SCANNING ALTERNATE SOURCES OF CALCIUM AND PHOSPHORUS SUPPLEMENTS AND THEIR AVAILABILITY & DISAPPEARANCE FROM RUMEN

A THESIS

Submitted to the Kurukshetra university
for the degree of

DOCTOR OF PHILOSOPHY

in the Faculty Of Dairying, Animal Husbandry and Agriculture

By

DALIP LALL

B.Sc. (D.H.), M.Sc. (Animal Nutrition)

DIVISION OF DAIRY CATTLE NUTRITION
NATIONAL DAIRY RESEARCH INSTITUTE
(I.C.A.R)
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I certify that the work reported in this thesis entitled "SCANNING ALTERNATE SOURCES OF CALCIUM AND PHOSPHORUS SUPPLEMENTS AND THEIR AVAILABILITY AND DISAPPEARANCE FROM THE RUMEN" was carried out by Shri DALIP LALL under my guidance for the requirement of the Degree of DOCTOR OF PHILOSOPHY, in the Faculty of Dairying, Animal Husbandry and Agriculture of the Kurukshetra University, Kurukshetra.

12/6/87

(T.Prasad)

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(Dalip Lall)

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ABBREVIATIONS USED

wks	weeks
wt	weight
kg	killogram
g	gram
mg	milligram
µg	microgram
ppm	parts per million
ml	millilitre
l	litre
hr	hour
$>$	greater than
$<$	less than
$\leq$	not more than
DM	dry matter
CP	crude protein
DCP	digestible crude protein
AIA	acid insoluble ash
SRL	strained rumen liquor
AAS	atomic absorbtion spectrophotometer
N	Nitrogen
NH_3-N	ammonia nitrogen
TVFA	total volatile fatty acids
OD	optical density
NS	not significant

CHAPTER - I

INTRODUCTION

INTRODUCTION

Mineral deficiency or toxicity is an area problem. The availability of different minerals through feeds and fodders to ruminants very with geographical location and the type of feeds or fodders available. Mineral supplements are nutritional devices to fortify the normal feeds and fodders in the areas to meet the mineral needs of livestock and poultry at the specific levels of animal productivity. The efforts to increase animal productivity by genetic manipulation further accentuates the problem of mineral nutrition and thus also the mineral supplements. In case of high producing animals, there is tremendous daily drainage of Ca and P through milk and adequate mineral supplements need be devised to replenish the daily loss inspite of the fact that internal regulatory mechaniems in animals can take care of the transient period of enhanced needs. ISI has laid down the specifications of mineral supplements and the same has been revised a number of times (ISI 1960, 1968, 1982). In certain countries, specific mineral supplements like high Cal, high Mag, high Phos, supplements are commercially manufactured to meet the demands of specific areas in question (Teethebarn, Animal feed supplements, U.K.).

The choice of a mineral supplement is determined by (i) per unit cost of the element, (ii) chemical forms in which the elements are blended which may influence mineral solubility and utilisability, (iii) the physical form such as fineness, and (iv) its freedom from harmful impurities. With Ca and P mineral supplements these factors can influence the choice of supplements. Mostly due to cost considerations and local availability the naturally available crude materials like, rocks, ores, bone meal are blended in mineral mixtures to serve as Ca and P sources. Apart from having variable utilisation of Ca and P, such sources are also likely to contain certain incriminating mineral elements in harmful quantities (Ammerman et al., 1977) and certain trace elements which may be of nutritional significance.

The natural resources like bone meal, dicalcium phosphate, rock phosphate and limestone are commonly used on wide scale by animal feed industry for making mineral mixtures. With the advancement of science and industry, there are possibilities to tap alternative sources from the available waste materials at relatively cheaper rates. Such materials may include gypsum, phosphogypsum, coal dolomite limestone, magnesite, rock phosphates and r phosphate (fertiliser grade) and possibly others cr used as sources of mineral supplements. In all p ties, such materials may be potential sources of and phosphorus supplements. Beside being chea

they may also provide other trace minerals of nutritional significance in utilisable form.

Before the use of such unconventional sources of mineral supplement is advocated, it is necessary to comperatively scan them for availability of useful elements and also ensuring the absence of toxic levels of incriminating minerals in them. Further, the mineral availability to ruminants not only depends upon the total supply of the mineral element in question but also on certain other factors, like the chemical form in which the element exists in the source, mineral solubility in the gut and availability to sustain a productive function of the animal. It is known that Fe present in ferrous sulphate is a better source for animal feeding than ferrous carbonate or ferric oxide (Ammerman et al., 1967). Availability of magnesium from dolonite limestone is much lower than that from pure magnesium oxide (Gerken and Fontenot, 1967). In addition, the reaction conditions in the rumen and other parts of the gut are also known to influence mineral solubility in the gastro-intestinal tract which may greatly modify mineral utilisation (Bremner, 1970). The natural sources discussed above and also animal feeds may contain 'ligands' which may form chelates with a particular mineral element modifying its availability. It is known that presence of oxalates and phytates in the feeds lower Ca and P utilisation (McDonald et al., 1978). Even certain amino acids may constitute 'ligands' for mineral

complexations (Georgievskii, 1982). Certain claims have been made that prechelated mineral supplements improve trace element utilisation (Darwish and Kratzer, 1965; Foll, 1966) and that addition of certain chelating agents like EDTA improves the utilisation of Zn and certain other trace minerals (Kratzer et al., 1959; Georgievskii, 1982).

The situations discussed above demanded detailed investigations about the suitability of alternate sources of Ca and P for use in mineral supplements which may be more economical and adaptable by cattle feed manufacturing industry and the other organised sector depending upon suitability. The proposed investigations have been broadly based on the objectives given below. Due to the multiplicity of approach, the area of interest was restricted to changes only in the rumen and sources contemplated at present included those which may potentially be suitable as Ca and P supplements. The objectives were:

1. To scan the suitability of different natural sources of Ca and P supplements.
2. To determine ruminal distribution and disappearance of Ca and P from alternate Ca and P supplements.
3. To assess the influence of Ca and P supplementation on rumen fermentation.
4. To determine the level of chelates influencing Ca availability in the rumen.
5. To compare the utilisation of certain alternate mineral supplements by growing ruminants.

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CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Adequate supply of mineral elements in the nutrition of livestock is known to be essential for animal health and production. In grazing ruminants, the mineral elements present in the dietary herbage are the only sources of minerals available to the animals. But in many locations in India, as also in different parts of the globe, the occurrence of frank clinical conditions either due to shortage or excess of one or more mineral elements, pose problems, needing urgent attention of animal nutritionists. Mineral nutrition problems, therefore, become area problems derived mostly due to soil conditions and the type of forages grown on such soils. In order to ameliorate the problems of mineral deficiency, free choice salt licks are provided in the grazing ranches in many countries.

In stall fed conditions, mineral blocks, licks, drenches or mineral mixtures prepared with the nutritionally essential mineral elements are commonly used to compensate for the inadequacy of mineral elements in the normal feeds and fodders. Such supplements are prepared from locally available cheaper mineral sources, e.g., ores, bone meal, chalk powder, oyster shells, etc. They are primarily used to supply major

mineral elements such as Ca, P and Mg, and are also fortified, in many cases, with common salt and pure trace mineral compounds.

The scientific basis for the formulation and use of mineral supplements has been reviewed with particular emphasis to Ca and P supplements for livestock feeding under the following headings:-

1. Mineral elements of nutritional significance
2. Physiological role of Ca and P
3. Regulation of Ca and P metabolism
4. The rationale of Ca and P requirements
5. Ca and P deficiency in livestock
6. Feed factors modifying Ca and P availability in the nutrition of livestock
7. Cri-teria for selection of mineral supplement
8. Conventional Ca and P supplements
9. Unconventional Ca and P supplements
10. Factors modifying mineral utilisation from mineral supplements
11. Interactions influencing Ca and P utilisation
12. Use of chelates in relation to mineral availability

1. Mineral elements of nutritional significance

There are about 21 mineral elements which are known to have essential metabolic roles in animals (Church and Pond, 1982). Out of these, 13 elements, namely, calcium (Ca), phosphorus (P), magnesium (Mg),

sodium (Na), potassium (K), sulphur (S), chlorine (Cl), iron (Fe), copper (Cu), zinc (Zn), cobalt (Co), iodine(I) and manganese (Mn) are known to be dietary essential nutrients. Although the functional roles of chromium(Cr), selenium (Se), molybdnum (Mo) and fluorine (F) have been demonstrated in many animal species, but their absolute essentiality has not been established in ruminants (Underwood, 1981).

The functions of different minerals in the physiology of animals are interrelated and seldom they can be considered individually as single functional components having quite independent and self-sufficient roles in the organised body processes (Hays and Swenson, 1970). A definite relationship exists between calcium and phosphorus in the formation of bones and teeth. The synergestic influence of iron, copper and cobalt in the synthesis of hemoglobin and red blood cells is also known(Cantarow and Schepartz, 1967). However, in some cases, individual elements serve specific roles, e.g., I in thyroxine and Co as integral part of vitamin B_{12} molecule.

Co, Cu and I inadequacies in soils and flora of certain parts of the world lead to the deficiencies of these minerals in domestic animals (Hansard, 1983). Also, selenium excess in the soils may result in high Se levels in plants which become toxic to animals (McDonald et al., 1980). Nutritional disorders involving mineral nutrition may arise as simple deficiency or excesses of particular

elements present in the diet. These conditions may themselves be either the reflection of the soil status on which the plants are grown or they may be related to the presence of specific plants which are seleniferous or goitrogenic.

2.2 Physiological roles of calcium and phosphorus

The skelton contains 99% of the total Ca and 80% of the total P present in the body. Boeltar and Greenberg (1941) suggested that Ca and P, which are predominent mineral elements of bones and teeth, exist primarily in the form of hydroxy apatite crystals having structural formulae

$$Ca^{++}_{10-x}(PO_4)_6(OH^-)_2(H_3^+O)_{2x}$$

Where, x may vary from 0-2. When x = 0, the compound is called octacalcium phosphate and when x = 2, the compound is termed as hydroxyapatite. In addition to their roles of supporting the structural frame of skeltal tissues, the bones also serve as body reservoir of these elements, mostly located in the bone trabeculae. There exists a dynamic equillibrium of Ca and P exchange between bones and body fluids. During the period of dietary deficiency of these minerals or in situations demanding increased Ca and P needs of particular physiological state, such as pregnancy and lactation, these minerals are readily mobilised from their stores in the bones and maintain near constant levels in body fluids, blood and other soft tissues(D'Souza and Flock, 1973; Wasserman,1977).

A small proportion of Ca, not located in the skeletal tissues, is widely distributed in the fluids and soft tissues of the body where it performs a number of essential functions. Forty five to fifty percent of the circulating Ca, present in plasma, is in soluble, ionic form, whereas the remainder is primarily bound to plasma albumin (Hays and Swenson, 1970). Ca present in soluble and ionic form is involved in several essential physiological functions including conduction of nerve impulses, contraction and relaxation of striated muscles and heart muscles (Church and Pond, 1982). Ca serves as an activator or stabiliser of certain enzymes and is required for normal blood clotting, i.e., in the conversion of prothrombin to thrombin, which reacts with fibronogen to form fibrin and blood clot (Cantarow and Schepartz, 1967).

About 17-20% of total body P (present in body) is widely distributed in fluids and soft tissues where it performs many biochemical functions related with growth and production (Church and Pond, 1982). Involved in the chemical structure of nucleic acids, P plays a very active biochemical role in the transmission of genetic information and in regulation of protein biosynthesis. Phosphoric acid is a component of ATP energy rich molecules and large number of coenzymes, and thus is actively involved in metabolism of proteins, lipids, carbohydrates, minerals and energy. Phosphorylation is another important step in the absorption of nutrients and renal excretion, transport of lipids, exchange of amino acids, etc. (Lehninger, 1977).

It is quite evident that inadequate supply of P to the body may result in reduced growth, production and overall animal performance (Georgievskii, 1981).

2.3 Regulation of Ca and P metabolism

Homeostatic control mechanisms very strictly influence Ca metabolism and plasma Ca levels but the control is not so effective on P metabolism. Radiotracer studies indicate that blood Ca and P keep exchanging with Ca and P pool to the extent of 35 and 100 times respectively than the quantities of Ca and P circulating in blood (Grace, 1981). Such high flux in the renewal of the pool mostly originate from intestinal absorption and bone reabsorption of these minerals, both of which are under the regulatory influence of potent calcitropic hormones - the parathyroid hormone and 1,25-dihydroxyvitamin D, a vitamin D metabolite produced in kidney.

Vitamin D(D_2 and D_3) circulates at relatively low concentration (1-3 ng/ml) in cows (Horst and Littledike, 1982) probably due to rapid conversion to 25-hydroxyvitamin D in liver (DeLuca, 1981). There are a number of 25-hydroxyvitamin D analogues which all are converted to 1,25 Dihydroxyvitamin D metabolites in the kidney. This final vitamin D metabolite together with parathyroid hormone interplay to regulate Ca and P entry rates to the body pools (Aarskog and Aksnes, 1980) and their exit through faeces, urine and bone in non-pregnant animals and through foetus and milk in case of pregnant and lactating animals.

Factors influencing Ca and P availability:- Several factors such as level of intake, age, acidity, presence of binding substances and endogenous excretion influence efficiency of Ca absorption from the intestines. Ramberg et al. (1984) found that reducing high dietary Ca load resulted in greater efficiency in Ca absorption. The absorption was found to be enhanced on increasing dietary acidity (Lomba et al., (1978). Experiments with rats suggested that on a dietary Ca load, the aged animals are less capable to increase their response of Ca absorption than the young animals (Horst et al., 1978). Both in ruminants and non- ruminants, dietary oxalates depress Ca availability (Ward et al.,1971). Nucleic acids produced by ruminal bacteria, dietary fat, high ingestion of fluoride and low supply of dietary P also have been found to reduce Ca availability for absorption (Braithwaite, 1976).

The absorption of dietary P in ruminants is reported to be in direct relation to P intake (Care et al., 1980). Unlike other animals, endogenous secretion of P is mediated largely through saliva flow and not through kidney, so that 70-80% of total endogenous P excretion is through saliva (Wadsworth et al., 1977). The quantity of P endogenously excreted into saliva recombines with dietary P before P from both the sources are available for absorption. Salivary P is mostly in the inorganic form. There is 4-5 times greater concentration of P in saliva than that in plasma of cattle (Clark, 1953) but the salivary P to plasma P ratio is much higher (5-19 times) in sheep (Tomas,

1974). Phosphorus turnover studies in sheep suggested that P absorption was inversely related to saliva flow (Tomas et al., 1967). Salivary P content is also influenced by parathyroid hormone which increases salivary P concentration by increasing endogenous P excretion (Wadsworth, 1977). In ruminants, it is the salivary P concentration which regulates P homeostasis. In turn, this regulation is mediated by plasma P levels, because low P diets which resulted in hypophosphataemia in cattle caused decrease in salivary P (Care et al., 1980).

2.4 The rationale of Ca and P requirements

Notwithstanding the benefits accruing with the use of suitable forms of Ca and P supplements, various agencies have detailed the total daily requirements of these mineral elements for feeding various categories of livestock. Such requirements must be viewed as tentative and rough guidelines only. Recommendations about Ca and P requirements are made periodically by Agricultural Research Council (ARC, 1965,1980) in U.K., National Research Council (NRC,1968,1975) in USA, Institut National de la Recherche Agronomique (INRA, 1978) and Sen and Ray (1964) in India. The recommendations vary considerably between the different sources and between publications from the same source. Table 2.1 presents an example of such variability for a 50 kg lactating ewe.

Table 2.1 Variation in recommendations of Ca and P requirements (g/day) for 50 kg ewe giving 1.36 l milk/day during first 8-10 wks of lactation

Recommendation	ARC		NRC		INRA
	1965	1980	1965	1975	1978
Ca	10.0	4.3	6.2	10.9	11.0
P	7.0	4.1	4.6	7.8	5.8

It was obvious that while ARC tried to reduce the recommendations of both Ca and P, NRC tried to raise the values. The recommendations of ARC (1980) were later objected and certain studies with radioisotopes and mineral balances also pointed about the inadequacy of ARC (1980) recommendations (Braithwaite, 1983).

Indian recommendations for Ca and P requirements are found in certain publications (Sen and Ray, 1964; Sen et al., 1978). Reviewing of recommendations did not bring in much difference. It is probable that variabilities in animal production levels and availability of feeds and fodders may initiate the necessity to review the recommendations.

Further, considering the changes in Ca and P requirements as per ARC or NRC in conjunction with the variabilities in biological availability of Ca (Hansard et al., 1957; Reid and Weber, 1976) and P (Fisher, 1978; Witt and Owens, 1983) to livestock and poultry, it is possible to speculate that the quantitative daily supply

of Ca and P from various sources of mineral supplements may differ considerably to meet the animal requirements.

2.5 Ca and P deficiencies in livestock

While unconditional Ca deficiency defects in livestock is not so predominant, P deficiency is very commonly encountered in growing ruminants. But due to their interdependence, it may be erroneous to consider them individually. Many disease conditions, as rickets, osteomalacia, osteodystrophia fibrosa, pica, etc. are very commonly associated with Ca and P deficiencies. Apart from frank clinical conditions, defects in animal performance and reproductive troubles also get manifested. Thus, Little (1968) found subnormal growth in young and unsatisfactory weight gains in mature ruminants when serum Ca and P concentrations started declining. Growing pigs, kept on maize and soyabean diets containing 0.34% P, showed lower weight gains and serum P concentration, in comparison to those kept on similar diets which was fortified with soft phosphate or dicalcium phosphate making total dietary P supply to the levels of 0.54 and 0.75% respectively (Harman et al., 1970). Inadequate Ca and P supply through diet also effects milk yield in cattle and egg production in poultry. On a P deficient and adequate protein ration supplied to cows in late pregnancy and early lactation, a significant depression in milk yield was noticed (Fishwick et al., 1977). In certain areas of South Africa, where P deficiency was

severe, exogenous supplementation of bone meal to the diet of lactating animals resulted 40-140% increase in the milk yield (Bisschop, 1964). However, such enhanced influence on milk yield cannot be attributed solely to P, since bone meal also provided protein. Lactating animals respond to dietary deficiency of Ca and P by reducing its milk yield without affecting its mineral concentration (Underwood, 1 981). In early stage of deficiency, or where the deficiency is moderate, the animal is able to draw from its own skeltal reserves of Ca and P in order to sustain the demands of lactation. But, when the same is prolonged or becomes severe, bone defects and other clinical signs become manifested and milk yield is also impaired (Bisschop, 1964). An almost complete failure of milk production has been observed in sows fed a Ca deficient diet during the previous pregnancy (Becker, et al., 1953).

2.6 Feed factors modifying Ca and P availability in the nutrition of livestock

The natural feeds and fodders wide differ in Ca and P content and to compensate for low mineral content in natural feeds and fodders, several inorganic compounds of geological or industrial origin are blended in the form of mineral supplements to feed livestock in order to sustain animal and health and productivity needs.

In early studies, Forbes et al. (1961) showed that high yielding cows fed on hay and concentrate diets showed negative Ca, P and Mg balance during lactation. Thomas et al. (1952) suggested that nonleguminous grasses

and forages grown under tropical condition are generally poor in Ca and P content and when fed to animals need be supplemented with additional Ca and P to overcome their deficiency. Morrison (1961) suggested that stall fed animals should be supplemented with Ca and P, into their whole mixed diet or into the concentrate portion so that intake of these minerals are adequate for the productivity of the animal. McDonald et al. (1981) reported that cereals and root crops are poor source of Ca and need supplementation under farm conditions.

Grazing sheep and cattle ingest variable amounts of soil and thus the dietary mineral supply from normal pasture herbage gets augmented. Field and Purves (1964) found that about 15% of total dry matter intake is contributed by ingestion of soil in sheep grazing on winter pasture which serves as additional source of minerals to such animals. Suttle et al. (1975) carried out studies with grazing sheep in Great Britain. They found that total soil ingestion may rise even upto 40% of total dry matter intake from winter pastures. Soil ingestion on grazing could be to the extent of 1,600 g/d in cattle and 400 g/day in sheep (Healy, 1967,1968; Mayland et al.,1975 and McGarth, 1982). Even then, P deficiency is very frequently seen in grazing ruminants of many parts of world (Cohen, 1980). It is possible that presence of high quantity of Al and Fe in such soils may be antagonistic to absorption and utilisation of dietary P (Rose et al., 1982).

Fertiliser applications to soils is likely to influence mineral uptake through soil contaminated herbage apart from bringing about improvement in herbage mineral content. Falade (1973) reported that superphosphate application to the soils in Australia, at the rate of 125 kg/ha increased the phosphorus content of the herbage by 50 percent in addition to the influence it made in doubling the pasture yield. In situations where fertilizer application to soils is uneconomical, additional phosphorus supplementation to animals can be achieved by regular drenching with mineral phosphates, use of phosphatic salt licks or by treatment of water supply with soluble phosphates (Underwood, 1981).

Mineral supplementation through drinking water is possible only where animals access to water supply is controlled, so that there is provision of pretreatment of water in a reservoir before supply to the animal. Hemingway and Fishwick (1976) described a procedure of such treatment of drinking water with soluble phosphates such as sodium phosphate or ammonium polyphosphate. But, since treatment to improve mineral supply through drinking water was quite expensive, Scharp (1979) further reduced the cost factor by using superphosphate, the cheapest material containing water soluble phosphate, as phosphorus source.

Under practical feeding conditions when young animals receive feeds containing roots, vegetables,

silage, hay or straw with little or no concentrate, P starvation also occurs. It is usual farm practice to supplement such feeds with P containing additives, which have beneficial effects on assimilation of nutrients, deposition of minerals and growth of the animals (Annenkov, 1982). However, good leguminous forages, when consumed in large quantities, may provide major proportion of this element (Kearl, 1982).

2.7 Criteria for selection of mineral supplements

Due to cost considerations and local availability, the mineral mixture manufacturers select Ca and P supplements not in the form of pure chemical compounds but in the form of locally available ores or other byproducts for blending the same into mineral mixtures. A wide range of compounds produced commercially from rock phosphate and other natural sources are available as Ca and P supplements. The merit on which the various mineral sources can be tested for their use as mineral supplement for animal feeding would depend upon (i) mineral composition, (ii) solubility in the gut, (iii) mineral availability, and (iv) presence of deleterious substance associated in these supplements.

Different grades of certain mineral supplements vary greatly in their chemical composition and mineral availability. Gillis (1954) while comparing the biological availability of phosphorus from untreated rock phosphate from different countries, found that only Florida rock phosphate was satisfactory in comparison to bone meal.

Dilworth et al. (1964) reported differences in Ca availability to chicks from five samples of feed grade phosphates, and attributed these differences to be due to variation in mineral composition. Reid and Weber (1976) analysed 24 samples of ground limestone and 5 samples of oyster shell and found wide variation in the Ca, Zn, Mn, Fe and Mg levels. They also reported that availability of Ca to chicks from five ground limestone samples ranged from 73.3 to 109.4 percent in comparison to Ca availability from pure analytical grade $CaCO_3$ which was taken as 100%. Ross et al. (1984) also found that Ca availability from different sources of calcitic limestone were not similar. Kearl (1982) has described wide variations in certain essential as well as contaminating mineral contents in samples of chalk powder, gypsum and rock phosphate. In case of chalk powder, Mg content ranged from 0.01 to 0.42% and Fe content ranged from 150-300 ppm. Apart from 2.5% Mg and 300 ppm Fe concentration, gypsum and limestone contained Zn, Cu and Mn. The samples of rock phosphate contained 16-32% Ca and 9-18% P. In addition, they contained fairly good amounts of Mg, Cu, Co, Fe and Mn. The F content even in defluorinated rock phosphate varied from 0.18% to 3.5%. Such findings suggested the existence of wide variations in the mineral composition of different Ca and P supplement sources.

Ammerman et al. (1977) suggested that conventional and unconventional mineral supplements need investigation as regard the accompaniment of various toxic

elements which are found to occur in natural sources of mineral supplement. They produced evidences for the presence of sufficient levels of toxic elements in certain many-mineral supplements. Thus, lead and arsenic levels in MnO samples were found to vary from 660-2180 ppm and 119-1400 ppm respectively. Concentrations of Hg, Cd, V and Sn were also found to be in toxic range in many salts of Zn, Mn, Fe and Cu. They found that few samples of phosphates contained impurities like Al, F, As and Hg which could be detrimental to the health of animals if used as supplement in mineral mixtures.

It is advantageous to fortify the diet of animals only with particular mineral element which is actually deficient in locally available feeds and fodder. Morrison (1961) pointed out that it is both uneconomical and injurious to use a supplement like dicalcium phosphate or bone meal, which supplied both Ca and P, to a ration which is deficient in Ca but has plenty of P. Underwood (1971) suggested that addition of bone meal to a chick ration which had plenty of phosphorus, caused the condition of slipped tendeon because P excess in the diet made Mn unavailable to the chicks. Diets consisting of brewer's mash and potato-pulp with a limited amount of roughage, become highly deficient in Ca, but adequate in P, whereas Bagasse feed contains excess Ca, but very low P (AnnenKov, 1982). Such diets also need specific mineral supplementation. P supplements are generally more

expensive than Ca supplements (Beamont, 1981) and, therefore, cost considerations also make judicious use of P supplements more imperative.

2.8 Conventional calcium and phosphorus supplements

Maynard and Loosli (1969) outlined the composition of certain Ca and P supplements for livestock feeding (Table 2.2). All the sources are not conventionally adapted in preparation of mineral supplements in India, partly because of availability considerations and partly because of lack of data about the relative merits of individual ingredients. Thompson (1980) indicated that

Table 2.2 Certain sources of Ca and P supplements

Supplements	Ca(%)	P (%)
Animal bone, steamed, Dehydrated	29.0	13.6
Dicalcium phosphate	26.5	20.5
Defluorinated phosphates	29-36	12-18
Limestone ground	33.8	-
Animal bone charcoal	22.0	13.1
Calcium phosphate	17.0	21.0
Sodium phosphate	-	22.4
Diammoniumphosphate	-	20.0
Oyster shell	35.0	-

calcium phosphates are the largest group, in terms of quantity used and there were many varieties within the

group. He subdivided the calcium phosphates into two major types: (1) natural and unprocessed materials, which include (a) rock phosphate from different sources and of varying composition, (b) collodial or soft phosphate (c) bone meal, and (2) chemically processed phosphates, which are subdivided into two groups:(a) dicalcium phosphate and (b) defluorinated phosphate.

In India, mineral supplements under various trade names are available in the market. The Indian Standards Institution (ISI) impose compositional standards of mineral mixtures for different category of livestock and modify the standards periodically. ISI (1960) recommended the use of Bone meal, chalk powder and dicalcium phosphate (DCP) in proportions of 45, 10 and 12 percent respectively, as Ca and P sources in the mineral mixture for cattle. Quality specifications of bone meal was also laid down (ISI, 1961). In its first revision, ISI (1968) allowed the use of calcined bone meal in addition to steamed bone meal, chalk powder and dicalcium phosphate. But, in the second revision, the ISI suggested the use of ground oyster shell, and replaced chalk powder with ground limestone in the recommended list of ingredients for use in the formulation of a mineral mixture and for the first time also made the provision of Zn supplementation to mineral mixture (ISI, 1982).

Ground limestone and chalk powder which contain around 40 percent Ca, are potential sources added

to mineral mixtures to fortify the need of this element. Kearl (1982) has suggested that steamed bone meal, dicalcium phosphate, ground limestone and defluorinated phosphate as good Ca and P supplements available in most regions.

Product which the feed industry refers to as "defluorinated phosphates" are produced by reaction of rock phosphate with phosphoric acid and sodium carbonate followed by calcination of the mixture at high temperature ($>1000^{o}C$). The product obtained is mostly tricalcium phosphate. Calcining drives off fluorine and converts the phosphate rock to a form which is biologically more useful to animals (Thompson, 1980). Incidently many materials which are used as P supplement supply significant amount of Ca also. Apart from bone meal and defluorinated phosphate, dicalcium phosphate is another supplement which can be used both as Ca and P source. Dicalcium phosphate is produced either chemically or from bones. In former, calcium carbonate and phosphoric acid are reacted together to form a mixture of dicalcium phosphate and monocalcium phosphate. In case of dicalcium phosphate prepared from bones, the material is treated with hydrochloric acid to form monocalcium phosphate which is further neutralised with lime water, and dicalcium phosphate is precipitated out (Thompson, 1980).

2.9 Unconventional Ca and P supplements

Inadequate availability of dicalcium phosphate or bone meal in certain areas may raise the cost of

manufacture of mineral mixtures due to expenses involved in transportation from a distance. With the development of industry and consequent availability of certain waste materials, there are possibilities for use of many of such waste materials in the manufacture of mineral mixtures. Morison (1961) suggested that oyster shell, ground clean shells, wood ashes, gypsum and dolomite limestone can be satisfactory Ca supplements. Gohl (1981) reported that calcitic limestone containing 36.4 percent Ca and can safely be fed free choice mixed with salt to livestock as mineral supplement. Few of the supplements that have more potentiality of being used as Ca sources, because of its availability, have been discussed below:

Unconventional Ca supplements:

Gypsum:- The possibility of using Gypsum, which contains $CaSO_4,2H_2O$ to the level of 87% (Sinha, 1967) as calcium supplement for livestock was considered by certain workers. Krogger and Carrol (1964) reported that gypsum feeding did not show any significant deterioration on ruminal microflorae population and activity or *in vitro* cellulose digestibility, but decreased the rate of passage of ruminal contents through G.I.tract. They also observed that on feeding high levels of gypsum, there was significant increase in blood plasma sulphur concentration and caused acidosis. Bouchred and Conrad (1973) used gypsum to increase calcium and sulphur content of the basal ration and found decrease in feed intake, due to excessive

accumulation of sulphur which eventually depressed the feed intake. On the contrary, Gartner and Rourke (1974) observed no influence of gypsum supplementation on nutrient utilisation from different roughages. However, Gypsum has not been found to be a suitable source of Ca in poultry (Morrison, 1961). Waldroup et al. (1964) observed that availability of Ca to chicks from pure $CaSO_4$ was essentially similar to $CaCO_3$, ground oyster shell, ground limestone and calcium gluconate.

Phosphogypsum:-This substance is produced as a byproduct in the industrial manufacture of phosphoric acid from rock phosphate which is heated with sulphuric acid to produce phosphoric acid and phosphogypsum. The annual production of phosphogypsum in India is to the tune of 2.8 million tons. It is considered as a superior grade gypsum having 80-90% purity as against 65-70% purity which is found in agricultural grade gypsum (Shrotria and Mishra, 1976). Phosphogypsum, however, does not contain very high phosphorus to merit a qualification for use as phosphorus supplement for ruminants, but phosphogypsum may be as good a Ca supplement as gypsum. Results of animal feeding experiments with phosphogypsum are too meagre.

Dolomite limestone:- Sinha (1967) reported that dolomite limestone contains 56 percent calcium carbonate and 46 percent magnesium carbonate. Therefore this level of Ca and Mg makes dolomite limestock as suitable Ca and

Mg source for ruminants. Morrison (1961) suggested that dolomite limestone is fairly satisfactory source for both Ca and Mg supplementation. He also indicated that due to presence of high amount of $MgCO_3$ it was not suitable for poultry. Most of the findings, however, seem to examine dolomite limestone as a potential Mg supplement source rather than a Ca supplement. Gerken and Fontenot (1967) found depression in carbohydrates digestibility and blood serum Mg values in the dolomite limestone fed group in comparison to pure MgO fed group. Rehnema and Fontenot (1983) observed greater utilisation of Mg from MgO than from dolomite limestone due to greater absorption of Mg in sheep fed MgO supplement. They reported that Mg absorption was 0.89 g/day in MgO fed group in comparison to 0.51 g/day in dolomite limestone group. Such depression in Mg absorption may also probably be due to the high Ca content of dolomite limestone.

