

**STUDIES ON THE TRACE MINERALS (COPPER AND COBALT)
SUPPLEMENTATION ON MILK PRODUCTION, BLOOD
COMPOSITION AND
REPRODUCTIVE PERFORMANCE IN CROSS-BRED COWS**

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(Indian Council of Agricultural Research)
BANGALORE
1973**

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A THESIS
submitted to the Punjab University
for the award of degree of
DOCTOR OF PHILOSOPHY
in Dairying and Animal Husbandry

by
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C E R T I F I C A T E

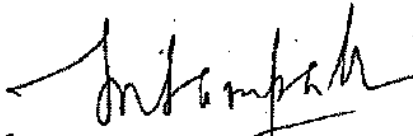
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Dated 14th Jun 1974

I certify that: (1) Shri. S.J.Kashirsagar has carried out research work under my direct supervision and guidance from September 1969 to December 1972 and that the manuscript of the thesis has been scrutinised by me; (2) The entire thesis comprises the work of the candidate's own personal achievement; (3) The thesis does not contain any co-joint research work with me or with any one else; (4) He has completed his research work to my entire satisfaction (5) The final typed copy of the thesis which is being submitted to the University office has been carefully read by me for its material and language and it is to my entire satisfaction; and (6) That it is worthy of consideration for the award of the Ph.D. degree.

It is certified that he has qualified in certificate examination in German in First Class of the Bangalore University.


(S.R. Sampath)

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PREFACE

P R E F A C E

Asia's teeming millions, particularly of the developing countries such as India, are constantly haunted by the fear of under-nutrition, owing to the paucity of balanced food, a fear that may become a reality with rising needs of the increased human population, adding on every year. Due to this explosive human population and the difficult agro-economic situation, the time is pressing hard on the direct use of land not only in India, but also in several other countries of the world. Dairy industry as it stands, primarily aims at providing sufficient milk for human consumption. Milk is the nearest approach to a most perfect single food so far known, which has also the largest measure of acceptance all over the world. There is now a growing awareness of the high nutritive value of milk and milk products. The civilised nations have been using milk and milk products as food or supplement to food, since times immemorial. Milk supplies exceedingly high quality protein that supplements the protein of the traditional vegetarian diet. Milk protein has a high biological value of 85 to 90 percent as against 60 to 70 percent for vegetable proteins. As the nearest approach to the perfect food, milk supplies easily digestible fat, lactose for the proper development and function of brain and tissue, valuable minerals for bone and blood formation and the vitamins which are essential for growth, reproduction, vigour and vitality of the human being.

It is an admitted fact that regardless of the great genetic potentialities, the animal cannot produce enough milk

or meat on an inadequate ration and the optimum reproductive performance is not achieved, which is necessary for the economic maintenance of the livestock. To get more milk and maximum economic returns from the animals, efforts in all directions are required to be made. The nutritional factors such as energy, protein, minerals and vitamins play a major role in determining their productivity. A ration can not be computed merely in terms of calories or of the old conventional concept of mere protein, fat and carbohydrates. It is fully recognised today that the mineral aspect of ration holds a position of equal importance with the so-called proximate principles or organic nutrients.

The animal body contains a large number of mineral elements. At present only fifteen of these can be described with certainty as nutritionally essential elements. They are calcium, phosphorus, sulphur, potassium, sodium, chlorine, magnesium, iron, zinc, copper, manganese, cobalt, iodine, molybdenum and selenium. The first seven i.e., from calcium to magnesium in this list are usually called as 'major or macro elements', because these are required in relatively large amounts by the animals. The remaining are frequently referred to as 'trace or micro elements', as these are required by the system in minute quantities and they occur in traces in the tissues. Out of these trace minerals, copper and cobalt are more important ones from the ruminant point of view.

The livestock malady which has been found to be associated with these trace mineral deficiency has been reported from

various regions of the world. Two major aspects with reference to these trace elements research in animal nutrition seem to be (i) the importance and use of these trace elements to the physiological process, and (ii) the relation of deficiencies or excess of these to diseases and disorders in the animals.

The first of these problems can be resolved into a number of definite phases that will give the outline of the type of research required. For instance, the information regarding effects and mode of action of various trace elements upon the formation and secretion of enzymes and hormones, as well as the changes brought about by trace elements in vital body fluids of complex nature, which influence the dependent physiological processes, is required to be obtained.

The second major problem is to acquire adequate knowledge of the actual requirements of the trace elements for different types of livestock. This knowledge is of great practical importance in order to get the optimum performance and maximum returns from the livestock enterprises in different regions under varied agro-climatic conditions.

In order to increase the milk production, cross-breeding is resorted to in the country. The cross-bred cows which yield good amount of milk also require fairly high level of nutrients. With a view to obtain optimum performance of these animals, it is of paramount importance to know their actual requirements of nutrients including the trace minerals. With a view to obtain the information on some of these aspects, a study was initiated and the findings are presented.

The effect of copper and cobalt supplementation on the performance of cross-bred cows forms the subject matter of the present studies. The various aspects related to the investigation that are dealt in different chapters are as follows.

A short introduction of the subject is given in Chapter I.

History and detailed review of literature on copper and cobalt in ruminant nutrition have been presented in Chapter II and III, respectively.

Materials and Methods those were used in the investigation have been incorporated in Chapter IV.

Results and Discussion on the effects of supplementation of Copper and Cobalt at three different levels on the blood composition, milk production, digestibility of nutrients and reproductive performance of cross-bred milch cows have been presented in Chapters V and VI, respectively.

Summary of the findings, Conclusions drawn and Recommendations made based on these are given in Chapter VII, which is followed by the Appendix and the Bibliography.

CHAPTER - I

INTRODUCTION

This will lead us to the time when the feeding standards began to appear, i.e., probably in 1810 when Thaer developed his 'hay values' as a measure of relative nutritive value (Maynard and Loosli, 1959).

Animals are utilised as converters of a wide varieties of coarse materials into palatable and nutritious food such as meat and milk. It is in this context that proper animal husbandry practice is of the greatest contribution to the well being of human race. For dairy cattle, the controlling factor in realizing the maximum efficiency is in the average daily milk yield per cow and the reproductive performance involving its rhythmicity and raising of an improved calf, with the lowest possible expenditure on it. This can be achieved only by the application of scientific methods of feeding and breeding of livestock, and thereby the socio-economic upgrading of the people in general and the peasantry in particular in an overwhelmingly agricultural country such as India is possible. It thus becomes imperative that feeding and breeding of the animals should be conditioned to a level, wherein the latest trends in the advances of the nutrition and physiology of animals' reproduction can be incorporated with maximum benefit.

All these productive and reproductive processes which are no doubt governed to some extent by inheritance, are to a great extent influenced by human factor in the modern days of improved feeding and breeding techniques, as also by general environmental conditions. Every animal in a herd that temporarily or permanently loses its conversion capacity or fertility due to

inadequate or faulty feeding or managerial imbalances, will result in a great loss to the owner. Delayed maturity, temporary sterility or low conception rate cause economic losses by making the animals unproductive or keeping them on low production level for comparatively longer time. There is a general agreement that infertility or even to some extent sterility is common among milch cows, wherever large numbers of them are brought together in an intensive programme of feeding, breeding and milk production and this failure in production and breeding is getting more prevalent. It is reported that occasionally whole herd will fail to breed in a particular period, without the apparent evidence of any disease condition. It is generally due to low or imbalanced state of nutrition. Failure to breed is very common, especially after heavy and protracted milk production, which is either due to over-drain of mineral reserves or inadequate level of some essential nutrients.

The endeavour of husbandry-men should, therefore, be to ward off the various causes, which may interfere with the normal inherent capabilities of the animal. These interference may otherwise be misunderstood as the limitations of animal system. Therefore, the feeding of animals according to their needs is important, both for effecting economy and obtaining maximum production and returns.

The ruminants are peculiarly different from other herbivorous animals because of their anatomical and physiological differences especially in the digestion of coarse and bulky fodders and that the microbial degradation of complex nutrients

and the subsequent synthesis of varieties of nutrients in their fore-stomach. The microbial break-down of complex carbohydrates yields readily available volatile fatty acids in the rumen. The feed nitrogen in the form of proteins, peptides, amino-acids, amides, urea, nitrates etc., is converted to ammonia by the action of rumen micro-organisms and subsequently microbial protein of high biological value is synthesised which in turn is utilized by the ruminant host. Therefore, the study of micro-nutrient requirements in the case of ruminant is complex. Primarily, the need is to carry out the essential physiological processes of the animal and secondarily to the micro-organisms in the rumen for their efficient growth and activity in breaking down a complex and undigestible materials such as cellulose and hemicellulose to a simpler digestible products.

The term micro-nutrient, in general is understood to be that the substances which are essential and required by the animal in microquantities. It includes both vitamins and trace minerals. During the last decade the importance of trace minerals have been increased prominently. The history of the trace elements is as stimulating and romantic as that of the vitamins. Their economic significance is as great as or greater than vitamins and they lie equally deep at the root of normal physiological processes of the living body. All the trace elements that are found in the animal body have not yet been proved to be nutritionally essential. A very large number of elements is known to occur in the tissues of higher animals. Many of them occur in such small quantities that the early workers were unable to measure their concentrations with the

methods then available, and therefore frequently referred to them as occurring in 'traces'. For this reason, they came to be known as 'trace elements'. Green (1941) suggested that the elements present upto 0.005 percent in the body may be considered as trace elements.

Essential trace elements are those which show (i) repeated demonstration of a significant growth response to dietary supplements of the element and that element alone, (ii) development of the deficiency state on diets otherwise adequate and satisfactory i.e., containing all the other known dietary essential in adequate amounts and proportions and free from toxic properties and (iii) correlation of the deficiency state with the occurrence of sub-normal levels of the element in the blood or tissue of animals exhibiting the response. At present, nine trace elements are considered as essential in animal nutrition. They are iron, iodine, copper, zinc, manganese, cobalt, molybdenum, selenium and chromium. Ruminants are the only class of animals for which cobalt can be called as essential. Other trace elements such as fluorine, bromine, barium, strontium and cadmium are classified as 'probably essential elements' because they have not so far been shown to meet all the above criteria (Underwood 1957).

Any element can be toxic to animal if consumed in large quantities or consistently for sufficiently long period. Fluorine, selenium, molybdenum, copper are the outstanding examples of these. Arsenic and lead are the other trace minerals which come under toxic elements. Further more, there are about 25 to 30 elements found in the animal body, mostly in

minute quantities but they are not yet proved to have significant physiological function or existence in the animal. They are termed as 'accidental' or physiologically inactive trace elements, present merely or accidentally.

The value of the trace elements is often confined to the part they play in the maintenance of the health of the animals and what disease conditions normally arise when a certain trace mineral is deficient, fails to function or is present in excess. Careful examination of the ration of livestock is, therefore, essential to determine quantity and the type of minerals contained therein as compared to the actual requirements of the animals concerned, in order to take the subsequent steps to supplement the deficiency accurately. Lack of sufficient good quality forage and grass, and deficiency of trace minerals in particular regions contribute to the failure of livestock enterprises. Hence the deficiency must be adequately supplemented with particular minerals, otherwise the health and production of the animals are affected.

Many attempts have been made abroad, especially in Western countries. Australia and New Zealand to study the various aspects of the trace minerals supplementation and their requirements in case of pure-bred as well as cross-bred dairy cattle. Very little work is reported in this regard on pure-bred or cross-bred animals in southern part of India, especially on the effect and requirements of vital trace elements such as copper and cobalt, where cross-breeding programme has been taken up since last two decades.

Most of the research work with copper and cobalt in cattle has dealt with the correction of deficiencies of these elements and little information is available on the role of copper and cobalt in promoting increased feed efficiency and production. The studies reported herein were conducted to determine the effect of added dietary levels of copper and cobalt on the performance of the cross-bred cows.

These investigations are important and vital not only for the maintenance of their optimum performance in the region, but also in other parts of the country, where the number of this type of animals are coming up very rapidly for increasing the milk production. The work embodies an attempt made towards a study on some of these important trace minerals such as copper and cobalt on the cross-bred cows. It is hoped that the revelations will add materially to the existing knowledge of these trace elements and will help to those engaged in management and feeding of cross-bred herds to keep their animals at optimum level of milk production and reproductive performance.

CHAPTER - II

REVIEW OF LITERATURE ON STUDIES WITH COPPER FOR RUMINANTS

CHAPTER - II

REVIEW OF LITERATURE ON STUDIES WITH COPPER FOR RUMINANTS

1. Introduction and History:

Copper is probably the first metal to be extensively used by man. Articles of copper were made as early as 6,000 years ago. Copper was obtained by Romans and Greeks from the island of Cyprus. The latin name 'Aes cyprum' or 'Cyprian copper', afterwards became 'Cuprium' and finally "Cuprum". The alchemists associate the metal with planet Venus, and designated it by the symbol ♀. Copper as a metal has been used since very early times, the oldest specimens of cast copper from Egypt and Babylonia dates back to 4,000 B.C. As a remedy for various ailments in man, it has been used since the time of Egyptians 1,500 B.C. The Greeks in the time of Hippocrates prescribed copper compounds in pulmonary affections.

In India, this metal is known since "vedic" times. It is considered to be sacred metal as its use is mentioned as a container for water storage in religious functions as well as for human consumption. Copper was used in Aurvedic medicine, since the time of Charaka and Sushruta, 1,800 to 1,400 B.C. The preparations and uses of copper in the form of 'Tanrabhasma' is mentioned by these authors. Aurvedic preparations containing copper compounds are prescribed in liver and spleen enlargements, inflammation of external secretory glands and in ascitis. It was also used in anaemia diarrhoea and dysentery.

Copper forms also an ingredient of other preparations of aurvedic medicines. Copper as copper sulphate appears to have been introduced by Wagbhat in Aurvedic medicine since 1,300 A.D. It was indicated for use in dressing wounds and in skin diseases, especially in syphilis in combination with other drugs.

In the western world, Paracelsus recommended copper for the treatment of mental diseases and lung affections and by 18th century, copper compounds were in wide clinical use. In veterinary practice, copper was used extensively in anti-helminthic preparations.

During the later part of 19th century, copper was recognised as a normal constituent of blood. Its presence was established much earlier in plant life. The real importance of this element in the nutrition of mammals was not appreciated, however, until 1920's, when Hart and co-workers demonstrated that the sucklings suffering from anaemia responded to copper supplements and that it was an essential element for the well being. As a result of this pioneer work, the copper deficiency in animals was studied in many countries including India, in 1930 onwards. Considerable amount of literature are available on this subject in the last 40 years. A review of the same follows in these pages.

2. Biological significance of copper:

Copper as a metal has been known for centuries and though occurring in soil, the presence of this metal in biological material and realisation of its importance in biological activities, however, remained undetected scientifically, until the

beginning of the nineteenth century. Bucholz^c (1816) and Meissner (1817) were probably the first to establish definitely the presence of copper in plant life. Later, Sarzeau (1830) detected the presence of copper in animals and determined the amount quantitatively. He found ox blood to contain 700 $\mu\text{g.}/\text{l.}$ While studying the copper content of vegetables, Deschamp (1848) observed a relationship between the copper in plants and that in the soil on which they were grown. The presence of copper in animal tissue was for the first time detected by Sarzeau (1830) and was recognized well over a one and quarter century ago by Boutigny (1833). Chevrueil (1838), in a review on the copper content of biological materials, suggested that the metal was quite widely distributed in organic matter, but offered no explanation for its presence. Its occurrence was assumed to be accidental. Lehman (1895) summarized the then known facts regarding the distribution of copper and added many results from his own investigations.

Bertrand (1920), Guerithault (1920), Fleurent and Levi (1920) established the universal distribution of copper in plant life and suggested that copper should be placed in the group of catalytic elements found in the plants. The results obtained by Fleurent and Levi showed that leaves contain the maximum amount, whereas Guerithault found seeds to be the richest source of copper. Maquenne and Demoussay (1920) detected the presence of copper in all the samples of plant they analysed, the proportion being greatest in young shrubs and leaves. They concluded that copper in some way or the other aids in the vital functions

and was an essential element in plant metabolism. They observed that treatment of soil with copper sulphate improved the yield and quality of the plants grown on it. The essentiality of copper as plant nutrient was established in 1931 by Sommer (1931) and Lippman and Mackinney (1931).

In animal kingdom, the concentration of this metal was considered to be higher in lower invertebrates than in plants and higher vertebrates. Harless (1947) detected the presence of copper in *Eledone* and *Halix Pomatia* (Snails) molluscan blood and showed that it existed in combination with blood protein. Fredericq (1878) established the fact that the copper containing pigment (haemocyanin) of the blood of octopus, behaved as a respiratory pigment. Lehman (1886) showed the presence of copper in whole blood and in serum. Church (1889, 1892) reported the discovery of pigment turacin in the feathers of the South African turaco birds. It was shown that turacin belongs to the group of porphyrin derivatives, normally found in plants and animals. This is the only species in which copper occurs combined with porphyrin. Dore (1915) has reviewed in detail the literature on the copper contents of marine animals. Rose and Bodansky (1920) suggested that possibly copper is an essential constituent of marine fish tissue.

Even though the presence of copper in animal tissues was first demonstrated by Sarzeau (1830) and Routigny (1833) and reviewed by Chevrul (1838), its occurrence was assumed to be accidental. But it was not until the investigations of Bodansky (1921) and McHargue (1925, 1926, 1927), however, that copper be-

both plants and animals were isolated. Many of them were shown to be enzymes with oxidative functions, such as tyrosinase, uricase, ascorbic acid oxidase, laccase and cytochromeoxidase, though the mechanism was not known (Keilme and Mann, 1933; Krebs, 1928; and Kubovitz, 1937). The specificity of copper as a supplement to iron in the regeneration of haemoglobin has since been demonstrated by Keil and Nelson (1931), Hamilton, *et al* (1933), Meyers *et al*, (1934), Potter *et al*, (1933) and Frost *et al* (1940). A specific effect on red blood cell production has been attributed to copper by Oda (1932), Schultz (1932) Stein and Lewis (1933), Binnet and Strunza (1934), Somogyi (1935) and others.

The livestock malady which has been found to be associated with the copper deficiency has been reported from widespread regions of the world in early 1930's. Investigations on naturally occurring diseases of grazing sheep and cattle showed that affected cattle responded to copper or copper and cobalt therapy. Neal and associates (1931) after their studies on "salt sick" cattle in Florida were the first to suggest that copper deficiency occurs naturally in livestock. The work was subsequently confirmed by Becker *et al*, (1931). Two years later Sjollema (1933) from Holland showed that copper deficiency is a causal factor of a wasting disease of sheep and cattle, called 'Lechsucht', characterised by anorexia, emaciation, anaemia and diarrhoea. In 1933, he attributed the salt sick condition in cattle to the poor quality of hay and wide spread disease of grain crops and observed that this condition was

checked by the copper sulphate fertilizer. Fens (1941) reported in Holland, a similar condition which too was remedied by copper administration.

Bennetts and Chapman (1937) found that a usually fatal condition of ataxia in new born lambs, enzootic in certain parts of Western Australia was associated with a deficiency of copper in the grazing mother and could be avoided by treating the ewes with the oral copper during the period of gestation. Confirmation of their discovery was reported from South Australia by Bull *et al.* (1938) and from England by Dunlop and Wells (1938), wherein a similar malady known as 'sway back' occurred in certain restricted areas. Marston *et al.* (1938) observed that 'ataxia' in lambs occurred on calcareous soil and he confirmed Sjollem's findings. A report of de-pigmentation, a new dietary deficiency disease, cured by copper supplementation has been made by Porter (1935) and McCollum *et al.* (1939).

Black *et al.* (1937) reported that adequate amount of copper in diet showed a more efficient utilisation of food, greater weight gain, greater energy storage and greater accumulation of fat and protein. Bennetts and Beck (1942) in their bulletin, reported low copper content of pastures in Australia and parts of New Zealand. Bennetts and Hall (1939) and also Bennetts *et al.* (1942-43) described in the same area a disease of dairy cattle 'falling disease', which is characterized by loss of condition, fibrosis of myocardium and a seasonal incidence of sudden death. Innes and Sheaver (1940) showed that the disease 'sway back' in lambs, wide spread in Great Britain was pathologically same as that of 'enzootic ataxia' noticed in Australia,

except for the fact that the copper contents of the grasses ranged from 7 to 23 ppm. Diarrhoea in cattle, varying in severity from a mild and transient condition to an acute, persistent and debilitating scouring occurred under conditions of copper deficiency. In Holland it was called 'scouring disease' (Brouwer *et al* (1938). In New Zealand, acute scouring condition was popularly known as 'peat scour' (Cunningham, 1946).

Davis *et al* (1946) stated that in Florida, cattle developed ~~mark~~ deficiency within 60 to 90 days and on copper supplementation, scour was arrested in 24 to 48 hours. Withdrawal of copper resulted in the reappearance of scour, within 2 to 4 weeks. Supplementation of ration with 3 gm of copper sulphate once in a fortnight or 0.5 to 2.0 gm of copper sulphate given daily maintained the animals in good condition.

Washburn (1946) in Colorado observed that symptoms of lameness, crooked feet and legs fragile bones, soft and eroded bone-articulative surfaces and occasional sterility showed by the animals could be prevented or cured by supplementation of copper at the rate of 15 ppm of the feed. The animals fed copper were more alert and active and presented a more desirable show and appearance. Smith and Ellis (1947) reported that copper deficiency in rabbit resulted in xopacia, dermatitis, achromotrichia, which could be cured by copper supplements.

These pioneer observations of Hart and associates, Neal and co-workers, Sjollema, Bennetts and Chapman etc., were

followed by the demonstration of copper deficiency of varying extent in many parts of the world, affecting both crop and livestock, and the laboratory studies with the different animal species revealed that copper participates in a remarkable varieties of functions in the living system. Simultaneously, the copper was shown to be vitally concerned in the processes of pigmentation, keratinization of wool, bone formation, myelination of spinal cord, reproduction and enzyme functions, in addition to that of hematopoiesis. It was possible to carry out these investigations, only because of the concurrent development of reliable analytical methods of sufficient sensitivity and precision to estimate the minute quantities of trace elements and their distribution in plant and animal tissues and their metabolic movements to be followed. All these gave a fillip to the overall knowledge of the problem and further development.

Indian workers were fascinated by these research findings abroad, initiated work in the country. The available references have been reviewed.

Capt. MacGuck (1931) commented on practical feeding tables for dairy cattle in India and suggested that the danger of mineral deficiency is ever present in the country. He suggested mineral mixture containing ground limestone, steamed bone meal and common salt besides a provision of Rock-salt by the side of the animal to overcome the mineral deficiencies.

Chaudhary (1931) mentioned the practice of feeding to cattle a very acid curd (dahi), in which the copper coils are

put, which results in the colour of the same to turn to blue. Krishnan and Iyer (1934) stated that copper induces better assimilation of lime. Krishnappa (1936) envisaged the possibility of the occurrence of 'ataxia' or 'sway back' in lambs in two villages. The affected 47 lambs showed shivering, convulsion and died in two days. Haemorrhages in grain and degeneration of spinal cord was also observed.

A systematic investigation on copper in relation to cattle nutrition was undertaken at Indian Veterinary Research Institute, Izatnagar in 1944. Results given in Annual Reports of 1946-47, 1952-53 and 1953-54 indicated that the

- (1) Normal copper requirement of cattle was estimated to be 45 to 77 mg per day. It will vary according to region (Sahai and Kehr, 1946-47),
 - (2) Average copper content of cow and buffalo blood per 100 ml was found to be $137 \pm 8.8 \mu\text{g}$ and $144.5 \pm 7.7 \mu\text{g}$, and that of sheep blood was $86.4 \pm 10.9 \mu\text{g}$,
- and (3) Haemoglobin values were discovered to be low on certain farms, and it was suggested that copper deficiency may be one of the causes for this. Effect of blood forming elements iron, copper and cobalt on fluorine toxicity was studied. Trace elements feeding to the animals, which had developed fluorine toxicity resulted in beneficial effect. The animals improved in their condition and amelioration of toxic symptoms was brought about.

Lal (1947) has mentioned common deficiency diseases of animals and suggested the likelihood of prevalence of mineral deficiencies in India. He suggested remedies such as the inclusion of copper and cobalt in mineral mixture. Sahai and Kehr (1951) determined the copper content of 72 common feeds and feeding stuffs as well as blood and tissues of six normal

bullocks. They reported that straws and hays were in general low in copper content (3.6 to 4.7 mg per kg dry matter). The greens and leafy fodders appeared to be better sources of copper (5.9 to 17.8 mg per kg dry matter). The copper content of concentrates ranged from 9.2 to 17.2 mg per kg. The copper content of blood ranged from 0.129 to 0.153 mg per 100 ml. The copper content of liver and other tissues also were examined.

At the same time, Datta and Datta Biswas (1961) at the Indian Agricultural Research Institute determined the copper and cobalt contents of grasses, forage crops, concentrates and mineral supplements. The copper content of all the samples in young stage were observed to be 'sound' (9.0 to 15.0 ppm dry matter). Some were found to be low or at 'critical' level at mature stage. Of the concentrates, the copper values of Bhusa, wheat bran, maize, barley, oats and grams were marginal or sound. They also observed the likelihood of the occurrence of copper deficiency.

The Indian Council of Agricultural Research undertook a scheme for the study of trace elements and survey of animal nutrition with respect to trace elements in the areas of trace mineral deficiencies in India (1962-69). A gross deficiency of copper in the feeds was observed at Bellary (Mysore State), Nagpur (Maharashtra), Bharari (Uttar Pradesh) and copper level in the feed appeared to be marginally deficient in other farms. Wide spread copper deficiency areas are seen all over India, on the basis of soil and crop conditions (Kanwar and Randhawa, 1967)

Mahadevan (1959) in the final report of the scheme 'Studies on the influence of trace elements on the health and productive capacity of livestock in India' recorded that it is reasonable to assume that sub-acute copper deficiency is wide spread throughout the length and breadth of the country. From the study of copper intake by cattle in 50 farms and the analysis of the copper content of 437 samples of feeds and fodders consisting of some 90 different items fed in these farms, these observations were made.

Dashpande (1959) reported the incidence of copper deficiency of a far wide spread nature in the former Bombay state. Existence of sub-clinical deficiency manifesting in stunted growth, low fertility and late maturity was reported.

Gopalkrishna and Mahadevan (1959) concluded from the digestibility studies on pigs that the digestibility of crude fibre improved significantly by the supplementation of the ration with copper. They observed linear relationship between intake and retention of copper.

Pachalag et al (1972) reported that the supplementation of copper and cobalt together in the ration had no effect on the packed cell volume, red blood cell count and haemoglobin in weaner lambs. A higher white blood cell count was recorded in the animals given the supplements.

3. Metabolism of copper:

The importance of copper in the nutrition of farm animals and its role in the prevention of certain nutritional diseases

is considerable. However, very little is known of the mechanism of the absorption, distribution, retention, functions and excretion of copper in the body as compared to that of iron. It appears to be absorbed at least by two processes, one being a first order reaction and the other through an enzyme mechanism, (Giffin et al, 1950). The absorption takes place markedly in pronounced acid reaction part of the gut. This was evident by the marked depression in the absorption of this element in sheep by high dietary intake of calcium carbonate, which rendered the reaction to alkaline side, as shown by Dick (1956). This indicates that absorption takes place from the upper part of the small intestine. Lassiter and Bell (1960) showed that the availability of copper to sheep is influenced by the chemical and physical form in which it is administered. Under the conditions of these experiments, the copper in the elemental and oxide form was found to be less available than in the sulphate, chloride, nitrate or carbonate forms. More soluble compounds are very well absorbed. As shown by Mills (1954, 1955, 1956) copper in the herbage and pastures exists to a major extent in the bound form of water-soluble organic complexes, and small quantity in the free ionic form, or positively charged complexes. He suggested that copper may be transported through the intestinal mucosa in both the forms mentioned above. The nutritional importance of it cannot yet be evaluated, but he suggested that the form in which copper occurred in feed and fodder is important and it needs more study, especially the aspect of the net available copper from the feed and fodder to the animal although the total copper content of these are sufficiently high.

There are many unrecognised dietary factors which can exert some influence on the absorption and utilization of copper. It has been shown by Lewis (1960) that the deficiency of copper in cattle and sheep occurred due to primarily the wide variations in the reaction of individual animals and secondarily the molybdenum and inorganic sulphate and copper contents of feeds and fodders. In fact he observed excessive copper storage in housed sheep on diets containing the concentrations of these three constituents similar to those of herbage on which neonatal ataxia can occur in lambs.

The absorbed copper is transported by the blood plasma in a loosely bound form with plasma protein to the organs and tissues of storage, utilization and excretion. The copper is either stored in these sites as liver, spleen, kidney or released for the incorporation of this into the various copper containing enzymes of the cells and such other compounds as haemocuprein and caeruloplasmin or it is secreted into the bile and excreted via this route to the intestinal contents. It may be excreted out or reabsorbed from the intestine. Little quantity may also pass directly back from the plasma through the intestinal wall in the intestine and little into the urine.

Comar (1950) found only 6% of a single dose of intravenously injected copper to be excreted by cattle in the first 5 days, as compared to 78% of a similar oral dose, which indicated that injected copper is better retained by the body than orally administered one. This finding was later confirmed by Lassiter and Bell (1960) in sheep and Bowland et al (1961)

per head per day. Mahadevan and Zubairy (1969) conducted balance trial on Hariann cows and found that retention of copper was 144 mg per day in animals given copper supplements as against 14 mg per day in animals receiving no supplement. Gopalakrishna and Mahadevan (1969) observed a significant linear relationship between copper intake and retention in the body of pigs.

Reddy (1966) studied the effect of copper supplementation on digestibilities of nutrients in lactating cows. In this study, the animals of control group received 6.99 - 8.23 ppm of copper in their feed, whereas the animals of experimental group received 21.95 - 33.49 ppm of copper in their feed. It was observed that the ingestion of dry matter, total digestible nutrients and water consumption by the animals were less in the experimental group compared with those of control group. The high level of copper feeding was observed to reduce significantly the digestibilities of crude protein and ether extractives. The digestibilities of dry matter and nitrogen-free extract were also found to be depressed slightly by the higher level of copper intake. However, digestibility of crude fibre was not affected. Copper supplementation was observed to favour significant retention of copper, calcium and nitrogen. The phosphorus seemed to be unaffected, though the average retention by the animals in the supplemented groups was little less than those in the control group.

While studying the growth rate of cow and buffalo calves on pastures, Gupta (1968) supplemented the feed of the calves with copper, cobalt and iodine at the rates of 1.5, 0.05 and 0.1 g,

respectively per week per head. It was observed that the introduction of trace elements did not bring about any change in the digestibility co-efficients of the nutrients either in the cow or buffalo calves.

Kapoor and Mahadevan (1970b) studied the effect of supplementation of the feed with copper alone and copper plus cobalt on the digestibilities of the nutrients and their retention in Haryana calves. The calves of two experimental groups were given the supplements at the rate of 12.64 mg copper and 12.64 mg copper plus 1.32 mg cobalt per 100 kg body weight. The supplementation of either copper alone or copper plus cobalt had no significant effect on either the balances of nitrogen, calcium, phosphorus, copper and cobalt or the intake of dry-matter, D.C.P. and T.D.N.

4. Copper in animal tissues and organs:

Rusoff (1941) noticed a marked differences in the distribution of copper in two new born calves. However, despite their great variability, the findings emphasized that large amounts of copper can exist in the bones, muscles, skin and liver of animals. Heart, brain and kidney were the other important organs of copper storage. Dick (1954) found the distribution to be 72-79% in the liver, 8-12% in the muscles, 9% in the skin and hairs and about 2% in the skeleton of adult sheep. The susceptibility of the individual tissues to variations in dietary copper intake also varies considerably. The endocrine glands, the muscles, the heart, the brain and the skin are not susceptible to constant

change, whereas the copper concentrations in the liver, kidney, spleen, and lungs can be greatly increased by high copper intakes and those of liver, kidney, spleen and blood readily reduced under conditions of dietary deficiency (Underwood, 1962). High concentration of copper occurs in the eye tissue, especially the pigment portion of many species. It is associated particularly with the pigments (melanin) in the eye and its role over there is not yet understood, (Howness et al 1962). Copper content of hair (wool) has got special importance and is reviewed elsewhere.

Since the liver is the main storage organ of the body for copper, and the levels in the liver provides an useful index of the copper status of the animal, liver copper concentrations have been extensively studied from all the angles in a wide range of animal species. The liver copper levels are related to four main factors, namely, the species, the age of the animal, the nature of its diet, and the occurrence of certain disease conditions. Individual variations in liver copper concentration is high in all species. The review of this subject is beyond the scope of these studies. However, it is worth mentioning that normal liver copper content of adult cattle is 100 to 300 ppm on dry matter basis (Cunningham, 1966).

5. Copper in blood:

Mann and Keilin (1938) isolated a copper-protein from the red blood cells of the cattle. They called this compound as 'haemocuprein' and showed that it contained 0.34% copper in the cupric form. It has a molecular weight of 35,000 and 2 atoms of

copper per molecule. It is a bluish pigment. Later, Tompsett (1940) suggested that copper in the red blood cells may occur in the cuprous form, combined with glutathione. Red cell copper can be increased to very high levels following the administration of massive doses of copper to animals (Cartwright, 1950). The function of haemocuprein is not yet fully understood.

Copper in the blood plasma exists in the form of a blue copper-protein compound known as 'caeruloplasmin' as reported by Cartwright (1950) and Wintrobe (1953). This compound is an α 2-globulin with a molecular weight of 151,000 and it contains 8 atoms of copper per molecule. It is a blue protein having true oxidase activity. Similar to haemocuprein, the important physiological function of caeruloplasmin is not known. The remaining copper in blood plasma reacts directly with dithionite, is nondialyzable and is loosely bound to protein, probably serum albumin. However, on addition of acid, it becomes dialyzable and can be completely extracted with tri-chloro acetic acid. (Bowland et al, 1961; Wintrobe et al, 1953).

In cattle, Eden and Green (1939) showed that copper in blood is normally divided equally between cells and plasma. Plasma copper is more labile than cellular copper and is more reliable indicator of changes in the copper status of an animal than whole blood copper content. Concentration of copper in the whole blood of healthy animal ranges widely. Beck (1956) reported for a wide range of species of animals, the copper content of whole blood to be 0.5 to 1.5 ^{mg}/per litre. Physical exertion increases the whole blood copper level, while sex or

the pregnancy does not show any effect on whole blood or plasma copper contents.

Copper concentration of whole blood as well as that of plasma are influenced by the level of dietary copper as well as the ratio of this copper to molybdenum and inorganic sulphate intakes. Hypocupremia was reported in sheep and cattle grazing on copper-deficient pastures by Eden (1940) and Bennetts and Beck (1942). Values as low as 10 μ g per 100 ml of whole blood have been reported in sheep and cattle. Hypocupremia can also result from heavy infestation of intestinal parasites due to both blood loss and interference with copper absorption (Brenner, 1959).

Hypercupremia has been reported to be caused either by high dietary copper intakes or transiently by single high dose of injection of copper (Boyden *et al.*, 1938; Cunningham, 1946; and Eden, 1939-40). It can also be produced by very high intakes of molybdenum and inorganic sulphate (Dick, 1952, 1953, 1954).

The report of Indian Veterinary Research Institute (Anon 1946-47) indicated that the copper content of blood of Kusunda bullocks was within the range recorded for cattle. Supplementation of the feed with copper sulphate increased their blood copper levels.

Dunkley *et al.* (1963) studied the effect of injecting the cows with copper glycinate on blood copper levels and found a prolonged elevation of blood copper level i.e., upto 175 μ g per 100 ml. Later, Dunkley *et al.* (1968) further investigated the

influence of dietary copper and ethylene-diamine-tetra-acetate (EDTA) on copper concentration in blood and concluded that treatments did not influence the concentration of copper in the blood of the cows. Feeding of the supplements other than copper such as molybdenum or sulphate was found to be accompanied by changes in the concentration of copper in blood and also milk (Riest *et al.*, 1957).

The normal copper content of blood of Kankrej and Gir breed of cattle was reported to be 170 μ g and 131 μ g per 100 ml respectively, by the Western Regional Animal Nutrition Station (1961-62, 1962-63). Dashpande (1959) reported the blood copper values for normal healthy field animals to be 90 to 120 μ g per 100 ml and that of sub-clinical deficient animals to be 60 to 90 μ g per 100 ml. He observed an increase in blood copper level from 50 μ g to 120 μ g per 100 ml within a month by supplementation of the feed of six heifers with 5 g copper sulphate per day.

The annual report of Indian Veterinary Research Institute (Anon., 1953-54) indicated the average copper content of blood of 15 cow calves and 15 buffalo calves to be 137 ± 8.6 and 144.5 ± 7.7 μ g per 100 ml respectively. The later report of the institute (Anon., 1954-55) gave the values to be 18.4 to 61.8 μ g copper per 100 ml of blood of calves. The effect of trace element mixture supplementation to calves on the blood copper level was studied in the same institute in the year 1955-56. It was seen that the blood copper level increased due to the supplementation.

The blood samples collected from 19 sterile cows in Bombay state were found to contain 71.56 to 85.26 μg of copper per 100 ml. Out of these 4 samples were having the blood copper below normal values. Similarly 60% of the 80 blood samples collected from animals in Assam had low copper contents, indicating sub-acute type of deficiency. About 50% calves on a farm were found to have sub-acute copper deficiency status (Anon, 1958-59).

Kumar (1960) analysed the blood of 27 male and 27 female calves and 25 cows for their copper content. He reported the range of blood copper contents to be 87 to 113 and 91 to 107 μg per 100 ml in the blood of male and female calves respectively. The blood copper level ranged in lactating and pregnant cows from 77 to 116 and 93 to 107 μg per 100 ml respectively. He concluded that copper content of the blood of calves of various age groups did not reveal any significant difference nor did they differ according to sex-wise. Dwarakanath *et al* (1962) showed the increase in blood copper levels of hypocupremic cows by supplementation with dietary copper. This observation was later confirmed by Agarwal and Majumdar (1963).

Reddy (1966) reported that the copper supplementation increased significantly within a month the whole blood copper content, which showed a decline later on. The blood copper concentration of the animals after 1, 2 and 3.5 months after copper supplementation for the supplemental group were 90.1 ± 1.7 , 104.5 ± 4.0 , 99.9 ± 4.4 whereas that of control group

were 62.0 ± 5.0 , 91.4 ± 4.2 and 86.3 ± 4.9 μg per 100 ml, respectively. Copper supplementation resulted in an increase of red blood cell count, haemoglobin and white blood cell counts significantly. However, Gupta (1968) observed no significant difference in haemoglobin, R.B.C., and W.B.C. counts in the blood of either cow calves or buffalo calves by supplementing their feed with trace mineral mixture containing copper, cobalt and iodine.

Kapoor and Mahadevan (1970a) observed a highly significant co-efficient of correlation between total copper in plasma and caeruloplasmin copper in the bovine blood.

6. Copper and milk:

The copper content of milk, as it is secreted by the animal is influenced by species, stage of lactation and the level and form of copper intake by the animal. Individual variations within the species exists for the reason unknown, but much of the individual variations can be attributed to faulty analytical methods and substantial contamination of the metal during milking, sampling, handling and processing (Davis, 1933 and Mulder et al 1964).

Presence of traces of metal compounds in milk is of fair importance from several points of view. Besides their physiological importance in nutrition, they are important for the activity and growth of micro-organisms in curd and cheese. They are also important in chemical processes as catalyst during processing, ripening and storage of dairy products.

The milk of rats appears to be unique in that it has the highest copper content compared to the milk of other mammals examined so far. Cox and Muller (1937) reported the copper content of milk of rat to be 7 mg per litre of pooled samples taken from rats at four different stages of lactation, whereas cow's milk contained only 0.6 mg copper per litre of milk similarly obtained and analyzed. The quoted figures of human milk is 0.5 mg copper per litre.

Even though there is no variation during one milking or between morning and evening milking, definite variation do exist during lactation. In all species colostrum is significantly richer in copper and in most cases there is a progressive fall throughout the lactation. According to Moulder et al (1964) the published figures from 1920 onwards are very highly variable and most unreliable due to high copper contamination. Hence, it is difficult to compare the results. However Beck (1941) found the copper content of the milk of normal ewes to vary from 0.30 to 0.64 mg per litre in early lactation to 0.04 to 0.16 mg per litre several months later. Similar fall of copper content was observed in the milk of cow during lactation by Mulder and Koppejan (1953). They also observed a tendency of the copper content of milk to increase at first for a period of 7 days of lactation and then to decrease in a later stage upto 6 weeks. The general trend observed was that colostrum contained about 100 μ g of copper per litre, which upto 7 days then increased to 200 μ g of copper per litre of milk and again decreased as the lactation advanced. After one month the copper

content is mostly between 40 to 80 μg per litre, which decreases to a level of 20 to 40 μg copper per litre and then onwards remains steady. Broek and Wolff (1935) were the first to give reliable figures for the copper content of cow's milk at three different stages of lactation. Recently King and Dunkley (1959) and King and Williams (1963) confirmed the earlier findings.

Broek and Wolff (1935) measured the copper content of cow's milk along with other trace elements. They found that neither feed nor season influenced the copper content of milk, which varied from 15 to 51 μg of copper per litre of milk. Similar findings were made by Kohler *et al.* (1935). Krauss and Washburn (1936) studied over a period of one year the copper and iron contents and the anaemia producing properties of mixed Jersey and Shorthorn milk and confirmed that it neither varied appreciably with the season nor with feed, even though the copper and iron intake of cows during stall feeding was about three times that when they were on pasture. The copper content of milk was shown to be 140 to 190 μg per litre.

Elvehjem *et al.* (1929) found the copper content of cow's milk to be about 100 μg per litre under normal feeding. After the copper content of the feed of cows was increased by 5 folds, no increase in the copper content of milk was found. Broke (1935) reported a feeding experiment in which cows received 3.0 or 5.0 g of copper sulphate per day per head. The natural copper content of the milk before the supplementation was 32 μg per litre and was about 35 μg per litre after 7 weeks. The copper content of the milk had not changed. He also reported that

there was no change in the copper content of milk when the cows were turned to pasture from stall feeding and vice-versa.

Kiermeier and Steger (1952) found a statistically significant dependence of the copper content of milk on the acidity of the soil on which the milk was produced. The copper content of the milk from an acid soil was about 47% lower than that of the milk from neutral or alkaline soil.

King and Dunkley (1959) observed no difference between pasture and dry feeding in the natural copper content in the resulting milk. They reported natural copper content of cow's colostrum to be 200 μg per litre and that for milk to be 20 to 40 μg per litre.

Mulder et al (1954) observed no effect of the feed on the copper content of milk. One of the authors added such quantities of copper sulphate to the feed of the cows that they showed unmistakable symptoms of copper poisoning. Even then the copper content of milk, however, remained unchanged. And also according to previous findings of Mulder and Koppejan (1953) cows when changed from pasture to stall feeding or vice-versa, the copper content of milk was not affected. By feeding copper, the deficiency symptoms disappeared in the cows when they were fed 2 gm copper sulphate per head per day. But the copper content of milk did not increase. On the other hand, deficient intake of copper were reflected in sub-normal levels in the milk of cows and ewes as shown by Beck (1941). He found the copper content of milk of cows and ewes grazing on copper deficient pasture containing 1 to 3 ppm copper on dry basis, fell to as low level as 10 to 20 μg per litre.

Contrary to the above findings, King and Dunkley (1959) observed that when the cows were drenched with large amounts of copper sulphate (10 gm) there was an increase of variable extent in the copper content of the milk, which ranged between 10 - 15 to 70 μg per litre of milk.

Dunkley *et al* (1964) reported that estrus cycle of cows had no effect on the natural copper content of milk.

Copper content of ewes' milk as reported by Thomas (1937); Bennetts and Chapman (1937); and Crimmer (1948) ranged from 40 to 64 μg per litre and that of goats' milk ranged from 150 to 900 μg per litre of milk, as reported by Grondel (1930), Renny (1932); and Maeno and Ardate (1951), which is very high as compared to that of cow or ewe's milk.

Significant differences were observed by Dunkley *et al* (1960) in the copper contents of milk produced by the cows fed rations containing alfalfa hay and oats hay, as the roughages. This was due to higher dietary copper provided by the alfalfa hay ration, which was 2.5 times that of hay ration. Dunkley *et al* (1963) reported that the natural copper content of milk increased for atleast 4 weeks as a result of administering 300 mg of copper as copper glycinate sub-cutaneously. Significant increase in the copper contents of milk was observed by feeding supplements of copper such as copper sulphate ($P < 0.05$) and copper EDTA ($P < 0.01$). Copper content of milk ranged from 40 to 60 μg per litre.

Melnikova and Ilyukhina (1969) showed that there was little increase in mean daily milk yield but not the fat percentage by

the supplementation of the ration of Black Pied cows with trace mineral mixture consisting of copper, cobalt, zinc, manganese and iodine. Babin (1967) observed an increase in milk yield by supplementing with 75 mg of copper sulphate per day of the ration of the animals fed on feeds from the soil deficient in copper, zinc, manganese, cobalt and iodine. The milk yield per lactation of the cows of supplemented and control groups were 2,692 and 2,455 kg, respectively. Shatokhina (1968) studied the effect of copper and cobalt supplementation of the ration of cows on the yield and composition of milk and found that there was no significant difference either in the milk yield or the fat percentage. But a slight increase in the protein content of milk was observed.

Zamarin *et al* (1962) reported the copper content of milk of cows grazing near a copper ore works to be 0.44 to 0.66 mg per litre, and that of cows located in other areas of the same country to be 0.41 mg per litre.

Barysheva (1966) found an increase in the milk yield, but not the fat percentage after supplementation of the feed of the milch cows with trace mineral mixture containing copper, zinc and molybdenum. Similarly, Pope (1966) studied the effect of copper sulphate supplementation of the feed of a group of seven cows on a farm, where deficiency of trace elements was thought to be the cause of abnormally low milk fat content and found that copper supplementation had no effect on either the milk production and fat percentage or the copper content of the milk produced.

The copper supplementation was found to increase the butter fat percentage within 3 days and the increase being significant ($P < 0.05$) after 18 days of supplementation. The increase in butter fat percentage was temporary and the same was not significant after 1 month of supplementation. However, the total milk yield of the animals were unaffected by copper supplementation (Reddy and Mahadevan, 1956).

Mirolyubov (1973) studied the mean daily milk yield, fat and protein contents of milk, before and after supplementation of the ration of Russian Black Pied cows with copper sulphate along with an enzyme preparation "Glyukavamin P 10 Kh" during the 5th and 6th months of lactation and observed that the supplementation helped in the maintenance of milk yield as well as fat and protein percentages.

7. Symptoms of copper deficiency:

Copper deficiency symptoms as described by various workers are mainly of two types, namely, severe or acute and mild or sub-acute in nature. In general, ruminants are reported to develop copper deficiency much more readily than horses and swine. The copper deficiency diseases in animals are classified as follows:

- (1) Absolute copper deficiency in feed or uncomplicated copper deficiency (Underwood, 1962)
- (2) Conditioned or physiological copper deficiency, not due to lack of copper in feed, but due to inefficient utilisation of copper by other interfering dietary factors having associative effects by or complicated copper deficiency (Abdellatif, 1968)

Copper deficiency is a regional disease and symptoms shown by the affected animals, as well as the name of the disease varies from region to region and species to species of animals. The terminology of the disease either due to copper deficiency or molybdenum and sulphate interference varied in different places. For instance in Australia it is known as

Ataxia,
Neonatal ataxia and Enzootic ataxia in lambs
(Bennetts & Chapman, 1937)

Falling disease in cattle
(Bennetts, 1939) and

Coast disease in sheep of south Australia

In New Zealand it is called as Feet scours in cattle
(Cunningham, 1944)

and in Holland, Scouring disease in cattle and Lechauchf in sheep and cattle
(Sjollem, 1933)

In England it is known as Sway-back or swing back in calves (Inns & Shearer, 1940), Teart, Wart in sheep and cattle
(Ferguson, *et al.*, 1938).

It is termed as Salt sick in cattle (Neal *et al.*, 1931) in Florida (USA)

A Wide varieties of symptoms have been associated with dietary deficiency of copper or the disease which responds to the copper therapy (Davis, 1951). They include anamia, depressed or stunted growth, loss of condition, deprived appetite, a characteristic pained and tucked up appearance and lethargic when driven (Bennetts *et al.*, 1941). Rough and faded hair coat, depigmentation of hair or wool, slow keratinization and abnormal

wool growth are also seen. Bone disorders with split or broken and fragile bones, particularly pelvis and rib bones are the common sites. Fibrosis of myocardium and heart failure are also seen. Gastro-intestinal disturbances and diarrhoea may vary from intermittent to severe are noticed (Becker et al 1953). Impaired reproductive performance, due to suppressed oestrus, sub-normal blood and liver copper levels and copper-containing enzymes, low haemoglobin levels, microcytic hypochromic anaemia, have been observed.

In young animals, beading of ribs and enlargement of the ends (epiphysis) of the lung bones occurs. Young calves may have straight postures and stand forward on their toes. Stunted growth and 'Goggled eye' are symptoms of copper deficiency in growing calves. General debility leading to sudden fall and death after little or no struggle, has been reported. The deficiency symptoms are marked after the weaning stage. Haemoglobin levels fall to 6 gm or below per 100 millilitres.

In sub-acute deficiency the animals remain in debilitated condition, appetite is fair and slight fading of body coat takes place. In females the oestrus cycle may be irregular and conception rate may be low. In males there is a destruction of germinal tissue of the testicles, which may be irreparable. Although only minute traces of copper are required, a deficiency of copper in feed and fodder and factor interfering in its assimilation will produce clinical symptoms of dietary deficiency, as mentioned by Allman and Hamilton (1958). Apart from the above mentioned symptoms, there may be the muscular

incoordination, pica, dead or weak off-spring, decreased or cessation of growth. The extent to which one or more these dysfunctions are revealed depends upon the species of animal and its age, and also upon the environmental factors and the severity and duration of the copper deficiency, whether it is uncomplicated or conditioned by other dietary factors (Underwood, 1962).

Dunlop and Wells (1938) investigated the disease known as 'sweeney' or 'swing back', 'warf' and 'Gingin rickets', which are widely distributed throughout Great Britain. They described the disease in North Derbyshire as being confined almost exclusively to lambs during the first month of life. Most were affected at birth and were unable to raise themselves to obtain milk from their dams. Others sway about in a peculiar manner and collapse or turn somersault, if they attempt to raise or walk. This was shown to be due to low copper level in the animals' body caused by interfering factors such as high amounts of lead, iron, calcium and zinc, but not due to the deficiency of dietary copper in pastures, which contained 12 to 27 ppm of copper.

The scouring disease on 'taart pastures' in cattle and sheep in Britain was found to be due to high molybdenum content, which ranged from 120 to 130 ppm while copper content of these pastures were 11 to 18 ppm. The scouring disease in cattle was also due to calcium deficiency. Pasture was not deficient in copper and also the molybdenum content was not abnormal, but it was found to be low in magnesium content.

However, the copper supplementation of the diet in all the above conditions mitigated the copper deficiency condition resulted either from low dietary intake or induced by interfering factors such as molybdenum, sulphate, heavy metals or even very high calcium and phosphorus ratio.

Subnormal levels of copper both in blood and liver and also occurrence of a whole range of copper deficiency symptoms were reported by Beck (1941) in both sheep and cattle grazing on pastures containing 3 to 4 ppm or less of copper and less than 1.5 ppm of molybdenum.

Despite the wide-spread use of copper licks, sway-back was found to prevail in North Derbyshire. Hunder, Eden and Green (1945) demonstrated the importance of ingestion by the ewes of the required amount of copper and indicated that some factors in the natural herbage other than the quantity of copper is responsible for the low copper status of grazing ewes.

Russel and Duncan (1956) observed a disease known as 'Enzootic ataxia' associated with anaemia and also stringiness of wool and attributed these to uncomplicated copper deficiency status in ewes and lambs, grazing on pasture containing less than 5 ppm copper. Sudden death and falling disease in Australia were seen in animals grazing on pastures containing less than 2 ppm copper. Similarly, in Europe licking disease in cattle, sheep and goat was found to be due deficiency of copper in pastures containing less than 5 ppm of copper.

Allcroft and Parker (1949) described hypocupraemia as a condition characterised by diarrhoea, low fertility, poor milk production and stunted growth. Cunningham (1950) reported the change of coat colour of red animals to be bleached to yellowish-red. The hair coat in adult cattle was found to be rough and staining and the colour of black animals to become tinged with dull red or grey colour and specially ground around the eyes. Scouring was particularly bad in animals fed on the bush pasture of spring and to a lesser state when the rains come in autumn. Marston (1952) listed specific coat changes in which the hair lost its capacity to produce pigment and became harsh and dishevelled. In mature cattle, heart failure may result due to atrophic lesions and fibrosis of myocardium.

Lately, a genetic influence on copper metabolism in sheep has been shown by Wiener *et al* (1968, 1969). In a flock, the level of copper in blood and liver as well as the susceptibility of new born lambs to sway back were related significantly to breed. Anke (1967) proposed a similar method of detecting copper deficiency in dairy cows. He proposed that 7 ppm copper in black hair is a border-line level for copper intake index. But Chauvaux *et al* (1965) concluded, after examining 535 hair samples from 31 farms, that copper levels in the hair were not effective for the diagnostic purposes. Seekles and Claessens (1967) indicated that copper content of the hair may be influenced by dietary copper intake. They also reported that positive correlation existed between the levels of blood and hair copper levels. The copper content

of the hair was found to be influenced by pregnancy (Anke, 1965) and hair colour (O'Mary, 1969). Binot *et al* (1970) found that the copper contents of hair and blood in cattle varied directly with the copper content of forage and inversely with the amount of iron and sulphate content of forage. Anke (*loc cit*) obtained a positive correlation between the molybdenum content of the pasture and the black hair of the cattle and reported that symptoms of molybdenosis resulted when molybdenum content of hair exceed 1 ppm.

8. Copper, blood formation and anaemia:

The well established need of copper in the diets of farm animals and its value in the prevention of certain nutritional diseases and the historical findings of Hart *et al* (1928) have stimulated the interest in the subject. Hart and co-workers established that copper had a definite function in the utilization of iron for the formation of haemoglobin. McHargue in the same year supported Hart's findings. In the absence of copper, dietary iron is absorbed and deposited in the liver or elsewhere but normal regeneration of blood does not occur, resulting in anaemia, which is to some extent is a frequent manifestation of copper deficiency in all species. When copper is added to the diet, haemoglobin synthesis is resumed.

The exact manner in which the copper plays its role and its absence affects haemoglobin formation is not yet completely understood. Present evidences suggest that a certain minimum supply of copper to the bone marrow is necessary for

the production and maturation of red cells, that have a normal survival time in the circulation and also for the synthesis of the haem-moiety of the haemoglobin.

The classical work of Hart *et al.* (1929) at Winconsin showed that nutritional anaemia could be produced by the exclusively feeding of milk, established beyond doubt that copper in addition to iron is essential for blood formation, i.e., haemoglobin and red blood cells. The development of the work on milk anaemia and the validity of these claims have been fully discussed by Elvehjem and Hart (1939). They have found out the relation of iron and copper to haemoglobin synthesis in the chick. It is probable that the role of copper in the formation of blood is secondary to its effect on cells. Schultze and his associates (1935) suggested the catalytic action of copper in haematopoiesis following their work on young swine. They suggested that rapid and continuous haematopoiesis cannot take place unless the copper content of the blood is maintained above a certain minimum level. Later this level has been suggested to be about 20 μ g per 100 ml of blood in pigs, (Lahey *et al.*, 1952). In sheep a level of 10 to 12 μ g copper per 100 ml of blood has been found to limit blood formation (Beck, 1941; and Marston *et al.*, 1948). If such low level is maintained for long time, anaemia occurs and death follows. Rats, pigs and dogs placed on a milk plus iron diet, quickly become anaemic due to the rapid fall in blood copper below the limiting levels.

The nature of anaemia caused by copper deficiency varies with the species. In cattle and ewes, it was found to result in hypochromic and macrocytic types of anaemia (Bennetts & Beck, 1942; Cunningham, 1946; and McDonald, 1947). Bennetts and Beck (1942) also showed that in lambs it is hypochromic and microcytic anaemia. Barer and Fowler (1937) found that some reticulocyte response occurred in milk anaemia on feeding copper alone (but not iron alone), which indicated that copper acts as catalyst both for the incorporation of iron into haemoglobin and for erythrogenesis and is not restricted especially to either function alone. Further evidence that copper is concerned in the erythrocyte maturation process come from the observation that copper anaemic pigs and dogs contained a lower percentage of reticulocytes in their blood than those with iron (Lehey et al, 1952). As well, Smith and Medlicott (1944) showed that addition of copper to the diet of rats, rabbits and pigs suffering from anaemia due to combined iron and copper deficiencies showed marked and persistent reticulocyte response, whereas iron has little effect on reticulocyte. Kumaoni bullocks fed on copper deficient diet had low erythrocyte count and showed a tendency towards development of what may be classified as macrocytic and hyperchromic type of anaemia (Anonym, 1946-47).

Schultz (1939, 1941) showed for the first time that there was a marked loss of cytochrome oxidase in the tissue (bone and liver) of the copper deficient rat, pig and chick. His findings later were supported by Gubler et al (1957),

who indicated that copper was especially concerned in the synthesis of the prosthetic group of cytochrome oxidase and that this must be regarded as one of the basic function of copper, unrelated to iron metabolism. Recently, Mills and Dalgarno (1970) and Poole (1970) also showed that the liver cytochrome oxidase activity decreased during copper deficiency and suggested that this could serve as a copper status of the animal.

Rush and associates (1956) observed that the survival time of the erythrocytes is shorter in copper deficient animals than normal ones. They suggested that copper was an essential component of adult λ red blood cell and that certain minimum of copper must be available both for their production and maintenance of their integrity in the circulation. Decrease in the absorption of iron from gastro-intestinal tract was also claimed by them under copper deficiency.

9. Influence of copper on iron metabolism:

Even though the amount of iron in the body is very small, its functions are very vital. The chromatic substance of the nucleus of every cell is an iron protein compound. The haemoglobin constitutes the outstanding material of the red blood cells and has the most important function of transport of oxygen to the nook and corner of the body and assisting in the maintenance of neutrality. Iron constitutes nearly one third of the haemoglobin. Hogen and Nicrman (1927) showed that about 0.004 percent, by weight of animal body consists of this element.

It is also an indispensable constituent of enzymatic oxidation system (Linton, 1950). Iron alone or in combination with other trace elements such as copper, cobalt, etc., prevented the occurrence of anaemia in growing animals (Krauss, 1929; Supplee *et al* 1930; Knoop *et al*, 1935, and Thomas and Okamoto, 1953).

Cunningham (1931) and Joseph (1932) did not observe any significant difference between the total body iron content of rats fed on milk diet plus iron and that of those on milk diet plus iron and copper. Later in 1935, Elvehjem reviewed the literature with regards to functions of copper in the absorption, utilization of iron and concluded that it is not concerned with the assimilation of iron except for the transformation of the ingested iron into haemoglobin in the animals. Five years later, Schultze (1940) concluded that copper is not necessary for the absorption and storage of iron in tissues, but it facilitates or is necessary for the utilization of iron by the blood forming organs and for the mobilisation of iron from the tissues. Marston and Allen (1967) observed that the amount of radioactive iron (Fe^{59}) absorbed was favoured by amounts of dietary copper upto levels of 0.25 to 0.50 μ g per rat per day. Above this amount, there was no greater iron absorption and on the other hand a some what reduction in the absorption of iron was observed. The influence of copper on the absorption of iron is not due to the simultaneous administration of copper with iron but appears to be correlated with the level of copper in the tissues. One of the specific effects of copper on iron metabolism appears to rest in the synthesis of the 'heme'

(iron-porphyrin), which is the prosthetic group of haemoglobin, cytochromes and other important biochemical compounds containing iron. They postulated that copper was required for the release of iron from its storage sites in the liver.

On the other hand, Houk *et al* (1946) found that addition of copper to copper deficient diets increased the dietary iron utilisation from about 30 to 70% as measured by the total body iron content of rats. Later, Chase and associates (1952) employing radio-iron observed that copper supplementation increased the iron absorption in rats and also the absorption of iron from gastro-intestinal tract was markedly reduced in copper deficient pigs. It was shown by Gubler *et al* (1952) that the deficiency of copper impaired the ability to mobilize iron from the tissues and to utilize iron in haemoglobin synthesis. Further more, from the subsequent studies of Bush *et al* (1956) and Jensen *et al* (1958) it became apparent that the transport and mobilization of iron was not impaired in copper deficient pigs as thought by the earlier workers. However, they still maintained that copper deficient pigs had a reduced ability to absorb iron from the intestinal tract.

Studies on the parallel lines as in rats and pigs have not been conducted with ruminant and hence little is known of copper - iron inter-relation in these species. On the other hand, the heavy deposits of iron that occur in the liver and other tissues of cattle and sheep confined to the pastures or areas which are deficient in copper suggested that copper does not markedly influence the absorption of iron in ruminants (Marston *et al*, 1948). Recently, as reported by Underwood

(1966) copper is apparently not directly involved in iron absorption, mobilization and transport to the bone marrow, as claimed by earlier workers (Matrone, 1960; Underwood, 1962). He further claimed that haemoglobin synthesis was blocked in copper deficient animals, so that absorbed iron could only enter the tissue iron reservoirs which in time causes a 'mucosal block', thereby decreasing iron absorption. Earlier, he felt that copper was apparently not necessary for proto-porphyrin synthesis, since normal or higher than normal amounts of free proto-porphyrin was found in the mature erythrocytes of copper deficient animals. Also there was no clear evidence that copper was concerned in the incorporation of iron into proto-porphyrin to form heme. The evidence so far available are therefore, not adequate to implicate copper unquestionably with any stage in the biosynthesis of haemoglobin. Therefore, the anaemia of copper deficiency could be explained for the time being, on the basis of impaired erythrocyte maturation and a reduced survival time of the mature erythrocytes produced. It should be emphasised nevertheless, that any factor such as copper that limits erythropoiesis also limits haematopoiesis, because haemoglobin is confined to the red blood cells and the haemoglobin content of the single cell cannot be increased beyond certain normal limit.

The studies of Standish *et al* (1969) demonstrated that excessive levels of dietary iron depressed liver copper in cattle, whereas Chapman and Kidder (1964) indicated that iron accumulated in the liver when copper was deficient. Rats fed

on copper deficient diet accumulated excessive liver iron, while those fed on iron deficient diet stored high levels of copper in their livers (Sourkes *et al.*, 1968).

