EFFECTS OF COBALT DEFICIENCY ON SOME RUMEN AND THYROID FUNCTIONS IN GOATS.

By

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE IN ANIMAL PRODUCTION, FACULTY OF VETERINARY MEDICINE COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES, UNIVERSITY OF NAIROBI.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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Date: 18th May 1992

This thesis has been submitted for examination with our approval as University Supervisors.

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PROF. J.M.Z. KAMAU

DEDICATION

This thesis is dedicated to my beloved parents

and

to Lydiah Wanjiru

for their encouragement.

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ABSTRACT.

Thirty small East-African goats, 19 females and 11 males aged between 13 and 36 months and weighing 14 to 30 Kg were divided into two treatments consisting of 15 goats each. They were fed *ad-libitum* on hay containing 0.02 mg Co/Kg DM and each goat was supplemented with 150 g of a commercial concentrate daily for a 23 weeks experimental period. Goats in treatment 1 were supplemented with an oral cobalt chloride drench to raise the dietary cobalt intake to 0.3 mg/Kg DM while those in treatment 2 were not supplemented. Goats in treatment 2 were considered cobalt deficient after 10 weeks on experiment when their serum vitamin B_{12} concentration fell below 200 pg/ml.

The effects of Cobalt deficiency on feed intake, digestibility of nutrients, rumen degradability of hay, liveweight change and body condition scores, blood parameters, serum levels of vitamin B_{12} and thyroid hormones plus the rate of resting metabolism in goats were studied.

For 23 weeks of the experiment, hay intake was not significantly ($P>0.05$) different between cobalt adequate and deficient goats. Rumen degradability of dry matter and acid detergent fibre in hay, studied by incubation of dacron bags over 72 hours in two rumen fistulated goats from each treatment, were not significantly ($P>0.05$) different between the treatments. Apparent digestibility coefficients of the dietary nutrients were not significantly ($P>0.05$) different between the treatments. However, the concentration of propionic, butyric, valeric and

isovaleric acids in the rumen liquor were significantly ($P<0.05$) lower in cobalt deficient than supplemented goats while there was no significant ($P>0.05$) difference in the concentration of acetic acid between the two treatments. Rumen liquor concentration of ammonia was significantly ($P<0.01$) lower and the liquor pH significantly ($P<0.01$) higher in cobalt deficient than adequate goats. Gas production from *in vitro* fermentation of rumen contents was significantly ($P<0.01$) lower in cobalt deficient than supplemented goats. Cobalt supplementation improved the rumen fermentation of the soluble components of the diet but not that of cellulose in the hay.

For the twenty-three weeks of the experiment, changes in bodyweight were not significantly ($P>0.05$) different between the two treatments but body condition scores were significantly ($P<0.01$) lower in cobalt deficient than adequate goats.

Although packed cell volume, hemoglobin concentration and erythrocyte counts were significantly ($P<0.05$) lower in deficient than cobalt adequate goats, these blood values were within the normal range for goats.

Total serum thyroxine was significantly ($P<0.01$) higher in deficient than in cobalt adequate goats but the free tetraiodothyronine, triiodothyronine and the rate of resting metabolism were not significantly ($P>0.05$) different between the two treatments.

A dietary cobalt level of 0.02 mg/Kg DM resulted in a subclinical vitamin B_{12} deficiency and a mild thyroid disorder in goats. The possibility that goats are more resistant to cobalt deficiency than sheep is discussed.

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LIST OF ABBREVIATIONS

ADF----------Acid detergent fibre.

DM----------Dry matter.

FT_3----------Free triodothyronine.

FT_4----------Free tetraiodothyronine.

mg---------- Milligram.

mMol---------- Millimole.

NDF----------Neutral detergent fibre.

nMol----------Nanomole.

pg---------- Picograms.

STPD----------Standard temperature and pressure at dry conditions.

TT_4----------Total tetraiodothyronine.

ul---------- Microlitres.

VFA----------Volatile fatty acids.

$W^{0.75}$-------- Metabolic bodyweight.

CHAPTER ONE

1. INTRODUCTION.

1.1 Cobalt deficiency.

In ruminants, inadequate dietary intake of the trace element cobalt leads to a deficiency syndrome clinically described as enzootic marasmus. Cobalt deficiency is widespread worldwide where soils are deficient of the element (Underwood, 1981). In Kenya, region lying in the great East African Rift Valley (Musewe and Gombe, 1980; Mwakatundu, 1977) and the adjacent areas (Chamberlain, 1957) are cobalt deficient. "Nakuruitis" -the local term coined for the deficiency- is derived from Nakuru; the deficient geographical region.

Ruminants obtain cobalt primarily through ingestion of the element in the herbage. Cobalt is used by the rumen microorganisms in the synthesis of vitamin B_{12} which becomes available to the host animal when the microbes are digested in the lower alimentary canal and the vitamin is absorbed at the level of the small intestines (Smith and Marston, 1970).

The vitamin B_{12} requirements of the host animal are met through ruminal vitamin synthesis when the dietary levels of cobalt are above 0.08 mg/Kg of dry matter; therefore, in ruminants, cobalt and vitamin B_{12} deficiencies refer to the same condition.

The development of vitamin B_{12} deficiency and the manifestation of deficiency signs are insidious processes. The major clinical sign, unthriftiness, is very similar to what is observed in undernutrition or chronic helminthiasis. A definite diagnosis of the

deficiency based on clinical signs is not possible without a recourse to biochemical indices (Mills, 1981) such as serum and liver vitamin B_{12} levels (Findlay,1972; Millar *et al.*, 1982), urinary levels of methylmalonic and formiminoglutamic acids (Rice *et al.*, 1987; Stebbings and Lewis 1986) and the analysis of the cobalt-content of the feed eaten by the ruminants.

Diagnosis of borderline cases of deficiency is difficult as there are disparities in the sensitivity of the indices regarding the definition of a "normal" and "deficient" range, the period in the course of deficiency when an index is appropriate and the suitability of their usage across various ruminant species (Mills, 1981). At present, there is no reliable, cheap and readily available diagnostic method.

Only 13% of the ingested cobalt is synthesized into true vitamin B_{12} in the rumen (Smith and Marston, 1970) while the greater portion is synthesized into cobamide compounds (Porter, 1953) which are thought to be essential for the normal functioning of rumen microflora (Georgievskii *et al.*, 1981). Little attention has been paid to the role that cobalt might play in rumen fermentation of nutrients.

Vitamin B_{12} is intimately involved in protein metabolism in all animal species and plays a major role in energy metabolism in ruminants (Gawthorne and Smith, 1974; Cannata *et al.*, 1965). In deficiency, these metabolic processes are impaired.

Vitamin B_{12} deficiency has been shown to cause pathological changes in the nervous tissue (Vincenti and Deane, 1977; Nishino and Itokawa, 1977) and the

thyroid gland (Mgongo *et al.*, 1981). However, the effects of these thyroid changes on the rate of resting metabolism are not known.

1.2. Objectives of the study.

The little attention previously paid to goats in studies on cobalt deficiency influenced the choice of this species as the experimental animals; furthermore, some evidence exists that goats could be more resistant to cobalt deficiency than sheep when the two species are put on the same cobalt deficient feed (Clark *et al.*, 1986).

In Kenya, goats are mainly reared in the low potential to marginally productive zones. For the greater part of the year, these animals are fed on low quality standing hay and browse. In the medium potential zones, the rapid growth in human population in recent years has necessitated fragmentation of hitherto large ranches into smaller holdings where cereals are produced for man and low-quality farm by-products are fed to ruminants. Among the areas affected by fragmentation are the cobalt deficient ones in the Rift Valley.

It was the intention of the present study to elucidate the effects of low-quality cobalt deficient hay on voluntary intake , degradability and other digestive processes in the goat rumen. The effects of low dietary cobalt intake in goats would be compared to those in other species in literature -especially in sheep- and the influence exerted by the documented thyroid disorders on resting metabolism would be looked into.

CHAPTER TWO.

2.0 LITERATURE REVIEW.

2.1. Minerals in ruminant nutrition.

Ruminants require about twenty-two minerals for normal growth and health (Underwood, 1981). In nutrition studies, these minerals are classified in accordance with their biological functions. Broadly, the minerals found in the body fall under three classes: essential, probably essential and elements whose functions are imperfectly known or unknown (Georgievskii *et al.*, 1981).

In the body, minerals are maintained within a narrow or "normal" range by homeostasis. Specific toxic or deficient conditions in animals develop in either excessive or inadequate dietary intake of the minerals. Deficient rather than toxic conditions are more common in most parts of the world (Underwood, 1981).

Subclinical mineral deficiencies cause vast economic losses through a reduction in animal production which is easily confused with undernutrition and helminthiasis (Underwood, 1981).

Mineral status in East Africa.

Only a few mineral deficiencies have been demonstrated in animals although soils and herbage contents of some minerals have been shown to be deficient or marginally deficient in East Africa. Sodium deficiency is probably the most widely distributed in East Africa (French, 1955; Howard *et al.*, 1959; Russel and Duncan, 1957), marginal phosphorus level is widespread (French, 1952, 1955;

Naik, 1965 and Mwakatundu, 1977) while low copper level (French, 1955) might result in "conditioned" copper deficiency in the Rift Valley where the soil molybdenum content is high (Mwakatundu, 1977).

2.2. Discovery of cobalt deficiency.

Cobalt was shown to be an essential nutrient for cattle and sheep in Australia (Marston, 1935, Underwood and Filmer 1935). Cobalt deficiency in goats was first reported in Kenya by Gombe and Verjee (1976).

Anaemia and ill-thrift observed in ruminants grazing on some pastures were alleviated by oral supplementation of an impure iron-ore extract - limonite. The condition was thought to be due to iron deficiency until Underwood and Filmer (1935), showed that supplementation of purified iron to deficient sheep failed to alleviate the condition and identified the curative impurity in limonite to be cobalt.

Ruminants have a higher requirement for cobalt than other animal species raised on the same deficient pastures (Underwood, 1981). Alleviation of deficiency occurs when cobalt is taken by mouth suggesting that it acts in connection with the rumen microorganisms (Marston and Lee, 1949). Kercher and Smith (1956) showed that orally administered cobalt was used by the rumen microorganisms to synthesize vitamin B_{12}.

Cobalt deficiency is essentially a vitamin B_{12} deficiency brought about by an inability of rumen microorganisms to synthesize sufficient vitamin B_{12}, when dietary cobalt is inadequate, to meet the metabolic needs of ruminant cells and tissues (Underwood, 1981).

2.3. Occurrence of cobalt deficiency.

Cobalt deficiency is widespread in many parts of the world with soil types low in cobalt (Underwood, 1981). The disease condition is known by different names in different enzootic areas e.g."Nakuruitis" in Kenya. Enzootic marasmus is the most appropriate description based on the condition's clinical manifestations (Underwood, 1981).