Industrial wastes as Ca supplement:- Because of their compositional qualities and easy availability, certain industrial wastes could serve as possible Ca supplement sources. These sources may be 'causticization sludge waste' from paper industries containing 12-16% Ca, dried filter press mud waste which contains 30-40% Ca and cement 'kiln dust' which contains 36% Ca. Gohl (1981) has reported that dried 'filter press mud waste' is a waste material of any sugarcane industries, and it can replace at least 50 percent of the concentrate mixture

in the diet of ruminants, because apart from Ca which is present in the form of calcium sulphate and calcium phosphate, it also contains proteins and carbohydrates in considerable amounts. Noller et al. (1980) found that 'cement kiln dust' which is also rich in Ca and many other trace elements, showed favourable response when fed to animals as Ca supplement in comparison to reagent grade $CaCO_3$.

Unconventional P supplements

In a mineral mixture, it is the phosphorus supplement which is quite an expensive ingredient. Different chemical forms of phosphorus compounds in a mineral supplement do not show similar phosphorus availability, if fed to livestock. Studies indicated that phosphorus present in bone meal, mono-di and tri-calcium phosphate, sodium pyrophosphate and curacao island phosphate were more available to chicks but colloidal phosphate or soft phosphate of colloidal clay were not available (Motzock et al., 1956). In ruminants, different P supplements such as urea phosphates, mono-ammonium phosphate and dicalcium phosphate gave good response of growth and P retention, when fed to P deficient sheep (Fishwick and Hemingway, 1973). Later, Fishwick (1978) found that two forms of magnesium phosphates, tri-calcium phosphate and feed grade dicalcium phosphate served as good P supplements in improving weight gains and P retention in P deficient sheep.

Soft phosphate of colloidal clay:- Soft phosphate of colloidal clay or colloidal phosphate is a mixture of fine particles of rock phosphate and clay and contains less F in proportion to P than that of rock phosphate. Morrison (1961) suggested that soft phosphate should not be used for a long period as the only phosphorus supplement. Plumlee et al. (1958) reported that availability of phosphorus to cattle was poorest from soft phosphate and it resulted in significant reduction in feed gain efficiency, feed intake and serum inorganic P levels, when compared to steamed bone meal, defluorinated rock phosphate, dicalcium phosphate and phosphoric acid as the source of P.

Rock phosphate:- Rock phosphate, which mainly contains tricalcium phosphate, had been invariably used as a phosphorus supplement for cattle. After the World War II, when bone meal was in short supply, the use of raw rock phosphate became more prevalent as Ca and P supplement source. Alipov (1955) observed that continued feeding rock phosphated supplement for more than 3-4 months in place of bone meal resulted in decline in animal performance and also deposition of F in teeth and tissues. If practically all the F present in rock phosphate is removed, then defluorinated rock phosphate is entirely a safe substitute for bone meal (Morrison, 1961). Maynard and Loosli (1969) also reported that Ca and P of raw rock phosphate are absorbable but feeding these products is

harmful because of F present in it. Gohl (1981) has also suggested that rock phosphate should not be used in animal feeds unless it is guaranteed to contain less than 0.4 percent F. Thus, the level of F is an important criteria for using rock phosphate as P supplement in animal feeding.

Heat treatment required to eliminate F present in raw rock phosphate resulted in loss of biological availability of the phosphorus (Underwood, 1981).

Witt and Owens (1983) observed that P from defluorinated rock phosphate was not so soluble and therefore was less available to ruminants in comparison to other sources of P such as dicalcium phosphate or sodium phosphate.

Fertiliser grade phosphate

The agricultural grade phosphatic fertilisers like superphosphate (essentially monocalcium phosphate), NPK fertiliser (essentially ammonium phosphate) and DAP (diammonium phosphate), are produced in our country from indigenous raw rock phosphate. On the basis of their mineral composition, these sources could be used as possible alternate sources of phosphorus supplements (Keerl, 1982). Agarwala et al. (1971) reported that calcined super phosphate was more satisfactory source of phosphorus in lambs ration in comparison to uncalcined superphosphate because of the F present in the latter. Calcination process removed F from such supplement. Gohl (1981) has suggested that fertiliser grade superphosphate can be used as mineral supplement if no other

source of P is available. In using this, he suggested that it should be thoroughly mixed in sufficient water, and the extract of the soluble phosphorus thus obtained by pouring off the supernatent liquid may be used as phosphorus supplement for ruminants.

2.10 Factors modifying mineral utilisation from mineral supplements

The value of a mineral supplement depends not only upon its mineral content, but also on the amount that the animal can extract and retain for its own use. Different chemical forms of a mineral element differ considerably in their availability due to differences in their solubility in the G.I.tract.

pH of the gut and solubility of minerals

The release of minerals from their compounds in diet or mineral supplements and their availability for absorption depends also upon the solubility, which in turn is influenced by pH of the gastrointestinal tract. Existence of binding 'ligands' in the gut also influence the net availability of minerals for absorption. The secretion of gastric juice helps in the release of Ca and Mg in the abomasum whereas decreased acidity is associated with a reduction in proportion of ultra-filterable Ca and Mg which is available for absorption (Story *et al.*, 1966). In abomasum, where the pH ranges from 2-3, there was virtually no bound Ca or Mg. Kroe *et al.* (1966) claimed that the apparent differences in

the absorptive capacity of the different regions of small intestine are related to pH and therefore to the solubilities of the minerals in the gut lumen.

Witt and Owens (1983) compared the availability of phosphorus from different supplement sources such as monocalcium phosphate, dicalcium phosphate, defluorinated rock phosphate and sodium phosphate. They found that phosphorus was available to the extent of 88, 62 and 40% from the respective sources in comparison to sodium phosphate. In vitro phosphorus solubility of these sources one hour after incubation in abomasal fluid were 71.6, 41.3 and 29.7% respectively compared to 100% for sodium phosphate. In the rumen liquor, these solubilities were still lower.

Chemical form

The availability of mineral elements to animals does not only depend on the total supply of the mineral elements in question. Variations in mineral availability are seen also with the chemical form in which the major or trace mineral's exist in a supplement.

Gillies et al. (1954) reported that pyro or metaphosphate were not as satisfactory as orthophosphate or mono, di-or tricalcium phosphate for chicks. Chapman et al. (1955) compared dicalcium phosphate, phytin phosphorus and steamed bone meal as P source for swine and found that P utilisation from phytin phosphorus was poorest. Tillman and Brethour (1958) reported that availability of P from monocalcium phosphate was 58% in

comparison to 37% in case of calcium phytate, and there were no significant differences in terms of apparent or true digestibilities of this element. Goodrich et al. (1967) reported that elemental sulphur is better utilised in ruminants than sodium sulphate. Ammerman et al. (1972) found that the apparent absorption of Mg from reagent grade $MgCO_3$ and feed grade MgO and MgSO in sheep ranged from 52-56% whereas that from magnesite ore ($MgCO_3$), it was only 9-14%.

Fisher (1978) did not find any effect on plasma P levels or on faecal P excretion while comparing the four supplemental forms of phosphorus: mono-calcium phosphate, dicalcium phosphate, monoammonium phosphate and mono sodium phosphate. However, the digestibility of acid detergent fibre fraction was 59.2% for monosodium phosphate compared to 56.5% for monoammonium phosphate. The production of propionate was also considerably higher with mono sodium phosphate compared to other sources. Brink and Steele (1985) compared two Ca sources, lime and dicalcium phosphate in the diet of ruminants to provide 0.7% Ca and found that post ruminal digestion of starch and NDF was significantly higher in limestone fed groups.

The influence of the chemical form of mineral supplement on their availability has also been demonstrated for certain trace elements. Mitchel and Schmidt (1926) demonstrated that dietary ferric chloride and ferric ammonium citrate were more effective in raising

haemoglobin levels of anemic rats than ferric oxide or ferrous carbonate forms. Ammerman et al. (1967) showed that biological availability of Fe from ferrous sulphate, ferrous carbonate, ferric chloride and ferric oxide was in decreasing order in calves and sheep when evaluated on the basis of tissue Fe^{59} deposition. They further suggested that lower solubility of ferrous carbonate and ferric oxide might be the reason of low Fe availability from these compounds. Fritz et al. (1970) determined the relative biological availability of iron from different iron compounds and suggested that iron sources such as ferric ammonium citrate, ferric chloride, ferric sulphate, ferrous ammonium sulphate and ferrous fumarate were superior when compared with a standard source, ferrous sulphate ($FeSO_4 . 7H_2O$); whereas sources such as ferric oxide, ferric orthophosphate, ferrous carbonate and sodium iron pyrophosphate were found to be inferior. Bremner and Delgarno (1970) also found that utilisation of iron from iron phytate in bulls was inferior to that of ferrous sulphate, ferric citrate or ferric EDTA. Sullivan (1961) added different levels of various sources of Zn to the basal diet of a-day old poults and found that Zn in technical grade $ZnCO_3$, USP grade Zinc sulphate ($ZnSO_4.7H_2O$) was more readily available than reagent grade $ZnCl_2$; while Zn from zinc oxide and zinc sulphate ($ZnSO_4.H_2O$) both technical grade were relatively unavailable. Seal and Heaten (1983) studied the chemical factors affecting the intestinal absorption of Zn in rats and found that

the uptake from gut segments varied with the form of inorganic salts used. They found that the uptake was in the following order $ZnSO_4 > ZnCl_2 > ZnPO_4$. Dewey et al. (1958) described an orally administered heavy pellet made of cobalt oxide and clay which remains in the reticulo-rumen for several months and releases Co at a rate slow enough to meet animal's requirement. Smith and Loosli (1957), Becker et al. (1965) have reported that the carbonate, chloride, sulphate and nitrate forms of cobalt have all been proposed to be satisfactory dietary sources of Co, but because of certain desirable physical characteristics, cobalt carbonate is the source of choice in feed industry. Chapman and Bell (1963) have suggested that both the physical characteristics of compound and biological value of Cu, cupric sulphate was the most suitable source for dietary supplementation. Physical characteristics sometimes become important consideration for feed manufacturer in selecting a compound as mineral supplements. Bandemer et al. (1940) compared the biological availability of Mn from 'Rhodocrosite' (a natural ore of Mn) and precipitated manganese carbonate, and found that even at high levels (125 ppm Mn) the former source was completely ineffective in preventing perosis in chick whereas at 40 ppm Mn supply through manganese carbonate, perosis was completely prevented. They suggested that the difference was because the one was soluble in dilute HCl at high temperature, whereas $MgCO_3$

was readily soluble in cold dilute HCl. These findings further indicated that it was not the presence of extrenous material such as silica in the ore which was responsible for the low availability of Mn, but the reason lied in its solubility. Although, biological availability assays for Mn have not been conducted in ruminants, but manganese either as chloride or sulphate may be effective sources of Mn for cattle (Bentley and Phillips, 1951; Rojas et al., 1965).

2.11 Interactions influencing calcium and phosphorus

Apart from the absolute quantity of dietary supply of Ca and P either through feed or mineral supplement and the chemically available forms of these material elements as discussed earlier, the utilisation of Ca and P is also under the influence of number of factors. The factors have been discussed as:

(i) Ca and P interaction and significance of Ca:P ratio.

(ii) Interaction with other mineral elements.

(iii) Interaction with certain organic nutrients

(i) Calcium and phosphorus interactions and significance of Ca:P ratio-

Certain studies suggest that calcium status of animals determine the major regulatory influence on P turnover, mostly mediated through hormones. While parathyroid hormone (PTH) increases salivary P concentration (Tomas, 1974), it maintains plasma Ca levels

stable largely by mobilisation from bones (Krownfield et al., 1976). There is a negative feedback of blood Ca level on PTH secretion. However, variabilities of plasma P levels causes no change of PTH secretion (Sherwood et al., 1968). The action of PTH on bone resorption is dependent on the presence of 1,25-dihydroxyvit.D metabolites (Omdahl and DeLuca, 1973) and is inhibited by calcitorin of thyroid gland (Rasmussen and Tenenhouse, 1967). At high Ca intake there is depression in production of $1,25(OH)_2D_3$ and consequent reduction in P absorption (DeLuca and Schroes, 1976). Conversely when dietary Ca is low, there is an increase in production of $1,25(OH)_2$-D_3, absorption of P, secretion of PTH, salivary P concentration, and mobilisation of P from bones to soft tissues (DeLuca and Schnoes, 1976).

Liberal supplies of vitamin D reduces the significance of adverse Ca:P ratio and enables the animal to make best use of limited intake of these minerals (Borle, 1974). A dietary Ca:P ratio between 1:1 to 2:1 is believed to be ideal for growth and bone formation since this is the ratio of the two minerals in the bones (Kearl, 1982). However, ruminants can tolerate a wide range of Ca:P ratio from 1:1 to 7:1 particularly when vitamin D status is high indicating that the regulation influence of Vit.D is mostly mediated through Ca and there is no strict regulation influence on P (Wise et al., 1963). But the P requirements must be met adequately. In the same study ratio of Ca and P beyond 7:1 decreased feed utilisa-

tion and growth rate significantly. Similarly the ratio of Ca:P lower than 1:1 in the diet of beef cattle had shown more serious adverse effects in terms of daily gain and feed efficiency (Wise et al., 1963).

(ii) Interaction with other minerals:-

Studies indicate that several other mineral elements are known to influence the absorption and utilisation of Ca and P in the diet. In such interactions the effects of Cl, Zn, Mg, F, NH_4Cl, Fe, Mo, Cu, Al, Be, and Mn have already been investigated. Lomba et al.(1978) observed that chloride and sulphate tended to stimulate Ca digestibility. Feeding of high levels of Zn to lambs was found to depress the net retention and true digestibility of dietary Ca and reduced the P absorption (Thompson et al., 1959). Ca and Mg are similar in many respects and there is an evidence in monogastric species that Mg may replace some Ca when it is lost from bone (Fontenot and Church, 1979). Adding 100 ppm F in drinking water depressed Ca absorption in calves (Ramberg et al, 1970). Supplementing of calves with NH_4Cl increased absorption and urinary excretion of Ca and decreased urinary pH (Braithwaite, 1972). Addition of Cu or Mo in the form of copper sulphate and sodium molybdate resulted in marked decrease in the urinary P excretion (Shirley et al., 1950). High Fe levels (1000 ppm) in the diet of cattle were found to have some depressing effect on the plasma P levels and also tended to decrease its apparent

absorption (Standish et al., 1971). Mineral elements like Al, Mn, BE and S are also known to influence P utilisation and absorption as reviewed (Jacobson et al., 1972).

(iii) Interaction with organic nutrients:-

The availability of Ca and P are also influenced by the presence of some mineral binding agents in the diet which might decrease the absorption in the gastro-intestinal tract. Presence of high amount of oxalates in paddy straw render the Ca unavailable by forming insoluble Ca-Oxalate in the gut (McDonald et al., 1981). The insoluble phytates which are found in soyabean meal, cotton seed, sunflower seed and seasam seed cake bind with P and certain micro-elements like Zn by forming insoluble complexes which are difficult to assimilate (Georgievskii, 1982). Dietary fat was shown to have a depressing effect on Ca utilisation by ruminants (Tillman and Brethour, 1958) due to the formation of insoluble Ca soaps in the gastro-intestinal tract (Roberts and McKirdy, 1965). Lactose and other sugars were found to stimulate absorption of Ca and some amino acids in some species, but the mechanism is not clear (Fontenot and Church, 1979).

Stilling et al. (1964) have shown improved retention of Ca and P in animals consuming high N forages as compared to low N forages, even though Ca intake was less. Wadsworth and Cohen (1976) have suggested that suitable response to P supplements can only be anticipated if intake of other nutrients like protein and energy are

adequate. Supplementation of P alone to a diet low in N content is able to provide little stimulus to animal production system (Cohen, 1976). The role of vitamin D in absorption and assimilation of Ca and P has already been discussed. Massive doses of vitamin D were found to cure 'rickets' and improve P retention in sheep (Ewer, 1951) and increase Ca and P absorption in cattle (Conrad et al., 1956; Braithwaite et al., 1972). Certain synthetic hormones like estrogens (diethylstillbestrol), progesterone or hexestrol were found to increase the absorption of Ca or P or reduce their urinary excretion(Whitehair et al., 1953; Shroder and Hansard, 1958; Braithwaite et al., 1972).

2.12 Use of chelating agents in relation to mineral availability

A very controversial aspect in the claim about better absorption of mineral elements by using chelated supplements needs further investigation.

In the digestive tract, different mineral elements form new bonds with various organic substrate. The strength of the bonds depend not only on the nature of the element in question but also on the nature of complexing substrates generally called the 'ligands'. Chelates, which are internally complex compounds, are found between metal and ligands due to primary and secondary forces of valencies caused by the presence of N, O and S atoms in the ligand (Georgievskii, 1982). These elements have electron pairs which are capable of

completing the electron shell of the central metal ion by forming coordinate linkages (Chernavina, 1970). There are claims that by chelation, the activity of the elements in such complexes become 10^5-10^7 times enhanced than the activity of the metal in ionic state giving beneficial effects to the organism (Chirnavina, 1970).

Many elements, Ca, Mg, Zn, Fe, Co, Ni, Cu, Cd and Hg, are known to form complexes with variable ligands such as amino acids, polypeptides, porphyrin, heterocyclic compounds, organic acids like aminoacetic, oxalic, citric, malic, formic and phytic acids and EDTA. Natural feeds with strong chelating property include dry malt residues, molasses and soya, cotton seed, sunflower seed and seasam seed cakes which contain insoluble phytates(Georgievskii, 1982).

Thus chelates function in two opposite ways in the digestive system:(i) they augment the deficiency of element in question by making them insoluble and thus unabsorbable (ii) they enhance the utilisability of the element by increasing the activity in comparison to the ionic state. Probably due to strong stability constant, the chelate picks up the metal ions from the feed and transports it through the intestinal wall and the tissues in better way. The stability of complexes formed by a livalent metal ion and a ligand is governed by the Irving-Williams ssies based on hard or soft nature of acids of metal cation.

$$Ca < Mg < Mn < Fe < Co < Ni < Cu < Zn$$

Stable bonds are formed only by hard acid with hard base or soft acid with soft base but weak bonds exist between hard acid and soft base or *vice versa*.

Mostly confining their studies to pig and poultry, many researchers claimed enhanced assimilation of dietary microelements by adding strong chelating agents (EDTA, aminoacetic acid derivatives and amino acids) and by prechelation of the mineral elements. Thus, Cu gets deposited in liver to a greater extent, when fed in the form of amino acid or peptide chelate than as copper sulphate (Kirchgessner and Grassman, 1968). Similar findings have also been reported with Zn and EDTA (Kratzer *et al.*, 1959; Scott and Zeigler, 1963; Kratzer and Starcher, 1963). While the advantageous effect was due to enhanced absorption, no effect was shown on the pattern of Zn excretion or DM digestibility in goats and calves (Powell *et al.*, 1967; Miller *et al.*, 1968 and Hiers *et al.*, 1968). *In vitro* studies with ruminal buffers indicated marked increase in solubility of Mn, Zn, Fe and Cu chelated premix mineral supplements than three different sources of commercial mineral mixtures in unchelated forms (Foll, 1966a). In separate studies, they suggested that in presence of molasses in diet of steers, the solubility of Mn, Fe and Zn with chelated supplements was 40-50% but with unchelated sulphate salts of the mineral element the solubility was depressed to 4-7% in presence of molasses than without it (Foll, 1966b).

Studies with Ca availability in presence of chelates are very scanty. Forbes (1961) observed that addition of EDTA to soya protein diet of rats showed no influence on Ca and Mg balance and deposition in bones but helped in better Zn retention and body wt. gains. They also suggested that EDTA complexed with certain minerals in acidic pH of the stomach and got dissociated in alkaline pH of the intestine for better absorption. Certain evidences suggest that better absorption of EDTA-Zn complex than protein Zn complexes found in feeds might be due to smaller molecular size of EDTA-Zn complex facilitating its absorption (Darwish and Kratzer, 1965). Under the circumstances, it was considered necessary to study the influence of EDTA supplementation on Ca distribution in rumen, its flow and utilisation together with the influence on the utilisation of other mineral elements from prepared mineral mixtures.

The literature cited above suggest that various mineral supplements have different potentialities in supplying both major and trace minerals. Such potentialities do not necessarily depend upon the chemical composition alone but also on the ruminal environment where new complexations may form. The different chemical form, pH and solubility of minerals influence the availability in the G I tract. Sometimes different forms also influences the utilisation of other nutrients. Such situations in the rumen with different mineral supplement

source need detailed investigations. In the investigation proposed the possible alternative sources of Ca and P supplements are contemplated for study in comparison to a standard source.

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CHAPTER-III

SCANNING VARIOUS ALTERNATE SOURCES OF Ca AND P SUPPLEMENTS FOR USE IN MINERAL MIXTURE FOR LIVESTOCK

EXPERIMENT-I COMPOSITIONAL SCANNING OF DIFFERENT SOURCES OF Ca AND P SUPPLEMENTS

In India, mineral mixtures for livestock feeding are marketed under various trade names and the quality standards of such products are regulated according to specification of ISI (1962,1968 and 1982). Mostly due to cost considerations, Ca and P sources in mineral mixtures are included in the form of ores, rocks and other locally available natural sources. Apart from compositional variabilities in such sources, presence of potentially toxic levels of one or more mineral elements (Ammerman et al., 1977) may limit the use of such ingredients. ISI standards (1982) recommended the use of bone meal, chalk powder and dicalcium phosphate as Ca and P supplements in mineral mixtures. With the development of industry and availability of industrial wastes, there are possibilities of using such waste materials also as Ca and P supplements for livestock feeding, provided compositional characteristics of such materials are satisfactory.

3.1 MATERIALS AND METHODS

3.1.1 Collection of samples

For this experiment certain conventional and unconventional materials such as Dicalcium phosphate(DCP), rock phosphate, Mussoorie rock phosphate, fertiliser grade

superphosphate, NPK fertiliser, Kiln dust, gypsum, phospho-gypsum, chalk pouder, marble, lime, lime sludge waste, filter press mud waste, fly ash, <u>usar mitti</u>,<u>kharia mittis</u> and plaster of paris were collected from various sources (Table 3.1).

Table 3.1 Sources and sampling of conventional and non-conventional Ca and P supplements

S. No.	Supplements	No.of samples	Sources
1.	Gypsum	3	i) Haryana land reclamation office, Karnal ii) Central Soil Salinity Res. Instt.,Karnal iii) Bhupindra Cement Works, Surajpur, Ambala
2.	Phosphogypsum	1	Hindustan Copper Ltd., New Delhi
3.	Rock phosphate ore	1	Geological Department, Punjab University, Chandigarh
4.	Mussorrie rock phosphate	2	Pyrites,Phosphates & Chemical Ltd.,Lucknow,U.P.
5.	Superphosphate	2	i) Hindustan Copper Ltd., New Delhi ii) Sri Ram Fertilisers and Chemicals, New Delhi
6.	Dicalcium phosphate	3	i) Rudra Trading Corp., New Delhi ii) Kakkar Enterprises, New Delhi iii) Locally procured
7.	Marble pouder	2	i) Maheshwari Chips Udyog Ltd., Shashdhara Road, Dehradun, U.P. ii) Locally procured

....contd.

....contd.(Table 3.1)

S. No.	Supplements	No.of samples	Sources
8.	Chalk powder	2	Locally procured
9.	Lime	1	Locally procured
10.	Plaster of paris	1	Locally procured
11.	Palai mittie	1	-do-
12.	Kharia mittie	1	-do-
13.	Fly ash	1	Thermal Power Station, HSEB, Panipat, Haryana
14.	Kiln dust	1	Bhupindra Cement Works, P.O. Surajpur, Ambala
15.	N.P.K. fertiliser	1	Locally procured
16.	Lime sludge	1	Ballarpur Paper Mills, Yamuna Nagar, Haryana
17.	Filter press mud waste	1	Saraswati Sugar Mills, Yamuna Nagar, Haryana

Efforts were made to procure as many batch samples as possible but in certain cases collection of more than one batch sample was not possible.

3.1.2 Analysis of samples

Different samples collected from various sources (Table 3.1) were analysed in duplicate for Dry Matter (DM), total ash, acid insoluble ash (AIA), Ca, P, Mg as major minerals and Fe, Mn, Zn, Cu and Co as trace minerals. In addition, they were also analysed for toxic elements like Pb, Cd and F. Total ash and AIA were analysed by

the method of AOAC (1970). Essential minerals like Mg, Fe, Mn, Zn, Cu and Co and toxic minerals like Pb and Cd were also analysed with the help of AAS as discussed in 3.1.3.2. F content in these samples was estimated as per the method of ISI (1975). Before the determination of Ca and P was carried out in the samples, a number of techniques described in 3.1.4 were evaluated on the basis of recovery and comparative efficiency and a suitable technique was selected and followed for all later determinations.

3.1.3 Selection of Analytical Techniques for determination of major and minor minerals in the mineral supplements

Accurate determination of various minerals of nutritional significance was considered to be important especially when various non-conventional mineral supplements are stipulated for use in feed formulations. Therefore, certain available methods were carefully evaluated regarding suitability for selection of a particular technique for analytical needs.

3.1.3.1 Analytical techniques and recovery trial for determination of Ca and P in mineral supplements

Ca and P are the two major minerals which are blended in mineral mixtures for livestock and poultry in the form of naturally available sources such as dicalcium phosphate, rock phosphate, bone meal, oyster shell, etc.

Certain commonly used methods for determination of Ca in feeds are based on: (i) precipitation of calcium

oxalate (AOAC 1970), (ii) atomic absorption spectrophotometric technique (AAS), and (iii) cresolphthalein complexone method (Sarkar and Chauhan, 1967). Similarly, for determination of P in feeds the techniques which were included in the study included (i) titrimetric procedure based on formation of phosphomolybdate (AOAC, 1970 and ISI, 1975) and (ii) certain colorimetric procedures based on the development of colour complexes, such as (a) with amino napthol sulphonic acid (Fiske and Subbarow, 1925), (b) with molybdate reagent (AOAC, 1980) and (c) Micro method using hydroquinone as reducing agent (AOAC, 1975). The reliance of these methods in determination of Ca and P in mineral supplements was tested by adopting following procedures: (a) by running recovery trial, and (b) by repeating the estimations of Ca and P in certain samples by different methods.

3.1.3.1.1 Ca recovery trial

Using chalk powder, the following procedure was adopted for carrying out the recovery trial. Three duplicate sets of crucibles marked, A, B and C were taken. In set A, B and C, 1 g of the sample in question was accurately weighed. To the set marked B, 5 ml of 10% pure $CaCl_2$ (= 180 mg Ca) solution was added after dry ashing at temperature 500°C, whereas to the crucible marked C, 5 ml of 10% pure $CaCl_2$(= 180 mg Ca) solution was mixed to the samples before dry ashing at same temperature. The remaining part of the procedure kept the same for all the three sets of crucibles. HCl extract was prepared, filtered through Whatman No.1 filter paper and washed until

acid free. The volume in each case was made to 250 ml. In suitable aliquots, Ca was estimated by precipitation of calcium oxalate method (AOAC, 1970) and with the help of AAS. The two methods have been discussed in detail in 3.1.4. The recovery percentage values, as estimated are given in table 3.2.

3.1.3.1.2 P recovery trial

For carrying out the P recovery trials by different methods, 1 g sample of DCP was accurately weighed in the three duplicate sets of crucibles marked A, B and C. 2 ml of 10% solution of disodium hydrogen phosphate (= 35mg P) was added in crucible marked B and C respectively. Ashing and extract preparation was carried out in the similar way as was done in Ca recovery trial. HCl extract thus prepared was used for the P estimation. The four techniques employed for P determination were those mentioned in 3.1.3.1 and have been discussed in detail in 3.1.4. These techniques were evaluated on the basis of recovery data and comparative efficiency studies carried out in number of samples and a suitable technique was selected for determination of P in different samples. Recovery percentage values by the four methods are given in table 3.3.

3.1.3.1.3 Comparative evaluation of techniques for Ca and P estimation

The comparative evaluation of efficiency of different techniques for Ca and P determination was carried out on gypsum, lime, phosphogypsum, rock phosphate, chalk

pouder, superphosphate and dicalcium phosphate samples and the values are presented in table 3.4.

3.1.3.2 Analysis of mineral elements other than Ca and P

Essential minerals other than Ca and P like Mg, Fe, Mn, Zn, Cu and Co and toxic minerals like Pb and Cd were analysed with the help of Pye Unicam SP-191, flame atomic absorbtion spectrophotometer (AAS) using the standard procedure as given in the instruction manual. Acetylene was used as fuel while air was used as oxidant. Specific hollow cathode lamps were used for the determination of individual elements. Conditions employed for determination of different elements by AAS and the recoveries obtained are given in table 3.5.

3. 1.4 Methods used for the estimation of Ca and P

3.1.4.1.1 Ca estimation by precipitation method(ISI,1975)

Reagents:

i) Hydrochloric acid - 25% v/v
ii) Methyl red indicator - 0.03% in distilled water
iii) Ammonium hydroxide reagent - 50% v/v
iv) Dilute ammonium hydroxide solution - 2% v/v
v) Ammonium oxalate solution - saturated soln.
vi) Concentrated sulphuric acid - sp.gr.1.84
vii) 0.1 N potassium permanganate solution

Procedure:(i) Preparation of HCl extract:- Accurately weighed 1 g of the material was moistened with 2 ml of dilute HCl in a silica basin and then dried in oven at 100$\pm$ 2^{o}C for over-

night. The sample was then ashed in a muffle furnace at 550 ± 10°C. The ash was taken in 40 ml of HCl and allowed to boil. After cooling, it was filtered on Whatman No.1 filter paper and the filterate was collected in a 250 ml volumetric flask. The residue was washed with hot water until the filterate was acid free and finally the volume was made to 250 ml mark.

Precipitation of Ca oxalate:- 10 ml of aliquot of HCl was taken in a beaker. Enough distilled water was added to make approximately 100 ml volume. To this 2 drops of methyl red indicator was added. Enough ammonium hydroxide reagent was added to bring down the pH to 5.6 with the appearance of brownish orange colour. To this was added 2 drops of dilute HCl solution such that the slight pink colour is developed with the appearance of ppt of Ca-oxalate. This solution was boiled slowly for the granular formation of the ppt and allowed to settle overnight.

Next day the supernatant liquid was filtered through a ashless filter paper (Whatman No.40) and ppt was washed with dilute ammonium hydroxide solution, and then thoroughly with hot distilled water. The filter paper containing ppt of Ca oxalate was carefully taken to side of a clean beaker and about 100 ml of distilled water and 5 ml of concentrated sulphuric acid was put in the beaker. The solution was warmed to about 70-80°C mixing the ppt in acid solution, and titrated with 0.1N $KMnO_4$ solution.

The Ca content in the sample was estimated, based on the following equation:

$$1 \text{ ml of } \frac{N}{10} KMnO_4 \text{ used} \equiv .002 \text{ g of Ca}$$

3.1.4.1.2 Calcium estimation by Atomic absorption spectrophotometer:

The estimation of Ca was also tried with the help of Atomic Absorbtion Spectrophotometer using Acetylene as fuel and air as oxidant in concentration ranges from 1-16 ppm. Proportional linear analytical response was observed in concentration range of 1-6 ppm. Since elements such as aluminium, beryllium, phosphorus, silicon, titanium and mineral acids mask the response of Ca in air acetylene flame (AAS manual), Strontium as strontium chloride solution as demasking agent was added in the process of dilution of HCl extracts prepared in 3.1.4.1., such that a concentration of 0.2% strontium is achieved in the test samples as well as the standards. Similar amounts of mineral acids were added in the standard, blank and test samples so as to release the masking influence of mineral acids on Ca response (AAS manual).