Pal *et al.* (1970) supplemented the ration with copper of growing haryana calves and did not observe any appreciable difference in the total red blood cell count, haemoglobin, packed cell volume and serum calcium and phosphorus. However, copper and cobalt mixed supplementation increased the white blood cell count. The increased white blood cell count was supposed to have resulted from the increased caeruloplasmin copper and low free reacting copper.

10. Copper, Enzymes and Vitamins:

The only common characteristic which all the traces elements have is their capacity to function in very minute quantities in the body. It is in this capacity that they must act as catalysts involved in enzymes, vitamin, hormone systems, either as constituent parts of the molecules of these or as enzyme activators. In fact, as Green (1941) suggested 30 years ago that 'enzymic catalysis is the only rational explanation of how a trace of some substance can produce profound biological effects'. Many examples of trace elements functioning in these ways are now known. Copper and iron are known to be irreplaceable compounds of the molecule of several oxidative enzymes. Cobalt has also been shown to be an integral part of vitamin B₁₂. These findings have little doubt that these trace elements function as activators or as catalysts in the

living cell, and that they lie at the root of living process. The metal ions, which include many of the trace elements, appear to act in two different ways in enzyme systems. They may be an indispensable part of the protein from which they can only be dissociated with difficulty viz., iron complex of the 'heme-protein' and the cobaltic complex of vitamin B₁₂. In these cases, it would be better, as Williams (1953) has suggested, to describe the function of metal ions, which are highly specific as one of ^{the} activators rather than that of catalysts of an enzymatic reaction. In these cases, ions are readily dialyzable from the enzyme, after which they become less active. The activating metal is loosely bound and can get readily lost during processing.

As described by Bowen (1966) the enzymes containing copper come under metallo-enzymes and due to their better defined chemical nature, they have been more intensively studied than metal activated enzymes. They are numerous in number and many more probably await discovery. Metallo-enzymes are divided into metallo-protein, metallo-prophyrin and metallo-flavin enzymes. Metallo-protein enzymes, which contain copper, are phenol oxidase, tyrosinase, urichase, laccase, mono-amine oxidase, ascorbic acid oxidase, ceruloplasmin, galactose oxidase, β -trans-sulphurase and benzylamine oxidase. The metal ions are often the catalysts for the reactions they take part in. For example, cupric ions catalyse the oxidation of phenols and ascorbic acid. Metallo-porphyrin enzyme containing copper is cytochrome oxidase having one atom each of copper and iron

with one protohaem as porphyrin, which is most important in the haemoglobin formation (Mahler, 1961). Cytochrome oxidase is reported to be reduced in the brain tissues of lambs with 'ataxia' due to 'conditioned' copper deficiency (Mills and Quarterman, 1963). Metallo-flavin enzymes containing copper atom are hyponitrite reductase, nitrous oxide reductase, and nitrite reductase type II (Mahler, 1961).

Furthermore, Owen (1963) listed the metallo-proteins, other than enzymes. The copper containing metallo-proteins are haemocyanin, cytoecyanin, erythrocuprin, hepatocuprin, cerebrocuprin, milk copper-protein etc. Cytoecyanin and haemocyanin are known as respiratory pigments. Therefore, the importance of copper in enzyme system is well understood from the above information. Hence its deficiency is bound to affect the normal physiological body processes.

Second only to the haemoglobin formation, is the role of copper in the activation of oxidation-reduction enzymes. Schultz (1939, 1941) from a series of experiments demonstrated that a high cytochrome activity of bone marrow was intimately associated with haematopoiesis. In copper deficiency, cytochrome oxidase activity of bone marrow was lowered. Upon starting the copper therapy, there was a rapid response, which reached a maximum in about 24 hours. A similar observation was made on the cytochrome oxidase activity of the liver and heart. Further, studies of Schultz and Juitzen (1941) suggested that the catalase activity of liver, kidney and blood was greatly influenced by copper and was found to be

decreased with copper deficiency and rapidly increased with copper therapy. Tyrosinase, associated with pigment formation is an enzyme activated by copper and deficiency of copper was shown to be exhibited through this by depigmentation of hair and wool.

Dawson (1950) reported that copper was concentrated within a limited area of its site of action in the body and was firmly bound to a specific or a type of protein of the enzyme. Lerner (1950) showed that certain compounds which combine with copper inhibit tyrosinase activity. The failure of pigmentation in copper deficiency resulted from a breakdown in the conversion of tyrosine to melanin, since that conversion is catalysed by copper-containing poly-phenyl oxidases (Raper, 1928).

Mahler (1953) identified butyl GSA dehydrogenase as a cupro-flavo-protein in which copper occurs as a part of prosthetic group. The activities of this enzyme was found to be dependent on the copper they contain. The mode of its binding with the protein, the way in which it functions and also takes part in the oxidative process in the intact living cell are not yet fully understood.

Relation of copper to enzyme activity is complicated by other deficiencies. In a comprehensive study of enzyme activities in copper deficient rats, Gallagher (1957) found that anaemia developed after ten weeks of starting the experiment and death occurred after fifteen weeks. Fourteen different enzymes were assayed, of which only cytochrome oxidase, the terminal enzyme of biological oxidation, showed a marked

decline as a result of copper deficiency. It was of interest that addition of copper salts *in vitro* did not restore the cytochrome oxidase activity, while normal liver cytochrome activity was ^{re}stored by feeding copper for ten days.

Copper had a role in the synthesis of iron containing heme-prosthetic group of cytochrome oxidase and D.P.N. cytochrome-c-reductase, a flavoprotein which contains iron, but not in heme form. The activities of these enzymes were not affected due to copper deficiency. In case of copper deficiency, it appeared that synthesis of the heme-prosthetic group of cytochrome oxidase was affected earlier, while that of haemoglobin somewhat later, and the catalase was found to be least sensitive. The exact mechanism of the action of copper in the synthesis of the iron porphyrin is not known (Mahler, 1958).

an indication that the copper is tied up with a vitamin such as pantothenic acid and ascorbic acid was observed by Singer (1949) and Singer and Davis (1950). This concept was somewhat expanded by the work of Hundley and Ing (1950), who suggested that pantothenic acid deficiency actually produced grey hair by blocking utilisation of copper in hair growth and melanin formation.

11. Copper and bone formation:

Changes in bones and deformities of bone have been reported to be very common in pigs, dogs, foals and chicks (Lahey et al, 1952; Teague and Carpenter, 1951; Bennetts,

1932; and Gallagher, 1957), but not in laboratory animals. A less incidence of spontaneous bone fracture was observed in cattle and sheep grazing on copper-deficient pastures in some areas (Bennetts and Beck, 1942; Cunningham, 1950; and Davis, 1950). The fractured bones are almost normal in appearance except for the osteoporosis and brittleness. Detailed study of the bones of the copper deficient dogs and pigs revealed a marked failure of deposition of bone in the cartilage matrix, accompanied by normal growth of cartilage. The bones were characterised by abnormally thin cortices, deficient trabeculae and wide epiphyses together with normal ash, calcium, phosphorus and CO₂ contents. These changes were distinct from those seen in rickets. It was suggested that studies of the levels of cytochrome oxidase or other copper enzymes in the ossification process may reveal the role of copper in bone formation (Underwood, 1962).

Copper is apparently necessary for the normal development of bone (Davis, 1950, 1951). Marston (1952), reported that in copper deficient areas, normal bone metabolism was inhibited and the cattle were unable to form bones properly without copper supplementation, with the result, that a condition 'very similar to ricket' was seen in young calves, whereas osteoporosis and rarification of bones resulted in older animals. In Australia and New Zealand, the bones of calves and lambs were found to have a tendency to fracture easily due to copper deficiency (Bennetts, 1932; Bennetts *et al.*, 1942; and Cunningham, 1950).

12. Copper in relation to hair and wool:

One of the most striking effects of the deficiency of copper is the loss of pigmentation in the hair coat. The achromotrichia of the hair is the earliest and most sensitive index of copper deficiency in all the animal species, except the pig. A related effect, apparently, is the interference with normal wool formation in sheep with loss of crimp, defective keratinization, lack of pigment production in black-wooled sheep and production of so-called 'steely or stringy wool' (Lee, 1951). This depigmentation during deficiency and pigmentation on an adequate copper level responds so quickly that, pigmented and depigmented bands in same fleeces are seen (Gorter, 1935; and McCollum, 1939). Even on fairly high copper intakes the functioning of copper in pigmentation processes can be blocked within two days by raising the molybdenum and inorganic sulphate intakes sufficiently (Dick, 1952, 1953 and 1954). The action of copper in the skin has been repeatedly emphasised and apparently this is one of the main sites of copper action. (Ferguson, 1943; Davis, *et al*, 1946; Hundley, 1950; Connor *et al*, 1948, 1949; Singer, 1949; Marston, 1950, 1952; and Davis, 1951).

According to Smith and Ellis (1947), the achromotrichia, alopecia and dermatosis syndrome is a more sensitive index of copper deficiency in rabbits as well as sheep, rather than the anaemia. The biochemical mechanism involving copper in the pigmentation process has not been defined. Raper (1928) suggested that breakdown in the conversion of tyrosin to

melanin may occur, since this is known to be catalysed by copper containing poly-phenol oxidases. Bennetts (1932) and Marston *et al* (1938) observed decline in quality and quantity of wool in copper-deficient sheep. An increase in quantity and improvement in quality was noticed by Marston and Lee (1948). This was attributed to the improved keratinization after copper supplementation. The abnormalities described were more obvious in the wool of breeds that are heavily crimped such as Merinos of Australia. However, they were demonstrated experimentally in Great Britain breeds of sheep (Lee, 1956). This type of lesions were not seen on copper-deficient pasture, other than those in Australia. These observations indicated that some other dietary factors in addition to copper may be involved and responsible for such abnormalities in that particular environment, where the lesions occur. Utilization of copper in hair growth and melanin formation was suggested to be blocked due to the deficiency of pantothenic acid (Hendley and Ing, 1950).

The copper content of hair and wool was determined by Kikkawa *et al* (1955), who found that it was significantly higher in those of pigmented (black) hair than those of white hair. However, Goss and Green (1955) were unable to confirm this finding. They examined black and white hairs of healthy animals of ten different species and reported 10 to 47 ppm of copper in the washed dry hairs. No relationship between pigmentation and copper concentration was observed. However a decrease in copper content was correlated with an increase

even though the other typical deficiency symptoms were noticed (Davis, 1950). Moreover, sheep and horses grazing on similar pastures did not scour. Laboratory animals, dogs and pigs did not develop diarrhoea even under severe copper deficiency status. However, scouring was reported in goats maintained on Dutch pastures that induced scouring in cattle (Underwood, 1966).

Molybdenum level in pastures was suggested to be the factor concerned in 'peat scours' (Cunningham, 1950). However, other dietary factors may also be involved, apart from deficiency of copper and higher levels of molybdenum in the pastures.

14. Copper, Molybdenum and sulphate inter-relationship:

Interest in the metabolic significance of molybdenum arose first from the investigations on the severe scouring disease of cattle, known as 'teart' in England, caused by excessive intake of molybdenum from the herbage by the cattle. This could be prevented or controlled by the treatment of animals with massive doses of copper supplements (Ferguson *et al.*, 1938, 1943). Dick and Bull (1945) showed that copper assimilation and retention in sheep and cattle could be limited by molybdenum. The interaction between these two elements and the inorganic sulphate is of great complexity, in which other elements and nutrients are reported to be involved. The quantitative relationships involved amongst these three constituents are yet to be fully understood. The inter-relationship between copper and molybdenum i.e., the toxicity due to a combination of excessive levels of molybdenum

and low levels of copper in forage was reported in Florida and California (Britton and Goss, 1946; and Davis, 1950).

Subsequently, peat scours of cattle in New Zealand was established as a molybdenosis, conditioned by a relative deficiency of copper (Cunningham, 1944). Simultaneous investigations on another problem of chronic copper poisoning of sheep in eastern Australia revealed further metabolic interrelations between molybdenum, copper and inorganic sulphates. In Great Britain, Jamieson and Russel (1946), Allcroft (1948, 1949) and Blakemore and Vearn (1950) reported deficiency of copper in animals maintained on field, which were found to contain sufficient levels of copper. This was attributed to high level of molybdenum in the herbage. Green (1951) did not observe any relationship between the levels of copper and molybdenum and hypocupremia. However, Dick (1952, 1953, 1954) showed that molybdenum exerted its limiting effect on copper retention in sheep only in the presence of inorganic sulphate and also neither molybdenum nor sulphate alone did interfere with copper retention. The effectiveness of either was found to be increased to a maximum as the intake of the other was increased. He further observed the molybdenum excretion in sheep to be profoundly affected by the dietary inorganic sulphate level. This finding was subsequently confirmed by Spais (1959).

Chronic copper poisoning associated with extremely high liver copper levels in sheep was observed under conditions of moderate copper intake and very low dietary levels of

molybdenum and sulphate. Conversely, depletion of animals' copper reserves, even to the extent of clinical copper deficiency was observed to take place after several months on normal copper and high molybdenum and sulphate intakes (Wynne, 1956). The administration of high doses of molybdenum (50 and 100 mg molybdenum per day as molybdate) to sheep, grazing on pasture with normal copper content, was found to decrease the copper concentration of the liver. However, a significant decrease in rate of depletion of liver copper, maintenance of significantly higher concentrations of copper in blood and precipitation of signs of severe copper deficiency were noticed in sheep grazing on copper-deficient pastures and receiving molybdenum supplementation. Dick (1952, 1954) demonstrated the characteristic lesions of copper deficiency in wool of sheep immediately after administering very high doses of both molybdenum and sulphate, despite the fact that liver copper was not depleted and blood copper levels were elevated above normal. These diverse and apparently conflicting observations were explained by Dick (1956) on the hypothesis that a membrane whose permeability to molybdenum is impeded by sulphate blocks copper transport.

Mills (1961) administered Cu^{64} intravenously to sheep and demonstrated that high intakes of molybdate and sulphate did not increase the rate of copper excretion. He suggested that these anions restrict copper utilization in sheep by depressing copper solubility within the digestive tract through precipitation as insoluble cupric sulphide. However, subsequently many drawbacks were noticed in this concept.

The other possibilities put-forth to explain the molybdenum induced metabolic disturbances involving copper were either inhibition of the activity of copper containing enzymes or the fixing of copper in an unavailable form in the tissues by the molybdeno-sulphur complexes (Underwood, 1962). There are indications that other dietary factors can exert a modifying influence on the inter-relationship between molybdenum and copper. Dick (1956) observed that high manganese intakes can block the limiting effect of molybdenum on retention of copper in sheep. He further showed that high protein diet with manganese and molybdenum together exert a severely limiting effect on copper retention.

The anti-molybdenum toxicity effect of copper has been repeatedly demonstrated in United States of America (Bennetts and Hall, 1939) and other parts of the world and it was found to be the only satisfactory method of overcoming the effect of high molybdenum intakes (Allertoft and Parker, 1949). It has been well demonstrated that higher intake of copper was necessary to prevent the toxic effect due to higher intake of molybdenum. With adequate copper and minimum molybdenum intake, the copper storage was found to be accentuated although the copper was not physiologically available to the animal.

Later studies were conducted by Vanderveen and Keener (1964) to evaluate the inter-relationship between these three constituents. It was observed that the heifers receiving diets low in copper and containing as much as 50 ppm of molybdenum and without added sulphate did not develop any

symptoms of molybdenum toxicity. Similar results were obtained with the diets containing 5 to 20 ppm molybdenum and 0.3% added sulphate sulphur. But the heifers receiving the diets containing 50 ppm molybdenum and 0.3% added sulphate sulphur developed severe symptoms of molybdenosis, which were alleviated by the supplementation of the diets with copper. Goodrich and Tillman (1966) obtained a lower body weight gains by increasing the sulphate sulphur level from 0.1 to 0.4 % of the diets, which contained 2 ppm molybdenum and 10 ppm copper. This did not occur, however, when the diets contained 3 ppm molybdenum. Liver copper was lowest when 0.4% sulphate sulphur was fed in combination with 3 ppm molybdenum. Working with sheep, Marcilless et al (1969) reported a response with 0.4% dietary sulphate as similar to that with the control diet as measured by liver copper accumulation, plasma clearance and urinary excretion of intravenously injected radio-active copper. The sulphate plus 50 ppm molybdenum, however, resulted in reduced liver copper accumulation, reduced ceruloplasmin formation, a delayed plasma clearance of the injected copper and increased urinary excretion of both stable and radio-active copper. Similar results were obtained with sulphate plus molybdenum (Suttle and Field, 1968; and Smith et al, 1968).

Hogan et al (1968) x from a study with sheep concluded that under conditions which facilitate excessive storage of liver copper, molybdenum plus sulphate depressed the storage of the same. Dowdy and Matrone (1968 and a & b) suggested the presence of copper-molybdenum complex, which renders copper

conditions. They are frequently ameliorated or accentuated i.e., 'conditioned' by other dietary factors peculiar to the conditions and environment where the disease occurs. Some of them have already been reviewed. Nevertheless it is apparent that dietary balance is no less important with regard to the trace elements than it is with other nutrients and the metabolic studies with these elements can be frustrating and misleading unless the nature of the whole diet is taken into account.

The possible relationship of copper to calcium and phosphorus metabolism was suggested due to the occurrence of fragile bones in copper deficient animals. This led to the investigations on this aspect of copper metabolism. Shirley *et al* (1964) demonstrated that high levels of molybdenum and low copper intakes by the cattle resulted in an interference with phosphorus metabolism, with an excessive excretion of the same. Davis *et al* (1947) in a study of alkaline blood phosphatase discovered that when the liver copper was depleted, there was a sudden rise in inorganic phosphorus and phosphatase activity of the blood. This was often rapidly followed by fractures of bones and even death of the animal, if copper therapy was not instituted promptly.

High intakes of zinc were reported to induce copper deficiency in rat, probably by reducing its absorption (Smith and Larson, 1946). Dynna and Harve (1961) observed a complex zinc-copper deficiency in both grazing and stall-fed cattle. The symptoms were typical of those expected for deficiencies

of both zinc and copper, and the condition was aggravated by the administration of zinc alone. A positive response was observed in cattle when supplemental zinc and copper were provided.

The interference of cobalt in copper absorption was observed by Jarston *et al* (1948). The Merino sheep grazing on the highly calcareous soils of South Australia, where herbage contained about 3 ppm copper, rapidly developed signs of copper deficiency when supplied with additional cobalt.

Bosman (1966) observed that intake of high digestible crude protein combined with a low starch equivalent increased the sulphide content of rumen liquor of grazing cows. This enhanced the copper sulphide formation resulting in poor absorption of copper. He suggested that high blood urea level associated with a surplus of organic sulphur in the rumen indicated the presence of high concentrations of sulphide in the rumen and serve as evidence of a copper deficiency in the animal. Increase of dietary protein resulted in reduced levels of liver copper in sheep, presumably by decreasing copper absorption. Soybean meal had a greater depressing effect on liver copper than did casein (Ammerman *et al*, 1963; McPherson and Henningway, 1965). Similar results were reported with swine by Combs *et al* (1966). Goodrich and Tillman (1966) obtained higher liver copper in sheep when urea served as the nitrogen supplement than with the purified soybean protein. High dietary protein intakes ameliorated toxic effects of

copper in rats and pigs (Wallace et al, 1960). Methionine also was found to be effective in this way.

Research of Hartmans and Bosman (1970) in Netherland, indicated that the availability of copper to the animal was more in the dry forages than in the fresh ones. Despite having a lower copper content, mature grass was more effective in maintaining liver copper level in cattle than young grass.

The continued ingestion of flourine at the level of 3 mg per kg live weight per day interfered with copper metabolism by increasing the minimum copper requirement to 133 percent (Anonym, 1946-47). Kaufman et al (1951) studied the relationship of copper to choline and found that choline either increased absorption and deposition of copper or else inhibited its excretion. Indications were there to show that carbohydrate metabolism may also be associated with copper absorption. The increased metabolic rate during pregnancy resulted in an increase of copper storage in both the dam and the foetus (Kozelka and Pendero, 1952).

16. Copper in relation to Reproduction:

The suppression of oestrus with low fertility and temporary sterility in cattle was associated with copper deficiency in several areas as reported by Bennetts and Hall (1939), Bennetts et al (1942, 1948), Russell (1944), Allcroft and Parker (1949) and Bennetts (1959).

A high incidence of infertility was reported by Bennetts et al (1948) to be associated with 'falling disease' in areas

where copper levels of the pastures were less than 3 ppm, which could be overcome by giving dietary copper supplements. Allcroft and Parker (1949) noticed low fertility in copper deficient cows and raised their blood copper levels by feeding copper sulphate supplement. Cunningham (1950) also considered that the reproductive capacity of cattle was reduced during copper deficiency condition. Hignett (1959) stated that hypocuprosis was sometimes, but not always, associated with reproductive disorders in female cattle. When fertility was affected, he found that administration of copper either orally or parenterally, usually resulted in a rapid and dramatic improvement in breeding performance. This was in agreement with the report of Van Rensburg (1961), but is not supported by the observations of Russell (1944), Cunningham (1950) and Bennetts (1959). Dutt and Mills (1960) in a preliminary study with rats fed on low copper diet, found that only one out of eighteen females produced a live litter and this rat immediately started to eat her young ones. Resorption of embryo was also observed. Eight rats receiving added copper at the rate of 2 ppm produced normal litters of 3 to 7 pups. However, no abnormality in the oestrous cycle of affected rats was noted. In South Africa, Van Rensburg (1961) recorded increased fertility in cows by the administration of copper.

Lower liver and blood copper levels were noticed in the dairy cattle given no supplements than compared with those given supplements of either copper alone or trace elements plus copper. However, differences were not observed in the growth, production

and reproductive performances of the animals of control or supplemented groups (Engel *et al*, 1964).

Donaldson *et al* (1964) studied the effect of copper and cobalt supplementation of the ration of Shorthorn heifers on their growth rate and fertility. A condition of ill-thriftiness was noticed in weaner cattle. A response was obtained by copper but not cobalt therapy. There was a significantly greater number of pregnancies in copper supplemented groups. Also there was no evidence of delayed onset of oestrus in non-pregnant heifers. The circumstances under which a supplement of copper improved the reproductive performance were reported. But the problem was found to be complex one according to Mills (1967), who pointed out that situations were known in which copper deficiency in young stock was severe and yet no adverse effects on breeding performance was apparent. Tossell Robin (1967) reviewed the subject on the effects of the minerals on the reproductive performances of sheep and cattle.

Sane *et al* (1958) described an interesting out-break of herd infertility in India, apparently due to deficiency with the typical symptoms of hypo-cupraemia in Gir cattle. There was a good response to the feeding of one gramme of copper sulphate daily per head. Kehar *et al* (1964) found that feeding mineral supplements including copper resulted in reduced inter-calving period. Further investigations at Indian Veterinary Research Institute, Izatnagar and National Dairy Research Institute, Bangalore, indicated that extra copper supplements may help in inducing early oestrus in heifers and dry cows (Ray, 1967)

Sampath *et al* (1969) observed a retention of 25% of the supplemented copper, fed to heifers. Consequently, the blood copper levels were also found to be increased. The animals receiving the copper supplement came into oestrus and conceived earlier than those receiving no supplement. Amrita Kumar *et al* (1973) studied the effect of feeding copper and cobalt supplements to cross-bred cattle at Madhavaram Milk Colony, Madras where low fertility was reported. They reported that feeding supplement of copper and cobalt resulted in 72% of heifers becoming pregnant and 75% animals settled within 1 to 6 months.

A batch of six heifers which had not conceived inspite of having regular oestrus cycle, were given daily 5 gm copper sulphate along with concentrate mixture. All of them were inseminated after 2 to 8 weeks of starting the supplementation. More than one insemination was not required except in case of one cow, which was served twice. All were found carrying when examined after 3 months (Deshpande, 1969).

Kehar *et al* (1959) observed the lower calving intervals by supplementing the ration with copper of cows, which were thought to be deficient in copper.

Mahadevan *et al* (1961) and Mahadevan (1963) found that the cows came into heat within 23 to 90 days of giving copper supplement. Mathur (1960-61) reported that Sindhi heifers aged about three years came into heat within 46 to 98 days of copper supplementation of their ration. Mathur (1960-61) and Mahadevan (1958, 1959) observed lower copper levels of 56

to 84 and 93 μg per 100 ml respectively, in the blood of Sindhi and Bariana heifers, ranging in age from 30 to 48 months. The low blood copper levels were associated with anoestrus. They stressed that the adequate level of copper was required for normal reproduction.

The continuous feeding of 0.5 gm copper sulphate per day per head for over six years to Mariana cows had no toxic or adverse effects. But it was found to be very beneficial in inducing oestrus and reducing the inter-calving period. The blood copper level was also found to be significantly higher than those receiving no supplement (Mahadevan and Zuberi, 1969).

Reddy (1966) concluded from his studies that supplementation of copper at the rate of 0.5 gm copper sulphate per day per animal for a period of 2 to 3 months after parturition to be beneficial for increasing the productive efficiency of the cattle in copper deficient or marginally deficient areas. Maciver and Foot (1972) observed higher ($P < 0.005$) percent of the embryo from the copper sulphate and steroid treated groups developed in vitro into blastocysts than those from L.H. treated group (92.0% vs 76.6%).

17. Copper toxicity:

The copper toxicity in ruminants is an important nutritional problem. It can be divided primarily into two types, namely (1) Natural chronic copper poisoning which is generally seen in sheep (Bull, 1949) due to (a) high copper content of soils and pastures, (b) normal copper content of soils and

pastures, but low level of molybdenum (Dick, 1952, 1953) and (c) in association with liver damage due to poisonous plants disturbing normal physiological function of liver and thereby copper storage and poisoning subsequently (Trautner and Neufeld, 1949). (2) Accidental copper poisoning as a result of ingestion of excessive amounts of copper due to increased use of copper compounds in agriculture and veterinary practices (Boughton and Hardy, 1934) and indiscriminate use of copper in the treatment of the various copper-deficiency diseases of the stock that occur in some areas (Jnderwood, 1962).

In all animals, continued ingestion of copper in excess of the requirement leads to some accumulation with the tissues, especially the liver. In cattle and sheep, liver has a remarkable ability to take up and retain very large amounts of ingested copper upto certain levels, which varies greatly among individuals and species, without any physiological harm to the animal. Above these levels, a catastrophic liberation of a high proportion of the liver copper into the blood stream occurs, with resultant extensive haemolysis and jaundice followed by death. Ammerman (1970) in his recent review has divided the chronic copper poisoning into two distinct phases, namely, (a) a period of passive accumulation of copper in the tissue (sepcially liver) during which no symptoms of toxicity are exhibited and (b) the toxic phase, which is an acute illness, frequently referred to as the 'copper crisis syndrome' or 'haemolytic crisis', which can cause death within few hours to 2 - 3 days (Guilbride, 1963). It is suggested that the

high liver copper concentration result in erythrocyte destruction in that organ (Goldberg et al, 1956). The common characteristic features are sudden hemoglobinemia and hemoglobinuria associated with marked acute hemolytic icterus, wide spread necrosis of liver, high levels of copper in liver and blood in fatal cases.

Sheep are more susceptible than cattle to copper poisoning. Cattle tolerate high dietary intake of copper than other species that have been studied. Very few references on chronic copper poisoning in the bovines are found. Adult cows were fed 1.2 to 2.0 gm copper sulphate daily for 5 to 10 weeks without harm (Ferguson, 1943). Cunningham (1946) fed 0.8 to 5.0 gm copper sulphate per day per head for 9 months to calves and adult cows without any ill effects. On the other hand, Kidder (1949) induced generalised haemolysis, icterus, haemoglobinuria and death in 500 lb steer by feeding 5.0 gm hydrated copper sulphate for 122 days. Shand and Lewis (1957) noticed typical signs of chronic copper poisoning in calves fed on milk substitute powder containing 115 ppm copper.

In large doses copper is extremely toxic and heavy losses due to copper poisoning were reported by Boughton and Hardy (1934) and Eden (1940). The toxic level, however, was reported by Eden (1940) and Pullar (1940) to vary with the type of diet as also with the species. Cunningham (1946) gave copper sulphate upto 80 gm in a single dose to yearling heifers, which did not develop toxic symptoms. A single dose of 400 gm of copper sulphate was lethal to all cattle, whereas the average lethal

dose (single) ranged between 200 to 400 gm. Border line toxic level for cattle was found to be approximately 1 gm of copper sulphate per 100 lb body weight (Boughten and Hardy, 1934).

Natural chronic copper poisoning can be alleviated by molybdate containing 'licks', which reduced the liver copper levels (Dick and Bull, 1954; Pierson and Aanes, 1958). The value of dosing with ammonium molybdate and sodium sulphate in preventing chronic copper poisoning in sheep was demonstrated by Ross (1964).

McCosker (1968) made a detailed study on blood copper during chronic copper poisoning in sheep. During the first stage, there was a transient elevation in red blood cell copper and in the second stage the level of whole blood copper essentially doubled from increases in red blood cell copper and also direct reacting copper. Plasma colour changed by the presence of bilirubin and there was a reduction in haematocrit value. A high level of 190 μ g copper per 100 ml blood appeared

during the 'hemolytic crisis' first in plasma as direct reacting copper and later in the red blood cells. Similar observations with calves subjected to chronic copper poisoning were reported by Todd and Thompson (1965) and Weiss and Baur (1968). The later authors reported dystrophy and cirrhosis of the liver and levels of copper in the liver at the time of death to vary between 838 to 2,091 ppm on a dry tissue basis.

The determination of serum glutamic oxaloacetic transaminase (SGOT) activity was suggested to be an useful index in

diagnosing and evaluating the treatment of copper poisoning (Ross, 1956; and Todd and Thompson, 1963). Concentrations of SGOT increased rapidly during approximately 4 weeks before death and decreased in relation to the effectiveness of the copper toxicity treatment. The author emphasized the value of plasma arginase as an aid in differential diagnosis, since this enzyme increased in liver damage while SGOT concentrations increased with either liver or other tissue damage. Todd and Thompson (1964) demonstrated that a single dose of intravenously injected copper (80 to 160 mg per head) caused death in sheep within a few hours to several days after injection and resulted in symptoms similar to gastro-enteritis rather than chronic copper poisoning. Repeated injections of smaller amounts of copper resulted in characteristic symptoms of copper toxicity.

The copper toxicity of sheep under natural grazing conditions in Western Australia was suggested by Beck and Bennetts (1963) to result from factors including native plants containing high levels of copper and hepatotoxic principles of fungal origin. The other toxic principles causing liver degeneration and thus, pre-disposing the animal to copper toxicity were recently discussed by Clemetson (1966) and Proyer (1965).

The degree of toxicity was found to be influenced by the form of copper administered. About 12 gm copper sulphate per head daily when administered as a drench to steers caused death in approximately 60 days, whereas same amount of copper sulphate administered in dry form in a gelatin capsule even for a period

of over a year did not indicate any toxic symptoms (Chaypman et al, 1962).

18. Copper Requirements and Supplementation:

Due to the presence of many dietary factors, which greatly influence the assimilation and utilization of copper in the body, the assessment of copper requirements based solely on copper intakes are of limited value. The copper requirements vary according to the presence or absence of these factors, and to the extent they are present in the normal diet of animals. Therefore, it is difficult to prepare a master table of the requirements of such a inter linked essential element. Secondly, the contamination as well as intrinsic and complex physiology of the element concerned pose problems in the assessment of its actual requirements. Apart from this, the feeding of purified diet to large animals to overcome these drawbacks, is not practicable and also this does not give the exact picture when the feeding is done under natural conditions.

The studies similar to the one undertaken will be more of practical value in overcoming the previously mentioned problems in the assessment of the requirement of the trace elements.

A basic minimum requirement can be assessed when all the other dietary conditions influencing copper adsorption assimilation are at the optimum and balanced. Such an exact requirement has not yet been given. However, attempts have been made to get as closer as possible to the requirement.

The minimum copper requirement of cattle and sheep were assessed to be 4 ppm of the diet (on dry matter basis) on pastures with less than 1 ppm of molybdenum and not containing calcium carbonate. Marino sheep, however, required nearly 6 ppm of copper on such pastures (Beck, 1951). Dick (1954) demonstrated that an intake of as low as 0.5 mg of molybdenum per day adversely affected the copper retention in sheep, provided the sulphate intake was high. This study indicated that if other interfering factors were present in the diet, they may adversely affect the absorption and retention or increase the excretion of copper. Such dietary factors were observed to be operative in many environments. (Marston and Lee, 1948a,b; Marston *et al.*, 1948; Anonym, 1942-46, 1946-47, 1948, 1952, 1953). Under such particular conditions, where there was high consumption of calcium carbonate and moderate intakes of molybdenum as well as sulphate from the herbage, it appeared that the minimum copper requirements of wool sheep were close to 10 mg per day (10 ppm of the diet).

A sub-normal status of copper in cattle (Allcroft and Parker, 1949; Lewis and Allcroft, 1960) and in sheep together with 'sway-back' in lambs (Allcroft, 1952; Allcroft and Lewis, 1957; Hunter *et al.*, 1945; Innes and Sheaver, 1940; Sheaver *et al.*, 1940; Sheaver and McDougall, 1944) were shown to arise on pastures containing 8 to 14 ppm copper or even more and normal levels of molybdenum and sulphate. It is evident that the assessment of the minimum copper requirement of sheep and cattle is one of great complexity.

Bennetts et al (1941) Maynard (1951) reported that minimum requirement of copper for cattle and sheep to be 50 - 60 mg and 5 mg respectively per day. The animals were observed to be healthy on forages containing 5 - 8 ppm copper when the interference from molybdenum and sulphate were minimum.

The copper requirement of the cattle was not known as reported by National Research Council Committee on Animal Nutrition, U.S.A. (1960). The addition of one percent of copper sulphate in salt, in copper deficient areas was recommended by the committee. However, on residual muck and high molybdenum mineral soils, higher levels of supplementation of copper were recommended.

Investigations carried out at Indian Veterinary Research Institute, Izatnagar, (Anon, 1946-47) on trace elements with Kumaoni bullocks revealed that the minimum requirement of copper for these bullocks ranged from 6.56 to 6.87 with an average of 6.69 mg of copper per 100 lb live weight per day. Further, it was noticed that optimum results were ^{obtained by supplementation} of the feed consisting of wheat straw and rape cake with copper at the rate of 7.38 mg of copper per day per 100 lb live weight. It was almost 10 percent higher than the minimum copper requirement recommended. It was further recommended that the minimum level of copper required by the cattle to be 7.3 to 8.0 ppm of the ration and the levels less than this was likely to result in the manifestation of the symptoms of copper deficiency. Based on the metabolism trials on cattle with different levels of copper intakes, Sahai (1947) suggested the requirement of this mineral to be 6.5 mg per 50 kg body weight.

The requirements of these species for copper in deficient areas can be met by any one of the three suggested methods: (i) by the application of copper containing fertilizers to the soil, which raises the copper content of the herbage to the adequate levels, (ii) by providing copper containing salt licks, drenching the animals at suitable intervals with soluble copper salts, or addition of 0.5 to 2 % of copper sulphate to salt/mineral mixture and (iii) by injecting various organic complexes of copper intramuscularly or sub-cutaneously at about 3 months intervals in doses of 120 to 240 mg for cattle (Allcroft and Jvarov, 1959) and 30 to 40 mg for sheep (Cunningham, 1959; Houle *et al.*, 1959) and Sutherland *et al.*, 1955). For this purpose copperglycinate was found to be safe and effective. Though this method of copper supplementation is not widely used, except in Great Britain, the workers in Australia also indicated its effectiveness (Alexander *et al.*, 1967, Cook *et al.*, 1966; Donaldson, 1964; and Dunkley *et al.*, 1963). Injectable copper glycinate, copper methionate and also Cu-EDTA, were effective in maintaining normal blood copper levels in pregnant ewes (Remington *et al.*, 1970). The injectable Cu-EDTA complexes currently available generally result in less localized reaction than that occur with copper glycinate. But they may have a greater toxicity potential than with glycinate (Ishmael *et al.*, 1970).

Copper deficiency or molybdenum toxicity was successfully treated by the use of dietary supplements of copper (Dye and O'Harra, 1959; Kide, 1962; Underwood, 1966). Top dressing of

copper deficient forages with copper salts was of limited success in various geographic locations (Allcroft and Varov, 1959; Underwood, 1960; and Spencers *et al*, 1958). The incorporation of copper salts in water or ration was often practiced in Western countries. However, the administration of dietary supplements of copper was found to be impracticable in the case of animals under pasture and range conditions. Hence, with the advent of injectible organic compounds such as copper glycinate suspensions, many ranchers and stock owners switched over to this preventive method of overcoming the deficiency of copper in their stock (Harvey and Sutherland, 1953; Cunningham, 1957; and Dye and O'Harra, 1959).

Many copper compounds were used as dietary supplements. The uptake of Cu^{64} by the blood of sheep was found to be greater in the case of labelled cupric chloride than either cupric nitrate or sulphate, administered orally (Lassiter, and Bell, 1960). Similarly, cupric carbonate was better utilized than cupric or cuprous oxide. Chapman and Bell (1933) while working on Hereford steers with radioactive copper compounds, noted that copper from cupric nitrate, cupric sulphate, cupric chloride and cupric carbonate were absorbed to a similar extent. More absorption into blood stream was found from cupric oxide in the powder form than from the ^dneedle form. The authors concluded that cupric sulphate was the most suitable source, out of those tested for use as a dietary copper supplements, from both physical and biological points of view. Buescher *et al* (1961) and Bunch *et al* (1961, 1965) also obtained similar results with the pigs.

19. Copper in Animal Feed and Fodder:

The copper content of animal feeds such as straws, green fodders and concentrates, which constitute the principle basal diet for the livestock in various parts of India, was estimated by Sahai and Kehar (1951). Seventy-two common feeding stuffs were analysed for their copper contents, of which some are listed in Table A (Appendix). The copper content of feeds ranged from 3.6 mg in oat straw to 17.8 mg per kg in Stereonarmus anaviolens (Podal) leaves. A critical examination of the results suggest that a tentative grouping of the feeds based on their copper contents is possible. When arranged in an ascending order of copper contents, cereal straws with 3.6 to 4.7 mg per kg constitute the first group. Green feeds containing 5.9 to 11.9 mg of copper per kg form the second group. The third group includes oil cakes, grains, seeds and their by-products, having a copper content of 9.2 to 17.2 mg per kg.

Similarly the data of the copper contents of pertinent animal feeds, published by Kanwar and Randhawa (1967) and also Elvehjem and Hart (1927) are also included in the Table A (Appendix).

The copper contents of crops could be increased within certain limits by manuring with a copper salt (Elvehjem and Hart, 1927; Wark, 1954; Joshi and Joshi, 1957 and Bhumbla et al., 1963-65). The copper content in the grasses was reported to be increased as a result of heavily fertilizing the

soils with the nitrogen or nitrogen and potash (Mudd, 1970). And this level of copper in grasses was in accordance with the A.A.C. standards for the requirements of dairy cows. But only in few cases, it exceeded the levels prescribed.

Levels of copper that gave satisfactory results in farm animals (Blaxter, 1952) are given in the following table.

Copper content of pastures in relation to requirements of sheep and cattle.			ppm of copper in HERBAGE
1.	Estimated mineral requirement		5
2.	Normal grazing, Great Britain		7 to 14
3.	Normal grazing, Australia		7 to 12
4.	For the areas where the diseases are cured by the administration of copper:		
1.	Sway-back area, Australia		1 to 14
2.	" " Derbyshire		12 to 27
3.	" " Scotland		4.4 to 9.0
4.	Peat-scour area, New Zealand		3.6
5.	" " , England		8 to 26
6.	Copper-pine areas, Caithness		6 to 21
7.	Falling disease areas, Australia		less than 2
8.	Polder disease areas, Holland		5 to 11

Deshpande (1959) analysed the samples of straws, greens and hay for their copper contents and reported the values to be 3.0 to 6.85 ppm for grasses and 3.39 to 5.23^{ppm} for cereal straws. Green jowar and maize contained 8.30 and 9.20 ppm copper, respectively.

Patel *et al* (1968) analysed about thirty samples of feeds and fodders collected from the different sources in Gujarat state, for their trace mineral contents. Their findings are also incorporated in Table A (appendix). Sawhney and Bedi (1972) and Sawhney *et al* (1973) analysed about hundred samples of feeds, fodders, tree leaves of hill area for their trace mineral contents. They reported that straws contained 5.8 to 15.8; grasses 5.8 to 19.8; green fodders 10.2 to 21.0; and tree leaves 7.8 to 17.0 ppm of copper on dry matter basis. Pertinent findings of theirs are also listed in Table A (appendix). Their study indicated that the distribution of copper varied widely in feeds and fodders grown in different regions. The concentration of copper in majority of fodders from hilly regions was found to be comparatively lower than those from the plains.

CHAPTER - III

REVIEW OF LITERATURE ON STUDIES WITH COBALT FOR RUMINANTS

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As early as fifteenth century a mineral ore of unknown composition was used by the Egyptian and Babylonian glass-makers for colouring the pottery. It was known also to European glass-makers in sixteenth century. The term 'kobold', a mischievous spirit was applied to certain ores in Hartz mountains. This mineral when treated with acids used to give blue coloured solutions, similar to that of copper ores. But it had the unusual property, unlike copper ores, of imparting a blue colour to glass. Some specimens of roman and venitian blue glasses owe their colour to cobalt. Because of this seemingly mysterious behaviour, the mineral was called as 'cobalt', the name being derived from German word 'Kobald', which means subterranean gnome - the little teasing earth spirit. Cobalt was used in colourlessinks. In 1735, Brandt, the genius Swedish chemist, examined the cobalt ore and found that it was a metal which had the property of imparting blue smelt to glass. After seperating this metal by fire assay, he named it as 'cobalt'.

In 1864, deposits of cobalt were discovered in many countries and 10 years later efforts to explore the mines started. Now Germany and Canada contribute the maximum to the total world's out-put of cobalt. No deposits are discovered in India, so far. According to geologists, cobalt forms 0.0001 percent of earth's crust and is generally found associated with nickel and arsenic. The cobaltous and cobaltic salts are

commonly used for colouring glasses, enamels, other silicates, as driers in paints and varnishes and in alloys.

The presence of cobalt was detected in the ash of certain plants about a century and a half ago. Although its presence in plant was detected as early as 1841 by Legrip, 75 years later Bertrand and Macheboeuf (1925-26) demonstrated its regular occurrence in plant and animal tissues (Dutoit and Zbinden, 1928; McHargue, 1947; Fox and Ramage, 1931). However, its significance and availability were not appreciated then. Comparatively high amounts of cobalt were reported in Archidoria tuberculata by Fox and Ramage (1931) and in tectibranch mollusk by Webb in 1937. The leaves of the swamp black gum contained very high amounts of cobalt i.e., 60 to 250 times the level that was found in grass growing under similar conditions (Beeson et al, 1955)

2. Cobalt in Nutrition:

None of the early studies could give any indication of the physiological role of this metal in the living tissues. Neither Bertrand and Nakamura (1927) nor Stare and Elvehjem (1933), the pioneer nutritionists, who used highly purified diets for experiments on mice and rats could put forth any convincing evidence of an essential nutritional role for cobalt in these species. Meanwhile Waltner and Waltner (1929) reported that cobalt in high doses stimulated erythropoiesis in rats and induced a condition of polycythemia. This discovery was of considerable historical significance and importance as it

created a great deal of interest in understanding the importance and role of this element in normal physiological processes of the body and for the treatment of some of the regional endemic diseases.

The occurrence of deficiency in 'bush sickness', a wasting disease of cattle and its correction with change of pastures was reported by workers in Scotland in 1907. Later, this proved to be due to cobalt deficiency. Aston as early as 1911 thought that wasting disease of cattle in New Zealand was due to mineral deficiency, and attributed it to iron deficiency. Aston (1924) summarized his studies in which it was claimed that ferric ammonium citrate or iron ores could either prevent or cure this condition. But it was actually due to cobalt deficiency. The finding that crude iron compounds were effective in curing or preventing similar maladies was however accepted all over the world (Dickinson, 1927; Becker *et al*, 1931; Orr and Holm, 1931 and Oreig *et al*, 1933).

Cobalt is one of the latest addition to the list of trace minerals known to be essential for the growth and health of ruminants. The demonstration of the biological significance of cobalt is one of the most interesting study of the recent developments in nutritional research. In many parts of the world symptoms of malnutrition or disease in livestock on certain areas have been traced to be deficiency of cobalt in the forages. In some of these areas, before the cause of ailments was discovered and remedial measures were taken, there used to be severe economic loss as a result of impaired

productivity and even death of the affected animals. Cobalt is required by ruminants in amounts so small as to baffle the scientist but the effect of deficiency is so devastating as to prevent the development of a healthy livestock industry over the vast areas. The response to traces of cobalt in the feed of ruminants was very remarkable that it was considered a wonder. In fact, it is fascinating to study the role of the element cobalt in animal health.

Earlier to the first conclusive evidence that cobalt was a dietary essential, Aston (1932) showed that a similar wasting disease of cattle called 'bush sickness' occurred in parts of New Zealand and that the same could be prevented or cured by oral administration of iron ores. Later on, Western Australian workers showed that the same type of disease could be cured by iron-free extract of crude limonite (Filmer, 1933; Filmer and Underwood, 1934). Thus the hypothesis was advanced that the disease was due to a deficiency of some trace element which occurred as a contaminant of iron compounds used. It was demonstrated by Underwood (1934) that the cobalt was the element in limonites that prevented the development of a wasting disease in Australia. It gave an impetus to the series of experiments and reports on the subject. First conclusive and indisputable evidence that cobalt was a dietary essential mineral required by ruminants was established, when the cause of 'cost disease' of sheep (Martson, 1935; Lines, 1935) and 'wasting disease' or 'enzootic marasmus' of cattle (Underwood and Filmer, 1935) was found to be due to the deficiency of cobalt.

This important discovery gave a great stimulus to studies of the nutritional significance of cobalt in ruminants. This led to the delineation of cobalt deficient areas in many parts of the world as far as soil and herbage was concerned. In United States, cobalt deficiency was first identified in Florida in cattle by Neal and Ahman (1937).

Analytical methods capable of estimating the minute amounts of cobalt in soil, plant and biological materials were developed after the original method of Stare and Elvehjem (1933), which could estimate 10 ug of cobalt. This facilitated the detection and correction of acute cobalt deficiency in ruminants.

Filmer and Underwood (1936, 1937) discovered that massive doses of iron alone was not the cure for wasting disease. The limonite containing some impurities, which owned its potency to cobalt, was described as the cure for the disease in cattle and sheep. Lines (1935) also reported miraculous effects due to cobalt supplementation of the ration of farm animals. Marston and coworkers (1938) reviewed the earlier investigations on the role of cobalt in animal nutrition.

Wunch (1935) classified areas of New Zealand as 'sound' and 'unsound' areas of with respect to cobalt content of soils as well as the crops grown on them.

Further, studies by Grimmett and Shortland (1934) as well as Rigg and Askew (1934, 1936, 1939 and 1940) contradicted the findings of Aston (1934) and postulated that some other element must be involved. They found that cobalt was absent in some

ores, which were ineffective against the disease. Grimmett and Shorland (1935) held that iron per se was a necessity for the prevention of 'bush sickness' in animals, but cobalt and other elements might have a stimulating effect.

Following the reports of the successful work on cobalt in Australia, Asher and Dixon (1936) in New Zealand demonstrated the effectiveness of cobalt salts in the treatment of 'bush sickness' at Glenhop and Morton Mains. They administered 4.0 mg cobalt per sheep twice a week to prevent the disease. Wall (1937) reported similar results with 1.0 mg per head per day. In Florida, the work was going on for long time since 1913, but it was attributed to 'nutritional anaemia', a nutritional deficiency of iron and copper. Following the results in Australia and New Zealand, Neal and Ahman (1937) experimented with calves in 'salt sick areas' of Florida. In their experiments, the disease was aggravated by iron and copper supplementation, but was cured by cobalt administration.

Meanwhile the composition of limonite ores that were effective and ineffective in correcting 'bush sickness' and 'salt sickness' in cattle were studied. McNaught, (1937) Becker and Gaddum, (1937) showed the presence of 5.2 to 281.0 ppm of cobalt in various effective ore samples, while it was not found in ineffective ore samples even by the spectrographic analysis. About 7 ppm of cobalt was present in ferric ammonium citrate which was effective in the treatment of cobalt deficiency diseases.

Patterson (1937) studied the problem in Daltmore, England and discovered that cobalt contents were higher in animals in 'healthy areas than in sick areas'. Mixtures of cobalt, magnesium and zinc salts cured the disease. Corner and Smith (1938) reported the striking benefits of cobalt administration for the disease - 'pine' in sheep. Underwood and Harvey (1938) analysed the soils and pastures of the affected areas and the liver of the affected animals. These samples contained sub-normal levels of cobalt. Howstead and Sackville (1939) reported from Western Canada that sheep developed some deficiency symptoms on a certain diet and the same could be corrected by the addition of cobalt to the diet. Russell (1944) and Maynard and Smith (1947) reviewed the importance of cobalt in the nutrition of animals.

Progress in the understanding of the mode of action of cobalt in the animal organism was slow until the independent discovery from two laboratories that the anti-pernicious anaemia factor in liver, which was subsequently designated as vitamin B₁₂, was a cobalt containing compound, that contained not less than 4 percent of the metal (Smith, 1948 and Rickes et al, 1948). The demonstration of the presence of cobalt in vitamin B₁₂ suggested that the vitamin might be the functional form of cobalt in ruminant metabolism and it was thought that the deficiency syndrome might originate from the deficiency of vitamin B₁₂. Within three years of that discovery in 1951, workers at Cornell University reported that adequate injections of vitamin B₁₂ effected complete remission of all signs of cobalt deficiency in lambs (Smith et al, 1951).

Soon it became apparent that cobalt deficiency in ruminants was in effect, the vitamin B₁₂ deficiency brought about by the inability of the rumen micro-organisms to synthesise this vitamin in sufficient quantities with the inadequate supply of dietary cobalt. An important and unique nutritional situation was revealed in which the ruminants appeared to utilize a trace element solely as an integral part of a vitamin and to be directly and completely dependent upon the symbiotic activities of their own micro-organisms for their requirement of this vitamin.

Studies in India, on cobalt in relation to cattle nutrition was started in 1946 at Indian Veterinary Research Institute, Izatnagar. The cobalt content of common animal feeds, effect of maturity, seasonal variations as well as the cobalt content of soil and its relation to cobalt content of vegetation, influence of irrigation were studied. Normal blood and tissue cobalt content, requirement of cobalt for cattle and its metabolism, morphological and biochemical constituents of blood in relation to cobalt intake and cobalt-manganese relationship were also studied.

According to Dube (1964), cobalt deficiency was recorded only in ruminants, under field conditions. As per Kanwar and Randhawa (1967), based on the total cobalt content of soils, cobalt deficiency exists in many soils of Delhi, Punjab, Gujarat and Maharashtra. No definite information was available yet regarding soils in other states of Indian territory. Total cobalt content in these states was found to vary from traces to 274 ppm.

Marston (1952) in his excellent review of cobalt, copper, molybdenum and vitamin B₁₂, observed that cobalt was nutritionally essential element. However, it was not known to serve any useful purpose in the animal body, except being a part of the necessary food factor i.e., vitamin B₁₂, which originated in nature only as metabolic product of certain micro-organisms and was absent in higher plants upon which the animals depend ultimately for subsistence. It was an essential factor in maintaining the optimum growth and activity of the mixed population of rumen micro-organisms. The nature and number of rumen flora was found to be affected adversely, if the concentration of cobalt in the ingesta fell below an optimum level. The mechanism of action of cobalt was not fully understood. The primary metabolic defect in the tissue of vitamin B₁₂ deficient sheep was later shown by Marston *et al* (1961) to be its inability to metabolize propionate.

Cobalt was considered to function in metabolism as vitamin B₁₂ and the symptoms of the deficiency of this vitamin were described as basically of those of starvation (Davis, 1958; Smith and Loosli, 1957). With regards to the sequence of deficiency symptoms, research with sheep indicated that a reduction in feed intake preceded the onset of normocytic, normochromic anaemia. On treatment with either cobalt or Vitamin B₁₂, feed intake improved immediately, but anaemia calves took long time to show improvement (Gawthorne *et al*, 1966).

Jones and Anthony (1965 & 1970) suggested that faecal vitamin B₁₂ may be used as an index of cobalt intake. In their studies on sheep, faecal vitamin B₁₂ decreased before weight

loss and other symptoms of cobalt deficiency were noticed. They developed a prediction equation for use in predicting oral cobalt intake from fecal vitamin B₁₂ concentration.

Supplementation with cobalt increased cobalt in the pigmented hair of dairy cow (Anke, 1956). Anke (1967) suggested that 0.05 ppm cobalt in the black hair of dairy cattle indicated the dietary intake of cobalt to be at the border line.

3. Species affected by cobalt deficiency:

Cobalt deficiency occurred in grazing ruminants. Cattle and sheep were the only species so far known to be susceptible to cobalt deficiency. Of the two species, the latter was more susceptible. Filmer and Underwood (1936) reported that the disease ran a more rapid course in sheep and calves, than in adult cattle. Horses and other non-ruminant species from cobalt-deficient areas were reported to be unaffected. Goats were equally affected as cattle. No cures for goats and hogs were reported. Underwood and Alvehjem (1938) were unable to produce deficiency in rats. Houk *et al* (1946) and Thompson and Ellis (1947) could find no evidence for the cobalt being beneficial for rats rabbits and guinea pigs. Marston and Lee (1949) during the course of studies with ruminants, observed similar results reported for ruminants.

The disease occurred in areas of acute cobalt deficiency. In such areas the ruminants of all ages and types suffered at all times and the mortality and wasting were usually severe. Much larger area existed in which the deficiency was mild or marginal.

In these areas only, the more susceptible type of stock exhibited signs of deficiency. The deficiency manifested in some years or seasons only, as sub-clinical unthriftiness, with little or no mortality might be the only evidence of inadequate supplies of cobalt. There was growing evidence that sub-clinical cobalt deficiency was of considerable economic importance. This is because it occurred over larger areas than was formerly believed and it was difficult to diagnose and it could readily be confused with the effects of parasitism or under-feeding. In fact, very mild cobalt deficiency could be positively identified only by observing the effects of cobalt or vitamin B₁₂ therapy (Underwood, 1956).

4. Symptoms of cobalt deficiency

The cobalt deficiency syndrome varies from the acute and fatal conditions, through a series of less acute stages, to a mild, ill-defined and often transient state of unthriftiness. The original work on deficiencies of cobalt in sheep was almost immediately followed by studies with cobalt on cattle with a description of cobalt deficiency. Most of the authors described the symptoms in cattle as 'almost similar to those in sheep', with one or two additional symptoms. Underwood (1962) described the symptoms in detail.

(a) Clinical symptoms: Perhaps the most characteristic symptoms of cobalt deficiency in livestock was the loss of appetite, followed by loss of body weight, emaciation, weakness, rapid wasting and severe anaemia (Neal and Ahman, 1937; Sackville

Allotriophagia was constant in cattle, there being a pronounced craving for harsh fibrous scrub. Anorexia was pronounced in calves, but not in sheep. Diarrhoea was also often seen in young calves, in which faeces used to be blood flecked in some cases. In older calves, diarrhoea was less frequent and alternated with constipation.

In cows, lactation was reduced or even totally suppressed. There was retarded development of the sexual characteristics. Oestrus rarely occurred and mating was seldom successful. Cows were found to abort between sixth and ninth months of pregnancy. Death occurred from sixth week to two years of the appearance of the first symptoms. The shorter course was usually observed in young animals. Cows frequently showed some recovery, with subsequent relapse. Muscular atrophy and in most instances a gauntness due to loss of appetite was generally observed (Filmer, 1933; Neal and Ahman, 1937). Dramatic response to dosing with cobalt was also taken as one of the symptoms (Filmer and Underwood, 1937).

(b) Haematological picture: The erythrocyte count was always low in the affected animals. Counts of 4 to 5 millions per cmm in cattle and 4 to 7 millions per cmm in the blood of sheep were quite common (Filmer, 1933). The lowest counts observed were 2.8 millions for cows and 3.7 millions per cmm for lambs. The mucous membranes were ²pl₂ and the haemoglobin concentration in the blood was low (Underwood, 1937). Contrary to it, Neal and Ahman (1937) reported the erythrocyte count may be above average and the haemoglobin concentration to be equal

to or above that of animal receiving cobalt. They reported the blood picture of affected animals to be similar to that observed by Filmer (1933), in which anisocytosis and poikilocytosis and an increase in the proportion of lymphocytes were noticed. No reticulocytosis was noticed.

The amount of haemoglobin per erythrocyte or per volume of erythrocytes was reduced. The condition could be classed as 'microcytic hypochromic anaemia'. Underwood (1958) reported red cell numbers and haemoglobin in blood were always low, in the affected animals in the field. Filmer (1933) found normocytic and hypochromic anaemia in lambs and microcytic, hypochromic in calves. Smith and co-workers (1950) reported a normocytic and normochromic anaemia in cobalt-deficient lambs, while Marston (1952) described the condition in sheep as a 'profound macrocytic anaemia with marked poikilocytosis and polychromasia'.

(c) Post-mortem findings: Post-mortem examination of severely affected animals revealed a picture of extreme emaciation, often with a total absence of body fat, paleness of all the muscles and membranes. The liver was fatty due to fatty degeneration. Spleen was small, fibrous and hemosiderised, and hypoplasia of erythrogenic tissue in the bone marrow was seen. Flabbiness of heart was observed with patchy myocardial degeneration and fibrous infiltration. Atrophy of the thymus earlier than normal and gelatinous exudate in the thoracic and peritoneal cavities were common (Smith et al, 1950; Filmer, 1933; Browning, 1937; Mal and Ahman, 1937). Post-mortem findings in cobalt deficiency animals had not been of much help in differential

diagnosis, since the pathological alterations were typically those of general inanition.

5. Occurrence and Terminology of Cobalt Deficiency Disease:

Cobalt deficiency in ruminants, with varying degree of severity, in particular areas occurs all over the world. If the affected flocks were shifted to the different type of land or country, there used to be rapid improvement in their condition. The earliest reference to this condition was that of Aston (1811) of Scotland, and since then it was reported and described by several investigators, from different countries.

The severe forms of cobalt deficiency in sheep and cattle have been given a variety of local names, some of which are descriptive of the symptoms of the disease, while some other merely reflect the area of deficiency. A short account of the terminology of the disease in various localities is presented here.

In south and west Australia, the disease is termed as 'coast disease' (Lines, 1935; Garston et al, 1935), and 'wasting disease' or 'enzootic marasmus' (Filmer, 1933), respectively. 'Bush sickness', 'Morton Mains disease' (Askew and Rigg, 1934) and 'Mairoa Dopiness' (McNaught, 1938) are some of the terms employed in New Zealand to denote the disease, which British workers have called as 'Pining, Daising or Vanquish' (Greig et al, 1933). Neal and Ahman (1937) from Florida (USA) have described it as 'salt sickness', while Baltzer et al (1941) of Michigan (USA) preferred to call it as 'GrandTraver's disease'. 'Makuritus' (Jack all) is the term

employed in Kenya (Africa) to denote the cobalt deficiency disease (Orr, 1929). Corner (1939) of New South Wales thought it to be 'nutritional anaemia'. In Germany and Holland, the disease is referred to as 'Lecksucht', which means licking disease. 'Mossjuka or moor disease is the term used in Estonia.

A condition similar to cobalt deficiency was encountered in the flock of University of Illinois (Wright, 1941). Keener *et al* (1944) reported in New Hampshire dairy cattle, a most troublesome disease, especially in young animals, but also existing in adult animals. Severe cases appeared in late winter and early spring. Quick improvement was noticed when the animals were given cobalt supplement/therapy or were turned on to pasture. The disease was called by the dairy-men as 'Burton-ail', which exhibited characteristic symptoms of cobalt deficiency. Geyer *et al* (1946) reported that the symptoms observed in cattle in north-eastern region of Wisconsin were no way different from those of cobalt deficiency in cattle in the Grand Traverse region of Michigan. The other reference to the condition characteristic to the cobalt deficiency was that of Bendixen and Penderson (1947) from Jutland, of the occurrence of similar conditions in livestock on an increasing number of farms in certain areas. The condition was prevented and cured by the addition of cobalt to the feed. It was the first record of such wide-spread cobalt deficiency in Denmark.

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soils, leached podsolized sands, sandy loams derived from granites, calcareous, wind blown shell sands and even ironstone gravels.

The cobalt content of the soils had been a subject of numerous investigations along with that of plant and animals. Bertrand and Mokragnatz (1924), Rigg (1935-36; 1936-37), Slater et al (1937), Harvey (1937), Kidson (1937), Patterson (1938), Underwood and Harvey (1938), Grimmett (1938-39), Stanton and Kidson (1939), Rogers et al (1939), Mitchell (1944), Malyuga (1944), Vinogradov (1945), Lazar and Baeson (1956), Walsh et al (1956) and many others analysed the soils from the places all over the world and found from traces to 300 ppm of cobalt in different soils.

The extensive studies of Slater et al (1937) revealed that the cobalt content of soils in six different horizons was surprisingly low. Rogers and associates (1939) could find no cobalt in Florida soils, even spectro-graphically. Some of the deficient soils of some regions were reported to contain as little as 0.1 ppm of cobalt (Underwood and Harvey, 1938), while the healthy soils seemed to range from 0.6 to 50 ppm of the element (Moore, 1938; Murnane, 1938).

Askew and Dixon (1936) reported 0.4 ppm of cobalt in soils of cobalt deficient areas in New Zealand and as much as 43 ppm in areas where the deficiency was not observed. The diseases such as 'Bush Sick' and 'Morton Main ailment' where prevalent, the soil contained 0.12 and 0.39 ppm of cobalt extractable in 0.1 N Hydrochloric acid, whereas the soils considered healthy had a level of 0.61 ppm to cobalt (McNaught 1937). A more

extensive soil study by Kidson (1937) showed a variation from 0.3 to 380 ppm of cobalt extractable with concentrated hydrochloric acid. Few soils contained more than 20 ppm, unless they were of basic origin.

Unhealthy soils from Dartmoor were reported to contain 1.9 to 6.7 ppm of cobalt, whereas healthy soils from Devon contained 19.02 to 38.20 ppm of cobalt (Patterson, 1938). In geochemical study of the biosphere, analysis were made of the soils of Russian plains from north to south along the 48 meridian, and the cobalt content was found to range from 0.1 to 1.2×10^{-3} per cent of the dry soil (Vinogradov, 1945).

Seekles (1948) while discussing biochemical approach to animal diseases, observed a real and conditional deficiency of cobalt in cattle on alluvial and diluvial soils of Netherlands. Kidson (1950) found more cobalt in soils developed from andesite than from either granite or rhyolite. Askew (1951) observed that soils developed on granite were deficient in cobalt and copper.

