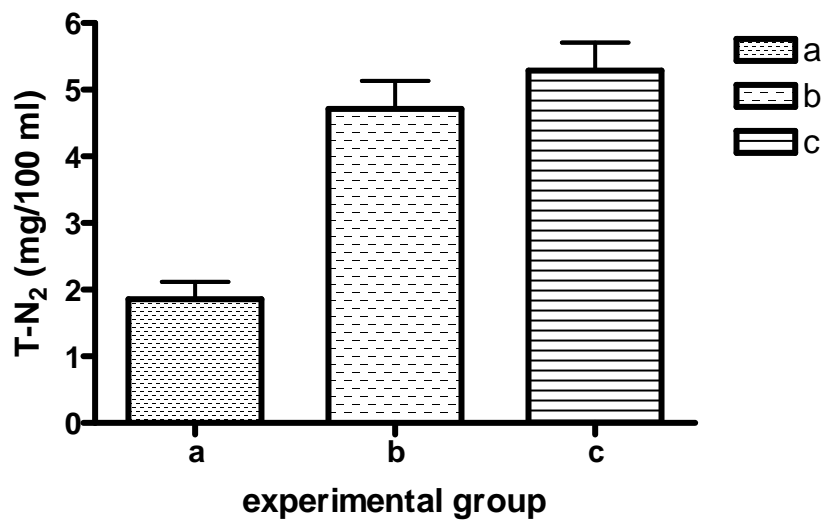
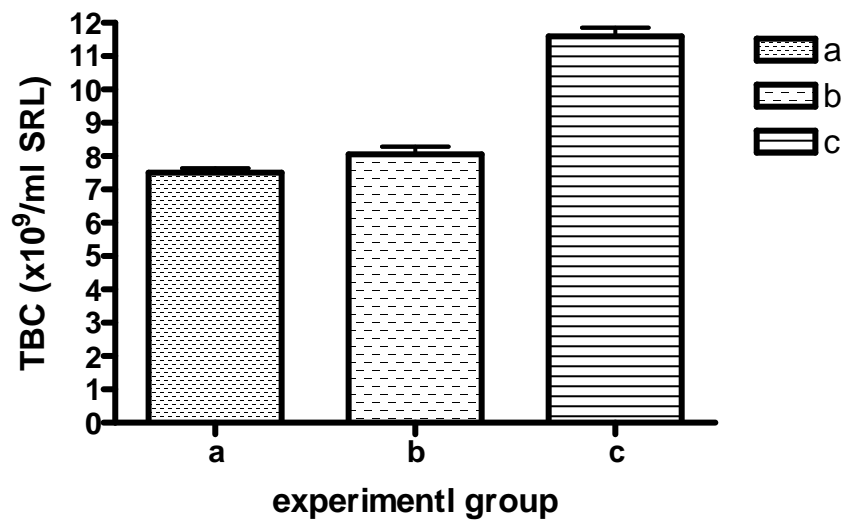


Fig. 14 Mean standard value of Total bacterial count



interesting picture as reflected by the results which revealed that the organic or inorganic forms of minerals might have accelerated the fermentative digestion of dietary protein which could be made available to the microbes for the protein synthesis, and as such the animals of these groups were found more active and healthier one. Hume *et al.*, (1970) suggested that total Nitrogen concentration of rumen increased in response to increase in nitrogen intake in sheep. But no results could be reported as how mineral supplementation could affect the total nitrogen concentration. In another study Leng (1984) reported a similar trends of nitrogen concentration as per the present study due to intake of organic minerals.

4.2.5 Total Bacterial Count (TBC):

The data of TBC has been given in Table No. 5 and Fig. 14. A significant difference has been observed amongst the group in which were fed diet supplemented with or without organic or inorganic minerals. However, difference were non-significant between group I and II. The higher values were obtained in group III where goats were fed diet supplemented with chelated minerals. It was found to be 12.05 ($\times 10^9$ /ml SRL) as against 8.06 or 7.59 in group II and I, respectively. The present study is in accordance to the result reported by Ogra (1980) and Lall and Prasad (1990). They further reported that supplementation of Ca did not affect the cellulolytic activity of rumen bacteria. The result indicated that the chelated minerals increase the total bacterial count, which increased the overall bacterial biomass required for meeting the protein requirement of animals.