3.1.4.2 Methods used for P estimation

3.1.4.2.1 P estimation by precipitation method (ISI,1975)

Reagents:(i) Concentrated nitric acid, AR.

(ii) Nitric acid solution - 2% w/v.

(iii) Potassium nitrate solution - 3% w/v

(iv) 0.1 N sodium hydroxide solution.

(v) 0.1 N nitric acid solution

(vi) Phenolphthalein indicator - 0.1% w/v in 60% rectified spirit.

(vii) Ammonium molybdate solution:- About 400 ml of distilled water was added to 100g ammonium molybdate taken in a 500 ml stoppered measuring cylinder. The contents were then shaken for 25 minutes to dissolve. Ammonium hydroxide solution (25% w/v) was gradually added until the molybdate solution became clear. Then sufficient distilled water was added to make up the volume 500 ml.

Procedure:-10 ml aliquot of the HCl extract prepared in 3.1.4.1.1 was taken in a 250 ml capacity beaker already containing 20-30 ml of distilled water warmed at $\geq 50^{o}C$. Phosphomolybdate precipitation was carried out by simultaneous addition of 10 ml of conc. nitric acid and 10 ml of ammonium molybdate reagent to the beaker containing aliquot of the HCl extract. The sample was immediately stirred with a glass rod and ppt formed was allowed to stand overnight.

The contents of the beaker were filtered slowly through Whatman No.42 filter paper, retaining the ppt in the beaker as far as possible. The ppt was washed twice with dilute nitric acid solution and then with potassium nitrate solution until the washings were free from acid. The ppt along with the filter paper was transferred back to the beaker and sufficient but known quantity of sodium hydroxide solution was added with the help of a burette to dissolve the yellow ppt. The volume of the standard

sodium hydroxide solution used was noted. The contents of the beaker were then titrated with standard nitric acid solution using phenolphthalein as an indicator. The P content in the sample was calculated by the following formulae based upon the equation that:

$$1 \text{ ml of } \frac{N}{10} \text{ NaOH used} = .000135 \text{ g of P}$$

$$P\ \% \text{ (on dry matter basis)} = \frac{336.75(AN_1 - BN_2)}{m \times DM\ \%}$$

Where,

A = Total volume of standard sodium hydroxide added to dissolve the ppt.

N_1 = normality of the standard NaOH solution used

B = volume of the standard nitric acid solution used to neutralise excess alkali.

N_2 = normality of the standard nitric acid used to neutralise excess alkali.

m = weight of the material taken

3.1.4.2.2 Phosphorus estimation by colorimetric measurement - Phosphomolybdovandate complex formation method(AOAC.1970)

Reagents:- Molybdovandate reagent - 10 g of ammonium molybdate was dissolved in 100 ml of hot distilled water in a beaker and cooled. In another beaker, 0.5 g of ammonium metavandate was dissolved in about 60 ml of hot distilled water and cooled. 60 ml of 70% perchloric acid was added gradually with constant stirring to metavandate solution. The molybdate solution was then slowly mixed in metavandate solution in a 500 ml capacity volumetric flask and volume was made upto the mark with distilled water

Preparation of standard curve:- Working standard solution containing 50 ug P/ml was used for the preparation of standard curve. A serial dilution was prepared by pipetting 0.5, 1.0, 1.5, 2.0, 2.5 and 3 ml of this working standard solution into six test tubes. The standard curve was prepared by adopting the similar procedure as discussed below.

Procedure:- Suitable aliquot of the HCl extract prepared (3.1.4.1.1) was taken in test tubes, to which 2 ml of the molybdovandate solution was added and volume was made to 10 ml using distilled water. It was allowed to stand for 10 minutes for the proper development of colour due to the formation of phosphomolybdovandate complex. The absorbance reading was taken in spectronic-20, spectrophotometer at a wave length of 400 nm. P content of the sample was calculated from the standard curve as follows.

From the standard curve (Fig.3.1)

$$0.45\ O.D = 150 \text{ ug P}$$

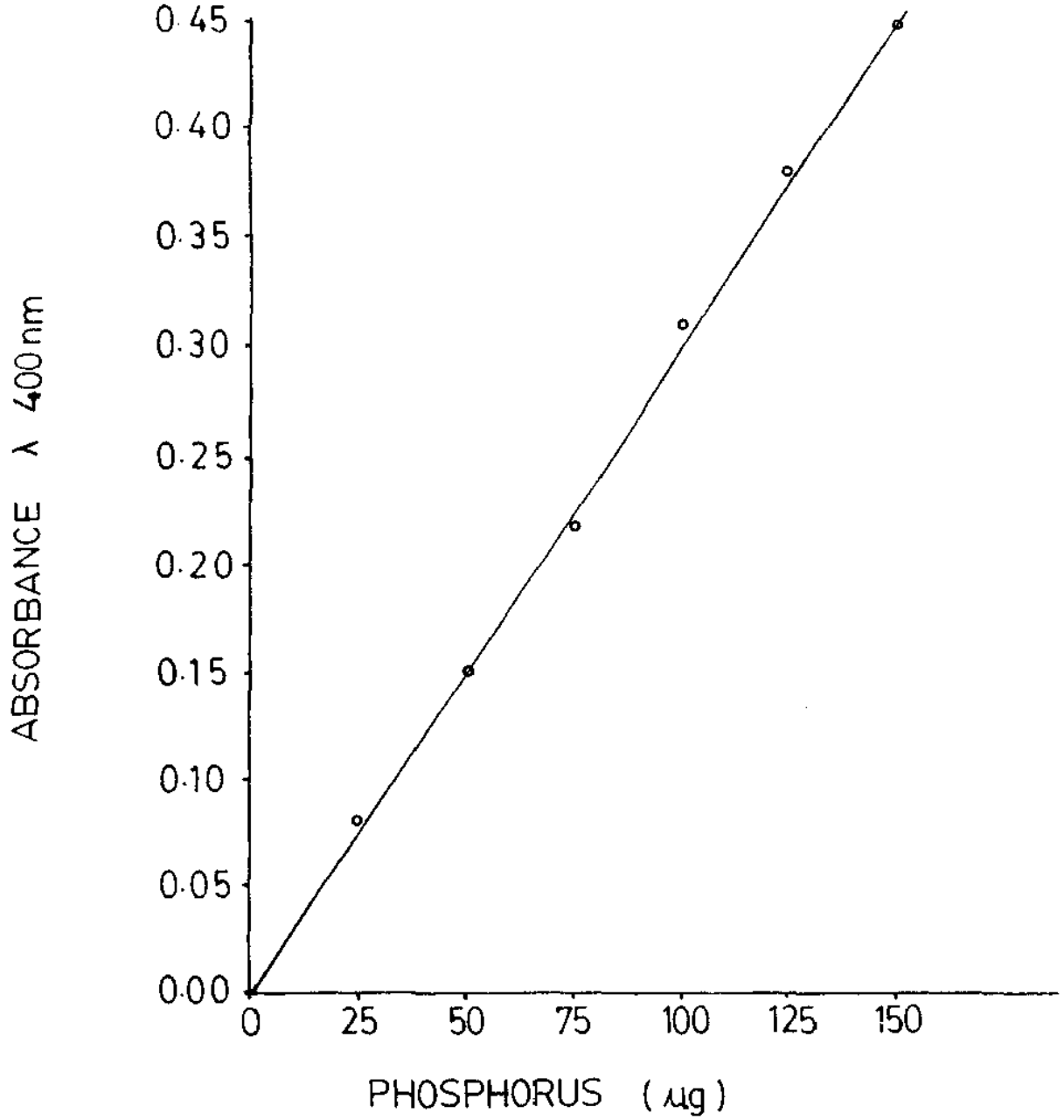
If O.D of the sample is $x = \frac{150}{0.45}x$ ug P

(If the aliquot taken is 'a' ml out of 250 ml of the HCl extract prepared by ashing 1 g of the sample)

then, P percentage in the sample $= \frac{150}{0.45}x \cdot \frac{250}{a} \cdot 100 \cdot \frac{1}{10^6}$

$$= 8.335 \frac{x}{a}$$

FIG-3.1 STANDARD CURVE FOR ESTIMATION OF PHOSPHORUS BY PHOSPHOMOLYBDOVANADATE COMPLEXATION METHOD (AOAC, 1970)



3.1.4.2.3 P estimation using hydroquinone(AOAC,1975)

Reagents:- (i) Ammonium molybdate solution:- 25 g of ammonium molybdate was dissolved in 300 ml of distilled water. In a seperate beaker 75 g of concentrated H_2SO_4 was diluted with 200 ml of distilled water. Ammonium molybdate solution was added to H_2SO_4 in a 500 ml volumetric flask and volume was made to the mark.

(ii) Hydroquinone solution:- 0.5 g of hydroquinone was dissolved in about 80 ml of distilled water in a 100 ml capacity volumetric flask. A few drops to conc. H_2SO_4 were added to retard oxidation before making the final volume.

(iii) Sodium sulphite solution - 10% w/v.

(iv) Stock P solution: This solution was prepared by dissolving 0.4394 g of pure dry KH_2PO_4 in distilled water and diluted to make up the volume to one litre. This solution gives the P concentration of 100 mg P/litre.

Preparation of standard curve

The stock solution prepared above was further diluted to give working standard solution containing 50 µg P/ml. A serial dilutions were prepared using 2, 4, 6, 8 and 10 ml aliquots of the working standard solution pipetted into five 50 ml capacity volumetric flasks and a standard curve was prepared as per the procedure given below.

Procedure:- A suitable aliquot (0.5-5.0 ml) of HCl extract was taken to 50 ml volumetric flask, to which 5 ml of

ammonium molybdate solution was added and mixed well. After about 10 seconds, 5 ml each of hydroquinone solution and sodium sulphite solution were added to the volumetric flask and volume was made to the mark. The contents were mixed well and allowed to stand exactly for 30 minutes for the proper development of blue colour which was measured at a wave length of 625 nm on spectronic-20, spectrophotometer.

Calculations:- The P content in the sample was calculated from the standard curve as shown below:

Standard curve (Fig.3.2)

0.36 O.D. = 200 ug P

Say x O.D. = $\frac{200}{0.36} \times$ ug P

= 555.6 x ug P

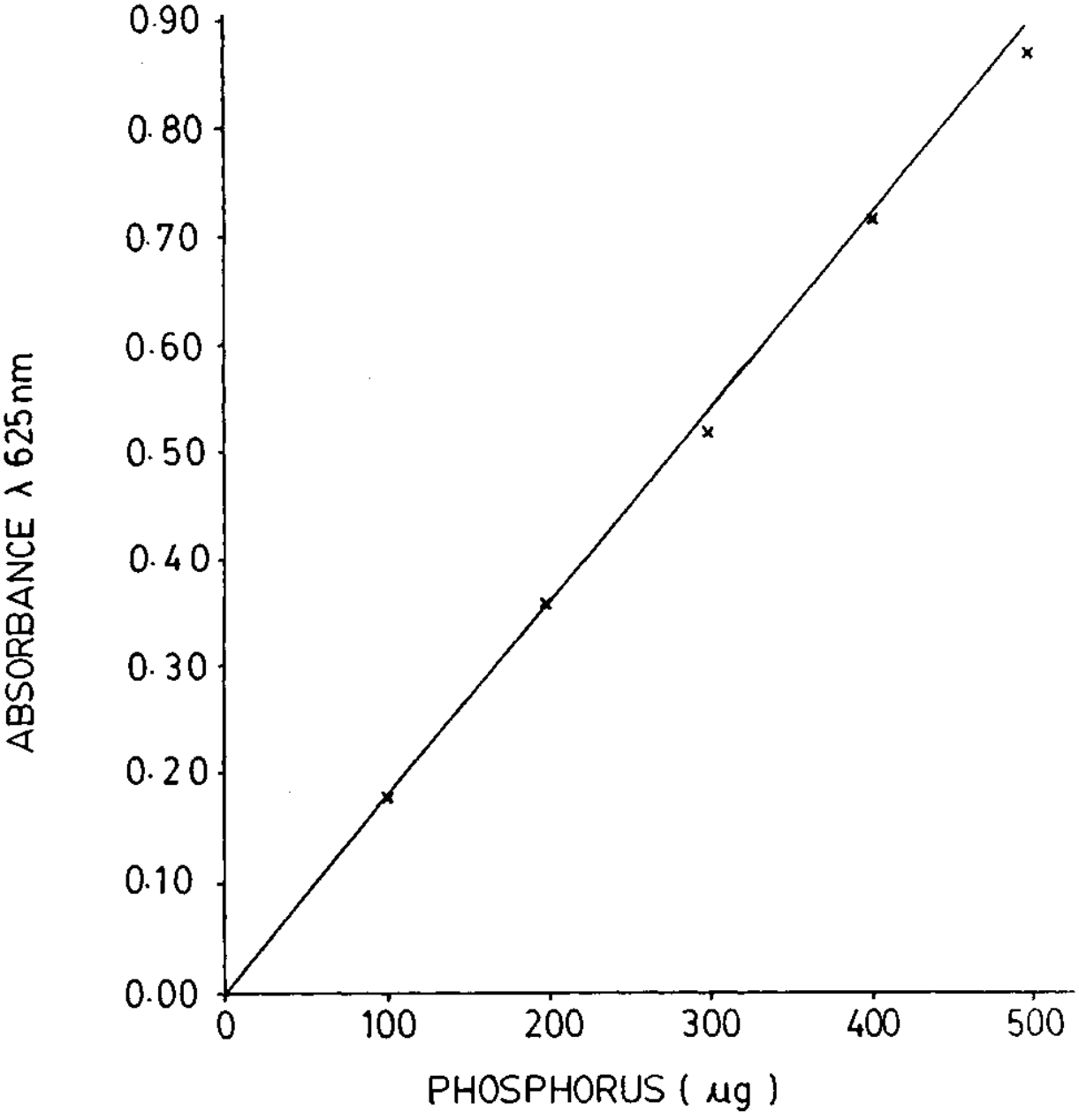
If the aliquot used is 0.5 ml out of 250 ml of the HCl extract prepared from 2 g of the sample ashed, then P (%age) in the sample

$$= 555.6 \times \frac{250}{0.5} x \times \frac{1}{10^6} \times \frac{100}{2}$$

$$= 13.88\, x$$

Where, x is the O.D. of the sample.

FIG-3·2 STANDARD CURVE FOR ESTIMATION OF PHOSPH--ORUS USING HYDROQUINONE (AOAC, 1975)



3.1.4.2.4 Phosphorus estimation by the method of Fiske and Subbarow (1925)

This method was originally designed for estimation of inorganic phosphorus in blood serum after deproteining it with TCA. The protein free serum filtrate was treated with acid molybdate solution to form phospho molybdic acid with P present in serum. Phosphomolybdic acid is then reduced by addition of 1, 2, 4 amino-naphthol sulphonic acid (ANSA) reagent which produces blue colour whose intensity is proportional to amount of phosphate present.

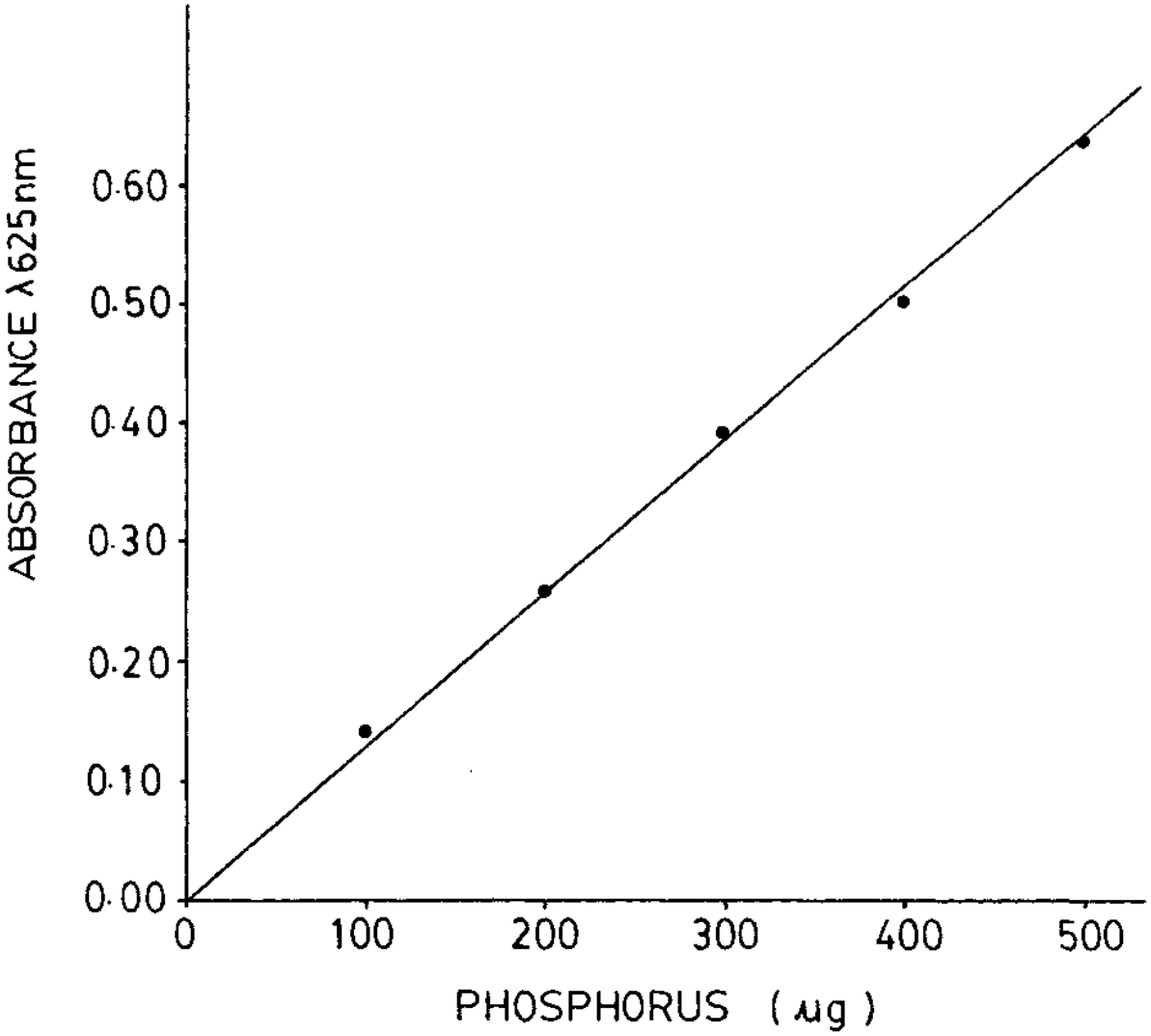
This method was also tried to estimate P in mineral supplement extracts. The only modification was that HCl extract described at 3.1.4.1.1 was used instead of TCA filtrate as adopted in serum.

Reagents:- (i) Ammonium molybdate solution - 25 g of ammonium molybdate was dissolved in 200 ml of distilled water and mixed with 300 ml of 10 N.H_2SO_4, in a 1000 ml capacity volumetric flask. The contents were well mixed and volume was made to the mark.

(ii) ANSA reagent:- 0.25 g of aminonapthol sulphonic acid was dissolved in 195 ml of 15% (w/v) solution of sodium sulphite, taken in a glass stoppered measuring cylinder. To this, 5 ml of (20% w/v) sodium bisulphite solution was added and the contents were mixed well till the ANSA reagent is completely dissolved. The contents were transferred to a brown bottle and stored in a cool place.

(iii) TCA solution - 10% w/v.

FIG-3·3 STANDARD CURVE FOR ESTIMATION OF PHOSPHORUS
(FISKE AND SUBBAROW METHOD)



Preparation of standard curve:- Working standard solution containing 50 µg P/ml was used for the preparation of standard curve. A seriel dilution having 2, 4, 6, 8 and 10 ml of the working standard solution was pipetted out into five different 50 ml volumetric flasks. The procedure described below was followed and a standard curve was prepared.

Procedure:- 5 ml of the ammonium molybdate solution was added to a 50 ml volumetric flask containing suitable aliquot of the HCl extract prepared. To this, 2 ml of ANSA reagent was added and volume was made to 50 ml mark. The contents were mixed well and allowed to stand for 5 minutes and absorbance reading was taken in spectronic-20, spectrophotometer at a wave length of 625 nm.

Calculations:

From the standard curve (Fig.3)

0.26 O.D. = 200xug P

If O.D. of sample is x = $\frac{200.x}{0.26}$ ug P

If the aliquot taken is 'a' ml out of 250 ml HCl extract prepared by ashing 1 g of the sample;

then P % = $\frac{200.x}{0.26} \cdot \frac{250}{a} \cdot \frac{1}{10^6} \cdot 100$

= $19.23 \frac{x}{a}$

3.2 RESULTS

3.2.1 Selection of analytical techniques for determination of Ca and P

3.2.1.1 Calcium:

The results of Ca recovery trial by (i) titrimetric method, and (ii) AAS using 0.2% strontium as demasking agent have been presented in table 3.2. The recovery percentage by these two methods was found to be 88.9 and 90.0% respectively when Ca was added before ashing, and 87.5 and 93.9% when Ca was added after ashing. This indicates higher recovery by AAS method. Likewise, Ca content of the eight mineral supplements estimated by these two methods (table 3.4) invariably showed higher values by AAS method than the titrimetric method. Therefore, the AAS method seemed to be the method of choice for Ca determination.

3.2.1.2 Phosphorus:

The results of P recovery trial by the four methods (i) titrimetric method (ii) AOAC (1970) using phosphomolybdate reagent (iii) AOAC (1975) method using hydroquinone and (iv) Fiske and Subbarow (1925) have been presented in table 3.3. The recovery percentage values of P by these methods was found to be 91.4, 94.3, 97.1 and 128.6% respectively, when external P was added before ashing, and 94.3, 94.3, 97.1 and 128.6% respectively when P was added after ashing. The data in table 3.3 revealed that except for method (i) the recovery values

Table 3.2 Ca recovery trial

Particulars	Titrimetric method		AAS method	
	Ca content (mg/g)	Recovery (%)	Ca content (mg/g)	Recovery (%)
1. Ca in $CaCO_3$ sample	390	-	431	-
2. Ca added	180	-	180	-
3. Total Ca determined (sample + Ca added before ashing)	550	88.9	593	90.0
4. Total Ca determined (sample + Ca added after ashing)	547	87.5	600	93.9

Table 3.3 Phosphorus recovery trial

Particulars	Titrimetric method		Molybdovandate method (AOAC, 1970)		Hydroquinone method (AOAC,1975)		ANSA method (Fiske and Subbarow, 1925)	
	P content (mg/g)	Recovery (%)	P content (mg/g)	Recovery (%)	P content (mg/g)	Recovery (%)	P content (mg/g)	Recovery (%)
1. P in dicalcium phosphate sample	194	-	175	-	242	-	288	-
2. P added	35	-	35	-	35	-	35	-
3. Total P determined (sample + P added before ashing)	226	91.4	208	94.3	276	97.1	333	128.6
4. Total P determined (sample + P added after ashing)	227	94.3	208	94.3	276	97.1	333	128.6

were the same in both the cases, i.e., when P was added after ashing or before ashing. In case of method (i), however, the recovery was higher (94.3%) when P was added after ashing than when P was added before ashing. In both the cases, the recovery was high, i.e., 128.6% when the determination was made by Fiske and Subbarow method. P content of eight such materials, estimated by the four methods also gave similar trend, presented in table 3.4. The hydroquinone method seemed to be the method of choice because of P recovery approached 100% (table 3.3) and because it was able to measure P in concentration as low as 0.02%.

3.2.2 Recovery trials of minerals other than Ca and P determined by AAS

Minerals like Mg, Fe, Cu, Co, Zn, Mn, Pb and Cd were estimated using Atomic Absorbtion Spectrophotometer against the suitable standards prepared as per the AAS-operation manual. The analytical conditions and recovery trials for individual elements have been present in table 3.5. It was evident that the technique followed had enough reliance as the recovery % for different elements ranged from 92.5 to 107.5%. The estimation of F was carried out by method of distilling hydrofluro-silicic acid and titration with thorium nitrate. The recovery for F determination was 109.3%.

Table 3.4 Comparative estimates of calcium and phosphorus content (% on DM basis) in various samples as determined by different methods

Samples	Calcium		Phosphorus			
	Titrimetric	AAS	Titrimetric	Molybdovanadate method (AOAC, 1970)	Hydroquinone method (AOAC, 1975)	ANSA method (Fiske and Subbarow, 1925)
1. Gypsum	13.06	14.03	0.13	0.02	0.02	nil
2. Lime	43.3	44.74	0.05	0.02	0.05	0.03
3. Phosphogypsum	11.24	12.54	0.34	0.24	0.37	0.40
4. Rock phosphate I	29.6	31.50	8.48	9.67	10.37	12.60
5. Rock phosphate II	24.52	25.63	9.45	12.73	12.16	14.47
6. Chalk powder	38.98	41.52	0.12	0.03	0.03	0.02
7. Superphosphate	16.88	17.01	9.43	11.33	12.47	13.00
8. Dicalcium phosphate	30.00	31.55	20.35	23.18	29.57	30.81

Figures are averages of two determinations for the same sample

Table 3.5 Analytical conditions and details of recovery trials in the determination of mineral elements other than Ca and P estimated by AAS

Particulars	Mg	Fe	Cu	Co	Zn	Mn	Pb	Cd
Conditions								
Wave length used for determination (λ)	285.2	248.3	324.8	240.7	213.7	279.5	283.7	228.7
Effective concentration range (ppm)	0.1-0.5	1-5	1-5	0.5-3.0	0.2-1.2	0.2-1.0	5	0.1-0.5
Fuel	----------acetylene----------							
Oxidant	----------air----------							
Recovery trial of different elements								
(i) Concentration in original sample (ug/g)	1043	1150	37.5	35	219.0	30.5	83	2.0
(ii) Quantity added to sample before digestion (ug/g)	1000	1000	10	20	100	10	100	2.0
(iii) Total concentration (sample + addition) determined (ug/g)	1997	2128	47.8	54.0	318.0	39.75	190.5	3.88
Percent recovery	95.4	97.8	103.0	95.0	99.0	92.5	107.5	94.0

3.2.3 Compositional scanning of different sources of Ca and P supplements for use in mineral mixtures

The mineral compositional qualities determining suitability of different conventional and non-conventional sources for being considered as Ca and P supplements have been presented in tables 3.6 to 3.10.

3.2.3.1 Ca sources

On reference to table 3.6 and Fig.3.4, it was evident that supplements having the carbonate and hydroxide forms of Ca, i.e., lime, chalk powder, marble powder and filter press mud waste were low in AIA (<2%) and high in Ca content ranging from 35-45%. Whereas, the sulphated forms of Ca sources, i.e., gypsum and phosphogypsum were found to have Ca content ranging from 12 - 35.6% and high AIA content (12 - 40%). Although the AIA content of lime sludge waste was low, its poor Ca and P values ascribed no merit to these substances for being considered as Ca supplement source. On the other hand, kiln dust which was poor in Ca content and high in AIA content also seemed unsuitable for consideration as a source of Ca supplement. Similar was the situation with plaster of paris, fly ash, kharia mitti and pelai mitti (table 3.7). They all were found to have low Ca content and high AIA content, which disqualified them as suitable Ca supplement. It was observed from table 3.8 that samples of dicalcium phosphate, a phosphatic form of Ca were low in AIA(0.5%) and high in Ca and P content (30% and 27% respectively).

Table 3.6 AIA, Ca, P, Mg and Fe content on percent DM basis of certain calcium supplements

Materials	Predominant chemical form	No.of samples analysed	AIA	Ca	P	Mg	Fe
Lime	$Ca(OH)_2$	1	1.91	44.70	0.05	8.26	0.07
Marble powder	$CaCO_3$	2	0.95 (0-1.9)	38.50 (35.4-41.5)	0.04 (.03-.05)	0.78 (.70-.86)	0.05 (.02-.08)
Chalk powder	$CaCO_3$	2	0.32 (.16-.48)	40.00 (39.5-40.5)	0.02 (.01-.03)	0.42 (.28-5.6)	0.03 (.015-.045)
Filter press mud waste	$Ca(OH)_2$	1	1.24	39.60	0.40	5.05	0.09

....contd.

.....contd.(Table 3.6)

Materials	Predominant chemical form	No. of samples analysed	AIA	Ca	P	Mg	Fe
Kharia mitti	-	1	81.50	3.60	-	0.33	0.18
Gypsum	$CaSO_4.2H_2O$	3	33.40 (12.1-34.6)	21.50 (14.0-35.6)	0.20 (.01-.04)	1.77 (.81-3.30)	0.55 (0.18-1.03)
Phosphogypsum	$CaSO_4.2H_2O$	1	41.70	12.50	0.30	0.06	0.02
Pelei mitti	-	1	67.60	2.00	0.30	0.08	5.53
Plaster of Paris	-	1	80.10	10.70	0.10	3.76	1.07
Lime sludge waste	mainly $Ca(OH)_2$	1	1.40	12.50	0.20	2.47	0.13
Fly ash	Ferro alumino silicates	1	90.20	1.00	0.10	0.11	0.23
Kiln dust	-	1	34.2	16.40	-	0.89	0.23

Figures are the average of two estimations. '-' indicates not detectable, The predominant chemical form of the materials wherever known, have been mentioned. Figures in parentheses indicate range.

Table 3.7 Mn, Zn, Cu and Co content (ppm) of certain calcium supplements

Materials	No. of samples analysed	Mn	Zn	Cu	Co
Kharia mitti	1	23	14	5	-
Gypsum	3	234 (53 - 435)	183 (116 - 288)	10 (0 - 15)	37 (25 - 40)
Phosphogypsum	1	12	14	25	-
Kalai mitti	1	65	28	25	23
Plaster of Paris	1	151	56	-	-
Lime sludge waste	1	76	77	25	60
Fly ash	1	40	23	10	8
Kiln dust	1	66	17	5	10

Figures are the average of two estimations. '-' indicate not detectable
Figures in parentheses indicate range

Table 3.8 AIA, Ca, P, Mg and Fe content (on percent DM basis) of certain phosphorus supplements

Materials	Chemical form	No. of samples analysed	AIA	Ca	P	Mg	Fe
Rock phosphate ore	tri-calcium phosphate	1	1.34	31.50	10.40	4.40	0.15
Mussoorie rock phosphate	tricalcium phosphate	2	11.80 (10.7-12.9)	26.60 (25.6-27.6)	11.72 (11.28-12.16)	2.70 (1.02 -4.37)	1.75 (1.61 -1.91)
Super phosphate	monocalcium phosphate	3	14.40 (13.9-15.0)	16.75 (16.0-17.3)	10.81 (9.67-12.50)	0.99 (0.67 -1.28)	0.41 (0.37 -0.45)
NPK-Fertiliser	ammonium phosphate	1	10.80	1.77	14.2	5.73	0.07
Dicalcium phosphate	-	4	0.56 (0.36-0.96)	30.41 (29.3-32.6)	27.83 (25.1-29.6)	0.66 (0.27-1.04)	0.11 (0.10-0.12)

Figures in parentheses indicate range

Besides, dicalcium phosphate also contained fairly good amount of Mg, Mn, Cu, Co and specifically Zn (Table 3.9).

3.2.3.2 P sources

The mineral composition of certain unconventional phosphatic forms of supplements like rock phosphate ore, Mussorrie rock phosphate, fertiliser grade super phosphate and NPK fertiliser have been presented in table 3.8 and the values were compared with those of dicalcium phosphate, which is a common and conventional Ca and P supplement. The AIA, Ca and P content of these P supplements have also been depicted in Fig. 3.5. The rock phosphate, Mussorrie rock phosphate and super phosphate had almost similar P content but AIA contents in both super phosphate and Mussorrie rock phosphate were moderately high. Calcium content in superphosphate was comparatively low. Although NPK had moderately high P, it contained little Ca and moderate amount of AIA.