Cobalt deficient soils and pastures are widely distributed in various ecological zones in East Africa (Mwakatundu, 1977) especially in the Rift Valley (Naik, 1965; French, 1955) and other areas such as Tana river, Narok and Sotik (Chamberlain,1957).

In a study aimed at relating the mineral status of grasses and soils in the cobalt deficient Mbulu district of Tanzania, Naik (1965) found that dry grasses contained higher cobalt than green ones and attributed the observation to contamination of the dry grasses by cobalt-containing wind-blown dust. During the wet season, as the dust was washed away, the cobalt content of the grasses declined which resulted in a deterioration in body condition of cattle grazing the deficient pastures during the wet season.

2.4. Cobalt sources and requirements by ruminants.

Ruminants obtain cobalt from the herbage and to a lesser extent from the soil (Underwood, 1981). The soil type, its cobalt level and pH determine cobalt uptake by the plants: high alkalinity, heavy liming and high manganese-oxide levels lower uptake (Georgievskii *et al.*, 1981). Generally, herbage cobalt levels below 0.08 ppm on dry matter basis leads to deficiency

(Underwood, 1981) while a mean soil concentration of 0.17 ppm acetic acid extractable cobalt is regarded as deficient (Russel *et al.*, 1975).

To a large extent, ruminants' requirements for cobalt has been based on sheep. ARC (1980) recommends a dietary level between 0.08 and 0.11 mg cobalt/kg DM of the diet; the lower level for adult and higher for young growing stock whose cobalt requirements are higher.

Requirements for cattle might be lower than for sheep as a dietary level of 0.08 mg Co/Kg of the diet only leads to slight deficiency signs in cattle (ARC, 1980).

Owing to the few trials conducted in goats, requirements for this species are less clearly defined. Mgongo *et al.*(1981) induced deficiency by feeding small East African goats on a diet containing 0.01 mg Co/Kg DM in which anaemia and weight loss were observed. However, Clark *et al.* (1986) failed to obtain a weight gain response by intramuscular injection of 1.5 mg hydroxycobalamin three times over a four months' experimental period to goats fed on a diet containing 0.035 mg Co/Kg. They suggested that vitamin B_{12} reference range for sheep cannot be used for goats as goats appeared not to be as prone to deficiency as the sheep.

2.5. Biochemical roles of cobalt.

Cobalt as the vitamin B_{12} cofactor.

The discovery and chromatographic isolation of vitamin B_{12} from liver extract and the finding that the vitamin contains cobalt was reviewed by Robertson (1971). Vitamin B_{12} refers to a group of cobalamins (Methylcobalamin, adenosylcobalamin, hydroxocobalamin, aquocobalamin and cyanocobalamin) considered to have an equivalent biological activity (Millar *et al.*,1982).

Cobalt exerts its biological activities in the form of vitamin B_{12} which functions as a co-enzyme in several biochemical reactions; the most studied being dimethylbenzimidazole cobamide (DBC) which functions as a co-enzyme of methylmalonyl-coA mutase (Cannata *et al.*,1965). The enzyme effects conversion of methylmalonate to succinate -the last reaction that make possible the utilization of propionate for provision of energy in animal tissues (Flavin and Ochoa, 1957). Methylmalonyl-coA mutase activity declines in cobalt deficiency (Cannata *et al.*,1965) leading to accumulation of methylmalonic acid in plasma early in deficiency (Rice *et al.*, 1989) and which is later excreted in urine (Rice *et al*,1987).

In deficient sheep, alternate hepatic metabolic pathways for accumulated plasma methylmalonic acid have been suggested (Peters *et al.*,1983). Excess methylmalonic acid is utilized in fatty acid elongation giving rise to branched-chain fatty acids while unmetabolised propionate becomes a "primer" for fatty acid biosynthesis giving odd-numbered fatty acids (Duncan *et al.*,1987).

Other biochemical reactions in which vitamin B_{12} participates include:

(a) Intrahepatic synthesis of methionine by transmethylation of homocystein catalyzed by the vitamin B_{12} containing enzyme, 5-methyltetrahydrolate-homocysteine transmethylase. Methionine synthesis falls in cobalt deficiency leading to reduced protein retention, wasting and low wool or hair growth (Gawthorne and Smith, 1974).

(b) Reduction of ribonucleotides to deoxyribonucleotides in erthropoiesis (Georgievskii,*et al.* ,1981).

(c) In vitamin B_{12} deficient rats, faecal thiamin levels rise suggesting that vitamin B_{12} enhances absorption of thiamine by active transport in the small intestines (Nishino and Itokawa, 1977). A concurrent thiamine deficiency may occur in chronic vitamin B_{12} deficient sheep in which nervous signs, similar to those observed in cerebrocorticonecrosis, occurs (Macpherson *et al.*,1976).

2.6. Synthesis of vitamin B_{12} and its analogues in the rumen.

In sheep, independent of the feed intake, a reduction in the number and types of bacteria in the rumen-liquor occurs in cobalt deficiency (Gall *et al.*, 1949). Most bacteria cover by synthesis their vitamin B_{12} requirements but some bacteria and protozoa require vitamin B_{12} or one of the vitamin B_{12} analogues for growth under some conditions (Porter, 1953).

Vitamin B_{12} is synthesized by some bacteria, especially *propionibacterium shermanii* (Williams, 1982), in the presence of adequate cobalt in the rumen-liquor (0.5 ng Co/ml) (Underwood, 1981). The presence of ciliates in the liquor appear to be necessary for bacteria to utilize organic cobalt for growth (Bonhomme *et al*, 1982).

Rumen contents contain a large number of factors, the vitamin B_{12} analogues, some of which are microbiologically active and essential for microbial functions (Smith and Loosli, 1957). Like vitamin B_{12}, the analogues are synthesized by rumen microorganisms: the most common analogues, pseudovitamin B_{12}, factor A and factor B are not active in mammalian tissues (Bigger *et al.*,1976).

2.7. Factors affecting synthesis of vitamin B_{12} and its analogues in the rumen.

The amount of vitamin B_{12} synthesized in the rumen is a function of the ingested cobalt (Smith and Loosli, 1957; Hedrich *et al.*,1973). With increasing cobalt intake, production of vitamin B_{12} is more rapid than that of analogues (Bigger *et al.*, 1976). The efficiency of vitamin B_{12} production is higher with smaller than higher levels of dietary cobalt (Smith and Marston, 1970). The production efficiency varies with the animal's vitamin status: about 15 and 3 percent of the dietary cobalt is synthesized to true vitamin B_{12} in deficient and cobalt adequate sheep respectively (Underwood, 1981). Preferential production of the analogues may occur at high cobalt intake (Somers and Gawthorne, 1969).

Irrespective of the dietary cobalt level, production of vitamin B_{12} is limited by the feed intake (Smith and Marston, 1970). Vitamin B_{12} production increases linearly with increase in dry matter intake (Sutton and Elliot, 1972) although the increase has only a small contribution to the total vitamin B_{12} production (Hedrich *et al.*, 1973).

Restriction of the amount of dietary roughage limits rumenal vitamin B_{12} production (Walker and Elliot, 1972) while a high proportion of concentrate in the diet reduces vitamin B_{12} production but not that of analogues (Sutton and Elliot, 1972). The enhancement of propionic acid production by addition of monensin to a roughage based diet reduces vitamin B_{12} synthesis in the rumen (Enev *et al.*, 1983).

Rickard *et al.* (1975) reported an increase in vitamin B_{12} production at the expense of the analogues when 5,6,Dimethylbenzimidazole (DMB) was added to a diet fed to cattle. DMB, the vitamin B_{12} moiety, is present in higher quantities in forages than in concentrates and, contrary to the above authors, has been reported to increase vitamin B_{12} and the analogues' synthesis to the same extent: feeding alfafa -which contains a high quantity of DMB- increases synthesis of vitamin B_{12}, factor A, and factor B (Bigger *et al.*,1976).

Dietary supplementation of cobalt lowers the rumen pH (Saxena *et al.*, 1980) but the effects of the rumen pH on vitamin B_{12} synthesis is not known.

2.8. Absorption, transport, storage and excretion of vitamin B_{12}.

Some vitamin B_{12} produced is degraded in the rumen while that in the bacteria is liberated into the fluid phase at the low abomasal pH (Smith and Marston, 1970). Absorption of the vitamin occurs in the upper small intestines and the amount absorbed is a function of the proportion of the vitamin to the analogues in the ingesta (Walker and Elliot, 1972; Bigger *et al.*, 1976). The mechanism of vitamin B_{12} absorption is not fully understood (Rickard and Elliot, 1978) but it has been suggested that Vitamin B_{12} and its analogues are absorbed as complexes of gastromucoproteins while the free ions of soluble cobalt salts pass through the walls of the small intestines (Underwood, 1975).

When in large amounts, factor B is absorbed to the same extent as vitamin B_{12} and stored in the liver but at levels encountered physiologically, factor B is unlikely to replace vitamin B_{12} in the liver during storage (Rickard and Elliot 1978; 1982).

About 5 percent of the vitamin B_{12} synthesized in the rumen is absorbed (Smith and Marston, 1970). Absorption increases as digesta flow decreases as might happen when feed intake declines with development of deficiency (Hedrich *et al.*, 1973).

Higher vitamin B_{12} production leads to higher absorption and retention: however, absorption efficiency increases when smaller quantities of vitamin B_{12} flows through the gut suggesting that an intrinsic mechanism of absorption might be involved (Rickard and Elliot, 1978).

When absorbed, cobalamins are carried in plasma by

proteins whose total binding capacity exceeds the endogenous cobalamin concentration (Millar *et al.*, 1982). Some of the absorbed vitamin B_{12} is secreted into the duodenum, a large proportion of which is reabsorbed in the ileum (Smith and Marston, 1970).

The liver, kidney and the bones serve as the major storage site (Georgievskii, *et al.*,1981). Hepatic distribution of vitamin B_{12} is uniform in the liver lobes (Osborn *et al.*,1983) and vitamin B_{12} activity in the organ is considered a reliable indicator of an animal's vitamin status (Millar *et al.*, 1982).

To a small extent, vitamin B_{12} is excreted through the faeces (Dawbarn *et al.*,1952; Phillip and Hugh, 1949) and mainly through the urine (Rickard and Elliot, 1981) while only a small amount through the milk (Georgievskii *et al.*,1981; Quirk and Norton,1982).

Ingested, unabsorbed and endogenously lost cobalt (through the bile and probably the intestinal walls) is excreted through the faeces (Georgievskii *et al.*,1981; Smith and Marston, 1970). The amount of cobalt in the faeces reflects the element's dietary intake (Jones and Anthony, 1970).

2.9. The role of cobalt in the digestion of nutrients.

The number of rumen microorganisms decline in cobalt deficiency but the particular organism implicated in the diminution is uncertain (Underwood, 1975). Reports on the effect of the deficiency on the fermentation of various dietary components differ.

Marston *et al.*,(1961) found the rate and course of fermentation of carbohydrates in deficient sheep to be unaltered. Production of volatile fatty acids in the

rumen during the initial stages of deficiency was unaffected (Marston *et al.*,1972).