Many investigations were conducted on the cobalt content of soils in India (Dakshinamurthy *et al.*, 1955; Satyanarayana, 1958; Datta Biswas and Dakshinamurthy, 1958; Iyer, 1959; Deshpande, 1959; Duarte *et al.*, 1961; Reddy and Mahta, 1961, 1962; Badhe and Zende, 1962; Mandhava and Kanwar, 1964; and Rajani, 1965). All these workers found the cobalt content of different soils to range from traces to 274 ppm. Ahmed and Shariff (1953) analysed 24 representative samples of soils and found the cobalt level ranged from 6 to 20 ppm. They observed that of these levels the utilisation of cobalt by the plants was excellent.

According to Davidson and Mitchell (1940) and Walsh *et al* (1956) the nutritional disorder among livestock could be expected in areas, where total cobalt content of the soil was less than 5.0 ppm. and Wunsch (1939) considered 2 ppm of cobalt in the soil to be low.

Neal and Ahman (1937), Underwood and Harvey (1937), Kidson (1937) suggested that the cobalt contents of forages should be relied upon for the estimation of deficiency and supplementation of cobalt required to the animals, and not the cobalt content of soil.

(b) Cobalt in plants: After Legrip (1841), a German Chemist, Bertrand and Makragnatz (1922, 1925, 1930) and McHargue (1927) were probably the first to report on the distribution of cobalt in plant materials. But after the recognition of the importance of cobalt in bovine nutrition, and with the development of suitable micro-techniques, considerable interest was developed in the study of the cobalt content of the herbage of both the healthy and unhealthy pastures all over the world. The cobalt contents of the pastures of the affected areas were found to be significantly low compared with those of healthy ones (Askew, 1936-37; Harvey, 1937; Askew and Dixon, 1937; Askew and Maunsell, 1937; Rigg, 1937-38 and 1938-39; McNaught, 1938; Underwood and Harvey, 1938; Askew, 1939; Ahmed and McCollum, 1939; Stanton and Kidson, 1939; Wunsch, 1939; Rao, 1940; Stewart *et al*, 1942; Anon, 1943; Hurwitz and Beeson, 1944; Russell, 1944; Mitchell, 1944 and 1945; Maunsell, 1945; Steward *et al*, 1946, and Beeson 1950). The cobalt content of the healthy pastures was usually

0.07 to 0.30 ppm and that of unhealthy pastures was 0.01 to 0.07 ppm on dry matter basis.

The most systematic work on this aspect was carried out in New Zealand, where the values of 'deficient' and 'good' forages have been worked out. The data summarized by Wunsch (1939) was as follows:

<u>Cobalt in forage</u>	<u>Effect on livestock</u>
0.01 ppm of dry matter	.. Serious bush sickness in sheep and cattle
0.04 to 0.07 " "	.. Some sickness in sheep; cattle fairly healthy
0.07 to 0.30 " "	.. Sheep and cattle healthy

Forage containing 0.10 to 0.13 ppm of cobalt on dry matter basis could supply a dietary requirement of 0.1 mg cobalt for sheep. In range above 0.07 ppm of cobalt, no supplement was necessary. Cobalt deficiency areas in U.S.A. were mapped out on this basis (Kubota, 1963). Young (1948) reported 0.1 to 0.17 ppm of cobalt as the minimum content in plants for normal health of cattle. However, Dube (1964) reported that a much lower content of cobalt was needed for the normal health of cattle.

Straws and Grasses: Limited surveys conducted by the animal nutrition division of the Indian Veterinary Research Institute, Izatnagar, suggested that cobalt deficiency was not common in India (Anon, 1946-47, 1964-55). Singh (1948) also reported the cobalt content of straws from different areas to range from 0.01 to 0.111 ppm of the dry matter. Of these samples of straws, 33 percent contained less than 0.04 ppm,

57 percent contained from 0.04 to 0.07 ppm while the rest had higher concentration of cobalt. Cobalt content of paddy straw ranged from traces to 0.064 ppm. Wheat straw had little higher value of 0.068 ppm and oat hay and gram bhoosa contained 0.185 ppm. Tree leaves showed higher cobalt content i.e., 0.660 ppm. Deshpande (1959) found rice straw to contain 0.06 to 0.14 ppm of cobalt with an average of 0.109 ppm, that of jowar straw ranged from 0.09 to 0.33 ppm, wheat straw contained 0.08 to 0.14 ppm, different types of hay ranged in their cobalt content between 0.06 to 0.08, while the greens had 0.05 to 1.2 ppm of cobalt.

Grasses exhibited a narrow range of cobalt than the straws. All the samples of grasses examined contained 0.028 to 0.075 ppm of cobalt on dry matter basis. The green feeds representing grasses, leafy fodders and cultivated plants showed a wider range from 0.025 to 0.092 ppm of cobalt (Singh, 1948). Some samples contained even upto 0.2 ppm (Wark, 1964).

Concentrates: Analysis of twenty samples of concentrates (cakes, grains, seeds and by-products) revealed that they contained cobalt ranging from 0.025 to 0.105 ppm. Among them, maize cake was the poorest and rice bran as well as linseed cake were the richest sources of cobalt.

Influence of maturity: Concentration of cobalt in wheat, barley, oats, jowar and gram at early stages ranged between 0.083 and 0.147 ppm. But with progress of maturity, it continued to decline till the final stage, when the cobalt content had fallen by more than 70 percent. Datta and Datta Biswas (1951) found that all grasses had high cobalt content

in the young stage, but with increasing age, there was a gradual decrease in the cobalt content.

Seasonal variation: The concentration of cobalt was found to vary significantly with advance in season from November to March. Sixteen pure species of grasses were examined for their cobalt content in four cuts at monthly intervals. The first cut was taken in early August. Of this 43.75 percent of the samples showed a continuous fall in their cobalt contents. The remaining samples exhibited an initial rise at the second stage of sampling and subsequently there was a sharp decline. In majority of the cases, the concentration of cobalt at the last cut was 50 percent of the first cut.

The cobalt content of plants from four different pastures ranged between 0.048 and 0.082, 0.052, 0.054 and 0.090, 0.048 and 0.085 and 0.055 and 0.093 ppm of cobalt on dry matter basis. In all the four pastures, the minimum cobalt content was uniformly recorded towards the beginning of October and with the advent of winter the concentration of cobalt began to increase till it attained its peak value towards the end of January. There after, it gradually declined throughout the hot summer months, reaching its lowest value towards the close of monsoon (Singh, 1948).

Influence of irrigation: There was a 25 to 53 percent increase in cobalt content in whole maize plants, 30 to 35 percent in leaves, 20 to 55 percent in stem, and 31 to 60 percent in roots, due to irrigation Singh (1948). Deshpande (1959) supported these findings with the wheat plant.

Soil and plant: The low levels of cobalt in soils were found to be associated with low cobalt content in vegetation grown on them, while no relationship was observed with higher concentration of cobalt.

Deshpande (1959) tried the top-dressing of soil with cobalt sulphate solution containing 8 oz on half acre plot near Bombay, before monsoon. No appreciable increase in grass (hay) crop yield was observed. Askew and Dixon (1937a) found the cobalt content of pastures increased to 6.7 and 74 ppm from 0.20 and 0.24 ppm by applying 10 and 100 pounds, respectively of cobalt chloride as top-dressing per acre, of the affected soil. Leguminous plants took up more cobalt than grasses. Phosphate favoured the uptake, and lime depressed it. Hudd (1970) showed that heavy doses of nitrogen or nitrogen plus potash had no effect on cobalt content of grasses grown on that soil.

For many years, it was believed that plants growing on cobalt deficient soils were unaffected with respect to other nutrients and growth. But recent studies indicated that cobalt probably plays an essential part in symbiotic nitrogen fixation of the root nodules of legumes (Ahmed and Evans, 1959; Hallsworth *et al.*, 1960; Reiserhauer, 1960) and significant growth responses to cobalt by clover growing under field conditions were reported in Australia by Powrie (1960). The essentiality of cobalt for leguminous plants was established recently by Delwiche *et al.* (1961). Scattered responses to the application

of cobalt for non-leguminous forages are available in literature, but its essentiality for them has not been established yet.

According to Iyer (1958) and Iyer and Satyanarayana (1958a,b) legumes has a higher level of cobalt than the grasses. Reddy and Mehta (1961) analysed twenty-four grasses and cereal straws and found them to contain 0.36 to 0.64 ppm cobalt, respectively. Average cobalt content of six fodders varied from 0.23 to 0.79 ppm. The cobalt content of some of the crops grown in India is given in the Table A (appendix).

(c) Cobalt in Animal Tissue and Organs:

The earliest reference to the cobalt content of animal organ was that of Bertrand and Macheboeuf (1925, 1926). Later on Shils and McCollum (1942) observed that cobalt occurred in highest concentrations in glandular organs especially the liver, pancreas, spleen and kidneys. Stare and Elvehjem (1933) could not detect cobalt in the organs of healthy animals unless cobalt had been administered. Yet Askew and Dixon (1937) reported the concentrations of cobalt in animal tissues suffering from cobalt deficiency.

Askew (1937) demonstrated that the concentrations of cobalt in the tissues were reduced below normal during cobalt deficiency and it could be increased beyond normal levels by supplementation of cobalt through the diets or by injections. He reported the average values to be 0.05 and 0.11 ppm in the liver before and after the treatment. Morton Mains experiments (Dixon, 1937) showed that the storage of cobalt took place mostly in the

(1948) reported mean of 0.09 and 0.34 ppm of cobalt in the liver of 'coasty' and 'healthy' sheep, respectively. From his extensive studies in New Zealand, McNaught (1948) suggested that 0.04 to 0.06 ppm cobalt or less in the liver of sheep and cattle indicated cobalt deficiency and 0.08 to 0.12 ppm or higher indicated a satisfactory cobalt status.

Singh (1948) examined the cobalt status of the organs of the healthy animals and reported the cobalt concentration to be 0.088 to 0.36 in liver, 0.028 to 0.064 ppm in spleen, 0.012 to 0.036 ppm in heart, 0.042 to 0.089 ppm in pancreas and 0.038 to 0.091 ppm in kidneys on dry basis. Of the different organs, liver, pancreas and kidneys showed consistently higher values for cobalt content throughout the period of experimentation. Deshpande (1959) reported 0.16 to 0.30 ppm of cobalt in liver samples of cattle and buffalo, collected from different slaughter houses in Bombay state.

Studies on the distribution of radio-active cobalt following oral and parenteral administration indicated that the retained cobalt was taken up by all the tissues (Braude *et al.*, 1949; Rothery *et al.*, 1953). The highest concentration occurred in liver and kidneys, while spleen and pancreas had lower concentration of cobalt (Lee and Wolterink, 1955).

7. Cobalt in Blood, Anemia and Haematopoietic Effect:

Askew and Dixon (1937) were probably the first to report the cobalt content of the blood of normal and affected animals.

They reported the blood cobalt content to be 0.025 and 0.07 ppm (2.5 to 7.0 µg per 100 ml) in the blood of control and treated animals.

Association of cobalt with haematopoietic function has now been generally accepted since the observations of Waltner and Waltner (1929). Subsequently, the phenomenon has been confirmed by Orten (1936), Seal and Ahman (1937), Corner (1939), Kleinberg *et al* (1939), Polonovski and Breskas (1939), Frost *et al* (1940), Killhaun (1941), Dorrance *et al* (1943), Maynard and Smith (1947), Marston (1952), Smith and Loosli (1957), Underwood (1962), Hammerman (1970) to mention only a few of the workers in the field.

Cobalt in the form of vitamin B₁₂ was seen to be inter-related with iron and copper in haematopoiesis and, thus, indirectly involved with molybdenum. Since the anaemia of cobalt deficiency characteristically followed by anorexia. It was frequently stated that the anaemia was probably not the primary sequela, but was rather secondary to appetite failure. It meant that there was no direct action of cobalt on haemoglobin and/or erythrocyte formation (Smith and Loosli, 1957). Secondly, the observations were in general agreement with that, the anaemia of cobalt deficiency responded slowly and variably, following either cobalt or vitamin B₁₂ administration. So much so, the criterion of treatment of it is considered to be secondary to appetite response, (Gawthorne, *et al* 1966).

Singh (1948) reported the cobalt content of blood to be 0.012 to 0.030 ppm for the cattle of northern India. An

appreciable amount of this metal must occur in blood in the forms other than vitamin B₁₂. In cobalt deficiency, the vitamin B₁₂ concentration in sheep blood fell rapidly and on oral administration of cobalt the level rose rapidly (Kercher and Smith 1955 and 1956).

Anken (1939) and Underwood and Beck (1941) did not observe any difference in haemoglobin level of sheep grazing in affected areas and that of control. While studying the necessity of cobalt in bovine nutrition, Hal and Ahman (1937) observed that the concentration of haemoglobin per cell volume was higher in animals receiving cobalt. No significant difference in the total number of leucocytes was observed.

Filmer and Underwood (1937) noted a rise in haemoglobin levels in affected animals as a result of cobalt treatment. Geyer *et al* (1945), Pope *et al* (1947), Smith *et al* (1951) observed that cobalt treatment affected haemoglobin formation and recorded a positive effect. Ray *et al* (1948) reported that administration of cobalt to sheep brought about a slow and often erratic haemoglobin response and indirect effect upon haemopoietic centres.

Keener *et al* (1949) noted a greater haemoglobin and haematocrit values when cobalt was given to calves at the rate of 50 mg per 100 lb body weight per day. Eganov (1958) gave 0.2 mg cobalt chloride per kg body weight to calves. The results of this experiment showed that the haemoglobin values and blood protein values were increased. Medulinskas (1959)

conducted experiments on sheep extending over a period of eight months. The animals were supplemented with 5 mg cobalt chloride per head per day. The increase in haemoglobin and blood volume was noted. Similar findings were reported by Rudin (1959), Kaminski and Jzig (1961) noted that when 10 healthy sheep were fed 20 mg of cobalt nitrate per head daily, the red cell count in the blood raised from 8.2 to over 10 million per cmm after 20 to 30 days, whereas haemoglobin increased from 65 to 74 per cent after 20 to 30 days. But the white blood cell picture was not affected. In sheep Kolesov *et al* (1961) observed increased values of haemoglobin and red cell count as well as serum protein on cobalt supplementation, which had no effect on the white cell count. On the other hand, Ganguli (1963) did not observe any statistically significant difference due to cobalt supplementation on growing Marwari heifers in the values of haemoglobin and packed cell volume.

No changes in the blood picture were observed consequent to feeding cobalt deficient diet for five months to the Kumaoni hill bullocks (Singh, 1948). The deterioration in the erythrocyte count was found to set in after 6 months of introducing the cobalt deficient ration. However, by the end of experimental period of eight months, the red blood cell count haemoglobin concentration and the corpuscular volume dropped by 24.80, 24.69 and 21.50 percent, respectively. However, at 0.083 ppm cobalt level of dry matter, the blood picture returned to normal limits. The mean corpuscular volume varied within the normal limits and was unaffected by further cobalt feeding.

The chemical constituents of blood, serum or plasma exhibited normal range throughout the experiment, irrespective of cobalt concentration in the ration. Serum protein was not affected. The first visible symptom of cobalt deficiency in the ration as judged in terms of cobalt concentration in the blood appeared during the fourth month of the experiment and continued with increasing rapidity with prolonged cobalt starvation. Supplementation of the ration with cobalt immediately resulted in the restoration of normal level of blood cobalt, which however, remained constant even though the level of intake was raised by more than 100 percent.

Singh (1964) reported after supplementing 0.5 and 1.0 mg of cobalt to calves per day, that cobalt had no statistically significant effect on haemoglobin, iron and serum protein values, upto three months of supplementation, after which the cobalt supplementation resulted in an increase of packed cell volume ($P < 0.05$). Annakar (1965) studied the effect of two levels of supplementation i.e., 0.5 and 2.0 mg of cobalt on growing kids, which were fed urea. There was little increase in the serum protein content of the 0.5 mg supplementation group.

Gupta (1968) did not observe any change in either red blood cell count or haemoglobin by supplementing the ration of cow and buffalo calves with 50 mg of cobalt per head per week. Ibbitson *et al* (1970) observed a decrease in cellularity of the bone marrow in sheep fed cobalt deficient diet, which was even supplemented with vitamin B₁₂. This was accompanied by the reduction in white blood cell count and low rate of erythropoiesis. Parental administration of cobalt had no effect on

this condition. Pal *et al* (1970) did not observe any appreciable difference in total red blood cell count, haemoglobin, PCV serum calcium and inorganic phosphorus on supplementation of the ration of Haryana calves with copper and cobalt. But white blood cell count and caeruloplasmin copper were found to be increased as a result of supplementation.

Pachalag *et al* (1972) reported that no significant increase was seen in haemoglobin, red blood cell count, PCV and white blood cell count as the result of supplementing cobalt along or cobalt and copper. Anrit Kumar *et al* (1973) also did not notice any significant increase in the haemoglobin, R.B.C. count, W.B.C. count and PCV in the blood of cross-bred heifer, as^a result of supplementing the ration of heifers with both copper and cobalt..

8. Cobalt in Milk:

Dingle and Sheldon (1938) felt that 'the complete absence of cobalt in the milk as reported by Dreight (1929) was somewhat unexpected, for it was an element with active biological properties'. In 1933, Stare and Elvehjem concluded that the cobalt content of milk was less than 0.01 mg per 100 mg. Underwood and Elvehjem (1938) reported a range of 8 to 16 µg with an average of 11 µg of cobalt per litre. Ellis and Thompson (1945) reported 0.64 µg cobalt per litre of milk. The other values recorded ranged from 1 µg (Sylvester and Limpitt, 1940) to 15 µg per litre of milk (Blumberg and Rask, 1933).

Askew (1942) studied the influence of cobalt in the feed or supplemental cobalt on the cobalt content of milk. He

reported that cows having access to cobaltized salt licks produced milk with 0.020 to 0.022 ppm as compared to 0.010 to 0.015 ppm of cobalt in the milk of the animals of control group. Comar *et al* (1946) observed a very small amount of cobalt in milk when the radio-active element was introduced into the blood stream of cows. However, when it was introduced directly into the rumen, cobalt was not detected in the milk produced. Archibald (1947) supplemented the ration of eight cows with 500 mg of cobaltous acetate per day for a period of two months and recorded consistent rise in the concentration of cobalt in milk. The average increase was found to be four folds. The milk from cows receiving the supplement averaged to 2.4 μ g of cobalt per litre, in contrast to 0.6 μ g of cobalt per litre, when the cows were on control ration.

The average cobalt content of colostrum of cows was 4 to 10 times higher than their milk (Lee and Wolternik, 1953). The concentration of cobalt in the colostrum of cows was found to be increased by prepartum cobalt supplementation of the ration (Thompson and Ellis, 1947).

Similarly, the vitamin B₁₂ content of milk could be increased by cobalt supplementation of the ration (Hart and Andrews, 1959; Moineuddin *et al*, 1953; O'Halloran and Skerman, 1961). Hart and Andrews found the vitamin B₁₂ content of the milk of ewes treated with cobalt pellet to average 10.3 μ g per litre as compared with a mean of 2.5 μ g per litre as compared with a mean of 2.5 μ g per litre of the ewes of control group.

Contrary to the earlier findings, supplementing the cobalt adequate rations of cows with cobalt had been shown to be ineffective in raising the vitamin B₁₂ activity of the milk (Hartman and Dryden, 1952).

Vsyakikh (1952) reported that some lambs that were not given cobalt supplement, developed equally well as those given the supplement. This was due to the fact that these lambs received equal cobalt from the milk of ewes, whose ration was supplemented with cobalt. This indicated that supplemented cobalt was secreted through milk.

Kiermeier and Winkelmann (1961) reported, after examining 500 samples of milk, that the range of cobalt content of milk of cows was 0.00 to 2.3 mg with the average of 0.84 mg per kg of milk. Pilon *et al* (1968) reported the cobalt content of ewes milk to be 1 to 6 μ g per litre, as a result of supplementing the diet with 8.0 mg of cobalt chloride per day per head. Gadzhiev *et al* (1971) reported 9.76 to 16.84 μ g of cobalt per 100 ml of ewes' milk, which was ten times that of cows' milk. They had analysed the milk samples of ewes on winter pastures, after one month of lambing.

9. Influence of Cobalt on Milk Production and Composition:

The influence of cobalt on the secretion of milk by dairy cattle and its composition has not been the subject of intensive study. However, Dixon (1957) reported that no significant benefit in increasing the milk yield, was seen, as a result of addition of cobalt to the ration of cows in sick areas of South

land of New Zealand. Contrary to these observations, Keener *et al* (1944) obtained an increase in the milk yield, consequent to the feeding of cobalt to the cows, which were described as completely 'run down' in a well managed herd and did not respond to other treatments. Similarly, Hamilton (1946) found an increase in the milk yield of a three year old cows from 13.5 litres to 18.0 litres per day, by feeding one table spoonful of cobalt sulphate solution, which contained one ounce of cobalt sulphate per gallon of water.

Skerman *et al* (1961) studied the effect of implementing cobalt bullets in the rumen on milk production on two dairy herd from cobalt deficient areas. But the cows did not exhibit any symptoms of cobalt deficiency. There was an increase in the milk production by 1,150 lbs (4% F.C.M.) per cow and also the vitamin B₁₂ activity of the milk of the bulletted cows was higher.

O'Halloran and Skerman (1961) treated the pregnant ewes with cobaltic oxide pellets and noticed the colostrum and milk to have higher vitamin B₁₂ activity than that of untreated ewes. They confirmed the earlier findings of Hart and Andrews (1959), who obtained higher vitamin B₁₂ activity in the milk by the administration of cobalt pellets to lactating ewes.

Supplementing with cobalt the maize silage fed to cows was found to increase the milk fat content by 0.5 to 0.6 percentage units. Similar but less pronounced effect resulted by the introduction of cobalt chloride solution directly into the

(Askew and Dixon, 1936; Wunsch, 1939) and the ease with which polycythaemia could be produced by the oral administration of cobalt (Josland, 1936) were the excellent proofs for the probable site of absorption of cobalt. Cobalt once absorbed, it is excreted partially by the kidney and partially by intestine and the route of excretion largely depends on the nature and mode of the administration of the supplement. Askew and Josland (1937) drenched sheep with large single dose of stable cobalt chloride. They found virtually the whole of the dose was excreted in the first 5 days. Out of this 2% was excreted in urine and remaining in faeces, apart from the little quantity retained in the body. Major portion of the excretion was within the first 24 to 48 hours.

Intersteiner (1931) found that divalent cobalt was more rapidly eliminated than trivalent cobalt. Simensen (1939) recovered in the urine 80% of $\text{Co}_3\text{Co}(\text{NH}_3)_4 \text{Cl}$, administered subcutaneously to rabbits, within the next 24 hours. This compound was excreted unchanged. Mascherpa and Perito (1931), who administered cobalt chloride subcutaneously to guinea pigs, recovered less than half the dose in the urine, during the following 10 days. The findings of Kent and McCance (1941) confirmed, in broader outline, the results of previous workers and was in close conformity with the results reported by Penati and Ruata (1940), Greenberg *et al* (1943); Montgomery *et al* (1943); Widdowson and McCance (1944) and Shelton *et al* (1946).

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The metabolic fate of cobalt was studied by various investigators in a wide range of species, using radio-active form of the

metal (Comar *et al.*, 1946; Comar and Davis, 1947; Keener *et al.*, 1950; Monroe, *et al.*, 1952; Rothery *et al.*, 1953; Lee and Wolferink, 1955). The salient findings of these studies were that salts when fed were eliminated mainly through the faeces (80%) with only minor amounts being excreted through urine (0.5%); the liver had the highest concentration of cobalt; and a significant excretion occurred through bile. When injected intravenously, cobalt rapidly disappeared from the blood and 65% of this was excreted in the urine and about 30% in the faeces. When small amounts of Co^{60} were injected, practically none was found in the rumen; whereas, much larger amounts permitted some accumulation in the rumen contents. The cobalt of both the bile and urine existed partly in bound forms other than as vitamin B_{12} (Lee and Wolferink, 1955).

In ruminants, the time required to reach 'uniform labelling' was longer following the injection (Comar, 1948). After the oral administration, Co^{60} was more slowly and less completely absorbed, indicating an immobilization of this element by the intestinal contents (Monroe *et al.*, 1952). It was made clear that this was due to the binding of cobalt by the rumen micro-organisms as vitamin B_{12} and related compounds. Ruminants were found to differ from other species also in that the injected cobalt accumulated in the liver, resulting in a very high concentration of cobalt in liver (Comar and Davis, 1947).

Singh (1948) reported the average absorption of the orally administered cobalt was of the order of 23.2% of the total intake at the minimum requirement level. When the

intake was raised to 0.083 ppm of the dry matter of ration, there was a corresponding increase in the absorption. However, further increase in the absorption was not noticed by increasing the level of intake to 0.128 ppm of the ration (on dry matter basis). About 60 to 70 percent of the absorbed cobalt was found in the urine and the rest was accounted for the retention by the animal.

11. Mode of Action of Cobalt:

It is of much interest to note that horses and other non-ruminants could thrive well on pastures, where sheep and cattle showed severe symptoms of cobalt deficiency. It was earlier pointed out that the laboratory animals failed to develop cobalt deficiency on the diets having only traces of cobalt. These indicate that function of cobalt is associated with the ruminant stomach.

Administration of liver and liver extract to animals suffering from cobalt deficiency was found to be beneficial. However, equivalent amounts of liver ash was found to be ineffective in this respect (Filmer, 1933; Filmer and Underwood, 1937). Further evidences were furnished by varying the route of administration of cobalt. As early as 1944, McCance and Widdowson showed that cobalt administered parenterally to cobalt deficient sheep was not therapeutically effective. Marston and Lee (1949) published their results, which demonstrated that 1 mg of cobalt fed per day per head to deficient lamb was of therapeutic value, whereas the same amount when administered parenterally was ineffective.

Even though the cobalt contents of blood and liver increased above normal, by the injection of cobalt, the same was not effective in alleviating the deficiency symptoms (Marston and Lee, 1949). This was confirmed by Gall *et al* (1949) and Smith *et al* (1950). On the other hand, Keener and Percival (1950), Kercher and Smith (1955) and Ray *et al* (1948) reported that there was slow response in deficient lambs given cobalt salts parenterally, but the quantities required were much larger than the oral doses i.e., more than 1 mg of cobalt per day per head.

These varying observations seem best explained on quantitative basis. These workers thought that with the high level of cobalt injections, enough of the element reached the rumen through rumen wall, or saliva to stimulate the slow response. The fact remains, however, that a quantity of cobalt which when fed orally elicits a good response and will not do so when given parenterally, and thus attention was focused on the intestinal tract as the probable site of

Furthermore, Phillipson found that 0.1 mg of cobalt per day per head, which was effective when fed and was not effective when introduced directly into the duodenum. Then the attention was focused on the probable area of the intestinal tract that was involved, namely the stomach. This was because of the fact that the lower part of the intestinal tract cannot be involved, as the path of excretion of cobalt is bile.

Meanwhile Gall *et al* (1949a) observed that the number of bacteria per gramme of rumen content were double, in the animals

receiving adequate dietary cobalt, as compared to those receiving inadequate cobalt in their diets. Call and Associates (1949b) in another study found both the number and type of rumen bacteria were adversely affected in cobalt deficient lambs. The species of bacteria of rumen that were affected were not known.

It was at this stage in studies on cobalt metabolism that the number of developments made it apparent that cobalt functioned in the rumen in the synthesis of a factor needed by the ruminants. Presumably, this was a compound in which cobalt was necessary or for which cobalt was required in its formation. The revelation that anti-pernicious anaemia factor of liver was a compound containing 4% of this metal (Smith, 1948; Rickers *et al*, 1948) was an important step in this study. This compound later designated as vitamin B₁₂, became the object of intensive investigation, culminating in the establishment of its total structural formula in 1955 (Hodgkin *et al*, 1956; Bonnett *et al*, 1955).

Subsequent work indicated that the effect of the anti-pernicious anemia factor of liver and vitamin B₁₂ was highly correlated in the treatment of sheep suffering from cobalt deficiency. Later it was found that vitamin B₁₂ was indeed effective, when injected to sheep suffering from cobalt deficiency (Becker and Smith, 1951a,b). And complete remission of all signs of cobalt deficiency in lambs was secured with parenteral administration of vitamin B₁₂ (Smith *et al*, 1951)

Thus, it was confirmed that cobalt deficiency in ruminants was actually that of vitamin B₁₂, due to the inability of the rumen micro-organisms to synthesize sufficient vitamin B₁₂ in the absence of adequate dietary cobalt. This provided strong presumptive evidence that the whole function of cobalt could be accounted for in terms of the activities of this vitamin. However, many other cobalt containing compounds also occurred in the rumen, in addition to vitamin B₁₂, ~~such as~~ ^a (cyanocobalamin) and these were found to be physiologically inactive in body tissues (Ford *et al*, 1952; Porter, 1953; Hine and Dawbarn, 1954; Johnson and Bentley, 1957). The effect of cobalt on the synthesis of vitamin B₁₂, by the rumen micro-organisms was estimated by Johnson and Bentley (1957). These workers in *in vitro* fermentation studies recorded a beneficial effect of cobalt on the synthesis of vitamin B₁₂ and similar compounds.

Marston *et al* (1951) showed that when the concentration of cobalt dropped below a critical level of 20 ug per ml of rumen liquor, and the rate of vitamin B₁₂ synthesis was also reduced below the animals' requirements. There was no evidence that the synthesis of vitamin B₁₂ was possible within the body tissues (Kercher and Smith, 1955). Hence, the ruminants ultimately depend entirely upon their symbiotic gastrointestinal flora, for the synthesis of vitamin B₁₂ (Hale *et al*, 1950; Gall *et al*, 1951; Hoekstra *et al*, 1952; Porter, 1953; Hine and Dawbarn, 1954).

Hine and Dawbarn (1954) showed that the rumen micro-organisms convert a considerable proportion of the cobalt

available to them into compounds similar to vitamin B₁₂, which were physiologically of no use to the animal. It was not clear whether these compounds were necessary for the normal microbial activity in the rumen, thus providing an additional function for cobalt in ruminant nutrition. This was pointed out by Ford et al (1952) and Porter (1953). However, it was quite obvious that only a small porportion of the total dietary cobalt was converted into true vitamin B₁₂, which could be utilized by ruminant tissues. Marston and co-workers (1961) attributed the non-disappearance of propionic^{acid} from the blood of cobalt deficient animals to be the ^{cause for} loss of appetite in cobalt deficient animals. And also the conversion of propionate to succinate was found to be inefficient as the result of low levels of vitamin B₁₂ in the rumen.

Since then numerous investigations were conducted, which indicated that in ruminants, cobalt exerted the physiological effect through vitamin B₁₂, the existence of which was established to be only in the rumen (Hale et al, 1950; Ford et al, 1951, 1952; Pfiffner et al, 1952; Porter, 1953).

12. Cobalt in relation to feed consumption and digestibility of nutrients:

The effect of cobalt supplementation of the ration of cobalt-deficient calves was studied by Neal and Ahman (1937). They showed that the animals receiving cobalt consumed much more feed than those of the same weight and receiving no cobalt. In cobalt-deficient sheep, administration of cobalt

salt at the rate of 1 mg of elemental cobalt per head per day brought about a rapid response in appetite and feed consumption (Ray *et al.*, 1948; Smith *et al.*, 1950).

Becker *et al.* (1949) observed that the ether soluble and nitrogen free fractions of the ration were digested more efficiently by the lambs, which were fed cobalt salts while significantly greater digestibility of crude fibre was seen in deficient lambs. From their observations, they concluded that cobalt deficiency did not markedly affect the cellulose splitting organisms. The higher digestibility of crude fibre in deficient lambs was attributed to the lower feed intake and also the retention of the ingesta in the rumen for longer time.

Keener and Percival (1950) noticed an increased feed intake and water consumption as well as gain in body weight as a result of supplementing the ration of cobalt deficient animals with cobalt. Bednjagin and Budnaja (1961) also reported similar observations. Stewart (1963) concluded that the effect of cobalt deficiency in diets of lambs was to restrict the appetite which was probably due to the reduced microbial digestion in rumen and consequent slower passage of ingesta from the rumen.

Salsbury *et al.* (1956) in *in vitro* experiments observed that cellulose digestion by rumen micro-organisms was slightly depressed by 12 ppm of supplemental cobalt, and when the cobalt level was 100 ppm, the depression was marked. Also in *in vitro* fermentation studies, Johnson and Bentley (1957) could

not increase the rate of cellulose digestion by adding cobalt at a level of 0.01 ppm and at a level of 1.00ppm the organisms were found to show inhibitory effects. Boganov (1958)

Boganov (1958) gave 0.2 mg of cobalt chloride per kg body weight to the calves and observed increased digestibility of crude protein and fibre as well as absorption and utilization of nitrogen. But the digestibility of nitrogen free extract was found to be reduced.

Hubbert *et al* (1958) using washed cell suspension showed that in vitro studies on addition of cobalt to the deficient medium, the cellulose digestion did not increase. On the other hand, as little as 5 ug of cobalt per ml of fermentation medium was found to depress the cellulose digestion by approximately 12% as compared to the amount digested in cobalt deficient medium. These results were in agreement with those of Hall (1955).

Modjanov (1959) observed an improvement in the digestibility of crude fibre and utilization of crude protein in sheep receiving 2 mg of cobalt chloride daily over a basal ration supplying 0.13 mg or above of cobalt daily. Suter (1962) reported that daily supplementation of 0.2 mg of cobalt per 100 lb body weight was favourable to rumen micro-flora in the digestion of cellulose.

Yel and Saw (1963) reported that feeding of 0.1 mg of cobalt chloride per kg body weight increased both the flow of saliva from the parotid gland as well as the amount of calcium, phosphorus, potassium, chloride, sodium bicarbonate

in the digestibility of ether extract, while that of dry matter, crude protein, and nitrogen free extracts were not influenced.

Singh (1964) and Singh et al (1970) reported that there were no significant differences in feed intake as well as digestibilities of dry matter, crude protein and ether extract between the calves receiving two levels of cobalt supplement (0.5 and 1.0 mg of cobalt daily) and those of control group. The digestibility of crude fibre was lowered due to the cobalt supplementation. Water intake was more in the supplemented animals. Nitrogen free extract digestibility was more in cobalt supplemented group, but was not statistically significant. Nitrogen retention was not statistically different in between the groups. Maheshwari (1964) also concluded that there was no significant difference between the treated and untreated sheep, as far as the average digestibility co-efficient of various organic nutrients were concerned. He supplemented the ration of 20 Bikaneri adult sheep on pasture with 70 mg of cobalt chloride at 15 day intervals.

Anaokar (1965) in urea and cobalt supplementation studies on growing lambs, observed that there was no significant difference in dry matter, crude protein, crude fibre and nitrogen free extract digestibilities at both the levels of cobalt supplementation. The nitrogen balances were not significantly different in the control and 0.5 mg supplemented groups. However, 2.0 mg supplementation resulted in a significantly higher nitrogen balance than that at 0.5 mg level of supplementation. Balances of calcium, phosphorus were positive in the animals of all the groups, without any significant differences between them.

Gupta (1968) supplemented 50 mg of cobalt to cow and buffalo calves on pasture per head per week and noticed no significant difference in the digestibility co-efficients of nutrients in either of the calves.

Kapoor (1968) and Kapoor and Mahadevan (1970b) reported that no significant difference as a result of supplementation of 12.64 mg of copper and 1.32 mg of cobalt per day on the balances of nitrogen, calcium, phosphorus, copper and cobalt in Haryana animals. All the balances were positive. Intake of dry matter, digestible crude protein and total digestible nutrients were also not affected.

Pal (1971) studied the effect of cobalt sulphate supplementation at the rate of 0.25 and 0.75 mg of cobalt sulphate per day per kg live weight on the feed utilization in sheep. Dry matter consumption and its digestibility were maximum at the low level of supplementation, followed in order by the 'control' and 'high' level of cobalt supplementation. Digestibilities of crude protein, ether extractives, crude h fibre, nitrogen free extract and total carbohydrates were higher in the supplemented animals than those of control group animals. Retention of nitrogen, calcium and phosphorus was higher in supplemented animals. However, statistical analysis of the results did not show any significant difference in the feed utilization by the animals of the supplemented groups. Ram (1971) supported the above findings in his studies with the supplementation level of 0.25 mg cobalt sulphate per kg body weight.

13. Cobalt requirements of ruminants:

As reported by Andrews (1956), young growing sheep were the most sensitive to cobalt deficiency, followed by mature sheep, calves between 6 and 18 months of age and lastly mature cattle, in the order mentioned. Severe cobalt deficiency was found to develop inevitably in sheep and cattle depending on herbage containing 0.02 to 0.05 ppm cobalt on the dry matter basis.

The levels of supplementation of cobalt to combat this deficiency as suggested from different laboratories were many and varied (Askew and Dixon, 1936; Filmer and Underwood, 1937; Deal and Ahman, 1937; Askew and Dixon, 1937; Browning, 1937; Hopkirk, 1938). These appeared to have originated consequent to abundant and accumulated empirical experience of various investigators in combating the deficiency disease prevalent in their region. Therefore, these signify the therapeutic doses, varying with the intensity of the deficiency in plant species, seasonal changes, grazing habits, soil contamination etc., for particular laboratories, rather than the minimum requirements of this element. However, extremely minute requirements of this element be, as judged by the size of the therapeutic dose, the response was quite remarkable.

Filmer and Underwood (1937) showed that supplementation of the diets of sheep in Western Australia, with 0.05 mg of cobalt daily was found to produce some improvement, while a supplementation level of 0.1 mg daily produced optimum growth and health of the sheep. The minimum cobalt requirement of sheep was closely approximated by New Zealand workers. These studies

well summarized by Russell (1944). It was found that pastures containing 0.07 ppm cobalt on dry matter basis were on the borderline of adequacy. Subsequently, Marston (1952) found that a supplement at the rate of 0.05 mg cobalt daily administered orally thrice a week was just sufficient for the growth of sheep on such pastures. An over-all dietary level of 0.08 ppm cobalt on dry matter basis in the ration was regarded as the minimum requirement for this species. This was in agreement with the observations of the other workers (Beck, 1958; Becker and Smith, 1951a; Maynard and Loosli, 1956 and Lee and Marston, 1969).

There is paucity of correct information on the specific requirement of cobalt for cattle. However, as indicated previously, the minimum cobalt requirements of bovine appear to be lower than those of sheep. But the exact extent is not precisely known. It has been shown, however, that supplements that furnish 0.3 to 1.0 mg of cobalt per day to cattle were found to be adequate for the maintenance of normal growth and health of cattle grazing on severely cobalt deficient pastures (Filmer and Underwood, 1937). Later, Richardson *et al* (1960) observed a significant improvement in the weight gain of dairy heifers by the cobalt treatment, when these animals were consuming such rations estimated to supply 1.2 mg cobalt per day.

The minimum requirement of cobalt for the bullocks has been found to be 0.0469 mg per 100 lbs body weight or 0.06 ppm. of the dry matter consumption. The values for growing calves and buffaloes in milk per 100 lbs body weight were 0.06 mg and 0.116

mg respectively. However the requirement in three groups of cattle was roughly varied within 0.05 to 0.07 ppm of dry matter consumption, (Singh, 1948). Brahm Dutt (1972) reported 0.1 ppm as the minimum requirement of cobalt in lacting cows.

However, according to National Research Council, U.S.A. (1963), the minimum cobalt requirements of cattle were not accurately defined.

14. Supplementation of cobalt - for prevention and treatment of cobalt deficiency:

Cobalt deficiency in ruminants is usually prevented or treated by either the application of cobalt containing fertilizer to the soils, top-dressing of pastures with cobalt salts, direct administration of supplementary cobalt to animals as drench or mixing with diet/mineral mixture or by injection of vitamin B₁₂. However, the last one is not practiced, as it is expensive and inconvenient method.

The most convenient, economical and widely practiced means of ensuring continuous and adequate supplies of cobalt to grazing stock in deficient areas was by the 'top-dressing' of the pastures with cobalt salts and the treatment of the deficient soils with the fertilizers containing cobalt salts. The latter helps the growth of leguminous component of the pastures (Hallsworth *et al.*, 1960 and Powrie, 1960). Application of 4 to 5 oz of cobalt sulphate biannually (Askew, 1949; and Rossiter *et al.*, 1948) or a single application of 20 oz of cobalt sulphate

per acre for five years was sufficient (Andrews, 1953), to bring the cobalt content of the herbage to the levels to meet the minimum requirements of animals fed on them.

The direct administration of the cobalt supplements was found to be easy, as the element could be easily incorporated into mineral mixture supplements. The inclusion of cobalt salt in such mixtures is now a common practice. However, there is no clear evidence that the unsupplemented rations were deficient in this element. Keener *et al* (1950) and Mittler (1954) working with radioactive cobalt carbonate and oxide found that cobalt was well absorbed and distributed all over the body of sheep and lambs. Only limited research work has been done concerning the biological availability of inorganic compounds, incorporated by this way. The carbonate, chloride, sulphate and nitrate forms of cobalt were proposed to be satisfactory dietary sources of the mineral (Ammerman, 1970; and Ammerman and Miller, 1972).

Under grazing conditions, providing cobalt-containing salt-licks or drenching of the animals with solutions of cobalt salts were also practiced since long, especially in sheep. Lee (1950) administered a drench containing 7 mg of cobalt once a week. Drenching can be done with large doses for longer intervals, but it was not found to produce optimum results (Filmer, 1941; Pope, 1971).

Contrary to iron and copper, which are stored in the body of the animals, the regular and frequent supply of optimum

doses of dietary cobalt was found to be most essential to overcome and prevent the deficiency symptoms in ruminants (Lee and Marston, 1969; Dewey *et al*, 1969; Marston, 1970; Griffiths, *et al* 1970). Since rumen is the primary site of action of cobalt, an adequate concentration of cobalt in the rumen liquor must be maintained to prevent, or cure the cobalt deficiency in the ruminants. Therefore, Dewey *et al* (1968) evolved a new procedure with advantages over the other to supply small amounts of cobalt constantly. The tendency of heavy foreign bodies to remain within the forestomach^{of}/ruminant was ingeniously taken advantage of, in this procedure. The pellets of cobalt oxide and clay known as 'cobalt bullets' having a specific gravity of 4.0, is delivered into oesophagus to be lodged in rumen or reticulum. This 'cobalt pellet therapy' was found to be equally effective (Skerman *et al*, 1959; and Richardson *et al*, 1960). Administration of a small screw and a pellet or two pellets at a time was found to keep the pellets clean and prevent coating of the pellet, which ensured constant and steady supply of cobalt.

Connolly and Poole (1967) designed a pellet containing layers of cotton gauze bandage impregnated with cobaltic oxide, which did not coat-over and thus remained effective for a longer period of time.

N.R.C. (1968) recommended that 60 to 100 gm of cobalt sulphate or 40 to 50 gm of cobalt carbonate per 100 kg of salt to be an effective supplement to feeds in most areas. Smith and Loosli (1957) suggested the addition of 0.5 to 1.0 oz of

cobalt salts per 100 lb of mineral mixture and added to the concentrate mixture at a level of 2 percent.

According to I.S.I. specifications, the mineral mixture should contain a minimum of 10.0 gm cobalt per 100 kg.

15. Cobalt in Reproduction:

Cobalt deficiency was reported by Russell and Duncan (1956) to result in infertility, abortion and the birth of weak calves. In these cases there was usually a loss of condition typical of cobalt deficiency. The 'border-line' cobalt deficiency also appeared to adversely affect the fertility in cows (Alderman, 1963). In a Shorthorn herd, grazing on cobalt-deficient land in which low blood copper levels among the calves had been demonstrated, the conception rate at first service of untreated cows was 53%, those given copper injection was 67% and those given cobalt bullet as well as copper injection was 93%. A beneficial effect of the additional cobalt may be obtained, where herds are fairly self-contained on soils derived from old red sand stone, particularly, if heavily limed or from certain lime-stone formations.

Donaldson et al (1964) studied the effect of cobalt bullet on fertility of Shorthorn heifers in Northern Coastal Queensland. They could not obtain any significant response to cobalt therapy.

Tossell Robin (1967) reviewed the effects of minerals including cobalt on the reproduction in many animals species.

16. Cobalt Toxicity and Polycythemia:

Cobalt, when administered orally or parenterally in amounts, excess of the normal requirements of the rats, induced polycythemia as was first shown by Waltner and Waltner (1929). This was confirmed by many research workers in all laboratory animals, pet animals birds and man. It also occurred in calves before the rumen development (Keener *et al*, 1949), but not in adult ruminants (Josland, 1937; Geyer *et al*, 1945; Becker and Smith, 1951). Polycythemia was found to be accompanied by augmentation of the blood volume, hyperplasia of bone marrow and reticulocytosis.

High dietary protein levels (Underwood, 1962) or Ethylenediamine-tetra-acetate (EDTA) (Child, 1951) was found to prevent the development of polycythemia. Concurrent injections of methionine, cystine, cysteine or histidine reduced the toxicity of high levels of orally ingested cobalt in rats or calves, by forming inactive complexes (Griffith *et al*, 1942).

Josland (1937) recorded a case of polycythemia in ewes with 5 mg cobalt per kg body weight daily. On the other hand, Geyer *et al* (1945) observed that the tolerance of cobalt in cattle was considerably high. Feeding as much as 50 mg per day of elemental cobalt to Holstein heifers weighing about 500 lb produced no polycythemia. Therefore, a very wide margin of safety, unlike copper, exists between toxicity level and the quantities of cobalt necessary to meet the nutritional requirements of sheep and cattle, as well as the treatment

levels. In fact, levels upto 100 times that normally supplied in diets were quite well tolerated by sheep and cattle (Ely *et al*, 1948; Becker and Smith, 1951; Dunn *et al*, 1952).

Dosages of 200 to 500 mg daily per 100 kg body weight of cobalt resulted in severely depressed appetite, loss of body weight in sheep. Anaemia was produced at higher levels of cobalt (Becker and Smith, 1951a,b).

Even though the adult animals very highly tolerant, the calves appeared to have lower tolerance than sheep. In calves more than 40 mg cobalt per 100 lb live weight usually resulted in a depression of appetite, lacrimation, salivation, dyspnoea and loss of weight (Ely *et al*, 1948; Keener *et al*, 1949)

A typical case of cobalt toxicity was recorded by Davis (1957) in Florida, due to mistake in the incorporation of cobalt salt in mineral mixture. After the feeding of this mixture, milk flow was depressed by more than 75 percent within 2 weeks. The animals went off feed and a dull, listless attitude was apparent. Removal of this mineral mixture resulted in rapid recovery within 5 to 6 days.

17. Cobalt in Relation to other Minerals and Dietary Factors:

The inter-relationship of cobalt with iron and copper through vitamin B₁₂ in haematopoiesis has been recognised (Underwood, 1962). An inter-relationship has been suggested between cobalt and selenium by Gardiner (1966). Cobalt-deficient sheep were more susceptible to selenium toxicity

than sheep with adequate cobalt nutrition. Andrews *et al* (1964) obtained definite responses to dietary supplementation of selenium, in areas of New Zealand, known to have soils deficient in cobalt. In further studies it was shown that sheep brought up on cobalt-deficient diets had significantly higher concentration of selenium in their kidneys. Wise *et al* (1968) demonstrated the metabolic relationships in sheep between cobalt and selenium in the white-muscle disease.

Chapman and Kidder (1964) studied interaction between supplemental cobalt and molybdenum in growing cattle. The administered cobalt (8 mg daily) resulted in higher blood haemoglobin and packed cell volume and significantly higher copper and lower iron levels in the liver. Increased dietary molybdenum (250 mg per day) resulted in decreased blood haemoglobin, packed cell volume and liver copper storage and increased iron in the liver.

CHAPTER - IV

MATERIALS AND METHODS

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MATERIALS AND METHODS

Studies on the trace mineral, copper and cobalt supplementation on blood constituents, milk composition, milk yield and reproductive performance of cross-bred cows were conducted at Southern Regional Station of National Dairy Research Institute, Bangalore, India.

The station is located at the latitude 77.6° and longitude 13.1° . The temperature varies from 8.0°C in winter to 40.0°C in summer, with an average of 27.0°C . The average rain fall is about 87.5 cm per annum and the relative humidity is about 70.0%. The elevation of Bangalore is 948.8 meters above the mean sea level.

MATERIALS:

A successful evaluation of the results of an experiment depends upon the accuracy and sensitivity of the experimental procedures, techniques employed and the materials used. In selecting the materials and employing the techniques for carrying out the investigation, this fact was always kept in view along with the limitations of the facilities.

(a) Selection and grouping of animals:

The experiments were conducted on half-bred Jersey-Tharparkar lactating cows, which were in their early lactation

period. Twenty pregnant cows due to calve in the subsequent three months were selected for the experiments from the dairy herd. They were divided into four groups of five animals in each, taking into account their age, number of lactations completed, milk yield in previous lactation, and probable date of calving. One group served as control, while the other three served as experimental groups. Animals were added on their fifteenth day of calving, to the experiment as and when they were available. All the animals were maintained on the basal balanced ration and experimental group of animals were given the supplements at three different levels, in addition.

(b) Selection of basal ration:

The selection of the basal ration is an important feature, which required special attention and consideration for the efficient working of the experiment. Since the animals in the experiment were cows in early lactation, their nutritional requirements were best served by a varied diet. The roughage and concentrate components of the ration were selected so as to adequately meet their nutritional requirements, considering the total digestible nutrients, digestible protein, minerals and vitamins. To avoid variations in the composition of the basal ration, the total ingredients of the concentrate ration and dry roughages required for the entire experimental period were stored at a time. Green fodders i.e., para grass, guinea grass or hybrid maize were drawn on daily basis and made available to the animals during the trial periods. Common salt was fed at the rate of 50 gm per animal per day along with the concentrate ration.

(c) Composition of concentrate mixture:

The concentrate mixture consisted of groundnut cake, crushed maize and wheat bran. Computation of ration was done considering the body weights and milk yields of the animals, according to the standards laid down by Sen and Ray (1971). The weight of the animals ranged from 300 to 450 kg. The requirements of the nutrients by the animals were as follows:

Nutrients for maintenance of adult cattle per head per day

Live weight Kg	D.C.P. Kg	T.D.N. Kg	Calcium gm	Phosphorus gm
300	0.197	2.36	7.0	7.0
350	0.227	2.70	8.0	8.0
400	0.254	3.03	9.0	9.0
450	0.282	3.37	10.0	10.0

The nutrients required for milk production were calculated on the basis of milk yield corrected to 5% butter fat and the nutrients required for every kg of milk produced were as follows:

Nutrients for production per kg of milk to be added to the maintenance ration

Fat percent in milk	D.C.P. Kg	T.D.N. Kg	Calcium gm	Phosphorus gm
5.0	0.051	0.353	2.0	1.4

The average nutritive values of the ingredients selected for computing the concentrate mixture were as given below:

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Nutrients for production per kg of milk to be added to the maintenance ration

Fat percent in milk	D.C.P. Kg	T.D.N. Kg	Calcium gm	Phosphorus gm
5.0	0.051	0.363	2.0	1.4

The average nutritive values of the ingredients selected for computing the concentrate mixture were as given below:

the experimental animals were divided into four groups consisting of five animals in each. Group I was kept as control, on basal ration without any supplement, in both the phases. The ration of the animals of remaining three groups, namely group I, II and III, were supplemented with three levels of copper in the form of copper sulphate, in the first phase and with three levels of cobalt in the form of cobalt chloride in the second lap of the experiment.

Completely randomized design was used in the copper supplementation experiment, while randomized-block design was used in the cobalt supplementation trial.

(c) Collection of samples and observations recorded:

Blood samples from all the animals were drawn in the beginning and at twenty day intervals commencing from the 15th day of lactation and were examined for haematological constituents such as (i) haematocrit (PCV) value, (ii) haemoglobin (Hb) percentage, (iii) total erythrocyte (R.B.C.) count, (iv) total leucocyte (W.B.C.) count, (v) specific gravity and also biochemical constituents such as (i) serum protein, (ii) blood iron, (iii) blood copper and (iv) blood cobalt contents.

Milk samples from all the animals were collected daily from morning, noon and evening milkings from the 15th day of lactation. Aliquots were taken from well prepared samples, proportionate to the milk yield and stored in refrigerator with a drop of formalin. The composite samples of 15 days milk of each animal was analysed for (i) fat, (ii) protein

(iii) total solids, (iv) solids not fat, and (v) total ash, percentages.

The morning, noon and evening milk production of each animal was recorded daily.

Reproductive performance of the animals was watched as (i) first heat symptom exhibited after parturition and (ii) fruitful service number.

A metabolism trial was conducted at the end of the experimental period on eight animals (two animals of each group of control and experimental groups) for assessing the influence of copper supplementation on digestibility of nutrients and to observe the balances of nitrogen, calcium, phosphorus, copper and cobalt in the body.

(f) Period of experiment:

The experimental period for each of the trials started from the 15th day after parturition and continued till 200 days of lactation. The first phase of copper supplementation was started in the month of January 1970 and lasted upto September 1970. The second phase of cobalt supplementation was started in July 1971 and lasted upto July 1972.

(g) Supplementation levels:

The animals from the groups I, II and III were supplemented with three levels of trace element copper and cobalt, over and above what they were getting through their feed and fodder. Supplements were given as normal requirement level, one and

half times and two times for this class of animals prescribed by the N.R.C. (1966). The levels of supplementation were as follows:

Control group	Group I	Group II	Group III
(a) Copper:			
Nil	52.0 mg of elemental copper (200 mg of copper sulphate)	78.0 mg of elemental copper (300 mg of copper sulphate)	104.0 mg of elemental copper (400 mg of copper sulphate)
(b) Cobalt:			
Nil	4.07 mg of elemental cobalt (16 mg of cobalt chloride)	6.10 mg of elemental cobalt (24 mg of cobalt chloride)	8.13 mg of elemental cobalt (32 mg of cobalt chloride)

(b) Feeding and management of animals:

Feeding of concentrate mixture was done twice in a day at the time of milking. The appropriate dose of the trace mineral supplement in the form of solution of known concentration, was incorporated in the concentrate mixture. A pre-experimental period of 15 days was allowed, wherein the animals of all the groups were maintained on the same feed, fodder and managerial conditions (without the administration of the supplement to the animals of the experimental groups) to eliminate the residual effect, if any, of the previous feeding regime.

Greens were chaffed and fed to the animals in the morning time at about 10.00 AM, while the ragi straw was offered at about 1.00 PM.

The animals were let loose, after each milking, in the compound with a shed for exercise, free movement and exposure to sun shine. During this time the animals were watched for the symptoms of oestrus. The animals showing the symptoms were examined and inseminated within 24 hours.

At the end of each experimental phase, a metabolism trial of 7 days duration (Staples and Dinasson, 1961) was conducted on two animals from each group, to study the effect of copper and cobalt supplementation, if any, on the appetite, digestibility of the nutrients and mineral metabolism. All possible precautions were taken to avoid the metallic contamination during feeding, watering and collection of faeces, urine and milk. Enamelled and polythelene-wares were used throughout the experimental period.

METHODS:

Collection of blood:

Blood samples were collected from the jugular vein of the animals in the morning hours at about 9.00 AM, allowing sufficient time after morning milking, watering and feeding, to avoid the changes that may occur due to these, in some of the blood constituents. The blood was collected from the animals in standing position, secured to a strong fixed pole, to avoid excitement and struggling due to casting, which might result

in changes in the blood composition. The area over the jugular vein was cleaned and sterilized with rectified spirit. Jugular vein was occluded by mild digital pressure just before inserting the 15 gauge sterile stainless steel bleeding needle, to prevent any possible haemoconcentration (Katiyar, 1952). Blood was allowed to flow freely and first few ml of blood was allowed to go out as an additional precautionary measure to avoid any little contamination.

Blood samples were collected separately in oxalated 100 ml Earlenmeyer flasks, prepared by drying a thin film of anti-coagulant solution in a clean double distilled water rinsed flask, in hot air oven at $100 \pm 2^{\circ}\text{C}$, without giving any chance of metallic contamination. (Anti-coagulant solution used was a mixture of one percent ammonium oxalate and one percent potassium oxalate in the proportion of 3:2 and one ml of this solution was used for the collection of every 5 ml of blood. This double oxalated anti-coagulant suggested by Heller and Pal (1934) has a definite advantage over other ones, in preventing shrinkage of erythrocytes, making it particularly useful for the determination of packed cell volume, and in determining specific gravity of blood samples. While collecting and immediately after collection of blood, the flasks were gently rotated to facilitate thorough mixing of the blood with anti-coagulant. Flasks were closed (plugged) immediately after the collection of blood to prevent evaporation of the sample. This blood was used for the estimation of packed cell volume, haemoglobin, total erythrocyte and leucocyte counts, specific gravity, blood iron, blood copper and blood cobalt contents.

Blood samples were also drawn at the same time for serum, from each animal separately, in a clean, and dried 10 ml centrifuge tubes. They were kept in a slanting position for about two hours at room temperature and then chilled in a refrigerator at 4°C for clotting and separation of serum. Tubes were plugged with cotton to avoid any contamination. A clear straw coloured supernatant serum was transferred to other similar centrifuge tube without disturbing the clot and was stored in refrigerator for subsequent estimation of serum protein content.

Blood Analysis:

(A) Haematological Constituents:

(1) Packed Cell Volume (Haematocrit value):

Determination of PCV was done by the method described by Napier and Dass Gupta (1946). The oxalated whole blood was shaken gently to ensure thorough mixing of the cells and plasma. The blood samples were then filled into Adam's make Wintrobe Haematocrit tubes with capillary pipette upto the mark ten (Wintrobe, 1953). Tubes were then centrifuged at 3,000 r.p.m., for half an hour in a International centrifuge. Readings were taken and the tubes were further centrifuged for ten minutes to note any change in the readings. When there was no change between the two successive readings, the final reading was noted. Results were expressed as percent packed cells for the total blood volume in the tube. While noting the cell volume, fluffy layer was not taken into account.

(ii) Haemoglobin:

'Sahli's acid haematin method' as described by Sehalm (1961) was followed for the estimation of haemoglobin using original H.S. Hellige make Sahli's Haemoglobinometer. Blood on dilution with dilute hydrochloric acid form the brown compound known as haematin hydrochloride. This was then matched against the provided glass standard in the haemoglobinometer.

Employing special haemometer pipette, 20 cm of oxalated blood was accurately pipetted out into 0.1 N hydrochloric acid filled in Sahli's graduated square haemoglobin tubes, upto 2 g mark. The contents were thoroughly mixed and kept aside for 10 minutes to ensure complete reaction. The column was matched in Sahli's haemoglobinometer to brown glass standard diluting with distilled water and stirring with glass rod. The haemoglobin values were straight away read on the graduated scale.

The values were expressed as gm haemoglobin per 100 ml blood, i.e., gm percent.

(iii) Total erythrocyte (R.B.C.) count:

The enumeration of red blood cells was done according to the method described by Sehalm (1961) using haemocytometer (Thoma-Zeiss). A clean dry red blood cell diluting pipette was used. Whole blood after thorough mixing was drawn upto 0.5 mark and the volume was made up to the mark of 101 with the diluting fluid. Gower's solution was used as erythrocyte diluting fluid as it prevents agglutination of

One crystal of aniline gentian violet was added to differentiate it from the other diluting fluid, and to stain the nucleus slightly. Acetic acid destroyed the red blood corpuscles and mercuric chloride makes the white blood cell conspicuous. The cells were counted employing the same technique as followed in R.B.C. count using low power, in four large 'corner squares' comprised of 16 small squares of improved Neubauer's double counting chamber.

Calculation, W.B.C. in thousands/cmm = $n \times 50$,

where n is the total number of leucocytes in all the four big squares.

The results were expressed in thousands per cmm. of whole blood.

(v) Specific gravity (sp. gr.) of blood:

Determination of specific gravity of oxalated whole blood was done by copper sulphate solution method of Phillips *et al* (1950) as described by Hawk *et al* (1954). Standard solution of known specific gravity of copper sulphate were prepared ranging from 1.042 to 1.062, with the difference of 0.001 between the two consecutive solutions, which were stored in air-tight bottles. A small drop of well mixed oxalated blood was allowed to fall by gravity into a series of these bottles, from a height of about 3 cm from the surface of the copper sulphate solution in each bottle to avoid variations due to gravitational force. The pipette used to deliver the drop, was kept constant throughout the experiment to ensure the uniformity of the drop delivered. Within 10 to 15 seconds, the solutions were examined from the sides to see whether the

drop floated, remained suspended or sank in the solution. Specific gravity of the solution holding the blood drop in suspension was noted and which represented the specific gravity of the whole blood. It was expressed in absolute units.

(B) Biochemical Constituents:

Analytical methods:- All the containers and glass-ware were of pyrex glass. The glassware were cleaned with washing soda and thoroughly rinsed with tap water and were allowed to drain in enamel trays kept in slanting position. Then they were rinsed with distilled water and again with double glass distilled water. They were dried in oven. Some important glasswares were treated with warm dilute nitric acid for 24 hours and rinsed as described earlier and dried.

The reagents or chemicals used in the analysis were of analytical grade and of well known standard companies.

Distilled water used in all the stages as well as for the preparation of reagents/solutions, dilutions and washings were distilled for the second time in a well used all-glass apparatus.

In all the steps and procedures utmost care and precautions were taken to avoid any metallic contamination.

(1) Estimation of serum protein:

Two ml of clear straw coloured serum was transferred to a 100 ml Kjeldahl flask and 10 ml of concentrated AR sulphuric acid was added to it, followed by 0.2 g of copper sulphate. The contents of the flask were digested on heater

till it became clear and for a further period of one hour, ensuring that no carbon particles were left in the flask. Contents were transferred after cooling to distillation flask, and ammonium sulphate thus formed was distilled by steam with excess of sodium hydroxide to liberate ammonia which was collected in a conical flask containing known quantity of standard sulphuric acid, in presence of a drop of methyl red indicator. The excess of acid was back titrated with $N/7$ standard sodium hydroxide solution. Thus the exact amount of $N/7$ acid neutralized by the liberated ammonia was calculated by taking 1 ml of $N/7$ sulphuric acid to be equal to 0.002 gm of nitrogen and multiplied by 6.25 to get the protein content.

The results were expressed as gm percent of total serum protein in serum.

(ii) Estimation of iron:

Estimation of iron in blood, feed and fodder, dung, urine and milk was carried out by employing the colour reaction of ferric iron with thiocyanate, colorimetrically according to the method of Kennedy (1927) as described by Allport and Keyser (1957).