The various forms of Ca and P supplements were also scanned for Mg, Fe, Mn, Zn, Cu and Co as elements of nutritional significance, and Pb, Cd and F as toxic mineral components. The results have been presented in table 3.9 and 3.10. It was evident that in Mg content, lime showed high status followed by NPK, filter press mud waste and rock phosphate. It was interesting to note that P supplements like rock phosphate, Mussorrie rock phosphate and superphosphate were conspicuously high in Mn. The

Table 3.9 Mn, Zn, Cu and Co content (ppm) of certain calcium and phosphorus supplements

Material	No.of samples analysed	Mn	Zn	Cu	Co
Lime	1	60	27	-	25
Marble	2	24 (19-29)	42 (29-55)	5 (5)	31 (30-33)
Chalk pouder	2	170 (150-190)	33 (30-36)	-	-
Filter press mud waste	1	103	67	30	25
Rock phosphate ore	1	545	25	-	20
Mussorrie rock phosphate	2	627 (622-633)	183 (106-261)	37 (35-38)	32 (30-35)
Super phosphate	3	961 (776-1070)	74 (57-108)	43 (35-60)	27 (15-35)
NPK fertiliser	1	26	637	20	5
Dicalcium phosphate	4	44 (31-62)	302 (219-528)	11 (5-24)	16 (8-32)

Figures in parentheses indicate range

'-' indicate not detectable

latter two sources were also high in Cu and Co. NPK and dicalcium phosphate had high Zn content. Presence of Pb and Cd in these samples ranged from 0 to 1.0 ppm and 0 to 5 ppm respectively. The F content in rock phosphate and Mussorrie rock phosphate ranged from 1000-2000 ppm (table 3.10).

3.3 DISCUSSION

3.3.1 Selection of analytical techniques for determination of Ca, P and other mineral elements in different supplements

Before resorting to compositional scanning of different sources of Ca and P supplements, it was considered desirable to have an appraisal about the comparative reliance of certain common analytical procedures to determine their suitability in estimation of mineral components in different Ca and P supplement sources. For this purpose, two procedure were followed (i) recovery of Ca and P using different techniques, (ii) relative estimation values for Ca and P in different supplement sources as obtained by different methods employed.

3.3.1.1 Calcium:

Table 3.2 depicts Ca recovery by the two techniques using chalk powder as the Ca supplement source. Recovery trials were carried out by titrimetric and AAS procedures. By using titrimetric technique Ca recovery ranged from 87.5-88.9% irrespective of the fact that

Table 3.10 Toxic mineral content (ppm) of certain calcium and phosphorus supplements

Materials	No.of samples analysed	Lead	Cadmium	Fluorine
Dicalcium phosphate	2	22 (20-25)	-	100 (25-175)
Gypsum	2	57 (15-100)	1.7 (1-2.5)	32 (0-65)
Phosphogypsum	1	25	1.0	-
& Lime	1	50	2.0	-
Chalk pouder	1	-	-	-
Marble powder	2	56 (45-68)	2.4 (2.3-2.5)	-
Filter press mud waste	1	-	-	-
Rock phosphate ore	1	55	1.5	2000
Mussorrie rock phosphate	2	82	1.5	1300 (1000-1600)
Super phosphate	3	61 (50-83)	1.0 (0.8-1.2)	670 (500-1000)
NPK fertiliser	1	15	5.0	-

Figures in parentheses indicate range
'-' indicate not detectable

external Ca was added before or after ashing of the original sample. Talpatra *et al.* (1940) had shown 100% recovery of Ca when tested in animal feed sample. However, titrimetric method when tested for mineral supplements gave lower values of recovery. Flame AAS is another common technique that can be used for the estimation of Ca. But in this method, Ca response is masked in presence of Na^+, K^+, Al^+, SO_4^{--} and PO_4^{+++} ions and gets corrected by the use of Lanthnum or Strontium as the demasking agent (Pye Unicam-AAS, Databook, 1976). Therefore, as described in 3.1.4, Sr at the level of 0.2% in the form of $SrCl_2$ was added to both the test samples and the standards to take care of demasking effect. Further, variation in the proportion of mineral acids used while making HCl extract or during digestion also influences the Ca response in AAS. Therefore, efforts were made to have similar concentration of mineral acids in both standard and test samples. Using this technique the recovery of Ca by AAS method ranged from 90.0-93.9%. It was, therefore, inferred that AAS method with suitable adjustments of mineral acids and use of Sr as demasking agent had a better reliance than the titrimetric method. Such inference was also supported by the results presented in table 3.4 in which Ca content in various supplements measured by AAS technique was always higher than the values obtained by titrimetric method. Therefore in all subsequent measurements, AAS method was considered as the method of choice and was employed to measure Ca in different supplements.

3.3.1.2 Phosphorus:

For the purpose of selecting suitable analytical technique for determination of P in mineral supplements, a titrimetric technique based on phosphomolybdate precipitate formation (AOAC, 1970) and three colorimetric techniques using (i) hydroquinone (AOAC, 1975), (ii) amino napthol sulphonic acid (ANSA)(Fiske and Subbarow, 1925) and (iii) phosphomolybdate complex formation (AOAC, 1970) were tried.

P recovery data (table 3.3) indicated that although hydroquinone method gave better recovery (97.1%) than molybdovanadate method (94.3%), both the methods accounted for similar recovery whether external P was added before or after ashing. By titrimetric method, P recovery approached molybdovanadate method where P was added after ashing (94.3%) but when P was added before ashing, P recovery was lower (91.4%). Since similar ashing techniques were followed, difference in the recovery values by titrimetric procedure indicates errors in washing of the ppts in the titrimetric procedure. But such differences were not evident either by molybdovanadate or hydroquinone method and the determined values seemed to be subjected to less analytical fluctuations. Again in Fiske and Subbarow method the recovery was 128.6% irrespective of the fact whether external P was added before or after ashing, indicating less changes of analytical fluctuations in the determined values. However, with this procedure more than 100% recovery was obtained which indicated

that certain components of dicalcium phosphate supplement other than phosphorus also were capable of forming complex with ANSA, making the technique less specific in so far as analysis of mineral supplements were concerned. Fiske and Subbarow method is basically meant for P estimations in blood but if the same is to be used for other materials, it should be standardised and checked (Lindberg and Ernster, 1956). Thus reliance cannot be placed on this method. Further, for most of the mineral supplement samples analysed for P (table 3.4), it was the hydroquinone method which give maximum analytical values. The results suggested the suitability of hydroquinone method which was followed in subsequent analysis.

3.3.3 Compositional scanning of different sources of Ca and P supplements for use in mineral mixtures

The compositional merit of any mineral supplement will be based on the fact that the supplement should have many times higher concentration of mineral in question than the levels present in common feeds. In addition, the mineral should be in available form and should be free from deleterious factors. In the present investigation, compositional quality of certain conventional sources of Ca and P supplements were compared with those of certain non-conventional sources available in the country either as industrial waste material or other uncommon sources. Chalk powder, lime and dicalcium phosphate are some of the conventional Ca sources for livestock and marble for poultry. The conventional P

sources is dicalcium phosphate. Bone meal has not been taken in the present investigation. The non-conventional sources selected for study included marble, gypsum, phospho-gypsum, rock phosphate, superphosphate, NPK fertiliser, plaster of paris, *kharia mitti*, industrial wastes such as fly ash, kiln dust, lime sludge waste and filter press mud waste.

3.3.3.1 *Calcium sources*:

The high AIA content (34-90%) and low Ca (/16%) content in *kharia mitti*, *nalai mitti*, plaster of paris, gypsum and phosphogypsum rendered them unsuitable for being considered as Ca supplements. Due to high concentration of Mg and Fe in gypsum (table 3.6), this source merits consideration as a Mg and Fe supplement and not as Ca and P supplemental source. But because of high AIA content (35%), even its consideration as Mg and Fe supplement becomes of limited value. The Indian Standards Institution (ISI, 1981) restricts AIA content to be 2.5-3.0% in final mineral mixture, probably because high level of AIA influences utilisation of nutrients and palatability. Ammerman *et al.* (1984) suggested that high levels of AIA in the ration of livestock depressed the utilisation of P and certain other micro-nutrients.

The carbonate and hydroxide forms of Ca such as lime, marble and chalk powder are good Ca sources. Lime was also found to be rich in Mg. This suggests that lime could be good alternate Ca and Mg source and in compounding mineral mixtures around lime, the high Mg and low Fe conten

in this material need be taken into account. Gohl (1981) suggested that calcitic limestone contained 36.4% Ca and can safely be fed free choice mixed with salt to livestock. However, due to $MgCO_3$ content (about 5% in dolomite limestone) it should not be used in the feeding of poultry.

High levels of Fe in pelei mitti and high Co content in lime sludge waste merited no significance as they contained no other mineral of nutritional use in appreciable quantities to categorise themselves as a mineral supplement.

The filter press mud waste, which is an industrial waste product of sugarcane mill had almost similar mineral composition as lime (table 3.6) and it contained low AIA, high Ca and sufficient Mg. Thus, filter press mud waste could be utilised with advantage as Ca supplement. Gohl (1981) suggested that dried filter press mud waste could constitute at least 50 percent of the concentrate portion of the diet of ruminants because, apart from calcium, it also contained proteins and carbohydrates. The filter press mud waste of Trinidad was shown to contain 10.4% CP, 12% CF, 1.9% EE and 23.9% ash, whereas Rotary filter mud of Mauritius contained 15.1% CP, 21.4% CF, 7.5% EE and 14.2% ash. However, the Ca and P content of the Mauritius variety were only 2.63 and 1.11% respectively (Devendra, 1977). Meade et al. (1965) has given entirely a different composition of filter press mud which contained 4% Ca as CaO and 2.5% P as P_2O_5, with 16% protein and 14% fibre. The filter press mud samples

analysed by Yam et al. (1983) contained 4.29% Ca, 3.87% P and moderate levels of Fe and Mg. These authors have also indicated that composition of any filter press mud varies greatly and depends upon the process used in the extraction of sugar. The samples of filter press mud waste of Yamuna Nagar sugar factory were considered suitable as Ca supplement.

3.3.3.2 Phosphorus sources:

The chemical composition of certain unconventional phosphatic forms of supplements like rock phosphates ore, Mussorrie rock phosphate (tricalcium phosphate as predominant form), fertiliser grade superphosphate (monocalcium phosphate as the predominant form), NPK fertiliser (ammonium phosphate as predominant form), and phosphogypsum (mainly calcium sulphate) was compared with dicalcium phosphate, a conventional Ca and P supplement. ISI (1962,1968) recommended the use of dicalcium phosphate in mineral mixture. With a moderate P content, and not very high AIA content, rock phosphate, Mussorrie rock phosphate and superphosphate may be considered as alternate replacement of dicalcium phosphate as P source in mineral mixture. The additional advantage is that these sources are invariably rich in Mn, Cu, Co and Zn. Although NPK may qualify as a P supplement on the basis of its P content, low AIA and high ZN content, but its use may become limited because of high Cd concentration (5 ppm).

Maynard and Loosli (1969) suggested that Ca and P content of rock phosphate varied from 29-36% and 12-18% respectively and that Ca and P of rock phosphate and superphosphate are absorbable. They suggested high fluorine

content present in these supplements to be harmful. In the present investigation samples of super phosphate were moderate in Ca in comparison to rock phosphate and Mussorrie rock phosphate. All the three were similar in phosphorus content. The F content in these phosphates was not higher than 2000 ppm. Maynard and Loosli (1969) suggested that curacao phosphate was safe (F) because it contained $\angle$0.4% F. Thus the samples of rock phosphate, Mussorrie phosphate and superphosphate tested had not high F content. However, the F content may vary in samples from different areas and need be checked before being incorporated in mineral supplements. Further, Witt and Owens (1983) reported that availability of P from different sources of phosphates were not the same. They compared phosphorus availability from sodium phosphate with monodicalcium phosphate containing 21% P, another monodicalcium phosphate containing 18.5% P and defluorinated rock phosphate and found P availability were 100, 88, 62 and 40% respectively. They ascribed the differences in availability to the solubility differences of different phosphate sources in the rumen.

From the findings presented, it could be concluded that the carbonate, hydroxide and phosphate forms of Ca in certain sources like lime, chalk powder, marble powder and filter press mud waste could be possible alternate sources of Ca supplements because of low AIA. High AIA and low Ca and P content in other sources like kiln dust, plaster of paris, kharia mitti and pelai mitti, limit the possibility of their use as Ca/P supplements for livestock. Thus, lime,

chalk powder, marble powder and filter press mud (> 30% Ca and ⩽2.0% AIA) have potentiality of being considered as alternative sources of Ca supplements, where rock phosphate and superphosphate (11% P and ⩽15% AIA) could be potential phosphorus supplements, provided F content was ⩽0.4%. However, actual use needed to be confirmed on the basis of solubility studied in rumen and availability of mineral to animals.

EXPERIMENT-II

3.4 RUMINAL SOLUBILITY OF DIFFERENT CALCIUM AND PHOSPHORUS SOURCES

Many mineral elements do not have similar solubility in different parts of gastro-intestinal tract. Solubility of any mineral element in the gastro-intestinal tract is an important criteria to assess its availability to the animal (Chicco et al., 1965). Thus, phosphorus from defluorinated rock phosphate was found to be less soluble and thus less available to ruminants, than P from other sources such as dicalcium phosphate and monocalcium phosphate (Ammerman et al., 1957). Similarly, ferrous sulphate and ferric chloride had higher biological availability to sheep than ferrous carbonate and ferric oxide because the latter two compounds were less soluble sources of iron (Ammerman et al., 1967). Certain research findings suggested that Mg from MgO is more utilised by sheep than Mg from dolomite limestone, because Mg from former source is more soluble (Rehnema and Fontenot, 1983).

Solubility of any mineral in the gastro-intestinal tract is related to the prevailing pH at that part of the gut. Storry et al. (1966) found that Ca and Mg present in the abomasal contents of sheep were in soluble form, where the pH ranged from 2-3. Bremner (1970) studied the changes in concentration and solubilities of Zn, Mn and Cu in the different parts of alimentry tract of sheep and found that a relationship existed between the solubilities of the metal and pH values of the gut contents. This pattern of

change could be reproduced *in vitro* by adjusting the pH of rumen and abomasal samples.

Keeping this background in view, the present experiment was planned to determine the *in vitro* solubility of Ca and P from different supplement sources in the conditions of changing pH of ruminal buffer.

3.4.1 MATERIALS AND METHODS

Trial I.- Calcium solubility of Ca supplements in ruminal buffer

In vitro experiments were conducted for studying the ruminal solubility of Ca from various Ca supplements at different pH. The Ca supplements included gypsum, lime, marble powder, superphosphate and dicalcium phosphate and the solubility data were compared with those of pure $CaCl_2$ (BDH grade). For solubility studies the procedure of Witt and Owens (1983) with slight modification as described below was followed.

Each of the sources mentioned above and $CaCl_2$ (control) was weighed into 250 ml conical flask to provide 50 mg Ca. A ruminal buffer solution was prepared by mixing strained rumen liquor (SRL) and McDoughall's buffer in the ratio of 1:2 (McDoughall, 1948). A volume of 100 ml of this mixture called ruminal buffer solution, was added to each conical flask containing weighed amounts of different Ca supplements. The contents were well mixed and the pH was adjusted to 4, 5, 6 and 7 using 0.1 N HCl with the help of ECIL digital pH meter. The amount of 0.1 N HCl added in

each of the flask was carefully noted and volumes of the flasks maintained at different pH were equalised with the addition of suitable quantity (few ml) McDoughal's buffer. In a separate set of conical flask the pH of the McDougall's buffer solution was also adjusted from 4 through 7 by addition of HCl so as to serve as blank. Immediately after the pH adjustment, the samples were shaken for 1 hr in metabolic shaker water bath maintained at 39°C. After allowing a mixing time of exactly one hr, 25 ml aliquot in duplicate was withdrawn from each of the conical flask. Aliquot I was centrifuged at 16000 x g and aliquot II was subjected to dry ashing. Supernatant obtained after centrifugation was diluted to 100 ml in a 100 ml volumetric flask. This was directly used for the estimation of Ca by AAS by the procedure described in 3.1.4.1.2. From the values obtained, Ca present in 25 ml of aliquot I was calculated. Total Ca present in aliquot II was also estimated. The 25 ml aliquot was dried at 100± 2°C on the waterbath. The dried material was ashed in muffle furnace at 440± 10°C. HCl extract of the ash was prepared as described in 3.1.4.1.1 and Ca determination made with the help of AAS by following procedure given in 3.1.4.1.2. Ca was also determined in McDougall's buffer by following similar procedure and the values served as blank. Supernatant obtained from 25 ml of buffer solution and that present in the same volume of uncentrifuged buffer solution was estimated in the similar way.

Ca solubility from different Ca sources was estimated by the method given by Yano et al. (1979) following the formulae given as under:

$$\text{Ca solubility in SRL (\%)} = \frac{\text{Amount of Ca present in the supernatant obtained from 25 ml of ruminal buffer (aliquot I)-blank}}{\text{Amount of Ca present in 25 ml of uncentrifuged ruminal buffer(aliquot II)-blank}}$$

Trial II: Phosphorus solubility of P supplements in ruminal buffer

P solubility in ruminal buffer was studied with materials like superphosphate, Mussorrie rock phosphate and dicalcium phosphate and the solubility data were compared with those obtained by using pure sodium dihydrogen phosphate (A.R.Grade). Each of the source tested was weighed in separate 250 ml conical flask to provide 50 mg of P. The samples were processed in similar way as described in trial I (for Ca). The P content in different aliquots (I and II) was measured spectrophotometry as per procedure described at 3.1.4.2.

3.4.2 RESULTS

Calcium solubility of Ca supplements in ruminal buffer

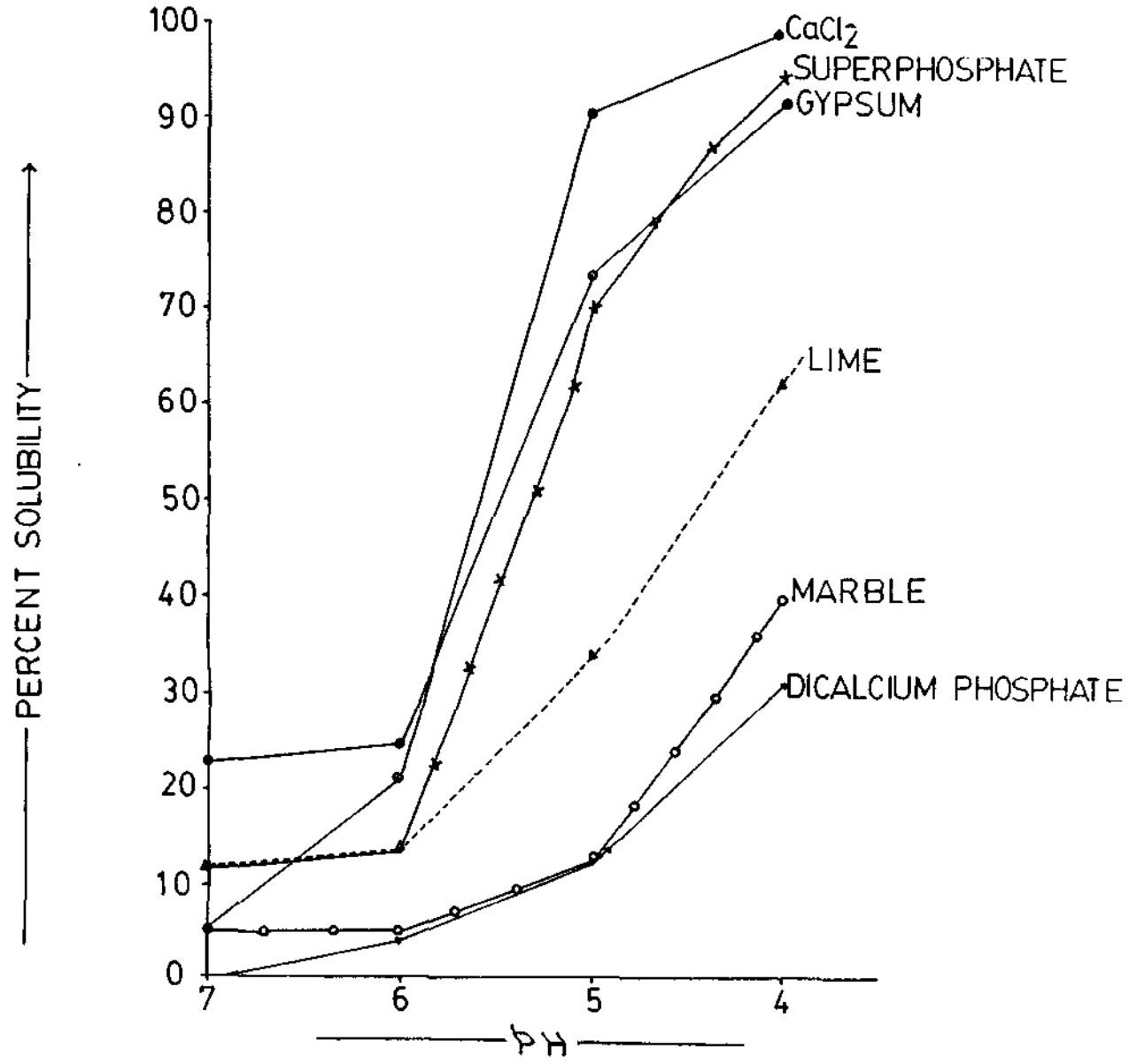
Materials like gypsum, lime, marble, dicalcium phosphate, superphosphate and rock phosphate were taken for Ca solubility studies and compared with pure $CaCl_2$

(A.R.Grade). The solubility data of different Ca supplement and pure $CaCl_2$ have been presented in table 3.11 and also depicted in Fig.3.4. It was observed that either at pH 7 or at any other pH, Ca solubility from different chemical forms of supplements was essentially dissimilar. At pH 7 that is approaching normal ruminal pH Ca from gypsum showed highest solubility of 23.0%, whereas Ca from dicalcium phosphate showed least solubility, i.e., 0.0%. At pH 4 that is near the abomasal fluid pH, $CaCl_2$ showed highest Ca solubility, i.e., 98.8% whereas dicalcium phosphate showed least Ca solubility, i.e., 32.6%.

Table 3.11 Ca solubility from different Ca supplements in relation to ruminal pH

Supplement	Predominal chemical form	Calcium solubility (%) pH			
		7.0	6.0	5.0	4.0
1. Calcium chloride (A.R.Grade)	$CaCl_2.2H_2O$	8.3	21.3	90.7	98.8
2. Gypsum	$CaSO_4.2H_2O$	23.0	24.0	73.0	91.5
3. Lime	$Ca(OH)_2$	12.0	14.0	34.0	62.0
4. Marble	$CaCO_3$	5.0	4.0	13.0	39.6
5. Superphosphate	$Ca(H_2PO_4)_2$	11.8	11.8	70.5	92.5
6. Dicalcium phosphate	$CaHPO_4$	0.0	4.0	13.0	32.6

FIG-3·4 IN VITRO CALCIUM SOLUBILITY IN RUMINAL BUFFER IN RELATION TO PH



Phosphorus solubility of P supplements in ruminal buffer

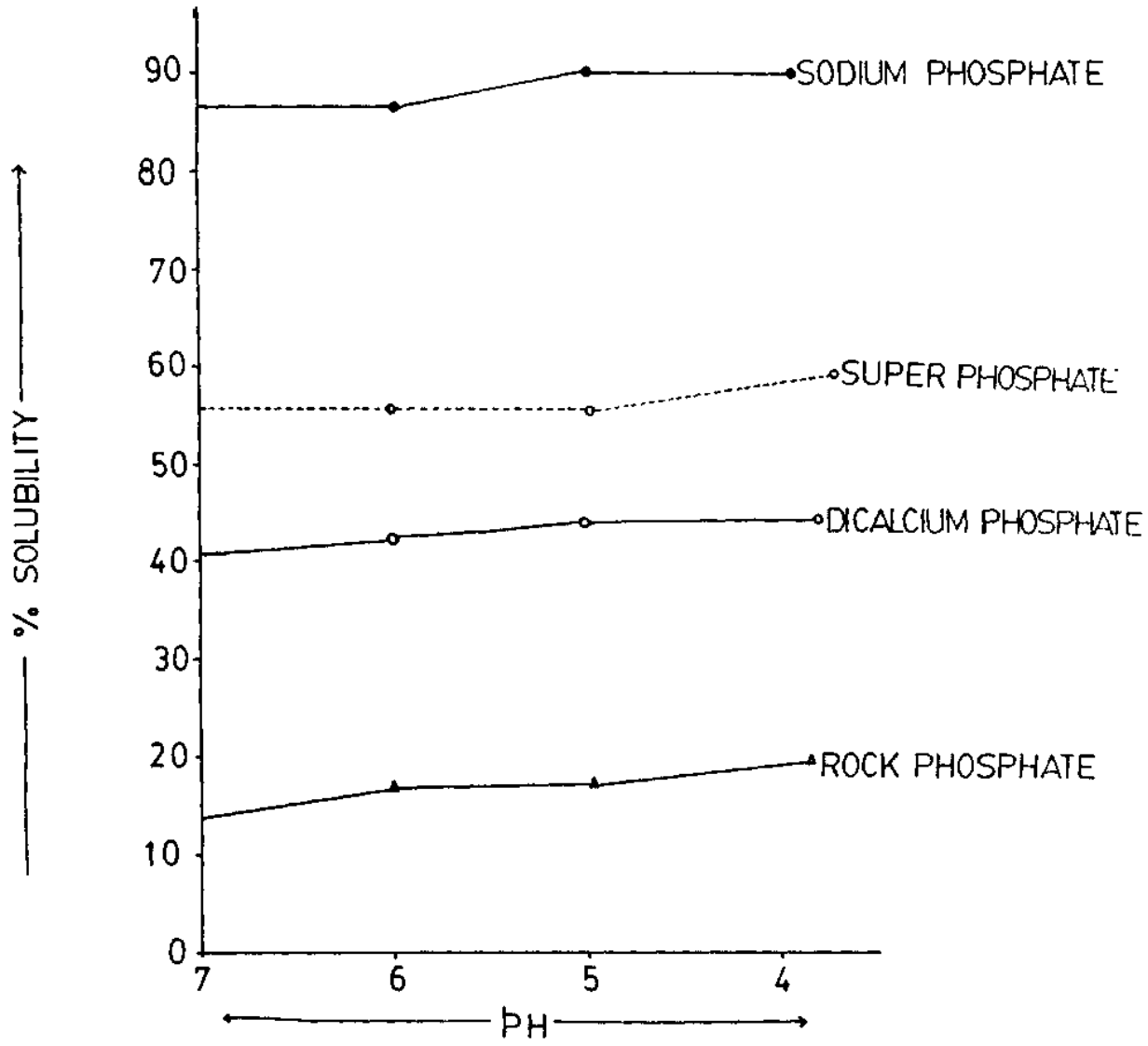
Materials like Mussorrie rock phosphate, fertiliser grade superphosphate, and dicalcium phosphate were taken for P solubility studies and the results were compared with those obtained from pure sodium phosphate. The results have been summarised in table 3.12 and also depicted in Fig 3.5. Unlike solubility of Ca sources, changing pH conditions of the ruminal buffer from 7.0 through 4.0 did not have any marked influence on P solubility of different sources investigated. It was observed that either at pH 7 or at any other given pH, P solubility from different chemical forms of these supplements was essentially dissimilar. Mussorrie rock phosphate showed least P solubility (14-19.1%), whereas sodium phosphate showed the highest P solubility (86.5 - 90.0%). The reason for such differences in solubility could be attributed to the influence of chemical form of the supplement on P solubility.

Table 3.12 P solubility from different forms of P supplements in relation to ruminal pH

Supplement	Predominant chemical form	Phosphorus solubility(%)			
		pH			
		7.0	6.0	5.0	4.0
1.Sodium phosphate A.R.Grade	NaH_2PO_4	86.5	86.5	90.0	90.0
2.Super phosphate	$Ca(H_2PO)_2$	55.9	55.9	55.9	58.1
3.Dicalcium phosphate	$CaHPO_4$	40.7	42.4	44.1	44.1
4.Mussorrie rock phosphate	$Ca_3(PO_4)_2$	14.0	17.4	17.3	19.1

These values are average of two determinations

FIG-3·5 INVITRO PHOSPHORUS SOLUBILITY IN RUMINAL BUFFER IN RELATION TO PH



3.4.3 DISCUSSION

As mentioned, solubility measurements were carried out by the procedure of Witt and Owens (1983). However, as suggested by Yano et al. (1979), the supernatant in the present investigation was separated by high speed centrifugation instead of by filtration. Witt and Owens (loc.cit) found that for P solubility studies 1 hr mixing time was optimum and that increasing the mixing time beyond 1 hr had no advantage. Therefore, in the present investigation, 1 hr continuous mixing time in a shaker waterbath was followed. Further, in the present procedure McDoughall's buffer containing 50 mg P and 38 mg Ca per 50 ml of buffer solution was used instead of Johnson's buffer containing 25.6 mg P per 50 ml. In the calculation, to determine Ca and P solubility, the contribution of Ca and P present in McDougall's buffer was accounted for by making suitable blank correction by determining Ca and P in the McDougall buffer processed in similar manner as the test samples. Yano et al(loc.cit.) also made similar blank corrections in their solubility trials.

Solubility of Ca sources

Solubility data (Table 3.11 and Fig.3.4) revealed that Ca solubility from various Ca supplements, such as, lime, marble, gypsum, dicalcium phosphate, super phosphate and the control (pure calcium chloride) in ruminal buffer were not similar at pH 7. At this pH of

the buffer, the solubility ranged from 0 in case of DCP and 23 in case of gypsum. It was amazing to find that pure $CaCl_2$ which is highly soluble in water, showed only 5.3% solubility at pH 7 in buffered ruminal fluid. By scaling down the pH of the ruminal buffer from 7 through 4, the Ca solubility increased in all the cases. In case of $CaCl_2$ it reached to 98.8%. It was further evident that at pH approached that of abomasal fluid, the chloride and sulphate forms of Ca showed similar solubility. The phosphate forms of Ca, superphosphate showed solubility similar to gypsum but dicalcium phosphate showed only 32.6% solubility at this pH. At pH 4 marble (carbonate form) and lime (hydroxide form) showed 39 and 62% Ca solubility respectively. Storry et al. (1966) have reported all the Ca and Mg present in abomasal content of sheep were in soluble form, where the pH was in the range of 2-3. Bremner (1970) has suggested that pH of gut influenced solubility and availability of certain trace elements. He studied the changes in the concentration and solubility of Zn, Mn and Cu in the different parts of the alimentry tract of sheep and found that a relationship existed between the solubilities of the metal and pH of the gut contents.

If reaction of the gut influenced the solubility of Ca from different Ca supplement sources to such a great extent, it may be anticipated that the distribution of Ca into soluble and particulate phases in the rumen and flow

rates of the two phases from the rumen may influence the rate at which Ca could be made available at the absorption sites lower down the gut. Therefore, different sources of Ca supplements, may not be of similar value so far as different Ca supplement sources were concerned. Evidently, there may be the need to modify the quantitative proportion of alternate sources of Ca in a mineral mixture not only on the basis of composition but also on the basis of solubility and net availability.