4.2.6 Total Protozoal Count (TPC):

The total protozoal count was done in the rumen liquor of all animals in the different groups. Data has been presented in Table No. 5 and Fig. 15. No

Fig. 15 Mean standard value of Total protozoal count

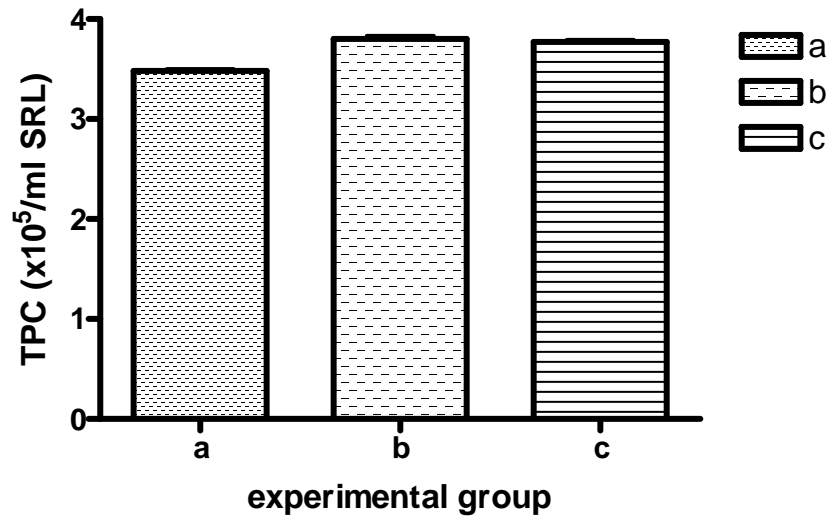
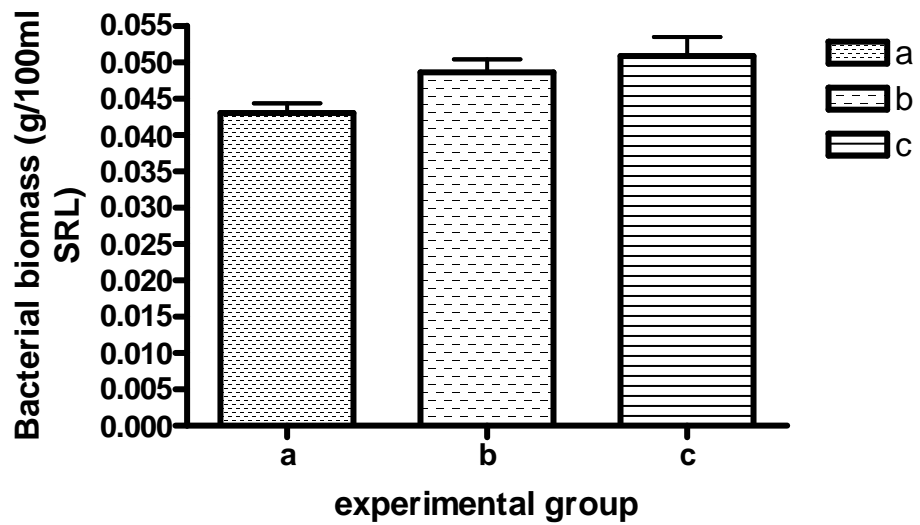


Fig. 16 Mean standard value of Bacterial Biomass



significant difference could be observed between inorganic and chelated mineral supplemented groups. However, the difference between group I and group II and group I and group III were highly significant ($P < 0.05$). This indicated that extrogenous minerals supplements have activated the growth of total protozoal count per milliliter of rumen liquor. It is well evident that minerals give a suitable media for the growth of ruminal protozoa. The values ranged from 3.4 ml ($\times 10^5$ /ml SRL) to 3.80 ($\times 10^5$ /ml SRL) being lowest in control group and highest in inorganic mineral supplemented group. The present findings are in accordance to Mathur *et al.*, (1991) and Ogra (1980) who reported a significant difference in mineral supplemented and control group. The mineral helps in development of flagellated and ciliated protozoan, which are predominant in the small ruminants, maintained on a mix grazing pasture deficient in most of the minerals.

4.2.7 Bacterial Biomass:

The bacterial biomass is the indicative of the availability of essential amino acid to the animals for the synthesis of tissue protein. Whenever there is defaunation which is being practiced especially in sheep and goats for the prolonged body weight gain and wool growth, there is compensatory growth of bacterial biomass in the rumen under a favorable environmental condition which could be attributed by the supplementation of essential minerals.