For the determination of iron, 1 ml of blood or known quantity of other samples was placed in (20 x 2.5 cm) test tube or Kjeldahl flask, depending upon the nature of the sample. To this 5 ml of concentrated AR sulphuric acid and 2 ml of 60 percent perchloric acid were added and the contents were digested over a low heat. In about 20 to 30 minutes the digestion mixture was colourless. Heating was continued till

a rapid ebullition with subsequent slow boiling and the characteristic dense fumes of sulphur dioxide, indicating the completion of digestion, appeared.

The digest was cooled, treated with a drop of concentrated nitric acid and diluted to 100 ml with water. To a 1 ml portion or appropriate quantity of diluted digest in a 50 ml volumetric flask were added 5 ml of 20 percent (w/v) aqueous solution of potassium thiocyanate, followed by accurately measured amyl alcohol. The contents were shaken for 4 to 5 minutes. The amyl alcohol layer was allowed to separate. Pink coloured amyl alcohol was taken out into test tubes and centrifuged for the removal of moisture for 5 minutes. Then the optical density of the pink coloured amyl alcohol was measured at 480 mμ in a Hilger Photo-electric Colorimeter set to zero with the reagent blank.

Standard for iron: In 100 ml of distilled water 0.7022 gm of ferrous ammonium sulphate was dissolved and to this, 5 ml of concentrated sulphuric acid were added. After warming slightly potassium permanganate (approximately 0.1 N) was mixed to this drop by drop while stirring till pink colour just appeared. The contents were transferred quantitatively to a 1 L volumetric flask and the volume was made upto the mark with distilled water. The flask was shaken to mix the contents thoroughly. This solution contained 0.1 mg of ferric iron/ml (Stock Standard solution).

To prepare the working standard solution, 10 ml of the stock standard solution (1 ml = 0.1 mg ferric iron) was

pipetted out into a 1 L volumetric flask and the volume was made upto the mark. This working standard solution contained 1 μg of ferric iron/ml.

Into series of 50 ml volumetric flasks were pipetted out the working standard solution from 1 to 10 ml (1 to 10 μg ferric iron) serially. The volume of the contents in all the flasks were made upto 10 ml with distilled water. In another flask 10 ml of distilled water was taken to serve as blank. A drop of nitric acid was added to all the flasks, followed by 5 ml of 20 percent (w/v) potassium thiocyanate and 10 ml of amyl alcohol. The contents of the flasks were shaken vigorously for about 4 to 5 minutes to extract the colour into amyl alcohol. After allowing to stand for two minutes for the amyl alcohol layer to separate, it was separated, then transferred to centrifuge tubes and centrifuged for 10 minutes to remove the moisture.

The optical densities of these amyl alcohol extracts of the standard solution were measured at 480 m μ in a Hilger Photo-electric colorimeter set to zero with the reagent blank.

Calculation:

$$\begin{aligned} \text{mg iron/100 ml blood (or } 100 \text{ g sample)} &= \frac{\text{Optical Density of unknown}}{\text{Optical Density of standard}} \times \frac{\mu\text{g iron in standard solution}}{1} \\ &\times \frac{100}{\text{amount of sample}} \times \frac{\text{Volume made up}}{\text{Aliquot taken}} \times \frac{1}{1000} \end{aligned}$$

(iii) Estimation of Copper:

Method of Eden and Green (1940) as described by Allport and Keyser (1957) with the modifications suggested by Sahai (1947) and Sahai and Kehar (1961) was adopted in the present studies. The exact procedure followed was as follows.

The method comprised of these four stages:

- (i) Digestion
- (ii) Deionization
- (iii) Colour development
- and (iv) Colour measurement.

Reagents: (All analar reagents were used)

- (a) Copper sulphate
- (b) Sulphuric acid
- (c) Perchloric acid (60%)
- (d) Nitric acid
- (e) Sodium pyrophosphate
- (f) Ammonia solution (sp. gr. 0.880)
- (g) Sodium diethyl-dithio-carbamate
- (h) Amyl alcohol

Standard copper solution: A solution containing 0.3928 gm pure copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) showing no sign of efflorescence was prepared with distilled water, and transferred to 1 L volumetric flask. To this, a drop of sulphuric acid was added to prevent the formation of basic copper sulphate on hydrolysis and the volume was made upto the mark with distilled water. This stock standard solution contained 100 μg copper/ml.

The stock standard solution was diluted to give 1 μ g of copper/ml by diluting 10 ml of this to 1 L in a volumetric flask.

Sodium diethyl-dithio-carbamate solution: Two gm of sodium diethyl-dithio-carbamate was dissolved in 100 ml glass distilled water. The same was heated to 50°C for half an hour to dissolve the reagent. The stock solution was stored in refrigerator away from light. Before use, it was filtered and diluted four times to get 0.5 percent solution.

(1) Digestion:

(a) Blood: Into a 20 x 3 cm pyrex digestion tube marked at approximately 25 ml, 5.0 ml of oxalated blood was pipetted out. To this 1 ml of sulphuric acid and 3 ml perchloric acid were added with mixing after every addition, and was allowed to stand overnight. The contents of the tube were digested first on very low heat and then on strong heat. Little initial frothing was controlled by low heat. Water was driven off by gentle boiling and heating was continued untill rings of darkened mixture began to ascend in the tube. The tube was removed from the heater and cooled for about a minute and then 2 ml nitric acid was added carefully, controlled heating was continued at once. The dark brown liquid in the tube gradually lightened in colour. The tube was then taken out again, cooled and 1 ml of nitric acid was added and heating was resumed again immediately. The heating was continued till the contents of the tube were yellowish, which turned to be colourless. The tube was then transferred to strong heater and heating was continued for a few minutes past the pale final stage to drive

off most of the residual perchloric acid and leave a final volume of about 1 ml. When the liquid became colourless and dense white fumes of sulphuric acid appeared, the tube was removed from the heater and cooled, after which 5 ml of distilled water was added by washing the sides. After mixing the contents of the tube were heated strongly to drive off all the perchloric acid fumes.

(b) Feeds and faeces: About 0.5 to 1.0 gm of the dry material was weighed out accurately into a clean 100 ml Kjeldahl flask, to which 5 ml glass distilled water and 2.0 to 3.0 ml sulphuric acid were added. About 2 to 3 glass beads were also added to the flask. The sample was carefully charred while gently shaking on a low heat in order to control the frothing. When the sample was completely charred, it was cooled. Then 2 ml of perchloric acid and 1 ml of nitric acid were added and heated at low temperature. As the liquid darkened and white fumes started coming out, flask was removed from the heater. After cooling for about 1 to 2 minutes 1 ml of nitric acid was added and heating was resumed at a high temperature. No further frothing took place and the dark brown colour gradually lightened to a faint greenish yellow and became colourless. The flask was then cooled. To ensure the removal of last traces of organic matter, 0.5 ml nitric acid and 10 drops of perchloric acid were added to the flask and heated strongly till perchloric acid was driven off. After the digestion was completed, 5 ml of distilled water was added to the flask and heated to dissolve the salts that separated out in the final stages of digestion.

(c) Milk: In a 100 ml Kjeldahl flask, 25 ml of milk was taken and to this 5 ml of sulphuric acid as well as two glass beads were added. The flask was heated slowly on bunsen burner on a low flame, while shaking continuously, so that the sample did not fath out of the flask. When the sample was completely charred, 2 ml of nitric acid and 2 ml of perchloric acid were added. Heating on a low flame was resumed till thick white fumes of sulphuric acid appeared. Flask was cooled for 1 to 2 minutes, 1 ml nitric acid was again added and heating was continued on low flame. This was repeated till the dark brown liquid lightened in colour passing through orange to faint greenish yellow colour. The flask was cooled. Then 0.5 ml of nitric acid and 10 drops of perchloric acid were added ^{and} heated on burner strongly till perchloric acid was driven off completely. While still hot, 5 ml of distilled water was added to keep in solution the salts separated out in the final stages of digestion.

(d) Urine: In a 20 x 3 cm pyrex test tube, 25 ml of urine was taken, to which 2 ml of sulphuric acid and 3 ml of perchloric acid as well as 2 glass beads were added. It was heated on a low power electric heater (specially designed to regulate the temperature) till white fumes of sulphuric acid started ascending from the black-brown liquid. The tube was taken out of heater and cooled for 2 minutes. The 2 ml of nitric acid was added and heating was resumed on medium heat, till white fumes re-appeared. Again the contents of the tube were cooled and 1 ml of nitric acid was added. It was then

heated strongly till the contents of the tube lightened in colour, and cooled. Then 0.5 ml of nitric acid and 10 drops of perchloric acid were added and this time heated on strong heater till all perchloric acid was driven off. When the liquid became colourless, little distilled water was added to keep the salts that were formed during the final stages of digestion, in solution.

(e) Water: In a 100 ml Kjeldahl flask, 100 ml of water was taken and was evaporated with glass beads, by heating on a slow heat, adding small quantities at a time. When about 2 ml of water was left, 1 ml of sulphuric acid and 1 ml of perchloric acid were added and water was evaporated off completely. When the white fumes started ascending from the liquid, 1 ml of nitric acid was added after cooling. The heating was continued on a strong heater, till all nitric acid and perchloric acid were driven off. About 5 ml of distilled water was added, when it was about to cool.

(ii) Deionisation:

Saturated solution of sodium pyrophosphate was used for deionisation of iron, a common interfering substance in plants and biological materials, which forms a brown complex with diethyl-dithio-carbamate. Pyrophosphate prevented the precipitation of calcium phosphate on alkalisation.

In the case of blood, in the digestion tubes, 10 ml of distilled water was added and mixed thoroughly and 5 ml of saturated solution of sodium pyrophosphate was added, after

which the volume was made up to 25 ml with distilled water. Then 5 ml of ammonia solution (Sp. gr. 0.880) was added. Ammonium sulphate thus formed promoted the deionisation. The tubes were allowed to cool in ice water. The blank was also treated this way.

In the case of feeds, faeces, milk and urine, where more than one ml of sulphuric acid was used for the digestion, required quantity of ammonia was added on the principle that 3 ml of ammonia was required for every 1 ml of sulphuric acid added and 2 ml of ammonia was added in excess to make the solution alkaline. For deionisation in the case of milk, 20% ammonium citrate was used instead of sodium pyrophosphate.

(iii) Colour development and extraction:

From the 2.0% solution of sodium diethyl-dithio-carbamate, 0.5% solution was prepared and filtered. This (2 ml) was added dropwise with constant shaking into the tubes. A yellow coloured complex of copper-diethyl-dithio-carbamate was formed. Exactly 5 ml of amyl alcohol was added to each tube. The tubes were closed with clean, well-treated and copper-free rubber stopper and vigorously shaken for two minutes. It was then allowed to stand to separate, the amyl alcohol layer having taking the copper complex. The amyl alcohol was then taken off carefully by means of a clean pipette leaving behind the watery portion and transferred to a 5 ml clean, dry test tubes, which was then centrifuged for 10 minutes to remove the adhering moisture. The density of the colour was measured in the Hilger Spekker absorptiometer.

Preparation of standard: Eleven clean 50 ml measuring cylinders with glass stoppers were taken and numbered serially from 1 to 10 and the eleventh one for blank, to which 10 ml of distilled water was added. To the rest of the cylinders standard copper solution were added, so as to get orange from 1 to 10 mcg of copper. To each of the cylinder, 1 ml of sulphuric acid and 10 ml of 4% sodium pyrophosphate were added. The contents of the cylinders were shaken after each addition. Later, 5 ml of ammonia was added to each of the cylinders and the contents were shaken and allowed to cool, after which the volumes were made upto 50 ml in all the cylinders with distilled water and transferred to clean separation funnels, with two washings of 3 ml each. Next 2 ml of freshly prepared from 2.0% solution and filtered 0.5% solution of sodium diethyl-dithio-carbamate was added dropwise to each of the funnels with constant shaking. The yellow coloured complex of copper diethyl-dithio-carbamate was extracted into 5 ml of amyl alcohol after shaking vigorously for two minutes. The amyl alcohol layer containing yellow coloured complex was taken in centrifuge tubes, after drawing out the lower portion of separating funnels. The tubes were centrifuged for five minutes to remove the moisture.

(iv) Colour measurement:

The readings were taken in Hilger Photo-electric colorimeter. The transmittance of the coloured complex was recorded at 430 m μ , after adjusting the colorimeter for 100% transmittance with the reagent blank, that had been prepared simultaneously.

Calculation:

μg copper per 100 ml of blood or 100 g of sample:

$$= \frac{\text{Density of unknown}}{\text{Density of standard}} \times \frac{\mu\text{g copper in standard}}{1} \times \frac{100}{\text{sample taken}}$$

(In the case of fodders, feeds and faeces appropriate dilution factor is also applied).

(iv) Estimation of Cobalt:**(A) Estimation of cobalt in feeds, fodders and faeces:**

The method of Kidson and Askew (1940) was followed for the estimation of cobalt in the feeds, fodders and faeces. The range of this method was between 1 to 30 mcg of cobalt (Allport, 1947).

Reagents: Nitric acid, 50% (1:1 diluted with distilled water)
 Hydrochloric acid, 4.5% (0.5 N solution)
 Hydrochloric acid, 33.3% (1 part of hydrochloric acid and 2 parts of distilled water)
 Sodium acetate
 Potassium hydroxide, 10% aqueous solution
 Phenolphthalein indicator, 0.2% solution
 Nitroso-R-salt, 0.1 g per 100 ml of distilled water
 Standard cobalt solution, 1 mcg of cobalt per ml of solution.

Procedure:

(1) **Ashing:** About 2 to 10gm of the sample (depending upon the type of sample) were weighed accurately in silica dish. To this 20 to 40 ml of 1:1 nitric acid was added, and kept

overnight. Then the contents of the silica dish were dried on water/sand bath and ignited on burner/hot plate. The silica dish was transferred to muffle furnace maintained at 600°C and kept there for about 2 hours. The ash was then moistened with 5 to 10 ml of 1:1 nitric acid, dried on sand bath and ignited again in muffle furnace till all the organic matter was destroyed.

(ii) Extraction and colour development: The ash was dissolved in 30 to 50 ml of 1:2 hydrochloric acid. Heated on sand bath for 30 minutes and filtered into a 100 or 150 ml beaker, the residue on the filter paper was washed with hot water repeatedly. The filtrate along with the washings was evaporated to dryness and 10 ml of distilled water and 1 ml of 1:2 hydrochloric acid, 10 drops of 1:1 nitric acid were added. It was then boiled for 10 minutes to oxidise any reducing substances, and the volume was maintained at 15 ml, cooled. Then 2 ml of nitroso-R-salt solution and 2 gm of sodium acetate were added. Heated to about 70°C and 6 drops of phenolphthalein solution, followed by dropwise addition of 10 percent potassium hydroxide until the pink colour was just appeared. Immediately, 0.5 N hydrochloric acid was added to just destroy the pink colour. Then 2 ml of concentrated nitric acid was added dropwise and boiled for two minutes. cooled under running water and the volume was made upto 25 ml in volumetric flask. The colour of the test sample was matched with that of freshly prepared standard solution at 420 m μ in a Hilger Photo-electric colorimeter.

Preparation of standard solution: In 100 ml of distilled water containing 10 ml of hydrochloric acid, 0.4037 gm of AR cobalt chloride was dissolved and transferred to 1 L volumetric flask. The volume was made upto the mark. This stock standard solution contained 0.1 mg of cobalt/ml.

To prepare the working standard solution, 10 ml of stock standard solution (0.1 mg cobalt/ml) was made upto 1 L in a volumetric flask. The solution contained 1 mcg cobalt/ml.

Preparation of standard: A series of aliquots from 1 to 10 ml of working standard solution of cobalt corresponding to 1 to 10 mcg of cobalt were pipetted out into series of 100 ml beakers. The volume of the contents in all the beakers were made upto 15 ml with distilled water. To each beaker 10 drops of 1:1 nitric acid and 1 ml of 1:2 hydrochloric acid were added, followed by 2 ml of nitroso-R-salt reagent and 2 gm of sodium acetate, when the colour was developed. Similarly, a reagent blank was also prepared. The optical densities of the colour developed in the standard solutions were measured at 430 m μ in a Hilger Photo-electric colorimeter, set to 100% transmission with the reagent blank.

Calculation: mcg cobalt in 100 gm sample =

$$\frac{\text{Density of unknown}}{\text{Density of standard}} \times \frac{\text{mcg of cobalt in standard}}{1} \times \frac{100}{\text{weight of sample taken}}$$

(B) Estimation of Cobalt in Blood, Milk and Urine:

For the estimation of cobalt content of blood, milk and urine, the method of Saltzman (1955) as described by Saltzman and Keenan (1957) was used. The separation of cobalt was made by forming the complex with 1-nitroso-2-naphthol and its extraction with chloroform. The final determination was made colorimetrically as the nitroso-R-salt complex. The determination of cobalt by this method was found to be accurate, because of good colour stability and very close adherence to Beer's law. Keenan and Kopp (1956) suggested that even 0.03 mcg cobalt can be accurately estimated by this method.

Reagents:

- (1) Nitric acid, AR
- (2) Methyl orange, 0.1 % solution in distilled water
- (3) Sodium citrate, 50% solution in distilled water (w/v)
- (4) 1-Nitroso-2-naphthol. In 125 ml of glacial acetic acid 2.5 gm of the reagent was dissolved and the volume was made upto 250 ml with distilled water.
- (5) Hydrochloric acid, concentrated
- (6) Hydrochloric acid, 1:99 in distilled water
- (7) Sodium sulphate, 10% aqueous solution
- (8) Phosphoric acid, 1:49 in distilled water
- (9) Nitroso-R-salt, 0.1% solution in distilled water
- (10) Sodium acetate, 50% solution of trihydrate salt
- (11) Standard cobalt solution (in 1:49 phosphoric acid)
- (12) Chloroform, AR

Procedure:

(a) ashing: In a silica dish, 50 ml blood/100 ml milk/100 ml of urine was evaporated to dryness on water bath. Five ml of nitric acid in the case of blood and 2 ml for milk were added to precipitate proteins before evaporation. It was ignited on hot plate for a while and then transferred to Muffle furnace maintained at 550 to 600°C and ashed for short time, after which the ash was cooled and moistened with 10 to 20 ml of 50% Nitric acid. It was again evaporated and ignited at 550 to 600°C for half an hour. The process of addition of nitric acid and ignition was repeated till all the organic matter was destroyed and the ash was obtained.

(b) Separation of cobalt: The ash thus obtained was dissolved in excess of 1:1 nitric acid and evaporated to dryness in 1 to 2 hours on steam bath. After drying the ash was immediately dissolved in 25 ml of 1:49 phosphoric acid and transferred to a separatory funnel, to which one drop of methyl orange indicator was added. Sodium citrate solution was added to this till it was orange in colour (pH 3 to 4). Then 5 ml of 1-nitroso-2-naphthol reagent was added and allowed to stand for one hour with occasional shaking.

The cobalt complex thus formed was extracted by shaking vigorously for 3 minutes, each time with 10 ml portions of chloroform until the final extract was colourless. The extracts were combined in another separatory funnels and the impurities were removed by shaking for 2 minutes with 25 ml of 1:99 hydrochloric acid. The chloroform extract was then

drained into a 100 ml beaker. Traces of cobalt complex remaining in the hydrochloric acid layer were recovered by extracting it with 5 ml chloroform, which was also drained into the same beaker.

The chloroform containing the purified cobalt extract was then evaporated off on steam bath and the residue was warmed with 0.5 ml of nitric acid to dissolve. Then 1 ml of sodium sulphate solution was added as an ashing base and the mixture was evaporated to dryness on sand bath, followed on hot plate at low heat. The beaker was covered with a watch glass and 1 ml of nitric acid was added. The residue was heated and agitated till it dissolved and distributed uniformly over the bottom of the beaker. Heating was continued until the salts again solidified and decomposed, liberating oxides of nitrogen. Ashing was completed by the addition of 0.5 ml portions of nitric acid and igniting at temperature upto 400°C. The process was repeated as in the case of original samples. A white ash was thus obtained without any oily-ness.

(c) Determination of cobalt: A clear solution was obtained by dissolving the ash in 5 ml of 1:49 phosphoric acid. To this 1 ml of nitroso-R-salt reagent and 2 ml of sodium acetate solution were added with mixing, after each addition. The solution was heated on the steam bath for exactly 3 minutes. Then 0.75 ml of concentrated hydrochloric acid was added and the heating was continued for exactly 2 minutes more. The beaker was then removed and placed in a tray of cold water. After cooling, the volume of sample was made upto

exactly 10 ml in a volumetric flask. The absorbancy of this solution was read at 520 m μ in a Hilger Photo-electric colorimeter set to maximum transmittance with distilled water as blank.

(d) Preparation of standard: To a series of 100 ml beakers, graduated amounts of standard solution of cobalt to get the concentration from 1 to 10 mcg in each were pipetted out. Then sufficient 1.49 phosphoric acid was added to each beaker or otherwise, evaporated on steam bath as the case was till the volume was 5 ml. The colour was developed and read, as described in the preceeding paragraphs.

(e) Calculations:

mcg of cobalt in 100 gm/100 ml sample =

$$\frac{\text{Optical density of unknown}}{\text{Optical density of standard}} \times \frac{\text{mcg cobalt in standard solution taken}}{1} \times \frac{100}{\text{Amount of sample taken}}$$

METABOLISM TRIAL

(A) Collection, Sampling and Preservation of Feeds, Fodders, Water, Faeces, Urine and Milk:

After an experimental period of 200 days, a metabolism trial was conducted on two cows from each group, to assess the effect of the supplementation of copper or cobalt, as the case was, on the digestibility of the nutrients and balances of some of the important minerals. The usual metabolism stalls were used for this purpose.

The samples of feed, fodders and water offered to the animals during the trial period were collected and preserved

residues of each if any, were also weighed every time and a record of this was maintained. A record of water intake by each animal per day was also kept.

(b) Output: After the preliminary period, the collection of 24 hourly excretion of faeces, urine and milk yielded was made for a period of 7 days with the help of trained personnel. The faeces and urine voided in every 24 hours by each animal were collected separately in polyethylene containers and carboys, respectively. These were weighed and measured daily between 9 A.M. and 10 A.M., and representative samples were drawn from each separately and transferred to suitable containers with lids for taking further aliquots in the laboratory.

After each milking into enamelled buckets, representative samples of milk of individual animals were collected separately in glass bottles with ground glass stoppers. These samples were used for taking further aliquots in the laboratory.

(B) Aliquots and Preservation of Samples of Fodder, Faeces, Urine and Milk in the Laboratory:

The representative samples of chaffed straw and green grass that were fed to the animals was taken each for the determination of the dry matter and the crude protein contents. The dried straw and grass samples were pooled together separately powdered and stored for further analysis.

From the representative well mixed sample 1/100th, by weight of total faeces of the previous 24 hours voided by each

animal, was weighed out into separate tared enamelled trays for the determination of moisture content daily. The samples were dried out $100 \pm 2^{\circ}\text{C}$ to constant weight. The dried faeces samples of each animal were pooled separately, powdered and preserved for further analysis. Similarly, for the determination of trace mineral content of faeces, a proportionate sample of the faeces of each animal was transferred into separate glass bottles, dried and preserved. For the determination of nitrogen outgo, $1/250\text{th}$, by weight, of total faeces of the previous 24 hours, voided by each animal, was transferred each to previously weighed and labelled wide mouth glass bottles, along with 10 ml of 20% sulphuric acid, for fixing the nitrogen. From each of the pooled sample of faeces with sulphuric acid, about 30 gm material was weighed out accurately in duplicate into 500 ml kjeldahl flasks, for the determination of nitrogen.

From the well mixed representative sample, $1/250\text{th}$, by volume, of the urine of previous 24 hours, voided by each animal was transferred by means of a burette into 500 ml kjeldahl flask and 1 L conical flask, to which about 50 ml sulphuric acid was added as well as in to 100 ml silica dish and 500 ml beaker. The contents of the latter two containers were used for the determination of minerals, after drying them daily, while those of former two were for the determination of nitrogen content of urine samples. Similarly, for the determination of traces mineral contents, proportionate samples of urine voided by each animal was transferred daily into separate glass stoppered bottles and preserved with formalin.

For the determination of nitrogen and dry matter as well as mineral 1/1000 th, by volume of milk yielded by individual animal during the previous 24 hours was transferred into separate 500 ml Kjeldahl flasks (in duplicate) containing 50 ml sulphuric acid, and 100 ml silica dish as well as 500 ml beakers, respectively. For the determination of trace minerals, 1/500 volume of milk yielded by each animal, was collected in separate glass stoppered bottles, and preserved at 4°C with formalin as the preservative.

(C) Analytical Procedures used for the Analysis of Feeding
Stuffs, Faeces, and Urine:

The methods for the analysis adopted were those recommended by A.O.A.C. (1970). The outlines of these are given below. The methods described by Talaptra *et al* (1940) were employed for the determination of total ash and major mineral constituents.

(1) Dry matter: The determination of dry matter content is necessary to express the results of the analysis of various samples on dry matter basis and also to calculate the dry matter intakes through feeds and dry matter outgo through faeces by the animals during the trial period.

The determination of dry matter contents were done by drying known weights of the representative samples of the materials, taken in suitable containers, to a constant weight at $100 \pm 2^\circ\text{C}$.

(2) Crude protein: The crude protein content of various material were arrived at by multiplying the nitrogen content determined by Kjeldahl method with 6.38 in case of milk and

6.25 in feed - fodders dung and urine.

For this determination $N/7$ sulphuric acid was used for absorbing the ammonia liberated by the distillation of ammonium sulphate, formed during the digestion of the samples with sulphuric acid. The fact that, 1 ml of $N/7$ sulphuric acid used up was equivalent to 0.002 gm of nitrogen in case of milk and 0.0014 gm of nitrogen in case of other samples, was employed for the calculation of nitrogen contents.

(3) Ether extractives: It was determined by extracting a known weight of sample taken in thimble with petroleum ether (40 - 60°C B.P.) into a previously weighted Soxhlet flask on a water bath for 8 hours.

(4) Crude fibre: The usual Weende method with slight modifications was employed for the determination of crude fibre content of the material, after taking out the ether extractives. The material was successively boiled with the 200 ml each of 1.25 % (w/v) sulphuric acid and sodium hydroxide, for half an hour and washed with hot water to make the residue free of acid and alkali, after each boiling. The residue was dried, weighed and ignited at 550°C for half an hour. The loss in weight after ignition of the residue, was used for the calculation of crude fibre content.

(5) Nitrogen-free extract and Total Carbohydrates: The values of these constituents of the various samples were arrived at by difference, by employing the dry matter, crude protein, ether extract, crude fibre and ash contents of the respective materials.

(6) Total ash: The total ash contents of the samples were determined by igniting known weights of the materials at 500°C for four hours in previously weighed silica dishes.

The ash samples thus obtained were extracted with 50 to 60 ml of 50% hydrochloric acid on boiling in beakers on a sand bath for about 2 hours. These extracts were filtered through Whatman filter paper No.1 into 250 ml volumetric flasks and the residue on the filter papers were washed with hot water till the filtrates were free from acid. The volumes in all the flasks were made upto the mark. These extracts were used for the determination of mineral contents of the respective samples.

(7) Calcium: The method of McCrudden modified by Talapatra et al (1940) was used for the determination of calcium contents of the feeds, faeces, urine and milk. The calcium in a known aliquot of ash extract, was precipitated as calcium oxalate which was washed with hot water till the filtrate was free from oxalates. The calcium oxalate was dissolved in 20% sulphuric acid and liberated oxalic acid was titrated against N/20 potassium permanganate solution. The calcium content was calculated using the value that 1 ml of N/20 potassium permanganate to be equivalent to 1 mg of calcium.

(8) Phosphorus: For the determination of phosphorus content of feeds faeces, urine and milk, the method of Newman, which was modified by Talapatra et al (1940) was used. The phosphorus in a known aliquot of ash extract was precipitated as ammonium phosphomolybdate, which was washed with 3% potassium nitrate solution till the filtrate was free of acid. The

precipitate was dissolved in a known excess of $N/7$ sodium hydroxide and the excess of alkali was back titrated against $N/7$ nitric acid. The phosphorus content was calculated using the value that 1 ml of $N/7$ sodium hydroxide is equivalent to 0.1927 mg of phosphorus.

(D) Analytical Procedures used for the Analysis of Milk:

(a) Milk fat: Gerber's method was employed for the determination of milk fat (Ling, 1966).

(b) Total solids, Protein and Total ash: The determination of total solids, protein and total ash contents of milk samples was done according to the procedures described for those of feeds, faeces, and urine, with appropriate changes in the quantities of the sample taken for each determination.

(c) Solids-not-fat: The difference between the total solids and the fat contents was taken as the solids-not fat content of the milk samples under analysis.

MILK PRODUCTION

The record of each milking upto an accuracy of 100gm per milking, was maintained for the complete lactation period or 305 days of lactation of each animal under the experiment. Salter's Milk Recording Scale was used for weighing the amount of milk yielded per milking. The milk yields of all the animals of the previous lactation were taken from the record, maintained by the institute. All the calves were weaned on the 5th day and hence the complete milk production was recorded accurately.

DETECTION OF OESTRUS:

The occurrence of oestrus in the cows was detected by engaging an experienced herdsman, who was well conversant with the detection and symptoms of heat in animals. Similar method for the detection of onset of oestrus in cattle has been followed by Miller et al (1931) and Triebberger (1944). The cows with doubtful symptoms of heat were examined clinically, or confirmed by employing the vasectomised bull. All the animals were bred artificially, with the semen of exotic bulls maintained at the Institute. They were inseminated within 24 hours of the onset of oestrus. The conceptions were assessed on actual calving or abortions as the case was.

STATISTICAL ANALYSIS OF THE RESULTS AND PRESENTATION OF THE DATA:

The results obtained are tabulated according to either groups or constituents of blood milk and feeds. The data are presented in the form of group and period averages with the respective standard errors. The averages of some of the observations are presented in the form of either graph or histograms.

The data collected for the constituents of blood and milk were subjected to statistical analysis to reveal significant differences, if any, between the treatments and intervals according to the methods suggested by Goulden (1959) and Snedecor and Cochran (1968) for the Completely Randomised and Randomised Block Designs.

The averages of the different observations recorded under the metabolism studies are presented group-wise and the results are discussed accordingly. The data on the milk production and reproductive performance of the animals under the experiment were

subjected to the co-variance test and analysis of variance and the results obtained are discussed.

CHAPTER - V

RESULTS AND DISCUSSION OF STUDIES ON COPPER SUPPLEMENTATION

CHAPTER - V.

RESULTS AND DISCUSSION OF STUDIES ON COPPER SUPPLEMENTATION

Twenty Jersey x Tharparkar lactating cows, which were the true representative of the institute dairy herd, were divided into four groups having five animals in each. The animals were allotted randomly to these groups. The distribution of animals is shown in Table 1. The experimental groups I, II, and III were given 200, 300 and 400 mg of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) i.e., 52, 78 and 104 mg of elemental copper, respectively, in addition to what they were getting through feeds and fodders. The studies on blood and milk constituents, milk production and reproductive performance of the cows were carried out.

(I) Influence of Copper on Blood Composition:

The cows of the control and experimental groups were bled once in twenty days during the course of the experiment and the various constituents of blood were examined. The haematological constituents studied included haematocrit value, haemoglobin, total erythrocyte and leucocyte counts and specific gravity of whole blood. The biochemical constituents such as serum protein, blood copper and blood iron were also studied. The results of these are summarised and discussed.

(A) Haematological Constituents:

(i) Haematocrit (PCV) values:

A-packed cell volume of the blood of the cows of control and experimental groups are presented in Table 2. The average

**Table 1. Allotment of Animals to Different Treatments.
Copper Supplementation.**

Groups	Animal No.	Age		No. of lactations completed	Previous lactation Milk yield
		Years	Months		
Control Group	75	5	6	2	Kgs. 2702
	76	4	8	2	2096
	934	9	6	7	2175
	954	9	5	6	2849
	981	7	7	5	1744
	Average	7	4	4.4	2313.2 ± 220.1
Group I	62	5	1	2	2448
	86	4	3	1	1578
	929	9	10	7	2912
	959	8	6	6	2791
	975	7	8	5	3014
	Average	7	1	4.2	2548.6 ± 276.4
Group II.	33	6	8	3	2391
	82	4	11	2	1846
	87	4	2	1	2177
	928	10	9	7	2881
	960	8	11	5	3012
	Average	7	1	3.6	2461.4 ± 224.2
Group III.	45	7	0	3	1033
	81	4	2	2	1697
	944	9	2	7	2403
	948	8	6	6	2552
	987	7	8	5	2408
	Average	7	4	4.6	2224.6 ± 430.3

values of PCV for control and experimental groups I, II and III were 35.99 ± 0.42 , 40.08 ± 0.65 , 38.45 ± 0.81 and 37.99 ± 0.47 percent, respectively. Holman (1955) and Smith (1959) reported 33.7 and 32.88 percent PCV values for normal lactating cows respectively. Values recorded by Schalm (1961) and Misra and Biswal (1961) were 40.0 and 38.0 ± 7.88 percent, respectively. Coffin (1953) and Dukes (1957) have given the range of 30 to 40 percent as normal PCV value of blood of cows. Values obtained in the present investigation are in agreement with those of Schalm and Misra and Biswal, and lie within the range given by Coffin and Dukes, while the values reported by Holman and Smith are lower. Khajuria (1933) reported 33.9 ± 3.57 percent PCV values for lactating Tharparkar cows. The present reported values for all the groups are found to be above this value.

The PCV values of the blood of the cows of all the groups has been shown in Fig. 1. It is seen from the graph as well as from the average values that the supplemented groups of animals recorded higher hematocrit values in blood as compared with those obtained for the animals of control group. The cows of the group, which received supplement of 200 mg of copper sulphate per day per animal, recorded the highest PCV values, after a supplemental period of two months, which further increased subsequently. However, the cows receiving the supplement of 300 and 400 mg per day recorded lower values compared with those receiving 200 mg supplement per day.

The statistical analysis of the data indicated that the difference between the treatments is significant at both one

Table 2. Average values of Haematocrit (PCV) percent with Standard Error.

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	36.4 $\pm$ 1.68	35.9 $\pm$ 1.72	36.1 $\pm$ 1.72	36.0 $\pm$ 1.48
20	36.1 $\pm$ 1.29	37.0 $\pm$ 1.82	36.8 $\pm$ 1.57	37.1 $\pm$ 1.98
40	34.7 $\pm$ 1.98	37.0 $\pm$ 1.86	36.0 $\pm$ 2.06	36.5 $\pm$ 0.78
60	34.9 $\pm$ 1.19	37.3 $\pm$ 1.27	35.9 $\pm$ 2.06	36.7 $\pm$ 1.47
80	36.4 $\pm$ 1.18	40.2 $\pm$ 1.78	35.4 $\pm$ 2.21	37.6 $\pm$ 1.47
100	35.0 $\pm$ 1.19	40.7 $\pm$ 1.96	37.6 $\pm$ 2.83	38.5 $\pm$ 2.06
120	37.0 $\pm$ 1.37	42.3 $\pm$ 1.96	41.8 $\pm$ 4.06	38.6 $\pm$ 1.92
140	37.1 $\pm$ 1.21	42.9 $\pm$ 1.86	41.3 $\pm$ 3.07	39.1 $\pm$ 1.76
160	37.3 $\pm$ 1.09	43.6 $\pm$ 2.32	41.3 $\pm$ 2.78	39.3 $\pm$ 1.66
180	37.0 $\pm$ 1.19	43.6 $\pm$ 2.40	41.3 $\pm$ 2.35	40.0 $\pm$ 1.57
Group Average	35.99 $\pm$ 0.42	40.06 $\pm$ 0.65	38.45 $\pm$ 0.81	37.99 $\pm$ 0.47

Table 3. Analysis of Variance for PCV of blood.

Source of Variance	Degrees of freedom	Mean Sum of squares	Variance ratio
Between Treatments	3	145.2046	8.5219**
Between Intervals	9	73.0818	4.5612**
Interaction	27	6.6963	0.3957
Error	160	17.1562	-

** Significant at 1 percent level.

Table 3A. Comparison of means of PCV/blood.

Critical Difference at 5% level	Group Average Values			
	Control	III	II	I
1.611	35.99	37.99	38.45	40.06

Note: Mean values under bar do not differ significantly.

and five percent levels (Table 3 and 3A). On comparison of means, of different groups, with the critical difference at $t = 0.05$, it is observed that all the supplemented groups are superior to that of control group. Treatments of group II and III as well as treatments of group I and II are not significantly different, while treatment of group I (200 mg per day) appear to result in giving higher PCV values over that of group III (400 mg per day). There exists a significant difference between intervals at both the percent levels, indicating the increase in haematocrit values as the lactation advanced. No significant difference due to interaction between treatment and interval was noticed.

Pal *et al* (1970) supplemented 12.64 mg of copper per 100 kg body weight in the diet of the growing Harijans calves. They did not observe any appreciable difference in the PCV value of the blood of the calves, after supplementation. However, they reported higher values after supplementation over the initial values. Pachalag *et al* (1970) reported similar findings while studying the effect of supplementation of copper and cobalt on weaner lambs of 6 months age. However, Thomson and Lawson (1970) observed the increase in the PCV value of the blood of sheep after supplementation. A similar observation has been recorded in the present study. Amrithkumar (1973) reported a slight increase in PCV value of blood of the animals supplemented with copper and cobalt over those of control group in Jersey x Tharparkar heifers from the same herd. They reported 50.0 percent as an average haematocrit value for the blood of supplemented heifers as against 49.6 percent for those of non supplemented

group. Their findings are in conformity with the trend observed in the present study.

It may, therefore, be concluded that supplementation of 200 mg copper sulphate per day per animal results in an increase of PCV value to the optimum, as compared to the levels of 300 and 400 mg supplementation.

(11) Haemoglobin:

The average haemoglobin percent values of blood of the cows from control and experimental groups during the period of copper supplementation have been presented in Table 4. From the perusal of the table, it can be seen that the average values were 9.20 ± 0.10 , 10.66 ± 0.28 , 10.29 ± 0.21 and 10.32 ± 0.16 gm percent, for the control and experimental groups I, II and III, respectively. Khajuria and Razdan (1956) reported an average of 10.30 ± 0.77 for Tharparkar lactating cows, while Dabadghao (1959) gave an average of 10.38 ± 0.25 for Tharparkar heifers. Amrith Kumar et al (1973) obtained 10.0 gm percent haemoglobin in the blood of Jersey x Tharparkar heifers. The present study confirmed the earlier findings. The values reported by Mullick and Pal (1943) for Indian Cattle and Coffin (1953) for Jersey cattle are not far from the values reported in the present study. However, the haemoglobin values reported by Holman (1955) and Schalm (1961) was 11.3 and by Dukes (1957) as 12.0 and by Misra and Biswal (1961) as 12.2 gm percent, were slightly higher compared with the values obtained here. But Smith (1959) has reported slightly lower haemoglobin value of 8.9 gm percent for this class of animals.

Table 4. Average values of Haemoglobin, gm percent with Standard Error.

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Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	9.1 ± 0.58	9.0 ± 0.40	9.0 ± 0.57	9.3 ± 0.65
20	8.9 ± 0.30	9.4 ± 0.40	9.5 ± 0.49	9.7 ± 0.67
40	8.9 ± 0.40	9.4 ± 0.10	9.4 ± 0.49	9.5 ± 0.58
60	9.0 ± 0.30	10.1 ± 0.20	9.4 ± 0.48	9.8 ± 0.58
80	9.4 ± 0.49	11.4 ± 0.20	10.5 ± 0.67	10.5 ± 0.58
100	9.2 ± 0.49	11.1 ± 0.19	10.3 ± 0.58	10.7 ± 0.59
120	9.7 ± 0.20	11.4 ± 0.49	11.4 ± 0.88	10.9 ± 0.49
140	9.2 ± 0.10	11.3 ± 0.59	11.2 ± 0.59	11.0 ± 0.58
160	9.4 ± 0.19	11.8 ± 0.58	11.3 ± 0.67	10.9 ± 0.39
180	9.2 ± 0.20	11.7 ± 0.49	10.9 ± 0.58	10.9 ± 0.67
Group Average	9.20 ± 0.10	10.66 ± 0.28	10.29 ± 0.21	10.32 ± 0.16

Table 5. Analysis of Variance for Haemoglobin.

Source of Variance	Degrees of freedom	Mean Sum of Squares	Variance ratio
Between Treatments	3	20.1146	19.921**
Between Intervals	9	9.7724	9.679**
Interaction	27	0.8864	0.858
Error	160	1.0097	-

** Significant at 1 percent level of probability.

Table 5A. Comparison of means of Haemoglobin of blood.

Critical Difference at 5% level	Group Average Values			
	Control	II	III	I
0.394	9.20	10.29	10.32	10.66

Note: Mean values under bar do not differ significantly.

The haemoglobin values of the blood of the cows of all the groups have been shown in Fig. 1. It is seen from the graph that the supplemented group of animals recorded higher haemoglobin as compared with those of control groups. As observed in PCV values, cows of the 200 mg supplemented group recorded the highest haemoglobin value after supplementation period of two months, which further increased subsequently. A little drop in the haemoglobin is observed after 40 days of initiating the supplementation, which may be due to the peak period of lactation. However, the cows receiving the supplement of 300 and 400 mg of copper sulphate per day recorded slightly lower values compared to those receiving 200 mg supplement. Control group of animals recorded uniform values as far as this parameter is concerned. This indicates that 200 mg per day is helpful in elevating the haemoglobin value of the blood. A concomitant raise in haemoglobin with the raise in the PCV and total erythrocyte count in the blood of supplemented animals is observed.

On statistical analysis of data, it is seen that there existed a significant difference (Table 5) between the treatments at both the levels of probability. On further analysis (Table 5A) it was observed that all the supplemented groups were superior to the control group. Cows of Group I recorded the highest value, while the control group recorded the lowest value for haemoglobin content of the blood. The experimental (Supplementation) groups have not shown any significant difference in their haemoglobin values. Supplementation of 300 or 400 mg of copper sulphate per day per animal did not result in any additional advantage

over 200 mg of supplementation i.e., the oxygen carrying capacity of the blood did not further improve as compared with that in cows receiving 200 mg supplement.

Statistically significant difference was observed between the time intervals. This and graphic representation show that the haemoglobin content of blood of experimental animal recorded trends as the lactation advanced. This trend is of higher degree in supplemented groups than that of control group. No significant difference was observed due to interaction between treatments and intervals.

Ray and Zubairy (1955) and Dutt and Ray (1956) observed that the addition of copper containing mineral mixture is effective in raising the haemoglobin content to normal values in animals on the farm where the subnormal copper intake existed. Kehar *et al* (1959) reported similar increase in the haemoglobin content after supplementation of copper along with other trace minerals to lactating cows and calves. A similar trend of increase in haemoglobin level of blood was observed in Haryana calves, when supplemented with copper and copper plus cobalt (Dwarkanath *et al*, 1962). Agarwal and Mijundar (1963) observed increase in haemoglobin level by about 50 percent, which was highly significant, when copper supplementation increased from 5 ppm to 45 ppm in Haryana calves. All these findings corroborate the results of present investigation, except for those of Paul *et al* (1970). They did not observe any appreciable difference in the haemoglobin content of the blood of growing Haryana calves, after copper supplementation.

Reddy and Mahadevan (1966) reported similar increase in haemoglobin content of Mariana lactating cows after supplementation with 0.5 gm copper sulphate per day per animal. Davis *et al.* (1946) observed that the haemoglobin values dropped down to 5 to 7 gm percent, when copper supplement was withheld in animals grazing on unfertilized pasture and supplementation brought them back to normal. Findings of Reddy and Mahadevan (1966) are in close conformity with the present findings. Recently, Felsman *et al.* (1972) reported marginal increase in haemoglobin content though insignificant, in the blood after supplementation of 300, 600 and 900 ppm of copper to calves.

(iii) Total Erythrocyte (R.B.C.) Count:

The average values with standard error for total erythrocyte count of blood of cows of control and experimental groups are presented in Table 6. The average values of total R.B.C. count were 6.139 ± 0.18 , 7.086 ± 0.37 , 6.82 ± 0.40 and 6.544 ± 0.43 millions per cumm. for control and experimental groups I, II and III, respectively.

The average values reported for R.B.C. count of blood of lactating cows by earlier workers ranged from 5.97 to 7.0 millions per cumm, (Smith, 1959; Holman, 1956; Coffin, 1953; Dukes, 1957 and Schalm, 1961) for exotic breeds. Erythrocyte count the Indian Cattle ranged from 5.67 to 7.90 millions per cumm. of blood. (Shankaramarayana, 1937; Mullick and Pal, 1943; Kehar and Murthy, 1943; Govindan Nair, 1949; Misra and Biswal, 1961; Khajuria and Rasdan, 1966; Dabodghao, 1969 and Anurith Kumar *et al.*, 1973). Out of these, values of Mullick and

Table 6. Average values of Total Erythrocytes (R.B.C.) millions per cmm. with standard Error.

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	5.832 $\pm$ 0.52	5.718 $\pm$ 0.24	5.748 $\pm$ 0.37	5.933 $\pm$ 0.81
20	5.690 $\pm$ 0.21	6.270 $\pm$ 0.29	5.948 $\pm$ 0.44	6.050 $\pm$ 0.47
40	5.788 $\pm$ 0.32	6.834 $\pm$ 0.43	6.543 $\pm$ 0.39	6.262 $\pm$ 0.48
60	5.980 $\pm$ 0.14	6.612 $\pm$ 0.23	6.376 $\pm$ 0.64	6.384 $\pm$ 0.47
80	6.124 $\pm$ 0.18	7.314 $\pm$ 0.51	7.080 $\pm$ 0.42	6.480 $\pm$ 0.42
100	6.178 $\pm$ 0.10	7.422 $\pm$ 0.38	7.166 $\pm$ 0.35	6.586 $\pm$ 0.56
120	6.180 $\pm$ 0.15	7.650 $\pm$ 0.39	7.226 $\pm$ 0.39	6.890 $\pm$ 0.59
140	6.192 $\pm$ 0.09	7.710 $\pm$ 0.38	7.600 $\pm$ 0.44	6.938 $\pm$ 0.51
160	6.358 $\pm$ 0.06	7.686 $\pm$ 0.44	7.264 $\pm$ 0.36	6.942 $\pm$ 0.58
180	6.989 $\pm$ 0.02	7.744 $\pm$ 0.40	7.672 $\pm$ 0.29	6.970 $\pm$ 0.49
Group Average	6.139 $\pm$ 0.18	7.088 $\pm$ 0.37	6.821 $\pm$ 0.40	6.544 $\pm$ 0.43

Table 7. Analysis of variance for Erythrocyte Count of Blood.
(Log. values)

Source of Variance	Degrees of freedom	Mean Sum of squares	Variance ratio
Between Treatments	3	0.25789	19.5046**
Between Intervals	9	0.10330	7.8130**
Interaction	27	0.00895	0.6772
Error	160	0.01322	-

** Significant at 1 percent level of probability.

Table 7A. Comparison of means of R.B.C. of blood.
(Log. values)

Critical Difference at 5% level	Group Average Values			
	Control	III	II	I
0.04403	1.73099	1.86583	1.90894	1.94851

Note: Mean values under bar do not differ significantly.

Pal and Kehar and Murthy are higher and that of Amrith Kumar et al are lower compared to the values recorded in the present study.

A graphic representation of the average total erythrocyte count values of blood of the cows of all the groups has been shown in Fig 1. It can be seen that the supplemented groups of animals recorded higher erythrocyte count values as compared to those obtained for control groups. The cows of the Group I, which received 200 mg copper sulphate supplement per day recorded the highest R.B.C. count after supplemental period of one month, which further increased subsequently upto four months and remained steady upto the end of the experimental period. The cows receiving the supplement of 300 and 400 mg per day recorded lower values as compared to those of 200 mg supplement. Control group has not recorded any appreciable change in this constituent. This constituent of blood has shown similar pattern of changes as observed in haematocrit value and haemoglobin content.

On subjecting the data to the variance test (Table 7), it is observed that the difference between the treatments is highly significant at both the percent levels. On comparing mean difference with critical difference (Table 7A), it is observed that all the supplemented groups have higher levels compared to the control group. Treatments of groups II and III as well as treatment of group I and II are not found significantly different from each other. Treatment of group I (200 mg) was found to record significant increase over the treatment of group III

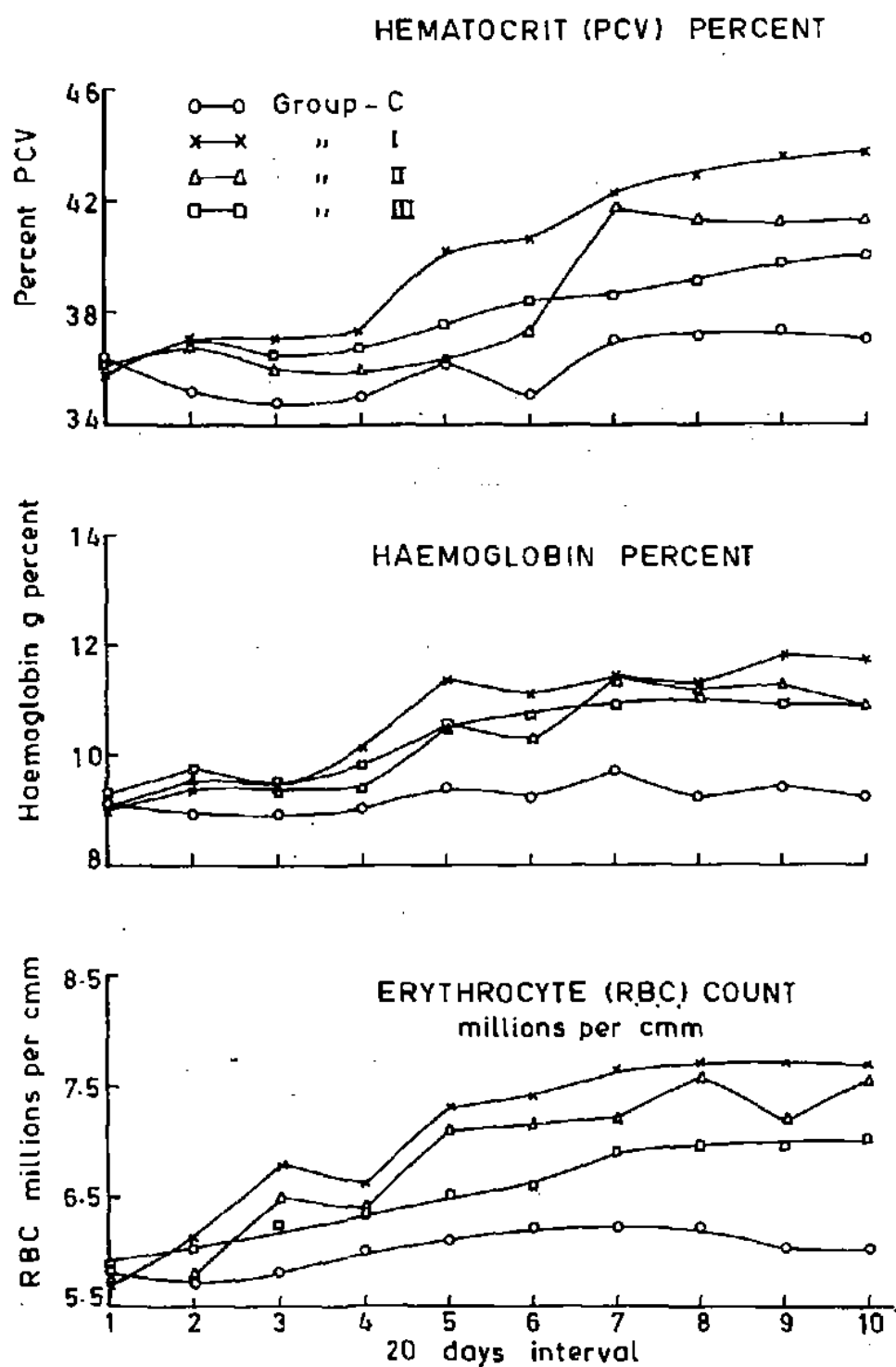


FIG.1-INFLUENCE OF COPPER SUPPLEMENTATION ON BLOOD COMPOSITION OF CROSS-BRED COWS

(40 mg). These results alongwith the results of PCV and Haemoglobin, further indicate that 200 mg of copper sulphate is an ideal level of supplementation of copper to lactating cross-bred cows. A significant difference was also observed at one percent level between the intervals. However, the interaction effect due to the treatments and intervals was found to be insignificant.

The increase in red blood cell count after supplementation of copper is in concurrence with the observations of Dwarkanath *et al* (1962). They observed similar trend of changes after supplementation of copper and copper plus cobalt to the ruminants. Reddy and Mahadevan (1966) reported the increase in the erythrocyte count of lactating cows after supplementation of 0.6 gm of copper sulphate. These are confirmed in the present study.

Pal *et al* (1970) and Pachalag (1972) could not observe any significant difference between the copper and copper and cobalt supplemented group over control group in Haryana calves and weaner lambs, respectively. Anurith Kumar *et al* (1973) reported slight increase in the R.B.C. count after supplementation of copper and cobalt tablets (125.00 and 17.61 mg of copper and cobalt, respectively) than before supplementation. They recorded 5.26 millions R.B.C. per cum. after supplementation, as against 4.47 millions R.B.C. per cum. before experiment.

(iv) Total Leucocyte (W.B.C.) Count:

The data for total leucocyte count in the blood of cows have been presented in Table 8. The average values with standard error for the total W.B.C. count in the blood for control and

experimental groups I, II and III were 8.483 ± 0.896 , 9.836 ± 0.731 , 9.798 ± 0.775 and 9.133 ± 0.614 thousands per cumm., respectively. These values are in close agreement with those of Mullick and Pal (1943) and Misra and Biswal (1961). These workers reported 9.0 and 9.44 thousands per cumm. as average values for this class of animal, respectively. The values obtained in this study falls well within the range of those reported by Coffin (1953), Hukes (1957) and Schalm (1961). They recorded a wide range from 4.0 to 13.0 thousands per cum for this constituent of blood in cattle. Govindan Nair (1949) reported similar observations on the South Indian cattle. Fraser (1930), Holman (1955), and Dabadghao (1959) reported lower values, while Shankaranarayana (1937, 1938), Smith (1959), Khajuria and Razdan (1966), and Amrith Kumar et al (1973) reported slightly higher values as compared to the values reported in the present investigation.

The total leucocyte count of the blood of the cows from all the groups has been shown in Fig 2. It is seen from the graph that the supplemented group of animals recorded higher W.B.C. count in blood as compared to those of control group. All the supplemented groups recorded the increase after supplemental period of two months, while that of control group did not increase. A slight variation observed in the values may be due to change factor. However, similar trend of increase in the values of supplemental animals as that for PCV, haemoglobin and R.B.C. count was observed.

Results of the Statistical analysis of the data are presented in Table 9. It is seen from the variance ratio that the difference

**Table 8. Average values of total Leucocytes (W.B.C.)
Thousands per cmm. with Standard Error.**

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	8.08 $\pm$ 0.81	7.97 $\pm$ 1.04	9.59 $\pm$ 0.91	7.54 $\pm$ 0.56
20	8.40 $\pm$ 0.65	8.74 $\pm$ 0.99	8.87 $\pm$ 1.95	9.42 $\pm$ 1.18
40	8.98 $\pm$ 0.99	9.06 $\pm$ 0.84	8.66 $\pm$ 1.02	8.57 $\pm$ 0.13
60	8.31 $\pm$ 1.05	8.63 $\pm$ 0.57	9.84 $\pm$ 0.69	8.72 $\pm$ 0.38
80	9.09 $\pm$ 1.24	10.33 $\pm$ 0.59	10.76 $\pm$ 1.09	9.43 $\pm$ 0.46
100	8.39 $\pm$ 0.98	10.24 $\pm$ 0.59	9.26 $\pm$ 0.62	9.13 $\pm$ 0.36
120	8.75 $\pm$ 0.83	10.33 $\pm$ 0.67	10.37 $\pm$ 0.34	9.91 $\pm$ 0.66
140	8.33 $\pm$ 0.78	10.92 $\pm$ 0.71	10.39 $\pm$ 0.88	9.82 $\pm$ 0.29
160	8.14 $\pm$ 0.85	10.23 $\pm$ 0.50	10.30 $\pm$ 0.52	9.35 $\pm$ 0.59
180	8.35 $\pm$ 0.81	10.41 $\pm$ 0.84	9.94 $\pm$ 0.45	9.43 $\pm$ 0.53
Group Average	8.483 $\pm$ 0.895	9.856 $\pm$ 0.731	9.798 $\pm$ 0.775	9.133 $\pm$ 0.514

**Table 9. Analysis of variance for Total Leucocyte Count of Blood.
(Log. values)**

Source of Variance	Degrees of freedom	Mean Sum of squares	Variance ratio
Between Treatments	3	0.23150	5.0074**
Between Intervals	9	0.07973	1.7214
Interaction	27	0.01837	0.3972
Error	160	0.04624	—

** Significant at 1 percent level of probability.

**Table 9 A. Comparison of means of W.B.C. of blood.
(Log. values).**

Critical Difference at 5% level	Group Average Values			
	Control	III	I	II
0.08429	2.11567	2.20248	2.25381	2.26494

Note: Mean values under bar do not differ significantly.

between the treatments is significant at both the percent levels. On further analysis (Table 9A), it is observed that all the supplemented groups are significantly higher over that of control group. This indicates that supplementation of copper has shown a positive effect on the white blood cell regeneration centres in the body. Treatments of group I, and III were not significantly different from each other. However, from the values in the table and the graph, it is seen that 200 mg copper sulphate supplemented group has shown slightly higher value amongst all the groups, which is in conformity with the previous findings. No significant difference between intervals and due to interaction effect between treatments and intervals was noticed.

Reddy (1966) and Reddy and Mahadevan (1966) observed similar increase in total leucocyte count of the blood of lactating Mariana cows after 0.5 gm copper sulphate supplementation per day per animal. Pal *et al* (1970) reported similar findings on the studies with growing Mariana calves, after copper supplementation. These investigators attributed the increase in white blood cell count to the increased caeruloplasmin copper and low free reacting copper in the blood, due to copper and copper plus cobalt supplementation. They reported an increase upto 13.92 thousands per cum. from 10.90 thousand per cum. initial W.B.C. count after supplementation. Asrith Kumar *et al* (1973) observed a marginal increase in the W.B.C. count in the blood of Jersey x Tharparkar heifers, after supplementation of 125 mg copper and 17.81 mg of cobalt per day. They reported 11.69 thousands W.B.C. per cum. of blood, before experiment and 11.83 thousands per cum. after supplementation.

(v) Specific Gravity:

The specific gravity of the whole blood of the animals of control and experimental groups are presented in Table 10. The average values of specific gravity for control and experimental groups I, II and III, were, 1.0484 ± 0.0003 , 1.0528 ± 0.0003 , 1.0511 ± 0.0004 and 1.0512 ± 0.0003 , respectively. Average values of specific gravity recorded in this experiment are in close agreement with those reported by Albritton (1952) and Coffin (1953), Dukes (1957), Schalm (1961) and Murty and Mullick (1960). The average value for specific gravity of whole blood of cows reported by these workers was 1.052, with the range from 1.046 to 1.058. The values reported in the present investigation lie well within this range. Khajuria and Razdan (1966) reported somewhat higher values (1.0591) for Tharparkar lactating animals in winter, which may be due to climatic variation. Recently Chaplin et al (1970) estimated the specific gravity of whole blood of various western dairy breeds. They reported 1.0510 as the normal average value for Jersey cows of 2 to 4 years of age. This is similar to values obtained in the supplemented group of Jersey x Tharparkar cross-bred cows.

A graphic representation of the average values of all the groups for specific gravity of whole blood of the cows is presented in Fig 2. It is seen from the graph that supplemented group of animals always recorded higher specific gravity values of the blood as compared to those obtained for control group.

The cows of the group I, (200 mg) recorded the highest specific gravity values, after 40 days of the supplemental

**Table 10. Average values of Specific Gravity of Blood
with standard error**

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	1.0493 $\pm$ 0.0013	1.0504 $\pm$ 0.0004	1.0506 $\pm$ 0.0012	1.0508 $\pm$ 0.0014
20	1.0484 $\pm$ 0.0008	1.0504 $\pm$ 0.0012	1.0506 $\pm$ 0.0012	1.0504 $\pm$ 0.0018
40	1.0483 $\pm$ 0.0008	1.0514 $\pm$ 0.0012	1.0506 $\pm$ 0.0014	1.0504 $\pm$ 0.0008
60	1.0480 $\pm$ 0.0006	1.0514 $\pm$ 0.0004	1.0504 $\pm$ 0.0018	1.0504 $\pm$ 0.0012
80	1.0482 $\pm$ 0.0006	1.0536 $\pm$ 0.0008	1.0514 $\pm$ 0.0014	1.0512 $\pm$ 0.0012
100	1.0484 $\pm$ 0.0006	1.0530 $\pm$ 0.0006	1.0512 $\pm$ 0.0012	1.0518 $\pm$ 0.0014
120	1.0483 $\pm$ 0.0006	1.0542 $\pm$ 0.0008	1.0518 $\pm$ 0.0016	1.0518 $\pm$ 0.0010
140	1.0482 $\pm$ 0.0006	1.0542 $\pm$ 0.0012	1.0516 $\pm$ 0.0016	1.0500 $\pm$ 0.0012
160	1.0478 $\pm$ 0.0004	1.0544 $\pm$ 0.0008	1.0510 $\pm$ 0.0018	1.0518 $\pm$ 0.0012
180	1.0478 $\pm$ 0.0004	1.0546 $\pm$ 0.0010	1.0516 $\pm$ 0.0014	1.0516 $\pm$ 0.0012
Group Average	1.0484 $\pm$ 0.0002	1.0528 $\pm$ 0.0003	1.0511 $\pm$ 0.0004	1.0512 $\pm$ 0.0003

Table 11. Analysis of Variance for Specific gravity of Blood

Source of Variance	Degrees of freedom	Mean Sum of squares	Variance ratio
Between Treatments	3	0.0001633	27.79**
Between Intervals	9	0.0000080	13.61
Interaction	27	0.0000041	0.693
Error	160	0.0000058	-

** Significant at 1 percent level of probability.

**Table 11 A. Comparison of Means of Specific gravity
of Blood**

Critical Difference at 5% level	Group Average Values			
	Control	II	III	I
0.00095	1.0484	1.0511	1.0512	1.0528

Note: Mean values under bar do not differ significantly.

period, which further increased subsequently upto four months of supplementation period and thereafter became steady. A similar trend is seen in the other two groups (300 mg and 400 mg). However, in these groups the rise in the values started at 2 months after supplementation. It may be due to the adjustment of rumen flora to a higher level of copper sulphate. However, these groups recorded lower values as compared to those of Group I (200 mg). Therefore, 200 mg supplementation seems to have maximum beneficial effect on the specific gravity of whole blood.