Solubility of P sources:

Solubility data in table 3.12 and fig.3.5 revealed that the solubility of P from the different P supplements such as superphosphate, dicalcium phosphate, Mussorrie rock phosphate and pure sodium phosphate (control) varied greatly at pH 7. The solubility values were not influenced by the decrease in pH of the ruminal buffer solution from pH 7 through 4. Mussorrie rock phosphate showing lowest P solubility of only 17-19%, whereas pure sodium phosphate registered highest P solubility. Earlier studies suggested that P from different sources were not equally soluble. Chapman et al. (1955) found that utilisation of Phytin phosphorus was not good in comparison to other sources of phosphorus such as dicalcium phosphate and steamed bone meal, because Phytin phosphorus was almost insoluble. Witt and Owens (1983) compared four different forms of phosphorus and found that P from pure sodium phosphate, monodicalcium

phosphate (containing 21% P), monodicalcium phosphate (containing 18.5% P) and defluorinated rock phosphate was 100, 72, 41 and 29.7 percent soluble in *in vitro* ruminal buffer solution 1 hr after incubation. But different pH and incubation time (> 1 hr) had no significant effect on P solubility. They further suggested that the method of P solubility in ruminal buffer could be employed to indicate the ranking of different P sources for ruminants.

CHAPTER-IV

RUMINAL DISTRIBUTION AND DISAPPEARANCE OF Ca AND P WITH DIFFERENT SUPPLEMENT SOURCES

EXPERIMENT - I : INFLUENCE ON CERTAIN Ca AND P SUPPLEMENTS ON RUMINAL DISTRIBUTION AND DISAPPEARANCE PATTERN OF Ca AND P, AND RUMEN FEED METABOLITES

There are reports to indicate the mineral availability to ruminants from different chemical forms of mineral elements present in certain mineral supplements are not similar and the availability does not depend only upon total overall supply of the minerals in diet. Ammerman et al. (1957) indicated that due to lower solubility, P availability from defluorinated rock phosphate was lower than that from dicalcium phosphate or steamed bone meal. Mg from dolomite limestone was only 14% available as against 52% availability from pure MgO (Gerken and Fontenot, 1967). If such differences in the availability of mineral elements exist, it is possible that different chemical forms of mineral elements present in various mineral supplements may influence the gut solubility of the mineral in question and also the distribution of the element in soluble, particulate and solid phases. Further, certain research findings also suggest that due to different chemical forms of Ca and P supplementation, the normal metabolic profile in the rumen gets altered.

Thus, Plumlee et al. (1958) have reported that soft phosphate of collodial clay as P supplement reduced feed intake, feed gain efficiency and lower blood P levels. Krogger and Carrol (1964) found that dietary gypsum supplementation decreased the rate of passage of ruminal contents through the gastro-intestinal tract and caused acidosis. Brink and Steele (1985) reported that lime as a Ca source influenced the buffering capacity of the rumen and also post ruminal digesion of starch and NDF.

It was, therefore, considered desireble to study the ruminal distribution of Ca and P from certain conventional and non-conventional Ca and P sources and to study the rate at which these elements leave the rumen. In this study, the influence of materials like marble, gypsum, Mussorrie rock phosphate and superphosphate were compared with dicalcium phosphate as a source of Ca and P supplement to cattle on the distribution and disappearance pattern of Ca and P from the rumen has been investigated. Further, the rumen-pH, ammonia-N and TVFA concentrations in the rumen liquor have been estimated so as to investigate their influence on rumen fermentation pattern.

4.1 MATERIALS AND METHODS

4.1.1 Experimental animals and their management

Four crossbred (Karan-Fries) male animals of about 2 years of age and weighing about 200-250 kg (table 4.1) were selected from the herd of the National Dairy

Research Institute.

Table 4.1 Showing details of experimental animals

Animal No.	Date of Birth	Weight (kg)
KF - 4357	1.5.83	200
KF - 4371	25.6.83	238
KF - 4346	5.4.83	264
KF - 4364	7.6.83	240

Animals were housed in individual pens and maintained under stall fed conditions in a clean and healthy environment. The stalls were plastic painted to avoid licking of walls. Daily washing the animals with clean water and disinfectants was routinely followed in order to control flies and other infection. The animals were dewormed using 'Panacure' from time to time to keep them free from any worms infestation. Clean tap water was provided free choice twice daily, to meet their water requirements.

Rumen fistulation and care of fistulae

Two stage surgical operations were carried out to fix rumen fistulae in each animal. Specially prepared ice bags having a metal plate at the outer sleeve was fixed in the process of fistulation. A hard rubber tubing was inserted inside the ice bag so that inner sleeve of the bag remained in perfectly expanded state making the rumen air tight as far as possible.

Special care was taken to maintain the fistulated animals. Apart from keeping the animals clean, the rumen fistulae were checked daily for its intactness and position. Application of 'Lorexane' and a mixture of phenyl and linseed oil (in the ratio of 1:1) around the rumen fistulae was routinely practiced biweekly in order to keep them free from maggots. In case of maggot infestation on the fistulated wounds, application of turpentine oil alone or mixed with weak tincture iodine solution was found very effective in treating the animals.

4.1.2 Experimental feeding

Preparation of basal concentrate mixture:

The basal concentrate mixture prepared by mixing appropriate quantities of maize, groundnut cake and wheat bran (table 4.2), supplied 20 percent CP (16% DCP) and 70% TDN. These figures were calculated from the standard values given in ICAR Bulletin No.25 (Sen and Ray, 1978). In addition, Rovimix as vitamin supplement, having the strength of 82,000 I.U. of vitamin A and 10,000 I.U. of vitamin D per gm of Rovimix powder, was mixed @ 10 gms/quintal of concentrate mixture. To this basal concentrate mixture, experimental mineral mixtures prepared with different Ca and P supplements (table 4.3) were added to respective batches for feeding of animals.

Table 4.2 Ingredients of concentrate mixture

Ingredients	Proportion (%)
Maize crushed	40
Groundnut cake	30
Wheat bran	30

Preparation of experimental mineral mixtures:

Based on the Ca and P content of various mineral supplements scanned (experiment-I, tables 3.6 and 3.8), materials like dicalcium phosphate, gypsum, marble powder, mussorrie rock phosphate and fertiliser grade superphosphate were selected as Ca and P source in the respective mineral mixtures for in vivo experiments.

Six different treatment groups (T_0 to T_5) of mineral mixtures were prepared (table 4.3) in a way that only the sources of Ca and P supplements was varied. Group T_0 served as negative control and contained no Ca and P source, whereas group T_1 made with conventional sources like dicalcium phosphate and chalk powder served as positive control. The calcium and phosphorus sources were marble powder and sodium phosphate in group T_2, gypsum and sod. phosphate in group T_3, rock phosphate and sod.phosphate in group T_4 and superphosphate and sod. phosphate in group T_5. The quantities were so adjusted that most of the Ca and P needs were met from the first

Table 4.3 Details of different Ca and P sources used in the preparation of mineral mixtures

Treatments	Ca/P source used	Ca & P content		Qty(kg) per 100kg of conc. mix.	Total supply of Ca and P through supplement in 100kg conc. mix.	
		Ca(%)	P(%)		Ca(g)	P(g)
T_0 (-ve control)	Nil	-	-	-	-	-
T_1 (+ ve control)	Dicalcium phosphate	32.0	28.0	1.650	528	478
	Chalk powder	41.0	-	0.331	136	-
					664	478
T_2	Marble powder	40.0	-	1.660	664	-
	Sod.phosphate	-	19.87	2.405	-	478
					664	478
T_3	Gypsum	16.0	-	4.150	664	-
	Sod.phosphate	-	19.87	2.405	-	478
					664	478
T_4	Rock phosphate	25.0	12.0	2.650	662	318
	Sod.phosphate	-	19.87	0.800	-	160
					662	478
T_5	Superphosphate	17.3	9.67	3.838	664	372
	Sod.phosphate	-	19.87	0.530	-	105
					664	477

In addition to the Ca/P source used, the following ingredients were used per 100 kg of conc. mix. for the preparation of complete mineral mixture. Sodium chloride 900 g; Magnesium carbonate 90g; Ferrous sulphate 1.6g; Copper sulphate 2.1g; Cobalt chloride 1.5g; Manganese dioxide 2.1g; Potassium iodide 0.3g and zinc sulphate 7.5g.

ingredient in the group and sodium phosphate was used only to marginally balance the remaining P needs. As evident from the table 4.3, all the test groups excepting the negative control supplied same quantity, i.e., 664g Ca and 478g P in 100 kg conc.mixture and the groups varied only with respect to the source.

To all the treatment groups the supply of other minerals including trace minerals were similar. 100 kg of concentrate mixture prepared with each treatment group was fortified with pure chemicals as shown in table 4.3.

Feeding schedule:

Concentrate and roughage (wheat straw) were the same in all the groups. The experimental treatments differed only with respect to supplementation of Ca and P from mineral mixtures as the mineral mixtures of different treatment group contained different sources of Ca and P supplements. The concentrate mixture prepared with the respective mineral mixtures were grouped in treatments T_0 to T_5 as shown in table 4.3.

The NRC feeding standards (1978) were followed in preparing the feeding schedule. Each animal was offered 2 kg of concentrate mixture daily at 9.00 A.M. and soon after its consumption wheat straw was provided ad libitum. The animals were fed in clean plastic troughs so as to avoid mineral contamination from external sources. All the four animals were fed under the each treatment for a period of 21 days (prefeeding)

at the end of which the samples of rumen liquor/ruminal contents/blood were taken for 6 consecutive days for the estimation of various parameters as described below. This constituted one treatment group. All the four animals were then switched over to the next treatment in a switch over design in which again experimental pre-feeding was followed for 21 days and samplings were made for next 6 days.

4.1.3 Experimental procedures

After following experimental feeding for 21 days, samples of rumen contents were collected for (i) 2 consecutive days for studying ruminal distribution of Ca and P and (ii) for next two days for ruminal metabolite, whereafter (iii) the animals were switched over to ruminal volume and ruminal fluid flow rate studies. Samples were collected as discussed later under the respective headings.

Sampling of ruminal contents for studies of Ca and P distribution

Animals were given 2 kg of experimental concentrate mixture according to the respective treatment at 9.00 AM followed by 2 kg of wheat bhoosa. Water was given ad libitum strictly at 11.00 AM so that samples of ruminal contents could be obtained at 1.00 PM, i.e., 4 hrs after feeding the concentrate mixture. For collecting the rumen digesta, the ice bags were removed from the rumen fistulae and rumen contents were mixed manually by inserting the

hand inside the rumen, so as to be able to collect a representative sample. About 500 ml of rumen digesta sample were collected in stoppered measuring cylinder from each of the fistulated animals and brought to the lab for further analysis.

Processing of ruminal digesta and calculation of distribution of Ca and P:

The samples of rumen digesta were filtered through four layers of cheese cloth by pressing it hard so as to squeeze out as much strained rumen liquor (SRL) as possible. The volume of the SRL obtained from 500 ml of the rumen digesta was recorded in each case and solid portion of the rumen digesta left after squeezing was kept in oven for drying at 80± 5^{0}C for 24 hours. This portion of the rumen digesta made the sampling for solid phase. The SRL was used to separate the soluble phase (supernatant) and the particulate phase (residue). For this separation 25 ml aliquot of the SRL was centrifuged at 16,000xg at room temp. and supernatant (soluble phase) was preserved in deep freeze for further analysis. Ca and P concentrations were measured both in the SRL and the supernatant as described in Chapter III (3.4.1).

Concentration of these elements in the particulate phase (residue) was calculated by difference in the following manner:

Total Ca in supernatant from 25ml SRL = X mg

Total Ca in 25ml of centrifuged SRL = Y mg

Therefore, Ca present in particulate phase of

25 ml of SRL = (X - Y) = Z mg

If 'A' is the volume (ml) of SRL obtained after squeezing 500 ml of the rumen digesta, then total Ca present in particulate phase in 500 ml of rumen digesta can be calculated as:

$$= \frac{Z \cdot A}{25} \quad \text{mg Ca}$$

Similarly, the amount of Ca present in soluble phase (i.e., in supernatant) in 500 ml of rumen digesta was calculated as:

$$= \frac{X \cdot A}{25} \quad \text{mg Ca}$$

Similar calculation for P distribution in supernatant and particulate was made.

The dry solid portion of the rumen digesta obtained was weighed and a representative sample was dry ashed. The HCl extract was prepared as in 3.1.4.1.1 Ca was estimated by AAS 3.1.4.1.2 and P was analysed 3.1.4.2.3. Total content in the solid phase of 500 ml of ruminal digesta was calculated. Finally, the proportion of Ca and P distributed in soluble, particulate and solid phase of the rumen digesta was presented.

Sampling and determination of rumen metabolites:pH, TVFA, individual VFA and NH_3-N concentration in rumen liquor:

After two days sampling for ruminal distribution, rumen samples were also collected for next two days while keeping the animals on same feeding regime. Samples were collected 4 hrs after concentrate feeding as described in 4.1.3. Rumen samples were collected by means of a hard

polythene tube. The samples were strained through a muslin cloth to collect 100 ml of SRL in a polythene bottle which was immediately brought to the laboratory for further analysis. The pH of the SRL was immediately recorded and then few drops of 1N H_2SO_4 was added to check microbial fermentation before the samples were transferred to deep freeze for further analysis of some metabolites.

pH:- pH was determined with the help of ECIL digital pH meter (Model EC-5651).

Ammonia-N:- Micro diffusion technique of Conway (1962) was followed to determine ammonia-N concentration in SRL. In the outer compartment of conway micro diffusion cell, 1 ml of SRL was taken on one side, whereas another 1 ml of saturated sodium carbonate solution was taken on the other side. Finally, 1 ml standard boric acid solution containing mixed indicator was placed in the inner well and lid was fixed making the system leak proof. The contents in the outer compartment were mixed by gently tilting and rotating the micro diffusion unit whereafter it was placed in an incubator adjusted at $39^{o}C$ and kept there for 3 hours. The contents of the inner chamber were then titrated using a microbiurette against 0.01 N H_2SO_4 to a light pinkish colour. The ammonia-N concentration was calculated as:

Ammonia-N(mg/100ml SRL) = ml of acid use x normality of acid x 14 x 100

Total volatile fatty acids (TVFA):

The concentration of TVFA in SRL was estimated by the method of Barnet and Reid (1957). 2 ml of SRL alongwith 2 ml of oxalate buffer (Scarsbrick, 1952) was steam distilled in a Markham's distillation apparatus. About 100 ml of distillate was collected, which was then titrated against 0.01N NaOH using phenolphethelin as an indicator. TVFA concentration in meq/100 ml in SRL was calculated as follows:

$$\text{TVFA(in meq/100 ml of SRL)} = \frac{\text{Vol. of NaOH used} \times \text{Normality of NaOH} \times 100}{2}$$

Partitioning of TVFA:

Individual VFA fractions were partitioned and estimated with the help of Nucan gas chromatograph series 5500 fitted with FID (Flame ionisation detector) and stainless steel column (2 MX0.25 cms) containing chromosorb-101, and using nitrogen as the carrier gas at a flow rate of 40-60 ml/minute.

Before injecting the samples into the gas chromatograph column, SRL was processed by the method of Erwin et al. (1961). 4 ml SRL was mixed with 1 ml of 20% metaphosphoric acid solution prepared in 5N H_2SO_4. Deprotonised sample of SRL was centrifuged at 4000 rpm for 10-15 min. The supernatant thus obtained was used for monitoring through the gas chromatograph.

0.2 ul of this supernatant was injected into the injection port of the gas chromatograph. The chambers

containing the column and injector were already equilibrated at 180 and 220°C respectively.

A standard containing acetate, propionate and butyrate in the molar ratio of 60:30:10 was also run into the gas chromatograph under the similar conditions, as those of the samples. The standard peaks obtained on the graph roll were used for identification and quantification of peaks in the test samples. Different proportions of acetate, propionate and butyrate in the samples of SRL were calculated on the basis of respective peak areas as shown below:

Peak area = ½ x height of the peak x width of base line

Rumen disappearance pattern of Ca and P:

The disappearance pattern of Ca and P from the rumen was calculated, based on the principle of disappearance pattern of N from rumen, from various protein sources, as described in the method given by Loerch *et al.* (1983).

Disappearance pattern of Ca or P through rumen fluid = Rumen fluid flow rate (l/hr) x mean Ca or P concentration of rumen fluid (mg/l)

The rumen fluid flow rate (l/hr) of all the animals under different treatments was calculated by estimating first their respective rumen volumes, the methodology of which has been discussed below. The concentration of Ca and P in the samples of rumen fluid, i.e., SRL taken at different time intervals was estimated in ruminal fluid

after ashing known amount of aliquot by the procedure described earlier in 3.4.1.

Rumen fluid volume and flow rate:

Rumen fluid volumes of all the animals under different treatments were estimated with the help of polythylene glycol (PEG) of mol.wt = 4000 using the method of Hyden (1956) as modified by Smith (1959). During rumen volume and flow rate determination the feeding pattern was slightly changed. In order to achieve steady state of rumen fermentation, the required quantity of feed was divided and given at periodical intervals. The animals were maintained in a steady state condition of rumen fermentation as far as possible, by offering them wheat straw and water distributed at hourly intervals and was given immediately after the collection of the rumen sample was made. The concentrate mixture was offered as usual in the morning at 9.00 AM. A sample of rumen liquor was collected just prior to feeding of the concentrate mixture and taken as zero hr collection. 100 ml of 25% solution of PEG was infused into the rumen just at the time of feeding of concentrate mixture. For better mixing of PEG, the rumen contents were stirred manually and one hr was allowed as the mixing time of PEG in the rumen as recommended by Smith (1959). Thereafter the samples of rumen liquor were collected at 1, 2, 3, 5 and 7 hours after the infusion of PEG solution, and subsequently stored in deep freeze for further analysis.

Estimation of PEG concentration:

The samples of rumen liquor were analysed for its PEG concentration by the method of Smith (1959) which has been described below.

Reagents used:(i) $ZnSO_4$ solution (5% W/V).

(ii) (0.3N) $Ba(OH)_2$ solution.
4.7325 g of Barium hydroxide was dissolved in 70 ml of hot distilled water and the volume was made to 100 ml.

(iii) $BaCl_2$ solution (10% W/V).

(iv) TCA - $BaCl_2$ solution. 75 g of the TCA and 14.75 g of $BaCl_2$ were dissolved separately in the two beakers containing 100 ml of distilled water. The two solutions were transferred to a 250 ml volumetric flask and the volume was made up. This gave the concentration of TCA (30 percent w/v) and $BaCl_2$ (5.9 percent w/v). The mixture was kept overnight and filtered before being used.

Preparation of standard curve:

The procedure followed for the preparation of standard curve is given below:

Steps	Test tubes						
	Blank	1	2	3	4	5	6
1. SRL taken(ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
2. PEG solution (25mg/100 ml)	0.0	0.5	1.0	1.5	2.0	2.5	3.0
3. 0.3N $Ba(OH)_2$	2.0	2.0	2.0	2.0	2.0	2.0	2.0
4. 5% $ZnSO_4$ soln.	2.0	2.0	2.0	2.0	2.0	2.0	2.0
5. 10% $BaCl_2$ son.	0.5	0.5	0.5	0.5	0.5	0.5	0.5
6. d.H_2O	4.5	4.0	3.5	3.0	2.5	2.0	1.5
Total(ml)	10.0	10.0	10.0	10.0	10.0	10.0	10.0

The contents of above tubes were well mixed and centrifuged at 3000 rpm for 10 minutes. 2 ml filtrate was taken and to it was added 5 ml of the TCA/$BaCl_2$ solution and 3 ml of distilled water so as to make it 10 ml. This was left exactly for 30 minutes for the development of turbidity and readings were taken in a spectrophotometer, spectronic-20, at 540 nm wave length. A standard curve was drawn for different concentration of PEG against OD readings (Fig.4.1).

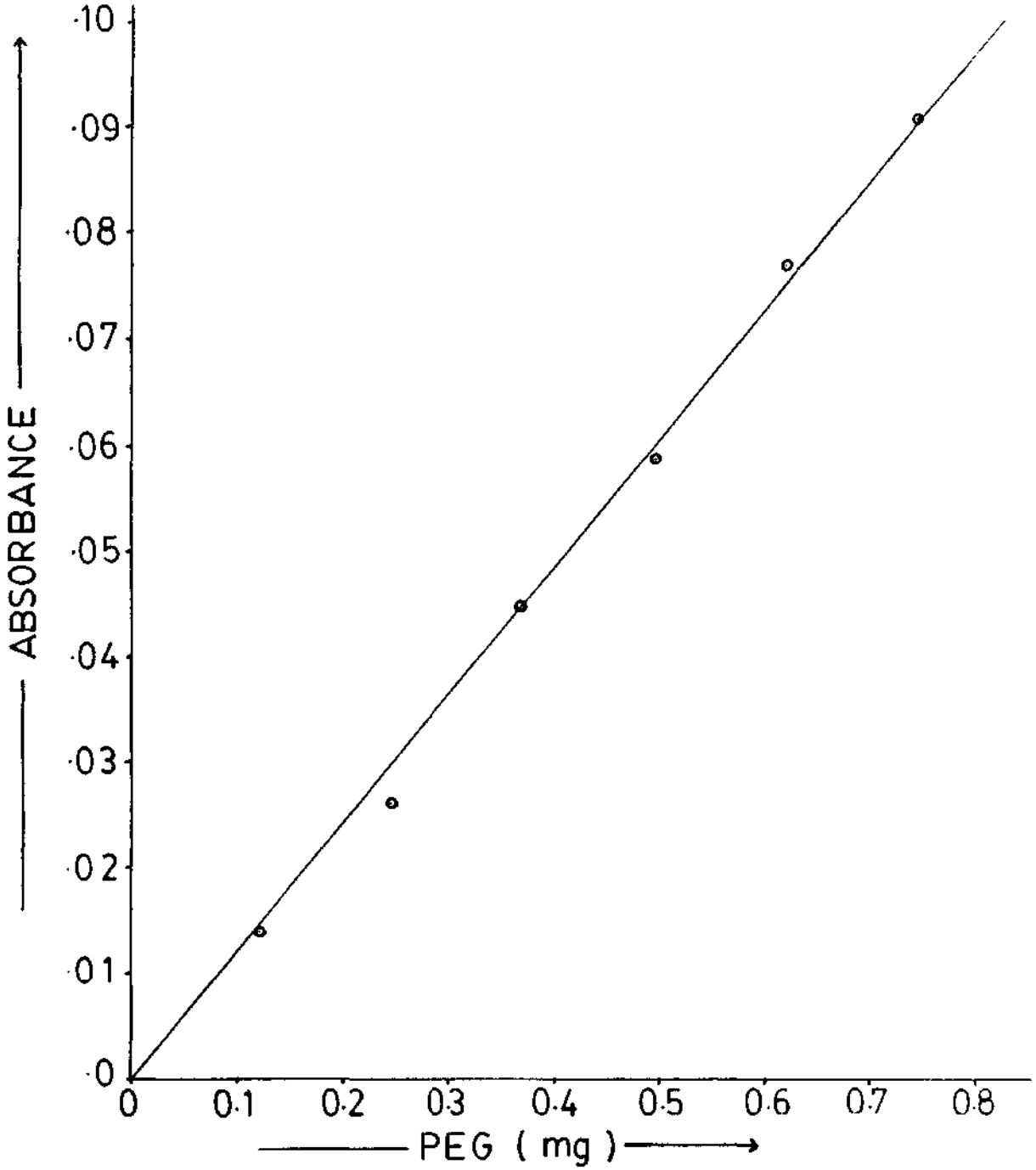
Calculations:

The PGE concentration/100 ml SRL was calculated with the help of standard curve (Fig.4.1) as follows:

From the standard curve

0.045 OD = 0.375 mg PEG

FIG -4.1 STANDARD CURVE FOR ESTIMATION OF PEG



For x OD (Vol.of SRL=1ml) $= \frac{0.375}{0.045}$. x mg PEG/ml of SRL

$= 8.33 \times$ mg PEG/ml of SRL

$= 8.33 \times$ mg PEG/100 ml of SRL

Using the above standard curve PEG concentrations for the five rumen collections made at 1, 2, 3, 5 and 7 hrs was estimated. The values of PEG concentrations against collection of 1, 2, 3, 5 and 7 hrs were plotted on a semilog graph paper and PEG concentration at zero hr was extrapolated. Rumen fluid volume was calculated at the time of infusion of PEG into the rumen by making use of the following equation:

$$\text{Rumen volume (ml)} = 100 \cdot \frac{b}{a}$$

Where, 'b' is the quantity of PEG (mg) infused into the rumen, and 'a' is the zero hr PEG concentration (mg/100 ml SRL).

Determination of ruminal fluid flow rate:

The ruminal fluid flow rate (l/hr) was calculated from the values of ruminal volume (RV) and exponential decline constant (b) in PEG conc. by using the following formula:

$$\text{Flow rate(l/hr)} = \text{RV(litres)} \times b \times 60$$

Ca and P concentration in blood serum:

The blood was collected in clean test tubes by jugular puncture. The blood samples were allowed

to clot. The clear serum was separated out and preserved in deep freeze for further analysis.

Serum Ca content was estimated by the method of Clark and Collip (1925) as modified by Sandroy (1944). The serum P concentration was estimated by the method of Fiske and Subbarow (1925).

4.2 RESULTS

4.2.1 Mineral composition of experimental mineral mixtures:

The mineral composition of experimental mineral mixtures T_1-T_5 prepared with different Ca and P sources have been presented in table 4.4A. Although there was wide variation in the Ca and P content of different mineral mixtures, quantities either of Ca and P per 100 kg of concentrate mixture were adjusted in a way that the respective concentrate mixtures supplied the same quantity of Ca and P (table 4.4B and 4.4C).

Except T_0 the negative control Ca and P content of different concentrate mixtures (T_1 to T_5) varied in the range of 1.05-1.09% for Ca and 1.08-1.15% for P, but T_0 contain 0.34% Ca and 0.56% P through the basal concentrate mixture. Wheat straw which was common for all the groups, contained 0.26% Ca and 0.1% P. There was only little variation in Mg, Fe and other trace minerals content in the concentrate mixtures under the respective treatment groups.

4.2.2 Dry matter, Ca and P intake of animals

Average live weight of animals and their total dry matter intake have been presented in table 4.5. The mean value for dry matter intake of animals in the six groups were 4.557 ± 0.112, 4.646 ± 0.106, 4.902 ± 0.098, 4.984 ± 0.142, 4.519 ± 0.101 and 4.947 ± 0.111 kg per day

Table 4.4A Mineral composition of experimental mineral mixtures prepared with different Ca and P supplemental sources

Treatments	Ca and P source	Mineral composition(% DM basis)									
		Ash	AIA	Ca	P	Mg	Fe	Mn	Cu	Zn	Co
T_0	Nil	93.77	-	0.36	-	4.60	0.33	0.054	0.075	0.270	0.051
T_1	Dicalcium phosphate	86.48	0.15	21.69	16.01	1.37	0.21	0.022	0.029	0.064	0.017
T_2	Marble powder+ sod.phosphate	95.77	0.13	12.46	8.53	1.53	0.17	0.015	0.021	0.056	0.007
T_3	Gypsum+sod. phosphate	86.36	15.05	8.78	6.08	0.91	0.12	0.015	0.031	0.031	0.006
T_4	Rock phosphate	96.07	10.34	14.72	10.28	1.37	0.67	0.046	0.023	0.071	0.006
T_5	Superphosphate	88.54	11.47	11.95	8.39	1.32	0.20	0.071	0.022	0.045	0.007

Above figures are mean of two determinations

Table 4.48 Mineral composition of experimental concentrate mixtures prepared with different sources of Ca and P supplements

Treatments	Ca and P source added to conc. mix.	Mineral composition									
		Ash	AIA	Ca	P	Mg	Fe	Mn	Cu	Zn	Co
		Percent DM basis						ppm			
T_0	Nil	4.61	0.12	0.34	0.56	0.085	0.054	69	27	81	20
T_1	Dicalcium phosphate	7.86	0.81	1.05	1.08	0.087	0.074	79	25	81	19
T_2	Marble + sod.phosphate	11.12	0.14	1.07	1.12	0.082	0.076	75	26	83	17
T_3	Gypsum + sod.phosphate	10.98	1.78	1.09	1.13	0.096	0.084	75	27	92	21
T_4	Rock phosphate	10.92	1.08	1.06	1.14	0.086	0.079	74	25	91	18
T_5	Superphosphate	10.12	0.88	1.08	1.15	0.091	0.061	89	31	91	21

Above figures are average of two determinations

Table 4.5 Average live weight, intake of dry matter, Ca and P of animals on using different sources of Ca and P supplements in concentrate mixtures

Particulars	Control		Treatments				F-value
	T_0 (-ve)	T_1 (+ve)	T_2 (marble) + sod.phos.	T_3 (Gypsum) + sod. phos.	T_4 (Rock. phos.)	T_5 (Super-phos.)	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Average live wt. of animals(kg)	238	241	238	234	236	232	0.18
DM intake (kg)							
Concentrate	1.731	1.718	1.725	1.734	1.742	1.724	-
Wheat straw	2.825	2.928	3.177	3.250	2.776	3.223	1.14
Total	4.557 ± 0.112	4.646 ± 0.106	4.902 ± 0.098	4.984 ± 0.142	4.519 ± 0.101	4.947 ± 0.111	1.71
DM intake/100kg body weight(kg)	1.907 ± 0.051	1.969 ± 0.053	2.065 ± 0.066	2.131 ± 0.077	1.910 ± 0.054	2.130 ± 0.063	1.26

....contd.

.....contd.table 4.5

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Intake of Ca(g/head/day)							
Concentrate	5.88	18.04	18.46	18.90	18.46	18.62	-
Wheat straw	7.34	7.61	8.26	8.45	7.22	8.40	-
Total	13.22^{a} ± 0.12	25.65^{b} ± 0.13	26.71^{b} ± 0.16	27.35^{b} ± 0.09	25.69^{b} ± 0.08	27.02^{b} ± 0.16	4.12*
Intake of P(g/head/day)							
Concentrate	9.69	18.55	19.32	19.59	19.85	19.82	-
Wheat straw	2.82	2.93	3.17	3.25	2.78	3.22	-
Total	12.51^{a} ±0.06	21.48^{b} ± 0.07	22.49^{b} ± 0.08	22.48^{b} ± 0.06	22.63^{b} ± 0.06	23.04^{b} ± 0.07	3.96*

Values with different superscripts(a,b) differ significantly

Each value is average of four figures

*P/0.05

respectively and differences were not significant ($P > 0.05$). Dry matter intake per 100 kg body weight of animals under the respective groups was 1.907± 0.051, 1.969± 0.053, 2.065± 0.066, 2.131± 0.077, 1.910± 0.054 and 2.130± 0.063 kg/day respectively. These differences were also not significantly different ($P > 0.05$). Intake of Ca and P through concentrate, through wheat straw and total have been presented in table 4.5, and it was evident that only group T_0(-ve control) was showing significantly lower value of total Ca and P intakes than all other groups which were statistically similar.

4.2.3 Effect of different sources of Ca and P supplements on rumen metabolites and blood serum Ca and P levels

4.2.3.1 Rumen metabolites:

The average values for rumen pH, NH_3-N concentration and total and individual VFAs levels in SRL collected 4 hrs after feeding, have been presented in table 4.6.

Rumen pH:- Rumen pH in different treatment groups T_0 to T_5 were 6.50± 0.09, 6.20± 0.13, 6.54± 0.06, 6.55± 0.07, 6.26± 0.11 and 6.56± 0.07, respectively, were statistically similar and reflected no influence of treatments.

Ammonia-N:-NH_3-N concentration in the rumen liquor of animals in treatments T_0 to T_5 were 11.83± 0.90, 16.43± 2.08, 16.31± 1.63, 15.26± 0.72, 12.03± 1.32 and 17.85± 0.69 mg/100ml SRL (table 4.6). These values were statistically similar in groups T_2, T_3 and T_5 and also in the control group (T_1)

but was significantly less ($P<0.05$) in group T_4(rock phosphate) and T_0 (-ve control), suggesting that rock phosphate may influence N-utilisation.