The data pertaining to the bacterial biomass has been presented in Table No. 5 and Fig. 16. It ranged from 4.0427 ($\times 10^9$ /ml SRL) to 0.0508 ($\times 10^9$ /ml SRL) from group I to group III being lowest in control and highest in-group III, where goats were supplemented with organic/chelated forms of minerals. The differences were significant ($P < 0.05$) amongst groups. Although there were no

Fig. 17 Mean standard value of TEC

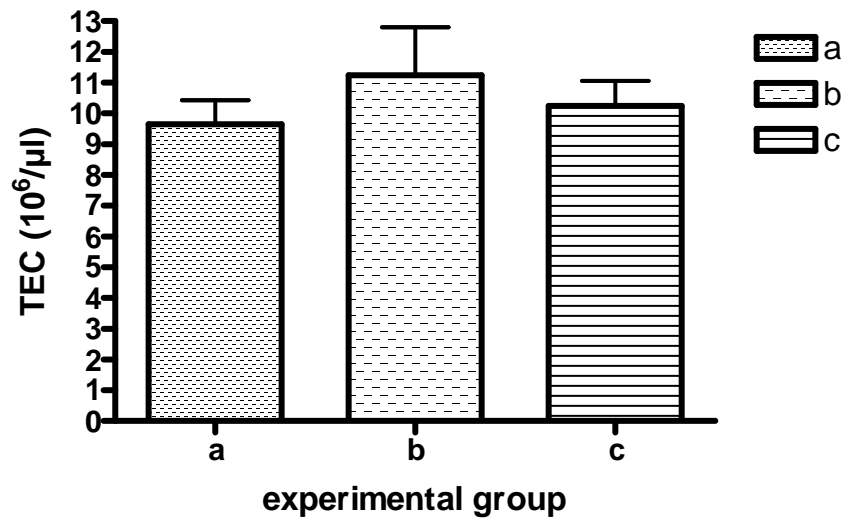


Fig. 18 Mean standard value of TLC

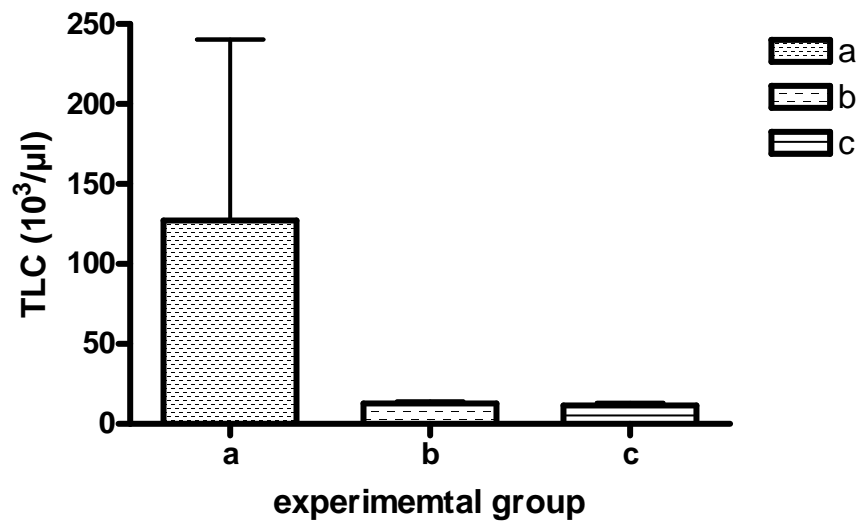


Fig. 19 Mean standard value of Hb

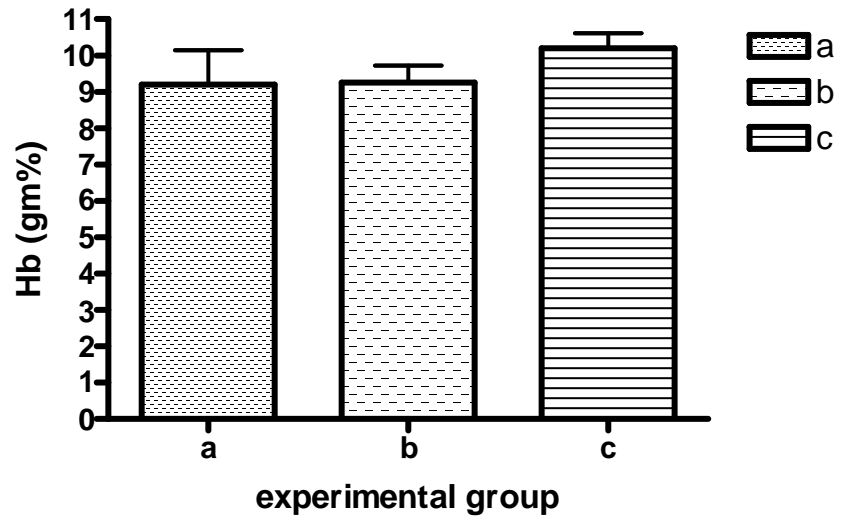


Fig. 20 Mean standard value of PCV

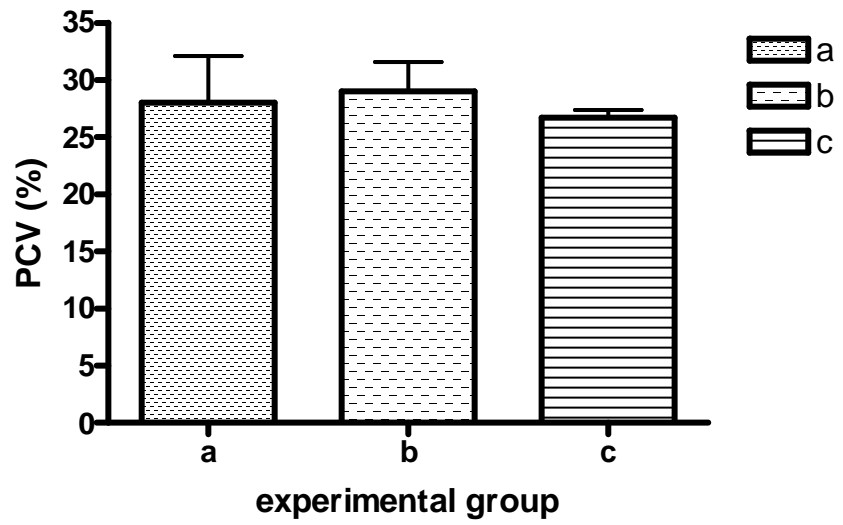


Fig. 21 Mean standard value of MCV

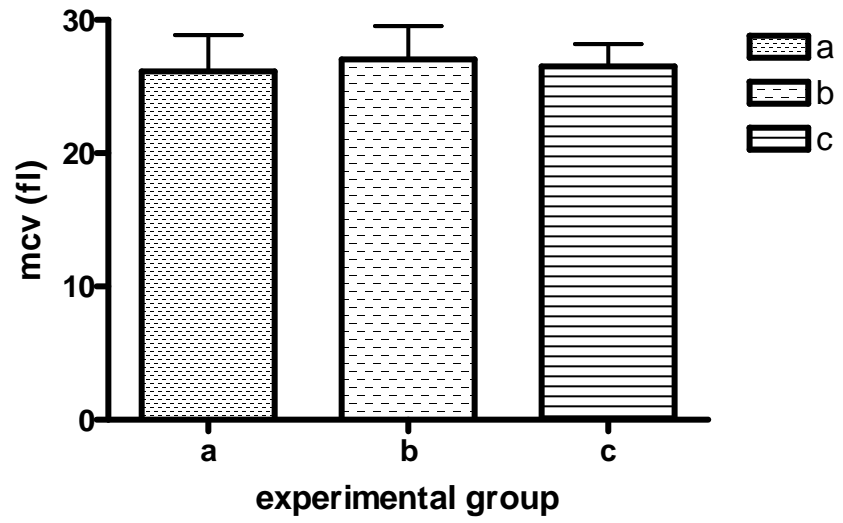


Fig. 22 Mean standard value of MCH