Beneficial effects of cobalt on cellulolytic activity is contradictory (Georgievskii *et al.*,1981) probably as the quality of feed offered to the ruminant appear to affect the course of fermentation.

When fed on cobalt deficient low-quality roughages such as wheat straw (Saxena *et al.*,1978; Pal *et al.*, 1980) or berseem hay (Pal *et al.*, 1980), oral supplementation of cobalt improved the fermentation of cellulose. This was reflected by an increase in the molar concentration of acetic acid in the rumen liquor of the supplemented calves compared with the unsupplemented controls. Fermentation of the rapidly degradable carbohydrates was unaffected as the molar concentration of propionic acid in the liquor from the two groups was similar (Saxena *et al.*, 1980). The improved fermentation of the fibrous portion of the diet was attributed to lower pH, higher bacterial and protozoal counts observed in the rumen liquor of the supplemented calves.

Retention of nitrogen.

Cobalt supplementation to deficient animals has been shown to rise the level of rumen protein nitrogen (Saxena *et al.*, 1980; Krasnodebska, 1984). Dosing sheep with cobalt enhances the pancreatic juice proteolytic activity (Georgievskii *et al.*, 1981) and in deficiency, higher excretion of faecal nitrogen occurs in sheep (Smith and Marston, 1970).

2.10. Thyroid disorders in vitamin B_{12} deficiency.

Hypertrophy of the thyroid gland occurs in malnutrition but the effects of this glandular change on the thyroid functions are variably reported by different authors. In vitamin B_{12} deficient adult goats, Mgongo (1979) demonstrated hypertrophy and hyperplasia of the thyroid epithelium and an elevation of plasma free thyroxine, T_3 and T_4 with advancement of deficiency as had earlier been reported (Bustad and Fuller, 1970; Smith *et al.*, 1972 and Burke and Eastman, 1974).

However, depression in the level of T_3, T_4 and TSH have been reported in malnourished children (Pimstone, 1976), protein deficient rats and farm animals (Srebnik *et al.*, 1963) suggesting that a breakdown of the negative feedback exerted by T_3 and T_4 on the hypothalamus occurs during stress.

Mgongo (1979) suggested that in vitamin B_{12} deficiency an initial hypothalamo-pituitary hyperactivity followed by hypoactivity in the latter stages of deficiency could explain the disparities in the hormone levels observed at various stages of the deficiency. Severity of the stress imposed and the animal's age could also influence the levels.

The dietary level of iodine and the iodine to cobalt ratio have been shown to affect the degree of thyroid dysfunction in man (Underwood, 1975).

2.11. Clinical signs of deficiency.

When put on a diet deficient in cobalt, the duration after which signs of deficiency appear depends on an animal's age, its vitamin B_{12} status and the

amount of cobalt in the diet (Underwood, 1981). When sheep are fed on a diet containing less than 0.02 ppm cobalt, for up to three months they show normal health and growth after which gradual loss of appetite occurs (Dawbarn *et al.*,1957), leading to reduced growth rate (Quirk and Norton, 1982), weight loss (Smith and Marston, 1970), extreme inappetance, rapid muscular wasting, pica or depraved appetite (Underwood, 1981) and anaemia which has been characterised as normocytic and normochromic in sheep (Sutherland *et al.*, 1979) and hypochromic and macrocytic in goats (Mgongo *et al.*, 1981). In lambs photosensitization occurs (Sutherland *et al.*, 1979; Mcloughlin *et al.*, 1984) while long standing cases in sheep show nervous signs (MacPherson *et al.*, 1976).

Young ruminants develop signs early: four week has been reported in lambs born to deficient ewes and raised on deficient pastures (Quirk and Norton, 1987). *Inutero* vitamin B_{12} storage is low and the young depend on the vitamin in milk provided during suckling (Grace *et al.*, 1986).

Subclinical deficiency is clinically undifferentiable from malnutrition or chronic helminthiasis (Musewe and Gombe, 1980).

Fisher and MacPherson (1986) observed high neonatal mortality and increased susceptibility to infection in deficient lambs which they attributed to reluctance by the weak lambs to suckle after birth.

Prolonged and mild stress such as that due to chronic naturally occurring worm infestation depresses vitamin B_{12} reserves in subclinically deficient sheep to levels low enough to cause overt deficiency (Downey,

1966).

Impaired immunity and high susceptibility to *Ostertagia* infection has been shown in deficient calves compared to supplemented controls (MacPherson *et al.*, 1987) while in chronically deficient sheep, incidences of diarrhoea and respiratory infections are frequent (MacPherson, 1982).

In advanced deficiency, plasma ascorbic acid levels fall probably due to reduced feed intake and failure to synthesize glucose -the precursor of ascorbic acid - from propionate and this may contribute to increased susceptibility of animals to infection in the late but not the early stages of deficiency (MacPherson, 1983).

CHAPTER THREE.

3.0 MATERIALS AND METHODS.

3.1. Experimental goats and their management.

3.1.1. Adaptation period.

Thirty small East Africa goats, nineteen females and eleven males were acquired from a research station at Isiolo in the North Eastern Province of Kenya in November 1989.

For a nine months' period that the study lasted, the goats were kept in individual pens in a well-sheltered concrete-floored house located at the field station, Kabete, University of Nairobi.

The goats were allowed a two months' period to acclimatize to the new environment and to being handled. Adequate cobalt-containing Rhodes grass hay (*Chloris gayana*) from Kabete was fed *ad-libitum* and a daily 200 g of a commercial concentrate supplement (dairy meal) containing 14% crude protein and 10.42% crude fibre was fed to each goat for the whole of the acclimatization period.

During this period, two goats developed pneumonia and responded to treatment with tetracyclin. At the end of the period, the goats weighed an average of 21.03 kg (range 14.4 to 30.2 kg) and were aged 19 months on average (range 13 to 36 months).

3.1.2. Experimental period.

The experimental period lasted seven months; from January to July, 1990. At the beginning of the period, the goats were assigned by sex to two treatments each

consisting of fifteen goats. Six males and nine females were cobalt supplemented controls (Treatment 1) while five males and ten females were unsupplemented (Treatment 2). The two groups of goats were seperated by an empty pen.

3.1.3. Feeding and feed analyses.

Cobalt deficient hay was purchased from Nakuru, a cobalt-deficient area in the Kenyan rift valley. At 10.00h daily, an amount of chopped hay, ranging from 400 to 800 g, was offered to each goat individually depending on the goat's previous week hay intake. At 9.00h the following day, the hay refusals were weighed and the goats'hay intake determined by difference. The average daily hay intake for 5 days in a week was calculated for each goat and hay offered the following week adjusted so that a daily refusal weighing between 15-50 % of the hay offered was recorded for every goat. Hay intake was thus determined for 23 weeks.

Daily, at 15.00h, 150g of the commercial concentrate (table 2) was fed to each goat. Clean drinking water was available to all the goats over the whole period.

Mineral supplementation.

One group of the goats (Treatment 1) were supplemented with cobalt as cobalt chloride ($COCl_2.6H_2O$; May and Baker LTD, Dagenham, London, MP 6270). 0.3126 g of the salt was dissolved in 1.2 litres of distilled water and each goat in the supplemented group was drenched orally with 10 ml of the solution three times a week so that 0.0291 g elemental cobalt was taken per week. This amount of cobalt raised the

cobalt-content of the basal diet to 0.3 mg/Kg DM which was above the minimum 0.08 Mg/Kg DM (Underwood, 1981).

Cobalt deficiency was induced in the other group of goats (Treatment 2) by feeding them on the cobalt deficient feed.

Goats in both the treatments had free access to cobalt-free salt blocks (supplied by Wellcome, Kenya Limited) whose composition is presented in table 2.

Chemical Analysis.

About 1 kg sample of hay was collected at random from ten bales of hay and 1 Kg sample of concentrate from three 70 kg bags. Seperately, the samples were ground by passing through a 1 mm screen.

Proximate composition of the hay and concentrate were determined according to the official methods of analysis of the AOAC (1984) while neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the methods of Van Soest (1973) and Van Soest and Wine (1967) respectively (Table 2).

Cobalt content.

2g Sample of either the hay or the concentrate was weighed into Kjeldahl flasks in triplicate and digested by wet-ashing: a 15 ml digesting solution containing 70% perchloric acid and concetrated nitric acid in the ratio 3:1 by volume, was added to each sample. The digesting mixture was brought to the boil, allowed to digest to clarity and the greater portion of the acid allowed to evaporate. The digested sample was

Table 1. Composition of the cobalt-free salt block.

Component	per cent (w/w)
Sodium chloride	86.89
Calcium	3.06
Phosphorus	1.09
Magnesium	0.45
Iron	0.30
Copper	0.20
Manganese	3.84
Zinc	0.30
Sulphur	3.85
Iodine	0.01
Selenium	0.005
Molybdenum	0.0002

Table 2: The chemical composition of the hay and concentrate (%).

	Hay	Concentrate
Dry matter	92.28	78.95
Ash	7.99	14.77
Crude Fibre	45.73	10.42
Crude Protein	7.05	14.00
Ether extracts	0.02	0.07
NDF	46.47	48.23
ADF	45.11	15.97

transferred to 100 ml volumetric flask and diluted to the mark with distilled water.

Standard solutions containing 0.05, 0.1, 0.15 and 0.3 ppm cobalt were prepared by diluting a cobalt chloride standard stock solution containing 1 ppm cobalt.

The standard solutions were aspired into a Perkin Elmer atomic absorption spectrophotometer (model 2380) and the absorbance read out with the instrument set at 240.7 nm wevelength and 0.2 nm slit diameter. An absorbance-against-concentration reference curve was plotted.

The diluted digested sample solutions were then aspired and the absorbance read out as for the standard solutions. Cobalt content of the hay and concentrate were calculated according to the Perkin Elmer analytical methods for atomic absorption spectrophotometry (1982). The hay and concentrate contained 0.02 and 0.05 mg co/kg DM respectively; both were below the critical 0.08 mg Co/Kg DM (Underwood, 1981).

3.2. Goat weighing and body condition scoring.

At monthly intervals during the experimental period, before the morning feeding, all the goats were weighed.

Body condition was assessed for all the goats on weeks 8, 10, 19 and 23. Each goat was assigned a score ranging from 1 to 4 after palpation of the lumber vertebral region by two independent observers. The coverage of the lumber vertebra by the overlying longissimus dorsi muscles, judged by the ease with

which the vertebral prominences were palpable and by the distance that the muscles extended along the horizontal and the spinous processes, were used as the scoring criterion according to the body condition scoring scheme for small East African goats (Honhold *et al.*, 1988).

3.3. Digestibility trial.

Six goats, two females and four males from each of the treatments, were selected at random during the thirteenth week of the experimental period. Three days before commencement of the eight days collection period, the goats were put on metabolic crates and allowed to adjust to the crate environment. During the trial the goats were fed as described in section 3.1.3 therefore, an adaptation period was not necessary.