Total volatile fatty acids:- The level of total VFAs in respective treatment groups, i.e., T_0-T_5 were 9.65± 0.78, 9.54± 0.72, 9.29± 0.69, 8.26± 0.48, 7.01± 0.59 and 8.15± 0.73 meq/100 ml SRL. Although having lower values in group T_4(rock phosphate) and group T_5(superphosphate), the ruminal TVFA showed no significant difference ($P > 0.05$) indicating that function of cellulytic organisms in the rumen may get affected by feeding certain sources of Ca and P supplements in the concentrate mixtures.

The relative percentage of individual VFA's, i.e., acetate, propionate and butyrate also showed no treatment effect.

4.2.3.2 Ca and P levelsiin blood serum:

The average values of serum for Ca and P levels under different treatment groups are presented in table 4.6. It was evident that serum Ca levels in treatments T_0 to T_5 were 10.90± 0.41, 10.70± 0.46, 9.25± 0.38, 10.85± 0.47, 9.35± 0.51 and 10.20± 0.42 mg Ca/100ml respectively. Some depression in blood Ca levels in group T_2(marble) and T_4(rock phosphate) were not significantly different ($P > 0.05$) and values of blood Ca levels in all the groups were statistically similar. The serum Ca levels in negative and positive control groups, T_0 and T_1 were also similar.

Table 4.6 Rumen metabolites and serum Ca and P levels in animals under various treatments

	Control		Treatments				F-value
	T_0 (-ve)	T_1 (+ve)	T_2 marble + sod.phos.	T_3 gypsum + sod.phos.	T_4 rock phos.	T_5 superphos.	
Rumen pH	6.50 ± 0.09	6.20 ± 0.13	6.54 ± 0.06	6.55 ± 0.07	6.26 ± 0.11	6.56 ± 0.07	0.76
Rumen NH_3-N (mg/100ml SRL)	11.83^a ± 0.90	16.43^b ± 2.08	16.31^b ± 1.63	15.65^b ± 0.72	12.03^a ± 1.32	17.85^b ± 0.69	3.43*
Ruminal VFA							
(i) Total volatile fatty acids (meq/100ml SRL)	9.65 ± 0.78	9.54 ± 0.72	9.29 ± 0.69	8.26 ± 0.48	7.01 ± 0.59	8.15 ± 0.73	2.06
(ii) Proportions of individual VFA's(%)							
Acetate	63.5	65.5	67.5	66.5	67.5	68.2	1.80
Propionate	25.7	23.2	21.8	24.2	23.7	22.5	0.77
Butyrate	10.8	11.0	10.7	9.3	8.8	9.3	0.19
Serum Ca (mg/100ml)	10.90 ± 0.41	10.70 ± 0.46	9.25 ± 0.38	10.85 ± 0.47	9.35 ± 0.51	10.20 ± 0.42	0.48
Serum P (mg/100ml)	8.61 ± 0.12	10.28 ± 0.18	10.36 ± 0.16	10.86 ± 0.16	9.79 ± 0.22	8.36 ± 0.12	2.07

Values with different superscripts (a,b) differ significantly *P/0.05
Each value is average of four figures

The serum P levels in different treatments (T_0-T_5) were found to be 8.61± 0.12, 10.28± 0.18, 10.36± 0.16, 10.86± 0.16, 9.79± 0.22 and 8.36± 0.12 mg P/100 ml serum respectively and all these values were statistically similar. Although serum P levels in group T_0 and T_5 (-ve control and superphosphate) were found to be slightly lower compared to control group(T_1), the differences were not significant ($P > 0.05$).

4.2.4 Distribution of Ca and P in rumina[illegible] contents

The distribution of Ca and P in soluble (centrifuged SRL), particulate (centrifugate mass) and solid phases (separated solid portion) of the rumen digesta collected 4 hrs after feeding, have been presented in table 4.7.

4.2.4.1 Calcium:

Ca ranged from 6.9-9.4% in soluble phase, 6.3 to 11.4% in particulate phase and 82.9-86.7% in solid phase of the rumen digesta respectively. The distribution of Ca was lowest in the soluble phase of rumen contents and had almost similar value in the particulate phase also. However, Ca was mostly located in the solid phase of all the treatment groups. Data in table 4.7 further revealed that in case of marble and rock phosphate, the distribution of Ca in soluble and particulate phase of the rumen digesta was significantly higher as compared to that in dicalcium phosphate (control).

Table 4.7 Ca and P distribution (%) in ruminal contents of animals under various treatments

	Control		Treatments				F-value
	T_0 (-ve)	T_1 (+ve)	T_2 (marble + sod.phos.)	T_3 (gypsum + sod.phos.)	T_4 (rock phos)	T_5 (super-phos)	
Distribution of Ca(%)							
(i) in soluble phase	6.9^a	7.4^{ab}	8.3^b	7.3^{ab}	9.4^c	8.4^b	5.93*
(ii) in particulate phase	6.3^a	7.0^a	11.4^b	7.4^{ac}	8.7^c	8.0^{ac}	6.12*
(iii) in solid phase	86.7^a	85.8^a	80.5^b	85.7^a	82.9^c	83.6^c	14.07*
Distribution of P(%)							
(i) in soluble phase	45.5^a	37.2^b	52.5^a	43.8^c	29.3^d	39.5^b	26.52*
(ii) in particulate phase	9.8^a	5.0^b	3.85^b	11.8^a	13.5^c	12.5^{ac}	11.51*
(iii) in solid phase	44.7^a	57.8^b	43.7^a	44.4^a	57.2^b	48.0^a	11.54*

Values with different superscripts a,b,c,d in the same line differ significantly ($P<0.05$) *$P<0.05$

4.2.4.2 Phosphorus:

It was evident from table 4.7 that unlike Ca, P was distributed to sufficient extent in soluble phase and ranged from 29.3 to 52.5 % in different treatment groups. In the solid phase also there was sufficient P distributed and ranged from 43.7 to 57.8%. However, the distribution of P in the particulate phase was lowest and ranged from 5.0 to 13.5%. Data in table 4.7 further revealed that distribution of P in the soluble phase was highest in case of group T_2 where marble and pure sodium phosphate were used as Ca and P supplements and lowest in rock phosphate group (T_4).

4.2.5 Calcium and Phosphorus concentration in SRL

Ca and P concentration in SRL samples collected at 0, 1, 3, 5 and 7 hrs after feeding of concentrate mixture are presented in table 4.8 and also depicted in Figs. 4.1 and 4.2. There is no marked variation in the Ca concentrations of SRL collected at different time intervals from animals fed different sources of Ca and P in their respective concentrate mixtures. The mean of the values of Ca concentration in different groups (T_0 to T_5) were 9.86 $\pm$ 0.17, 14.60 $\pm$ 0.18, 14.20 $\pm$ 0.24, 14.04 $\pm$ 0.38, 13.92 $\pm$ 0.43 and 13.75 $\pm$ 0.42 mg Ca/100 ml SRL respectively. These values were similar in all the groups except group T_0 which showed lower value 9.86 mg Ca/100 ml SRL as no Ca supplementation was made in this group.

P concentration in the SRL samples was much higher as compared to Ca. The mean of the values of P

Table 4.8 Ca and P concentration (mg/100 ml) in SRL of animals under various treatments, as a function of time

Hours	Treatments					
	T_0	T_1	T_2	T_3	T_4	T_5
			Calcium			
0	9.3	14.0	13.7	12.7	13.0	12.7
1	9.7	14.5	14.0	15.0	15.0	14.5
3	10.0	15.0	15.0	14.0	14.3	14.7
5	10.0	14.5	14.5	14.0	14.5	14.0
7	10.3	15.0	13.8	14.5	12.8	12.8
Mean	9.86	14.60	14.20	14.04	13.92	13.75
SE±	± 0.17	± 0.18	± 0.24	± 0.38	± 0.43	± 0.42

....contd.

.....contd.table 4.8

Hours	Treatments					
	T_o	T_1	T_2	T_3	T_4	T_5
			Phosphorus			
0	49.3	48.8	62.2	53.1	49.8	65.15
1	50.2	49.2	61.8	54.8	48.2	63.33
3	51.4	50.3	63.2	55.6	48.5	63.66
5	50.6	50.2	62.6	55.5	49.4	65.81
7	51.6	50.0	60.4	54.0	48.7	64.81
Mean	50.62	49.78	62.04	54.60	48.92	64.600
SE±	± 0.41	± 0.30	± 0.47	± 0.47	± 0.29	± 0.4459

Each value is average of four observations

FIG-4.2 CALCIUM CONCENTRATION IN SRL

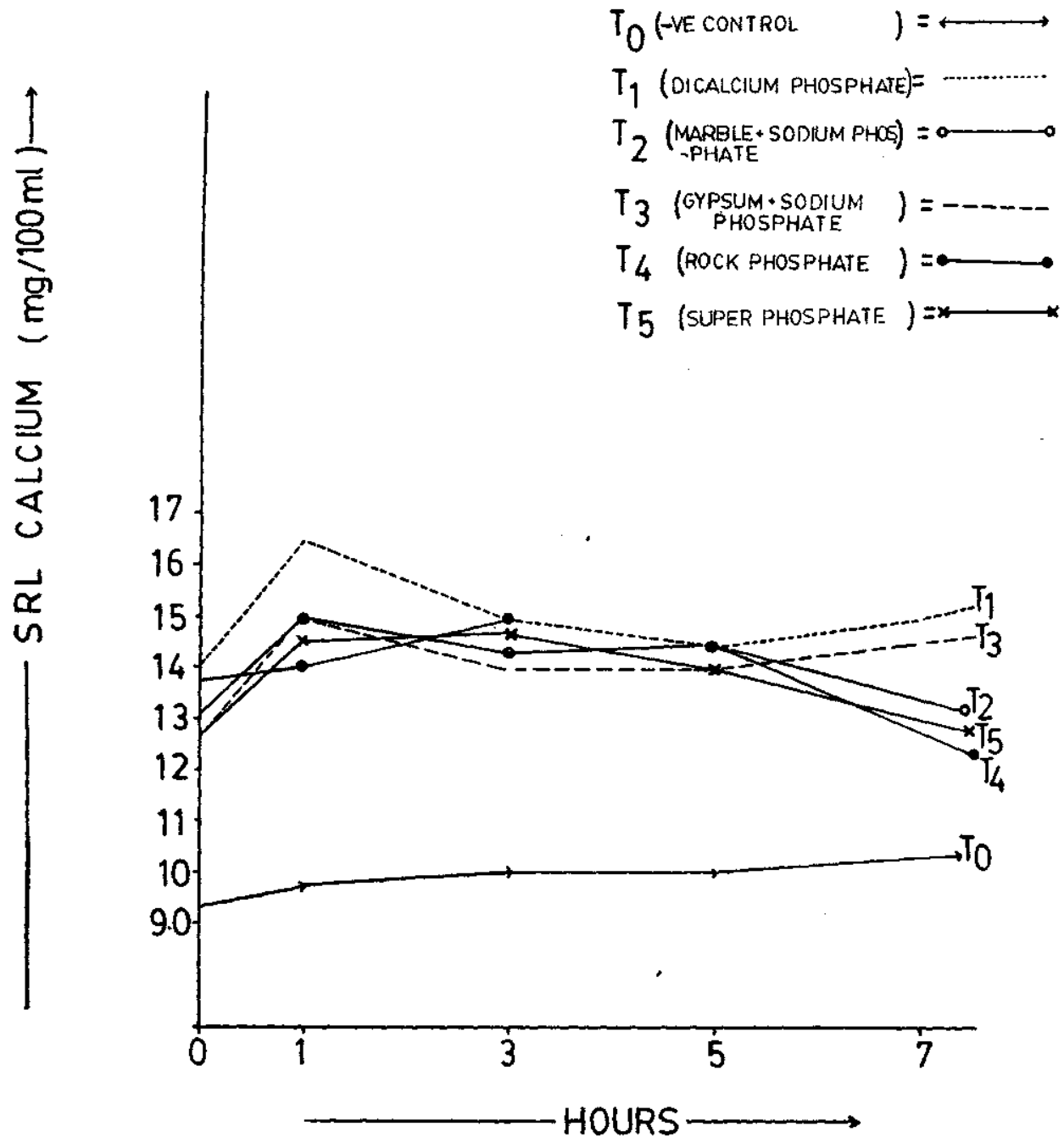
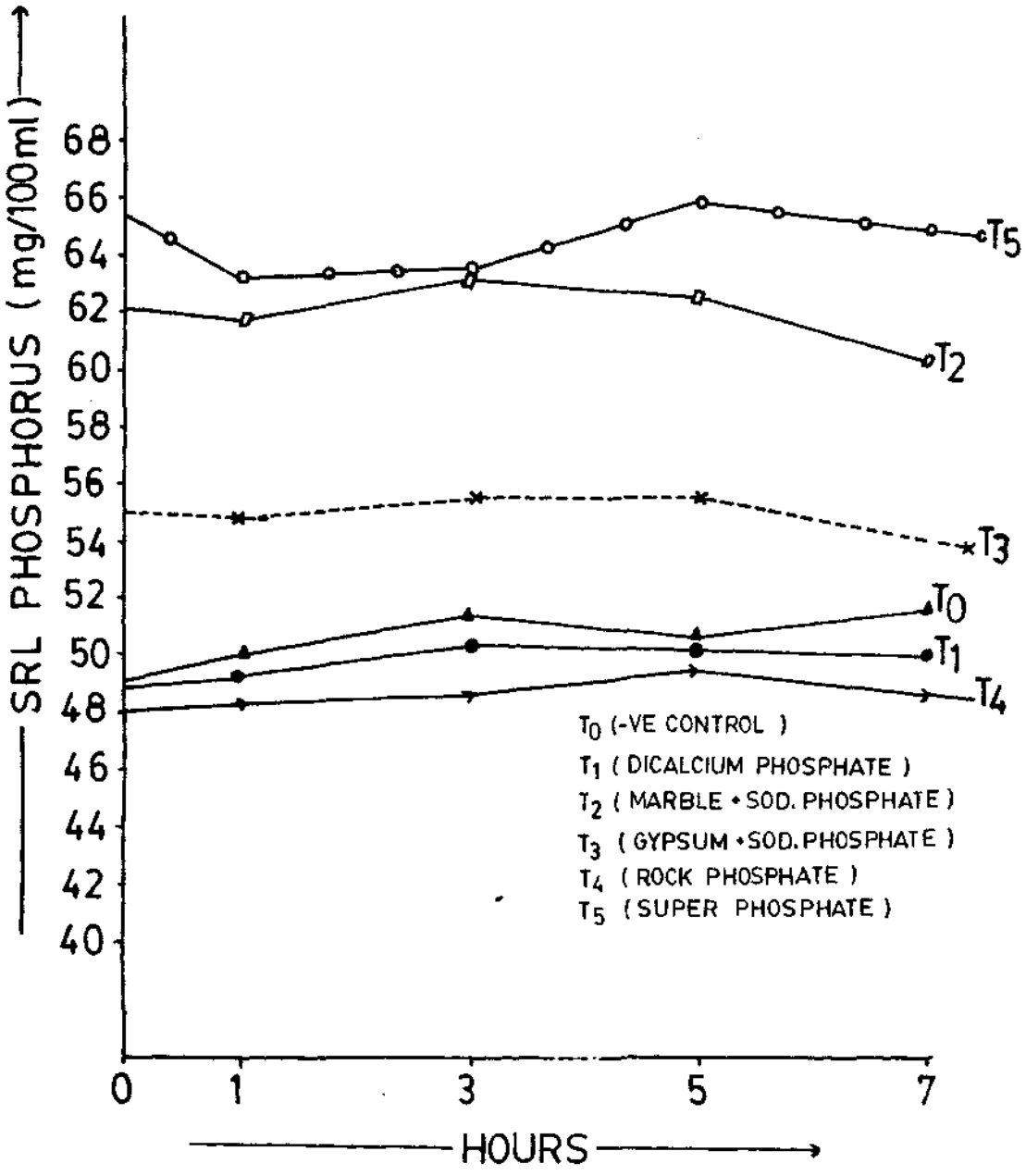


FIG-4.3 PHOSPHORUS CONCENTRATION IN SRL



concentration in various groups (T_0 to T_5) were 50.62 ± 0.41, 49.78 ± 0.30, 62.04 ± 0.47, 54.60 ± 0.47, 48.92 ± 0.29 and 64.60 ± 0.49 mg P/100 ml SRL respectively. The values were similar in groups T_0, T_1, T_3 and T_4 but only marginally higher in groups T_2 and T_5, indicating that the proportion of total P that is present in soluble plus particulate phases (representing SRL) was greater in these two groups as compared to others. Such findings were also supported by the P distribution studies in the rumen digesta (Table 4.7) where to total of soluble and particulate phases in these groups were high. Unlike Ca, P concentration in group T_0 to which no P supplementation was made, also showed similar value in comparison to control group.

4.2.6 Ruminal fluid volume, flow rate and Ca and P disappearance pattern

The disappearance rate of Ca and P from rumen was calculated as product of rumen fluid flow rate (l/hr) and mean rumen fluid concentration (mg/litre) of Ca and P respectively. The rumen fluid flow rate in animals under various treatments was calculated by estimating first the respective rumen volumes and then utilising this data in the estimation of rumen fluid flow rate as described in 4.1.3.

4.2.6.1 Rumen fluid volume and its flow rate:

The data on rumen fluid volume, rumen fluid flow rate and water intake of animals have been presented

in table 4.9. The mean values of rumen fluid volume under various treatments (T_0 to T_5) were 25.83± 0.63, 25.01± 1.17, 25.71± 1.30, 22.83± 1.36, 25.26± 1.33 and 21.55± 1.39 litres respectively. The mean values of rumen fluid flow rate in the respective groups were 0.993± 0.049, 0.874± 0.084, 0.950± 0.032, 0.856± 0.033, 0.833± 0.089 and 0.797± 0.04 l/hr respectively. There was no significant difference (P 0.05) in the values of rumen fluid volume, rumen fluid flow rate and water intake of animals under various treatments, indicating that the treatments had no effect on ruminal volume and flow rates.

4.2.6.2 Rumen disappearance rate of Ca and P:

The rumen disappearance rates of Ca and P through rumen fluid under various treatments have been presented in table 4.9. These values were 92.5± 4.2, 131.7± 5.6, 135.0± 4.5, 120.5± 3.6, 115.2± 7.2 and 109.0± 2.2 mg/hr for Ca and 474± 14, 436± 16, 579± 8, 473± 18, 413± 24 and 516± 10 mg/hr for P respectively. Disappearance rate of Ca through rumen fluid was significantly lower ($P < 0.05$) in group T_0 as compared to other groups (T_1 to T_5), but the values in groups T_1 to T_5 were statistically similar showing no variability in rumen disappearance rates of Ca on using different Ca/P supplements. Rumen disappearance rate of P under these conditions were statistically similar in all the groups including group T_0.

Table 4.9 Water intake, rumen fluid volume, rumen fluid flow rate and disappearance pattern of Ca and P through rumen fluid

	Control		Treatments				F-value
	T_0 (-ve)	T_1 (+ve)	T_2 (marble)	T_3 (gypsum)	T_4 (rock phos)	T_5 (superphos)	
Water intake (litres/day)	29.19 ± 2.19	26.83 ± 1.67	31.45 ± 2.13	28.88 ± 1.89	33.36 ± 1.56	28.59 ± 1.64	0.89
Rumen fluid volume(litres)	25.83 ± 0.63	25.01 ± 1.17	25.71 ± 1.30	22.83 ± 1.36	25.26 ± 1.33	21.55 ± 1.39	2.10
Rumen fluid flow rate(litres/hr)	0.993 ± 0.049	0.874 ± 0.084	0.950 ± 0.032	0.856 ± 0.033	0.833 ± 0.089	0.797 ± 0.040	0.93
Rumen disappearance rate (mg/hr) through rumen fluid							
Ca	92.5^{a} ± 4.2	131.7^{b} ± 5.6	135.0^{b} ± 4.5	120.5^{b} ± 3.6	116.2^{b} ± 7.2	109.0^{b} ± 2.2	3.39*
P	474 ± 14	436 ± 16	579 ± 8	473 ± 18	413 ± 24	516 ± 10	2.19

Rumen disappearance rate of Ca and P through rumen fluid is calculated as product of rumen fluid flow rate(litres/hr) and mean rumen fluid concentration(mg/litre) of Ca and P respectively.
Values with different superscripts(a,b) differ significantly *P/0.05
Each value is mean of four observations

4.3 DISCUSSION

4.3.1 Based upon scanning studies (Chapter III), Ca and P supplements like marble, gypsum, Mussorrie rock phosphate and fertiliser grade superphosphate were selected for ruminal studies. The parameters were distribution, ruminal concentration and rates at which Ca and P left the rumen compartment. In addition, the influence on normal metabolites in the rumen and blood Ca and P concentrations were also studied. The positive control consisted of dicalcium phosphate as a commonly accepted Ca and P supplement source and the negative control, consisted of no extra supplement for Ca and P. Two Ca supplement sources, i.e., marble (T_2) and gypsum (T_3) and two P supplement sources, i.e., rock phosphate (T_4) and superphosphate (T_5) were included in experimentation. In T_2 and T_3 groups P supplementation was made through sodium phosphate. Since both rock phosphate and superphosphate contained enough Ca, no extra calcium supplementation was needed. The mineral mixtures were computed in a way to supply similar quantity of Ca and P in treatments T_1 to T_5 through the ration (Table 4.3). In addition, the trace mineral supplementation were also made similar. The animals were adult, non-producing and of similar age, hence, the metabolic demands of these animals either for mineral elements or for feed nutrients were essentially similar. Since the animals were housed in plastic painted individual stalls, possibility of

extraneous Ca and P supply through leaking of walls was avoided. Since the basal diet was same in all the groups, the supply of organic nutrients were also similar.

Under the condition, the treatment effect stipulated was only due to the different sources of Ca and P supplements used and compared with dicalcium phosphate as an accepted Ca and P supplement source.

4.3.2 Intake of dry matter, Ca and P of animals using different sources of Ca and P in the concentrate mixtures

Data in table 4.3 reveals that only 0.34% Ca and 0.56% P was present in the basal concentrate mixture(T_0) in which no Ca/P source was added. In the treatment groups T_1 to T_5, the dietary total calcium content ranged from 1.05-1.09% and P content ranged from 1.08-1.15%. It meant that about 66% of Ca and 50% of P in the respective concentrate mixtures were from externally added supplement sources (4.3.1). These different sources of Ca and P fed to animals in the concentrate mixtures did not show any significant differences in the total dry matter intake or dry matter intake per 100 kg body weight of animals(table 4.4) The results suggested that any of the treatments had no adverse effect on palatability of the diet. Plumlee et al. (1958) reported that soft phosphate with colloidal clay resulted in decrease in feed intake, feed gain efficiency and lower serum inorganic P levels. Bouchrad and Conrad (1973) found decrease in feed intake when agricultural grade gypsum was used to increase Ca and S content of the

basal ration. However, only with rock phosphate groups the animals in beginning showed some reluctance in acceptance of concentrate mixture but in couple of days during the pre-experimental days they soon got adjusted and the intake was similar to other groups. The low Ca and P through concentrate and thus the total intake of Ca and P in group T_0 in comparison to other groups was only the reflection that the P basal diet in group T_0 contained low Ca and P because it was unsupplemented.

4.3.3 Effect of different sources of Ca and P supplements on rumen metabolites and blood serum Ca and P levels

4.3.3.1 Rumen metabolites:

Rumen pH:- The major minerals play important role in maintaining physiochemical characteristics of rumen medium. The main buffer components in rumen are Na, P, K and VFA's, and any decrease in these components in rumen results in change in rumen pH (Durand and Kawashima, 1980). In the present experiment no significant effect on rumen pH was observed as there was no difference in the supply of dietary P in the respective groups except group T_0. In group T_0 also, animals maintained ruminal P concentration similar to control and other groups because of recycling of P through saliva (Cohen, 1980). VFA contents in different groups also did not differ.

Ammonia-N:- Ruminal NH_3-N concentration showed no variation in groups T_1, T_2, T_3 and T_5 but the values were significantly ($P/0.05$) lower in T_0 and T_4 groups. Inter se

comparison of T_0 and T_4 groups suggested that T_4 group behaved in similar way as T_0 group where the concentrations in SRL were low (table 4.8). It was evident that only P but not Ca from rock phosphate (T_4) was comparatively less soluble (table 4.7) as its distribution in the soluble phase was low. Considering this low level of P solubility in the SRL (T_4) and low level of P supply through diet (T_0), the two groups behaved similarly in ruminal NH_3-N concentration. There are only suggestive evidences to content that lower P concentration in rumen liquor either due to low dietary supply or less solubility in the SRL, the activity of proteolytic organisms in the rumen is affected. No direct evidence is available to support this contention although there are findings to suggest that P requirements of ruminal organisms are much higher than Ca, and that ruminal P concentration affected the activities of Bacteriods succinogenes (Bryant et al., 1959).

Volatile Fatty Acids:- Although statistically non-significant, the TVFA concentrations in SRL were slightly lower in treatment groups T_3, T_4 and T_5 as compared to other groups T_0, T_1 and T_2. Since T_0 and T_1, i.e., negative and positive control groups showed similar TVFA concentration, the slightly lower values of T_3, T_4 and T_5 groups cannot be attributed to lower Ca and P concentrations in the SRL or the treatment effect and can only be interpreted as normal variabilities of animals.

It was also observed that the treatments did not influence the relative proportions of acetate, propionate and butyrate. Therefore, it was evident that different forms of mineral supplements exerted no influence on ruminal energy metabolites. Chicco *et al.* (1965) used different sources of P, such as Ca orthophosphate, Na ortho- and meta phosphate and observed no difference in cellulytic activity of rumen bacteria. Fisher (1978) also reported that total VFA's level in animals fed four supplemental forms of P in their diet - monocalcium,phosphate, dicalcium phosphate, monoammonium phosphate and monosodium phosphate were similar. However, in the same study the production of propionate was considerably higher with monosodium phosphate compared to other sources.

4.3.3.2 Blood Ca and P levels:

There are evidences to suggest that concentration of P in the blood of young animals fall rapidly when P intake is inadequate (Wise *et al.*, 1961) and also when P source is poorly utilised (Hemingway and Fishwick, 1976). Such inadequacies in dietary sources from different mineral supplements were not observed in the present investigations, since serum Ca and P values were not influenced due to treatments. No differences in Ca and P levels in T_0 and T_1 groups suggested that even at lower dietary Ca and P supply in T_0, the interplay of homeostatic mechanisms (Cohen, 1980) were sufficient to maintain serum Ca and P concentrations. However, there was marginalk depression in

serum P in case of T_0, T_4 and T_5 groups suggesting that prolonged feeding on these treatments may outbalance the homeostatic regulatory mechanisms in course of time.

Distribution of Ca and P in ruminal digesta:

Due to the complex nature of the rumen, recycling of mineral elements in the rumen, pH conditions of the rumen, rumen volume and rate of digesta flow, it was felt necessary to study the relative distribution of Ca and P in the ruminal digesta as influenced by the supplemental sources. Ca and P distribution was studied in these phases of ruminal digesta.

(i) Soluble phase - represented the ionic form of the mineral element, uncomplexed with binding ligands, unadsorbed on feed particles and the portion not held up as insoluble moiety in the feed particles. This was accounted for as the supernatant portion of the strained ruminal digesta.

(ii) Particulate phase - represented portion of the mineral elements bound to ligands as insoluble complexes and utilised by microorganisms which could be accounted for as the residue remaining after centrifugation of strained ruminal digesta.

(iii) Solid phase - which represented the portion of mineral elements held up as insoluble mineral particle or adsorbed on the feed particle. This portion was accounted for by taking the residue of the strained ruminal digesta.

It was observed in *in vitro* studies with ruminal buffer that various mineral supplement sources were not having similar solubility in the rumen. The Ca solubility but not the P solubility was influenced by pH changes (Chapter III). Even the different mineral supplements had not same P solubility in ruminal buffer. Such findings had indicated that there may be variability of Ca and P distribution in solubles, particulate and solid phases in the rumen.

The present studies (table 4.7) suggested that Ca from different sources was soluble in the ruminal fluid to the extent of 7-9% as compared to P which was soluble to the extent of 30-50%. Yano *et al.* (1979) came up with similar observations with their studies on sheep and reported that in comparison to Ca, Mg and K, the concentration of P and Na in the digesta and supernatant of rumen and omasum were higher. Ca in the soluble phase was lower in group T_0 than the overall distribution from supplemented treatment groups (T_1 to T_5), but Ca from rock phosphate (T_4) showed highest solubility. Its distribution in the particulate phase followed almost similar pattern, although the highest distribution in the phase was in marble supplemented group (T_2). The proportion in the solid phase was the resultant effect of its distribution in the soluble and particulate phases and was about 80.5 to 86.7% of total Ca present in the rumen contents. In contrast to Ca, there was significantly higher distribution of P in the soluble phase, the lowest

value being in group T_4 (rock phosphate). The lower values of P distribution in the soluble phase in T_1, T_3, T_4 and T_5 groups than the basal feed group T_0 was due to higher P intake in supplemented groups than the basal group T_0. Data in table 4.5 suggested that P intake was significantly high in all the supplemented groups than the basal group T_0. Witt and Owens (1983) have reported that ruminal P solubility is decreased with increase in level of its dietary supply. Sufficiently depressed values of soluble P in T_4, T_1 and T_5 groups might be due to lower solubility of the respective forms of supplemental P. Higher value in group T_2 could bea because of highly soluble source of P, i.e., sodium phosphate, was used as P supplement. The results suggested that in terms of P solubility in the rumen, rock phosphate was the least soluble source. The distribution of P in the particulate phase was lowest in group T_2 where a soluble form of P, sodium phosphate was used. Higher values in T_3, T_4 and T_5 suggested that there was presence of enough binding ligands in these groups as the AIA content were also higher. However, comparatively a higher value of P in the particulate phase of groups T_0 than T_1 and T_2 is difficult to explain.

4.3.5 Ca and P concentration in SRL

Ca and P concentration in the ruminal fluid are known to be influenced by dietary level of these elements and their solubilities in the rumen contents (Yano *et al.*,

1979; Witt and Owens, 1983). In the present experiment dietary intake of Ca and P were similar in all the groups except group T_0 (the control) which supplied Ca and P of feed origin only. Ca concentration in group T_0 was significantly less ($P<0.05$) in comparison to all other groups which were showing statistically similar values (table 4.8). In contrast the P concentration in all the groups were similar. It is known that there is continuous turnover of P through saliva to the rumen liquor. Tomer et al. (1967) have indicated that P secretion from both parotid glands of sheep exceeded by 1.3-7.2 times the intake of P through feed which ranged from 0.4 to 4 g P/day. This contribution of P through salivary recycling was the predominant factor by which P concentration in SRL is essentially maintained irrespective of dietary supply. Clark (1953) have reported that even in case of clinical hypophosphorosis in cattle, P concentration in the ruminal fluid did not fall below 200 mg/litre.