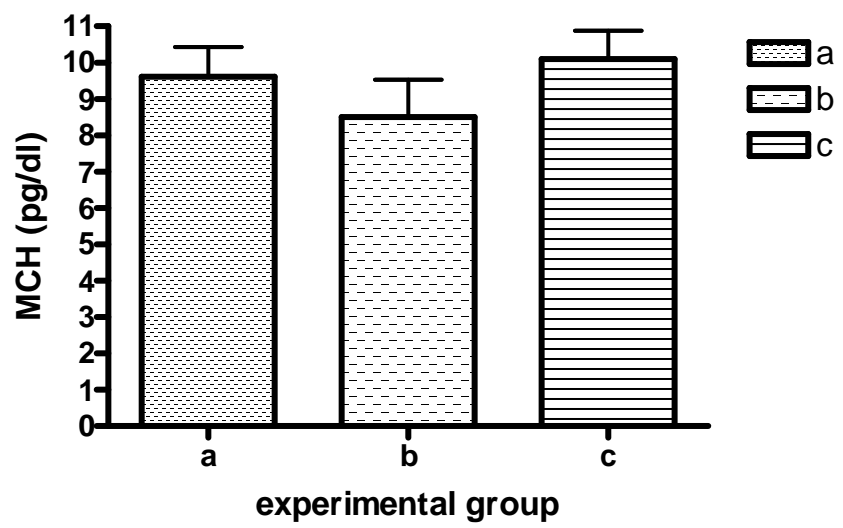
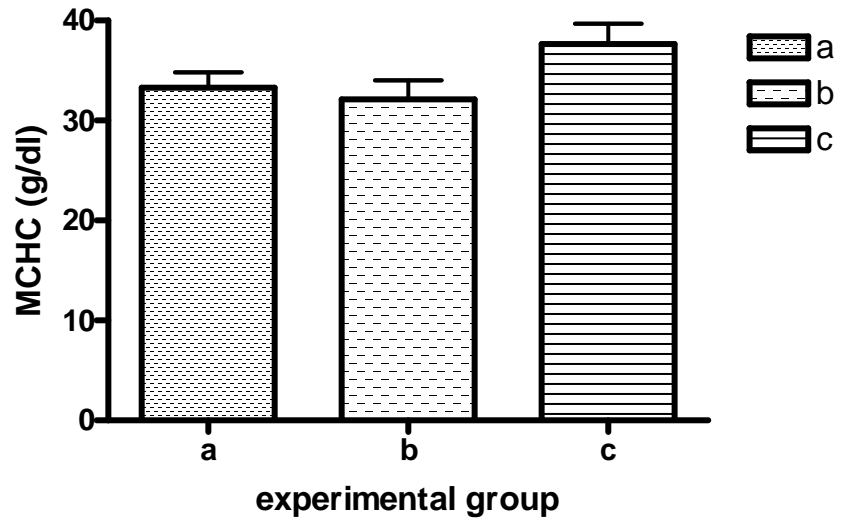


Fig. 23 Mean standard value of MCHC



significant difference between group I and group II where inorganic form of mineral was supplemented, however, it was highly significant when chelated mineral was supplemented in-group III. This might have contributed besides minerals some of the essential amino acids to the growth of bacterial cells. The chelated form of mineral also facilitates the bioavailability of minerals for the better growth performance and for the other physiological functions of the body. The study was in accordance with the findings of Hungate (1966).

4.3 Hematological Parameters:

The hematological parameters (TEC, TLC, Hb, PCV, MCV, MCH and MCHC) throughout the experimental period were similar in all the treatment groups i.e. group I group II and group III, shown in Table No. 6 and Fig. 17 to 23. The present study was supported by Dutta, *et al.*, 2006.

The study was contradicted by Tola, D. *et al.* 2003 that Hb concentration decreases as the Mo concentration in diet increases and for TEC and TLC did not differ much.

4.4 Biochemical Parameters:

4.4.1 Alkaline Phosphatase:

Plasma alkaline activity (KA units) was found to be maximum in group III 161.4 (KA units) and minimum in group I 93.145 followed by group II 101.870 (KA units) as shown in Table No. 7 and Fig. 24. The values were significantly ($P < 0.05$) different in all the three groups. This was probably due to the deposition of protein and energy in muscular tissue of body, in addition to increased rate of deposition of calcium in bone tissue. Osteoblasts cells secrete a large quantity of alkaline