On statistical analysis of the data, 'F' value for treatments was found highly significant (Table 11) at one percent level. This indicates that the treatments are significantly different from each other and the supplementation of copper has an effect on the specific gravity of blood. Further analysis (Table 11A) by comparing the differences between the means of four groups with the critical difference at 5 percent level revealed that treatments of group I, II and III are superior to treatment of control group. This shows that copper supplementation has a positive effect on specific gravity of blood. Further it was observed that the treatment of group I (200 mg) appeared to be better over treatments of group II (300 mg) and group III (400 mg), while in group II (300 mg) and group III (400 mg) similar effect was noticed. No significant difference was noticed between intervals and due to interaction effect between the treatments and intervals indicating thereby that the treatments did have an effect on the specific gravity of whole blood, independent of time interval factor.

There are no known references as far as the effect of copper supplementation on specific gravity of blood of lactating cows. However, the observations of Raghavan *et al* (1961) are in agreement with the trend of specific gravity with other constituents of blood, i.e., PCV, erythrocyte count and haemoglobin, noticed in the present study. They reported a positive correlation between specific gravity and PCV, erythrocyte count and haemoglobin. Earlier, Holman (1955) also reported similar findings. Chaplin *et al* (1970) reported 1.0510 as the normal average value for specific gravity of blood of Jersey cows. In the present investigation, the supplemented groups have shown the same average values, while the un-supplemented group has shown a lower value. This indicates that copper supplementation has brought the specific gravity of the blood of lactating cows to normal reported values for Jersey breed. The 200 mg copper sulphate supplementation appears to be the suitable level.

(B) Biochemical Constituents:

(vi) Serum Protein:

The average serum protein values of the cows of control and experimental groups are presented in Table 12. The average values of serum protein for the control and experimental groups I, II and III were 7.515 ± 0.08 , 7.592 ± 0.07 , 7.563 ± 0.06 and 7.541 ± 0.07 percent respectively. From the table, it is seen that there was a steady increase in the serum protein values from the beginning of the experiment to the end. The values of serum of all the supplemented groups have shown a slightly increasing trend than those of control group. Group I animals that received

Table 12. Average values of Serum Protein Percentages with Standard error

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	7.217 $\pm$ 0.13	7.192 $\pm$ 0.25	7.08 ⁴ $\pm$ 0.28	7.106 $\pm$ 0.19
20	7.381 $\pm$ 0.17	7.482 $\pm$ 0.30	7.292 $\pm$ 0.26	7.354 $\pm$ 0.21
40	7.423 $\pm$ 0.20	7.597 $\pm$ 0.25	7.416 $\pm$ 0.19	7.472 $\pm$ 0.24
60	7.549 $\pm$ 0.19	7.614 $\pm$ 0.21	7.601 $\pm$ 0.17	7.513 $\pm$ 0.15
80	7.582 $\pm$ 0.21	7.607 $\pm$ 0.17	7.723 $\pm$ 0.16	7.544 $\pm$ 0.19
100	7.515 $\pm$ 0.23	7.660 $\pm$ 0.19	7.633 $\pm$ 0.12	7.567 $\pm$ 0.18
120	7.594 $\pm$ 0.19	7.651 $\pm$ 0.24	7.708 $\pm$ 0.19	7.678 $\pm$ 0.22
140	7.647 $\pm$ 0.18	7.721 $\pm$ 0.28	7.752 $\pm$ 0.14	7.693 $\pm$ 0.23
160	7.639 $\pm$ 0.22	7.703 $\pm$ 0.21	7.713 $\pm$ 0.18	7.702 $\pm$ 0.21
180	7.623 $\pm$ 0.24	7.754 $\pm$ 0.17	7.706 $\pm$ 0.17	7.771 $\pm$ 0.15
Group Average	7.515 $\pm$ 0.08	7.692 $\pm$ 0.07	7.583 $\pm$ 0.06	7.541 $\pm$ 0.07

Table 13. Analysis of Variance for Serum Protein

Source of Variance	Degrees of freedom	Mean Sum of Squares	Variance ratio
Between Treatments	3	0.161	0.285
Between intervals	9	6.621	3.306**
Interaction	27	0.077	0.016
Error	160	30.294	-

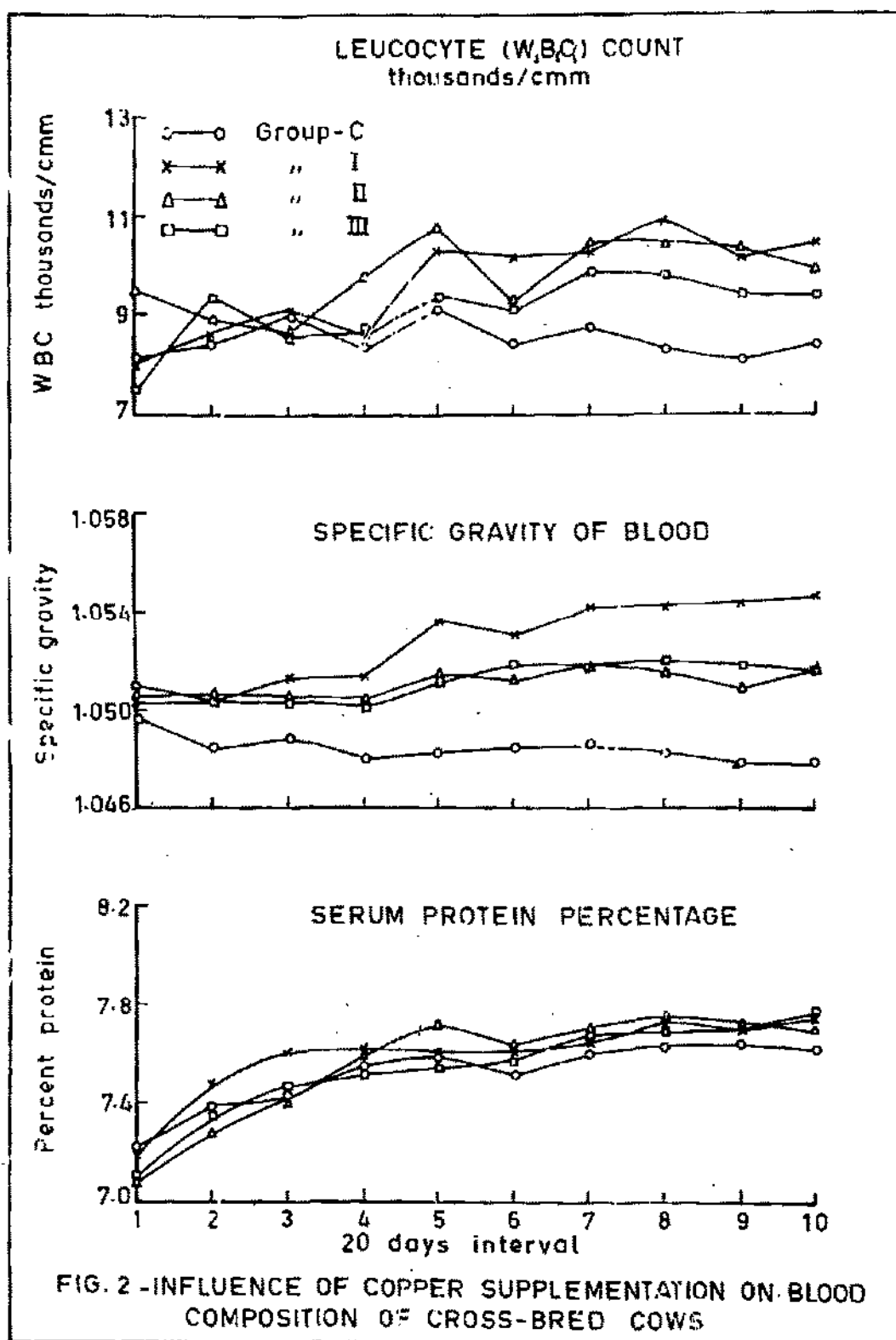
** Significant at 1 percent level.

200 mg copper sulphate supplementation recorded the highest value, followed in order by those in group II and group III. A narrow range of 7.08 to 7.77 gm percent was observed in all the groups throughout the experiment. Kehar and Murty (1946) reported slightly higher values, but Pal *et al* (1945) reported similar values of serum protein in dairy cattle. Sahai and Kehar (1947) reported similar values of serum protein in Kumaoni bullocks.

The serum protein values of the cows of all the groups are shown in Fig 2. It is seen that the supplemented groups of animals recorded higher serum protein values after supplemental period of four months as compared to those observed for control group. The initial lower values of serum protein of supplemented groups as compared to control group went above that of control group values in latter half of the experiment, and became almost steady in last two months of the experiment.

It is seen from the statistical analysis of the data, that there is no statistically significant difference between the values of serum protein of different groups (Table 13). However, a significant difference at one percent level was observed between the intervals, which is also evident from Fig 2. The interaction effect due to treatments and interaction however, was found to be statistically significant.

Amrith Kumar *et al* (1973) reported similar observations on serum protein values, after supplementation of 125 mg of copper and 17.81 mg of cobalt per day to the Jersey x Tharparkar heifers, from the same dairy herd. They reported 6.20 gm



percent as a value for serum protein before the experiment as against 6.80 gm percent after the experiment.

(vii) Blood Iron:

The average values of blood iron contents of the cows of control and experimental groups are presented in Table 14. The average values of iron content of blood were 38.81 ± 0.62 , 47.70 ± 0.68 , 45.80 ± 0.68 and 46.98 ± 0.67 mg per 100 ml blood of the animals of control and experimental groups I, II and III, respectively.

The values reported in the present investigation are well within the range (30.0 to 50.0 mg percent), given by Dukes (1957) for this class of animals. Mullick and Pal (1943) and Pal *et al* (1945) reported 39.8 mg percent iron as an average value for Indian cattle. The value observed for control group is in close agreement with this value, while the values recorded for supplemental groups were higher. Kumar (1960), Patel *et al* (1961) and Razdan and Setty (1968) reported values ranging from 32.59 to 48.83 mg of iron per 100 ml of blood.

The blood iron values of the cows of all the groups are shown in Fig. 3. It is seen from the graph that the animals of supplemented group recorded higher blood iron values as compared with those of control group of animals. The cows of the group I (200 mg) recorded the highest blood iron values, after supplemental period of one month, which is followed in order by the group III (400 mg) and then group II (300 mg). A steady increase was observed only after two months of supplementation. Slightly lower values recorded at two months of supplementation

Table 14. Average values of Blood iron in mg percent with Stand. Error*

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	39.52 $\pm$ 3.86	43.94 $\pm$ 1.38	41.47 $\pm$ 1.76	44.56 $\pm$ 1.12
20	40.97 $\pm$ 2.57	43.12 $\pm$ 1.68	43.16 $\pm$ 0.78	42.57 $\pm$ 1.95
40	43.40 $\pm$ 1.48	46.93 $\pm$ 0.98	45.12 $\pm$ 2.51	47.13 $\pm$ 1.06
60	36.66 $\pm$ 2.06	44.23 $\pm$ 2.33	42.29 $\pm$ 3.83	41.46 $\pm$ 2.42
80	39.27 $\pm$ 1.21	48.38 $\pm$ 2.21	46.32 $\pm$ 3.07	45.95 $\pm$ 2.53
100	36.31 $\pm$ 1.66	46.79 $\pm$ 2.60	46.53 $\pm$ 1.92	49.21 $\pm$ 2.23
120	39.69 $\pm$ 1.05	51.90 $\pm$ 1.10	46.54 $\pm$ 2.97	50.53 $\pm$ 1.88
140	38.86 $\pm$ 1.25	48.61 $\pm$ 1.31	49.57 $\pm$ 0.75	49.89 $\pm$ 1.36
160	38.85 $\pm$ 1.39	51.39 $\pm$ 1.83	48.41 $\pm$ 1.50	49.41 $\pm$ 1.84
180	38.63 $\pm$ 1.64	50.66 $\pm$ 1.66	49.03 $\pm$ 1.86	49.12 $\pm$ 1.75
Group Average	38.81 $\pm$ 0.62	47.70 $\pm$ 0.66	45.80 $\pm$ 0.68	46.98 $\pm$ 0.67

Table 15. Analysis of Variance for Blood Iron*

Source of Variance	Degrees of freedom	Mean Sum of Squares	Variance ratio
Between Treatments	3	832.318	52.287**
Between Intervals	9	106.080	6.664**
Interaction	27	24.006	1.508**
Error	160	15.918	-

** Significant at 1 percent level of probability.

Table 15 A. Comparison of Means of Blood Iron.

Critical Difference at 5% level	Group Average Values			
	Control	II	III	I
1.664	38.81	45.80	46.98	47.70

Note: Mean values under bar do not differ significantly.

period may be due to the active physiological status of animals in the heavy milk production stage. However, the other undulations may be due to chance factor.

On subjecting the data to the variance test (Table 15), it is observed that the iron content of blood of different groups of animals differed significantly from each other due to treatments. The difference was found highly significant at both the percentage of probability. Further analysis (Table 15A) revealed that all the supplemented groups are superior over that of control group. Treatments of group II and III and group I and III were not found significantly different from each other. While treatment of group I (200 mg) was found to be superior over treatment of group II (300 mg), indicating the maximum utilization of iron by 200 mg supplemented animals. Further, a significant difference between the intervals at both the percent levels of probability was observed. This is also evident from the Fig 3, that as the time advanced, the rise in the iron content of blood is seen in supplemented animals. In control group, however, with initial slight rise, the values remained more or less constant. An interaction effect between treatments and intervals was also observed to be statistically significant, showing thereby the contribution of both the factors to the increased blood iron values.

Kehar et al (1959), Dwarkanath et al (1962), Agarwal (1963) and Reddy and Mahadevan (1966) reported the increased haemoglobin percentage of the blood of cattle after copper supplementation, which is in conformity with the present findings.

Amrith Kumar et al (1973) observed similar increase in blood iron content of Jersey x Tharparkar heifers, after supplementation of copper and cobalt. They reported 41.6 mg of iron per 100 ml of blood as against only 30.0 mg, before supplementation. Their findings are corroborated in this study.

(viii) Blood Copper:

The concentration of copper in whole blood is very similar to that in plasma. Cartwright (1950) has confirmed it by the compilation of the data on distribution of copper in whole blood, plasma and cells. Therefore, in this study the effect of copper supplementation on whole blood copper is undertaken.

The blood copper values of the animals of all the groups are shown in Table 16. The average values of blood copper with standard error were 87.58 ± 1.46 , 117.88 ± 3.17 , 109.60 ± 3.09 and 107.72 ± 2.63 mcg per 100 ml blood of the animals of control and experimental groups I, II and III, respectively. The concentration of copper in whole blood of they healthy animals is wide. The range observed by Beck (1956) was 50 to 150 mcg per 100 ml of blood. In the present investigation, the values were found to be between 80 to 130 mcg per 100 ml of blood, which is in conformity with the findings of Beck (1956). Values reported by Sahai and Kehar (1951) for the Kumaoni bullocks were 129 to 153 mcg with an average of 141 mcg per 100 ml of blood. These values were high as compared to the values obtained after supplementation in this study. Similarly high values were reported for Kankrej and Gir cattle by Western Animal Nutrition

Table 16. Average values of blood copper in ug percent with Standard Error.

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	84.43 $\pm$ 6.10	96.29 $\pm$ 6.80	87.89 $\pm$ 7.21	94.21 $\pm$ 7.50
20	82.14 $\pm$ 3.21	98.23 $\pm$ 8.02	95.84 $\pm$ 6.10	101.39 $\pm$ 9.78
40	90.33 $\pm$ 8.22	116.76 $\pm$ 5.63	118.66 $\pm$ 10.16	113.99 $\pm$ 10.16
60	93.61 $\pm$ 5.31	131.27 $\pm$ 7.46	121.85 $\pm$ 11.32	113.86 $\pm$ 8.00
80	37.24 $\pm$ 2.85	130.61 $\pm$ 13.01	118.78 $\pm$ 6.34	121.45 $\pm$ 10.35
100	89.28 $\pm$ 4.42	139.63 $\pm$ 12.10	131.98 $\pm$ 8.15	120.80 $\pm$ 10.17
120	88.49 $\pm$ 4.40	121.07 $\pm$ 10.17	114.53 $\pm$ 10.75	108.48 $\pm$ 11.28
140	85.92 $\pm$ 4.48	115.27 $\pm$ 8.94	110.45 $\pm$ 11.72	106.23 $\pm$ 8.85
160	83.59 $\pm$ 6.74	116.78 $\pm$ 12.11	97.22 $\pm$ 2.51	99.18 $\pm$ 3.42
180	87.73 $\pm$ 5.98	118.37 $\pm$ 11.89	97.80 $\pm$ 2.88	100.52 $\pm$ 3.43
Group Average	87.58 $\pm$ 1.46	117.88 $\pm$ 3.70	109.50 $\pm$ 3.09	107.72 $\pm$ 2.63

Table 17. Analysis of Variance for Blood Copper.

Source of Variance	Degrees of freedom	Mean Sum of Squares	Variance ratio
Between Treatments	3	8215.509	27.233**
Between Intervals	9	1665.083	5.514**
Interaction	27	202.484	0.671
Error	160	301.678	-

** Significant at 1 percent level of probability.

Table 17A. Comparison of Means of Blood Copper.

Critical Difference at 5% level	Group Average Values			
	Control	III	II	I
6.809	87.58	107.72	109.50	117.88

Note: Mean values under bar do not differ significantly.

Station, Anand (Annon, 1951-52, 1952-53). The values reported by Deshpande (1959) and Kumar (1960) are similar to those reported under the present investigation for the very same class of animals.

The copper content of blood of the cows of all the groups are shown in Fig. 3. It is seen from the graph that the supplemented groups of animals recorded higher blood copper values as compared with those of control groups. The cows of the Group I, which received 200 mg copper sulphate supplement per day, recorded the highest blood copper values after supplementation for a period of 40 days, then a slight fall in values after 4 months and subsequently became steady. However, those cows receiving the supplement of 300 and 400 mg copper sulphate per day recorded lower values compared with those of 200 mg supplemented group. Therefore, 200 mg supplement seems to be optimum to get the increased copper level in blood. A slight drop in the blood copper values has been observed at 105 days of supplementation, which may be attributed to the influence of initiation of pregnancy (Butler, 1953).

Statistical analysis of the data to assess the effect of supplementation of dietary copper on blood copper levels is presented in Tables 17 and 17A. It is seen from the 'F' values of the analysis of variance, that there exists a highly significant difference between the treatments for the blood copper levels in different groups. This indicates that the supplementation of copper has positive effect on the blood copper level. Further analysis by critical difference revealed

that all the supplemental treatments recorded significantly higher values over control groups. Treatment of group I (200 mg) was not found significantly different from treatment of group II (300 mg). Similarly, treatments of group II and III (400 mg) were not significantly different from each other. However, group I is significantly higher over group III. This indicates that either 300 or 400 mg of copper sulphate supplementation has no additional advantage over 200 mg. The increase in the copper level in the blood after supplementation of dietary copper to lactating cows is in close agreement with the findings of Ferguson (1943), Dashpande (1959), Reddy (1966) and Sampath *et al* (1969). They observed similar trend of increase in the blood copper level, after supplementation of copper. A significant difference at 1 percent level, between intervals is also noticed. However, the interaction between the treatments and intervals was not significant.

Allcroft and Parker (1949) observed an increase in blood copper levels in dairy cows of hypocupraemia from 28 to 80 mcg copper per 100 ml blood, in a month by dietary supplementation of 2 gm copper sulphate per day. Increase in whole blood copper concentrations were also observed after copper or copper and cobalt supplementation to Haryana calves by Dwarakanath *et al* (1962). Agarwal and Hujundar (1963) observed a temporary increase in blood copper levels on copper supplementation in Haryana calves. Similar increase was observed by Engel *et al* (1964) in the concentration of circulating whole blood, on copper supplementation. However, Felsman *et al* (1978) did not notice such an increase in serum copper level after copper supplementation in calves.

Kapoor (1968) and Kapoor and Mahadevan (1970a) reported the similar beneficial effect of copper and copper plus cobalt supplementation in Haryana cattle on total copper and caeruloplasmin copper in bovine plasma and serum. Observations of Dasgynska *et al* (1970) are still interesting. They estimated the copper and caeruloplasmin levels in circulating blood of pregnant cows at monthly intervals. They observed an increase of copper and caeruloplasmin upto fifth month of pregnancy and these returned to normal levels before calving. There may be a natural rise in blood copper level of lactating cows upto seventh month of lactation. Recently, Arith Kumar *et al* (1973) reported 35 to 40 percent increase in the copper content of blood of Jersey x Tharparkar heifers, from the same herd, after supplementation (125 mg copper and 17.81 mg cobalt) with one copper-cobalt tablet per day per head.

(II) Influence of Copper on Milk Composition:

An attempt has been made to find out the effect of supplementation of copper to the lactating cows on their milk composition and the proportions of different constituents of milk. Daily morning, noon and evening, milk samples were collected from each individual cow. Aliquots were taken proportionate to the milk yield and were pooled for a period of fifteen days. These pooled samples were stored at 4°C with formalin as preservative. Then the composite samples were analysed fortnightly, for milk fat, protein, total solids and ash percentages. The solids not fat were calculated by difference. The results are summarized and discussed below.

(1) Milk fat:

The average values for fat content of milk of all the groups are presented in Table 18. The fat percentages for control and experimental groups I, II and III were 5.47 ± 0.06 , 5.25 ± 0.10 , 5.44 ± 0.11 and 5.83 ± 0.12 percent, respectively. Values for butter fat recorded in this study were all within the normal range (4.12 to 5.92%) reported by various workers (Sen, 1963; Ghosh, 1956; Ling, 1966; Jenness and Patton 1959; Armstrong, 1959 and Murthy, 1969-1972) for Jersey and Tharparkar cows. However, the average values reported by Sukhatme et al (1948) for Tharparkar animals was found to be slightly lower (4.6%) than the values recorded here. This may be due to the cross-breeding with Jersey, which is known for its high milk fat percentages.

The fat percent of the milk of the cows for all the groups has been shown in Fig. 3. It is seen from the figure that cows of the group III, that received a supplement of 400 mg of copper sulphate per day recorded higher milk fat as compared to those of control and other experimental groups. In all the supplemented groups, the normal trend of decrease in the butter fat at the beginning of the lactation did not occur, while it was observed in the control group upto two months of lactation period. The animals of group II and I, getting 300 and 200 mg of copper sulphate respectively per day per animal have shown proportionately more increase in the butter fat percent of milk, as compared to their initial fat percent values, as against that in control group. Cows of group II recorded higher milk fat than the control group at 5th month of lactation and that of group I, at the end of the studies.

Statistical analysis of the data for the fat percentages of milk has been presented in Table 19 and 19A. The difference between the treatments is highly significant at one percent level. On further analysis, it is seen that the treatment of group III was found significantly different from other treatments. Treatments of group I and II as well as that of control and II were not significantly different from each other. The control group values were found significantly higher than that of group I. This may be due to the initial high values of milk fat in cows of control group. A significant difference between the intervals was also noticed, which may be due to the normal trend of this constituent of milk, as the

Table 18. Average values of fat percentage of milk with standard error.

Fortnights Intervals in days	Groups			
	Control	I Experimental	II Experimental	III Experimental
1	5.28 ± 0.13	4.80 ± 0.31	4.80 ± 0.32	4.94 ± 0.28
2	5.14 ± 0.19	4.94 ± 0.23	4.94 ± 0.21	5.34 ± 0.17
3	5.10 ± 0.11	4.88 ± 0.24	4.94 ± 0.14	5.32 ± 0.21
4	5.16 ± 0.13	4.86 ± 0.13	5.02 ± 0.11	5.30 ± 0.28
5	5.22 ± 0.13	5.06 ± 0.30	5.20 ± 0.16	5.46 ± 0.26
6	5.42 ± 0.10	5.14 ± 0.36	5.26 ± 0.14	5.68 ± 0.23
7	5.42 ± 0.09	5.12 ± 0.30	5.34 ± 0.15	5.68 ± 0.23
8	5.44 ± 0.10	5.16 ± 0.30	5.42 ± 0.10	5.76 ± 0.24
9	5.50 ± 0.12	5.28 ± 0.30	5.48 ± 0.15	5.78 ± 0.26
10	5.56 ± 0.12	5.30 ± 0.31	5.52 ± 0.25	5.82 ± 0.23
11	5.62 ± 0.11	5.42 ± 0.32	5.72 ± 0.30	5.14 ± 0.24
12	5.72 ± 0.12	5.54 ± 0.32	5.88 ± 0.24	6.34 ± 0.45
13	5.76 ± 0.12	5.62 ± 0.30	5.86 ± 0.22	6.44 ± 0.48
14	5.84 ± 0.14	5.82 ± 0.32	6.14 ± 0.24	6.60 ± 0.54
15	5.88 ± 0.14	5.82 ± 0.34	6.18 ± 0.23	6.64 ± 0.49
Group Av.	5.47 ± 0.08	5.25 ± 0.10	5.44 ± 0.11	5.83 ± 0.12

Table 19. Analysis of Variance for Milk Fat.

Source of Variance	Degrees of freedom	Mean Sum of Squares	Variance ratio
Between Treatments	3	4.3382	10.77**
Between Intervals	14	3.0213	7.50**
Interaction	42	0.0943	0.21
Error	240	0.4028	-

** Significant at 1 percent level of probability.

Table 19 A. Comparison of means of Milk Fat Percentages.

Critical Difference at 5% level	Group Average Values			
	I	II	Control	III
0.203	5.25	5.44	5.47	5.83

Note: Mean values under bar are not significantly different.

lactation advanced (Sukhatme ¹⁹⁵⁶ et al, 1948, Ghosh, and Ghosh, and Anantakrishnan, 1954). No significant difference due to the interaction between the treatments and intervals was noticed.

Reddy (1956) and Reddy and Mahadevan (1956) reported similar findings with 0.5 gm of copper sulphate supplementation in Hariana cows. Earlier, Dinlop (1953) studied the effect of copper on butter fat production in cows. He supplemented the ration with 10.0 gm of copper sulphate daily and reported a significant increase in butter fat production. Later, King (1955) studied the effect of copper supplementation on the fat content of milk of cows having normal blood copper levels. He supplemented the basal ration with 1.0 and 2.0 gm of copper sulphate and 10.0 gm of sodium sulphate, daily. The fat percentage rose from the first to the second week after supplementation of copper sulphate. Fat globule number increased and diameter of these decreased significantly. Sodium sulphate had no effect on the fat percentage of milk, meaning thereby sulphate was not responsible for the changes in fat percentages. In the present study, an increase was observed in the fat content of milk produced by the cows of supplemented groups, during the first fortnight, after which it was steady for a period of 4 weeks. This observation is in conformity with that of King (1955).

On the other hand, Barysheva (1956) and Poppe (1956) could not observe any significant increase in the butter fat content of milk produced by the cows receiving supplements of either copper alone or copper plus other trace minerals. While

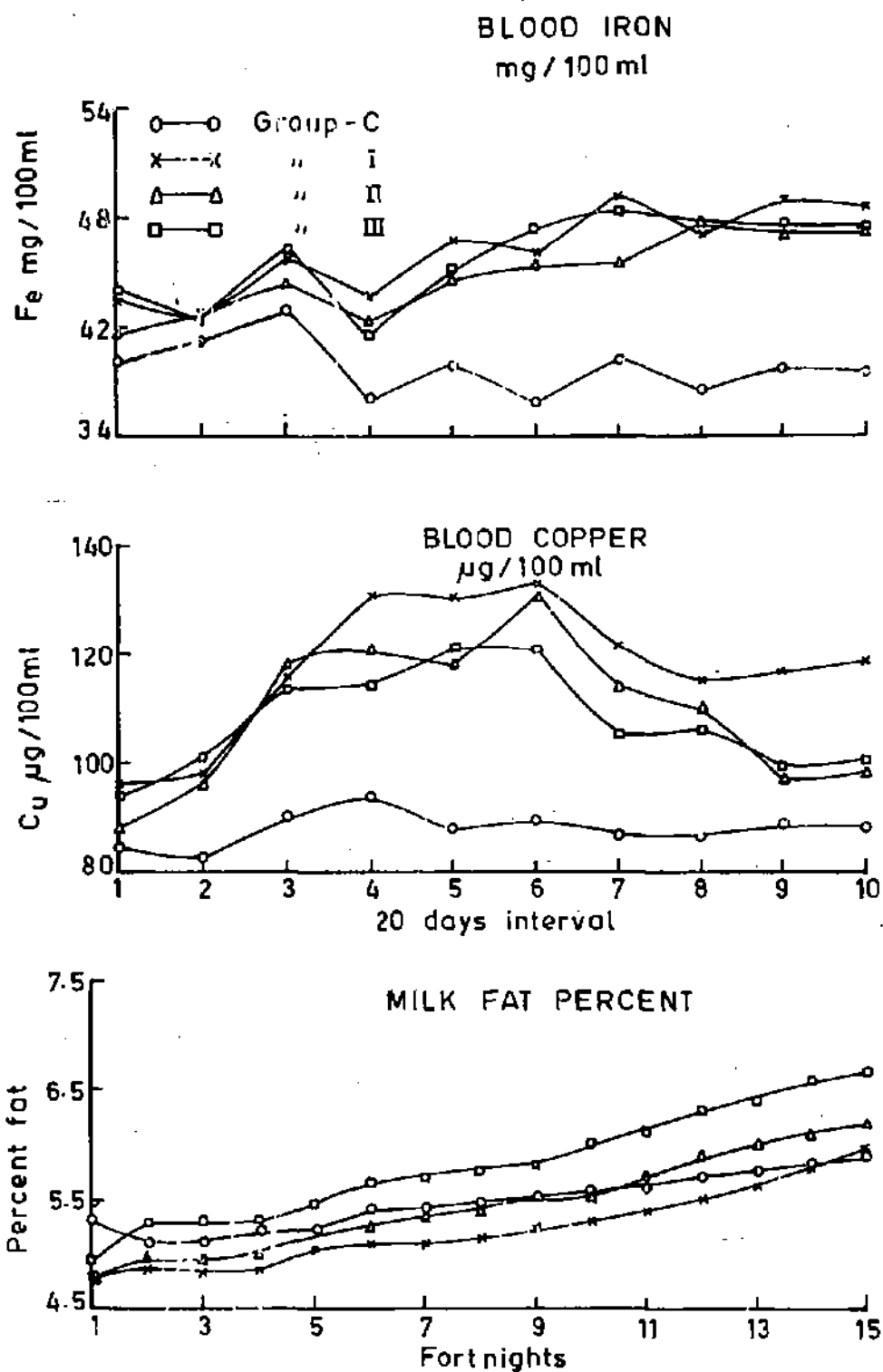


FIG.3-INFLUENCE OF COPPER SUPPLEMENTATION ON BLOOD AND MILK CONSTITUENTS

A graphic representation of the milk protein percentages for all the groups is shown in Fig. 3. It is seen from the figure that the supplemented groups recorded higher milk protein values as compared with those obtained for control group. Similar to the milk fat percentage, cows of group III (400 mg) recorded the highest milk protein values inspite of having lowest initial values as compared to those of the other two supplemented groups.

A normal tendency of the content of this constituent of milk to decrease during the initial stages of lactation (Sukhatme *et al.* 1948) is not observed in supplemented groups to that extent as it is observed in control group. The values of group I and II have shown almost similar increase as in control and group III. Ekles and Shaw (1913) observed that the protein content of the milk of Jersey cows gradually decreased to a minimum of 3.06 percent from the initial figure of 3.25 percent, during the second and third months of lactation. After the third month, it increased from 3.32 and at the end of 56 weeks it was 3.91 percent. In the present study also similar observations have been recorded in the case of Jersey x Tharparkar cows.

A statistical analysis of the data is given in Table 21 and 21A. A significant difference between the treatments was observed at 1 percent level. On comparing the differences of means with the critical difference at 5 percent level, it is observed that all the supplemented treatments recorded higher values of protein over those of control. However no significant

Table 20. Average Values of Milk Protein Percentage with S.E. 17

Fortnights	Groups			
	Control	Experimental I	Experimental II	Experimental III
1	3.292±0.06	3.292±0.13	3.292±0.13	3.241±0.06
2	3.262±0.10	3.297±0.14	3.313±0.15	3.338±0.10
3	3.200±0.10	3.267±0.15	3.272±0.15	3.418±0.12
4	3.251±0.08	3.277±0.11	3.302±0.12	3.374±0.11
5	3.276±0.04	3.318±0.13	3.338±0.11	3.425±0.12
6	3.323±0.06	3.435±0.12	3.466±0.11	3.466±0.11
7	3.389±0.08	3.517±0.13	3.466±0.08	3.578±0.15
8	3.430±0.06	3.563±0.13	3.578±0.08	3.598±0.09
9	3.465±0.04	3.634±0.12	3.659±0.07	3.654±0.12
10	3.506±0.04	3.660±0.12	3.660±0.08	3.721±0.08
11	3.537±0.03	3.726±0.10	3.690±0.06	3.813±0.13
12	3.593±0.06	3.751±0.10	3.797±0.10	3.797±0.14
13	3.578±0.06	3.761±0.09	3.777±0.01	3.853±0.10
14	3.669±0.06	3.828±0.08	3.915±0.06	3.899±0.12
15	3.663±0.03	3.815±0.10	3.884±0.06	3.945±0.11
Group Av.	3.428± 0.027	3.549±0.046	3.561±0.042	3.615±0.046

Table 21. Analysis of Variance for Milk Protein Percentages.

Source of Variance	Degrees of freedom	Mean Sum of Square	Variance ratio
Between Treatments	3	0.46613	9.526**
Between Intervals	14	0.81474	16.644**
Interaction	42	0.01466	0.298
Error	240	0.04898	-

** Significant at 1 percent level of probability.

Table 21A. Comparison of Means of Milk Protein percentages.

Critical Difference at 5% level	Group Average Values			
	Control	I	II	III
0.0708	3.428	3.549	3.561	3.615

Note: Mean values under bar do not differ significantly.

difference between the treatments of supplemented groups was noticed. Supplementation of copper at different levels did not result in much variation in the protein contents among the treated groups. A highly significant difference was observed between the intervals, which is a normal trend. The interaction effect due to the treatments and intervals was not significant.

Khidekel (1967) and Shatokhina (1968) reported similar observations in lactating cows after copper and copper plus cobalt supplementation.

(3) Total solids:

The total solids percentage of the milk of cows of control and experimental groups are presented in Table 22. The average values for total solids of milk were 14.850 ± 0.098 , 14.772 ± 0.184 , 14.932 ± 0.156 and 15.376 ± 0.163 percent, for the control and experimental groups I, II and III respectively. The values are within the normal range reported by Overman *et al* (1939) and Warner (1961), Agarwala and Sharma (1961) and Armstrong (1959) for milk of Jersey and Tharparkar cows. Sen and Dastur (1947) reported 12.98 percent as the average total solids in the milk of Tharparkar cows. The values obtained in the present investigation are somewhat higher. This may be due to the higher fat percentage in the milk of these cross-bred cows. Sukhatme *et al* (1948) reported a range of 12.712 to 15.166 percent total solids in the milk of the Indian cows.

The total solids contents of milk of the cows of all the groups are shown in Fig 4. It is seen from the figure that

the animals of supplemented groups recorded higher total solids in their milk as compared to those of control group, except in the case of animals of group I (200 mg), during the latter half of experimental period. Similar to fat and protein percentages, the animals of group III (400 mg) recorded the highest values for total solids in their milk throughout the experiment. Moreover, a normal feature of total solids content of milk to decrease upto second or third month of lactation (Sukhatme et al, 1948), which is observed in the milk of cows of control group, is not seen in the animals of supplemented groups. Instead of that, a slight rise in the total solids percentage values during the first month of lactation is recorded. This was not observed in the control group. The rate of increase in the total solids contents of milk was faster in supplemented groups than the control group as the stage of lactation advanced.

On subjecting the data to analysis of variance a significant difference was observed (Table 23) between the group treatments at one percent level. Further analysis revealed that the total solids values of milk of cows of group III (400 mg) were significantly higher than those of control and experimental I and II groups (Table 23 A). Treatments of control groups I and II did not differ significantly from each other. A significant difference between the intervals was observed. No significant difference due to the interaction effect between the treatments and intervals was noticed.

No reference on the effect of copper supplementation on total solids content of milk of lactating cows are available.

Table 22. Average Values of Total Solids Percentage with S.E.

Fortnights	Groups			
	Control	Experimental I	Experimental II	Experimental III
1	14.405±0.21	14.005±0.24	13.834±0.99	14.056±0.34
2	14.303±0.17	14.334±0.26	14.424±0.46	14.610±0.26
3	14.062±0.21	14.365±0.22	14.105±0.29	14.476±0.26
4	14.234±0.18	14.260±0.25	14.495±0.21	14.661±0.29
5	14.284±0.18	14.395±0.41	14.556±0.14	14.745±0.31
6	14.518±0.07	14.566±0.42	14.708±0.19	15.169±0.32
7	14.548±0.15	14.637±0.38	14.850±0.10	15.273±0.42
8	14.353±0.20	14.640±0.32	15.051±0.22	15.450±0.29
9	15.094±0.24	14.867±0.40	15.241±0.26	15.539±0.41
10	15.233±0.19	14.896±0.41	15.270±0.23	15.733±0.42
11	15.241±0.15	14.983±0.35	15.307±0.22	15.823±0.40
12	15.281±0.20	15.128±0.39	15.471±0.31	15.926±0.50
13	15.372±0.20	15.231±0.41	15.542±0.31	16.022±0.49
14	15.856±0.18	15.540±0.43	15.911±0.41	16.409±0.73
15	15.678±0.26	15.727±0.43	16.100±0.42	16.743±0.40
Group Av.	14.850±0.098	14.772±0.184	14.938±0.156	15.376±0.163

Table 23. Analysis of Variance for Total Solids Percentages.

Source of variance	Degrees of freedom	Mean Sum of Square	Variance ratio
Between Treatments	3	5.3941	8.952**
Between Intervals	14	7.5190	12.478**
Interaction	42	0.5472	0.908
Error	240	0.6026	-

** Significant at 1 percent level of probability.

Table 23A. Comparison of Means of Total Solids in Milk.

Critical Difference at 5% level	Group Average Values			
	I	Control	II	III
0.2485	14.772	14.850	14.938	15.376

Note: Mean values under bar do not differ significantly.

However, the effect of supplementation of copper on fat and protein percentages will also hold good for this, as the total solids of milk includes these two constituents.

(4) Solids not-fat:

The non-fatty constituents of cows milk which makes up more than 70 percent of the total solids contain the most important animal protein, lactose, and minerals required for muscle and tissue growth as well as bone development, besides the essential vitamins required for the metabolic activities of the young calf and the human infant. Therefore, its importance is well recognised. The solids not-fat content of the milk of the cows of control and experimental groups I, II and III were 9.379 ± 0.055 , 9.528 ± 0.076 , 9.524 ± 0.081 and 9.562 ± 0.059 percent respectively (Table 24). Sen (1953) reported 9.53 percent as the average solids not-fat content in the milk of Tharparkar cows. While Aggarwala and Sharma (1961) reported 9.37 percent as the solids not-fat content of cows milk. These values are in close conformity with values observed in this present experiment. Values reported by Drakeley (1927), Overman (1939, 1945) Warner (1951) and Armstrong (1959) for the S.N.F. content of milk of Jersey animals (9.1 to 9.8 percent) are also not far from the values observed in the present experiment. Sukhatme (1948) reported 9.17 percent as the S.N.F. value for milk of Tharparkar cows, which is slightly lower than those obtained for Jersey x Tharparkar crosses.

The S.N.F. contents of milk of the cows of all the four groups are shown in Fig. 4. It is seen from the graph that the

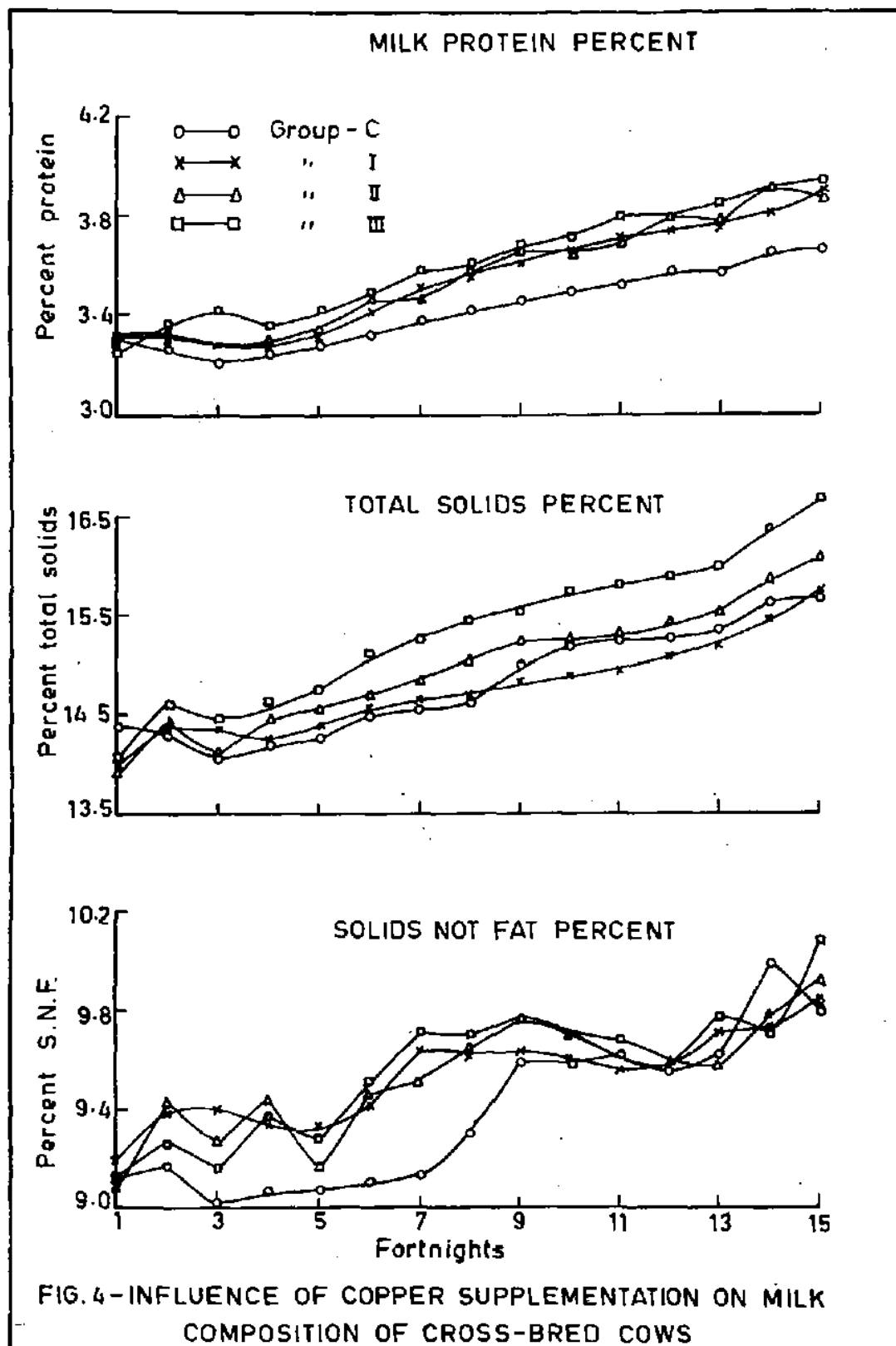
Table 24. Average Values of Solids not fat percentage with S.E.

Fortnights	Groups			
	Control	Experimental I	Experimental II	Experimental III
1	9.125 $\pm$ 0.17	9.205 $\pm$ 0.20	9.084 $\pm$ 0.22	9.116 $\pm$ 0.22
2	9.163 $\pm$ 0.08	9.394 $\pm$ 0.31	9.424 $\pm$ 0.31	9.270 $\pm$ 0.13
3	8.997 $\pm$ 0.14	9.405 $\pm$ 0.29	9.265 $\pm$ 0.20	9.155 $\pm$ 0.19
4	9.074 $\pm$ 0.07	9.340 $\pm$ 0.30	9.439 $\pm$ 0.11	9.361 $\pm$ 0.20
5	9.084 $\pm$ 0.16	9.335 $\pm$ 0.23	9.156 $\pm$ 0.29	9.285 $\pm$ 0.31
6	9.099 $\pm$ 0.07	9.425 $\pm$ 0.21	9.446 $\pm$ 0.15	9.609 $\pm$ 0.11
7	9.127 $\pm$ 0.11	9.637 $\pm$ 0.12	9.510 $\pm$ 0.22	9.713 $\pm$ 0.32
8	9.295 $\pm$ 0.16	9.620 $\pm$ 0.10	9.631 $\pm$ 0.13	9.690 $\pm$ 0.21
9	9.594 $\pm$ 0.08	9.627 $\pm$ 0.09	9.761 $\pm$ 0.16	9.759 $\pm$ 0.22
10	9.578 $\pm$ 0.08	9.596 $\pm$ 0.15	9.697 $\pm$ 0.14	9.712 $\pm$ 0.20
11	9.621 $\pm$ 0.06	9.663 $\pm$ 0.14	9.687 $\pm$ 0.17	9.683 $\pm$ 0.15
12	9.561 $\pm$ 0.08	9.588 $\pm$ 0.08	9.591 $\pm$ 0.09	9.585 $\pm$ 0.11
13	9.612 $\pm$ 0.09	9.711 $\pm$ 0.11	9.582 $\pm$ 0.10	9.782 $\pm$ 0.22
14	10.016 $\pm$ 0.22	9.720 $\pm$ 0.14	9.771 $\pm$ 0.22	9.710 $\pm$ 0.25
15	9.799 $\pm$ 0.19	9.847 $\pm$ 0.12	9.920 $\pm$ 0.32	10.103 $\pm$ 0.13
Group Av.	9.379 $\pm$ 0.055	9.528 $\pm$ 0.076	9.524 $\pm$ 0.081	9.562 $\pm$ 0.069

Table 25. Analysis of Variance for Solids not fat percentages.

Source of Variance	Degrees of freedom	Mean Sum of Square	Variance ratio
Between Treatments	3	0.49367	8.387
Between Intervals	14	1.16498	5.491*
Interaction	42	0.18247	0.719
Error	240	0.21218	-

** Significant at 1 percent level of probability.



supplemented groups recorded the higher S.N.F. contents as compared with those obtained for control group, upto 5 months of lactation. Thereafter, all the groups have shown similar values.

The results of statistical analysis did not reveal any significant difference between the treatments, indicating that supplementation of copper sulphate at levels of 200, 300 and 400 mg per day per animal did not have much influence on the S.N.F. content of milk produced (Table 25). A significant difference at 1 percent level between the intervals has been noticed. This indicates that S.N.F. content of milk increases with the advance of lactation period. The trend reported in this present experiment confirms the findings of Sukhatme *et al* (1948). Interaction effect due to the treatments and intervals was not significant.

(5) Total ash:

The average ash contents of the milk of the animals of control and experimental groups with standard error are presented in Table 26. The average ash contents of milk were 0.718 ± 0.007 , 0.745 ± 0.007 , 0.756 ± 0.008 and 0.763 ± 0.007 percent for the control and experimental groups I, II and III respectively. Bunce (1932) gave the average ash content of cows milk as 0.75 percent. Brahmachari (1934) and Sukhatme *et al* (1948) reported the average ash content of cows milk to be 0.74 percent. Ray Sarker (1943) reported 0.77 percent as the ash content of cows milk. Warner (1951) reported 0.743 percent ash in cows milk. All these values compare well with those of the present findings.

Anantakrishnan *et al* (1943) found cow milk to contain 0.71 percent ash. This corroborates with that of unsupplemented group value. However, all the reports experiment lie within reported by Aggarwala for the milk of Jersey animals by Overman (1939), Davies (1939) as 0.7 ± 0.04 is slightly lower than those reported for supplemented groups and nearer to the value obtained for control group animals.

A graphic representation of the values of ash content of milk for all the groups is shown in Fig. 8. It is evident from the graph that the supplemented groups recorded higher milk ash values as compared with those obtained for control group. Cows of the group II and III, (300 and 400 mg) showed higher values, while those of group I recorded in between the control and groups II and III. A trend of rise as the lactation advanced was noticed similar to that reported by Sukhatme *et al* (1948).

It is seen from the statistical analysis of the data that the difference in ash content of milk due to different treatments is highly significant at one percent level (Table 27). It is also observed that the treatments of groups I, II and III were significantly different from control group (Table 27A). The treatments of groups II and III (300 and 400 mg) were found to be similar, but significantly superior over treatment of Group I (200 mg). A significant difference was also observed between intervals at one percent level. This is due to the normal increase in the ash content of milk as the lactation advanced

Table 26. Average values of Total Ash Content of Milk with S.E.

Fortnights	Groups			
	Control	Experimental I	Experimental II	Experimental III
1	0.689±0.023	0.697±0.030	0.691±0.032	0.686±0.041
2	0.702±0.007	0.716±0.010	0.746±0.010	0.738±0.020
3	0.704±0.021	0.709±0.012	0.747±0.011	0.719±0.012
4	0.702±0.011	0.721±0.007	0.742±0.017	0.737±0.016
5	0.702±0.028	0.730±0.021	0.745±0.015	0.742±0.015
6	0.713±0.020	0.728±0.016	0.777±0.022	0.748±0.008
7	0.723±0.026	0.756±0.024	0.764±0.016	0.786±0.011
8	0.722±0.018	0.761±0.023	0.779±0.015	0.788±0.007
9	0.724±0.020	0.760±0.021	0.777±0.021	0.784±0.006
10	0.724±0.005	0.763±0.022	0.794±0.020	0.799±0.006
11	0.722±0.006	0.766±0.017	0.786±0.023	0.794±0.014
12	0.726±0.015	0.757±0.011	0.796±0.025	0.782±0.014
13	0.748±0.016	0.762±0.015	0.787±0.009	0.780±0.017
14	0.746±0.015	0.770±0.016	0.791±0.015	0.782±0.027
15	0.749±0.019	0.772±0.017	0.800±0.017	0.787±0.021
Group Av.	0.718±0.007	0.745±0.007	0.766±0.008	0.763±0.007

Table 27. Analysis of Variance for Milk Ash Percentages.

Source of variance	Degrees of freedom	Mean Sum of Square	Variance ratio
Between Treatments	3	0.03710	19.370**
Between Intervals	14	0.01603	8.373**
Interaction	42	0.00096	0.050
Error	240	0.00191	0 -

** Significant at 1 percent level of probability.

Table 27A. Comparison of Means of Ash Content of Milk.

Critical Difference at 5% level	Group Average Values			
	Control	I	III	II
0.014	0.718	0.745	0.763	0.766

Note: Mean values under bar do not differ significantly.

(Sharp and Struble, 1935), after two months of lactation. No significant difference due to the interaction effect was noticed.

Khidekel (1967) observed a significant difference ($P \leq 0.01$) between the ash content of milk of copper supplemented and unsupplemented cows. The present observations confirm the same.

III. Influence of Copper on Digestibility of Nutrients and Balance of Minerals:

Varying amounts of copper in the ration may affect the digestion of nutrients either due to associative or additive effects or through the microbial population and biochemical processes. Hence a digestibility trial was conducted to study the effect of different levels of supplemental copper on the digestibility co-efficients of the nutrients towards the end of the six and half months supplementation period, as detailed in Chapter IV.

The percentage chemical composition of feed and fodders fed to the animals during metabolism trial is given in Table 28 and the average body weights dry matter and water intakes of animals during metabolism trial are presented in Table 29. The intake of dry matter and nutrients on dry matter basis through feeds and fodders by the animals per day during the digestion trial and the digestibility co-efficients of the nutrients are given in Tables 30 and 31. The T.D.N. ingestion of individual animal per 100 kg body weight and per 100 gm dry matter intake are presented in Table 32. The balance of nitrogen and minerals of the individual animals along with the group averages are worked out in Tables 35 to 39. It is seen from the tables that the animals received 95 to 120 mg of copper from different sources of feeds and water per head per day. On an average the animals of control group were getting 108.0 mg of copper per head per day, while the animals of experimental groups received 171.0, 193.3 and 212.0 mg copper, after

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Table 28. Percentage Chemical Composition of Feed and Fodders on Dry matter basis.

Feeds	Dry matter	Organic matter	Crude protein	Ether extratives	Crude fibre	Nitrogen free extract
Concentrate mixture	89.80	94.576	22.433	5.475	6.699	59.969
Guinea grass (green)	23.83	89.014	10.085	2.510	36.453	36.966
Ragi straw	91.45	92.237	3.950	1.277	36.336	50.674

Feeds	Total carbohydrates	Total ash	Calcium gm %	Phosphorous gm %	Iron mg %	Copper mg %
Concentrate mixture	66.668	5.425	0.223	0.875	27.181	1.289
Guinea grass (green)	76.419	10.936	0.679	0.189	69.616	1.072
Ragi straw	87.010	7.763	0.870	0.091	57.505	0.749
Water	-	-	0.035 gm per litre	-	110 ug per litre	3.61 ug per litre

(b) Dry matter intake and T.D.N. ingestion:

From the Tables 29 and 30, it is seen that the average feed consumption per 100 kg body weight increased in all the supplemented groups over that of control group. The animals of control group recorded 2.570 kg of dry matter consumption as against 3.017, 3.055 and 2.744 kg per 100 kg body weight in the animals of supplemented groups I, II and III respectively. The highest feed consumption was recorded by the animals of group II (300 mg). The dry matter intake by the animals of group I was found to be near to that of group II. The dry matter intake by the animals receiving the highest level of supplement (400 mg) was lower among the animals of experimental groups, while the animals of control group recorded the least dry matter intake amongst the animals of all the four groups. In the supplementation at higher levels resulted in a slight depression in dry matter intake, while the lower levels of copper supplementation resulted in higher dry matter intake. This is in conformity with the findings of Felsman *et al* (1973), who studied the effect of different levels of copper supplements in the ration of Holstein bull calves.

Sahal (1946) observed an increase in roughage consumption and live weight gain by adult hill bullocks on copper supplementation level of 7 ppm and above this level the intake was either stationary or depressed. Katiyar (1983) observed an improvement in appetite and drymatter consumption, by supplementing the ration of cattle with copper as well as cobalt and iodine. Reddy (1986) recorded depressed dry matter consumption on supplementation of 500 mg copper sulphate in the ration of Haryana lactating cows.

Table 29. Average body weights, Dry matter and Water intake per day.

Exptl. group	Animal No.	Average body wt. in kg.	Average DM intake kg.		Group average per 100 kg. body wt.	Average water intake lit.		Group average per 100 kg. body wt.
			per day	per 100 kg. body wt.		per day	per 100 kg. body wt.	
Control	76	356.5	10.112	2.840	2.570	31.64	8.875	7.770
	934	449.0	10.333	2.300		29.93	6.666	
Group I	62	367.6	10.778	2.930	3.017	31.21	8.492	8.750
	929	356.0	11.050	3.104		32.07	9.008	
Group II	87	314.6	10.554	3.356	3.055	28.36	9.176	9.556
	928	399.0	10.993	2.755		39.64	9.935	
Group III	81	320.0	9.329	2.915	2.744	25.14	7.856	8.188
	944	443.6	11.411	2.573		37.78	8.519	

Consequent to high dry matter intake the T.D.N. ingested per 100 kg body weight and per 100 g dry matter intake by the animals of different groups also revealed the same trend (Table 32 and 33). Reddy and Mahadevan (1966) recorded less ingestion of T.D.N. per 100 kg body weight of the animals on 500 mg of copper sulphate supplementation. Kapoor and Mahadevan (1970b) reported the statistically insignificant increase in the T.D.N. ingestion by the animals of copper supplemented group.

(c) Water intake:

Table 29 gives the details of the total water consumption and the water intake of individual animals per 100 kg body weight. It is observed that water consumption is higher in the animals of supplemented groups. The animals of group II recorded the highest water intake i.e., 9.553 litres of water per 100 kg body weight, while the cows of control group recorded the lowest i.e., 7.770 litres of water per 100 kg body weight. Group III (400 mg) recorded lowest water consumption amongst the supplemented groups. The higher water intake by the animals of supplemented groups indicated the greater metabolic activity in those animals. Reddy (1966) did not notice any significant difference in water intake between the animals of supplemented and un-supplemented groups. Kapoor (1968) observed an increased water intake by Mariana calves given copper supplement compared to those animals of control group.

Table 30. Intake of Nutrients in Feed and Fodders by the animals in gm per day, D.M. basis.

Animal No.	Feed	Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total carbohydrates
Control Group								
76	Conc. mix.	4082.0	3880.912	916.715	233.490	273.453	2447.935	2721.383
	Ragi straw	3462.0	3193.246	136.749	44.210	1257.962	1754.334	3012.286
	Guinea grass	2568.0	2286.979	288.983	64.457	336.113	949.287	1962.440
	Total	10112.0	9339.716	1311.447	342.157	2467.518	5151.556	7696.114
924	Conc. mix.	3924.0	3766.962	893.606	213.069	266.821	2398.565	2655.396
	Ragi straw	3596.0	3316.842	142.042	45.921	1306.643	1822.237	3128.880
	Guinea grass	2754.0	2451.446	277.741	669.125	1003.916	1018.044	2104.579
	Total	10333.0	9535.250	1313.289	333.115	2577.380	5228.846	7888.846
Group I								
62	Conc. mix.	5600.0	5296.256	1256.248	306.600	375.144	3358.264	3733.408
	Ragi straw	2922.0	2769.731	118.184	39.208	1087.173	1516.166	2603.339
	Guinea grass	2186.0	1946.846	226.458	64.869	796.863	808.077	1671.516
	Total	10778.0	10001.833	1694.890	399.677	2259.180	5682.507	8007.266
929	Conc. mix.	4911.0	4644.627	1101.685	263.877	328.938	2945.078	3274.066
	Ragi straw	3674.0	3296.550	141.173	45.640	1298.649	1811.089	3109.737
	Guinea grass	2566.0	2283.209	268.690	64.281	935.019	948.179	1960.147
	Total	11050.0	10224.386	1501.538	373.898	2562.656	5704.345	8343.950

continued ...

Table 30 Contl.---

Animal No.	Feed	Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total carbohydrates
<u>Experimental Group II.</u>								
87	Conc. mix.	4911.0	4644.528	1101.635	268.877	328.988	2945.078	3274.066
	Ragi straw	3278.0	3023.529	129.481	41.860	1191.014	1661.094	2852.138
	Guinea grass	2365.0	2105.181	238.510	59.362	852.113	874.246	1807.309
	Total	10554.0	9773.337	1469.676	369.699	2382.215	5480.418	7933.563
928	Conc. mix.	4554.0	4306.991	1021.599	249.332	305.072	2730.998	3636.061
	Ragi straw	3930.0	3624.914	155.235	50.186	1428.005	1991.488	3419.498
	Guinea grass	2509.0	2233.381	253.033	62.975	914.606	927.477	1917.353
	Total	10993.0	10165.266	1429.867	362.494	2647.683	5649.963	8372.907
<u>Experimental Group III.</u>								
81	Conc. mix.	3279.0	3101.147	735.578	179.526	219.660	1966.384	2186.044
	Ragi straw	3833.0	3535.444	151.403	48.947	1392.759	1942.334	3335.093
	Guinea grass	2217.0	1973.440	223.594	56.647	808.163	819.536	1694.209
	Total	9329.0	8610.031	1110.565	284.119	2420.528	4728.254	7215.346
944	Conc. mix.	4733.0	4476.282	1061.744	259.132	317.094	2838.333	3155.396
	Ragi straw	4083.0	3786.037	161.278	52.140	1433.599	2069.019	3552.618
	Guinea grass	2595.0 12411.0	2309.913 16392.232	261.706 1484.798	65.125 376.407	945.955 2748.618	959.268 5858.266	1983.073 3391.047
	Total	11411.0	10552.232	1484.738	376.407	2746.618	5866.260	8691.087

(d) Digestibility of dry matter and organic matter:

Table 31 shows the intake through feeds and fodders, outgo through faeces as well as digestion coefficients of both dry matter and organic matter by the animals. The average digestibility coefficients for the different groups are recorded in Table 34. It is seen from the tables that the digestibility coefficients of the dry matter for the animals of control and experimental groups I, II and III were 67.30, 68.15, 67.70 and 63.87 percent and that of organic matter were 69.44, 70.62, 69.80 and 68.91 percent, respectively. The digestibility coefficient of dry matter and organic matter were found to be highest in group I (200 mg), followed in order by group II (300 mg), control and group III (400 mg). Group III (400 mg) recorded the depressed percent digestibility of dry matter confirming the findings of Reddy and Mahadevan (1966). However lower levels of supplementation of copper has been shown a favourable effect.

Sahai and Kehar (1946, 1947) recorded no appreciable variation in the digestibility of dry matter in Hill bullocks on copper supplementation. On the other hand, Kapoor and Mahadevan (1970b) found lower digestibility of dry matter in Mariana calves of copper supplemented group, as compared to those of control group. The calves received 63.71 ppm of copper in their feed on dry matter basis. This level is higher than the highest level of copper supplied in the present investigation (20.6 ppm).

Table 31. Digestibility Coefficients of the different Constituents of Feed.

Animal No.		Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total carbohydrates
Control Group								
76	Intake	10112.0	9339.716	1311.447	342.157	2467.518	5151.556	7696.114
	Outgo	3250.00	2803.710	486.720	33.508	806.780	1476.703	2283.483
	Balance	6862.00	6536.006	824.727	308.649	1660.738	3674.853	5412.631
	% digesti- bility	67.860	69.981	62.387	90.207	67.304	71.335	70.329
934	Intake	10333.00	9535.250	1313.289	333.115	2577.380	5223.846	7888.845
	Outgo	3439.00	2964.862	391.221	34.528	863.292	1676.825	2539.117
	Balance	6894.00	6570.388	922.068	298.587	1714.088	3553.021	5349.728
	% digesti- bility	66.718	68.906	70.210	89.635	66.505	67.950	67.814
Experimental Group I								
62	Intake	10778.00	10001.833	1594.890	399.677	2259.180	5682.507	8007.266
	Outgo	3584.00	3063.030	474.271	43.402	760.561	1784.796	2545.357
	Balance	7194.00	6938.803	1120.619	356.275	1498.619	3897.711	5461.909
	% digesti- bility	66.747	69.699	70.263	89.141	66.336	68.591	68.212
929	Intake	11050.00	10224.386	1501.638	378.898	2562.666	5704.345	8343.950
	Outgo	3364.00	2897.985	409.231	36.735	760.869	1691.150	2462.020
	Balance	7686.00	7326.399	1092.307	342.163	1801.797	4013.195	5881.930
	% digesti- bility	69.556	71.653	72.746	90.306	70.309	70.353	70.613

continued ...