On their floors, the crates were fitted with two openings that allowed for separate collection of faeces and urine voided by the male goats. Faeces were collected in a polythene bag suspended at the crates'rear opening while urine was collected in a plastic bowl placed under a central opening. 10 ml of 50% sulphuric acid were added to trap the urinary ammonia.

For the female goats, the faeces and urine were collected through the rear-crates' opening. The urine-collecting bowl was covered by a fine wire-mesh and then placed in a larger basin. The mesh allowed the urine to pass through while the faecal pellets rolled into the basin.

The Faeces and urine were collected for eight days. After twenty-four hours collection, the feaces

were weighed and the volume of the urine determined after which a 10% faecal and urine samples were taken separately for every goat. The samples were stored frozen at -20^{o}C. At the end of the trial, the eight days' faecal and urine samples were pooled separately for every goat.

About 1 Kg sample of the bulked hay and the same quantity of the concentrate offered during the trial were taken for proximate analysis. A 10% sample of hay refused daily was taken and stored at -20^{o}C. At the end of the trial, refusal samples for each goat were pooled. The hay, concentrate and refusal samples were bulked separately and milled through a 1 mm screen.

The feaces were dried at 70^{o}C for 48 hours and the dry matter content determined after which the faeces were also milled. proximate analysis was performed for the hay, refusal, concentrate and faecal samples while urinary nitrogen was determined by the Kjeldahl method (AOAC, 1984).

Cummulative digestibility coefficients of the proximate components of the feed offered for the eight days were calculated and expressed in percentages. The digestibility of dry matter, organic matter, crude fibre, crude protein and ether extract were determined.

3.4. Rumen function studies.

Surgical fistulation of the goats.

During the fourth week of the experimental period Six female goats, three from the cobalt' adequate and three from the deficient groups were selected at random and fasted for 30 hours before fistulation.

An area measuring about 10 by 15 cm on the left paralumber fossa was shaved, washed with soap and water and disinfected with 70% alcohol. The goat was laid on right lateral recumbency and, on the dorsal third of the fossa, a ring block local analgesia was induced to the skin and underlying abdominal muscles.

A circular skin incision, 3.5 cm in diameter was made and the skin flap enclosed by the incision excised. The abdominal muscles were seperated by blunt dissection and the peritoneum incised to expose the dorsal sac of the rumen. A small portion of the rumen was exteriorised and held by doyen's intestinal clamps. To prevent contamination of the abdominal cavity by rumen ingesta, the rumen was sutured to the skin by six horizontal mattress stay-sutures.

A portion of the rumen, approximately the same diameter as the skin incision was excised and the rumen margins sutured to the skin incision using nylon suture number 2-0 in a simple continous pattern broken at three points.

A circular plastic cork, made in the Department of Animal Production, was used to close the fistula. The cork had a hole about 2 cm in diameter at its centre which was covered with a rubber bang and through which the rumen cavity could be accessed.

Intramuscular injection of an antibiotic (Penicillin- Steptomycin combination) was administered daily for five days following the surgery. In three weeks, the surgical wound had healed and sutures were removed.

Slight leakage of rumen-contents occured between the cork and the margins of the fistula due to an

increase in fistula diameter with time making it necessary to replace the corks with slightly wider ones.

Three female goats were fistulated as replacements after two goats from treatment 2 and one from treatment 1 died. The cause of death was identified as pneumonia for the cobalt adequate goat but no specific cause was identified for the cobalt deficient goats.

3.4.1 Rumen degradability of hay.

Dacron bags measuring 140 x 90 mm and having a mesh-pore diameter 40 um were washed in water and dried at 70^{o}C. A nylon thread was passed through a seam in the open-end of every bag which was made such that it could be clossed in a purse-like manner. The bag was labelled and then weighed together with the string.

Cobalt-deficient hay was milled and approximately 5 g weighed into each bag and the nylon thread securely fastened thereafter.

Two fistulated goats from either treatment were used to study the degradability of hay in cobalt deficient goats during week 21 of the experimental period.

Bags incubated in the same rumen were tied along a nylon string about 25 cm long and 4 mm in diameter to ease introduction and removal of the bags through the fistula and to prevent entangling of the bags when in the rumen.

The bags were incubated over 6, 12, 24, 48 and 72 hour periods. For every period, two bags were incubated in the same goat and no more than six bags were incubated at the same time in a goat. The

experiment lasted six days during which the incubation for each of the five periods was replicated twice (Table 3). On removal, bags were washed under a running tap until the water dripping from the bags was clear. The bags and their contents were dried at 70°C for 48 hours and then weighed.

Four "Zero hour" bags were treated as above but they were not incubated.

The weight difference between the hay incubated and that left in the bags after incubation for various periods of time were computed as the dry matter (DM) disappearance. The acid detergent fibre (ADF) in the hay before and after incubation was determined by the method of Van Soest (1973) and the ADF disappearance computed by difference.

Percentage DM and ADF disappearance were calculated and the disappearance rate constants were calculated according to the following equation (Orskov *et al.*, 1980):

$$P = a + b(1-e^{-ct})$$

where

P: The actual percentage degradation of DM or ADF after incubation for t hours

a: The instantaneous loss at t=0; i.e soluble DM or ADF relative to the degradation of the component described by $b(1-e^{-ct})$

b: The potential degradability of DM or ADF which will, in time t, be degraded

(a+b): The total DM or ADF degradation in the rumen

c: The rate constant for the degradation of 'b'.

Table 3. Scheme for incubation of the hay samples.

DATE	TIME Incubated	Commulative hours	Bag put in	Bag Removed	Time removed
18/6/90	9.00 h	0	6a, 72a	6a	15.00 h
19/6/90	9.00 h	24	12a, 48a	12a	21.00 h
20/6/90	9.00 h	48	24a	-	-
21/6/90	9.00 h	72	-	24a, 48a	9.00 h
22/6/90	9.00 h	0	6b, 72b	6b	15.00 h
23/6/90	9.00 h	24	12b, 48b	12b	21.00 h
24/6/90	9.00 h	48	24b	-	
25/6/90	9.00 h	72		24b, 48b 72b	9.00 h

Key: a: Sample incubated during the first 72 hours;
b: Sample incubated during the second 72 hours period.

3.4.2. Concentration of volatile fatty acids and ammonia in rumen liquor.

A day after removal of the last nylon bags, rumen liquor samples were collected from the four fistulated goats for two consecutive days at 9.00h, 12.00h, 15.00h, 17.00h, 20.00h and 24.00h for each of the days.

A 100 ml syringe was connected to one end of a cylindrical stainless-steel strainer whose other end was rounded and bore holes about 2mm in diameter. The strainer was introduced into the rumen through the fistula and 70 ml of the liquor was drawn from three points: anterior, middle and caudal regions of the dorsal sac. The liquor was strained through four layers of cheese cloth and divided into a 20 and 50 ml portions.

The pH of the 20 ml portion was read from a pH meter (Pye Unicam, PW 9418) after which a few drops of 50% sulphuric acid were added to this portion to trap ammonia. The liquor was stored in screw-top plastic bottles at $-20^{o}C$ for determination of ammonia concentration later.

The 50 ml portion was poured into screw-top plastic bottles and stored at $-20^{o}C$. From this portion, the concentration of volatile fatty acids were determined.

3.4.2.1 Volatile fatty acids in liquor.

About 10 ml of each thawed rumen liquor sample was centrifuged for 15 minutes at 2000g. 5ml of the supernatant was transferred into clean tubes and 1 ml of 20% orthophosphoric acid was added to all the samples. After standing for 30 minutes, the samples

were centrifuged for 10 minutes at 2000g and the supernatant transferred to clean tubes.

The volatile fatty acids concentration in the liquor were determined by gas-liquid chromatography using a model 427 Packard GLC (Supelco, Inc. GC Bulletin 749F). The acids were seperated by a 2M glass column packed with chromosorb W AW as the support phase and supelco 1200 treated with 1% phosphoric acid as the stationary phase. The column was maintained at 130^{O}C, the injection pot at 150^{O}C and the flame ionization detector at 200^{O}C. Hydrogen, nitrogen and compressed air were used as the fuel, carrier and oxidant gases respectively.

Exactly 10.7 ul of a standard solution containing acetic acid, 140 mMol/l; propionic acid, 40 mMol/l; butyric acid, 30 mMol/l; isovaleric acid, 15 mMol/l and valeric acid, 15 mMol/l was injected into the injection pot and a chromatogram obtained for the standard acids.

After stable peaks for standard acids were obtained, 10.7 ul of the prepared liquor samples were injected in duplicate. The volatile fatty acid concentrations in the liquor were calculated by comparing corresponding peak-areas of chromatograms for the standards and liquor samples.

3.4.2.2 Free ammonia determination.

Rumen liquor was thawed and 10 ml of each sample taken into labelled tubes and centrifuged at 800g for 5 minutes to sediment feed particles.

Free ammonia in the clear rumen ' fluid was determined by steam distillation using a 46MC Markham semi micro-ditillation unit. 5 ml Of the liquor was

taken into the inner jacket of the unit and mixed with 10 ml of 40% (w/v) NaOH. Steam was passed through the outer jacket and the mixture in the inner jacket allowed to boil for 5 minutes. Ammonia liberated from the liquor was trapped in 10 ml of boric acid after which the free ammonia, in gm/ml of liquor, was determined by titrating the trapped ammonia against 0.01N hydrochloric acid.

NH_3 (g/ml)= 0.00304 x HCL titre (ml)

3.4.2.3 Gas production from *in vitro* fermentation of rumen contents.

Artificial saliva was prepared by dissolving 5 g N_aHCO_3, 0.5 g $(NH_4)_2SO_4$; 0.5 g K_2HPO_4; 0.2 g KH_2PO_4; 0.05 g $MgSO_4.7H_2O$ in 1 litre of distilled water and the pH adjusted to 6.8-6.9 by bubbling carbon-dioxide (Hungate, 1965).

About 100 g of fresh rumen contents were taken after a goat was killed (see section 3.6), placed on four layers of surgical gauze and rumen liquor squeezed out of the contents. The pH of the liquor was determined.

In vitro fermentation of rumen contents was carried out by weighing, in duplicate, 50 g of the fresh rumen contents into fermentation flasks of 250 ml capacity after which 100 ml of artificial saliva were added into each flask. The flasks were sealed and immersed in a water bath at 37^{o}C and connected to a water manometer with graduated limbs.

The fermentation flasks were gassed with carbon-

dioxide for 3-5 min at a rate of 3-10 ml/sec to reinstate anaerobic condition. The atmospheric pressure and pressure in the flasks were equilibrated and after the gas production stabilised, the total gas produced at 10 min intervals was read from the manometer for a period of 90-120 minutes. The rumen contents were dried at 105^{0}C for 48 hours and weighed. Correction was made for atmospheric pressure and ambient temperature and results expressed as micromoles gas STPD/gm dry matter.h (Hoppe *et al.*, 1977).