4.3.6 Rumen fluid volume, flow rate and Ca and P disappearance pattern

Data in table 4.9 indicated that there was no effect of feeding different Ca and P supplements on water intake, rumen fluid volume and flow rate. Ingestion of certain inorganic salts like sodium chloride or sodium bicarbonate was found to increase water intake, rumen fluid volume and flow rate (Hemsley et al., 1975; Thompson et al., 1978) and decrease the dry matter intake and ruminal digestion of organic nutrients (Rogers et al., 1979). But

this was not the case with Ca and P supplements used in the present investigation as the addition of Ca/P supplements brought no significant change in water intake, rumen fluid volume and flow rate as compared to basal group (T_0). The availability of Ca and P to the animals from the lower gut would depend upon the ruminal fluid concentration and the digesta flow rate as depicted by disappearance pattern. Since all the treatments except T_0 had similar Ca concentration in the SRL, the different mineral supplement groups may not bring about any difference in Ca availability to the animal particularly because the digesta flow rate and ruminal disappearance of Ca were not affected due to the type of supplements used. However, in unsupplemented group T_0, due to lowered Ca concentration in the SRL, the Ca disappearance rate was also lower suggesting decreased mineral availability to the lower gut for absorption. In case of P, this was not the case. With a similar P concentration in SRL in all the groups T_0 to T_5 and similar P disappearance pattern in all the groups, there may not be any influence on the P availability at lower gut. It was thus probable that the gut factors exerted no influence on regulation of P status of the animals until the change in dietary supply was so severe to cause dimunition in ruminal P concentration or salivary recycling of phosphorus.

EXPERIMENT-II

4.4 INFLUENCE OF EDTA SUPPLEMENTATION ON RUMINAL Ca SOLUBILITY AND ITS DISAPPEARANCE PATTERN

EDTA is a strong chelating agent. Its use in mineral supplements has been claimed to improve utilisation of certain trace minerals by non-ruminants (Scott and Zeigler, 1963; Oberleas et al., 1963; Kratzer and Starcher, 1963). But no advantage in Zn utilisation was noticed when EDTA was added in the diet of ruminants (Powell et al., 1967; Miller et al., 1968; Hiers et al., 1968). However, in vitro and in vivo solubility of Mn, Zn and Fe and thus their utilisation was found to be improved by feeding chelated primix trace element supplements in comparison to other commercial trace mineral mixture (Foll, 1966). The extent of binding of any mineral with EDTA in the rumen is more in case of heavy metals like Cu and Zn than with Ca and Mg (Georgievskii, 1982).

With this background in view, the influence of different levels of EDTA supplementation was seen on Ca utilisation from mineral supplements as evident from ruminal Ca solubility and disappearance rates.

4.4.1 MATERIALS AND METHODS

Experimental animals

The same four fistulated animals used in previous experiment (4.1.1) were switched over to for the present investigation.

Experimental feeding

The composition of concentrate mixture and that of mineral mixture were the same as used in control group (T_1) of previous experiment (4.1.2). The animals were fed concentrate mixture and wheat straw in similar way as described earlier (4.1.2). The experimental treatments differed in four levels of EDTA supplementation, in the diet of respective groups, following the switch over design as followed in 4.1.2, differing only with respect to EDTA dose levels.

In treatment T_1, no EDTA was mixed in the concentrate mixture, while in treatments T_2, T_3 and T_4 ratio of EDTA:Ca added through mineral supplement was kept as 0.2:1, 0.5:1 and 1:1 (on g/g basis). All the four animals were fed under each treatment starting from T_1, for a period of 21 days at the end of which samples of rumen liquor were collected for Ca solubility measurement studies and for rumen fluid volume. All the four animals were then switched over to the next treatment (i.e., T_2 then T_3 and so on) in a switch over design.

Experimental procedures

Sampling of rumen liquor for the measurement of pH, solubility of Ca in ruminal fluid and rumen fluid volume/flow rate was made in the similar way as described in 4.1.3.

Ca solubility in the SRL of animals fed different levels of EDTA in their diet was estimated by the method of Yano *et al.* (1979) as described in 3.4.1.

Samples collected at 4 hrs postfeeding were used as a representative sample to provide mean value of Ca concentration. Procedures employed for the estimation of rumen fluid flow rate, Ca concentration in the SRL and Ca disappearance pattern were the same as followed in 4.1.3.

4.4.2 RESULTS

Rumen pH, Ca solubility and its concentration in SRL:

The values of ruminal pH, Ca solubility and concentration in samples of SRL collected are presented in table 4.10. The values of ruminal pH in different treatments were 6.58 ± 0.04, 6.51 ± 0.02, 6.42 ± 0.04 and 6.50 ± 0.03 respectively and were quite similar to each other. The solubility of Ca in the ruminal fluid were 43.02 ± 2.94, 38.55 ± 2.63, 30.42 ± 0.81, 29.87 ± 0.98 % respectively and increased significantly ($P < 0.05$) with the increasing level of EDTA in the diet of animals in treatment groups T_1 to T_4. Ca concentration in SRL in different treatments did not vary significantly and the values ranged from 11.30 to 12.50 mg Ca/100 ml SRL.

Ruminal fluid flow rate and Ca disappearance from rumen:

There was no effect of addition of different level of EDTA in treatments T_2, T_3 and T_4 over treatment T_1, on ruminal flow rate or on disappearance rate of Ca from rumen. Values for ruminal flow rate were 0.930 ± 0.050, 0.868 ± 0.064, 0.918 ± 0.072 and 0.916 ± 0.042

litres/hr; and for Ca disappearance rate through ruminal fluid were 104.7± 2.2, 107.4± 2.6, 114.7± 3.6 and 111.8± 4.2 mg Ca/hr respectively (Table 4.10). Both these parameters did not show any significant difference in different treatments.

4.4.3 DISCUSSION

It was evident from the table 4.10 that by changing the EDTA:Ca ratio in the mineral supplements the ruminal pH remained constant and was not affected by treatments. However, the Ca solubility declined as a function of treatment contrary to the expectations. Ca concentration in SRL, the ruminal fluid flow rate and thus Ca disappearance rate did not change with the treatments. Since the SRL represents the soluble and particulate phases, decrease in Ca solubility and constant concentration in SRL suggested that there was increase in the particulate phase Ca. This particulate Ca might be because of formation of complexes of Ca-EDTA, since the chelating bonds are very strong. It is difficult to presume that there will be dissociation of Ca from its ligands in the chelate in acidic conditions of pH in the abomasum or proximal intestine. Therefore, despite the same Ca disappearance rate from the rumen, supplemental Ca in the presence of EDTA may not render complete Ca availability for absorption.

-o-o-o-

Table 4.10 Ruminal pH, Ca solubility, concentration and its disappearance rate from rumen on feeding different levels of EDTA to the animals

	Treatments				F-value
	T_1	T_2	T_3	T_4	
Ratio of EDTA:Ca(w/w basis) in mineral mixture	0:1	0.2:1	0.5:1	1:1	-
Rumen pH	6.58 ± 0.04	6.51 ± 0.02	6.42 ± 0.04	6.50 ± 0.03	0.04
Ca solubility(%)	43.02^{a} ± 2.94	38.55^{b} ± 2.63	30.42^{c} ± 0.81	29.87^{c} ± 0.98	6.56*
Ca concentration (mg/100ml) in SRL	11.30 ± 0.34	12.50 ± 0.26	12.50 ± 0.40	12.20 ± 0.30	1.90
Rumen fluid flow rate (litres/hr)	0.930 ± 0.050	0.868 ± 0.064	0.918 ± 0.072	0.916 ± 0.042	0.87
Ca disappearance through rumen fluid (mg/hour)	104.7 ± 2.2	107.4 ± 2.6	114.7 ± 3.6	111.8 ± 4.2	1.62

Values in the same line with different superscripts a,b and c, differs significantly at ($P<0.05$).

CHAPTER-V

INFLUENCE OF CERTAIN CALCIUM AND PHOSPHORUS SUPPLEMENTS ON NUTRIENT UTILISATION AND GROWTH IN RUMINANTS

INFLUENCE OF CERTAIN CALCIUM AND PHOSPHORUS SUPPLEMENTS ON NUTRIENT UTILISATION AND GROWTH IN RUMINANTS

Evidences suggested that different sources of mineral supplements were not similar in their effects on nutrient utilisation by animals.

Soft phosphate with collodial clay was found to reduce the feed intake, feed gain efficiency and blood P levels (Plumlee et al., 1958). Gerken and Fontenot (1967) compared dolomite limestone and MgO as source of Mg to sheep. They found that Mg supplementation by dolomite limestone depressed carbohydrate digestibility and serum Mg value than MgO. Bouchard and Conrad (1973) found that on feeding agricultural grade gypsum as Ca supplement, there was decrease in feed intake. Witt and Owens (1981) reported that P from defluorinated rock phosphate is not so soluble, and less available to ruminants as compared to other sources of phosphorus like dicalcium phosphate or sodium phosphate. Gohl (1981) has suggested that fertiliser grade superphosphate can be used as mineral supplement if no other source of P is available.

In the present experiment, two alternative sources of mineral supplement - rock phosphate and superphosphate have been taken and efforts are made to evaluate their use as Ca and P supplements in the mineral mixtures in growing crossbred calves. Dicalcium phosphate was chosen

as a conventional source of Ca and P supplement. Certain reports suggested that inclusion of chelating agent in mineral mixture, improved the utilisability of certain trace element like Zn (Darwish and Kretzer, 1965). Since EDTA is good chelating agent for Ca, in one of the experimental treatment groups EDTA was fed in conjunction with dicalcium phosphate and the influence on growth and mineral utilisation was recorded. With a view to compare the influence of the sources of Ca and P supplements, in different treatment groups all the nutrients supply including Ca and P was kept similar in each case. A growth trial on 16 animals divided into 4 groups of 4 animals each was done for a period of 16 weeks duration. In order to see utilisation of nutrients and balance of Ca, P and other minerals from these mineral mixtures, a metabolism trial of six days duration was also done at the termination of this experiment.

5.1 MATERIALS AND METHODS

5.1.1 Experimental animals and their management

Sixteen crossbred (Karan Fries) male calves of 4-6 months of age and weighing about 60-70 kg were taken from the Institute herd. These calves were randomly divided into four groups, I to IV (Table 5.1) in RBD design.

Housing:- Well ventilated concrete floored calf pens were used to house the experimental animals. The location of the calf pens were such that it permitted easy access

Table 5.1 Initial body wt and age of experimental animals in different treatment groups

S.No.	Animal No.	Initial body wt. (kg)	Date of birth
		Group-I	
1.	KF-4603	69	10.5.85
2.	KF-4608	50	14.5.85
3.	KF-4628	57	2.8.85
4.	KF-4639	53	19.9.85
		Group-II	
5.	KF-4616	50	29.6.86
6.	KF-4623	58	23.7.85
7.	KF-4642	70	5.10.85
8.	KF-4647	51	21.10.85
		Group-III	
9.	KF-4600	59	1.4.85
10.	KF-4606	53	8.5.85
11.	KF-4632	65	16.8.85
12.	KF-4643	60	10.10.85
		Group-IV	
13.	KF-4601	57	1.4.85
14.	KF-4602	57	5.4.85
15.	KF-4621	50	22.7.85
16.	KF-4646	60	19.10.85

to sun-shine most of the day. They were sufficiently large for the easy movement of the animals. The walls of the pens were plastic painted periodically to avoid extra mineral supply by licking.

Care of animals:- Clean and healthy environment was provided for the animals. The animals were tied individually. After tieing their mouth, the animals were washed daily with clean water and given exercise in the open sun-shine for 1 hour. The animals were duly protected from cold stress during the winter. During the night time whole of the calf shed was enclosed with terpolene sheets. Before the start of the experiment the animals were dewormed with full course of "Penacure". Clean tap water was provided free choice, twice daily in plastic buckets. Feed was offered in plastic tubs.

5.1.2 Experimental feeding

Preparation of mineral mixtures:- Four different mineral mixtures were formulated using commercial grade dicalcium phosphate, Mussoorie rock phosphate and fertiliser grade superphosphate as Ca and P sources, and added in the concentrate of groups I, III and IV respectively(table 5.2). In group II, dicalcium phosphate was used in similar way as group I but in addition EDTA was mixed at the rate of 0.1% w/w in the final concentrate mixture. The supply of other mineral elements were met by addition of pure salts (table 5.3). By using appropriate quantities based on its

Table 5.2 Ca and P supply from different mineral supplements used in preparation of experimental mineral mixtures

Groups	Mineral supplement source used	Ca supply g/100 kg conc. mixture	P supply g/100 kg conc. mixture
I (control)	Dicalcium phosphate 1.65 kg, chalk powder 0.330 kg + trace mineral mixture *	664	478
II	-do- + EDTA(0.1%) in concentrate mixture	664	478
III	Mussorrie rock phosphate 2.65 kg + sodium phosphate 0.8 kg + trace mineral mixture*	664	478
IV	Super phosphate 3.838kg, + sodium phosphate 0.56kg + trace mineral mixture*	662	477

In addition to the Ca/P source used, the following ingredients were used per 100 kg of concentrate mixture for the preparation of complete mineral mixture. Sodium chloride 900 g; Magnesium carbonate 90g; ferrous sulphate 1.6g; copper sulphate 2.1g; potassium iodide 0.3g and zinc sulphate 7.5g

composition, different Ca and P sources were blended in mineral mixture so as to supply same amount of Ca and P per 100 kg of the concentrate mixture in all the groups. Rovimix was added in appropriate quantities (4.1.2) to meet the reqáirements of vitamin A and D.

Table 5.3 Composition of concentrate mixture

Ingredients	Proportions	CP(%)	TDN(%)
Maize	45	5.00	40.00
G N C	32	14.40	22.40
W.bran	20	2.00	9.80
Min.mix.	3.00	-	-
Total	100	21.40	72.00

Preparation of concentrate mixtures:- The concentrate mixture was prepared by mixing appropriate quantities of maize, ground cake and wheat bran (table 5.3). These ingredients alongwith different mineral mixture were mixed on a plastic sheet, laid down on the floor, so as to avoid any loss of mineral mixture used in the concentrate mixture. The values of CP and TDN were taken from ICAR Bulletin No.25 - K.C.Sen and S.C.Ray (1978). The samples of concentrate mixtures prepared were analysed for its proximate principles from time to time. The composition of concentrate mixtures in four groups were essentially the same, except that it differed in different mineral mixtures used in the respective groups.

Feeding schedule:- NRC feeding standards (1978) were followed in preparing the feeding schedule (table 5.4). The calves were offered concentrate mixture in the morning at 9.00 AM, and green fodder maize at 2.00 PM.

Table 5.4 Feeding schedule of animals

Body weight (kg)	Concentrate offered (kg)	Green maize (kg)	
		Fresh basis	DM basis
50	0.8	4.0	0.8
60	1.0	5.0	1.0
70	1.2	5.0	1.0
80	1.4	6.0	1.2
90	1.5	7.0	1.4
100	1.6	8.0	1.6
110	1.7	8.5	1.7
120	1.8	9.0	1.8
130	1.6	10.0	2.0
140	1.8	11.0	2.2

Roughage to concentrate ratio was kept as 1:1

5.1.3 Experimental procedures

Recording of body weights and feed intake:- After allowing an adaptation period of 2 weeks the experimental calves were switched over to experimental study. Body weight was recorded at the start of the experiment and thereafter at weekly intervals. Their requirements for

concentrate and green fodder were periodically changed as per body wt gain. The calves were fed individually weighed quantity of concentrate mixture and green fodder as per the schedule given in table 5.4 and feed residue if any were collected and weighed to calculate the daily dry matter intake. Dry matter content of the feeds and fodders were analysed at weekly intervals.

Determination of growth rate(b value):- The growth rate was calculated by regression analysis of the cumulative weight gains during the 16 weeks period as 'b' value, as shown below:

$$\text{b value (kg/week)} = \frac{\Sigma xy - \frac{\Sigma x \cdot \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}$$

Metabolic trial:- After the completion of 16 weeks of growth period on different treatment groups, a metabolic trial of 7 days collection period was conducted on all the sixteen calves in the metabolic shed. The calves were properly harnessed and given two days adaptation period before the actual sampling of feeds, faeces and urine was made.

Collection of feed, faeces and urine samples:- Samples of feed offered and residue left, if any, were taken each morning for dry matter and chemical analysis. The quantity of faeces and urine voided by individual animal were recorded every morning (24 hr collection) for 7 days, and representative samples were drawn for further analysis.

Aliquoting the faeces and urine:- For the purpose of analysis of proximate principles and nitrogen, 1/100th aliquots of faeces were taken separately. For mineral analysis, the faeces samples were dried daily at 100°C in hot air oven. For nitrogen, however, wet faeces for individual calves were preserved with 25% H_2SO_4 in a pre-weighed plastic bottles. After 7 days of collection, the contents were weighed, mixed thoroughly and about 10g accurately weighed material was taken in a Kjeldhal's method.

Similarly with urine also an aliquot of 1/100th size of total urine voided by individual animals was taken separately daily and pooled for 7 days in Kjeldhal's flask containing 50 ml of concentrated H_2SO_4. The nitrogen estimation was done as usual after the pooled samples were digested.

Analytical procedures:- Samples of feed and faeces were analysed for proximate principles - Dry matter, total ash, acid insoluble ash (AIA) and nitrogen content as described by AOAC (1970). Acid detergent fibre (ADF) was estimated in all the samples as per the method described by Goering and VanSoest (1970).

Minerals:- Mineral content and balances were studied of feeds, faeces and urine samples. The different mineral elements included Ca, P and Mg as major minerals and Fe, Mn, Zn and Cu as trace elements. The samples were dry ashed at 550 ± 10°C in a muffle furnace and acid extract

for the estimation of minerals was prepared as described in 3.1.1.3. The representative pooled samples of urine were evaporated to dryness in a boiling waterbath and then dry ashed and processed by similar method for estimation of different mineral elements.

Ca in the above samples was estimated by precipitation method as per ISI (1975) and P was determined by the method using hydroquinone (AOAC (1975). The analytical procedures have been described at 3.1.1.2.

Essential mineral elements other than Ca and P like Mg, Fe, Mn, Zn and Cu were estimated in the samples with the help of Atomic Absorption Spectrophotometer, as by the method described in 3.1.1.1.

5.2 RESULTS

5.2.1 Composition of feeds

5.2.1.1 Organic nutrient composition of feeds

The organic nutrient composition of feeding stuffs, i.e., concentrates fed to different groups and green maize offered as sole roughage source is given in table 5.5. It was evident that the composition of the concentrate mixtures in the four treatment groups were essentially similar. The concentrate mixtures differed only in their mineral mixtures prepared with different sources of Ca and P. This was the reason why the total ash content in the four concentrate mixtures was different. Only groups I and II were similar because Ca and P source was the same in these two groups. The crude protein content varied from 22.35 to 24.10% and ADF content from 11.13 to 12.10%.

5.2.1.2 Mineral composition

Mineral composition of the four concentrate mixtures used in the experiment are presented in table 5.6. It was evident that the mineral ingredients in the concentrate mixture was so adjusted that the mineral content of different concentrates fed to calves were not different. Thus the Ca content ranged from 1.02-1.09%, P content ranged from 1.11 to 1.16% and Mg content ranged from 0.087

Table 5.5 Organic nutrient composition (percent DM basis) of different concentrate mixtures and green fodder

	DM	OM	CP	ADF	Ash
Concentrate					
Group I	93.00	92.49	23.53	11.96	7.51
Group II	93.04	92.47	22.82	11.13	7.53
Group III	92.87	89.82	22.35	11.28	10.18
Group IV	92.95	90.22	24.10	12.10	9.78
Roughage					
Green maize	23.00	89.04	9.39	39.67	10.96

Table 5.6 Mineral composition of experimental concentrate mixtures and green maize

Concentrate	Ca/P source used in mineral mixture	Total Ash	AIA	Ca	P	Mg	Fe	Mn	Zn	Cu	Co
		(% DM basis)					(ppm)				
Group I	Dicalcium phosphate	7.51	0.79	1.07	1.12	0.09	640	69	91	29	26
Group II	-do- + EDTA	7.53	0.77	1.09	1.11	0.09	620	67	91	28	23
Group III	Rock Phosphate	10.18	1.09	1.05	1.16	0.09	820	72	93	27	21
Group IV	Superphosphate	9.78	0.93	1.02	1.13	0.09	530	92	90	32	22
	Green maize	10.96	3.86	0.77	0.42	0.32	330	42	35	9	-

The figures are average of two determinations in case of concentrate mixtures and average of four samples in case of green maize

to 0.089%. However, Fe content was highest in group III (820 ppm) in comparison to the lowest value (530 ppm) in group IV. Although Mn content different in different groups, the concentration of Zn, Ca and Co were essentially similar.

5.2.2 Dry matter intake, nutrient utilisation and N-balances

The mean values of dry matter intake, nutrient digestibility and N balances of calves of the four treatment groups have been presented in tables 5.7 and 5.8.

5.2.2.1 Dry matter intake

The daily dry matter consumption of calves in groups I to IV were 3.520± 0.14, 2.747± 0.13, 3.171± 0.18 and 2.848± 0.24 kg respectively. Figures for dry matter intake per 100 kg body weight were 3.59± 0.07, 3.58± 0.06, 3.36± 0.11 and 3.52± 0.16 kg in the respective groups. The figures suggested that the rations were acceptable to the animals and there was no significant different ($P > 0.05$) in the four treatment groups.

5.2.2.2 Digestibility coefficient of organic nutrients:

The digestibility coefficients of DM, CP and ADF were determined and have been presented in table 5.7. Its values in the four groups were 70.65± 1.11, 74.03± 2.15, 71.07± 2.32 and 71.54± 2.65% respectively for DM; 72.01± 0.75, 70.85± 3.76, 72.78± 1.21 and 76.82± 2.16% respectively for C.P. and 63.31± 1.39, 63.34± 3.79,

Table 5.7 Daily DM intake and nutrient utilisation by calves in different groups

	Groups				F-value
	I	II	III	IV	
A. Daily DM intake(kg)					
Concentrate	1.520± 0.14	1.356± 0.13	1.449± 0.16	1.356± 0.21	1.37^{NS}
Roughage	2.000± 0.19	1.391± 0.12	1.722± 0.08	1.491± 0.17	3.13^{NS}
Total	3.520	2.747	3.171	2.848	
DM intake per 100kg body wt(kg)	3.59± 0.07	3.58± 0.06	3.36± 0.11	3.52± 0.16	0.374^{NS}
Nutrient					
B. Digestibility(%)					
Dry matter	70.65± 1.11	74.03± 2.15	71.07+_ 2.32	71.54± 2.65	0.43^{NS}
Crude protein	72.01± 0.75	70.85± 3.76	72.78± 1.21	76.82± 2.16	0.58^{NS}
Acid Detergent Fibre	63.31± 1.39	63.34± 3.79	62.69± 3.34	60.00± 3.02	0.36^{NS}
C. DCP intake (g/head/day)	397± 40	312± 18	358± 34	371± 31	1.07^{NS}

Table 5.8 N-balances of calves in different groups(g/head/day)

	Groups				F-value
	I	II	III	IV	
Intake					
Roughage	30.8	21.0	26.7	23.1	-
Concentrate	57.2	49.5	51.8	53.7	-
Total	88.0± 8.2	70.6± 1.9	78.5± 6.3	76.8± 5.7	1.45^{NS}
Outgo					
Faecal	24.5± 1.7	24.8± 2.3	21.1± 1.4	17.5± 1.8	2.16^{NS}
Urinary	21.4± 2.1	24.8± 2.2	23.3± 1.2	25.2± 2.3	1.56^{NS}
Balance	+42.1± 5.64	+22.3± 5.80	+34.0± 9.8	+34.0± 9.9	1.59^{NS}

62.69 ± 3.34 and 60.00 ±3.02% respectively for ADF. These values of digestible coefficients for DM, CP and ADF did not show any significant difference in the experimental treatments.

The DCP intake by calves in the four respective groups being 397 ± 40, 312 ± 18, 358 ± 34 and 371 ± 31 g/head/day, showed similar trend as the DM intake (table 5.7), and were not significantly different (P 0.05).

5.2.2.3 Nitrogen balance

The N balances of calves fed rations containing different mineral mixtures according to respective treatments ranged from + 42.1 ± 5.64, + 22.3 ± 5.80, + 34.0 ± 9.8 and + 34.0 ± 9.9 g/head/day and were positive in each case suggesting that they could support growth (table 5.8). These values for N intake and N balance did not differ significantly (P ≥ 0.05). Data of faecal outgo and urinary outgo have also been presented in the table which also were statistically similar. Although N-balances in group II, III and IV were showing lower values in comparison to control, but did not differ significantly due to large variation within groups as shown by standard error. However, on perusal of the N-balance values of group I and group II, it seemed evident that the values may be non-significantly only at marginal levels and had tendency to attain significantly different levels.

5.2.3 Calcium and Phosphorus balances

The mean Ca balances in the respective groups (table 5.9) were 15.54± 1.78, 15.01± 1.12, 12.65± 1.28 and 13.41± 0.96 g/head/day, against the mean intake of 31.78± 2.86, 25.58± 1.36, 28.49± 1.96 and 25.41± 1.25 g/head/day. Likewise the average P balance in the four groups were 8.12± 0.55, 7.45± 0.42, 7.21± 0.61 and 6.24± 0.72 g/head/day against the mean intake of 26.82± 0.82, 21.86± 0.73, 25.25± 0.66 and 22.64± 0.96 g/head/day. It was evident that both Ca and P were positive in each case and did not differ significantly for all the experimental treatments. However, the figure for Ca balance in group II and IV tended to be non-significantly different only at marginal levels as compared to other groups.

5.2.4 Blood Ca and P levels

The mean blood Ca and P levels of calves under different groups determined for three consecutive months during the period of growth trial are presented in table 5.10. It was evident that the values were normal in each case and none of the treatments rendered the serum Ca and P levels below normal levels. Neither the monthly average values nor overall means for treatments differed significantly suggesting that there was no condition of hypocalcaemia or hypophosphataemia.

5.2.5 Utilisation of other essential minerals (Mg, Fe, Zn, Mn and Cu) from different mineral mixtures

The utilisation of other minerals, i.e., Mg, Fe, Zn, Mn and Cu are presented in tables 5.11 to 5.15 respec-

Table 5.9 Daily intake and balance of Ca and P of animals in different groups (g/head/day)

	Groups				F-value
	I (DCP)	II (EDTA)	III (Rock phos)	IV (Superphos)	
CALCIUM					
Intake					
Roughage	15.52± 1.06	10.80± 0.92	13.28± 0.93	11.57± 0.75	2.96^{NS}
Concentrate	16.26± 0.91	14.78± 0.79	15.21± 0.82	13.84± 0.52	1.39^{NS}
Total	31.78± 2.86	25.58± 1.36	28.49± 1.96	25.41± 1.25	1.56^{NS}
Outgo					
Faecal	15.74± 0.82	10.24± 1.21	15.43± 1.16	11.68± 0.26	3.36^{NS}
Urinary	0.50± 0.12	0.33± 0.08	0.36± 0.09	0.34± 0.10	0.82^{NS}
Ca-balance	+15.54± 1.78	+15.01± 1.12	+12.65± 1.28	+13.41± 0.96	0.148^{NS}
PHOSPHORUS					
Intake					
Roughage	9.80± 0.96	6.82± 0.87	8.44± 0.72	7.30± 0.86	1.45^{NS}
Concentrate	17.02± 0.78	15.05± 0.58	16.80± 0.52	15.34± 0.32	1.12^{NS}
Total	26.82± 0.82	21.86± 0.73	25.25± 0.66	22.64± 0.96	0.76^{NS}
Outgo					
Faecal	13.89± 1.67	11.06± 1.13	15.03± 0.97	13.36± 1.06	1.76^{NS}
Urinary	4.94± 1.01	3.35± 0.86	3.01± 0.82	3.04± 0.93	0.56^{NS}
P-balance	+8.12± 0.55	+7.45± 0.42	+7.21± 0.61	+6.24± 0.72	0.94^{NS}

Table 6.10 Serum Ca and P levels (mg/100 ml) of calves in different treatments

	Groups				F-value
	I (Control)	II (EDTA)	III (Rock phos)	IV (Superphos)	
Serum Calcium					
1st month	9.75± 0.36	10.0± 0.46	8.50± 0.62	8.75± 0.35	2.25^{NS}
2nd month	9.88± 0.28	10.88± 0.49	9.25± 0.52	9.88± 0.42	2.06^{NS}
3rd month	10.38± 0.45	11.63± 0.60	10.0± 0.55	10.75± 0.52	0.58^{NS}
Mean	10.0± 0.41	11.08± 0.56	9.25± 0.56	9.79± 0.46	1.43^{NS}
Serum phosphorus					
1st month	8.14± 0.12	7.74± 0.16	8.37± 0.17	9.29± 0.16	2.86^{NS}
2nd month	8.14± 0.16	8.85± 0.15	8.37± 0.13	8.55± 0.47	2.41^{NS}
3rd month	8.71± 0.22	8.49± 0.08	8.26± 0.12	8.54± 0.42	1.20^{NS}
Mean	8.33± 0.13	8.36± 0.12	8.33± 0.15	8.80± 0.36	1.52^{NS}

Table 5.11 Daily intake and balances of Mg of animals in different groups (g/head/day)

	Groups				F-value
	I (Control)	II (EDTA)	III (Rock phos)	IV (Superphos)	
Intake					
Roughage	6.300	4.382	5.424	4.696	-
Concentrate	1.338	1.193	1.275	1.194	-
Total	7.638 ± 0.142	5.574 ± 0.136	6.699 ± 0.103	5.890 ± 0.163	1.66^{NS}
Outgo					
Faecal	5.948 ± 0.00	3.938 ± 0.072	5.029 ± 0.129	4.931 ± 0.132	1.35^{NS}
Urinary	0.325 ± 0.052	0.315 ± 0.036	0.329 ± 0.060	0.243 ± 0.037	0.83^{NS}
Balance	$+1.365^{a} \pm 0.072$	$+1.321^{a} \pm 0.068$	$+1.341^{a} \pm 0.051$	$+0.716^{b} \pm 0.094$	4.56 *
Percent absorbed	22.12	29.35	24.92	16.28	-

Values with different superscripts a,b differ significantly($P < 0.05$)

Table 5.12 Daily intake and balances of Fe of animals in different groups (g/head/day)

	Groups				F-value
	I (Control)	II (EDTA)	III (Rock phos)	IV (Superphos)	
Intake					
Roughage	0.660	0.459	0.568	0.492	-
Concentrate	0.972	0.840	1.188	0.719	-
Total	1.632 ± 0.098	1.299 ± 0.072	1.756 ± 0.069	1.211 ± 0.116	1.22^{NS}
Outgo					
Faecal	0.955 ± 0.061	0.521 ± 0.102	0.960 ± 0.112	0.777 ± 0.068	2.33^{NS}
Urinary	0.007	0.006	0.006	0.005	-
Balances	$+0.670^{a}$ ± 0.052	$+0.772^{a}$ ± 0.066	$+0.790^{a}$ ± 0.043	$+0.429^{b}$ ± 0.055	3.86*
Percent absorbed	41.48	59.89	45.33	35.83	

Values with different superscripts, a, b, differ significantly ($P \leq 0.05$)

Table 5.13 Daily intake and balances of Zn of animals in different groups (mg/head/day)

	Groups				F-value
	I (Control)	II (EDTA)	III (Rock phos)	IV (Superphos)	
Intake					
Roughage	70.00	48.68	60.27	52.18	-
Concentrate	138.32	123.39	134.75	122.13	-
Total	208.32 ± 8.23	127.07 ± 6.22	195.03 ± 5.06	174.31 ± 3.47	0.095^{NS}
Outgo					
Faecal	39.07 ± 0.56	33.32 ± 0.99	36.36 ± 0.76	28.79 ± 0.67	0.68^{NS}
Urinary	3.97	6.76	9.27	3.51	-
Balances	+165.28 ± 5.23	+132.00 ± 4.13	+149.39 ± 3.46	+142.08 ± 2.39	1.98^{NS}

Table 5.14 Daily intake and balances of Mn of animals in different groups (mg/head/day)

	Groups				F-value
	I (Control)	II (EDTA)	III (Rock phos)	IV (Superphos)	
Intake					
Roughage	84.00	58.42	72.32	62.62	-
Concentrate	104.88	90.85	104.33	124.84	-
Total	188.88 ± 6.88	149.27 ± 5.46	176.65 ± 3.26	187.46 ± 4.64	1.76^{NS}
Outgo					
Faecal	62.14 ± 2.32	47.10 ± 1.56	56.22 ± 1.96	62.74 ± 1.89	1.32^{NS}
Urinary	0.37 ± 0.07	0.32 ± 0.16	0.44 ± 0.09	0.33 ± 0.11	0.04^{NS}
Balances	+126.37 ± 2.32	+101.94 ± 4.01	+119.99 ± 1.98	+129.39 ± 2.11	1.83^{NS}

per cent absorption

Table 5.15 Daily intake and balance of Cu of animals under different groups (mg/head/day)

	Groups				F-value
	I (Control)	II (EDTA)	III (Rock phos.)	IV (Superphos)	
Intake					
Roughage	18.00	12.52	15.50	13.42	-
Concentrate	44.08	37.97	39.12	43.42	-
Total	62.08 ± 1.32	50.49 ± 1.76	54.62 ± 1.26	56.84 ± 1.49	2.13^{NS}
Faecal outgo	25.13 ± 1.98	15.32 ± 2.78	16.75 ± 2.56	22.59 ± 1.85	2.96^{NS}
Urinary outgo	2.59 ± 0.56	2.17 ± 0.23	1.78 ± 0.32	1.35 ± 0.46	2.86^{NS}
Balance	+34.36 ± 3.68	+33.00 ± 2.62	+36.85 ± 1.62	+31.55 ± 2.90	1.76^{NS}

tively. In case of Mg the balances in the four groups were 1.365± 0.072, 1.321± 0.068, 1.341± 0.051 and 0.716± 0.094 g/head/day respectively against the total intake through concentrate and green fodder in the range of 7.64± 0.14, 5.57± 0.136, 6.70± 0.103 and 5.89± 0.16 g/head/day respectively. The daily intake of Mg although was low in group II and IV, they were not significant and thus resulted in similar values for Mg balances in group I, II and III and significantly lower in group IV. In case of Fe, animals in different groups exhibited a daily balance of 0.670± 0.052, 0.772± 0.066, 0.790± 0.043 and 0.429± 0.055 g/head/day respectively against the mean intake of 1.632± 0.098, 1.299± 0.072, 1.756± 0.069 and 1.211± 0.116 g/head/day. The Fe balances were statistically similar in group I, II and III respectively, but was significantly low in group IV, as also reflected by lower intake in this group. The daily intakes, faecal and urinary outgo, and balances of Zn, Mn and Cu in the calves in different treatment groups showed no significant differences as shown in tables 5.13, 5.14 and 5.15. The balances were 165± 5.23, 132± 4.13, 149± 3.46 and 142± 2.39 mg/head/day for Zn; 126± 2.32, 102± 4.01, 120± 1.98 and 124± 2.11 mg/head/day for Mn, and 34.36± 3.68, 33.0± 2.62, 36.09± 1.62 and 31.55± 2.90 mg/head/day for Cu respectively. The balances were positive in each case.