Fig. 24 Mean standard value of Alkaline Phosphatase

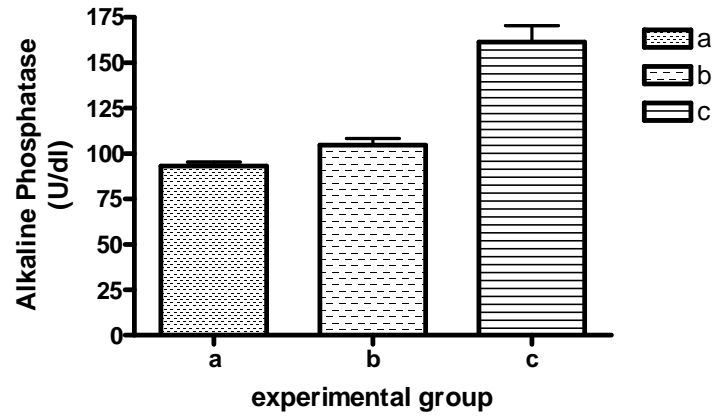


Fig. 25 Mean standard value of Albumin

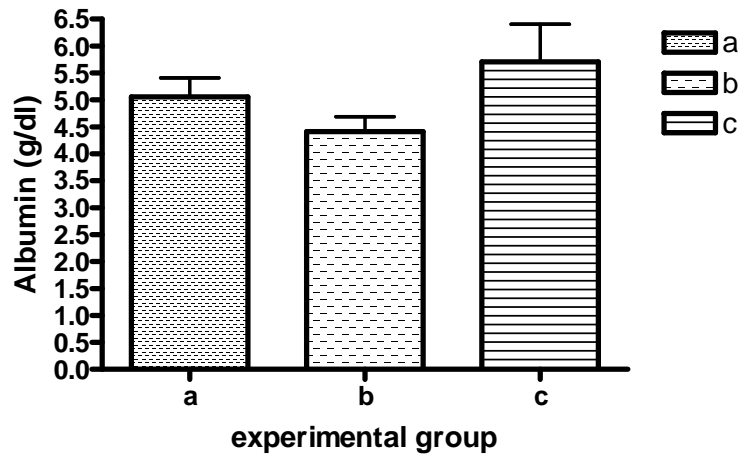


Fig. 26 Mean standard value of Total Protein

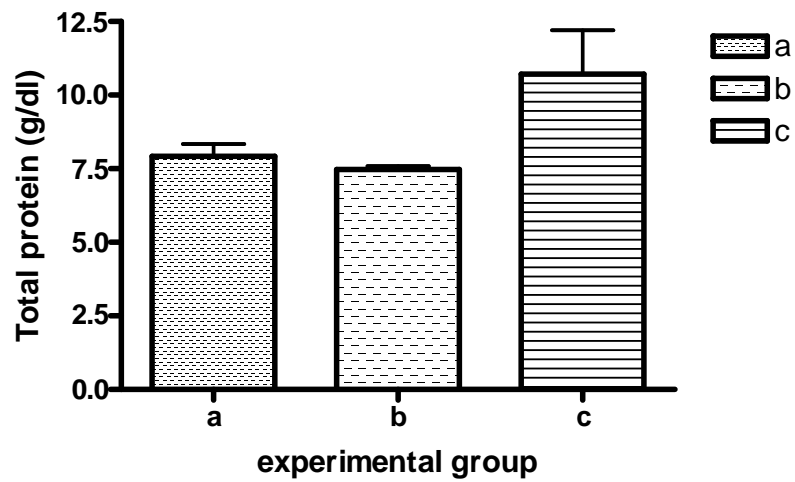


Table 6: Mean values and standard error of Hematological parameters in three groups in goats

S.No.	Parameters	Group I	Group II	Group III
01.	TEC	9.64±0.78	11.22±1.56	10.24±0.81
02	TLC	13.17±1.23	12.72±1.2	11.72±1.79
03.	Hb	9.4 ±0.84	9.25 ±0.46	10.11 ±0.40
04.	PCV	24.28±0.92	29.00±2.58	26.57±0.71
05.	MCV	26.14±2.71	27.64±2.50	26.51±1.66
06.	MCH	9.61±0.8	11.74±0.6	10.10±0.77
07.	MCHC	33.28±1.14	32.12±1.88	37.27±2.04

phosphate when they are actively depositing in bone matrix (Guyton and Hall, 1988). This enzyme is believed either to increase the local concentration of inorganic phosphate or to activate the collagen fibers in such a way that they cause deposition of calcium salts. Since some alkaline phosphate diffuses into the blood stream, its blood level is increased. Thus this enzyme is usually a good indicator of the rate of bone tissue formation in growing animals. Also it might be possible due to supplementation of Zn mineral in the diet of animals, as alkaline phosphate is a zinc dependent enzyme. The availability of zinc in the body of animals participates in increasing level of alkaline phosphatase. On contrary Rao *et al.* (2004) found that the trace elements in salt did not improve the biochemical profile.

Hatfield *et al.* (2001) documented that serum enzyme activity did not differ between control group and group fed with supplements of zinc and copper.

4.4.2 Albumin:

No significant difference was obtained amongst the group with regards plasma albumin concentration in goats. However, group III showed highest numerical value (5.70) as compared to the rest of the group, the groups I 5.05 and group II 4.04 (Table No. 7 Fig. 25) (Rao *et al.*, 2004).

Table 7: Mean values and standard error of Biochemical parameters in three groups in goats

S.No.	Parameters	Group I	Group II	Group III
01.	ALP	93.14±2.17 ^{bc}	101.87±3.72 ^{bc}	161.41±5.81 ^a
02	ALB	5.05±0.35	4.40±0.27	5.70±0.69
03.	T Protein	7.91±0.42 ^{bc}	7.46±0.12 ^{bc}	11.14±1.33 ^a

Mean values in each row carrying common superscript do not differ (P<0.05) significantly

4.4.3 Total protein:

In group I, II and III the average concentration of total protein (gm/100 ml) in serum were 7.91, 7.46 and 11.14 respectively (Table No. 7 and Fig. 26). These values were differ significantly ($P < 0.05$) (Rao *et al.*, 2000). This increase in total protein value might have favored the defensive mechanisms against certain unidentified diseases.