3.5 Blood sampling and analyses of serum vitamin B_{12} and thyroid hormones.

3.5.1 Collection of blood samples.

During the first six weeks of the experimental period, at three week intervals, 8 ml of blood was collected from each goat by jugular venipuncture before the morning feeding. The blood was transferred into sterile universal bottles, allowed to clot and after 4 hours, the serum was decanted into sterile bijoux bottles and stored at -20^{0}C until thawed for vitamin B_{12} analysis. 10 ml of blood was collected during weeks 8, 10, 15, 19 and 23 and divided into two portions: 2 ml was transferred into sterile receptacles containing an anticoagulant, K-EDTA, while serum was obtained from the 8 ml portion.

From the 2 ml sample, mean corpuscular volume (MCV) the Leucocyte and erythrocyte counts were determined using a coulter counter (coulter electronics Icn., Hialeah, Florida), hemoglobin

concentration by a hemoglobinometer (Coultronics, France S.A. 95580, Margency) packed cell volume (PCV) by the capillary method and total blood protein by a refractometer (Atago SPR-T2, Japan).

3.5.2 Vitamin B_{12} assay.

Serum samples were analysed for vitamin B_{12} concentration by radioimmunoassay (RIA) using a vitamin B_{12}/folate dual RIA kit, code CT.302 purchased from Radiochemical centre, Ammersham, U.K. The kit pack-size allowed for analysis of standards and 200 serum samples.

Radioassays are based on the competition of the substance to be measured and a fixed amount of the radioactively labelled tracer for a limited number of specific binding sites. Serum vitamin B_{12} competes with ^{57}Co-labelled vitamin B_{12} for sites on the binding protein -the purified hog intrinsic factor. The amount of ^{57}Co-labelled vitamin B_{12} that binds to the intrinsic factor is inversely proportional to the serum vitamin B_{12} concentration in the reacting medium.

The assay reagents were provided in the kit. Precision pipettes were used to transfer serum, standards and reagents into polystylene assay tubes.

(a) The serum samples and six vitamin B_{12} standards containing 0, 50, 150, 400, 1000 and 2000 pg vit. B_{12}/ml were brought to room temperature before pipetting.

(b) 200 ul aliquotes of the standards and samples were pipetted in duplicate into appropriately labelled assay tubes arranged in a rack.

(c) 100 ul of the working tracer solution

(containing ^{57}Co-labelled vitamin B_{12}) was added into all tubes. Serum vitamin B_{12} was released from serum binding proteins by addition of 100 ul of an alkaline denaturation agent in the presence of potassium cyanide at pH 12.8-13 to all tubes. The tubes were vortex mixed and allowed to stand for 15 minutes at room temperature so that the serum binding proteins were irreversibly denatured.

(d) 1000 ul of a binding protein solution (containing purified hog intrinsic factor) was pipetted into all tubes which were then vortex mixed and incubated for 45 minutes at room temperature.

(e) The tracer and vitamin B_{12} bound to the intrinsic factor were separated from the free vitamin B_{12} and tracer fractions by addition of a tablet of coated charcoal to each tube. The charcoal was allowed to dissolve for 5-10 seconds. The tubes were vortex mixed and incubated at room temperature for 15 minutes to allow adsoption of the free vitamin B_{12} and tracer fractions onto the charcoal.

(f) All tubes were centrifuged at 1500g for 15 minutes and the supernatant decanted into a clean set of tubes.

(g)The supernatant was counted for 60 seconds in a scintillating gamma counter for counting ^{57}Co (Nuclear Enterprises NE 1600).

A curve was plotted for the mean ^{57}Co counts against vitamin B_{12} concentration in the standards (Figure 1). Serum vitamin B_{12} concentration (y) was

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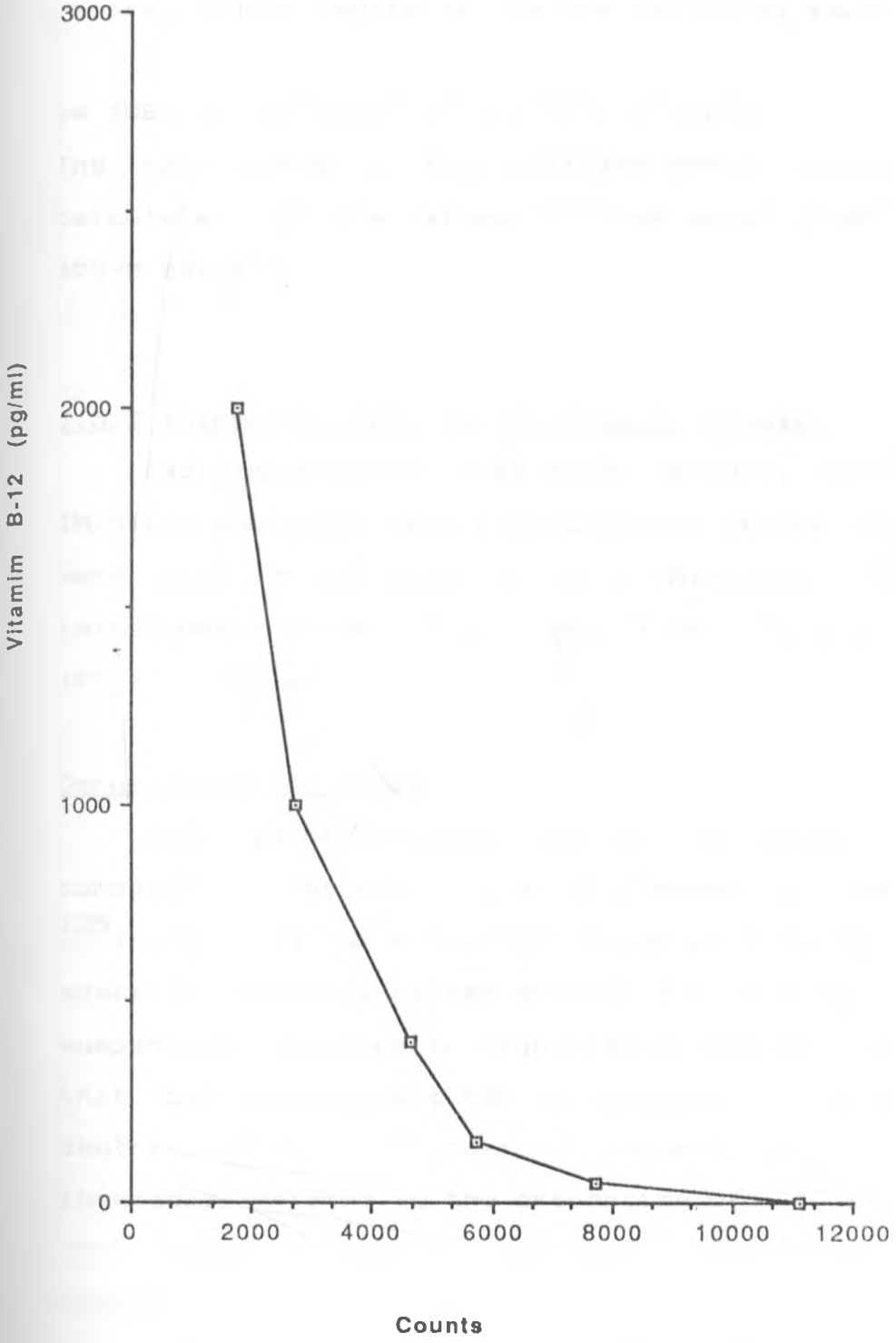

Fig. 1. Standard curve for vitamin B-12 determination

related to the counts (x) by the following equation:

$y = 4086.6 - 1.5x^{2} + 1.9^{-4}x^{2} - 8.1^{-9}x^{3}$; $R^{2} = 0.98$

The mean counts for the duplicate serum samples were calculated and the values obtained substituted in the above equation.

3.5.3 Thyroxine, free T_4 and Free T_3 assays.

Radioimmunoassay kits codes IM.3011, IM.5051 and IM.3101, purchased from radiochemical centre, Ammersham were used for the assay of serum thyroxine (T_4) free tetraiodothyronine (FT_4), and free triiodothyronine (FT_3) in serum.

Serum thyroxine assay.

The radioimmunoassay method is based on the competition between thyroxine present in serum and ^{125}I-thyroxine for a limited number of binding sites on specific antibody raised against T_4. The T_4 antibody suspension is bound to magnetisable polymer particles that can be precipitated by a magnetic field or by centrifugation. Thyroxine present in serum is inversely related to the proportion of ^{125}I-thyroxine that binds to the T_4 antibody. By measuring the proportion of ^{125}I-thyroxine bound to the antibody in the presence of reference standard concentrations of T_4, the serum T_4 concentration is determined.
Assay reagents were provided in the T_4 kit for analysis of 100 serum samples and 12 standards.

(a) 50 ul standards containing 0, 30, 60, 120, 200 and 320 nmol T_4/l in human serum were pipetted

in duplicate and 50 ul serum samples in singleton were pipetted into appropriately labelled assay tubes.

(b) 500 ul of ^{125}I-thyroxine was dispensed into each tube.

(c) 500 ul of the T_4 antibody was pipetted into the tubes, vortex mixed and incubated at room temperature for 45 minutes.

(d) The tubes were centrifuged at 1500g for 15 minutes, the supernatant discarded and the tubes inverted over absorbent tissue for 5 minutes. Droplets adhering to the tube rims were blotted with tissue pad.

(e) All tubes were counted in ^{125}I gamma scintillating counter for 60 seconds (Nuclear Enterprises NE 1600).

The mean counts for duplicate standards were plotted against their T_4 concentrations (Figure 2). Serum T_4 concentration (y) was related to the mean counts (x) by the equation:

$$y = 4105.2 - 2.2x + 4.4^{-4}x^2 - 3.8^{-8}x^3 + 1.2^{-12}x^4 ; \quad R^2 = 0.99$$

From the equation, the serum concentration of TT_4 was calculated.