5.2.6 Growth rate and feed conversion efficiency of calves fed different mineral mixtures

5.2.6.1 Growth rate:- Average body weight of calves for the period under study are shown in table 5.16. The growth rate was calculated by regression analysis of the cumulative weight gains during the 16 weeks period of 'b' values which are shown in table 5.16. The 'b' values in groups I to IV were 3.18 ± 0.37, 2.18 ± 0.24, 2.83 ± 0.34 and 2.39 ± 0.31 kg/week respectively. The average daily gains in the four groups were 451 ± 44, 323 ± 27, 403 ± 34 and 349 ± 13 g/head/day respectively. The relative pattern of growth under the 4 experimental treatments of feeding have been shown in table 5.17 and Fig.5.1. As evident from Fig.5.1, the growth rate of calves was lowest in group II and highest in group I. However, the statistical differences were not significant ($P > 0.05$).

5.2.6.2 Feed conversion ratio:- It is seen from table 5.16, the body weight gained per kg of feed consumed in groups I to IV were 156 ± 6.8, 116 ± 6.0, 146 ± 5.8 and 134 ± 5.6 g/kg. These values were significantly lower in group II in comparison to all other groups, but there was no significant difference ($P > 0.05$) in groups I, III and IV, suggesting possible depression in feed conversion efficiency in EDTA supplemented diets.

Table 5.16 Growth rate and feed conversion efficiency of calves under different treatment groups

	Groups				F-value
	I (OCP)	II (EDTA)	III (Rock phos)	IV (Superphos)	
Initial body wt.(kg)	57.25± 4.17	57.25± 4.61	56.75± 4.53	56.0± 4.53	0.03^{NS}
Final body wt.after 16 weeks (kg)	107.25± 9.26	92.25± 7.81	101.75± 4.76	93.75± 3.75	1.08^{NS}
Mean weight gain (kg)	50.0	35.0	45.0	37.75	-
Average daily gain (g)	451± 44	323± 27	403± 34	349± 13	3.17^{NS}
Growth rate 'b' value (kg/week)	3.18± 0.37	2.18± 0.24	2.83± 0.34	2.39± 0.31	3.20^{NS}
Gain to feed ratio(g/kg)	156^{a}± 6.8	116^{b}± 6.0	146^{a}± 5.8	134^{ab}± 5.6	5.38*

Value with different superscripts, a, b, ab differ significantly at ($P \leq 0.05$)

FIG-5·1 BODY WEIGHT OF CALVES UNDER DIFFERENT TREATMENT GROUPS

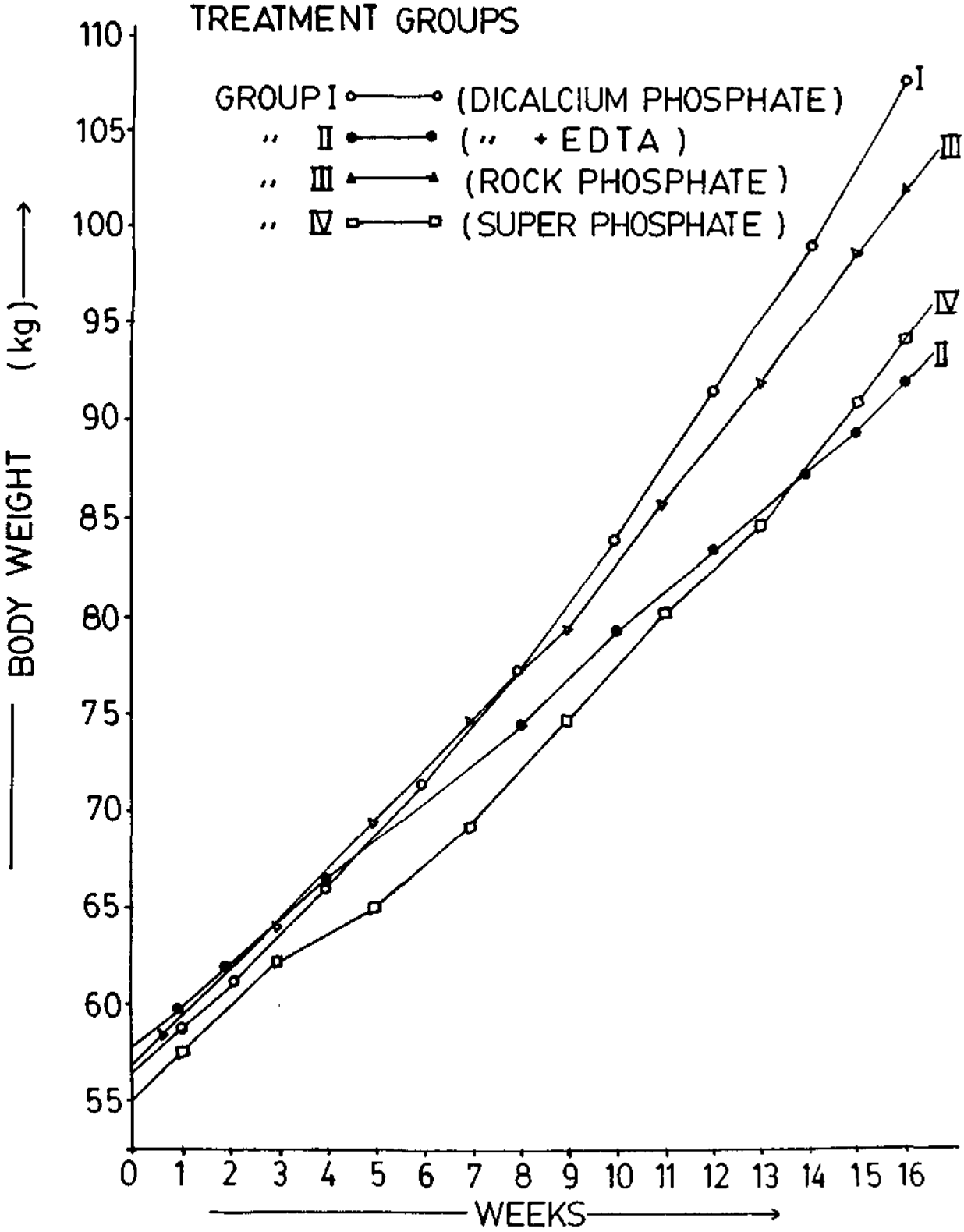


Table 5.17 Cumulative body weights (kg) of calves under different treatment groups

Weeks	Groups			
	I	II	III	IV
1	58.7	60.0	59.4	57.6
2	61.1	62.0	61.9	59.9
3	63.4	64.0	64.2	62.2
4	66.0	66.4	66.4	63.6
5	67.9	68.7	69.5	64.9
6	71.5	71.4	73.5	67.6
7	74.5	73.1	74.7	69.4
8	77.2	74.2	76.4	71.5
9	80.4	76.5	79.5	74.7
10	83.9	79.2	83.7	78.5
11	87.9	81.2	86.1	80.2
12	91.5	83.6	88.9	81.7
13	95.2	85.1	92.1	84.7
14	99.1	87.4	95.4	88.5
15	103.5	89.6	98.7	91.1
16	107.2	92.2	101.8	93.8

5.3 DISCUSSION

5.3.1 Composition of feeds

5.3.1.1 Chemical composition of feeds

Based upon the scanning studies (Chapter III) and ruminal studies (Chapter IV), three sources of Ca and P supplements, viz., dicalcium phosphate, rock phosphate and superphosphate were selected for comparative studies on nutrient utilisation and growth on crossbred calves fed on a basal diet containing concentrate mixture and roughage diet. Due to certain claims (Kratzer and Strarcher, 1963; Foll, 1966) that chelated mineral supplements improve mineral utilisation, another group of EDTA was also included in the studies. The chemical composition of the concentrate mixtures used in the four treatment groups were kept essentially the same and they differed only in type of mineral supplement (table 5.2). As presented in table 5.5, the variation in their protein, fibre and organic matter contents was kept minimum in the experimental groups. Although the contents of Ca, P and other trace mineral were not the same in these materials, but their proportions used in the preparation of mineral mixtures were so adjusted (table 5.2) that they provided similar level of Ca and P, i.e., 664g Ca and 478g P per 100 kg of concentrate mixture. In group II, EDTA was

mixed thoroughly in the concentrate mixture @ 0.1% w/w of concentrate mixture (i.e., 1000 ppm). The amount of EDTA added in the ration of group II was calculated on the basis of the findings in Experiment-2, Chapter IV so that the overall concentration of EDTA in the total diet was 500 ppm under the conditions where the DM ratio in concentrate and roughage was maintained as 1:1. EDTA was found to improve the utilisation of Zn and several other trace minerals in non- ruminants (Darwish and Kratzer, 1965; Forbes, 1961) but no advantageous observations could be made in literature for ruminants (Powell et al., 1967; Miller et al., 1968 and Hiers et al., 1968).

5.3.2 Dry matter intake, nutrient utilisation and N-balance of calves

5.3.2.1 Dry matter intake and nutrient utilisation

As evident from table 5.7, the total daily dry matter intake or dry matter intake per 100 kg body weight did not vary in the respective treatment groups. Also the digestibility of organic nutrients, i.e., OM, ADF and CP were statistically similar. This indicates that the compositional characteristics of rock phosphate or super phosphate had no influence when used as Ca/P source on intake and utilisation of dietary organic nutrients and compared to be similar to dicalcium phosphate supplementation group I.

Fisher (1978) compared different supplemental forms of P such as dicalcium phosphate and certain pure

phosphatic compounds like monocalcium phosphate, monoammonium phosphate and monosodium phosphate by including them as P supplement source in cattle ration. He found that neither dry matter intake nor the digestibilities of dry matter or crude protein were influenced by various chemical forms of P. However, the digestibility of acid detergent fibre fraction was reported to be higher on using monosodium phosphate as the P source.

Certain other studies were conducted with commercial grade supplements like gypsum and dolomite limestone. Bouchrad and Conrad (1973) found that on including agricultural grade gypsum in ruminant ration, not only there was depression in feed intake but also condition of acidosis developed in animals. Gerken and Fontenot (1967) included dolomite limestone as commercial grade Ca and Mg supplement in ruminant ration. They found that on using such supplements of Ca and Mg, there was depression in feed intake and carbohydrate digestibility.

In the present investigation, no significant difference in feed N intake, faecal or urinary outgo caused no difference in N-balances also (Table 5.8). However, in case of EDTA supplemented group II, there was lowered N-intake through roughage than other groups but the total N-intake was not significantly different. Probably this might have resulted in lowered N-balance in this group compared to other groups. The figures were

suggestive that they reached non-significant values only marginally, otherwise they were tending to show significant difference. The findings, therefore, suggested that except for group II, other groups compared to be almost similar in terms of dry matter intake, digestibilities of proximate principles and N-balances.

5.3. 3 Calcium and Phosphorus utilisation

The Ca and P utilisation from different sources of Ca and P supplements by growing calves were found to be similar in all the 4 groups as the intake, outgo and the balance figures were statistically not different (table 5.9). Studies presented in earlier Chapter III suggested that either in terms of composition or *in vitro* solubility in the ruminal buffer, rock phosphate and super phosphate were not similar to dicalcium phosphate, and therefore when judged on weight for weight basis they were not similar sources of Ca and P supplementation. As presented in Chapter IV also suggested that particularly the P distribution in soluble phase was lower in case of rock phosphate. But such distribution did not influence the net ruminal disappearance rate of Ca and P, for reasons already discussed. Therefore, *in vivo* utilisation of Ca and P from these three sources were also essentially similar as observed in this experiment. This was particularly because the rations were constructed not on the basis of weight to weight quantity of the supplements but the total Ca and P supply through feed from the

three different sources were made similar, which raised the quantity of rock phosphate and superphosphate used (Group III and IV) in comparison to dicalcium phosphate (Group I).

The calcium and P balances were statistically similar in EDTA supplemented group II inspite of the fact that both Ca intake from roughage and faecal Ca outgo values were very much depressed in this group in comparison to other groups.

Many workers have tried to compare the utilisability of inorganic mineral elements on using different naturally crude and pure chemical sources of certain Ca and P supplements. Thus, Ammerman et al. (1957) compared various sources of inorganic phosphorus in steer and lamb balance trials. Based on P retention and maintenance of blood P level, dicalcium phosphate, calcined defluorinated phosphate, bone meal, soft phosphate with collodial clay and curacao island phosphate were of equal value for steers. But in lambs, soft phosphate and defluorinated phosphate were poorly utilised in comparison to the other two sources.

Researchers differ with respect to their findings on the use of various sources of Ca and P as an alternative to dicalcium phosphate. In many instances defluorinated rock phosphate was found to be inferior to dicalcium phosphate (Witt and Owens, 1983); Fishwick, 1976), but the same has also been reported to be of equal value when compared to dicalcium phosphate or steamed bone meal

(O'Donovan et al., 1965 and Hemingway and Fishwick, 1975). It is possible that the supplemental sources obtained for investigation differed in compositional quality. The findings could also differ because of species differences of experimental animals on which the materials were tested.

Superphosphate was not found to be a satisfactory P supplement for sheep because of its high F content, and decreased daily gain and retention of Ca and P compared to a standard P supplement (Agarwala et al., 1971). In the same study, calcination of superphosphate at 600° C for 2 hours lowered F content from 23,500 ppm to 1600 ppm. However, feeding of such treated material of superphosphate produced no such effects as mentioned above in case of untreated superphosphate. In the present study, rock phosphate and superphosphate used, contained F in the range of 1500-2000 ppm and showed statistically similar balance of Ca and P in the respective groups. Both the alternative sources used here appeared to satisfy the Ca and P needs of the animals. Gohl (1981) had suggested that superphosphate can be used as P supplement, if other source of P is not available.

5.3.4 Blood Ca and P levels

Certain findings suggested that concentration of phosphorus in the blood of young animals capable of rapid growth falls rapidly when P intake is inadequate (Wise et al., 1961) and also when the P source is poorly utilised (Hemingway and Fishwick, 1976). Results of the

present investigation as indicated in table 5.10 revealed that serum Ca and P levels under different groups during the entire period of experiment were quite similar and differed neither with the treatments nor with the duration of a particular treatment. As the animals under different groups showed similar Ca and P balances (table 5.9), it was evident that both these alternative supplement sources, i.e., Mussorrie rock phosphate and fertiliser grade superphosphate were able to satisfy the Ca and P needs of the growing animals in the present study suggesting that these sources could be good alternative to dicalcium phosphate as Ca and P supplement sources. Further, EDTA supplementation produced no significant advantage in so far as the metabolisms of Ca and P are concerned.

5.3.5 Utilisation of other essential mineral elements from different mineral supplements

Data presented in table 5.11 revealed that Mg balance in group IV was significantly low as compared to the values in groups I, II and III. The lower intake of Mg in group IV might be one reason, but comparatively lower percent absorbtion of Mg in this group was not clearly understood. Supplementation of EDTA in group II has resulted in an increase of Mg absorbtion if compared with group I, II and III. The reason for this increased Mg absorption with EDTA supplementation is not clearly understood and needs further investigation. The animals maintained positive Mg balances in all the groups and

their requirements seem to have been met from the roughage and concentrate offered. Blaxter and McGill (1956), Field et al. (1958) have reported that minimum needs of sheep and cattle for growth can generally be met by pastures on rations containing 0.07 to 0.1% Mg on air dry basis.

It is evident from table 5.12 that although the animals in group IV showed significant (P/0.05) lower Fe balance in comparison to control group, the balances were + ve in each case and were sufficient to meet Fe requirements of calves. Hansard (1983) presented that intake of 100 ppm of Fe was adequate for calves upto 3 months of age, and requirement was still less for older calves. NRC (1978) suggested that 30-40 ppm Fe in ration may be adequate for growing calves. The balances shown in group I, II and III (table 5.12) are quite high and may be because of higher levels of intake in these groups.

Addition of EDTA in group II has increased the Fe absorbtion by one and a half times. Underwood (1981) has reported that organic form of iron is better absorbed than inorganic form of iron which forms various chelates in the diet. Chelating agent like ascorbic acid improve its absorbtion, while EDTA inhibit the same. Foll (1966), Forth and Rummel (1974), Hansard (1983) have shown that ferric chelates to be better utilised than ferrous sulphate. The reports in relation to the use of chelating compounds are contradictory and depends upon the type of chelate used as well as dietary conditions. In the

present study use of chelating agent, i.e., EDTA has not brought any advantageous effect in terms of body wt gain as discussed later.

The daily intake of Zn, Mn and Cu by ahimals under different treatment groups were commensurate with the DM intake. There was no statistical difference in terms of their availability or balances (table 5.13-5.15). Only in case of Cu, addition of EDTA in the ration marginally increased the absorbtion of this element(table 5.15). However, the studies suggested that Zn, Mn and Cu utilisability from different experimental rations containing different mineral sources were not greatly altered.

5.3.6 Growth rate and feed conversion efficiency of calves

The growth rate and feed efficiency data (table 5.16) suggested that starting from similar initial body wts, the different treatment groups, showed non-significant differences in growth rate. However, figure 5.1 suggested that although the growth differences between the groups were non-significant, groups II and IV showed somewhat lower growth than groups I and III. The different supplements supported medium daily body wt gain of calves ranging from 350 to 450 g/day. However, the gain to feed ratios (g/kg) was significantly lower in EDTA supplemented group II than other groups. In EDTA supplementation group showed negative influence on feed consumption. The faeces of the animals were of loose consistency. Similar observations

were made in our laboratory on feeding goats at the level of 900 ppm EDTA supplementation.

It was evident from the discussions presented above that although there were no significant differences in terms of feed and mineral utilisation, serum Ca and P levels and growth rate of animals, if rock phosphate or superphosphate was used as Ca and P supplement than when dicalcium phosphate was used, there was possibility that these alternative sources could sustain only medium growth rate. But it is difficult to claim that these supplements are enough to meet out the requirements for high growth rate or high level of lactation, which needs further investigation.

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CHAPTER-VI

SUMMARY AND CONCLUSION

SUMMARY

On tracing the history of utilisation of Ca and P supplements for use in mineral mixture during the century, it appeared that the use of different sources remained changing. Around 1902, meat by-products, meat scrapings and tankages were recognised as useful feed ingredients for livestock which might serve as mineral supplements apart from supplying other nutrients. Shortly thereafter, bone meal emerged as a Ca and P supplement source for animal feeds. By 1940, supply of bone meal became inadequate and search for other sources of phosphorus were made. Since then various chemically processed phosphates originating from raw rocks came into practice as Ca and P supplements and dicalcium phosphate emerged as good potential source.

Presently, in this country, chalk powder and dicalcium phosphate are the widely used Ca and P supplements sources for animal feeding. With the development of science and industry in this country and consequent availability of industrial wastes, there are possibilities of finding certain alternative potential sources of Ca and P supplements which may become available at relatively cheaper cost and may be innocuous in use as animal feed supplements provided compositional characteristics of such material was able to sustain demands of increased

animal production in terms of growth, milk production and animal reproduction. In the experiments discussed below, attention has been focussed on medium growth of animals.

Experiment-I : Compositional scanning of various sources of Ca and P supplements

Samples of dicalcium phosphate, rock phosphate, Mussorrie rock phosphate, superphosphate, NPK fertiliser, kiln dust, chalk powder, marble, lime, lime sludge waste, filter press mud waste, fly ash, pelei mitti, kharia mitti and plaster of paris were collected and analysed for different essential and certain incriminating elements of nutritional consideration.

Before resorting to the compositional scanning of different materials mentioned above, it was felt desirable to verify comparative reliance of techniques used for Ca and P determination. Two techniques of Ca and four techniques of P determinations were evaluated using few samples of Ca and P supplements. The recovery trials and the estimated values by different techniques suggested that out of the two methods tested for Ca estimation, AAS method using strontium as demasking agent proved to be superior to the titrimetric method. Out of the four methods tried for P estimation, the AOAC method using hydroquinone proved to be the most superior because of 97% recovery by this method as compared to 93, 93 and 126% recoveries in titrimetric, molybdovanadate complex

formation (AOAC, 1970) and Fiske and Subbarow methods respectively.

Compositional scanning of different materials included in the study suggested that lime, marble, chalk powder and filter press mud waste had the potentiality of being used as Ca supplements as they contained > 30% Ca and /2% AIA. Materials like kharia mitti, pelei mitti, plaster of paris and kiln dust were unsuitable as Ca or P supplements because of very low Ca and P content and very high (34-90%) AIA content. Samples of rock phosphate and superphosphate with moderate P content (>11%) and not very high AIA content (/15%) could be the potential sources of P supplements for animals, but F concentration needed to be checked in every sample before they could be put to use as mineral supplement.

Experiment-II : Ruminal solubility of different calcium and phosphorus sources

In vitro experiments were carried out to test the solubility of Ca from various Ca supplement sources such as lime, marble, chalk powder, gypsum and dicalcium phosphate in ruminal buffer solution. The solubility results were compared with that of pure Ca Cl_2. At pH 7, the solubility values ranged from 0% in case of dicalcium phosphate to 23% in case of gypsum. Even the Ca solubility in case of pure $CaCl_2$ was only 5.3% at pH 7. With the increase in buffer pH, these values increased to as high as 98.8% in case of pure $CaCl_2$ at pH 4. On decreasing

the buffer pH from 7 to 4, the solubilities of all the Ca supplements increased to variable proportions. Similarly, P solubility from various P supplements such as rock phosphate ore, Mussorrie rock phosphate, dicalcium phosphate and pure sodium phosphate in ruminal buffer solution ranged from 14% in case of rock phosphate to as high as 86.5% in sodium phosphate at pH 7 of the ruminal buffer. Contrary to the findings of Ca solubility, there was little improvement in P solubility from different sources by scaling down the buffer pH to 4.

Experiment-III : <u>Influence of certain calcium and phosphorus supplements on ruminal metabolites and distribution and disappearance pattern of Ca and P in the rumen</u>

Based on the scanning studies in Experiment I and II, Ca and P supplements like marble, gypsum, Mussorrie rock phosphate and superphosphate were selected for ruminal studies, and compared with dicalcium phosphate as a standard source of Ca and P supplement. For this study, a switch over design was followed by using 4 fistulated animals. While the basal feed consisting of conc.mixture and straw remained same in each case, the groups differed only in terms of Ca and P supplements used in mineral mixture preparation. Calculated quantities of different sources mentioned above were compounded in the mineral mixture in a way that the concentrate containing respective mineral mixtures supplied same level of Ca and P. In group T_0 no Ca/P supplement was used and termed as negative

control, while dicalcium phosphate served as positive control in group T_1. Marble and gypsum served as Ca supplement sources in groups II and III respectively. In these groups, sodium phosphate was used as P supplement. Mussorrie rock phosphate and fertiliser grade superphosphate served as both calcium and phosphorus supplement in groups IV and V respectively. Distribution of Ca and P in rumen digesta and their disappearance from rumen were investigated. In addition, the influence of different Ca and P sources on rumen fermentation pattern was studied by investigating changes in the ruminal energy and protein metabolites like VFA and NH_3-N concentration together with ruminal pH.

The results indicated that there was no significant effect ($P > 0.05$) on dry matter intake of animals or on the ruminal pH and TVFA concentrations and individual VFA proportions in rumen liquor. However, rumen NH_3-N concentration was significantly ($P < 0.05$) lower in rock phosphate group for reasons not clearly understood. Blood serum values for Ca and P were also statistically similar in all the groups and they ranged from 9.35-10.90 mg/100 ml in case of Ca, and 8.36-10.86 mg/100 ml in case of P. The values indicated that serum levels of Ca and P were not lower than normal in any treatment group. Distribution of Ca and P in soluble, particulate and solid phases of the rumen digesta differed significantly ($P < 0.05$) between the treatment groups. While about 7-9% of Ca was distributed on the soluble phases of different treatment

groups, Phosphorus distribution in this phase ranged from 30-50% approximately. Ca distribution in soluble phase from rock phosphate was highest (9.4%) whereas P distribution in this phase was lowest (29.3%) among the different treatment groups. Inspite of these differences in distribution, the rumen disappearance rates of Ca and P did not differ for the reasons discussed.

Experiment-IV : <u>Influence of EDTA supplementation on ruminal Ca solubility and disappearance pattern</u>

The influence of different level of EDTA supplementation in the diet of animals on ruminal solubility of Ca in SRL and disappearance rate from rumen was investigated. The same four fistulated animals used in earlier experiment were switched over to the present experiment. EDTA was mixed in the concentrate mixture in the ratio of EDTA:Ca (added through mineral mixture) as 0.2:1, 0.5:1 and 1:1 on w/w basis in treatments T_2, T_3 and T_4 respectively, and no EDTA was added in treatment group T_1. Concentrate mixture containing dicalcium phosphate as Ca and P supplements in the four treatment groups (T_1 to T_4) differed only with respect to level of EDTA added.

Results of this experiment indicated that changing the EDTA:Ca ratio did not affect rumen pH but Ca solubility declined as a function of treatment. The values were 40% in group T_1 and 29.9% in group T_4. However, the Ca concentration in SRL and its disappearance rate were not altered statistically with the addition of different levels of EDTA in the diets of animals.

Experiment-V : Influence of certain calcium and phosphorus supplements on nutrient utilisation and growth in ruminants

Based on the results of earlier experiments on scanning and ruminal studies, three sources of Ca and P supplements, viz., dicalcium phosphate, Mussorrie rock phosphate and superphosphate were selected for comparative studies on nutrient utilisation and growth in ruminants. Different mineral mixtures were prepared using these Ca/P supplements in proportions so adjusted, that they supplied same level of Ca and P, i.e., 664g Ca and 478g P per 100 kg of concentrate mixture in all the groups. An additional group of EDTA supplementation was included in the studies to investigate its effect on utilisation of Ca and other minerals. EDTA was mixed @ 0.1% in the concentrate mixture containing dicalcium phosphate as Ca/P source.

For this purpose, 16 crossbred calves were divided equally into 4 groups (T_1 to T_4). The basal diet was essentially the same and was made around green maize as roughage and concentrate mixture which differed only with respect to the sources of Ca and P. The net mineral supply through diet as also energy and protein contents were maintained at similar level. A growth trial of 16 weeks duration was conducted at the end of which a metabolic trial was carried out to study the effect of different Ca and P sources on nutrient utilisation.

Results of this experiment indicated that there was no significant difference in any of the parameters

like dry matter intake, digestibility of nutrients, mineral balances including that of Ca and P, and blood serum Ca and P levels of animals if rock phosphate and superphosphate were used as Ca and P supplements than when dicalcium phosphate was used. Although Mg and Fe balances in superphosphate group were significantly lower ($P < 0.05$) but were positive enough to sustain the growth. Growth rate which was in the range of 350-450 g/day in the groups having used three type of supplements, feed to gain ratio was also statistically similar. However, growth rate in EDTA fed group was depressed though non-significantly ($P > 0.05$) but could bring about significantly lower values in the same group.

CONCLUSION

1. Certain analytical procedures for determination of Ca and P in mineral supplements were comparatively evaluated. On the basis of recovery, the Atomic absorption spectrophotometer method for Ca estimation, and method using hydroquinone as reducing agent for P determination were adjudged to be most suitable.

2. Compositional scanning of materials like marble, gypsum, phosphogypsum, chalk powder, lime, plaster of paris, kharia mitti, pelsi mitti, fly ash, kiln dust, rock phosphate ore, Mussorrie rock phosphate, superphosphate, filter press mud waste, lime sludge waste and dicalcium

phosphate, suggested that chalk powder, marble, lime and filter press mud waste could be good Ca supplements because of high Ca content (> 30%) and low AIA content (< 2%), while rock phosphate and superphosphate could be used as an alternative to dicalcium phosphate.

3. *In vitro* Ca solubility in ruminal buffer was low at pH 7, but increased considerably by scaling down the pH to 4. P solubility was not dependent upon ruminal pH, although different P supplements showed variable solubilities.

4. Although distribution of Ca and P in soluble, particulate and solid phase of the rumen digesta were variable with certain Ca and P supplements, the supplements did not influence the disappearance pattern of Ca and P from the rumen. Except for slight effect on ruminal NH_3-N concentration in rock phosphate group, these supplements had no influence on other rumen metabolites.

5. With increasing level of EDTA supplementation in the diet, decreased the Ca solubility in the ruminal fluid from 43.0 to 29.8%, but had no influence on the disappearance pattern of Ca.

6. Ruminal solubility of Ca and P from different supplements suggested that they have no bearing on post-ruminal availability of these minerals.

7. With some initial dietary adaptation period needed for proper intake, rock phosphate and super-

phosphate could sustain medium growth in animals as an alternative to dicalcium phosphate, if supplied in a quantity to give similar Ca and P levels in the diet, as they showed no differences in growth or utilisation of organic and inorganic nutrients.

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