Table 31. cont.

Animal No.		Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total carbohydrates
<u>Experimental Group II</u>								
87	Intake	10554.00	9773.337	1469.678	369.699	2382.215	5480.418	7933.563
	Outgo	3180.00	2722.748	399.090	34.280	725.704	1563.670	2289.377
	Digested	7374.00	7050.589	1070.586	335.419	1656.507	3916.748	5644.186
	% digestibility	69.869	72.141	72.845	90.728	69.536	71.468	71.143
928	Intake	10993.00	10166.266	1429.867	362.494	2647.683	5649.953	8372.907
	Outgo	3790.00	3307.495	453.095	39.909	875.793	1938.699	2814.492
	Digested	7203.00	6857.771	976.772	322.585	1771.890	3711.254	5558.415
	% digestibility	65.524	67.463	68.312	88.990	66.922	65.686	66.386
<u>Experimental Group III</u>								
81	Intake	9329.00	8610.031	1110.565	284.119	2420.582	4728.254	7215.346
	Outgo	3384.00	2967.785	338.483	28.899	839.943	1700.460	2540.403
	Digested	5943.00	5652.246	722.082	255.220	1580.639	3027.794	4674.943
	% digestibility	63.705	65.647	65.019	89.828	65.300	64.036	64.792
944	Intake	11411.00	10582.232	1484.738	376.407	2746.618	5866.620	8691.087
	Outgo	4103.00	3569.159	470.327	31.716	1022.796	3044.320	3067.116
	Digested	7308.00	6993.073	1014.411	344.691	1723.822	3822.300	5623.971
	% digestibility	64.043	66.176	68.122	91.574	62.762	65.153	64.710

(e) Digestibility of crude protein:

The digestibility coefficients of crude protein are tabulated in Tables 31 and 34. It is seen from the tables that the digestibilities are improved with the 200 and 300 mg copper sulphate. The control group recorded a digestibility of 66.55 while the experimental groups I, II and III recorded 71.50, 70.58 and 65.57 percent for crude protein respectively. The higher level of supplementation depressed the digestibility of crude protein. The digestibility of crude protein by the animals of group III (400 mg) was almost equal to that of the animals of control group. Sahai and Kahar (1946, 1947) working with adult hill bullocks observed a decrease in the digestibility of crude protein when the ration of the animals was supplemented with copper at a level of 50 ppm.

Reddy and Mahadevan (1956) reported a significant depression in crude protein digestibility after supplementation of 500 mg of copper sulphate per day to lactating cows. These observations are confirmed in the present study.

(f) Digestibility of ether extract:

The digestibility coefficient of ether extracts were 89.92, 89.72, 89.86 and 90.70 percent respectively for the control and experimental groups I, II and III (Tables 31 and 34). The results show that the digestion coefficient of ether extracts are slightly lowered in the animals due to supplementation of copper at lower level. However the difference is negligible and insignificant, indicating that the differences in values may not be due to the biological variations. The animals of group III (400 mg) on the other hand, had a slightly

Table 32. Ingestion of Total Digestible Nutrients in gm. per day.

Animal No.	Group	Body wt. in Kg.	T.D. N. intake	T.D. N. intake per 100 kg. body wt.	Group average	Dry matter intake	T.D. N. intake per 100 gm. dry matter	Group average
76	Control	356.5	6931.877	1944.42	1745.43	10112.0	68.551	67.874
934	group	449.0	6943.569	1546.45		10333.0	67.198	
62	Exptl.	367.5	7384.223	2009.31	2093.71	10778.0	68.512	69.342
929	Group I.	356.0	7754.116	2178.12		11060.0	70.173	
87	Exptl.	314.5	7469.488	2375.03	2097.41	10554.0	70.774	68.421
928	Group II.	399.0	7260.988	1819.80		10993.0	66.051	
81	Exptl.	320.0	5371.213	1866.00	1768.83	9329.0	64.007	64.489
944	Group III.	443.5	7413.841	1671.66		11411.0	64.971	

higher value for the digestibility of this component of the feed as compared to those of control group.

Reddy (1965) observed a depressed digestion coefficient for the ether extracts after supplementation of the ration of Mariana lactating cows with 500 mg of copper sulphate per day per animal. He further observed that the total digestible nutrients of the two groups were the same, showing no appreciable change in the digestible energy due to the lowering of the digestibility coefficient of the ether extracts.

Similarly, it is seen from the table 33, that the digestible ether extracts per 100 gm dry matter intake has been improved from 6.88 to 7.20 in group I over the control group. Group II also showed improved digestible ether extracts (6.89) over the control group, but was less than in group I. Group III recorded the lowest figure i.e., 6.475 for digestible ether extract signifying the depressed digestibility at higher levels (400 mg) of copper supplementation. Kapoor and Mahadevan (1970b) reported the similar depressed digestibility of ether extracts in a group of animals receiving 63.71 ppm of copper supplement, over the control group receiving 56.27 ppm copper, on dry matter basis of the feed.

(g) Digestibility of crude fibre:

The digestibility coefficients and group averages of digestibility of crude fibre are presented in Tables 31 and 34. The digestibility coefficients of crude fibre were 66.90, 68.32 68.23 and 64.03 percent for the control and experimental groups I, II and III respectively. It shows that the digestibility of

Table 32. D.C.P., D.E.E., D.T.C., & T.D. per 100 gm. dry matter intake.

Animal No.	Digestible crude protein	Digestible ether extract	Digestible total carbohydrates	Total Digestible nutrients	Group average
<u>Control group</u>					
76	8.156	6.869	53.627	68.551	
934	8.923	6.502	51.773	67.198	
Aver.	8.539	6.685	52.650	67.874	
<u>Experimental Group I</u>					
62	10.397	7.438	50.677	68.512	
929	9.885	6.957	53.321	70.173	
Aver.	10.141	7.203	51.999	69.342	
<u>Experimental Group II</u>					
87	10.144	7.151	53.479	70.774	
928	8.886	6.603	50.563	66.051	
Aver.	9.515	6.877	52.021	68.412	
<u>Experimental Group III</u>					
81	7.740	6.155	50.112	64.007	
944	8.390	6.796	49.285	64.971	
Aver.	8.315	6.475	49.698	64.489	

Table 34. Digestibility Coefficients of ration. Group Averages. (Copper Supplementation)

Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	N.F.E.	Total carbohydrates
<u>Control Group</u>						
67.289	69.443	63.548	89.921	66.904	69.642	69.071
<u>Experimental Group I.</u>						
68.151	70.516	71.504	89.723	68.322	69.472	69.412
<u>Experimental Group II.</u>						
67.696	69.802	70.878	89.859	68.229	68.577	68.764
<u>Experimental Group III.</u>						
63.874	65.911	66.570	90.701	64.031	64.694	64.751

crude fibre is improved slightly by the supplementation of copper at low level (52.0 mg). Increase in the level of copper supplementation depressed the crude fibre digestibility. The animals of group III (104.0 mg) have recorded the lowest digestibility which was even less than those of control group.

Reddy and Mahadevan (1936) observed that in lactating cows the supplementation of copper up to 125 mg (500 mg copper sulphate) per day per head did not result in any significant depression in the digestibility of crude fibre. They recorded negligible increase in the digestibility of crude fibre due to the copper sulphate supplementation. Morier Williams (1949) observed a temporary inhibition of the bacterial activity occurred in the rumen after higher dosage of copper (70 to 80 ppm) supplementation. The crude fibre digestion in ruminants is mainly dependent on the micro-flora of the rumen. Hence, the depression in crude fibre digestibility is very likely at higher levels. Boganov (1958) observed the increased digestibility of crude fibre after copper supplementation in cattle, which is in conformity with the present findings.

Kapoor and Mahadevan (1970b) observed depressed crude fibre digestibility after supplementation at a level of 12.64 mg of copper per 100 kg body weight in 8 to 12 months old Haryana calves. However, the effect was not significant.

(h) Digestibilities of nitrogen-free extract and total carbohydrates:

It will be seen from the Tables 31 and 34, that the dietary copper supplementation has a depressing effect on the

digestibility of these constituents of feed. The average values have shown the descending trend from the control group to Group III. Haddy and Mahadevan (1968) reported the similar findings on the Haryana cows which were given 500 mg of copper sulphate supplement per day per animal. Since these values are arrived at by the method of difference, not much emphasis is warranted in the discussion of the digestibilities of these nutrients. Kapoor (1968) and Kapoor and Mahadevan (1970b) reported that the depressing effect of copper and copper plus cobalt on the digestibilities of these nutrients in Haryana calves was however, not statistically significant.

(B) Balances of Nitrogen and Minerals:

The effect of copper supplementation on the balances of nitrogen and minerals was studied. The major minerals studied were calcium and phosphorus, and trace elements were iron and copper. The intakes and outgo of these constituents are tabulated in Tables 35 to 39.

(1) Balance of nitrogen:

The nitrogen intake through feeds and fodders, outgo through faeces, urine and milk as well as the balances of nitrogen in each animal are presented in Table 35. All the animals were in positive balance. This indicates that the ration has provided enough protein for the maintenance as well as for production of milk. The group averages show that the retention of nitrogen is greater in animals of copper supplemented groups I and II and in Group III, it was found slightly lower compared with that of control group. The average values

Table 35. Nitrogen intake, outgo and balance in gm per day (Copper Supplementation)

Animal No.	Conc. mixture	Ragi straw	Guinea grass	Total Intake	Faeces	Urine	Milk	Total Outgo	Balance	Group Average
Control Group										
76	146.544	23.926	41.435	211.905	74.894	75.000	33.914	183.798	+28.107	
934	142.990	24.852	44.436	212.278	62.597	81.249	40.571	184.417	+27.861	+27.933
Experimental Group I.										
62	201.040	20.678	35.271	256.989	75.836	102.874	51.800	230.560	+26.429	
929	176.305	24.700	41.386	242.391	65.430	87.866	53.343	206.679	+35.712	+31.070
Experimental Group II.										
87	176.305	22.654	38.159	237.118	63.354	87.553	43.514	204.921	+32.197	
928	163.489	27.160	40.483	231.132	72.495	84.236	45.143	201.923	+29.209	+30.703
Experimental Group III.										
81	117.716	26.490	35.771	179.977	62.155	62.196	33.143	157.494	+22.483	
944	169.915	28.218	41.870	240.003	75.254	100.588	49.243	225.185	+14.818	+18.650

Table 36. Calcium intake, outgo and balance in gm per day (copper supplementation)

Animal No.	Conc. mixture	Ragi Straw	Guinea grass	Water	Total Intake	Faeces	Urine	Milk	Total Outgo	Balance	Group Average
Control Group											
76	9.111	30.119	17.447	1.107	57.784	47.222	0.500	8.646	56.368	+1.416	
934	8.891	31.286	13.710	1.047	59.933	58.119	0.214	10.248	68.581	-8.648	-3.616
Experimental Group I.											
62	12.800	26.030	14.851	1.032	54.473	42.234	1.214	14.511	57.959	-3.486	
929	10.962	31.094	17.426	1.122	60.604	49.913	0.518	14.172	63.703	-3.099	-3.292
Experimental Group II.											
87	10.962	28.519	16.067	1.010	56.558	46.442	0.804	12.677	59.923	-2.366	
928	10.166	34.191	17.046	1.387	62.789	51.885	1.250	11.823	64.958	-2.169	-2.267
Experimental Group III.											
81	7.319	33.347	15.062	0.930	56.608	46.428	0.357	10.782	57.567	-0.959	
944	10.566	35.522	17.630	1.322	65.039	57.401	0.429	10.090	67.920	-2.881	-1.920

2
12
15

recorded were 27.983, 31.070, 30.703 and 18.350 gm per day per animal of control and experimental groups I, II and III, respectively. Group I had the highest nitrogen retention in the system, followed in order by the group II. The group III recorded the lowest retention of nitrogen.

Sahai and Kehar (1946, 1947) noticed an improved retention of nitrogen with the increasing level of copper feeding i.e., upto 7 ppm of copper per animal per day beyond which a decline in the nitrogen retention was noticed with the increase of dietary copper level upto 50 ppm. These observations were in close agreement with those of the present investigation.

Reddy and Mahadevan (1966) reported similar findings after supplementation of the ration of Mariana lactating cows with 500 mg of copper sulphate, during the early lactation.

However, the differences were statistically insignificant. Kapoor (1968), Kapoor and Mahadevan (1970b) and Arith Kumar et al (1973) reported high but insignificant nitrogen retention in the copper supplemented group, as compared to control^{group}. These observations are in agreement with those reported in the present study.

(2) Balance of calcium:

Intake through feeds, fodders and water and outgo through faeces, urine and milk as well as balances of calcium along with the group averages of these are presented in the Table 36. All the animals except one in control group, have shown negative balances of calcium, although the intakes were

sufficient. This may probably be due to the fact that these animals were high yielders and this phenomenon is not uncommon. Copper supplementation has narrowed down the negative gap, as compared to that of control group. The animals of control group have shown an average as -3.515 gm of calcium as against -3.292, -2.267 and -1.920 gm in the animals of experimental groups I, II and III, respectively. It is seen that there is a better retention of calcium in the supplemented groups as compared to that of control group.

Sahai and Khar (1946, 1947) observed with Kumaoni bullocks, that the animals fed at 7 ppm of copper retained more calcium than the animals of control group receiving 5 ppm of copper. On increasing the level of copper feeding to 50 ppm, the animals showed a lower retention of calcium. Reddy (1966) observed similar negative balance in some of the animals and reported more excretion of calcium in control group. He reported better retention of calcium in the supplemented group, which was statistically significant at 5 % level. Kapoor (1968) and Kapoor and Mahadevan (1970b) found better retention of calcium in the copper supplemented group of Hariana calves, as compared to those of control group. Arith Kumar *et al* (1973) reported a slightly higher but statistically insignificant retention of calcium in the copper plus cobalt supplemented group of Jersey x Tharparker heifers, as compared to those of control group which are confirmed by the results obtained in this study.

(3) Balance of phosphorus:

The balances of phosphorus presented in the Table 37 reveal that all the animals exhibited positive balance with respect to phosphorus. The retention of phosphorus in the control and experimental groups I, II and III were 8.397, 6.994, 7.931 and 3.326 gm per day per animal, respectively. The average retention of phosphorus is less in the animals of copper supplemented groups as compared to those of the control group, whereⁱⁿ the retention was highest. However, the differences in the balance is not pronounced, except in group III.

Sahai and Kehar (1946, 1947) observed similar trends. Reddy (1966) reported the similar findings with Haryana cows fed 500mg copper sulphate per day per head. He obtained positive phosphorus balance in all the animals. But the average retention of phosphorus was less in the copper supplemented groups as compared to that of control group. However, the differences between these two were not statistically significant. Kapoor and Mahadevan (1970) and Harith Kumar et al (1973) also reported less retention of phosphorus in the copper supplemented groups than that of control group. All these earlier findings are in agreement with that observed in present investigation.

(4) Balance of iron:

The intake, outgo and balances of iron in individual animals as well as the group average are presented in Table 38. The average balance of iron were 1095.08, 1090.53, 955.02 and 939.75 mg for the control and experimental groups I, II and III respectively. It is seen from the figures that supplementation

Animal No.	Conc. Mixture	Ragi straw	Guinea grass	Total intake	Faeces	Urine	Milk	Total Outgo	Balance	Group Average
<u>Control Group</u>										
76	38.718	3.161	4.853	43.732	28.242	0.255	5.551	34.048	9.682	8.397
934	34.851	3.283	5.205	43.339	27.650	1.778	6.800	36.228	7.111	
<u>Experimental Group I.</u>										
62	49.000	2.732	4.131	55.863	41.395	0.241	8.438	50.074	5.789	6.994
929	42.971	3.263	4.847	51.682	32.395	0.279	10.209	42.893	8.199	
<u>Experimental Group II.</u>										
87	42.971	2.993	4.470	50.434	33.835	0.386	7.584	41.805	8.629	7.981
928	39.847	3.583	4.742	48.177	32.503	0.186	3.155	40.844	7.333	
<u>Experimental Group III.</u>										
81	28.691	3.500	4.190	36.381	26.092	1.830	5.930	33.952	2.429	3.326
944	41.414	3.728	4.904	50.046	37.685	0.365	7.792	45.822	4.223	

Table 33. Iron intake, outgo and balance in mg per day (Copper Supplementation).

Animal No.	Conc. Mixture	Ragi straw	Guinea grass	Water	Total intake	Faeces	Urine	Milk	Total Outgo	Balance	Group Average
Control Group											
76	1109.53	1990.83	1787.74	3.43	4891.58	4056.13	15.62	3.43	4076.98	816.40	1095.08
934	1082.62	2067.89	1917.22	3.29	5071.02	3674.06	20.83	2.38	3697.27	1373.75	
Experimental Group I.											
62	1522.14	1720.56	1521.80	3.43	4767.93	4134.18	19.34	3.57	4157.09	610.84	1090.53
929	1334.86	2055.24	1785.65	3.53	5179.28	3573.44	31.25	4.17	3608.96	1570.42	
Experimental Group II.											
87	1334.86	1835.02	1646.42	3.17	4920.97	4231.98	58.93	5.54	4306.45	684.52	955.02
928	1237.83	2259.96	1746.66	4.36	5248.81	3964.61	31.62	6.99	4003.22	1245.59	
Experimental Group III.											
81	891.27	2204.18	1543.38	2.76	4641.59	3485.88	76.79	18.21	3580.86	1060.73	939.75
944	1286.48	2347.94	1806.53	4.16	5446.11	4545.51	75.00	6.84	4627.35	817.76	

of dietary copper has no favourable effect on the retention of iron when fed over and above the levels available from the basal ration. A diminished retention of iron in the body of animals is seen at the increased levels of dietary copper supplementation.

Elvehjem (1935) and Schultze (1940) reported that copper is not concerned with the assimilation of iron. Similar findings are reported as far as absorption of iron is concerned in the present study. It will be seen from the Table 38 that the balances of iron are not much different in the animals of all the groups, irrespective of supplementation of copper. But the excretion of iron through urine has been found to increase with the increasing intakes of dietary copper supplement. This aspect needs further investigation to explain in detail the iron metabolism and as to how it gets affected by the dietary levels of copper supplementation. It may, however, be due to higher amounts of iron being released from the storage sites in the liver, as a result of increased concentration of copper in the blood, subsequent to dietary supplementation of copper, as suggested by Marston and Allen (1967). They further reported in case of rats that the amount of radio-active iron absorbed was favoured by amounts of dietary copper upto levels of 0.25 to 0.35 mg per rat per day. Above this concentration of copper, there was no greater absorption, and on the other hand there was some what reduced absorption of iron. It is evident from the results that a similar trend was noticed in the present investigation also.

Houk *et al* (1946), Chase *et al* (1952), Gubler *et al* (1952) Bush *et al* (1956) and Jensen *et al* (1956) reported similar effects of copper on the absorption of iron in the copper-deficient rats and pigs. Studies on the same line as in rats and pigs have not been extensively conducted on ruminants so far. Hence, little is known of copper-iron inter-relationship in these animals. Marston *et al* (1948) Matrone (1960), Underwood (1952, 1956) suggested that in copper-deficient animals there was 'mucosal block' to the iron absorption; and when the diets of these animals were supplemented with copper, the absorption of iron increased due to the removal of 'mucosal block'.

Anarith Kumar *et al* (1973) reported similar findings in the cross-bred heifer, when the ration of these were supplemented with copper.

(5) Balance of copper:

The average balances of copper in the animals of control and experimental groups I, II and III were 37.20, 59.25, 55.67 and 38.11 mg, respectively (Table 39). The average intake of copper by the animals of these corresponding groups were 108 mg (10.5 ppm), 171 mg (15.6 ppm), 193 mg (18.0 ppm) and 212 mg (20.6 ppm), respectively. The intakes were quite adequate in line with the recommended allowance (Maynard and Loosli, 1969; Anon, 1946-47).

It will be seen from the figures in Table 39, that the retention of copper in the body of the animals was highest

Table 32. Copper intake, output and balance in mg per day (copper supplementation)

Intake							Outgo					
Animal No.	Conc. mixture	Ragi Straw	Guinea grass	Supple- ment	Water	Total	Faeces	Urine	Milk	Total	Balance	Group Average
Control Group												
76	52.576	25.944	27.529	-	1.143	107.192	64.935	2.463	1.389	68.787	38.405	37.205
934	51.301	26.948	29.523	-	1.081	108.853	69.971	1.984	0.893	72.848	36.016	
Experimental Group I.												
62	72.128	22.422	23.434	52.00	1.127	171.111	111.642	2.033	1.389	115.054	56.047	59.250
929	63.254	26.783	27.497	52.00	1.158	170.692	104.822	1.239	2.133	108.244	62.448	
Experimental Group II.												
87	63.254	24.565	25.353	78.00	1.042	192.214	129.346	1.994	1.488	132.818	59.396	55.670
928	58.656	29.461	26.897	78.00	1.431	194.435	139.320	1.140	2.034	142.494	51.941	
Experimental Group III.												
81	42.234	28.724	23.766	104.00	0.908	199.632	158.161	2.746	1.483	162.394	37.248	38.111
944	60.961	30.598	27.818	104.00	1.364	224.741	180.820	2.815	2.133	185.768	38.973	

in Group I, receiving 200 mg copper sulphate (52 mg copper) supplement, while the lowest balance is seen in the control group that received no supplement of copper. It is also seen that the retention of copper was higher in all the supplemented groups than in control group. These observations are in conformity with those of Reddy (1968) on Haryana lactating cows, Saspath *et al* (1969) on Jersey x Tharparkar heifers, Kapoor and Mahadevan (1970a) on Haryana cattle of different ages and also Arith Kumar *et al* (1973) on Jersey x Tharparkar heifers. The main path of excretion of copper was found to be through faeces, as reported by several workers. The excretion through urine and milk has also slightly increased consequent to higher dietary copper intakes, as shown earlier by Mitchell and Hamilton (1949) Bell (1960), Bowland (1961), Joshi (1964) and King and Dunkley (1969).

Mahadevan and Zubairy (1969) reported the average retention of copper in the animals of supplemented group was about 114.0 mg per day, while that in the control group was about 14.0 mg per day. Similar were the copper retention figures in the present investigation, showing thereby the additional requirement of copper for increasing the production and fertility as well as decreasing the calving intervals.

IV. Reproductive Performance of cows in relation to Copper Supplementation:

Van Kemsburg (1961) stated that although copper deficiency symptoms in cattle have been known and observed for several years, in the affected areas, the role of copper in

lowering the fertility of stock has only been fully revealed by the recent investigations. The functional or physiological type of infertility is generally reported from the areas of copper-deficient soils and pastures or even border-line copper contents of the feeds and fodders. It is seen that the copper deficiency causes impaired reproductive activities, before any evidence of clinical deficiency becomes apparent. A very clear example of copper deficiency and reproductive performance was observed in East London District, when 50 percent of the animals of a dairy herd were showing irregular oestrus cycles or no signs of oestrus. The condition of all the animals was set right by simple supplementation of dietary copper within three months and the animals started showing regular post-partum oestrus within three months of calving.

Therefore, the study of this nature was undertaken along with the other observations.

It is seen from the Table 40 that all the animals of copper supplemented groups came into oestrus earlier than those of control group i.e., within an average period of 43 to 48 days, indicating that the copper supplementation helps to bring about the occurrence of early oestrus in cows. However, the statistical analysis (Table 41) did not reveal any significant difference between the treatments.

Reddy and Mahadevan (1958) reported similar findings with Haryana cows fed 500 mg of copper sulphate supplement.

**Table 40. Reproductive Performance of Cows under Copper
Supplementation Experiment.**

Animal No.	First oestrus after calving No. of days	Fruitful insemination number	Days from calving to successful insemination
<u>Control Group</u>			
75	91	16	448
76	54	2	75
934	55	4	118
954	82	8	258
931	48	1	48
Average	65±8.6	6.0±2.8	197.4±80.0
<u>Experimental Group I.</u>			
62	35	2	77
86	53	5	158
929	45	3	108
989	41	1	41
975	41	1	41
Average	43±3.6	2.4±0.8	85.0±23.4
<u>Experimental Group II.</u>			
33	40	2	103
82	36	1	36
87	69	8	258
928	40	3	124
950	55	3	118
Average	48±6.6	3.4±1.6	127±44.4
<u>Experimental Group III.</u>			
45	46	2	88
81	33	1	33
944	41	2	83
948	57	5	183
987	63	1	63
Average	48±6.0	2.2±0.4	90.0±30.0

Table 41. Analysis of Variance for Reproductive Performance of cows (Copper Supplementation)

Source of Variance	Degrees of freedom	Mean Sum of Squares	Variance Ratio
<u>First Oestrus After Calving</u>			
Between Treatments	3	511.25	2.734 N.S.
Within Treatment	16	157.00	-
Total	19		
<u>Successful Insemination After</u>			
Between Treatments	3	3333.1	1.360 N.S.
Within Treatment	16	2455.6	-
Total	19		
<u>Days from Calving to Fruitful Insemination</u>			
Between Treatments	3	13457.7	1.214 N.S.
Within Treatment	16	11081.6	-
Total	19		

N.S. - Not Significant.

The number of inseminations required for the successful fertilisation and pregnancy were also recorded and studied. The average number of inseminations required were 6.0, 2.4, 3.4 and 2.2 for the control and experimental groups I, II and III, respectively, which thereby show that the supplemented groups were superior over the control group. In this respect Group I and III were almost similar, while group II required on an average one more insemination. Statistical analysis (table 41) did not reveal any significant difference between the groups that received the copper supplement. Wishy *et al* (1965) reported similar findings in buffalo heifers that received 1.0 gm copper sulphate supplement per head per week. They observed that the buffalo heifers of supplemented group required on an average 1.6 services, while those of control group required 4.0 services.

Barysheva (1966) observed that the dietary supplementation of copper along with molybdenum and zinc improved the reproductive performance of the cows. He reported that 14% of the animals of supplemented group required 4 or more services as against 34% of the animals of control requiring this number of services. These observations supported the earlier ones of Dzenik's (1965).

Another aspect in this regard of study was the observation of the number of days from the calving to successful insemination. Table 40 reveals that the animals of control group required on an average 197.4 days, while those of experimental Groups I, II and III required 85.0, 127.8 and 90.0 days respectively to get

settled. In this respect, the animals of Group I (200 mg) were superior over the animals of all the other groups. However, statistical analysis did not reveal any significant difference between the treatments.

After supplementing the ration of Mariana cows for over 6 years Mahadevan and Zubairy (1969) observed that the inter-calving period for the animals receiving 500 mg copper sulphate per day per head was 408 ± 12 days, while those of control group receiving no supplement was 511 ± 12 days. The difference between the animals of these two groups was statistically significant ($P < 0.01$). The present findings are in conformity with the above observations. The post-partum oestrus in the experimental group was 70 ± 8 days while that of control group was 258 ± 46 days; during the first few years of the experiment. Sampath et al (1969) observed that the animals that received copper supplement at the rate of 500 mg copper sulphate per day per animal, came into oestrus and conceived earlier as compared to those of control group.

The observations of these experiments, in general, seem to be that the copper supplement of the range of 200 mg of copper sulphate per day per head, was beneficial to induce early oestrus, increase fertility and reduce the inter-calving period.

Munro (1960) found sub-normal blood copper levels ranging from 30 to 60 mcg per 100 ml in some of the animals in a herd. Such low values caused an-oestrus and sub-oestrus conditions in heifers, as well as repeat breeding. The administration of 1 to 2 gm of copper sulphate per day per head for a period of 3 months each year improved the fertility.

Donaldson et al (1964) gave 400 mg copper glycinate subcutaneously and studied its effect on fertility of short-horn heifers. He observed that after 4 months, 64 percent of the copper treated heifers were pregnant as compared to 42 percent of heifers of control group ($P < 0.01$) and at 6 months, the values were 74 percent and 56 percent, while that at 9 months were 93 percent and 83 percent, respectively.

Hignett (1950), and Tossell Robin (1967) while reviewing the subject of influence of nutrition on fertility in farm animals stated that the gross deficiency of copper results in inaction and an-oestrus was not uncommon. However, in less severely affected animals, ovarian activity was maintained. They observed that in majority of the herds with low blood copper values, there was an impaired female fertility. Dutt and Hills (1960) observed that the oestrus cycles as shown by vaginal smears to be normal in rats on copper-deficient diets, and the cause of reproductive failures in copper-deficient conditions was due to the resorption of foetus.

Dzennik (1968) observed that the cows given an additional supplement of copper, cobalt and zinc in their ration came into heat on an average within 35 days of calving and conceived within 60 days of calving as against 40 and 91 days for those cows, which were not fed the supplement. Of the former 55 percent conceived by insemination during post-partum oestrus as against 18 percent in the latter. Exactly similar was the trend recorded in the present investigation, when copper was supplemented.

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Table 42. Milk yields of cows during previous and experimental lactations (Copper Supplementation)

Animal No.	Previous lactation Yield in Kg	Experimental lactation yield in Kg
<u>Control Group</u>		
75	2,702	2,633
76	2,096	2,593
934	2,175	2,470
954	2,849	3,076
981	1,744	1,778
Average	2,313.2 $\pm$ 220.1	2,510.0 $\pm$ 249.6
<u>Experimental Group I.</u>		
62	2,448	3,816
86	1,578	2,505
929	2,912	3,484
959	2,791	2,844
975	3,014	3,829
Average	2,548.6 $\pm$ 276.4	3,295.4 $\pm$ 254.6
<u>Experimental Group II.</u>		
33	2,391	3,013
82	1,846	2,155
87	2,177	2,410
928	2,831	2,920
960	3,012	3,466
Average	2,461.4 $\pm$ 224.2	2,792.8 $\pm$ 252.1
<u>Experimental Group III.</u>		
45	1,063	2,369
81	1,697	1,979
944	3,403	3,300
948	2,552	3,060
987	2,408	2,273
Average	2,224.6 $\pm$ 430.3	2,396.2 $\pm$ 424.8

(Fig. 5), showing the average milk yields of the previous and present lactations of the animals of different groups show that there is a slight increase in the milk production by the animals of supplemented groups. The highest increase was shown by the animals of group III (400 mg) and the next was by group I (200 mg). Similar results were obtained by Reddy and Mahadevan (1966) with Mariana cows, which were given supplement of 500 mg of copper sulphate per day per head.

Engel *et al* (1964) did not observe any difference in the milk yield on copper supplementation. Melnikova and Ilyukhina (1969) and Kulikov (1971) reported slight increase in the mean daily milk yield by the supplementation with either copper alone or copper along with other trace elements to Black Pied and Russian Simmental cows, respectively. Similar observations were recorded by Babin (1967) and Khidkel (1967) when they supplemented the ration of cows with 75 mg and 62.5 mg of copper sulphate per day per cow respectively. Shatokhina (1968) on the other hand could not observe any significant difference in the milk yield of cows with copper supplementation.

Kehar *et al* (1959) observed a significant increase in the lactation yield of experimental buffaloes, after supplementation of their ration with trace mineral mixture containing copper. Poole and Walshe (1970) studied the copper status and milk yield of dairy cows, by administering copper and Cu-EDTA parenterally to half of the herd. They reported that copper supplementation had no significant effect on the total milk yield.

Table 43. Analysis of Co-variance for Milk Production (Copper Supplementation).

Source of Variance	Degrees of freedom	Sum of Squares X	Sum of Squares Y	S P XY	byx	xyxbyx	$\sum Y^2 - byx \sum Y$	Adjusted degrees of freedom
Between treatments	3	317351	1645898	345399	1.088	375794.1	1270104	2
Within Treatments	15	6289020	6525089	4465309	0.710	3170389.4	3354720	15
Total	19	6606371	8170987	4810708	0.728	3502195.4	4668792	18

Table 43A. Adjusted Analysis of Variance for Milk Production.

Source of Variance	Degrees of freedom	Sum of Squares	Mean Sum of Squares	Variance Ratio
Total	18	4668792	-	
Within Treatment	15	3354720	223648	1.968 N.S.
Between Treatments	3	1314072	438024	

N.S - Not Significant.

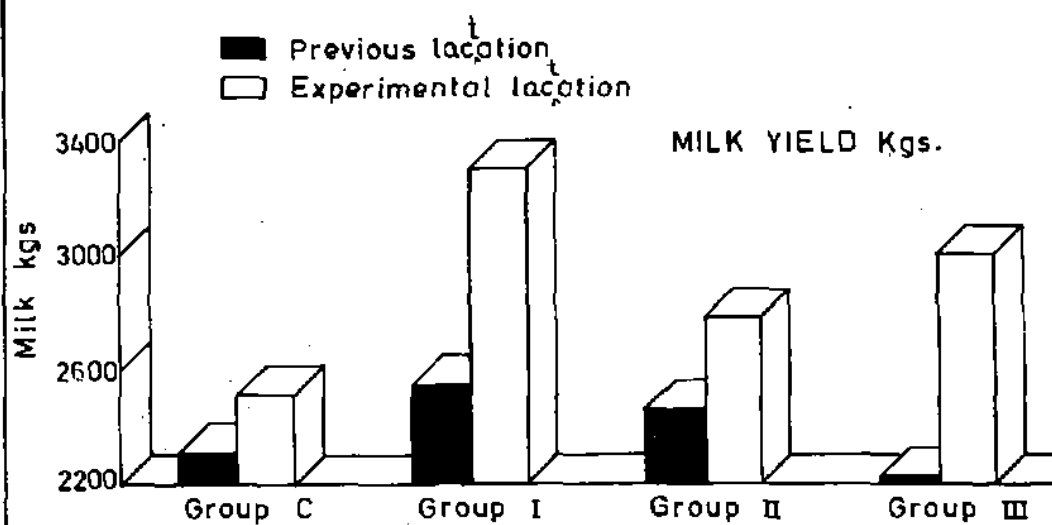
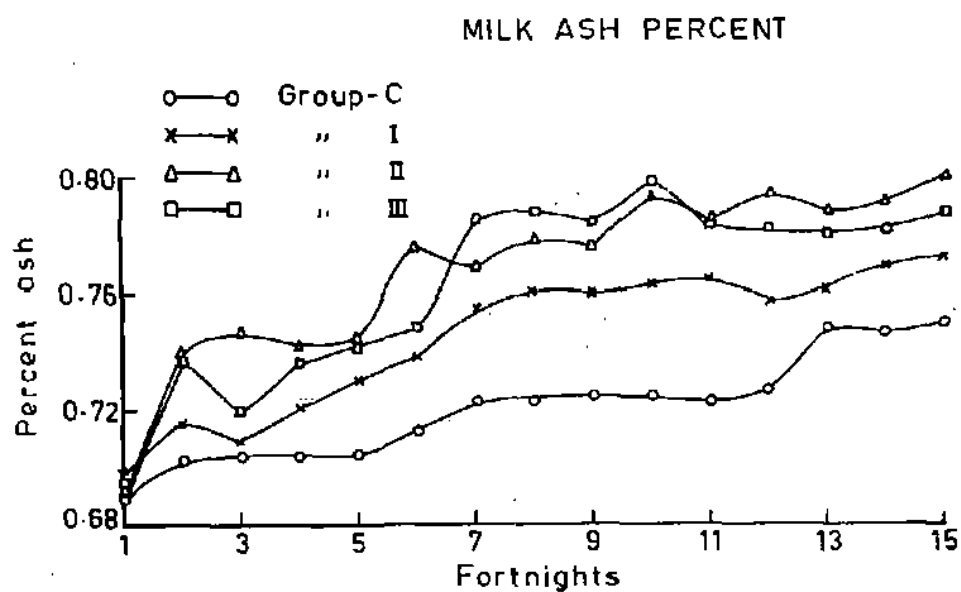


FIG. 5-INFLUENCE OF COPPER SUPPLEMENTATION ON MILK OF CROSS-BRED COWS

CHAPTER - VI

RESULTS AND DISCUSSION OF STUDIES ON COBALT SUPPLEMENTATION

CHAPTER - VI.

RESULTS AND DISCUSSION OF STUDIES ON

COBALT SUPPLEMENTATION

The first indisputable evidence that cobalt is an essential trace mineral nutrient for ruminants came from the investigation into the cause of the naturally occurring diseases of sheep and cattle in Australia (Warston, 1935; Lines, 1935; and Underwood and Filmer, 1935). The supplementation of cobalt has given a wonderful and fascinating results among ruminants suffering from anti-pernicious anaemia, as cobalt is the metallic component of vitamin B₁₂, the cyanocobalamin. It is an important trace element next to copper and study on the effects of supplementation of cobalt, similar to that of copper was undertaken, in the second phase of the investigation.

As already outlined in Chapter IV, twenty Jersey x Tharparkar lactating cows were selected from the institute herd. They were divided into four groups to constitute one control and three experimental groups, containing five animals in each. They were taken in groups of four, having similar or close characteristics such as age, number of lactations completed, date of calving and previous lactation milk yield. Then they were randomly assigned to the four groups of the experiment, so that the groups were identical as far as possible with the limitations of the facilities and resources. A randomized block design was followed for the allotment of animals to different groups, in this part of the investigation.

The distribution of animals is shown in Table 44. The animals of experimental groups I, II and III were given 16.0, 24.0 and 32.0 mg of cobalt chloride ($6\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) to supply 4.07, 6.10 and 8.13 mg of elemental cobalt per day per animal, respectively in addition to the amount they were getting from the basal ration. The observations were recorded on the similar lines as that of copper supplementation studies. The observations recorded are discussed.

(1) Influence of cobalt on Blood Composition:

In the animal husbandry and nutritional practices, the examination of blood has served as an important guide for the diagnosis of various nutritional and physiological conditions and body responses of any deficiency or excess of mineral elements. Hence, blood studies were conducted to find out the effect of cobalt supplementation through feed to the lactating cows. The haematological constituents examined were haematocrit (PCV) value, haemoglobin, total red blood cell and white blood cell counts, specific gravity of the whole blood and levels of biochemical constituents such as iron, copper and cobalt contents of blood were studied. Total serum protein content was also examined. The results with discussion are presented in the following text.

(A) Haematological Constituents:

(1) Haematocrit (PCV) value:

The average PCV of the blood of cows of control and experimental groups are presented in Table 45, with standard errors and over all group averages. The average values for

Table 44. Allotment of Animals to Different Treatments.
Cobalt Supplementation.

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Groups	Animal No.	Age		No. of lactations completed	Previous lactation Milk yield
		Years	Months		
Control Group	6	9	4	6	Kgs. 2097
	88	5	10	2	2446
	936	10	11	8	3516
	970	10	4	7	3914
	984	10	4	6	3442
Average		9	4	5.8	3043.0 ±342.8
Group I	29	8	2	5	2443
	89	5	8	2	2376
	933	11	3	8	3010
	945	11	0	8	2699
	972	9	10	6	3042
Average		9	2	5.8	2714.0 ±125.6
Group II	76	6	7	4	2935
	87	5	8	2	2410
	943	11	5	8	2949
	956	10	6	7	3354
	976	9	2	6	3558
Average		8	8	5.4	3021.2 ±212.6
Group III	47	7	8	5	3457
	73	7	2	4	1844
	96	6	11	2	2394
	940	11	0	9	3516
	954	10	9	7	3076
Average		8	8	5.4	2857.4 ±399.6

control and experimental groups I, II and III were 39.42 ± 0.54 , 40.85 ± 0.49 , 37.92 ± 0.61 and 37.71 ± 0.49 percent respectively. The average values for PCV of blood of cows reported in the present investigations are in close agreement with those of Coffin (1953), Dukes (1957), Schalm (1961) and Misra and Biswal (1961). The values reported by Holman (1953) and Smith were 33.7 and 32.88 percent, respectively. Similarly, Khajuria (1963) reported 33.9 ± 3.57 percent as the PCV value for the blood of Tharparkarcows. The values reported in the present experiment are slightly higher than those reported by the both workers.

The graphic representation of PCV values of all the groups is shown in Fig. 6. It is seen from the graph that the cows of the group I (16 mg) recorded the highest throughout the experiment, as compared to those of other groups. The group II (24 mg supplement) and group III (32 mg supplement) animals recorded the lower values throughout the experimental period as compared with those of control group. This may be due to the initial lower values of the animals of these groups for this constituent of blood or the depressing effect due to the higher level of supplementation.

On subjecting the data to the analysis of variance test, a significant difference was observed between the blocks and between the treatments, at 1% level (Table 46). Further analysis (Table 43A) revealed that group I (16 mg) was found to be superior over that of both control and experimental groups II and III, while these two latter groups were similar and control group was superior over the groups II and III. Therefore, it is obvious that

Table 45. Average values of Haematocrit (PCV) percent with standard Error.

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	38.3±2.92	39.2±0.80	37.7±2.92	36.3±1.21
20	38.0±1.51	40.7±1.20	36.9±3.31	37.5±1.00
40	38.6±2.72	41.0±2.67	38.0±2.02	37.6±2.31
60	40.8±1.10	41.3±2.68	38.3±1.51	37.4±1.44
80	40.9±1.21	41.6±2.70	38.3±2.73	37.6±1.06
100	40.4±1.60	41.4±1.81	37.6±1.74	37.4±1.31
120	40.2±1.70	41.0±1.61	37.4±1.34	37.7±1.52
140	38.8±2.60	40.6±2.01	38.6±1.61	37.7±1.72
160	39.4±2.00	40.6±2.11	38.1±1.34	39.1±2.11
180	39.8±1.20	41.3±1.72	38.2±1.14	38.8±1.56
Group Average	39.42±0.54	40.86±0.49	37.92±0.61	37.71±0.49

Table 46. Analysis of Variance for PCV of blood.

Source of Variance	Degrees of freedom	Mean Sum of squares	Variance ratio
Between Blocks	4	216.164	19.523**
Between Treatments	3	107.741	9.731**
Between Intervals	9	8.498	0.768
Interaction	27	3.594	0.325
Error	156	11.073	-

** Significant at 1 percent level of probability.

Table 46A. Comparison of means of PCV of blood.

Critical Difference at 5% level	Group Average Values			
	C III	II	Control	I
1.3044	37.71	37.92	39.42	40.86

Note: Mean values under bar do not differ significantly.

higher levels of supplementation has no additional advantage over the 16 mg supplement. A highly significant difference between the blocks was noticed, which may be due to the age differences of animals in the different blocks. There was no significant difference between intervals as well as due to interaction between treatments and intervals.

Keener *et al* (1949) noticed an increase in haematocrit values when cobalt was supplemented in the ration of calves at the rate of 50 mg per 100 lb body weight. However, Singh (1948) reported that the mean corpuscular volume varied within the normal limits and was not affected by the cobalt supplementation in Kumaoni bullocks. Ganguli (1963) and Gupta (1968) reported the higher PCV values in cobalt supplemented groups of cows and calves, respectively, though statistically insignificant. Singh (1964) observed a significant increase ($P < 0.05$) after three months of cobalt supplementation in the PCV values of the blood of Haryana calves. Medalinkas (1969) also noticed the increased PCV values on supplementing the diet with 5 mg of cobalt chloride in calves. Chapman and Kidder (1963) too reported similar ^{findings} after administration of 8 mg of cobalt daily to growing cattle. Recently, Pal *et al* (1970) and Arith Kumar *et al* (1973) reported higher PCV values over the initial values in Haryana calves and Jersey x Tharparkar heifers, respectively, after supplementation of the ration with copper and cobalt. However, the difference was statistically insignificant.

(ii) Haemoglobin:

Cobalt in the form of vitamin B₁₂ is inter-related with iron and copper in haematopoiesis and thus indirectly involved

in the haemoglobin and red blood cell regeneration. Therefore, the effect of dietary supplementation of cobalt on the haemoglobin levels in the animals was studied.

The average haemoglobin values of the blood of animals of control and experimental groups during the investigation are presented in Table 47. The average values with standard error for the control and experimental groups I, II and III were 10.10 ± 0.12 , 10.43 ± 0.10 , 9.78 ± 0.11 and 10.13 ± 0.09 g percent respectively. The average values reported by Khajuria and Hazdan (1966) as 10.30 for lactating cows, by Dabudghao (1969) as 10.28 ± 0.25 for heifers and by Amrith Kumar *et al* (1973) as 10.00 gm percent for Jersey x Tharparker heifers are in close agreement with those reported in the present experiment. Earlier reported values of Mullick and Pal (1943) for Indian cattle and Coffin (1953) for Jersey animals do not vary from those of the present investigation. However, average values reported by Holman (1955) and Sachala (1961) as 11.3, by Dukes (1957) as 12.0 and by Misra and Biswal (1961) as 12.2 gm percent are slightly higher.

The haemoglobin values of the blood of the cows of control and experimental groups have been shown in Fig.6. From the graph, it is evident that the animals of group I (16 mg) recorded higher haemoglobin values as compared to those in the control and other experimental groups. The trend is similar to that observed in PCV values of blood. The control group animals have shown higher values than those of group II (24 mg) which may also be due to their initial high values. However, the

Table 47. Average values of Haemoglobin gm percent with Standard Error.

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	10.3±0.58	10.3±0.57	9.9±0.47	10.0±0.27
20	10.2±0.56	10.6±0.32	9.8±0.49	10.3±0.26
40	10.0±0.51	10.7±0.51	10.0±0.59	10.2±0.31
60	10.2±0.27	10.2±0.27	9.7±0.24	10.4±0.42
80	10.1±0.19	10.2±0.25	9.7±0.27	9.9±0.25
100	10.0±0.18	10.4±0.21	9.6±0.20	10.1±0.32
120	10.2±0.27	10.3±0.32	9.6±0.18	10.1±0.34
140	10.0±0.41	10.3±0.24	9.9±0.23	10.0±0.26
160	10.1±0.32	10.6±0.27	9.8±0.25	10.2±0.19
180	9.9±0.19	10.7±0.22	9.8±0.26	10.1±0.25
Group Average	10.10±0.12	10.43±0.10	9.73±0.11	10.13±0.09

Table 48. Analysis of Variance for Haemoglobin.

Source of Variance	Degrees of freedom	Mean Sum of Squares	Variance ratio
Between Blocks	4	8.1481	17.677**
Between Treatments	3	8.5300	7.658**
Between Intervals	9	0.1811	0.392
Interaction	27	0.1618	0.350
Error	186	0.4609	-

** Significant at 1 percent level of probability.

Table 48A. Comparison of means of Haemoglobin of blood.

Critical Difference at 5% level	Group Average Values			
	II	Control	III	I
0.2562	9.78	10.10	10.13	10.43

Note: Mean values under bar do not differ significantly.

animals of group III (32 mg) recorded slightly higher values than those of control group. Such variations in the biological experiments with large animals is not uncommon.

The statistical analysis of the data has been presented in Table 48. By the perusal of the 'F' value, it is quite evident that there exists a statistically significant difference between the treatments at one percent level. On further analysis (Table 48A) it was observed that treatment of group I (16 mg) was superior over that of all the other groups. The treatment of group III (32 mg) was found similar to that of control group, while treatment of group II $\bar{Z}$ (24 mg) was found to record significantly lower haemoglobin values. From these observations as well as from the graphic representation, it is seen that a somewhat uncertain response is shown by the animals to this supplement. Ray et al (1948) also observed that administration of cobalt supplement brought about a slow and often erratic haemoglobin responses and indirect effect upon the haemopoietic centres.

A statistically significant difference was noted between the blocks at both the levels. This indicates that block to block variation was present, which may be due to the age difference in the animals of different blocks. However, this was removed from the treatment to render the treatments more sensitive. No significant difference between intervals and due to interaction between treatments and intervals was noticed.

Filmer and Underwood (1937) reported a rise in haemoglobin levels in affected animals in response to cobalt supplementation.

Similar observations were recorded by Neal and Ahman (1937), that concentration of haemoglobin per cell volume was increased by cobalt supplementation. Geyer *et al* (1945), Pope *et al* (1947) and Smith *et al* (1951) observed that cobalt treatment of affected animals influenced the haemoglobin formation and showed a positive effect on haemoglobin level. This was confirmed by Chamberlain *et al* (1948) in lambs. On the other hand, Askew (1939) and Underwood and Beck (1941) did not notice any change in haemoglobin level in deficient animals as a result of cobalt supplementation.

Keener *et al* (1949), Boganov (1958), Medalinskas (1959), Rudin (1959), Kaminski and Jtzig (1961) and Kolesov *et al* (1961) reported positive response by calves and sheep to cobalt supplementation of haemoglobin level. On the other hand Ganguli (1963) in Mariana heifers, Singh (1964), Gupta (1968) and Pal *et al* (1970) in calves and Pachalag *et al* (1972) in lambs reported no significant difference with cobalt supplementation on haemoglobin levels of blood. However, from the results obtained by these investigators, it was evident that there was a trend of increase in the levels of haemoglobin in animals as a result of cobalt supplementation. A slow response after 3 months of supplementation was observed by Singh (1966) along with PCV values. Chapman and Kidder (1963) also observed similar increase in haemoglobin content of blood after the administration of 8 mg of cobalt per head per day in growing calves.

(iii) Total Erythrocyte (R.B.C.) Count:

In Table 49, the average values of red blood cell count of the blood of cows of control and experimental groups have been presented. The average values of erythrocyte count

of blood of the animals of control and experimental groups I, II and III were 6.397 ± 0.257 , 6.607 ± 0.283 , 6.463 ± 0.275 and 6.111 ± 0.244 millions per cmm. respectively. These values are slightly lower than those reported by Mullick and Pal (1943) and Kehar and Murty (1945) for the blood of Haryana and Dhanni breed of cattle. The values reported at present are well within the range reported by Coffin (1953), Holman (1955) and Schalm (1961) and are in close conformity with those reported by Smith (1959), Dukes (1957), Govindan Nayer (1949), Misra and Biswal (1961), Khajuria and Rastan (1966), and Dabadghao (1969), for this class of animals.

The erythrocyte count values of blood of the cows of all the age groups have been shown in Fig.6. It is seen from the graph that at majority of the points, the animals of group I (16 mg) recorded the highest values, and at all the points, the animals of all the supplemented groups recorded higher values, as compared to those of control group. This indicates that the supplementation has a positive effect on the total R.B.C. count of the blood. Of course, it is a known fact that PCV value is a function of erythrocyte number and size of the cells per unit volume of blood. Therefore, this constituent has also been affected, similar to PCV values.

Statistical analysis of the data of total erythrocyte count is presented in Table 50. From the variance ration, it is observed that there is a significant difference between the treatments at one percent level. This means that supplementation of cobalt has significant effect on the total erythrocyte number of the blood of the cows. When the difference between the means of the

Table 49. Average values of Total Erythrocytes (R.B.C.) in millions per cmm. with standard error.

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	6.284 $\pm$ 0.41	6.312 $\pm$ 0.22	6.002 $\pm$ 0.54	5.432 $\pm$ 0.22
20	6.438 $\pm$ 0.37	6.634 $\pm$ 0.23	6.026 $\pm$ 0.56	5.834 $\pm$ 0.12
40	6.226 $\pm$ 0.26	6.292 $\pm$ 0.30	6.380 $\pm$ 0.37	6.006 $\pm$ 0.30
60	6.220 $\pm$ 0.16	6.294 $\pm$ 0.31	6.426 $\pm$ 0.31	6.145 $\pm$ 0.28
80	6.288 $\pm$ 0.21	6.298 $\pm$ 0.25	6.134 $\pm$ 0.42	5.990 $\pm$ 0.21
100	6.478 $\pm$ 0.18	6.654 $\pm$ 0.30	6.108 $\pm$ 0.25	5.956 $\pm$ 0.25
120	6.494 $\pm$ 0.21	6.700 $\pm$ 0.29	6.402 $\pm$ 0.39	6.346 $\pm$ 0.12
140	6.478 $\pm$ 0.27	7.016 $\pm$ 0.41	6.872 $\pm$ 0.37	6.650 $\pm$ 0.31
160	6.560 $\pm$ 0.15	6.932 $\pm$ 0.27	7.118 $\pm$ 0.22	6.466 $\pm$ 0.20
180	6.704 $\pm$ 0.35	6.940 $\pm$ 0.25	7.158 $\pm$ 0.32	6.492 $\pm$ 0.23
Group Average	6.397 $\pm$ 0.267	6.607 $\pm$ 0.283	6.453 $\pm$ 0.275	6.111 $\pm$ 0.244

**Table 50. Analysis of variance for Erythrocyte Count of Blood.
(Log. values)**

Source of Variance	Degrees of freedom	Mean Sum of Squares	Variance ratio
Between Blocks	4	0.231625	29.987**
Between Treatments	3	0.047667	6.169**
Between Intervals	9	0.045778	5.925**
Interaction	27	0.005184	0.671
Error	156	0.007726	-

** Significant at 1 percent level of probability.

**Table 50A. Comparison of means of R.B.C. of Blood.
(Log. values)**

Critical Difference at 5% level	Group Average Values			
	III	Control	II	I
0.03445	1.80536	1.85155	1.85466	1.87914

Note: Mean values under bar do not differ significantly.

two groups were compared with the critical difference at 5 percent level (Table 50 A) it is observed that the treatment of Group I (16 mg) was superior over the group III (32 mg). While group I and group II (16 and 24 mg) were found to have a similar effect on the erythrocyte count of blood. However, they were found to be significantly different from the control group. It is seen from the table and graph that animals of group I and II recorded higher values for this parameter of the blood. This reveals that 16 mg of cobalt chloride appears to be a suitable level of supplementation.

A highly significant difference between the blocks has been noticed at 1 percent level of probability, which is in parallel with that of PCV value and Haemoglobin percentage of blood. The statistically significant difference was observed in the values between the intervals at one percent level. This indicates that as the location advances, the R.B.C. count also increases, as can be seen from the graph. Interaction between the treatments and intervals did not show any significant difference.

Kaminki and Staig (1961) reported that when ten healthy sheep were fed 20 mg of cobalt nitrate per day per head, the erythrocyte count was raised to 10.0 from 8.2 millions per cum. Kolesov (1961) supported these findings. On the other hand, Gupta (1968), Pal *et al* (1970) in calves and Pachalag *et al* (1972) in sheep did not observe any significant difference due to cobalt supplementation, in the erythrocyte counts of blood. So were the findings of Anrith Kumar *et al* (1973) with

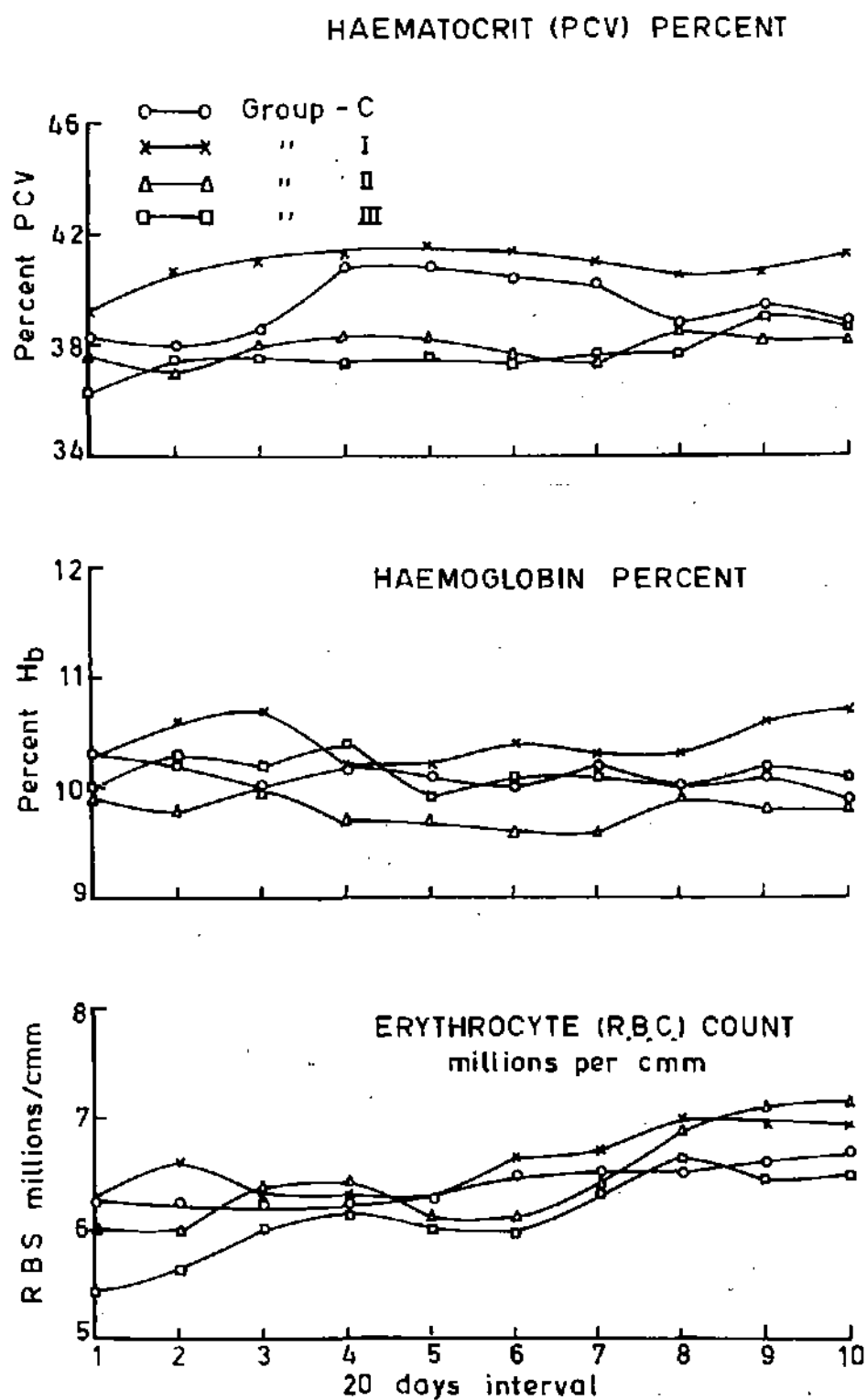


FIG.6-INFLUENCE OF COBALT SUPPLEMENTATION ON BLOOD COMPOSITION OF CROSS-BRED COWS

copper and cobalt supplementation in Jersey x Tharparkar heifers. They reported 5.28 millions per cm. as the count of R.B.C. after supplementation as against 4.47, before supplementation.

(iv) Total leucocyte (W.B.C.) Count:

The average values with the standard error were 9.959 ± 1.16 , 8.928 ± 1.24 , 9.650 ± 1.22 and 8.843 ± 0.77 thousands per cm., in the blood of the animals of control and experimental groups I, II and III, respectively (Table 51). These values lie well within the range reported by Govindan Nayar (1949), Coffin (1963), Dukes (1967) and Schalm (1961) for this class of animals. The values reported in this experiment are slightly higher than the average values reported by Mullick and Pal (1943) for Mariana and Bhanni cattle, Babadghao (1969) for Tharparkar cattle, and Holman (1965) for exotic breed of cattle. The values reported by Smith (1969) for Jersey, Shankaranarayana (1937, 1938), Khajuria and Razdan (1966) for Tharparkarcows and Amrith Kumar et al (1973) for Jersey x Tharparkar heifers are higher than those reported in this experiment. The values reported by Misra and Biswal (1961) are in close agreement with the values reported in the present study.

The total leucocyte count values of blood are also shown in Fig. 7. It is seen from the graph that the animals of supplemented groups recorded lower values of W.B.C. count in blood as compared to those of control group. This may be due to the initial higher values of W.B.C. count in the animals of control group. There was a slight increase in all the groups in the initial stages upto 40 days of supplementation. Subsequently, no definite trend was observed.

**Table 51. Average values of total leucocytes (W.B.C.) in
Thousands per cmm. with Standard Error.**

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Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	8.85±0.96	8.78±1.71	8.71±1.27	8.43±0.51
20	9.70±1.10	8.82±1.30	9.44±1.11	8.61±0.56
40	10.58±1.25	9.10±1.37	9.82±1.31	8.92±0.76
60	10.17±0.90	8.97±1.42	9.53±0.92	8.95±0.81
80	10.35±1.00	9.17±1.12	9.69±1.31	8.85±0.83
100	10.23±1.32	9.70±1.34	10.09±1.63	9.16±0.92
120	9.86±1.37	9.22±1.16	10.17±1.28	9.02±0.96
140	9.62±1.19	8.69±1.01	9.48±1.07	9.00±0.88
160	9.85±1.31	8.23±1.06	9.60±1.14	8.60±0.78
180	10.40±1.19	8.52±0.95	9.97±1.12	8.89±1.69
Group Average	9.959±1.16	8.923±1.24	9.650±1.22	8.843±0.77

**Table 52. Analysis of variance for Total Leucocyte count
of Blood
(Log. values)**

Source of Variance	Degrees of freedom	Mean Sum of Squares	Variance ratio
Between Blocks	4	1.19952	33.434**
Between Treatments	3	0.18393	5.118**
Between Intervals	9	0.02624	0.731
Interaction	27	0.00642	0.018
Error	156	0.03588	-

** Significant at 1 percent level of probability.

**Table 52A. Comparison of means of W.B.C. of Blood.
(Log. values)**

Critical Difference at 5% level	Group Average Values			
	III	I	II	Control
0.0742	2.1442	2.1580	2.2314	2.2715

Note: Mean values under bar do not differ significantly.

The statistical analysis of the data is presented in Table 52. By the perusal of the results, it is seen that a significant difference existed between the treatments. On further analysis (Table 52 A), it is observed that the treatments of group III and I, I and II as well as II and control group are not statistically different, while the control group is seen to be significantly different from group III and I. A significant difference was observed between the blocks, which may be ascribed to the individual variation or variation due to age factor. The difference between the intervals and interaction due to treatments and intervals was not statistically significant. This indicates that cobalt supplementation had no effect on the total leucocyte count of the blood of cows.

Neal and Ahman (1937) observed no significant difference after cobalt supplementation on total ~~leucocyte~~ ^{glucocyte} count in the blood of cattle. Kaminski and Utzig (1951) reported similar findings, that supplementation of 20 mg of cobalt nitrate per day per head to healthy sheep had no effect on the white blood cell picture of the animals. Pal *et al* (1970) reported that the supplementation of copper and cobalt together to Hariana calves increased the white cell count of blood, though not statistically significant. The increase was attributed to the increase in caeruloplasmin copper content of blood, due to supplementation of copper alongwith cobalt.

Amrith Kumar *et al* (1973) reported no significant difference between the values of W.B.C. count before and after supplementation of copper and cobalt to Jersey x Tharparker heifers. Their observations are in conformity with the present findings.

(v) Specific Gravity of Blood:

The average values of specific gravity of whole blood of cows of control and experimental groups are presented in Table 53. The average values observed were 1.0516 ± 0.0005 , 1.0520 ± 0.0004 , 1.0500 ± 0.0004 and 1.0514 ± 0.0003 for the whole blood of animals of the control and experimental groups I, II and III respectively.