<u>FT_4 and FT_3 assays.</u>

The radioassay principles applied for FT_4 and FT_3 are similar. Reagents used for the assays are chemically treated to minimise disturbance to the existing equilibrium between the free and protein bound

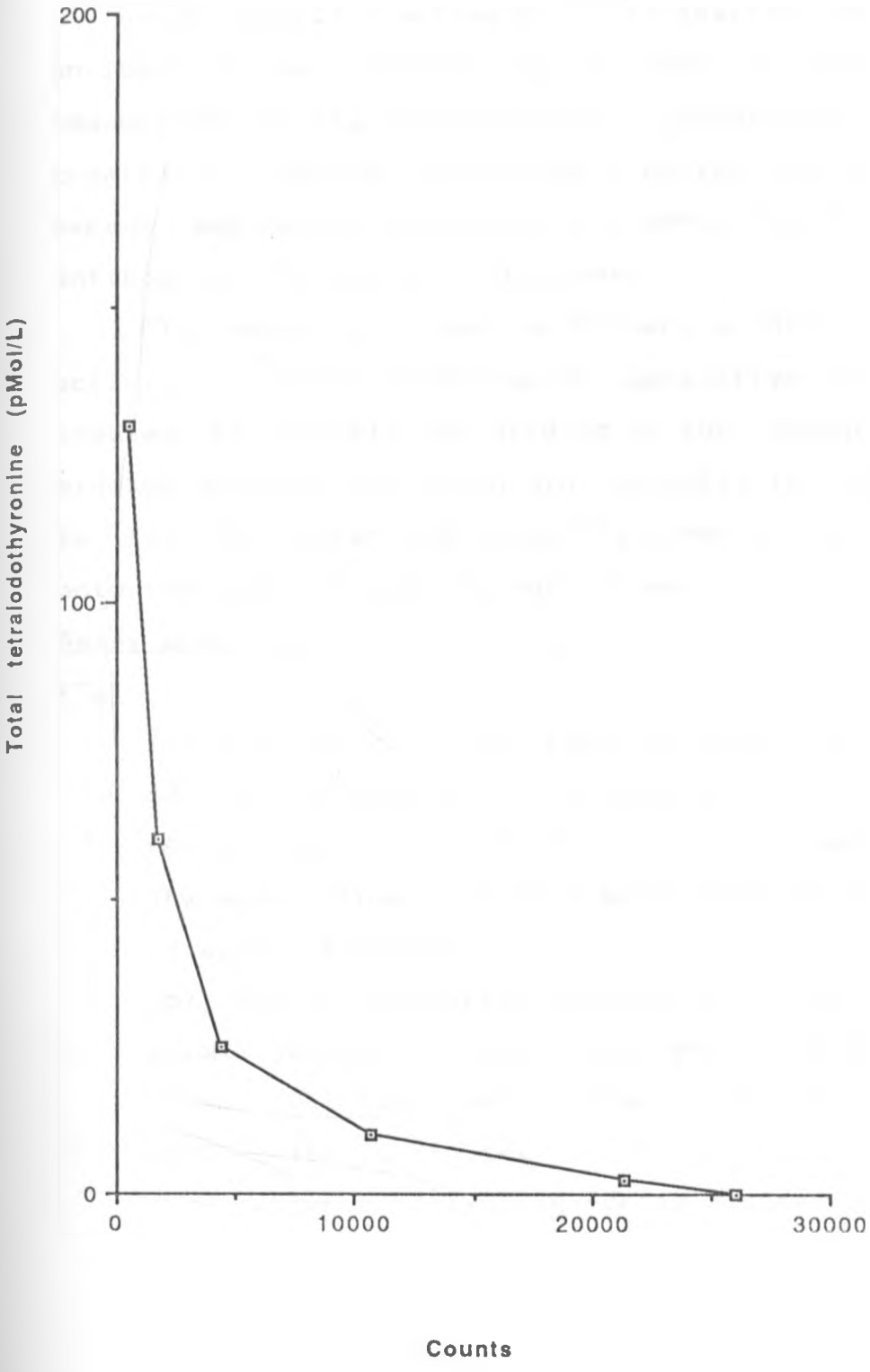

Fig. 2
Standard curve for tetraiodothyronine determination.

fractions of the thyronines in serum.

High specific activity ^{125}I-labelled monoclonal antibody raised against T_4 is used as tracer for measurement of FT_4 concentration. Competition between chemically modified magnetisable polymer particles in excess separation suspension and serum FT_4 for the T_4 antibody is the basis of the assay.

FT_3 assay utilizes, as tracer, a high specific activity ^{125}I-triiodothyronine derivative chemically treated to inhibit its binding to the endogenous T_3 binding proteins but which bind normally to antibodies to T_3. The tracer and serum FT_3 compete for binding onto the high affinity T_3 antibodies.

Assay protocol.

FT_4.

(a) Into appropriately labelled tubes was pipetted 50 ul aliquotes of standards , in duplicate, containing 0, 2.5, 10, 25, 60 and 130 pmol FT_4/l. The same volume of single serum samples were taken in seperate tubes.

(b) 500 ul separation suspension followed by an equal volume of the tracer were added to all tubes. All tubes were vortex mixed and incubated at $37^{O}C$ for 30 minutes.

(c) After centrifuging for 15 minutes at 1500g, the supernatant was discarded and the tubes inverted over absorbent tissue for 5 minutes then pressed firmly over the tissue to drain droplets at the mouth of tubes.

FT_3.

(a) 100 ul aliquotes of standards in duplicate, containing 0, 1.0, 2.5, 10, 20 and 40 pmol FT_3/l

and a similar volume of single serum samples were pipetted into appropriate tubes.

(b) 500 ul ^{125}I-triiodothyronine derivative solution was added to each tube followed by a similar volume of T_3 antibody suspension. All tubes were vortex mixed and incubated at 37^0C for 2 hours.

(c) The tubes were centrifuged and the supernatant discarded.

The tubes for both assays were counted in an ^{125}I gamma counter (Nuclear enterprises NE 1600). Seperate FT_4 and FT_3 standard curves were plotted (Figure 3 and 4). The concentrations of the two thyronines (y) in the standards were related to the mean counts (x) by the following equations:

FT_3; $y= 1.1^4-871.6x+37.4x^2-0.5x^3$; $R^2=0.99$

FT_4; $y=2.5^4-1402x+23.6x^2-0.1x^3$; $R^2=0.98$

Serum concentration of the two thyronines were calculated by substituting the mean counts (x) in the above equations.

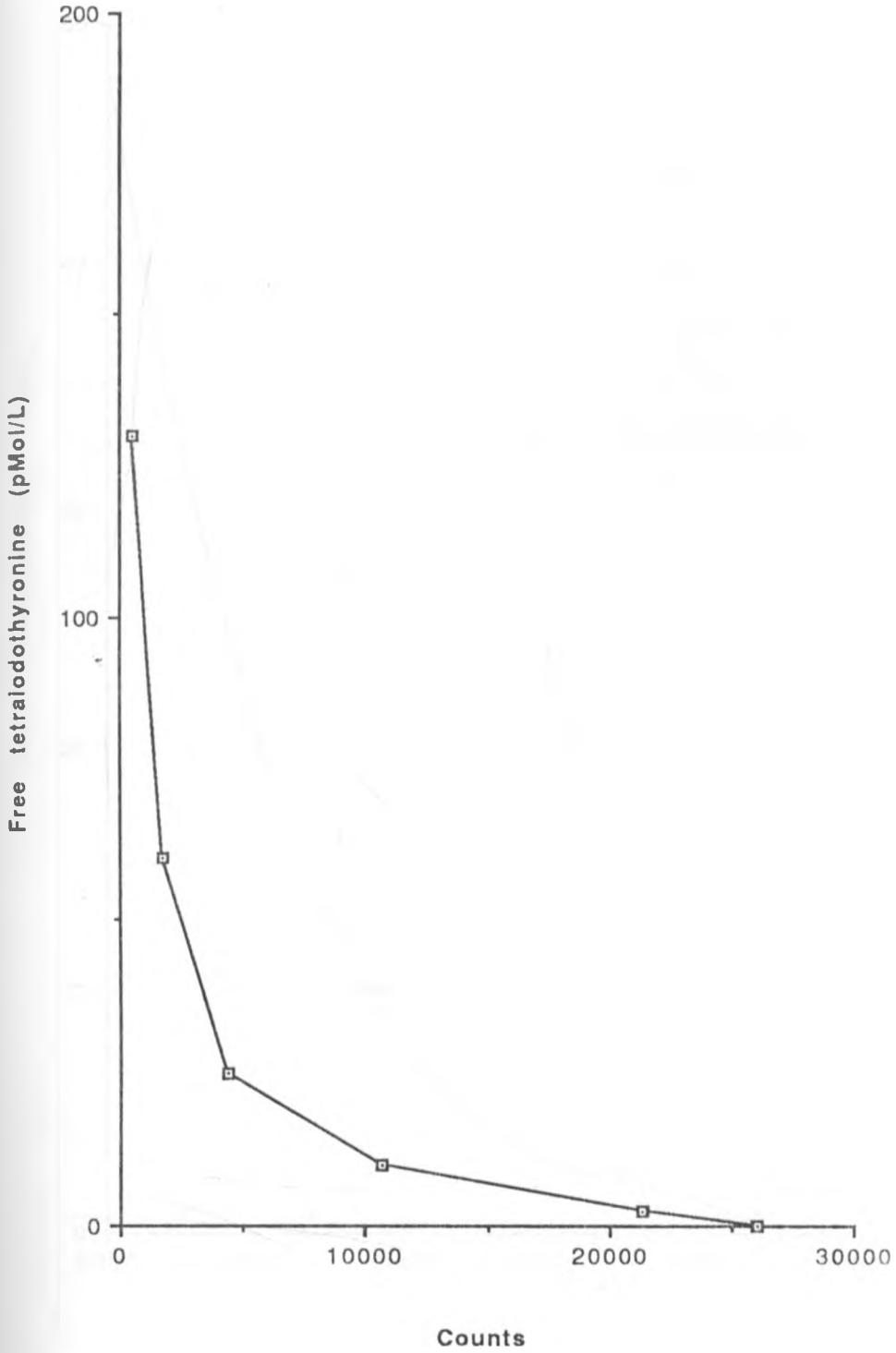

Fig. 3 .
Standard curve for free tetraiodothyronine determination.