CHAPTER-V

SUMMARY, CONCLUSION AND SUGGESTION FOR FUTURE WORK

In the present study, readymade organic and inorganic trace minerals were supplemented to the goat. Twenty one non-descript goats of either sex were randomly divided according to body weight into three groups (I, II and III) of seven goats in each. They were fed with concentrate feed @ 150 ml/goat/day along with mineral mixture. Group I served as control which was without any supplementation, group II was supplemented with in-organic mineral mixture whereas, group III was supplemented with organic mineral mixture. Feeding trail was conducted for twenty one days which was followed by collection of blood on 22nd, 23rd and 24th day. The strained rumen liquor was collected from all goats using stomach tube for determination of pH, total volatile fatty acid, total nitrogen, ammonia nitrogen, total protozoal count, total bacterial count and bacterial biomass. The blood sample were collected for determination of alkaline phosphatase, albumin and total protein in serum. Blood was also used to perform the complete haematology.

The serum sample were analyzed for determining the trace minerals status of each goat in Atomic Absorption Spectrophotometer (AAS). The blood serum of these goats revealed that there was no significant difference regarding the macro mineral status. As for calcium status, however the value of group III was slightly higher from the other two groups. But incase of phosphorus the significant ($P < 0.05$) value was found. Group III having lowest value (6.12 ± 0.39) and group

III having highest value (8.61 ± 0.18). This reveals that the dietary supplement did not influence serum phosphorus level.

Regarding micro mineral analysis the results revealed that, in zinc, iron, manganese and iodine the values did not differ significantly. The results of the present study investigate no significant effects amongst groups irrespective of the organic and inorganic form of dietary supplementation in goats. But the picture was different in case of copper, cobalt and molybdenum where the difference in the status was found to be significant ($P < 0.05$). The data of copper varied from 0.901 ± 0.02 to 1.062 ± 0.08 . The difference was found to be non significant at ($P < 0.01$). Copper and zinc share a common pathway of absorption and hence the removal of one element from the site of absorption would lead to the enhancement in the absorption of the other. The level of cobalt was found to be significantly different in all the three groups. In grazing animals cobalt supplementation affected its level in serum and other vital organs.

The pH of SRL was increased from 6.88 ± 0.04 in group I to 6.95 ± 0.004 in group III. The increase was in accordance to the level of dietary protein. However, it was closer to the neutrality which could not adversely affect the buffering capacity of rumen as reflected by normal health condition of goats. The values of TVFA, ammonia nitrogen, total nitrogen, bacterial biomass, total protozoal count and total bacterial count in SRL was found to be increased in group II and group III.

The average values of blood calcium, phosphorus, total protein, albumin, alkaline phosphatase follows the same pattern as that of SRL. These values were highest in group III followed by group II were goats were fed organic and

inorganic trace minerals. There was no significant difference amongst group with regard to albumin.

The values of bacterial ($\times 10^9/\text{ml SRL}$) and protozoal ($\times 10^5/\text{ml SRL}$) in groups I, II and III were found to be 7.58 ± 1.2 and 3.4 ± 0.1 , 8.06 ± 0.21 and 3.80 ± 0.29 , 12.06 ± 0.29 and 3.77 ± 0.208 respectively. It was noted that group II and III has the highest value as compared to group I. It indicates that the supplementation of organic trace minerals increases the bacterial count but decreases the protozoal count.

The haematological values of serum sample was found to be non-significant throughout the experiment in all the experimental groups.

The present study concluded that production potential and health status of goats can be improved by supplementing the diet with a source of organic trace minerals with concentrate feed. The inclusion of such diet with a catalytic amount of chelated trace minerals further improved the growth rate and performance of goats.

Conclusion:

It is inferred from the present study that supplementation of chelated minerals with the concentrate feed appears to improve the bioavailability of mineral as compared to the non-chelated as evident by the increase in the value of the parameter influencing by these viable trace minerals and economical terms of cost. The feeding of mineral supplementation suggested that they met the requirement of goats adequately. Increase in TFVA, protozoal and bacterial population in the rumen reveals that the overall digestibility and utilization of micro nutrients has increased, indicating positive effects of these minerals availability and utilization.

Suggestions for future work:

The present study has been conducted in goats however the study is on bioavailability of organic trace minerals, hence it should have also been conducted in cattle and buffalo, in view of its importance in proper utilization of the nutrients and milk production. The following suggestions are recommended for future work.