The average values reported in this experiment are observed to be well within the normal range (1.046 and 1.058) reported by Dukes (1957) and Schalm (1951) and these are in close agreement with the average values reported by Albritton (1952), Coffin (1953), Holman (1955) and Murty and Mullick (1960). Khajuria and Rasdan (1956) reported a higher value as 1.0591 for the blood of Tharparkar lactating cows. However, Chaplin *et al* (1970) recorded 1.0510 as the normal average specific gravity of whole blood of Jersey cows. This is in conformity with those of the present findings. The values of specific gravity were found to vary in line with those of PCV and haemoglobin percent of the whole blood as reported by Raghavan *et al* (1961).

The specific gravity values of the whole blood of the cows of all the groups are shown in Fig. 7. It is seen from the graph that animals of group I recorded higher specific gravity values of whole blood compared with those of the other groups. A slight increase in the beginning of the supplementation period upto 40 days was noticed in all the supplemented groups. There was an increase in the value of blood of animals of control

Table 53. Average values of Specific Gravity of Blood
with standard error.

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Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	1.0518±0.0022	1.0518±0.0011	1.0500±0.0014	1.0504±0.0012
20	1.0526±0.0020	1.0532±0.0010	1.0498±0.0017	1.0518±0.0013
40	1.0514±0.0023	1.0528±0.0014	1.0500±0.0015	1.0520±0.0011
60	1.0518±0.0011	1.0518±0.0012	1.0504±0.0019	1.0508±0.0007
80	1.0516±0.0013	1.0516±0.0009	1.0494±0.0012	1.0506±0.0009
100	1.0514±0.0009	1.0512±0.0007	1.0494±0.0009	1.0512±0.0007
120	1.0514±0.0019	1.0512±0.0011	1.0494±0.0015	1.0510±0.0007
140	1.0512±0.0018	1.0516±0.0016	1.0500±0.0018	1.0508±0.0009
160	1.0516±0.0021	1.0518±0.0015	1.0504±0.0020	1.0516±0.0013
180	1.0512±0.0017	1.0516±0.0012	1.0508±0.0015	1.0514±0.0017
Group Average	1.0516±0.0006	1.0520±0.0004	1.0500±0.0004	1.0514±0.0003

Table 54. Analysis of Variance for Specific gravity of blood.

Source of Variance	Degrees of freedom	Mean Sum of squares	Variance ratio
Between Blocks	4	0.00016175	28.850**
Between Treatments	3	0.00004000	7.600**
Between Intervals	9	0.00000333	0.633
Interaction	27	0.00000074	0.141
Error	156	0.00000526	-

** Significant at 1 percent level of probability.

Table 54A. Comparison of Means of Specific gravity of Blood.

Critical difference at 5% level	Group Average Values			
	II	III	Control	I
0.00073	1.0500	1.0514	1.0516	1.0520

Note: Mean values under bar do not differ significantly.

group, but this was noticed only after 20 days. At the end of the experiment, the values were pretty close to each other, inspite of the variation earlier. Graph as well as the values shown in the table, show that the animals of supplemented groups II and III recorded an increase in the specific gravity values of blood over their respective initial values. The animals of control and group I had the same initial values, while after supplementation, higher values in the blood were recorded in group I, and indicating some response.

On subjecting the data to analysis of variance test, a significant difference was observed in the values between the groups (Table 54). In order to find out the superiority of the different group treatments, the mean differences were compared with the critical difference at five percent level (Table 54A). This revealed that the treatment of group I (16 mg) was found to be superior over the rest of the groups. However, the mean values of control group and group I as well as control group and group III were not significantly different from each other. The animals of group II have recorded the lowest values, which may be due to their initial low value. Even then they have shown the tendency of having higher values especially at the end of the experimental period.

The differences in the values were also noticed between the blocks, which were highly significant, at both the percentages of probability. However, no significant difference was observed between the intervals and due to interaction between the treatments and intervals.

There are no known references regarding this aspect of study, in the literature. Therefore, results were discussed based on the present findings only. In the present study, it was found that cobalt supplementation did not reveal any consistent effect on the specific gravity of the whole blood of lactating Jersey x Tharparkar cows. However, supplementation of cobalt at the rate of 16 mg of cobalt chloride was found to have optimum effect on this parameter of blood.

(B) Biochemical Constituents:

(vi) Total serum protein:

The average serum protein percent values of the blood of cows of all the four groups are presented in Table 55, with the respective overall group averages and standard errors. The average values of serum protein content in the blood of the animals of control and the experimental groups I, II and III were 7.678 ± 0.071 , 7.678 ± 0.063 , 7.69 ± 0.066 and 7.556 ± 0.062 gm percent, respectively. These values are in close agreement with those reported by Singh (1948). A narrow range from 7.1 to 8.1 gm percent was observed in all the groups. Kehar and Murthy (1945) reported slightly higher values for this constituent of blood. But Patel et al (1945) for dairy cattle and Sahai and Kehar (1947) for Kumaoni bullocks reported values comparable with those obtained in this study.

The serum protein values of the blood of the cows of all the groups are shown in Fig.7. It is seen from the graphic representation of the data that the animals of supplemented groups recorded the sharp rise in the serum protein percentages

Table 55. Average values of Serum Protein Percentages with Standard error.

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	7.150 $\pm$ 0.16	7.250 $\pm$ 0.14	7.075 $\pm$ 0.16	7.092 $\pm$ 0.25
20	7.400 $\pm$ 0.19	7.638 $\pm$ 0.11	7.463 $\pm$ 0.20	7.350 $\pm$ 0.21
40	7.513 $\pm$ 0.27	7.650 $\pm$ 0.19	7.612 $\pm$ 0.22	7.576 $\pm$ 0.32
60	7.775 $\pm$ 0.12	7.750 $\pm$ 0.16	7.638 $\pm$ 0.18	7.525 $\pm$ 0.11
80	7.763 $\pm$ 0.19	7.775 $\pm$ 0.18	7.713 $\pm$ 0.20	7.850 $\pm$ 0.14
100	7.688 $\pm$ 0.20	7.701 $\pm$ 0.16	7.688 $\pm$ 0.18	7.650 $\pm$ 0.16
120	7.587 $\pm$ 0.20	7.650 $\pm$ 0.19	7.688 $\pm$ 0.14	7.575 $\pm$ 0.10
140	7.614 $\pm$ 0.28	7.751 $\pm$ 0.18	7.800 $\pm$ 0.12	7.663 $\pm$ 0.11
160	6.638 $\pm$ 0.26	7.751 $\pm$ 0.19	7.783 $\pm$ 0.14	7.738 $\pm$ 0.19
180	7.600 $\pm$ 0.21	7.853 $\pm$ 0.18	7.728 $\pm$ 0.11	7.838 $\pm$ 0.12
Group Average	7.572 $\pm$ 0.071	7.678 $\pm$ 0.053	7.619 $\pm$ 0.056	7.556 $\pm$ 0.062

Table 56. Analysis of Variance for Serum Protein.

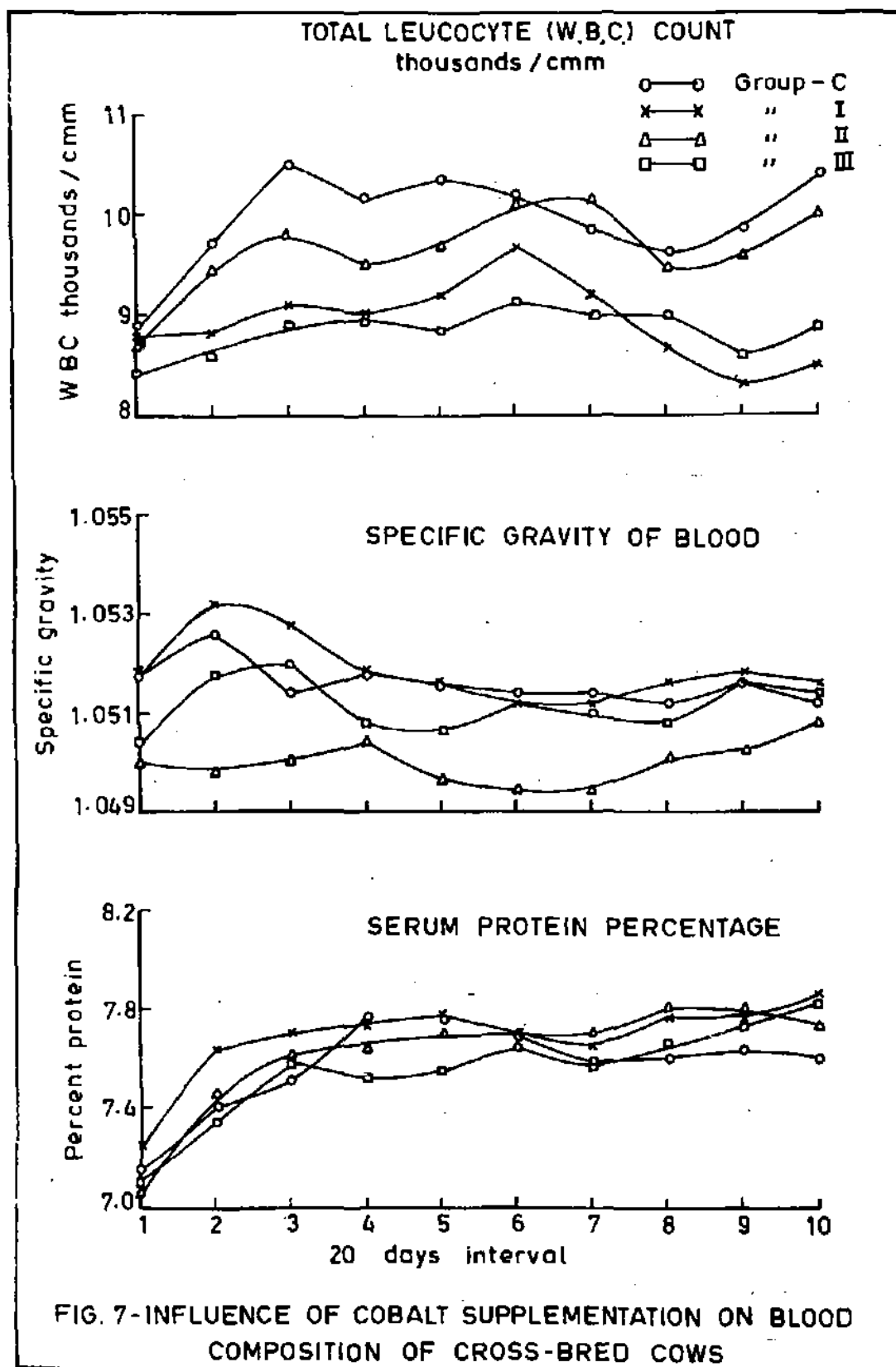
Source of Variance	Degrees of freedom	Mean Sum of Squares	Variance ratio
Between Blocks	4	0.3878	2.318
Between Treatments	3	0.1834	1.094
Between Intervals	9	0.7766	4.626**
Interaction	27	0.0341	0.203
Error	156	0.1876	-

* Significant at 1 percent level of probability.

as compared with those of control group, upto 40 days of supplementation. Again in the last two months of the experiment, cobalt supplemented group of animals have shown higher serum protein values as compared with those of control group.

The results of the statistical analysis of the data with the variance ratio are shown in Table 56. As can be seen from the table that there is no significant difference between the treatments as well as between the blocks. However, significant difference was observed between the intervals. This is also seen from the graph that the serum protein values of the blood of the cows of all the groups have shown an increasing trend upto three months of the experimental period, followed by a decline for some time and again a gradual increase upto six months of lactation. Interaction effect due to treatments and intervals was also not significant. Therefore, the supplementation of dietary cobalt to lactating cows had very little effect on serum protein values of the blood of cows. From the group averages, it is seen that 16 mg of cobalt chloride supplemented group recorded the highest value for this constituent of blood, which is followed by 24 mg supplemented group. The animals, which received 32 mg cobalt chloride per day per head recorded the least value, indicating that higher level has no marked effect.

Boganov (1958) observed similar increase in the serum protein values in the blood of calves, after supplementation of cobalt at the rate of 0.2 mg cobalt chloride per kg body weight. Later, Kolesov *et al* (1961) confirmed his findings with



cobalt supplementation in sheep. Singh (1948) and Singh (1964), however, could not observe any statistically significant difference due to cobalt supplementation on serum protein values in bullocks and calves respectively.

Anandar (1965) studied the effect of two levels of cobalt supplementation on growing kinds along with urea supplementation. He reported that 0.5 mg per day of cobalt showed slight increase, while 2.0 mg level did not bring about any such apparent increase in the serum protein contents. Similar type of results are recorded in the present investigation. Babin (1967) also reported similarly a higher blood protein value in the blood of animals receiving 20 mg of cobalt chloride than those of control group that did not receive any supplement of cobalt.

(vii) Blood iron:

The association of cobalt with haematopoietic functions has since been generally accepted, after the observations of Waltham and Waltham (1929). Cobalt in the form of vitamin B₁₂ is inter-related with iron. A study of blood iron level on supplementation of cobalt was made. The iron content of blood of the cows of control and experimental group I, II and III were 31.58 ± 0.58 , 34.17 ± 0.2 , 32.83 ± 0.49 and 33.08 ± 0.45 mg per 100 ml of blood, respectively (Table 57).

The average values reported by Mullick and Pal (1943) for the Indian breeds of cattle and by Setty (1963) for Tharparker cows are comparable with those obtained in the present study. The range given by Dukes (1957) for this constituent of blood as 30.0 to 50.0 mg per 100 ml of blood, covers the present

reported values. The average value of 39.8 mg per 100 ml of blood, reported by Pal et al (1945) is somewhat higher. Kahar and Murthy (1945), Kumar (1980), Patel et al (1981) and Razdan and Setty (1986) reported that the values ranged from 32.59 to 48.83 mg per 100 ml blood for different breeds of Indian cattle. The average value of blood iron content reported by Amirth Kumar et al (1973) for Jersey x Tharparkar heifers from the same herd as 30.0 mg per 100 ml blood was found to be slightly lower than those reported in the present study.

The iron content of blood of cows of all the groups are shown in Fig.8. It is seen from the graph that the animals of supplemented groups have recorded higher blood iron values than those of control group. The cows of group I (16 mg) recorded the highest blood iron values one month after the initiation of cobalt supplementation. Group II and III (24 and 32 mg) were found to have the blood iron values ranging between those of the control and group I (16 mg).

It is seen from the statistical analysis of the data (Table 58), that the difference between the blocks and between the treatments are significant at 1 percent level. On comparing the mean differences with critical difference at 5 percent level (Table 58 A), it is observed that all the supplemented groups were found to be significantly different from the control group. The cows of control group recorded the lowest blood iron values, while those of group II and III (24 and 32 mg) were next in the order to that of control, but below group I (16 mg). The treatments of group II and III were found to be similar, while

Table 57. Average values of Blood Iron in mg percent with Standard Error.

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	31.40 $\pm$ 1.78	31.50 $\pm$ 0.80	31.32 $\pm$ 3.96	34.13 $\pm$ 0.30
20	33.14 $\pm$ 2.54	33.93 $\pm$ 1.72	33.43 $\pm$ 2.09	34.41 $\pm$ 1.62
40	30.52 $\pm$ 2.43	35.99 $\pm$ 1.64	30.78 $\pm$ 1.26	32.64 $\pm$ 1.30
60	29.90 $\pm$ 2.15	33.78 $\pm$ 1.81	32.42 $\pm$ 1.21	32.42 $\pm$ 2.02
80	30.64 $\pm$ 1.03	34.21 $\pm$ 1.51	31.35 $\pm$ 1.19	31.56 $\pm$ 1.51
100	31.28 $\pm$ 1.23	33.20 $\pm$ 1.59	30.86 $\pm$ 1.02	32.36 $\pm$ 0.82
120	31.57 $\pm$ 2.14	33.56 $\pm$ 1.74	31.21 $\pm$ 1.03	32.64 $\pm$ 1.12
140	31.57 $\pm$ 2.24	33.71 $\pm$ 1.53	33.28 $\pm$ 1.09	33.07 $\pm$ 1.80
160	32.99 $\pm$ 1.71	35.78 $\pm$ 1.70	31.99 $\pm$ 1.30	33.56 $\pm$ 1.75
180	32.79 $\pm$ 0.81	35.99 $\pm$ 1.21	32.63 $\pm$ 1.07	33.99 $\pm$ 2.02
Group Average	31.58 $\pm$ 0.58	34.17 $\pm$ 0.52	32.83 $\pm$ 0.49	33.08 $\pm$ 0.45

Table 58. Analysis of Variance for Blood Iron.

Source of Variance	Degrees of freedom	Mean Sum of Squares	Variance ratio
Between Blocks	4	159.1543	15.514**
Between Treatments	3	80.6101	7.852**
Between Intervals	9	15.8785	1.548
Interaction	27	5.1050	0.498
Error	156	10.2584	-

** Significant at 1 percent level of probability.

Table 58A. Comparison of Means of Blood Iron.

Critical Difference at 5% level	Group Average Values			
	Control	II	III	I
1.2558	31.58	32.83	33.08	34.17

Note: Mean values under bar do not differ significantly.

that of group I was found to have marked response over group II. No significant difference between the intervals and due to interaction between the intervals and the treatments were observed.

Singh (1964) reported a parallel increase in the iron content of the blood along with PCV and Haemoglobin percentage in the blood, after three months of cobalt supplementation in Hariana calves at levels of 0.6 and 1.0 mg per day. Similar observations were recorded by Amrith Kumar *et al* (1973) after supplementation of copper plus cobalt in the ration of Jersey x Tharparker heifers. An appreciable increase was recorded after supplementation, though statistically insignificant. These observations are in support of those of the present study.

(viii) Blood copper:

The average values with standard errors of the copper content of blood of cows of all the groups are presented in Table 59. The values of copper contents in the blood of cows of control and experimental groups I, II and III were 115 ± 1.23 , 125 ± 1.33 , 120 ± 1.53 and 120 ± 1.41 mcg per 100 ml of blood respectively. The blood copper content in the whole blood of healthy animals vary considerably. The range reported by Beck (1956) is from 50 to 150 mcg per 100 ml of blood. It is seen from the values reported earlier under copper supplementation experiment (Chapter V), that these values are well within the range reported by many workers. Still higher values were reported in normal and healthy cattle by Sahai and Kehar (1951), which ranged from 129 to 153 mcg per 100 ml of blood. Similar values were reported by workers at Western Animals Nutrition

that of group I was found to have marked response over group II. No significant difference between the intervals and due to interaction between the intervals and the treatments were observed.

Singh (1964) reported a parallel increase in the iron content of the blood along with PCV and Haemoglobin percentage in the blood, after three months of cobalt supplementation in Hariana calves at levels of 0.5 and 1.0 mg per day. Similar observations were recorded by Amrith Kumar *et al* (1973) after supplementation of copper plus cobalt in the ration of Jersey x Tharparkar heifers. An appreciable increase was recorded after supplementation, though statistically insignificant. These observations are in support of those of the present study.

(viii) Blood copper:

The average values with standard errors of the copper content of blood of cows of all the groups are presented in Table 59. The values of copper contents in the blood of cows of control and experimental groups I, II and III were 115 ± 1.23 , 125 ± 1.33 , 120 ± 1.53 and 120 ± 1.41 mcg per 100 ml of blood respectively. The blood copper content in the whole blood of healthy animals vary considerably. The range reported by Beck (1956) is from 50 to 150 mcg per 100 ml of blood. It is seen from the values reported earlier under copper supplementation experiment (Chapter V), that these values are well within the range reported by many workers. Still higher values were reported in normal and healthy cattle by Sahai and Kehar (1951), which ranged from 129 to 153 mcg per 100 ml of blood. Similar values were reported by workers at Western Animals Nutrition

Table 59. Average values of blood copper in ug percent with Standard Error.

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	108 $\pm$ 7.51	110 $\pm$ 4.02	111 $\pm$ 4.15	104 $\pm$ 3.87
20	116 $\pm$ 4.67	114 $\pm$ 3.37	113 $\pm$ 5.62	114 $\pm$ 5.28
40	116 $\pm$ 4.72	123 $\pm$ 4.72	120 $\pm$ 5.27	119 $\pm$ 2.19
60	115 $\pm$ 3.14	130 $\pm$ 2.01	122 $\pm$ 6.04	122 $\pm$ 4.93
80	116 $\pm$ 1.41	129 $\pm$ 3.31	124 $\pm$ 4.35	120 $\pm$ 4.02
100	113 $\pm$ 2.78	128 $\pm$ 1.01	122 $\pm$ 7.03	124 $\pm$ 4.09
120	115 $\pm$ 1.14	128 $\pm$ 2.19	122 $\pm$ 3.16	125 $\pm$ 2.38
140	116 $\pm$ 1.71	130 $\pm$ 1.96	123 $\pm$ 2.37	124 $\pm$ 2.96
160	117 $\pm$ 2.00	128 $\pm$ 4.12	121 $\pm$ 3.35	123 $\pm$ 2.25
180	116 $\pm$ 4.11	128 $\pm$ 4.31	124 $\pm$ 4.34	122 $\pm$ 3.16
Group Average	115 $\pm$ 1.23	125 $\pm$ 1.33	120 $\pm$ 1.53	120 $\pm$ 1.41

Table 60. Analysis of Variance for Blood Copper.

Source of Variance	Degrees of freedom	Mean Sum of Squares	Variance ratio
Between Blocks	4	695.6700	10.923**
Between Treatments	3	865.1517	13.585**
Between Intervals	9	438.2528	6.892**
Interaction	27	60.5268	0.950
Error	156	63.6803	-

** Significant at 1 percent level of probability.

Table 60A. Comparison of Means of Blood Copper.

Critical Difference at 5% level	Group Average Values			
	Control	III	II	I
9.8917	115	120	120	125

Note: Mean values under bar do not differ significantly.

superior over that of control group. The differences among all the supplemented groups were found statistically not significant. The treatments of groups II (24 mg) and III (32 mg) were also found not significantly different from that of control group.

A significant difference noticed between the blocks may be due to the individual variation and that of intervals due to the increasing trend of values in the animals of supplemented groups with the advancement of lactation period. No significant difference due to interaction between the treatments and the intervals was noticed.

Chapman and Kidder (1964) reported a significantly higher levels of copper in the liver of growing cattle after supplementation of 8 mg of cobalt per day per animal. Kapoor (1968) reported beneficial effect of cobalt supplementation in maintenance of higher levels of caeruloplasmin in blood, indicating higher copper levels in blood as total copper is the direct index of caeruloplasmin in blood. However, there is paucity of references regarding this particular aspect of study in relation to dietary cobalt supplementation in cattle.

(ix) Blood cobalt:

Cobalt content of the blood of cows of all the four groups are presented in Table 61. The average values of cobalt content in the blood of cows of control and experimental groups I, II and III were 0.990 ± 0.014 , 1.600 ± 0.116 , 1.598 ± 0.134 and 1.584 ± 0.124 μg per 100 ml of blood respectively. The normal values for the cobalt content of blood were reported probably

for the first time by Askew and Dixon (1937). They reported 2.5 mcg per 100 ml blood as the cobalt concentration of affected or cobalt-deficient animals, while 7.0 μ g of cobalt per 100 ml of blood in treated animals. These values are slightly higher than the ones obtained in the present study for the blood of cows of control as well as supplemented groups. In India, Singh (1948) reported 2.3 to 3.5 mcg per 100 ml of blood, as the concentration of cobalt in the blood of Kumaoni cattle. The values are not far from the present observed values in Jersey x Tharparker lactating cows.

A graphic representation of the blood cobalt values observed during the experimental period for all the four groups is given in Fig. 8. From the perusal of the graph, it is seen that the animals of supplemented groups recorded higher cobalt values in their blood, as compared to those of control group. The cows of group I (16 mg) recorded the highest values for the cobalt content in their blood, up to two months of supplementation. Thereafter, the animals of group II (24 mg) recorded the highest cobalt concentration in their blood upto almost the end of the experimental period. The animals of all the supplemented groups recorded an increase in the blood cobalt content.

It is seen from the statistical analysis of the data, that the difference between the treatments is significant at one percent level (Table 62). On further analysis, it was seen that treatments of all the supplemented groups were superior and significantly different from that of control group (Table 62A). The differences among the supplemented groups I, II and III

**Table 61. Average values of blood cobalt in ug percent
with Standard Error.**

Intervals in days	Groups			
	Control	Experimental I	Experimental II	Experimental III
0	0.64±0.10	0.68±0.08	0.42±0.10	0.52±0.08
20	0.78±0.12	1.00±0.15	0.66±0.12	0.92±0.23
40	0.70±0.15	1.20±0.28	0.94±0.20	0.98±0.21
60	0.70±0.14	1.18±0.21	1.20±0.32	1.08±0.24
80	0.90±0.15	1.30±0.20	1.38±0.30	1.25±0.26
100	1.02±0.17	1.52±0.22	1.52±0.34	1.35±0.25
120	1.34±0.18	1.88±0.23	2.06±0.23	1.68±0.30
140	1.22±0.09	2.26±0.21	2.52±0.20	2.34±0.31
160	1.35±0.12	2.48±0.17	2.76±0.27	2.50±0.23
180	1.24±0.10	2.60±0.22	2.62±0.20	2.60±0.19
Group Average	0.990±0.014	1.600±0.116	1.592±0.134	1.524±0.124

Table 62. Analysis of Variance for Cobalt Content of Blood.

Source of Variance	Degrees of freedom	Mean Sum of Squares	Variance ratio
Between Blocks	4	0.3557	1.424
Between Treatments	3	4.3257	17.323**
Between Intervals	9	7.7048	30.455**
Interaction	27	0.3539	1.417
Error	156	0.2497	-

** Significant at 1 percent level of probability.

Table 62A. Comparison of Means of Blood Cobalt.

Critical Difference at 5% level	Group Average Values			
	Control	III	II	I
0.186	0.990	1.524	1.592	1.600

Note: Mean values under bar do not differ significantly.

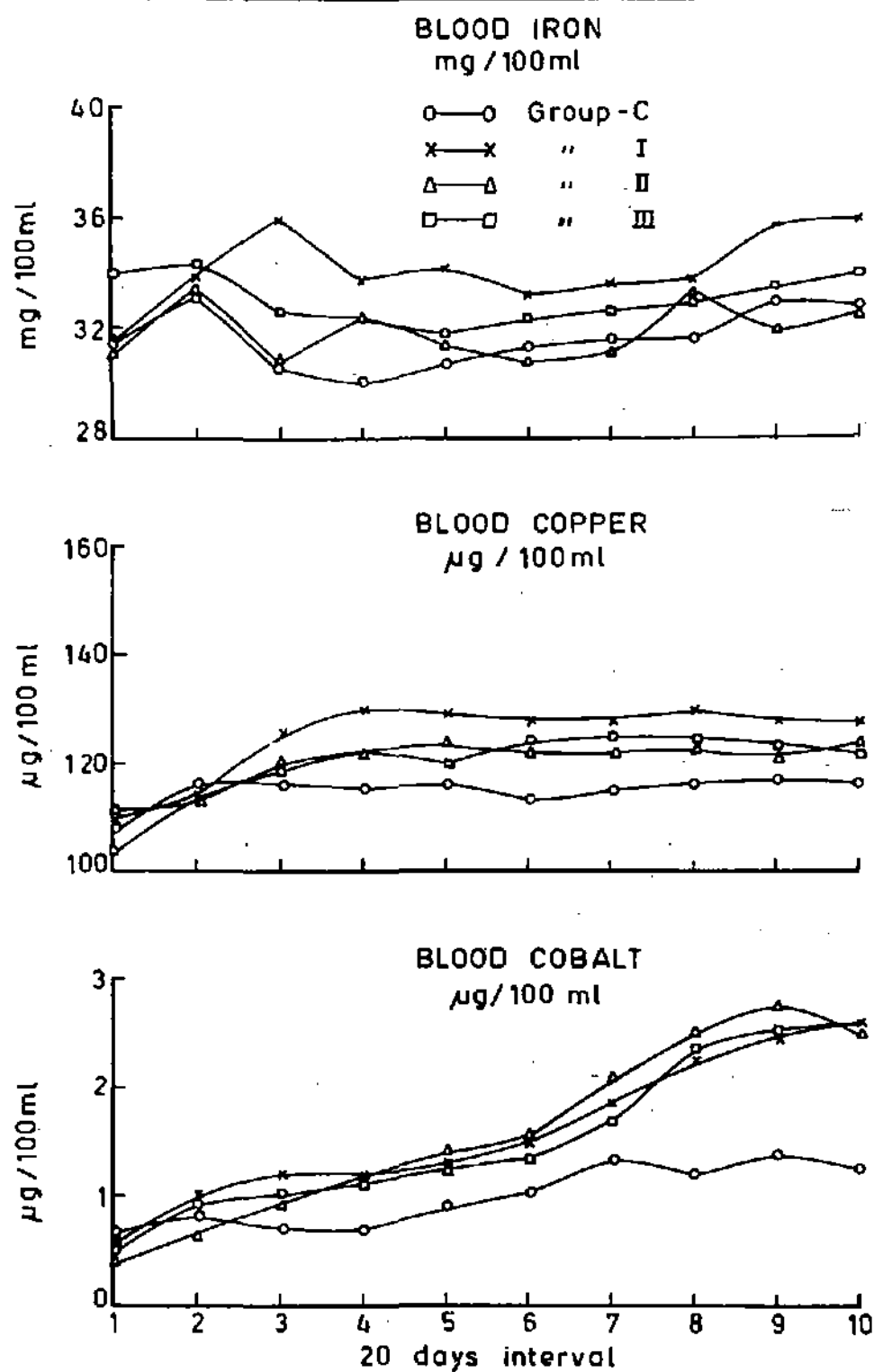


FIG. 8-INFLUENCE OF COBALT SUPPLEMENTATION ON BLOOD COMPOSITION OF CROSS-BRED COWS

were not significantly different from each other. However, from the group average values, it is seen that group I (16 mg) has shown the highest values followed in order of groups II and III. A significant difference between the intervals was noticed. This is evident from the graph too. As the time advanced, there was a steady increase in the cobalt concentration of blood, except in control group. A slight increase was observed in control group upto 4 months of lactation, thereafter it became almost steady. No significant difference between the blocks and due to interaction between the treatments and intervals were noticed.

(II) Influence of Cobalt Supplement in the Feed on Milk Composition:

Cobalt as a factor in influencing the milk composition has so far not been a subject of intensive study. Only very little work has been carried out to find the effect of cobalt supplementation of the feed on the milk composition. In this study, this aspect has been investigated.

(1) Milk fat:

Average values with standard error for fat percentages of the milk of cows of all the four groups are presented in Table 63. The group average values observed for the fat content in the milk of cows of control and experimental groups I, II and III were 5.15 ± 0.031 , 5.14 ± 0.050 , 5.09 ± 0.035 and 5.20 ± 0.063 percent, respectively. The values for milk fat percentages recorded by Overman *et al* (1939) as 5.18, Richmond (1952) as 5.53, Ling (1956) as well as Jeenness and Patton (1959) as 5.18 percent in the milk of Jersey breed of cattle are not far from those observed in the present study for the Jersey cross bred-cows. Davies (1939) and Armstrong (1959) also reported similar values for the milk fat percentage of Jersey cows. Sukhatme *et al* (1948), Ghosh (1950) and Murthy (1969, 1972) reported values ranging from 4.60 to 5.53 percent for the milk of different cross-bred cows. Kothavalla and Kartha (1939), Sukhatme *et al* (1948) and Sen (1953) reported the average values of fat percentage of milk of Tharparkar cows as 4.55, 4.60 and 4.60 respectively. The milk fat values obtained for the milk of Jersey x Tharparkar cross-bred cows

**Table 63. Average values of fat percentage of milk
with standard error.**

Fortnights	Groups			
	Control	Experimental I	Experimental II	Experimental III
1	4.98 $\pm$ 0.31	4.82 $\pm$ 0.16	4.74 $\pm$ 0.22	4.78 $\pm$ 0.21
2	4.94 $\pm$ 0.22	4.82 $\pm$ 0.20	4.74 $\pm$ 0.20	4.86 $\pm$ 0.13
3	4.90 $\pm$ 0.24	4.86 $\pm$ 0.17	4.78 $\pm$ 0.14	4.94 $\pm$ 0.22
4	4.98 $\pm$ 0.29	4.88 $\pm$ 0.14	4.78 $\pm$ 0.18	4.92 $\pm$ 0.23
5	5.02 $\pm$ 0.29	5.00 $\pm$ 0.09	4.88 $\pm$ 0.21	4.94 $\pm$ 0.24
6	5.08 $\pm$ 0.26	5.02 $\pm$ 0.16	5.00 $\pm$ 0.14	5.22 $\pm$ 0.21
7	5.10 $\pm$ 0.23	5.12 $\pm$ 0.18	5.00 $\pm$ 0.20	5.30 $\pm$ 0.30
8	5.08 $\pm$ 0.31	5.12 $\pm$ 0.21	5.08 $\pm$ 0.18	5.30 $\pm$ 0.25
9	5.20 $\pm$ 0.40	5.16 $\pm$ 0.14	5.16 $\pm$ 0.19	5.31 $\pm$ 0.27
10	5.26 $\pm$ 0.41	5.22 $\pm$ 0.16	5.30 $\pm$ 0.21	5.36 $\pm$ 0.26
11	5.24 $\pm$ 0.40	5.28 $\pm$ 0.20	5.36 $\pm$ 0.30	5.40 $\pm$ 0.28
12	5.30 $\pm$ 0.38	5.38 $\pm$ 0.18	5.38 $\pm$ 0.29	5.34 $\pm$ 0.21
13	5.30 $\pm$ 0.32	5.48 $\pm$ 0.14	5.38 $\pm$ 0.32	5.36 $\pm$ 0.22
14	5.40 $\pm$ 0.34	5.52 $\pm$ 0.15	5.38 $\pm$ 0.25	5.44 $\pm$ 0.23
15	5.44 $\pm$ 0.22	5.56 $\pm$ 0.14	5.40 $\pm$ 0.17	5.50 $\pm$ 0.24
Group Av.	5.149 $\pm$ 0.081	5.143 $\pm$ 0.050	5.095 $\pm$ 0.085	5.196 $\pm$ 0.063

Table 64. Analysis of Variance for Milk Fat.

Source of variance	Degrees of freedom	Mean Sum of squares	Variance ratio
Between Blocks	4	11.8145	92.445 ^{**}
Between Treatments	3	0.1320	1.033
Between Intervals	14	1.1284	8.829 ^{**}
Interaction	42	0.0445	0.348
Error	236	0.1278	-

^{**} Significant at 1 percent level of probability.

compare favourably with these values reported by various workers for Jersey-Tharparkar, crosses and indigenous stock of cows.

The fat percent values of the milk of cows of all the groups are shown in Fig. 9. It will be seen from the figure that milk of the cows of all the groups indicated more or less similar values for the fat content of milk. The cows of cobalt-supplemented groups recorded a rapid rise in their milk fat content and they crossed the control group values at about 5 months of lactation period, inspite of their initial lower fat percentages. Amongst the supplemented groups, cows of group III (32 mg) recorded the highest values, followed in order by cows of group I (16 mg) and those of group II (24 mg).

However, the statistical analysis of the data revealed that there is no significant difference between the treatments (Table 64). A highly significant difference was noticed between the blocks as well as between the intervals. A significant difference between the blocks may be due to the differences in the lactation numbers and the age of the animals in different blocks. This fact is supported by the findings of Barlett (1934). There was no significant interaction effect due to treatments and intervals.

Nagorskaya et al (1966), Palian (1968) and Melnikov and Ilyukhina (1969) studied the effect of cobalt and cobalt plus other trace minerals on the fat percentage of milk in cows and ewes. They did not find any significant difference in the fat content of milk. On the contrary, Rudometkii (1962), Nikitin

(1956), Poppe (1956) and Shatokhina (1958) reported a favourable effect of dietary cobalt supplementation on milk fat in cattle.

(2) Milk protein:

The results of the examination of this constituent of milk of cows of all the four groups are presented in Table 65. The average values of milk protein percentages in the milk of cows ^{of} control and experimental groups I, II and III were 3.428 ± 0.024 , 3.427 ± 0.020 , 3.344 ± 0.017 and 3.407 ± 0.031 gm percent, respectively. The values reported by Brahmachari (1934) as 3.50, Seshan (1937) as 3.02, Sukhatme *et al* (1948) as 3.30 and Ghosh (1956) as 3.33 per cent for the milk of pure-bred and cross-bred animals are quite close to those observed in the present experiment. Roder (1938), Davies (1939), Overman (1945) and Armstrong (1959) reported the average values for the protein content in the milk of Jersey cows. Their values were slightly higher than the present observed values. The values reported under copper supplementation experiment (Chapter V) were also slightly higher than those observed in the present investigation.

The milk protein percentages of the milk of cows of all the four groups are presented in Fig. 9. Sukhatme *et al* (1948) and Davis (1939) reported that the percentage of protein in milk of cows tends to decrease in the beginning upto 2-3 months of lactation period normally. Similar type of decrease in the value of protein contents of milk has been observed and supplementation of cobalt is found to have no effect on this constituent of milk. The animals of group III (32 mg) have shown

Table 65. Average Values of Milk Protein Percentage with S.E.

Fortnights	Groups			
	Control	Experimental I	Experimental II	Experimental III
1	3.287±0.13	3.389±0.14	3.346±0.03	3.236±0.10
2	3.276±0.13	3.382±0.07	3.281±0.02	3.190±0.11
3	3.287±0.11	3.272±0.04	3.200±0.04	3.175±0.12
4	3.312±0.09	3.318±0.04	3.211±0.03	3.205±0.08
5	3.353±0.06	3.328±0.07	3.231±0.03	3.276±0.13
6	3.389±0.05	3.372±0.07	3.317±0.05	3.263±0.12
7	3.389±0.03	3.394±0.07	3.343±0.05	3.394±0.13
8	3.471±0.06	3.364±0.09	3.333±0.05	3.445±0.11
9	3.486±0.06	3.399±0.07	3.343±0.06	3.460±0.11
10	3.481±0.11	3.455±0.05	3.369±0.04	3.522±0.11
11	3.511±0.11	3.483±0.07	3.399±0.04	3.547±0.14
12	3.527±0.10	3.532±0.05	3.414±0.03	3.557±0.13
13	3.552±0.10	3.588±0.05	3.491±0.07	3.583±0.12
14	3.548±0.07	3.583±0.04	3.517±0.07	3.578±0.14
15	3.547±0.06	3.629±0.05	3.527±0.07	3.573±0.13
Group Av.	3.428±0.024	3.427±0.020	3.344±0.017	3.407±0.031

Table 66. Analysis of Variance for Milk Protein Percentages.

Source of Variance	Degrees of freedom	Mean Sum of Square	Variance ratio
Between Blocks	4	0.4534	16.659**
Between Treatments	3	0.1172	4.310**
Between Intervals	14	0.2810	10.331**
Interaction	42	0.0080	0.294
Error	236	0.0272	-

** Significant at 1 percent level of probability.

Table 66A. Comparison of Means of Milk Protein percentages.

Critical Differences at 5% level	Group Average Values			
	II	III	I	Control
0.0528	3.344	3.407	3.487	3.428

Note: Mean values under bar do not differ significantly.

quicker increase in protein content of milk as compared to those observed in either control or other supplemented groups, inspite of their low initial values to begin with.

The analysis of variance test conducted on the milk protein percentage values showed significant difference between the treatments (Table 66). On further analysis (Table 66A), it is observed that the treatments of groups control, I and III were similar and not significantly different from each other. Group II has shown slightly lower values from the beginning of the experiment. However, the trend of increase in protein percentage values in group II was found parallel with those of other groups and the average values were well above the reported normal average for this constituent in cross-bred animals. Therefore, these lower values as compared to control and other supplemented groups does not appear to be due to any physiological effect consequent to cobalt supplementation.

Bagorskaya *et al* (1966) and Melnikov (1969) reported no significant effect of cobalt supplementation on the milk composition in cattle. However, Shatekhina (1968) reported 3.51 percent of protein in the milk of the animals receiving 16 mg cobalt chloride per day per head as against 3.33 percent in the milk of animals of control group receiving no supplement of cobalt.

A significant difference between the blocks and intervals was noticed. The former may be due to the variation in the lactation number and age of the animals of different blocks and latter being due to the normal trend of this constituent

of milk with the advancement of lactation. Barlett (1934) and Ghosh (1956) recorded similar variations in this constituent of milk. No significant difference due to interaction between treatments and intervals was noticed.

(3) Total solids:

The average fortnightly values of total solids content of milk upto seven and half months of lactation period of the animals of control and experimental groups are presented in Table 67. The group average values of total solids with standard error for the milk of animals of control and experimental groups I, II and III were 14.624 ± 0.077 , 14.697 ± 0.039 , 14.624 ± 0.060 and 14.689 ± 0.108 percent, respectively. These values are comparable to the average value (14.69 ± 1.11 percent) reported by Overman *et al* (1939) for the milk of Jersey cows. The value reported by Armstrong (1959) is also close to the values observed in this experiment. Davies (1939) reported on an average 14.73 percent total solids in the milk of Jersey animals, which is also not far from the present observed values. Sen and Dastur (1947) reported an average value of 12.93% of total solids content in the milk of Tharparkar cows which is slightly lower as compared to the present values obtained for the milk of Jersey-Tharparkar cross-bred cattle. Murthy (1969-1972) recorded similar values as observed in the present investigation for the milk of Jersey x Tharparkar cows.

In Fig. 9, the total solids content of milk of cows of all the four groups are presented graphically. It is seen from the figure that the animals of supplemented groups have not shown much higher values in their milk, as compared to

Table 67. Average Values of Total Solids Percentage with S.E.

Fortnights	Groups			
	Control	Experimental I	Experimental II	Experimental III
1	14.381 $\pm$ 0.54	14.433 $\pm$ 0.42	14.186 $\pm$ 0.43	14.159 $\pm$ 0.42
2	14.382 $\pm$ 0.37	14.471 $\pm$ 0.22	14.381 $\pm$ 0.24	14.425 $\pm$ 0.30
3	14.485 $\pm$ 0.30	14.480 $\pm$ 0.14	14.294 $\pm$ 0.20	14.430 $\pm$ 0.39
4	14.516 $\pm$ 0.22	14.486 $\pm$ 0.17	14.280 $\pm$ 0.22	14.268 $\pm$ 0.43
5	14.527 $\pm$ 0.25	14.625 $\pm$ 0.11	14.453 $\pm$ 0.31	14.440 $\pm$ 0.48
6	14.532 $\pm$ 0.30	14.650 $\pm$ 0.10	14.470 $\pm$ 0.31	14.581 $\pm$ 0.47
7	14.558 $\pm$ 0.21	14.670 $\pm$ 0.10	14.444 $\pm$ 0.28	14.791 $\pm$ 0.45
8	14.509 $\pm$ 0.31	14.751 $\pm$ 0.20	14.405 $\pm$ 0.30	14.843 $\pm$ 0.42
9	14.550 $\pm$ 0.38	14.678 $\pm$ 0.21	14.403 $\pm$ 0.31	14.855 $\pm$ 0.43
10	14.794 $\pm$ 0.31	14.715 $\pm$ 0.23	14.523 $\pm$ 0.34	14.883 $\pm$ 0.41
11	14.820 $\pm$ 0.53	14.760 $\pm$ 0.21	14.635 $\pm$ 0.41	14.913 $\pm$ 0.42
12	14.844 $\pm$ 0.51	14.853 $\pm$ 0.27	14.633 $\pm$ 0.38	14.925 $\pm$ 0.49
13	14.788 $\pm$ 0.37	14.824 $\pm$ 0.20	14.806 $\pm$ 0.31	14.888 $\pm$ 0.50
14	14.815 $\pm$ 0.36	14.957 $\pm$ 0.22	14.903 $\pm$ 0.22	14.827 $\pm$ 0.46
15	14.850 $\pm$ 0.39	15.031 $\pm$ 0.27	14.987 $\pm$ 0.22	14.897 $\pm$ 0.43
Group Av.	14.624 $\pm$ 0.077	14.697 $\pm$ 0.039	14.624 $\pm$ 0.080	14.689 $\pm$ 0.108

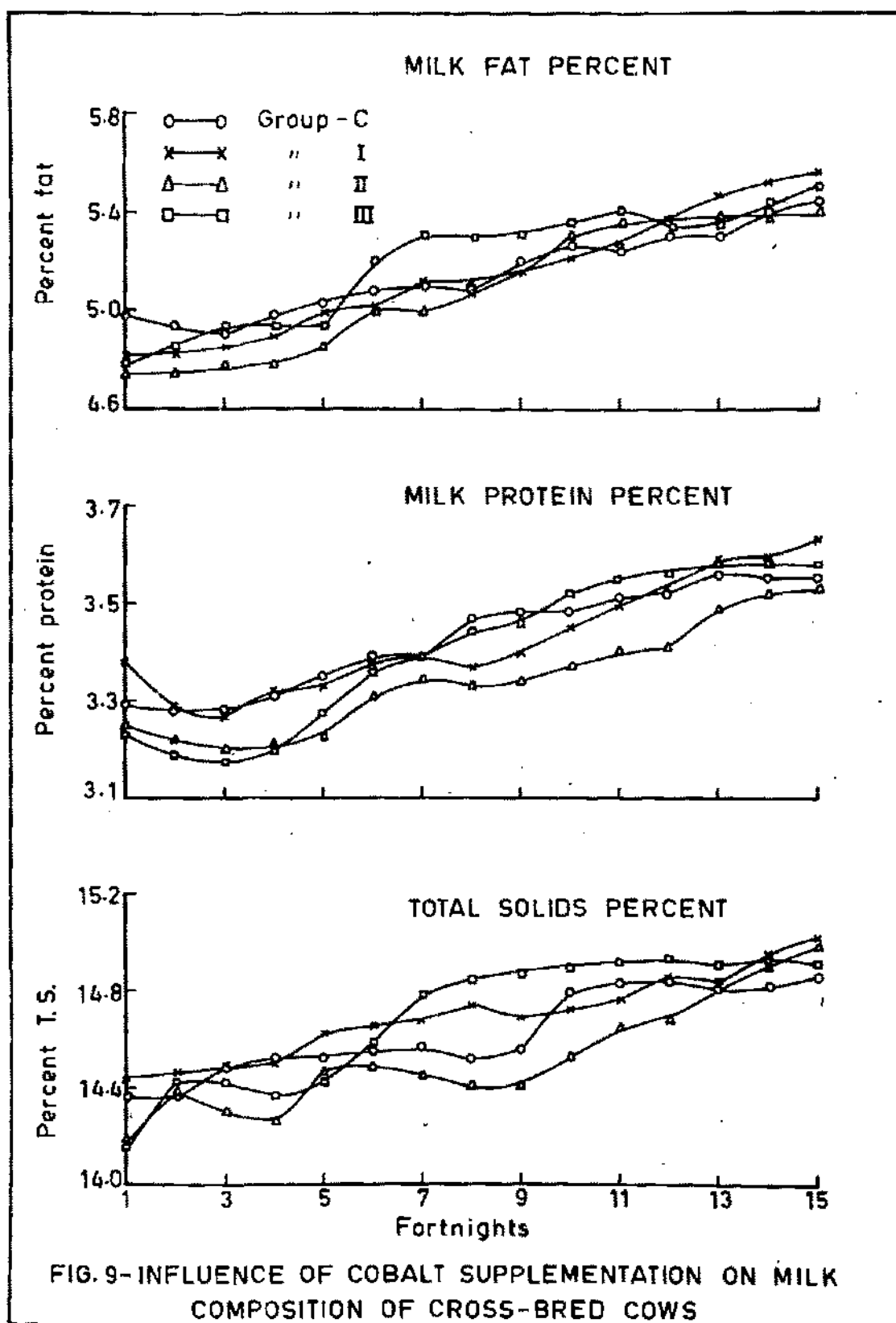
Table 68. Analysis of Variance for Total Solids of Milk.

Source of variance	Degrees of freedom	Mean Sum of Square	Variance ratio
Between Blocks	4	17.6906	74.466**
Between Treatments	3	0.4828	2.033
Between Intervals	14	0.9175	3.837**
Interaction	42	0.0620	0.261
Error	836	0.2372	-

** Significant at 1 percent level of probability.

those obtained in control group. However, all the supplemented groups have recorded an increase within 15 days of initiating the supplementation of cobalt, wherein normally the values for total solids show a decreasing trend (Sukhatme et al., 1948). This effect was seen temporarily, as subsequently the values fell down except in group I (16 mg). The cows of group I (16 mg) recorded higher values almost throughout the experiment, over those of control group. The cows of group II and III (24 and 32 mg) crossed the control group values at six and half months of lactation period, inspite of their low initial values as compared with those of control group.

It is seen from the statistical analysis of the data that there is no significant difference between the treatments (Table 68). Therefore, it shows that supplementation of cobalt at these three levels did not affect the total solids content of milk. A highly significant difference between the blocks has been noticed. This can be attributed to the normal variation found due to the individual, age and lactation number. Similarly, the significant difference observed between the intervals is normal feature of this component of milk, as the lactation advanced (Sukhatme et al., 1948). The interaction effect due to the treatments and intervals was not significant.



Agorskaya et al (1966) and Malinikov and Ilyukhina (1969) reported similar observation of insignificant difference between the supplemented and un-supplemented animals with cobalt and other trace mineral supplementation on the chemical composition of milk. There are no known references in the literature as far as the total solids and cobalt supplementation alone is concerned.

(4) Solids-not fat:

From the Table 69, it is seen that the average values for solids-not fat content of milk were 9.499 ± 0.037 , 9.536 ± 0.041 , 9.438 ± 0.024 and 9.481 ± 0.055 percent, respectively, for the milk of the cows of control and experimental groups I, II and III. These average values are in close agreement with those reported by Overman (1939, 1945), Warner (1951) and Armstrong (1959) for the milk of Jersey breed of cattle, as well as those reported by Sakhatue et al (1948), Sen (1953) and Aggarwala and Sharma (1961) for the milk of Tharparkar breed of cattle. Murthy (1969, 1973) recorded 9.11 percent, as the average solids-not fat content in the milk of Jersey x Tharparkar animals, which is slightly lower than the values recorded in this experiment.

Table 69. Average Values of Solids not fat percentage with S.E.

Fortnights	Groups			
	Control	Experimental I	Experimental II	Experimental III
1	9.381 $\pm$ 0.32	9.413 $\pm$ 0.41	9.445 $\pm$ 0.31	9.279 $\pm$ 0.26
2	9.524 $\pm$ 0.12	9.551 $\pm$ 0.09	9.541 $\pm$ 0.13	9.445 $\pm$ 0.15
3	9.501 $\pm$ 0.13	9.520 $\pm$ 0.13	9.537 $\pm$ 0.15	9.500 $\pm$ 0.17
4	9.535 $\pm$ 0.11	9.515 $\pm$ 0.22	9.500 $\pm$ 0.08	9.448 $\pm$ 0.20
5	9.507 $\pm$ 0.10	9.525 $\pm$ 0.09	9.508 $\pm$ 0.09	9.540 $\pm$ 0.25
6	9.472 $\pm$ 0.08	9.530 $\pm$ 0.09	9.494 $\pm$ 0.21	9.361 $\pm$ 0.24
7	9.458 $\pm$ 0.08	9.570 $\pm$ 0.18	9.444 $\pm$ 0.15	9.491 $\pm$ 0.20
8	9.428 $\pm$ 0.04	9.531 $\pm$ 0.29	9.325 $\pm$ 0.20	9.543 $\pm$ 0.21
9	9.350 $\pm$ 0.05	9.538 $\pm$ 0.11	9.343 $\pm$ 0.17	9.505 $\pm$ 0.15
10	9.534 $\pm$ 0.11	9.425 $\pm$ 0.15	9.353 $\pm$ 0.15	9.527 $\pm$ 0.14
11	9.580 $\pm$ 0.15	9.453 $\pm$ 0.10	9.325 $\pm$ 0.13	9.593 $\pm$ 0.14
12	9.544 $\pm$ 0.14	9.473 $\pm$ 0.10	9.353 $\pm$ 0.171	9.585 $\pm$ 0.27
13	9.488 $\pm$ 0.15	9.444 $\pm$ 0.12	9.425 $\pm$ 0.06	9.524 $\pm$ 0.25
14	9.415 $\pm$ 0.19	9.438 $\pm$ 0.12	9.573 $\pm$ 0.08	9.487 $\pm$ 0.27
15	9.410 $\pm$ 0.22	9.471 $\pm$ 0.15	9.557 $\pm$ 0.11	9.397 $\pm$ 0.17
Group Av.	9.499 $\pm$ 0.097	9.535 $\pm$ 0.041	9.438 $\pm$ 0.024	9.481 $\pm$ 0.055

Table 70. Analysis of Variance for Solids not fat percentages

Source of variance	Degrees of freedom	Mean Sum of Square	Variance ratio
Between Blocks	4	1.4565	10.054**
Between Treatments	3	0.1244	0.853
Between Intervals	14	0.0520	0.425
Interaction	42	0.0465	0.320
Error	235	0.1459	-

** Significant at 1 percent level of probability.

The solids-not fat contents of milk of the animals of control and experimental groups have been presented in Fig.10. It is seen from the graph that the animals of all the groups have recorded a sharp rise in the beginning followed by a slight drop and then an uncertain trend subsequently till the end of experimental period of seven and half months. Similar observations were recorded by Sukhatme *et al* (1948) and Ghosh (1956) in the case of milk from pure and cross-bred animals, respectively.

After subjecting the data to the statistical analysis, it was observed that the treatments did not differ from each other significantly (Table 70). Similarly, no significant difference between the intervals and due to interaction was noticed. However, a significant difference between the blocks was observed. This may be due to the individual variation, as reported earlier. Therefore, the supplementation of cobalt at three different levels has not shown any effect on the solids not fat content of the milk of cows. A perusal of the table and the graph indicates that the animals of group I (18 mg) recorded higher values for the solids-not fat content of their milk upto four and half months, as compared to those of the other groups. No known references regarding this component of milk and the dietary cobalt supplementation are available at present.

(5) Total ash:

The average ash contents of the milk of cows of control and experimental groups with standard error have been presented in Table 71. The group average values for the milk of cows of control and experimental groups I, II and III were 0.770±0.003,

0.787 $\pm$ 0.004, 0.76 $\pm$ 0.003 and 0.770 $\pm$ 0.005 percent, respectively. The average value^{reported} for this constituent of milk by Overman (1939, 1945) and Armstrong (1939) as 0.7 $\pm$ 0.004 percent, for the milk of Jersey cows is slightly lower than those reported in this study. Davis^e (1939) and Hance (1932) reported 0.75 percent as an average value for the ash content of the milk of Jersey cows. This is pretty close to the values observed in the present investigation. Ray Sarkar (1943) and Sukhatme et al (1948) reported similar values for cows' milk. However, the value reported by Ananta-Krishnan et al (1943) was slightly lower (0.71 percent) than those observed in this experiment.

The total ash content values of milk of animals of control and experimental groups have been shown in Fig.10. It is evident from the graph that the animals of group I (16 mg) reported the highest values, almost over the entire experimental period. A gradual increase of this constituent in the milk of the animals of all the groups was noticed after one and half months of lactation period.

The statistical analysis of the data (Table 7B and 7BA) revealed a highly significant difference between the blocks, treatments and intervals and due to interaction between the treatments and intervals, too. On comparing the mean difference with critical difference at 5 percent level, it is observed that the treatment of group I (16 mg) was found to be significantly different and superior over all the other treatments. The treatments of control and group III were similar, but these were superior to that of group II. This shows that supple-

Table 71. Average Values of Total Ash Content of Milk with S.E.

Fortnights	Groups			
	Control	Experimental I	Experimental II	Experimental III
1	0.760±0.022	0.765±0.020	0.748±0.019	0.746±0.021
2	0.755±0.019	0.767±0.015	0.739±0.013	0.739±0.031
3	0.753±0.020	0.770±0.030	0.737±0.014	0.735±0.028
4	0.769±0.020	0.775±0.011	0.760±0.009	0.750±0.021
5	0.771±0.016	0.774±0.014	0.766±0.015	0.753±0.015
6	0.774±0.008	0.778±0.007	0.754±0.016	0.765±0.021
7	0.770±0.011	0.787±0.011	0.757±0.014	0.772±0.015
8	0.772±0.009	0.784±0.017	0.765±0.013	0.777±0.009
9	0.772±0.007	0.780±0.021	0.761±0.009	0.765±0.018
10	0.768±0.007	0.782±0.014	0.764±0.007	0.789±0.006
11	0.774±0.014	0.791±0.010	0.766±0.011	0.783±0.006
12	0.769±0.013	0.806±0.009	0.773±0.009	0.779±0.016
13	0.780±0.009	0.815±0.012	0.776±0.008	0.785±0.011
14	0.782±0.009	0.815±0.008	0.787±0.006	0.799±0.010
15	0.774±0.010	0.819±0.008	0.783±0.007	0.792±0.005
Group Av.	0.770±0.003	0.787±0.004	0.762±0.003	0.770±0.005

Table 72. Analysis of Variance for Milk Ash Percentages.

Source of Variance	Degrees of freedom	Mean Sum of Square	Variance Ratio
Between Blocks	4	0.008525	36.393**
Between Treatments	3	0.007967	33.616**
Between Intervals	14	0.004586	19.350**
Interaction	42	0.004126	17.410**
Error	235	0.000237	-

** Significant at 1 percent level of probability.

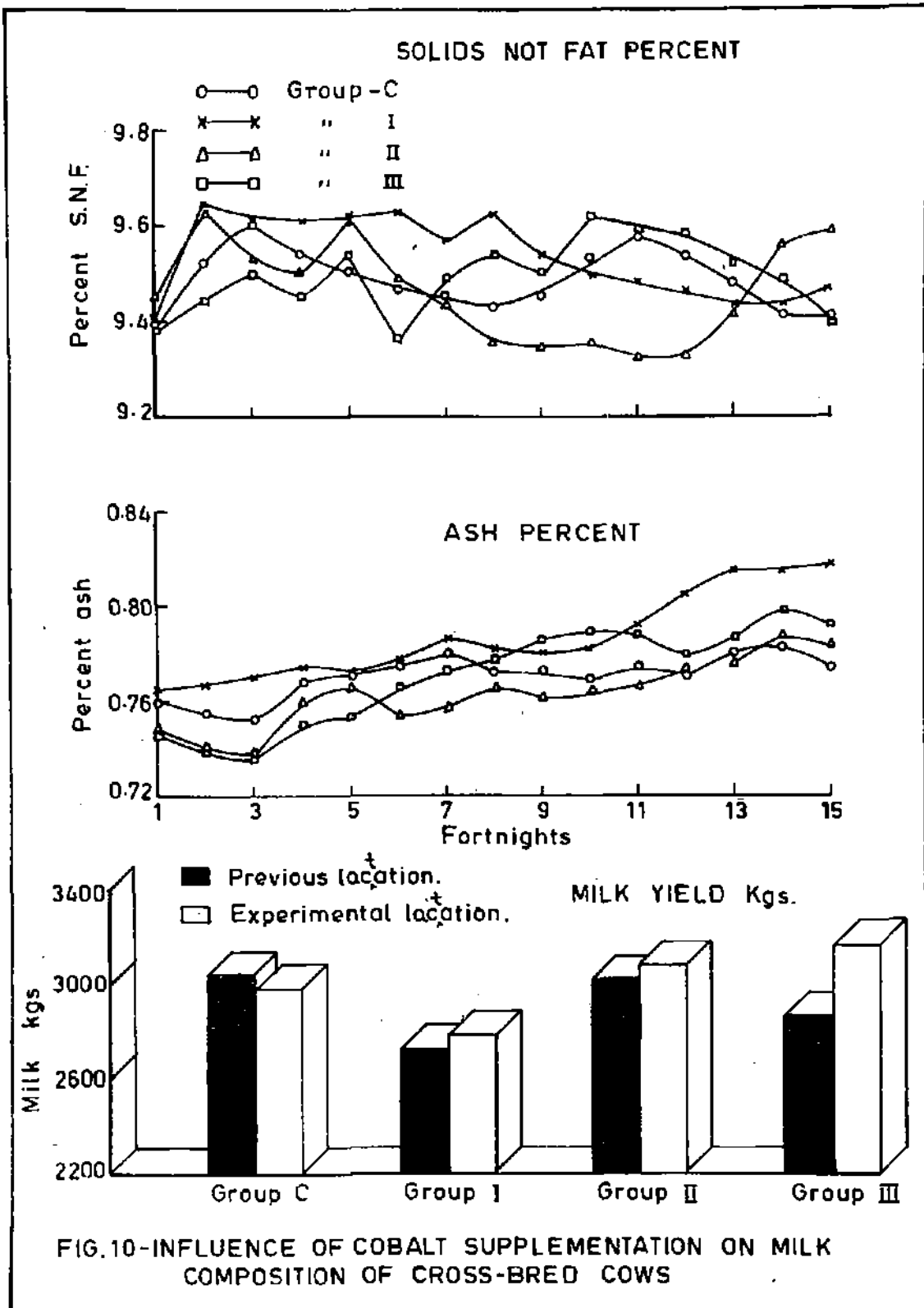
Table 72A. Comparison of Means of Ash Content of Milk.

Critical Difference at 5% level	Group Average Values			
	II	Control	III	I
0.004927	0.762	0.770	0.770	0.787

Note: Mean values under bar do not differ significantly.

mentation of cobalt at the rate of 15 mg of cobalt chloride per day per animal has a beneficial effect on the total ash content of the milk. The low ash content of the milk of animals of group II was well above the normal reported values for this constituent of milk. The low values observed may be due to chance factor. However, after six months of lactation period, the ash content of milk of group II animals was higher than that of control group animals. Highly significant difference between the treatments indicated that the cobalt might have influenced the secretion of some major minerals in the milk, with a positive correlation. No known reference are, however, available on this aspect of study.

The significant difference between the blocks, as explained earlier, may be due to the variation in the age and lactation number of the animals of different blocks. A normal ascending trend of total ash content of milk due to the increasing chloride content of milk explains the significant difference between the intervals (Sharp and Struble, 1935, and Sukhatme et al, 1948).



III. Influence of Cobalt on Digestibility of nutrients and Balances of Minerals:

Cobalt is a growth promoter for the rumen micro-organisms, besides being an integral part of vitamin B₁₂, synthesised by the rumen micro-organisms. Call et al (1949) reported that both the total concentration and the principle types of the rumen micro-flora change according to the cobalt status of the feeds and fodders fed to the ruminants. This in turn influences the entire digestive and metabolic processes in the animal. A study of the influence of dietary supplementation of cobalt fed over and above the amount available through feeds and fodders to the animals on the digestibility of nutrients and the balances of minerals was, therefore, undertaken. Hence, a metabolism trial was conducted at the end of the six and half months of experimental period.