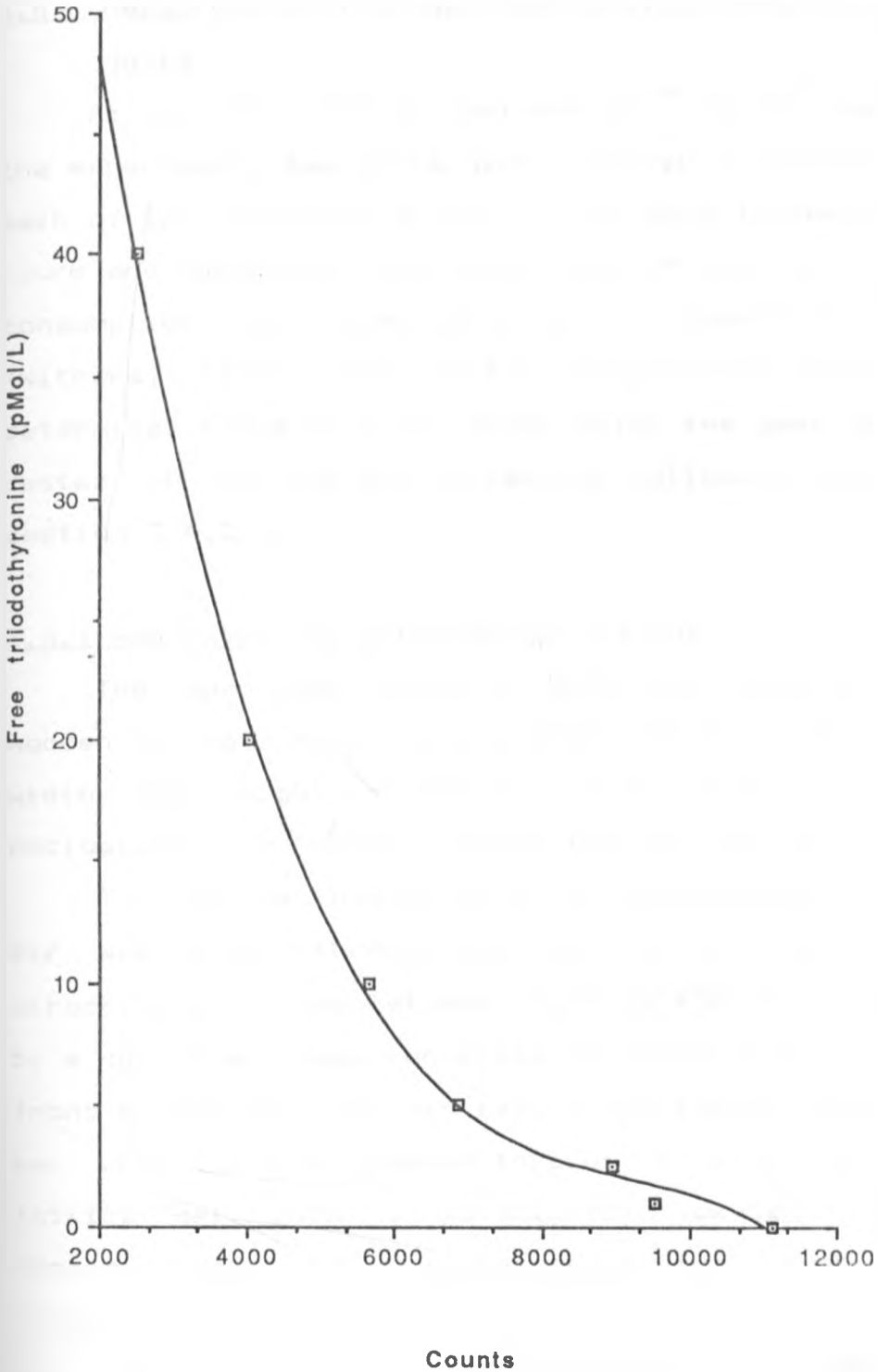

Fig.4.
Standard curve for free triiodothyronine determination.

3.6 Measurement of oxygen consumption by the resting goats.

At the 7^{th}, 11^{th} and between 23^{rd} to 27^{th} weeks of the experiment, two goats were selected at random from each of the treatment groups. They were fasted for 24 hours and thereafter the goats'rate of resting oxygen-consumption was measured using an open-flow system (Withers, 1977). Each goat's oxygen-consumption was determined twice in a day after which the goat was fed, rested for one day and killed the following day (see section 3.4.2.3).

3.6.1 Apparatus and experimental layout.

The open mask (Withers, 1977) was replaced by a wooden box measuring 96.6 x 50.5 x 74.5 cm in length, width and height respectively that acted as a mask enclossing the whole animal (Taylor *et al.*, 1982).

The box was placed in a room maintained at $23^{o}C$. Air was drawn through the box in a rear-to-front direction at a rate between 3600 to 4500 lh^{-1} at STPD by a downstream pump connected by rubber tubing to the front of the box. An air sample was tapped between the box and the pump, passed through a water absorbent (silica gel) into a paramagnetic oxygen analyser (Beckman Model F3) calibrated at full span of 1% oxygen.

At the start of the experiment, air was drawn through the box for a minimum period of two hours and the fractional oxygen concentration in the room air, F_I, recorded. The air flow was maintained constant for the whole day.

Oxygen consumption was determined for two goats in

a day, one from each treatment. A goat was weighed and enclossed in the box for a period of 90 minutes. The goat consumed oxygen from the air entering the box at a rate, $\dot{V}O_2$ Lh^{-1}, thus reducing the fractional oxygen concentration of air leaving the box to F_E'. The difference, $(F_I - F_E')$ was recorded by an automatic recorder connected to the oxygen analyser for the 90 minutes. Two 90 minute measurements, one in the morning and the other in the afternoon, were conducted for each goat. The fractional oxygen concentration change for the first 15 minutes of each 90 minutes run was ignored as the goats tended to be restless when first placed in the box. Ten representative points for $(F_I - F_E')$ on the recording chart, were read for the remaining 75 minutes of a run.

Daily, the air flow through the box, V_{box} was determined by 'replacement' of the goat with a tube that introduced nitrogen into the box at a known rate, $\dot{V}_{N2}$, from an N_2 gas-cylinder. The N_2 "diluted" oxygen in the box so that a fractional O_2 concentration, F_E, in the air leaving the box when N_2 was passed, and which was almost equal to that due to oxygen consumption when the goat was in the box, was recorded. In this case, $\dot{V}_{N2}$ was maintained at 23.8021 lh^{-1} at STPD until F_E recorded stabilised for a period of 10 minutes.

By applying the universal gas law, all gas mesurements were converted to standard temperature and pressure (STP) taking barometric pressure at Nairobi to be 632 mmHg and the appropriate room temperature on the Kelvin scale.

3.6.2 Calculations.

According to Taylor, C.R. *et al.*, (1982),

If F_I = Fractional O_2 concentration in room air (STPD).

F_E = Fractional O_2 concentration in room air when N_2 is passed through the box (STPD).

$F_{E'}$ = Fractional O_2 concentration in room air when goat is in the box (STPD).

$\dot{V}_{N2}$ = Nitrogen flowing into the box in $1h^{-1}$ (STPD).

$\dot{V}_{box}$ = Air flow through the box in $1h^{-1}$ (STPD).

$$\text{then } F_E = \frac{F_I (V_{box} - V_{N2})}{V_{box}}$$

and solving the equation for V_{box};

$$\dot{V}_{box} = \frac{F_I \dot{V}_{N2}}{(F_I - F_E)}$$

The oxygen consumption by the goat, $\dot{V}_{O2}$, in $Lkg^{-1}h^{-1}$;

$$\dot{V}_{O2} = \frac{\dot{V}_{box} (F_I - F_{E'})}{\text{Goat wt. (Kg) x 0.96.}}$$

The constant 0.96 is obtained assuming an $R.Q = \dot{V}_{CO2}/\dot{V}_{O2} = 0.8$

$\dot{V}_{O2}$ was calculated for the ten representative points for $F_{E'}$ taken over the 75 minutes period. The

difference ($F_I - F_E$) was read directly from the analyser therefore, it was not necessary to determine the absolute value of F_I (Withers, 1977).

3.7 Statistical analyses.

The data collected were subjected to regression analysis using the Least-squares and maximum likelihood computer program (Harvey, 1987). Two models were fitted:

(a) At the start of the trial, the goats varied in age. To control the variation in the dependent variables due to the age differences, each goat's age at the beginning of the trial was fitted as a covariate (Steel and Torry, 1987).

The dependent variables were:

(i) Hay intake as a percentage of intake at the start of the trial;

(ii) Liveweight as a percentage of liveweight at the start of the trial;

(iii) Body condition scores;

(iv) Serum vitamin B_{12} levels;

(v) Serum levels of the thyronines;

(vi) Rate of oxygen consumption.

The following general model was fitted:-

$$Y_{ijklm} = u + T_i + S_j + W_k + b(A_{ijkl} - B) + e_{ijklm}.$$

Where,

Y_{ijklm}: A dependent observation for the m^{th} goat;

u: An underlying constant;

T_i: Effect of the i^{th} treatment;

S_j: Effect of the j^{th} sex;

W_k: Effect of the k^{th} week on experiment;

b: Partial regression coefficient of the age

of a goat at the beginning of the experiment on the dependent variable;

B: The mean age of the goats at the beginning of the trial;

A_{ijkl}: The age of a goat (months) during the i^{th} week of experiment

e_{ijklm}: The residual component.

(B) The data on rumen functions and blood parameters were analysed using the same general model where the dependent variables were:-

(i) Digestibility coefficients of the nutrients;

(ii) pH, concentrations of volatile fatty acids and ammonia in the rumen liquor;

(iii) Disappearance of dry matter and acid detergent fibre from the nylon bags;

(iv) Gas production from *in vitro* fermentation of rumen contents;

(v) Blood parameters (erythrocyte and total leucocyte counts, hemoglobin concentration, mean cell volume, packed cell volume and total plasma protein).

$$Y_{ijkl} = u + T_i + W_j + H_k + e_{ijkl}.$$

Where;

Y_{ijkl}: A dependent observation for the l^{th} goat;

u: An underlying constant;

T_i: Effect of the i^{th} treatment;

W_j: Effect of the j^{th} week on experiment;

H_k: Effects of the k^{th} hour of sample collection (where applicable);

e_{ijkl}: The residual component.

The least-squares means and their standard errors were reported for all the dependent observations.

CHAPTER FOUR

4.0. RESULTS AND DISCUSSION.

4.1. Feed intake in vitamin B_{12} deficiency.

The age and sex of the goats affected intake of hay regardless of the Treatment ($P<0.01$). The mean daily hay intake was higher for older goats and ranged between 208.4 ± 35.68 g and 664.1 ± 38.00 g for goats aged 13 and 42 months at the beginning of the experiment. Owing to the wide age differences, the mean daily hay intake varied widely within the same Treatment. On average, males had a higher daily intake than females (table 4). The daily hay intake, expressed as a percentage of the intake at the beginning of the experiment for each goat, was not significantly ($P>0.05$) different between the two Treatments (Fig.5). The average daily intake, expressed as a percentage of the intake at the beginning of the trial for each goat, was 90.5 ±0.87% and 91.7 ± 0.95% in cobalt adequate and deficient goats respectively, for the whole experimental period.

When intake was expressed as dry matter of hay per metabolic body weight (g $DM/W^{0.75}$), there was no significant difference ($P>0.05$) between the cobalt supplemented and deficient goats (Fig. 6). In both Treatments, males had significantly higher daily total feed intake ($P<0.01$) and intake per metabolic body weight ($P<0.05$) than females (Table 4).

In ruminants, development of vitamin B_{12} deficiency leads to a gradual loss of appetite (Underwood, 1981). When the liver vitamin B_{12} in sheep falls below 0.15 ug/gm, feed intake declines as a consequence of an inability by the animal tissues to

Table 4. Daily feed intake

	Intake g (DM)/goat/day	
	Co adequate	Co deficient
Hay	417.8±23.17	425.9±20.35
Concentrate	127.8± 0.00	127.8± 0.00
Total intake	545.6±23.17	552.9±20.35
Intake per metabolic body weight	41.9±1.52	43.8±1.33
	Males	Females
Total feed intake		
Co adequate	620.9±31.49**	519.4±29.28
Co deficient	670.1±24.79**	439.5±29.69
Intake per metabolic body weight		
Co adequate	46.2±2.06*	41.6±1.32
Co deficient	47.4±1.63*	40.2±1.95

Key: ** Intake for males was higher than for females at (P<0.01) and * at (P<0.05).