01. Similar study should be conducted in cattle and buffaloes.
02. Similar study should also be conducted in growing animals.
03. The study on effect of organic trace mineral supplementation on reproductive performance of the cattle and buffaloes should also be conducted.
04. A study needs to be conducted involving trace minerals of soil, plants and animals.

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ABSTRACT

Studies on Effect of Trace Mineral supplementation on Haemato-biochemical profiles in Goat

By

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In the present study, readymade organic and inorganic trace minerals were supplemented to the goat. Twenty-one non-descript goats of either sex were randomly divided according to body weight into three groups (I, II and III) of seven goats in each. They were fed with concentrate feed @ 150 g/goat/day along with mineral mixture. Group I served as control which was without any supplementation, group II was supplemented with in organic mineral mixture whereas, group III was supplemented with organic mineral mixture. Feeding trail was conducted for twenty-one days, which was followed by collection of blood on 22nd, 23rd and 24th day. The strained rumen liquor was collected from all goats using stomach tube for determination of pH, total volatile fatty acid, total nitrogen, ammonia nitrogen, total protozoal count, total bacterial count and bacterial biomass. The blood samples were collected for determination of alkaline phosphatase, albumin and total protein in serum. Blood was also used to perform the complete hematology.

The serum samples were analyzed for determining the trace minerals status of each goat in Atomic Absorption Spectrophotometer (AAS). The blood serum of these goats revealed that there was no significant difference regarding the macro mineral status. As for calcium status, however the value of group III was slightly higher from the other two groups. But incase of phosphorus the significant ($P<0.05$) value was found. Group III having lowest value (6.12 ± 0.39) and group III having highest value (8.61 ± 0.18). This reveals that the dietary supplement did not influence serum phosphorus level.

Regarding micro mineral analysis the results revealed that, in zinc, iron, manganese and iodine the values did not differ significantly. The result of the present study investigates no significant effects amongst groups irrespective of the organic and inorganic form of dietary supplementation in goats. But the picture was different incase of copper, cobalt and molybdenum where the difference in the status was found to be significant ($P<0.05$). The data of copper varied from 0.901 ± 0.02 to 1.062 ± 0.08 . The difference was found to be non significant at ($P<0.01$). Copper and zinc share a common pathway of absorption and hence the removal of one element from the site of absorption would lead to the enhancement in the absorption of the other. The level of cobalt was found to be significantly different in all the three groups. In grazing animals cobalt supplementation affected its level in serum and other vital organs.

The pH of SRL was increased from 6.88 ± 0.04 in-group I to 6.95 ± 0.004 in-group III. The increase was accordance to the level of dietary protein. However, it

was closer to the neutrality, which could not adversely affect the buffering capacity of rumen as reflected by normal health condition of goats. The values of TVFA, ammonia nitrogen, total nitrogen, bacterial biomass, total protozoal count and total bacterial count in SRL was found to be increased in group II and group III.

The average values of blood calcium, phosphorus, total protein, albumin, alkaline phosphatase follows the same pattern as that of SRL. These values were highest in-group III followed by group II were goats were fed organic and inorganic trace minerals. There was no significant difference amongst group with regards to albumin.

The values of bacterial ($\times 10^9/\text{ml}$ SRL) and protozoal ($\times 10^5/\text{ml}$ SRL) in group I, II and III were found to be 7.58 ± 1.2 and 3.4 ± 0.1 , 8.06 ± 0.21 and 3.80 ± 0.29 , 12.06 ± 0.29 and 3.77 ± 0.208 respectively. It was noted that group II and III has the highest value as compared to group I. It indicates that the supplementation of organic trace minerals increases the bacterial count but decreases the protozoal count.

The hematological values of serum sample were found to be non-significant throughout the experiment in all the experimental groups.

The present study concluded that production potential and health status of goats can be improved by supplementing the diet with a source of organic trace minerals with concentrate feed. The inclusion of such diet with a catalytic amount of chelated trace minerals further improved the growth rate and performance of goats.

It is inferred from the present study that supplementation of chelated minerals with the concentrate feed appears to improve the bioavailability of mineral as compared to the non-chelated as evident by the increase in the value of the parameter influencing by these viable trace minerals and economical terms of cost. The feeding of mineral supplementation suggested that they met the requirement of goats adequately. Increase in TFVA, protozoal and bacterial population in the rumen reveals that the overall digestibility and utilization of micro nutrients has increased, indicating positive effects of these minerals availability and utilization.