The percentages chemical composition of feeds and fodders fed to the animals during the metabolism trial is given in Table 73. The average body weights, dry matter and water intakes of the animals per day during the metabolism trial are presented in Table 74. Table 77 contains the T.D.N. ingested per 100 kg body weight and per 100 gm dry matter intake by the animals. Intake of different nutrients on dry matter basis through feed and fodders by the animals per day during the digestion trial and their digestibility co-efficients are given in Table 75 and 76. The average digestible crude protein, ether extractives total carbohydrates and total digestible nutrients per 100 gm dry matter taken by the animals of control and experimental

groups are incorporated in Table 78, while the group average of digestibility coefficients for different nutrients have been assigned to Table 79. In Tables 80 to 86, intake, outgo and balances of nitrogen and minerals are presented for individual animal along with group averages.

It will be seen from the tables that the animals received 6.36 to 6.26 mg of cobalt from the feeds and fodders per day. This works out to an average of 5.932 mg (0.616 ppm of dry matter of ration) of cobalt per day. In the trial period on an average, control group animals received 6.077 mg of cobalt per day, while those of supplemented groups received 9.954, 11.994 and 13.602 mg of cobalt, through feed, fodder and supplementation of 4.07, 6.10 and 8.13 mg of cobalt as cobalt chloride for the animals of experimental groups I, II and III, respectively. This works out to 0.656, 1.010, 1.231 and 1.481 ppm of cobalt on dry matter basis for the ration of the cows of control and experimental groups I, II and III respectively.

(A) Intakes and Digestibility of Nutrients:

(a) Chemical composition of feed and fodders:

The chemical composition of feed, fodders and water offered to the animals is presented in Table 73. On examination, the chemical composition of concentrate mixture revealed that its crude protein percentage (25.08) was well above the minimum level (20.0%) and crude fibre (6.85 %) content was below the permissible level (13.0%), specified by the Indian Standard Institution (1968) for the compounded concentrate cattle feed. The crude protein percentage of guinea grass (14.35%) was

nearer to its maximum value and that of ragi straw (8.58 %) was close to the average value reported by Sen and Ray (1971). The crude fibre contents of guinea grass (34.99 %) and ragi straw (32.49%) were found slightly below the average values reported by Sen and Ray (1971). This indicates that the quality of feed and fodders given to the animals were quite good. The mineral matter contents were also found well within the range reported by Sen and Ray (1971). The iron, copper and cobalt contents of feeds, fodders and water were also found normal as compared with those reported by other workers (Singh, 1948; Sahai and Keher, 1961; Reddy and Mehta, 1961; Joshi, 1964; Dube, 1964). The values for these minerals in guinea grass were found to be higher than those reported by Patel *et al* (1966), while they were well within the range reported by Sawhney *et al* (1972, 1973).

(b) Dry matter and T.D.N. ingestion:

The average feed consumption per 100 kg body weight of the animals of control and experimental groups I, II and III were 2.757, 2.910, 2.786 and 2.824 kg, respectively (Table 74). It is seen that the average dry matter intake per 100 kg body weight increased in all the supplemented groups. The highest feed consumption was recorded by the animals of group I (16 mg), followed in order by those of group III (32 mg) and group II (24 mg). The dry matter consumption by the animals of control group was slightly less.

Singh (1948) reported the loss of appetite, low dry matter and T.D.N. consumption by the Kumaoni bullocks, when kept on basal ration low in cobalt. When the minimum level of cobalt

was supplemented, he observed an increase in the dry matter intakes as also the T.D.N. Katiyar (1963), Singh (1964), Singh *et al* (1970) observed the increased feed intake in calves after cobalt supplementation. Pal (1971) and Ram (1971) reported similar findings with sheep. They reported low level of cobalt supplementation (0.25 mg of cobalt sulphate per kg body weight) increased the dry matter intake, while the higher level (0.75 mg) had slight depressing effect as compared to the low level of supplementation. Similar findings were recorded in the present investigation.

Table 77 and 78 shows the total T.D.N. ingestion in g per day per 100 kg body weight and per 100 gm of dry matter intake with the corresponding group averages. The ingestion of T.D.N. per day per 100 kg body weight were 1793.95, 2024.95, 1919.88 and 1683.88 gm for the animals of control and experimental groups I, II and III, respectively. The ingestion of T.D.N. per 100 kg body weight was found highest by the animals of group I (16 mg); less by those of group II (24 mg), but higher than those of control group. The lowest T.D.N. ingestion was by the animals of group III (32 mg). The T.D.N. per 100 g of dry matter intake were 65.24, 69.15, 68.91 and 67.99 gm per cent respectively, for the control and experimental groups I, II and III. An increased level of cobalt supplementation decreased the T.D.N. per 100 gm per dry matter intake. Therefore, 16.0 mg of cobalt chloride (4.1 mg Co) supplementation was found to be suitable.

Pal (1971) and Ram (1971) reported similar observations for the T.D.N. intakes in sheep with high and low levels of

Table 74. Average body weight, Dry matter and Water intake per day by the animal.

Exptl. group	Animal No.	Average body wt. in kg.	Average DM intake kg.		Group average per 100 kg. body wt.	Average water intake lit.		Group average per 100 kg. body wt.
			per day	per 100 kg. body wt.		per day	per 100 kg. body wt.	
Control	88	325.5	9.011	2.758	2.757	20.93	6.436	7.010
	936	347.0	9.532	2.747		26.33	7.59	
Group I	89	283.0	9.853	3.482	2.910	24.38	3.62	7.37
	933	404.0	9.440	2.337		24.74	6.12	
Group II	87	340.0	9.284	2.726	2.785	23.53	6.92	7.67
	956	356.0	10.287	2.846		29.93	8.41	
Group III	96	328.6	9.145	2.784	2.824	19.59	5.96	7.40
	940	422.0	9.220	2.863		37.29	8.83	

cobalt supplementation, while Maheshwari (1964) and Gupta (1968) did not observe any significant difference due to cobalt supplementation in the case of sheep and calves respectively. The higher levels appear to have a depressing effect on the dry matter as well as T.D.N. ingestions.

(c) Water intake:

Total water consumption per day and the water intake of individual animal per 100 kg body weight with the group averages are shown in Table 74. It is observed that water consumption is higher by the animals of supplemented groups. The highest water intake of 7.67 litres per 100 kg body weight per day was observed in the animals of group II (24 mg), while lowest of 7.01 litres per 100 kg body weight per day was found in the animals of control group. Amongst the supplemented groups, the animals of group I (16 mg) recorded the least intake of water (7.37 litres per 100 kg body weight per day). Maheshwari (1964) did not observe any effect on water consumption by cobalt supplementation in sheep. The findings of Singh (1948) are in agreement with those of the present findings. Keener and Percival (1950) found double the quantity of water intake in cobalt supplemented animals. Kapoor (1968) reported similar findings as observed in the present study with the copper plus cobalt supplementation in Haryana cattle.

(d) Digestibility of dry matter and organic matter:

The total dry matter that was ingested, excreted in faeces, total digested and the digestibility co-efficients for dry matter and organic matter are presented in Tables 75 and 76.

**Table 75. Intake of Nutrients in Feed and Fodders by the animals in gm per day on D.W.basis.
(Cobalt supplementation)**

Animal No.	Feed	Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total carbohydrates
Control Group								
88	Conc. mix.	2794.0	2638.853	700.819	169.037	191.472	1577.325	1768.798
	Ragi straw	4023.0	3679.154	143.826	59.640	1307.113	2168.638	3475.751
	Guinea grass	2194.0	1922.822	312.711	63.604	767.658	778.848	1546.507
	Total	9011.0	8240.630	1157.392	292.182	2266.244	4524.811	6791.056
936	Conc. mix.	3992.0	3770.045	1001.313	241.516	273.571	2253.644	2627.215
	Ragi straw	3368.0	3080.137	120.340	49.846	1094.297	1815.554	2909.851
	Guinea grass	2172.0	1903.641	309.576	62.966	759.961	771.038	1530.999
	Total	9532.0	8753.723	1431.328	354.329	2127.830	4840.236	6968.066
Experimental Group I								
89	Conc. mix.	4417.0	4171.416	1107.916	267.228	302.697	2493.573	2796.270
	Ragi straw	3267.0	2978.624	116.470	48.204	1058.232	1755.713	2913.950
	Guinea grass	2179.0	1909.676	319.573	63.169	768.410	773.523	1535.940
	Total	9863.0	9057.716	1534.959	378.601	2123.339	5022.816	7146.160
933	Conc. mix	3876.0	3660.494	972.217	234.498	265.622	2188.157	2453.779
	Ragi straw	3374.0	3085.624	120.664	49.936	1096.246	1918.788	2915.036
	Guinea grass	2190.0	1919.316	312.141	63.488	766.259	777.428	1543.687
	Total	9440.0	8665.436	1405.012	347.921	2128.128	4787.373	6912.501

continued ...

Animal No.	Feed	Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total carbohydrates
<u>Experimental Group II</u>								
87	Conc. mix.	4056.0	3830.486	1017.366	245.388	277.958	2289.774	2567.732
	Ragi straw	3033.0	2773.769	108.460	44.888	985.452	1634.939	2820.421
	Guinea grass	2195.0	1923.693	312.854	63.633	768.008	779.230	1647.212
	Total	9284.0	8527.954	1438.680	353.909	2031.418	4703.946	5735.364
88	Conc. mix.	4777.0	4511.399	1198.215	289.008	327.358	2696.807	3024.175
	Ragi straw	3475.0	3177.992	124.266	61.430	1129.032	1873.233	3002.296
	Guinea grass	2136.0	1871.990	304.444	61.923	747.365	758.259	1506.624
	Total	10387.0	9561.381	1626.925	402.361	2203.795	5328.300	7532.095
<u>Experimental Group III</u>								
96	Conc. mix.	3876.0	3660.494	972.217	234.498	265.622	2188.157	2453.779
	Ragi straw	3141.0	2872.639	112.322	46.487	1020.542	1693.186	2713.720
	Guinea grass	2129.0	1865.866	303.446	62.935	744.916	755.774	1510.690
	Total	9145.0	8398.999	1387.965	343.980	2031.080	4637.118	5668.190
940	Conc. mix.	4236.0	4000.478	1052.516	256.273	290.293	2391.391	2681.684
	Ragi straw	3811.0	2570.744	100.521	41.603	913.322	1515.292	2428.620
	Guinea grass	2173.0	1904.417	309.718	61.720	760.311	771.393	1531.704
	Total	9220.0	8475.639	1472.755	359.600	1963.926	4678.082	5642.008

It is seen from the tables that the digestibility coefficients of dry matter in the case of animals of control and experimental groups I, II and III, respectively, were 65.09, 68.51, 68.18 and 68.83 percent, and that of organic matter were 67.38, 71.04, 70.59 and 69.51 percent (Table 79). The digestibility coefficients were least in the case of control group, moderate in group III (32 mg), slightly more in group II (24 mg) and the highest in group I (16 mg). This shows that the digestibility of dry matter and organic matter was higher in the supplemented groups. It is also noticed that the same reduced with the increased level of cobalt supplementation. The level of 16 mg per day of cobalt chloride supplementation was found optimum which gave the better dry matter assimilation.

Singh (1948) showed that when the animals were fed on cobalt deficient diet, the digestibility of whole ration was adversely affected. The digestibility of the ration improved greatly by the supplementation of lower levels of cobalt through the feed. Stewart (1953) attributed it to the failure of microbial digestion and slowness of passage of the ingesta from the rumen.

Ganguli (1963), Singh (1964), Maheshwari (1964), Gupta (1968) and Singh *et al* (1970) reported no significant difference due to cobalt supplementation in dry matter digestibility in Haryana animals.

Anaokar (1965) found higher digestibility coefficients with 2 mg of cobalt supplementation in sheep but this was not

Table 76. Digestibility Coefficients of the different Constituents of Feed (Cobalt Supplementation)

Animal No.		Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total carbohydrates
<u>Control Group</u>								
88	Intake	9011.000	8240.629	1157.392	292.181	2266.244	4524.811	6791.056
	Outgo	3363.000	2891.373	350.771	59.592	935.450	1515.637	2481.087
	Digested	5648.000	5349.256	806.621	232.589	1300.794	3009.174	4309.969
	% digesti- bility	62.679	64.913	69.698	79.604	57.398	66.504	63.465
938	Intake	9532.000	8753.723	1431.328	354.329	2127.930	4840.236	6968.066
	Outgo	3007.000	2638.489	351.429	68.506	839.101	1379.466	2218.567
	Digested	6435.000	6115.233	1079.899	285.823	1288.729	3460.770	4749.499
	% digesti- bility	67.509	69.859	75.447	80.636	60.565	71.500	68.168
<u>Experimental Group I</u>								
89	Intake?	9853.000	9059.715	1534.959	378.601	2123.339	5022.815	7146.160
	Outgo	2847.000	2412.975	362.292	65.111	736.078	1253.506	1985.583
	Digested	7006.000	6646.740	1172.667	313.490	1391.262	3769.309	5160.577
	% digesti- bility	71.105	73.366	76.397	82.802	65.522	75.044	72.215
933	Intake	9440.000	8665.435	1406.012	347.921	2128.128	4784.373	6912.501
	Outgo	3218.000	2710.650	348.384	61.689	868.924	1412.348	2300.880
	Digested	6222.000	5955.064	1056.628	286.232	1259.203	3372.025	4611.621
	% digesti- bility	71.122	73.557	76.080	83.220	65.773	75.420	72.511

continued ...

Table 76. cont.

Animal No.		Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total carbohydrates
<u>Experimental Group II</u>								
87	Intake	9284.000	8527.954	1438.690	353.910	2031.418	4703.946	6735.365
	Outgo	2681.000	2255.016	344.133	59.384	695.291	1156.208	1951.499
	Digested	6603.000	6272.938	1094.137	294.525	1336.128	3547.738	4883.866
	% digestibility	71.122	73.557	76.090	83.820	65.773	75.420	72.511
956	Intake	10387.000	9561.381	1628.926	402.361	2203.795	5328.300	7532.095
	Outgo	3611.000	3095.746	433.810	66.225	1019.691	1575.129	2596.020
	Digested	6776.000	6465.634	1193.416	336.135	1183.904	3752.170	4936.075
	% digestibility	65.235	67.622	73.350	83.541	53.721	70.420	65.533
<u>Experimental Group III</u>								
98	Intake	9145.000	8398.339	1387.936	345.980	2031.090	4637.118	6668.199
	Outgo	3146.000	2702.477	375.613	58.882	931.913	1354.101	2288.214
	Digested	5999.000	5695.862	1012.373	286.118	1099.167	2383.017	4400.184
	% digestibility	65.599	67.823	72.938	82.389	54.117	70.800	65.988
940	Intake	9220.000	8475.639	1472.756	359.600	1963.926	4675.082	6642.008
	Outgo	2944.000	2424.413	323.846	51.962	800.797	1247.814	2048.612
	Digested	6276.000	6051.226	1148.909	307.638	1163.129	3430.268	4593.396
	% digestibility	68.069	71.396	78.011	85.550	59.225	73.326	69.157

statistically significant. Kapoor (1968), and Kapoor and Mahadevan (1970b) found depression in dry matter digestibility after copper plus cobalt supplementation in Haryana cattle.

Pal (1971) and Ram (1971) reported similar observations with sheep i.e., percent digestibility of dry matter increased at low level of cobalt supplementation and decreased at higher levels of supplementation.

Similar observations are recorded regarding the digestibility of organic matter. Therefore, 16 mg of cobalt sulphate is found to be ideal level of supplementation to the normal healthy dairy animals to maintain the rumen flora in flourishing conditions (Marston, 1962).

(c) Digestibility of crude protein:

Table 76 records the data of crude protein intake through feeds and fodders, and outgo through faeces along with the balances and the digestibilities. The average values of protein digestibilities are presented in Table 79. The digestibilities of crude protein observed in the control and experimental groups I, II and III, were 72.57, 75.80, 74.72 and 75.47 percent respectively. It is seen from the values that the digestibility of crude protein improved due to cobalt supplementation. The digestibility of protein was better in the case of animals receiving 16 mg cobalt chloride supplement per day and also it was found that higher the level of supplementation, lower was the digestibility of crude protein.

Singh (1948) reported that the crude protein digestibility was adversely affected by cobalt deficient diet and improved

immediately after cobalt supplementation. Ganguli (1963) reported slight depressing effect on the digestibility of crude protein as a result of cobalt supplementation. On the other hand, Singh (1964) reported higher average value of crude protein digestibility with 1 mg of cobalt supplementation in calves. The difference was, however, not statistically significant. Similar increase was noticed by Ananekar (1965) with 2 mg cobalt supplementation in sheep. Maheshwari (1964) and Gupta (1968) observed no effect of cobalt supplementation of crude protein digestibility in sheep and calves respectively. Contrary to this, Singh (1964) and Ananekar (1965) reported slight depressing effect on the digestibility of crude protein due to low level (0.5 mg of cobalt) of cobalt supplementation in urea fed sheep. This may be due to the N.P.N. compound incorporated in the feed. Supplementation of cobalt has shown depressing effect on N.P.N. utilization in *in vitro* studies (McNaught *et al.*, 1960; and Johnson and Bentley, 1967).

Boganov (1958) observed an increase in the digestibility of crude protein on supplementation with 0.2 mg of cobalt chloride per kg body weight to calves. Modjanova (1959) reported similar rise in the digestibility of crude protein in sheep after supplementing 2 mg of cobalt per day per head. Khorkhin (1959) confirmed it by reporting 12 percent rise in the protein digestibility after cobalt chloride supplementation in ruminants. Sommer (1965) too observed increased (70.28%) digestibility of crude protein after supplementation of cobalt sulphate over unsupplemented group (50.68%) in young spotted bulls.

Kapoor (1968) and Kapoor and Mahadevan (1970b) reported lowered digestibility of crude protein on supplementation of copper with cobalt in Haryana cattle. The difference was not statistically significant. Contrary to this, Pal (1971) Ram (1971) reported high level (0.75 mg of cobalt sulphate/kg body weight) recorded high digestibility in sheep and low level has shown improved crude protein digestibility in sheep.

The digestible crude protein per 100 gm dry matter intake also shows similar trend (Table 78). Higher values were recorded in the supplemented groups over that of control group. An increasing trend was observed with the increasing level of supplementation.

The results of the present investigation increased that supplementation at the rate of 16 mg cobalt chloride per day per head recorded beneficial effect on the digestibility of crude protein.

(f) Digestibility of other extractives:

The other extract digestibility data, the total intake through feed and fodders and outgo through faeces along with the retentions are presented in Tables 76 and 79. The average digestibility values of other extractives were 80.13, 82.54, 83.38 and 84.22 percent, respectively, for the control and experimental groups I, II and III. It is seen that the supplementation of dietary cobalt resulted in the increased other extractives digestibility. Becker and Smith (1961) observed that on cobalt supplementation to deficient lambs, there was a significant increase in the digestibility of other extractives.

Singh (1964) and Singh *et al* (1970) reported similar findings, when cobalt was supplemented to calves fed urea. They reported an increased value showing the tendency towards significant difference. Similar were the findings of Becker *et al* (1949) in lambs.

Ganguli (1963) and Annakar (1965) reported significant increase in the digestibility of ether extractives as a result of cobalt supplementation to calves and sheep on high and low energy and urea ration, respectively. Annakar (1965) reported an increase from 54.3 to 73.2 percent on 0.5 mg cobalt supplementation. However, with 2 mg cobalt supplement along with urea, a slightly lower values were recorded for the digestibility of ether extractives. These observations are in agreement with those of Boganov (1958). Khorkhin (1959) also supported these findings by showing the increase of 6.6% in the digestibility of ether extractives by cobalt chloride supplementation.

Maheshwari (1964) and Gupta (1968) reported no effect on the digestibility of ether extractives after supplementation of cobalt and trace mineral mixture with cobalt in sheep and calves respectively. Kapoor (1968) and Kapoor and Mahadevan (1970b) on the other hand reported depressed digestibility of ether extractives, when cobalt was supplemented along with copper to Haryana cattle. But the difference was not statistically significant. Pal (1971) and Ram (1971) reported increased digestibility of ether extractives in sheep after supplementing the high and low levels of cobalt, respectively.

From the results, it can be summarized that supplementation at the rate of 16 mg of cobalt chloride per day per head is adequate to get the optimum results, as higher levels depress the digestibility of other nutrients (Table 78).

(g) Digestibility of crude fibre:

The digestibility of crude fibre by individual animal are recorded in Table 78. The group averages are presented in Table 79. The group averages for the digestibility coefficient of crude fibre for the control and experimental groups I, II and III, respectively, were 58.98, 62.35, 59.75 and 56.67 percent. These values show that the digestibility of crude fibre by the animals of supplemented groups receiving 16 mg/and 24 mg (Group I) cobalt chloride per day per head was found to be higher than those of group III (32 mg) and control group. The highest digestibility was recorded by the animals of group I (16 mg), followed in order by those of group II (24 mg), control group and group III (32 mg). Therefore, it indicates that increased digestibility can be attributed to cobalt supplementation at low level i.e., 4.07 to 6.10 mg per day per head.

Similar results were reported by Singh (1948) after supplementing optimum levels cobalt to Kumaoni bullocks fed washed wheat bhoosa and rape cake (Brassica campestris) diet. Gall et al (1949) recorded decrease in the number and type of fibre digesting micro-organisms in the rumen, whereas Becker (1949) reported that cobalt deficiency did not affect the cellulose splitting organisms markedly. Williams (1949) observed temporary inhibition of the bacterial activity. On continued

feeding of cobalt supplement, the micro-flora of the rumen gets adjusted to the changed environment. The crude fibre digestibility in the case of ruminants is completely dependant upon the rumen micro-organisms. Hence, the change in the rumen flora due to cobalt deficiency or excess as shown by Marston (1952) is important in supplementing this element.

In *in vitro* fermentation studies, Johnson and Bentley (1957) could not observe the increase in the rate of cellulose digestion by adding cobalt. Similar observations were recorded by Hall (1955), Salisbury *et al* (1956) and Hubbert *et al* (1958). Contrary to these, Boganov (1958) observed improved digestibility of crude fibre on the addition of 0.2 mg of cobalt chloride per Kg body weight of calves. Modjanov (1959) reported similar findings on the supplementation of 2 mg of cobalt chloride daily in sheep. Outer (1962) reported that daily supplementation of 0.2 mg of cobalt per 100 lb body weight was favourable in rumen micro-flora favouring digestion of cellulose. This was in conformity with the earlier findings of 8% rise in cellulose digestibility after cobalt chloride supplementation by Khorkhin (1959).

Ganguli (1963) reported that cobalt supplementation had a depressing effect on the digestibility of crude fibre. Andres and Church (1969) with *in vitro* studies with washed suspension of rumen micro-organisms and 7 ppm cobalt addition to the medium, supported his findings. Maheshwari (1964) and Gupta (1968) reported no effect of cobalt supplementation on the digestibility of crude fibre in sheep and calves,

respectively. Gupta (1968) fed cobalt along with copper and iodine. Contrary to this, Andres and Church (1969) reported stimulating effect of 3 ppm of cobalt supplementation in *in vitro* studies.

Singh (1964) and Singh *et al* (1970) supplemented two levels of cobalt along with urea in the ration of calves. They observed a depressing effect on crude fibre digestibility in first trial, which was significant at one percent level. No significant difference was noticed in the second trial. Anacker (1965) similarly supplemented two levels of cobalt in the ration of sheep along with urea. Contrary to the findings of Singh, he reported equal digestibility in first trial and increased digestibility in the second trial over the control group values. The difference was, however, not statistically significant. Later, Kapoor (1968) and Kapoor and Mahadevan (1970b) supported these findings with Mariana cattle on copper plus cobalt supplementation. They observed high crude fibre digestibility as a result of cobalt supplementation.

Findings of Pal (1971) and Ham (1971) support the present ones obtained in this investigation in toto. They supplemented ration of sheep at three levels of cobalt.

Therefore, based on the observations recorded in the present investigation, it can be concluded that supplementation at the rate of 16 mg of cobalt chloride (4.07 mg cobalt) per day per animal is the optimum level that can be recommended for cross-bred lactating cows.

Table 77. Ingestion of Total Digestible Nutrients in gm. per day.
(Cobalt Supplementation)

Animal No.	Body wt. in Kg.	T.D.N. intake	T.D.N. intake per 100 kg. body wt.	Group average	Dry matter intake	T.D.N. intake per 100 gm. dry matter	Group average
<u>Control Group</u>							
88	325.5	5639.896	1732.687		9011.0	62.599	
936	347.0	6472.326	1865.222	1798.954	9532.0	67.901	65.245
<u>Experimental Group I.</u>							
89	283.0	7038.788	2487.204		9853.0	71.438	
933	404.0	6312.528	1562.507	2024.886	9440.0	66.870	69.154
<u>Experimental Group II.</u>							
87	340.0	6641.031	1953.244		9284.0	71.532	
933	365.0	6886.750	1886.507	1919.876	10387.0	66.232	68.912
<u>Experimental Group III.</u>							
96	328.5	6064.081	1842.947		9145.0	66.201	
940	422.0	6434.546	1524.773	1683.860	9220.0	69.789	67.996

Experiment

Table 78. D.C.P., D.E.M., D.T.C., & T.D.N. per 100 gm dry matter intake (Cobalt Supplementation)

Animal No.	Digestible crude protein	Digestible ether extract	Digestible total carbohydrates	Total Digestible Nutrients
Control Group				
88	8.952	5.807	47.830	62.589
936	11.329	6.745	49.827	67.901
Average	10.140	6.276	48.820	65.245
Experimental Group I.				
89	11.902	7.150	52.375	71.438
933	11.193	6.822	48.855	66.870
Average	11.648	6.991	50.616	69.154
Experimental Group II.				
87	11.790	7.137	52.605	71.532
956	11.489	7.281	47.522	66.292
Average	11.639	7.209	50.063	68.912
Experimental Group III.				
96	11.070	7.015	48.115	66.201
940	12.461	7.508	49.820	69.789
Average	11.765	7.261	48.968	67.925

Table 79. Digestibility Co-efficients of Ration, Group Averages. (Cobalt Supplementation)

Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	N.P.E.	Total carbohydrates
Control Group						
65.094	67.385	72.572	80.135	58.981	69.002	65.467
Experimental Group I.						
68.508	71.044	75.801	82.535	62.346	72.762	69.467
Experimental Group II.						
68.178	70.589	74.717	83.380	59.747	72.920	69.022
Experimental group III.						
66.834	69.609	75.474	84.219	56.671	72.053	69.572

(h) Digestibility of nitrogen-free extract and total carbohydrates:

The data of the digestibilities of nitrogen-free extract and total carbohydrates are presented in Table 76. The average digestibilities of nitrogen-free extract and total carbohydrates by the animals of control and experimental groups I, II and III were 69.00, 72.75, 72.92 and 72.06; and 65.47, 69.47, 69.02 and 67.57 percent respectively. All the supplemented groups recorded higher digestibility of both N.F.E. and total carbohydrates as compared to those of control group. The highest digestibility was recorded by the group II (24 mg) for N.F.E. and by group I (16 mg) for total carbohydrates while the lowest figures are recorded by the control group. The high level (32 mg) of cobalt supplementation was found to have a depressing effect on the digestibilities of these two components of ration. However, these were still higher than that of control group.

Becker *et al* (1949) observed that the N.F.E. fraction of the ration were digested more efficiently by the lambs which were fed cobalt salts. Ganguli (1963), Maheshwari (1964) and Gupta (1968) supplemented cobalt and cobalt plus copper and iodine to the growing heifers, calves and sheep, respectively. They reported no influence on these supplements on the digestibility of either N.F.E. or Total carbohydrates.

Boganov (1958) reported that in younger bulls, the supplementation of cobalt chloride at the level of 0.2 mg per Kg body

weight reduced the digestibility of N.F.E. The level of cobalt used in these experiments was much higher than that used in the present studies. A similar low figure was observed at 8.13 mg of cobalt per day per animal in the present study also.

Singh (1964) and Singh *et al* (1970) reported the higher digestibility of N.F.E. in 1 mg cobalt supplemented group in both the trials, while 0.5 mg level showed little less digestibility in the first trial, but not in the second metabolism trial. Anacker (1965) reported no significant difference either due to 0.5 or 2 mg cobalt supplementation levels in the case of sheep fed urea.

Kapoor (1968) and Kapoor and Mahadevan (1970b) found little depressed digestibility of N.F.E., when copper and cobalt were fed together to Mariana cattle. Contrary to this, Pal (1971) reported an upward trend in the digestibility of N.F.E., as a result of cobalt supplementation. Similar was the trend in the case of digestibility of Total carbohydrates. The figures reported in Tables 76, 78 and 79 show that the digestibilities in the case of supplemented groups are higher than those of control group. Amongst the supplemented groups, low level (16 mg) has shown the highest digestibility for Total carbohydrates.

The N.F.E. and Total carbohydrates being the calculated values from the other determinations, a similar effect of cobalt supplementation is anticipated. Hence, it can be concluded that lower level (16 mg) of cobalt supplementation to these animals is adequate for the better utilization of these nutrients.

(B) Balances of Nitrogen and Minerals:

(a) Balance of Nitrogen:

Table 80 furnishes the total nitrogen intake through different sources, outgo through faeces, urine and milk and the retention for the individual animals as well as for the group averages. The average retention of nitrogen for the control and experimental groups I, II and III were 35.993, 59.137, 58.201 and 59.155 gm, per day per animal, respectively. The supplemented groups showed higher retention of nitrogen as compared to those of the control group. Group I (16 mg) and group III (33 mg) showed almost equal retention which are not statistically different from that of group II (24 mg). However, an appreciable low figure was recorded by the control group, indicating the favourable effect of cobalt supplementation on the nitrogen retention. The higher retention in the supplemented groups was found to be due to the higher intake of total nitrogen alongwith the other nutrients.

Singh (1948) reported a fall in the nitrogen retention of Kusaoni bullocks on semi-purified diet with respect to cobalt and found that it increased appreciably as a result of cobalt supplementation. Poganov (1958) on feeding 20 mg cobalt chloride per 100 kg body weight observed increased absorption and utilization of nitrogen. Similar findings have been reported by Modjanov (1959) in sheep on supplementation of 2 mg of cobalt chloride daily. This was supported by Khorkhin (1969) by showing 13.8% increase in the nitrogen utilization after cobalt chloride supplementation. Burke and Schwartz (1960)

observed an improved nitrogen retention after about 6 months of initiating the supplementation of 0.6 mg of cobalt to sheep on low cobalt diet and urea. They observed less retention in cobalt rich diet. Sommer (1965) reported 51.21 gm nitrogen retention in cobalt supplemented bulls against 30.19 gm in the unsupplemented group.

Singh (1964) and Singh *et al* (1970) observed a slightly higher retention of nitrogen in cobalt supplemented calves in the first trial. In the second trial, higher retention was noticed in 1 mg cobalt supplemented animals. Their findings are in support of the results of the present studies. Anzakar (1965) reported no beneficial effect of cobalt at 0.5 mg level in the case of sheep given 15% nitrogen in the form of urea. On the other hand when the level of supplementation was raised to 2 mg, there was an appreciable increase in the amount of nitrogen retention as compared to the control group. The nitrogen retention was significantly higher (at 5 % level) in 2 mg supplementation group as against that of 0.5 mg supplementation group. His observations are also in support of the present findings.

Kapoor (1968) and Kapoor and Mahadevan (1970b) reported similar observations in Haryana cattle, with copper plus cobalt supplementation. Pal (1971) also reported the positive effect of cobalt supplementation by recording slight increase in the nitrogen retention by sheep fed two levels of cobalt supplement.

Table 80. Intake, Outgo and Balance of Nitrogen in g per day (Cobalt Supplementation)

Animal No.	Conc. mixture	Ragi straw	Guinea grass	Total Intake	Faeces	Urine	Milk	Total outgo	Balance	Group Average
Intake					Outgo					
Control Group										
88	112.130	23.020	50.034	185.274	56.113	72.678	29.141	157.932	27.342	35.993
926	160.208	13.272	49.533	229.013	56.228	87.856	40.284	184.368	44.645	
Experimental Group I.										
89	177.266	18.637	49.692	245.594	58.269	73.392	46.283	177.944	67.650	59.137
923	155.553	19.306	49.943	224.802	55.750	92.142	26.284	174.176	50.626	
Experimental Group II.										
87	162.777	17.255	50.067	230.139	55.072	81.249	39.284	175.605	54.534	58.201
966	191.712	19.867	48.712	260.291	69.370	85.535	48.569	198.474	61.817	
Experimental Group III.										
95	155.553	17.973	48.552	222.078	60.093	68.928	37.712	166.733	55.345	59.155
940	170.001	16.085	49.555	235.641	51.819	104.285	16.571	172.675	62.966	

Table 81. Intake, Outgo and Balance of Calcium in g per day (Cobalt Supplementation)

Animal No.	Conc. mixture	Ragi Straw	Guinea grass	Water	Total Intake	Faeces	Urine	Milk	Total Outgo	Balance	Group Average
88	6.901	32.381	16.580	0.994	56.856	54.212	0.750	6.251	61.213	-4.357	-3.028
926	9.860	27.109	16.438	1.251	54.658	46.621	1.607	9.106	56.334	-1.704	
Experimental Group I.											
89	10.910	26.216	16.467	1.158	54.751	43.987	0.455	11.876	56.318	-1.567	-1.151
923	9.574	27.157	16.550	1.033	54.314	46.021	1.178	5.849	55.048	-0.734	
Experimental Group II.											
87	10.018	24.412	16.538	1.118	52.136	41.134	0.701	9.643	51.478	+0.658	-1.982
956	11.799	27.970	16.142	1.422	57.333	49.152	1.732	11.071	61.955	-4.622	
Experimental Group III.											
95	9.574	26.282	16.089	0.930	51.875	46.990	0.643	8.483	55.116	-3.341	-0.195
940	10.463	22.626	16.421	1.770	51.280	44.899	0.317	3.214	48.430	+2.850	

The investigation carried out in this experiment revealed that supplementation at the rate of 16 mg of cobalt chloride per day per animal gives high retention of nitrogen. The higher doses of supplementation had no added advantage.

(2) Balance of calcium:

The total ingestion of calcium through feeds and ^{urine} fodders, excretion through faeces, and milk and also the retention for the individual animal as well as the group averages are given in Table 81. Except two animals, one each from groups II and I.I, all the rest of the animals recorded negative balance. The group averages were -3.028, -1.151, -1.932 and -0.195 gm per day per animal of control and experimental groups I, II and III, respectively. All the groups recorded negative balances, even though apparently the calcium intakes were adequate. The negative balances might be due to the fact that the cows were high yielders in lactation. However, the cobalt supplemented groups have recorded marginal negative balances as compared to the control group and also higher level of cobalt supplementation (32 mg) has shown better retention of calcium in the body amongst the supplemented groups.

Bogdanov (1958) observed that cobalt supplementation of the ration improved the calcium absorption and utilization in dairy calves. On the other hand, Anacker (1965) reported no significant difference in calcium retention as a result of cobalt supplementation in sheep, fed urea. While Kapoor (1968) and Kapoor and Mahadevan (1970b) reported depressed retention due to the copper plus cobalt supplementation in Mariana cattle.

However, this depressing effect was not statistically significant. Pal (1971) reported higher retention of calcium in sheep after cobalt supplementation, as compared to the animals of control group.

The present study also indicated that cobalt supplementation at three different levels, did not have any significant effect on the calcium retention in lactating cows.

(3) Balance of phosphorus:

The total intake, outgo and balances of phosphorus for individual animal as well as the group averages are presented in Table 82. All the animals, except one in control group, were in positive phosphorus balance. The average retention of phosphorus by the animals of control and experimental groups I, II and III were 0.079, 2.218, 1.669 and 0.779 gm per day per animal. All the group averages were positive. It seems from the results that the supplemented groups have shown on an average higher retention than those of control group. Highest retention was observed in group I (16 mg) and the lowest in control group. The higher level of supplementation of cobalt seems to have a depressing effect on the retention of phosphorus, rather ^{than} by the lower levels.

Boganov (1958) found that supplementation of cobalt at the rate of 20 mg per 100 kg body weight in calves improved the phosphorus absorption and utilization. On the other hand, Anaskar (1955) and Kapoor (1958) noticed no significant differences due to cobalt and cobalt plus copper supplementation of the ration of sheep fed urea and cattle respectively. Pal (1971)

Intake					Outgo				Balance	Group Average
Animal No.	Conc. mixture	Ragi straw	Guinea grass	Total Intake	Faeces	Urine	Milk	Total outgo		
Control Group										
88	21.240	4.474	5.735	31.449	27.407	0.145	4.619	32.171	-0.722	+0.079
938	30.347	3.745	5.678	39.770	31.994	0.217	6.580	39.891	+0.879	
Experimental Group I.										
89	33.578	3.621	5.696	42.895	32.542	0.124	8.271	40.937	+1.958	+2.218
933	29.465	3.752	5.725	38.942	31.960	0.179	4.326	36.465	+2.477	
Experimental Group II.										
87	30.834	3.373	5.737	39.944	30.113	0.598	6.394	37.596	+2.348	+1.669
966	36.315	3.884	5.583	45.762	36.331	0.172	8.269	44.772	+0.990	
Experimental Group III.										
96	29.465	3.493	5.565	38.523	31,992	0.133	6.358	38.483	+0.040	+0.779
940	32.202	3.126	5.680	41.008	37.097	0.165	2.229	39.491	+1.517	

Table 83. Intake, Outgo and Balance of Iron intake per day (Cobalt Supplementation)

Animal No.	Intake					Outgo				Balance	Group Average
	Conc. mixture	Ragi. Straw	Guinea grass	Water	Total Intake	Faeces	Urine	Milk	Total outgo		
Control Group											
88	783.62	1670.17	958.48	2.30	3419.57	2407.49	2.77	22.96	2433.22	986.35	1027.95
935	1126.77	1398.24	948.87	2.89	3476.77	2361.37	2.25	43.57	2407.19	1069.58	
Experimental Group I.											
89	1246.73	1352.16	961.92	2.68	3563.49	2515.33	2.89	18.37	2536.59	1016.90	1032.24
933	1094.02	1400.74	956.73	2.39	3453.88	2369.73	2.33	34.23	2406.29	1047.59	
Experimental Group II.											
87	1144.83	1259.37	958.91	2.59	3364.70	1995.02	1.86	19.62	2015.40	1349.30	1409.33
966	1348.24	1442.67	933.14	2.29	3727.44	2220.88	1.96	35.15	2257.99	1469.45	
Experimental Group III.											
96	1094.02	1204.01	930.08	2.15	3230.26	1828.37	3.40	83.50	1909.28	1420.98	1363.52
940	1186.64	1167.00	949.30	4.10	3316.04	2008.19	3.88	19.90	2029.77	1286.07	

reported better retention of phosphorus in cobalt supplemented groups of sheep.

Therefore, these results further support that the lower level of cobalt (4.1 mg) supplementation results in better retention of phosphorus as compared to that of the either higher levels of supplementation or control group.

(4) Balance of iron:

The intakes through feeds and water, outgo through faeces, urine and milk, retention for individual animals and the group average of iron retention are presented in Table 83. All the animals have shown positive balance. The group averages of iron balances were 1.028, 1.032, 1.409 and 1.353 g per day per animal of control and experimental groups I, II and III, respectively.

It will be seen from the results that all the supplemented groups have shown increased assimilation and retention of iron. The highest balance was observed in medium level (24 mg) of supplementation (Group II), while the lowest was in the control group. The retention of iron by the animals of control and group I (16 mg) were almost similar. Therefore, it is seen that supplementation of 6 to 8 mg of cobalt per day per animal had a favourable effect on the iron absorption and retention.

No known references of the work earlier in this line are available.

(5) Balance of copper:

Intake, outgo and retention data of the copper for individual animals and group averages are presented in Table 84.

All the animals were in positive balance. The average balances of copper in the animals of control and experimental groups I, II and III were 15.569, 34.459, 25.437 and 20.190 mg per day per animal, respectively. It is obvious from the results that the cobalt supplementation has shown a significantly high retention of copper in the system. The highest retention was observed at the lowest level of cobalt supplementation (16 mg) and this was more than twice the retention observed in the animals of control group. The next higher retention was noticed in the animals receiving moderate level (24 mg) of cobalt supplement, followed by the highest level of supplementation. The control group recorded the least copper retention. A gradual decline is seen due to higher levels of cobalt supplementation.

Similar observations were recorded by Kapoor (1968) and Kapoor & Mahadevan (1970b). They reported higher retention of copper in copper-cobalt supplemented group, which was statistically significant at 5 percent level. Further, they observed that cobalt helped in maintaining high copper levels in animals (Kapoor and Mahadevan, 1970a). Arith Kumar *et al* (1973) reported the similar observations after supplementation of the ration of Jersey x Tharparkar heifers with copper plus cobalt.

(6) Balance of cobalt:

The total ingestion through feeds, excretion through faeces, urine and milk, retention in the body by the individual animal and the group average of retention of cobalt are presented in Table 85. The average values for the balances of cobalt in the body of the animals of control and experimental

respectively. The animals of group I (200 mg) recorded the minimum number of days in this respect.

Statistically significant differences were not seen in any of the three parameters of the reproductive performances of the animals, as a result of supplementing their ration with copper at three different levels.

7.60 Effect of supplementation of copper to lactating cows on the milk yield:

7.61 The average milk yields of the previous lactation were $2,313.2 \pm 220.1$, $2,548.6 \pm 276.4$, $2,461.4 \pm 224.2$ and $2,224.6 \pm 430.3$ kg, while that of the experimental lactation were $2,610.0 \pm 249.6$, $3,285.0 \pm 254.6$, $2,792.8 \pm 252.1$ and $2,995.2 \pm 428.8$ kg for the animals of control and experimental groups I, II and III, respectively. The increase in the milk yields of the animals of the supplemented groups during the experimental lactation over the previous lactation was higher than that of cows of control group. However, the differences observed were not statistically significant.

8.00 STUDIES ON COBALT SUPPLEMENTATION

8.10 Effect of supplementation of cobalt to lactating cows on the haematological constituents of blood:

8.11 The average values of the packed cell volume of the blood of the animals of control and experimental groups I, II and III were 39.42 ± 0.54 , 40.86 ± 0.49 , 37.92 ± 0.61 and 37.71 ± 0.49 percent, respectively. Dietary supplementation of cobalt at

the rate of 16 mg of cobalt chloride per day per animal was found to result in a significantly higher ($P < 0.01$) PCV of the blood of cows than those of control group. Higher levels of cobalt seem to have an adverse effect on PCV of blood.

8.12 The blood of the animals of control and experimental groups I, II and III contained on an average 10.10 ± 0.12 , 10.43 ± 0.10 , 9.78 ± 0.11 and 10.13 ± 0.09 gm percent ^{of haemoglobin}, respectively. A similar trend as in the case of PCV was noticed in the case of haemoglobin content of blood also.

8.13 The average red blood cell counts of the blood of the animals of control and experimental groups I, II and III were 6.397 ± 0.257 , 6.607 ± 0.283 , 6.463 ± 0.275 and 6.111 ± 0.244 millions per cmm respectively. A significant difference ($P < 0.01$) was noticed between the treatments. The R.B.C. values of the blood of the animals of control and experimental groups I and II were statistically similar and were superior over those of group III.

8.14 The average values of white blood cell counts of the blood of the animals of control and experimental groups I, II and III were 9.359 ± 1.16 , 8.928 ± 1.24 , 9.650 ± 1.22 and 8.843 ± 0.77 thousands per cmm respectively. The values of W.B.C. counts were found to be significantly different ($P < 0.01$) due to treatments. A slight depressing effect was observed on this constituent of blood due to cobalt supplementation.

8.15 The average values of the specific gravity of the whole blood of the animals of control and experimental groups I, II and III were 1.0516 ± 0.005 , 1.0520 ± 0.0004 , 1.0500 ± 0.0004 and

1.0514 $\pm$ 0.0003, respectively. The specific gravity of the blood of the animals of group I was similar to that of animals of control group but significantly higher ($P < 0.01$) than that of the animals of both the groups II and III.

8.20 Effect of supplementation of cobalt to lactating cows on the biochemical constituents of blood:

8.21 The blood of the animals of control and experimental groups I, II and III contained on an average 7.572 $\pm$ 0.071, 7.678 $\pm$ 0.053, 7.619 $\pm$ 0.056 and 7.556 $\pm$ 0.062 gm percent of serum protein, respectively. A slight increase was observed in the blood of the cows of groups I and II which was not significant.

8.22 The average iron content of the blood of the animals of control and experimental groups I, II and III were 31.58 $\pm$ 0.58, 34.17 $\pm$ 0.52, 32.83 $\pm$ 0.49 and 33.08 $\pm$ 0.45 mg per 100 ml blood, respectively. The blood of the animals of all the supplemented groups recorded significantly higher ($P < 0.01$) iron content than that of animals of control group. The blood of the animals of group I recorded the highest values.

8.23 The blood of the animals of control and experimental groups I, II and III contained on an average 115 $\pm$ 1.23, 125 $\pm$ 1.33, 120 $\pm$ 1.53 and 120 $\pm$ 1.41 mcg of copper per 100 ml blood, respectively. A significant difference ($P < 0.01$) was observed between the treatments. The blood of the cows of group I recorded significantly higher copper content than those of control group. All the supplemented groups were statistically similar.

8.24 The average cobalt content of the blood of the animals of control and experimental groups I, II and III were 0.990 ± 0.014 , 1.600 ± 0.116 , 1.598 ± 0.134 and 1.524 ± 0.124 mcg per 100 ml of blood, respectively. Supplementation of cobalt at three different levels was found to be beneficial in raising the cobalt content of blood significantly ($P < 0.01$). All the supplemented groups were not significantly different from each other. Highest blood cobalt content was recorded in the blood of the animals of group I.

8.30 Effect of supplementation of cobalt to lactating cows on the composition of milk:

8.31 The average values of milk fat recorded were 5.15 ± 0.08 , 5.14 ± 0.06 , 5.10 ± 0.03 and 5.20 ± 0.06 percent in the milk of the cows of control and experimental groups I, II and III, respectively. The dietary supplementation of cobalt did not seem to have any significant effect on the butter fat content of milk.

8.32 The milk of the animals of control and experimental groups I, II and III contained on average 3.43 ± 0.02 , 3.43 ± 0.02 , 3.34 ± 0.02 and 3.41 ± 0.03 gm percent of protein, respectively. A statistically significant difference ($P < 0.01$) between the treatments was observed. The animals of group II recorded the lowest value for the protein content of their milk. All the other groups were statistically similar.

8.33 The average total solids content of the milk of the animals of control and experimental groups I, II and III were 14.624 ± 0.08 , 14.697 ± 0.04 , 14.624 ± 0.08 and 14.689 ± 0.11 gm percent respectively. Similar to milk fat, the cobalt supplementation

had no effect on the total solid content of milk produced.

8.34 The average values observed for the ash content of the milk of the animals of control and experimental groups I, II and III were 0.770 ± 0.003 , 0.787 ± 0.004 , 0.782 ± 0.003 and 0.770 ± 0.005 gm percent, respectively. The dietary supplementation of cobalt resulted in a significant increase ($P < 0.01$) in the ash content of milk. The ash content of the milk of cows of control and group III were statistically similar but significantly higher than that of cows of group II. The ash content of the milk of the cows of group I was significantly higher than that of animals of rest of the groups.

8.400 Effect of supplementation of cobalt to lactating cows on the intake and digestibility of nutrients and balances of minerals:

8.401 It was evident from the metabolism trial that the supplementation of cobalt resulted in an increase in the ingestion of dry matter and T.D.N. as well as water consumption. The average dry matter intake per 100 kg body weight of the animals of control and experimental groups were 2.757, 2.910, 2.786 and 2.824 respectively. The highest values for the ingestion of dry matter and consequently T.D.N. were recorded by the animals of group I. The average water consumption by the animals of control and experimental groups I, II and III were 8.01, 7.37, 7.67 and 7.40 litres per 100 kg body weight, respectively. The water consumption was highest by the animals of group II.

I, II and III were 58.98, 62.35, 59.75 and 56.67 percent, respectively. The supplemented groups I and II recorded higher digestibility of crude fibre of the ration than that of control or of group III. Highest level of supplementation of cobalt resulted in a lowering of digestibility of crude fibre of the ration.

8.407 The average balances of nitrogen in the animals of control and experimental groups I, II and III were 35.99, 59.14, 58.20 and 59.16 gm per day per animal, respectively. Higher values of retention of nitrogen were recorded by the animals of all the supplemented groups.

8.408 The average balances of calcium were -3.03, -1.15, -1.98 and -0.20 gm per day per animal of control and experimental groups I, II and III, respectively. A slightly increased retention of calcium was observed in the animals of supplemented groups as compared to those of control group.

8.409. The average values of retention of phosphorus per day per animal were 0.08, 2.22, 1.67 and 0.73 gm in the animals of control and experimental groups I, II and III, respectively. Similar to calcium, the animals of all the supplemented groups retained higher phosphorus than those of control group.

8.410 The animals of control and experimental groups I, II and III retained on an average 1.028, 1.032, 1.409 and 1.353 gm of iron in their body, respectively. Supplementation of cobalt was found to be slightly beneficial in the assimilation and retention of iron in the body of the animals.

8.411 The average retention of copper in the body of the animals of control and experimental groups I, II and III were 15.569, 34.489, 25.437 and 20.190 mg per day per animal, respectively. Supplementation of cobalt to the lactating cows was found to favour the assimilation and retention of copper in the body. The highest retention was found in the animals of group I, and the lowest in those of control group.

8.412 The average retention of cobalt in the body of the animals of control and experimental groups I, II and III were -0.380, 2.173, 1.690 and 1.898 mg per day per animal, respectively. The animals of all the supplemented groups were in positive balance with respect to cobalt and those of group I recorded the highest retention of cobalt. Higher levels of supplementation did not result in a proportional increase in the retention of cobalt in the body of the animals.

8.50 Effect of supplementation of cobalt to lactating cows on their reproductive performance:

8.51 The post-partum oestrus was noticed in the animals receiving the cobalt supplement earlier than those of control group. The average number of days required by the animals of control and experimental groups I, II and III were 69.0 ± 12.1 , 49.6 ± 4.03 , 48.2 ± 1.8 , and 42.2 ± 2.1 respectively, for the occurrence of post-partum oestrus. The animals of supplemented groups showed post-partum oestrus within an average period of 46.7 days, as against 69.0 days in the case of the animals of control groups. The animals of group III recorded the minimum number days in this respect.

8.52 A similar trend was observed in the case of fruitful insemination number, the averages of which for the animals of control and experimental groups I, II and III were 2.2 ± 0.6 , 2.6 ± 0.5 , 1.8 ± 0.4 and 1.5 ± 0.5 respectively.

8.53 The average interval in days from calving to successful insemination of the cows of control and experimental groups I, II and III were 95.2 ± 20.3 , 101.8 ± 23.4 , 78.2 ± 12.2 and 64.0 ± 19.4 respectively. The animals of group III recorded the minimum number days in this respect.

Statistically significant differences were not observed in any of the three parameters of the reproductive performances of the animals, as a result of supplementing their ration with cobalt at three different levels. However, the higher levels of supplementation of cobalt have shown the better performance.

8.60 Effect of supplementation of cobalt to lactating cows on the milk production:

8.61 The average milk yield of the previous lactation were 3,043.0 $\pm$ 342.8, 2,714.0 $\pm$ 125.6, 3,041.2 $\pm$ 212.6 and 2,857.4 $\pm$ 309.6 kg, while that of the experimental lactation were 2,985.6 $\pm$ 303.7, 2,772.2 $\pm$ 122.3, 3,084.0 $\pm$ 155.5 and 3,163.2 $\pm$ 294.2 kg for the animals of control and experimental groups I, II and III respectively. The milk yield of the animals were found to be almost unaffected in the supplemented groups, except in group III, where a slight increase in the milk yield during the experimental lactation was observed, over the previous lactation. However, the statistical analysis of the data did not reveal any significant difference between the treatments.

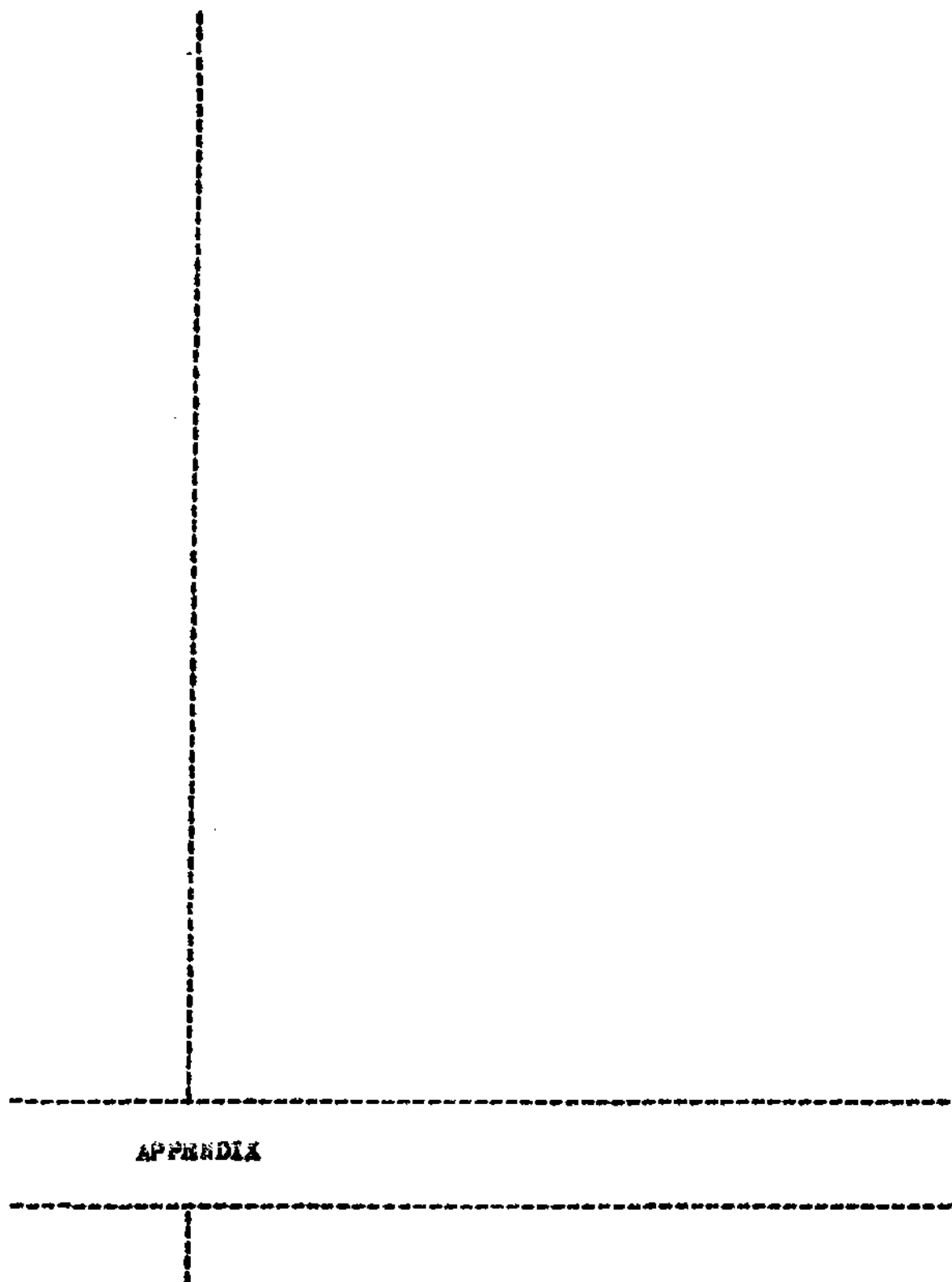
9.00 It is evident from the results of the present investigation that the supplementation of the ration of cross-bred lactating cows with trace minerals such as copper and cobalt is beneficial in improving their physiological status, production and composition of milk, assimilating^o and retention of essential nutrients as well as the reproductive performances.

Supplementation of the ration of cross-bred lactating animals with copper at the rate of 200 mg of copper sulphate (52 mg copper) per day per animal has been found to be the optimum level of supplementation under good management and feeding, as supported by the observations on certain parameters studied in the present investigation.

Similarly, feeding of 16 mg of cobalt chloride (4.1 mg cobalt) per day per animal along with ration to the cross-bred lactating cows has been found to be beneficial in improving the physiological status of these animals, their productive and reproductive performances.

10.00 It is apparent that confinement raising of dairy animals together with the continuing improvement in their genetic inheritance by either selective or cross-breeding for rapid growth, early maturity and higher productivity is likely to change the requirements of nutrients including those of trace minerals. Therefore, it is recommended that supplementing the ration of cross-bred lactating cows with 52 mg of copper and 4.1 mg of cobalt per day per animal will be highly beneficial in improving their physiological status and performances. This would go a

long way in meeting the problems such as the probable higher requirements of nutrients, especially the trace minerals such as copper and cobalt, by these cross-bred animals and the reproductive problems encountered among the high yielding cross-bred animals.



APPENDIX

APPENDIX

Table - A.

Copper and Cobalt contents of some common animal feeds.*

S1. No.	Common name	Botanical name	Copper mg/Kg.	Cobalt mg/Kg.
<u>Greens</u>				
1.	Bajra	.. Pennisetum typhoides	9.6	0.20-0.33
2.	Barley (young)	Hordeum vulgare	11.9	-
3.	Jowar	.. Andropogon sorghum	8.2-19.1	0.20-0.50
4.	Berseem	.. Trifolium alexandrinum	10.4-21.0	0.28-1.20
5.	Berseem, silage	-do-	7.7	-
6.	Blue grass	..	8.3	-
7.	Ber grass	.. Ischaemum aristatum	3.44	0.07
8.	Babul leaves	.. Acacia arabica	9.9	-
9.	Dub grass	.. Cyanodon dactylon	4.9-9.6	0.10-0.20
10.	Elephant grass	Pennisetum purpureum	9.0	-
11.	Guinea grass	Panicum maximum	9.2	0.14-0.18
12.	Green plant	Cicer arietinum	15.6	-
13.	Giant star grass	Cyanodon plecostachyum	6.8	-
14.	Hill tree leaves	-	7.8-17.0	-
15.	Jowar	.. Andropogon sorghum	8.2-19.1	0.20-0.50
16.	Lucerne	.. Medicago sativa	8.5-15.9	0.20-0.50
17.	Maize	.. Zea mays	6.1-11.5	0.28-1.80
18.	Moshi	.. Iscilema weightii	3.66	0.09

* Elvehjem and Hart (1927, 1929); Sahai and Kehar (1961); Datta and Datta Biswas (1961), Patel et al (1966), Kanwar and Randhawa (1967) and Sawhney et al (1972, 1973).

Sl. No.	Common name	Botanical name	Copper mg/Kg	Cobalt mg/Kg.
19.	Napier grass	Pennisetum purpureum ..	10.5-16.10	0.30-0.45
20.	Neem leaves	Asadirantha indica	8.4	-
21.	Oat plant	Avena sativa	7.9-11.7	0.39
22.	Pea plant	Pisum sativa	7.6	-
23.	Para grass	Brachiaria mutica	3.1-4.9	-
24.	Phool grass	Themeda cymbaria	4.20	0.07
25.	Pippal tree leaves	Ficus religiosa	9.10	-
26.	Spear grass	Andropogon contortus	8.30	-
27.	Sudan grass	Andropogon sorghum var sudanensis	7.10	-
28.	Sugar cane leaves	-	2.8-3.9	-
29.	Sugar cane sheaths	-	2.3-6.8	-

Straws and Hays

1.	Bajra straw	Pennisetum typhoides	4.5-9.5	0.11-0.24
2.	Barley straw	Hordeum vulgare	3.9	-
3.	Gram straw	Cicer arietinum	4.0-4.7	-
4.	Jowar straw	Andropogon sorghum	4.4-9.2	0.11-0.16
5.	Jungle hay	-	5.1	0.23
6.	Lucerne hay	Medicago sativa	4.6-14.8	0.24
7.	Maize stover	Zea mays	4.0	-
8.	Oat straw	Avena sativa	3.6	-
9.	Pea plants & straw	Pisum sativa	7.1	0.42
10.	Paddy straw	Oryza sativa	3.6-20.8	0.03-0.30
11.	Red clover hay	-	17.6	-

Sl. No.	Common name	Botanical name	Copper mg/Kg	Cobalt mg/Kg.
12.	Rye straw ..	-	4.4	-
13.	wheat straw ..	Triticum vulgare	1.7-11.0	0.23
14.	Thiathy hay ..	-	2.2	-
<u>Concentrates</u>				
1.	Arhar grain ..	Gajanus indicus	17.2	-
2.	Bajra grain ..	Pennisetum typhoides	12.4-16.8	0.23
3.	Barley grain ..	Hordeum vulgare	2.9-3.4	0.10
4.	Cotton seed ..	Gossypium indicum	12.1-18.8	0.23
5.	Cotton seed cake	-do-	9.6-17.8	0.49
6.	Groundnut cake	Arachis hypogea	7.5-21.8	0.56
7.	Guar grain ..	-	12.7	0.61
8.	Gram chuni ..	Cicer arstinum	13.0	0.34
9.	Gram grain ..	-do-	3.6-15.0	0.83
10.	Jowar grain ..	Andropogon sorghum	9.8	-
11.	Linseed ..	Linum sitatissimum	11.6	0.81
12.	Linseed oil meal	-do-	21.8	-
13.	Maize grain ..	Zea mays	4.4-10.9	0.04-0.10
14.	Mango seed kernel	Mangifera indica	12.4	-
15.	Masoor grain ..	Arum lens	13.4	-
16.	Mung grain ..	Phaseolus mungo var. glabar	14.5	-
17.	Rape cake ..	Brassica campestris	10.6	-
18.	Oat grain ..	Avena sativa	7.6	0.07
19.	Rice grain ..	Oryza sativa	9.5	-
20.	Rice bran ..	-do-	4.6-12.5	0.07

Sl. No.	Common name	Botanical name	Copper mg/Kg	Cobalt mg/Kg
21.	Soya bean ..	-	24.07	-
22.	Sarson (Mustard) cake	Brassica alba	12.30	-
23.	Sugar beet ..	-	8.5	-
24.	Til cake ..	Sesamum indicum	9.2-44.1	-
25.	Tur chuni ..	Cajanus indicus	17.4	0.35
26.	Urad grain ..	Phaseolus mungo var. radiatus	13.3	-
27.	Wheat grain ..	Triticum vulgare	11.4	-
28.	Wheat bran ..	-do-	3.5-14.7	0.23
<u>Animal By-products</u>				
1.	Blond meal ..	-	13.1	-
2.	Tankage ..	-	23.1	-

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