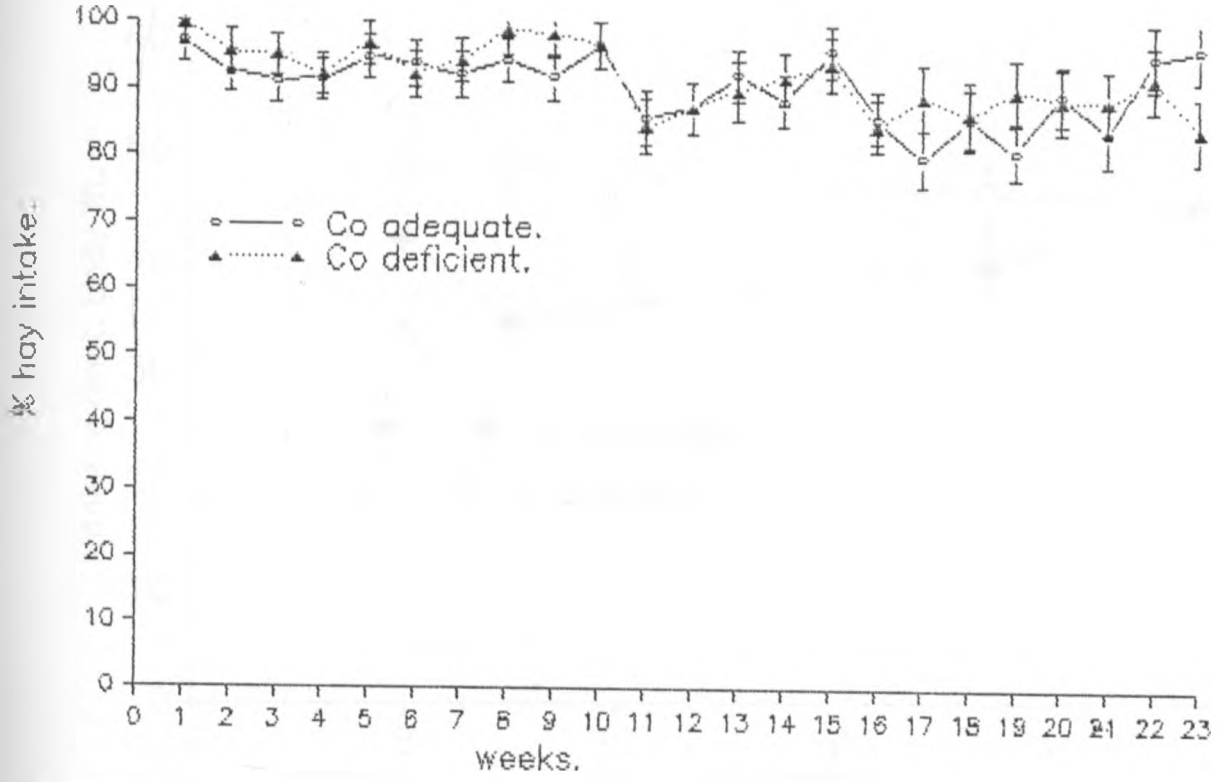

Fig. 5. Hay intake as a percentage of intake at the start of the trial. Vertical bars represent the mean standard errors.

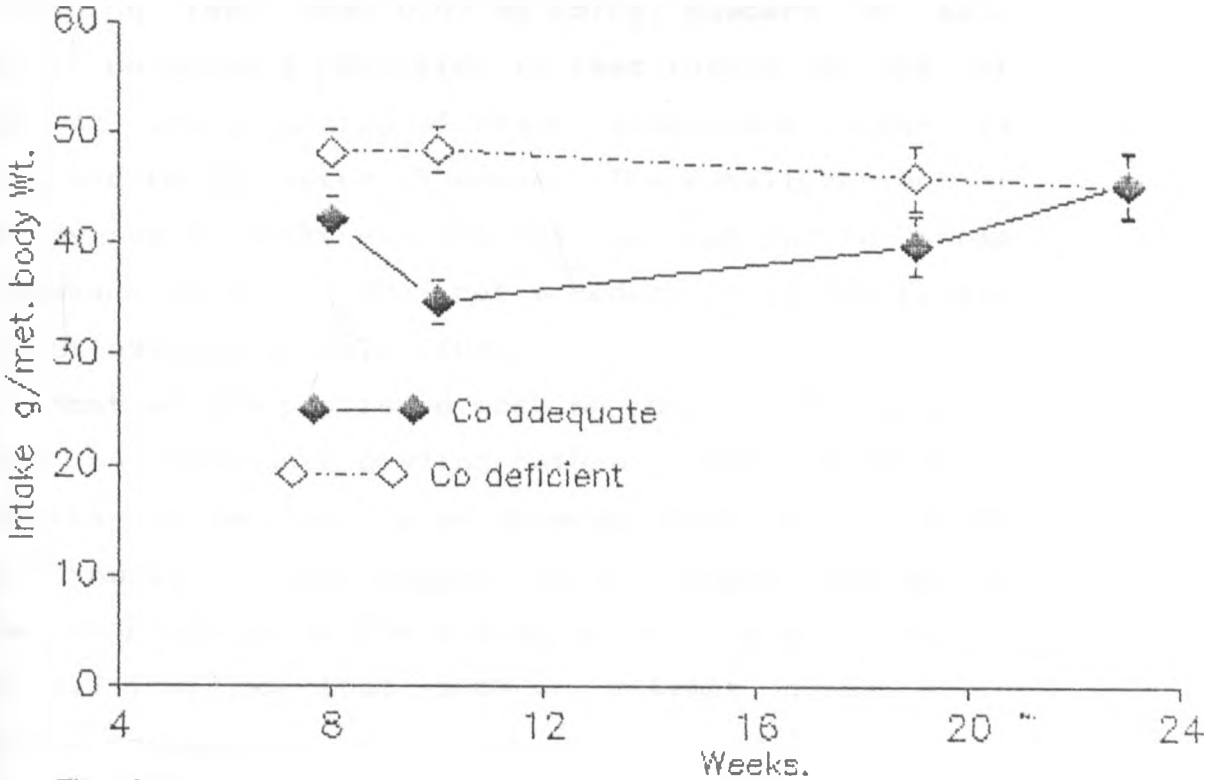

Fig. 6. Hay intake per metabolic body weight.
Vertical bars represent the mean standard errors.

metabolise propionate produced by fermentation in the rumen (Marston *et al.*, 1961).

The duration after which a loss of appetite is observed when an animal is put on a cobalt-deficient feed is dependent on the animal's age and the cobalt level of the diet (Underwood, 1981). In lambs on a cobalt deficient ration, anorexia has been reported after 10 to 12 weeks (Bremner *et al.*, 1988; Jones and Anthony, 1970) while in adult sheep fed on a diet containing less than 0.02 mg Co/Kg, Dawbarn *et al.*, (1957) observed a reduction in feed intake to 87% of that at the beginning of their experiment after 21 weeks and to 66% after 35 weeks. The cobalt content of the hay fed to goats was the same as that fed to sheep by Dawbarn *et al.*, (1957) but a reduction in hay intake did not develop in this study.

Most of the published work on cobalt dificiency is based on ruminants grazing natural pastures in which anorexia is reported as an observed sign (e.g. Jones and Anthony, 1970 and Bremner *et al.*, 1988) but do not show quantitatively the reduction in intake owing to the difficulties that such an attempt presents in grazing conditions.

Feed intake in cobalt-deficient goats has not been documented previously. A loss of appetite directly attributable to vitamin B_{12} deficiency did not develop in this trial. It is doubtful that diminishing vitamin B_{12} status in goats on feed containing 0.02 mg Co/Kg for twenty-three weeks results in a loss of appetite. Anorexia, as a sign of deficiency in goats, might occur at a later stage than it does in sheep.

4.2 Effects of cobalt deficiency on the digestion of nutrients.

The coefficients of apparent digestibility of nutrients in the feed were higher in the cobalt adequate than deficient goats (Table 5) although not significantly ($P>0.05$).

Dietary intake of nitrogen and faecal nitrogen loss were comparable between the two treatments but the urinary nitrogen loss was significantly ($P<0.05$) higher and nitrogen retention significantly ($P<0.05$) lower in the deficient than in vitamin B_{12} adequate goats (Table 6).

Figures 7 and 8 and Tables 7 and 8 shows percentage disappearance of dry matter (DM) and acid-detergent fibre (ADF) from the nylon bags incubated in the rumen of cobalt deficient and supplemented goats for periods between six and seventy-two hours, after correcting for the 'zero-hour' disappearance. The disappearance of the two components was not significantly different ($P>0.05$) between the two Treatments. In both deficient and cobalt adequate conditions, less than 20% of the incubated DM and ADF had left the bags after incubation for 72 hours.

However, the potential DM degradability (b) and the total DM degradation (a+b) were significantly ($P<0.01$) higher for the cobalt supplemented than deficient goats (Table 7).

The effects of cobalt on digestibility of nutrients are variedly reported by different authors: Becker and Smith (1949) found cobalt supplemented lambs digested more efficiently the ether-soluble, nitrogen-free extracts, organic and dry matter fractions of the

Table 5. Coefficient of apparent digestibility of feed nutrients.

Component	coefficient of apparent digestibility (%)	
	Co adequate	Co deficient
Dry matter	57.1 ± 1.64	55.9 ± 1.48
Organic matter	51.3 ± 1.49	48.8 ± 1.33
Crude fibre	62.9 ± 2.39	62.9 ± 2.12
Ether extract	65.5 ± 2.66	60.6 ± 2.41
Nitrogen-free extract	43.5 ± 2.30	41.9 ± 2.08
Crude-protein	59.3 ± 2.32	58.0 ± 2.17

Key: Means between the two treatments were not significantly ($P>0.05$) different.

Table 6. Nitrogen balance.

Parameter	No of goats	Co adequate	Co deficient
N Utilization			
(g per day)			
N intake		15.2±2.18	15.7±7.58
Faecal N		11.9±2.19	12.8±7.20
Absorbed N		3.1±1.14	2.9±1.05
Urinary N		2.9±1.15	4.2±2.02*
Retained N		0.14±0.06*	-1.28±0.94
Retained N/N intake		5.6×10^{-3}	-4.9×10^{-2}
Retained N/absorbed N		4.5×10^{-2}	-4.4×10^{-1}

Key: *; Urinary N loss higher and retained N lower in Co deficient than in Co adequate goats at ($P<0.05$).

Table 7 . Disappearance of hay (DM %) incubated in the rumen.

	Percent DM disappearance	
Incubation time (h)	Co adequate	Co deficient
6	8.4±1.58	7.9±1.57
12	8.8±1.57	8.5±1.58
24	9.7±1.56	11.8±1.55
48	12.6±1.57	13.8±1.57
72	16.9±1.59	19.8±1.57
a: soluble DM	6.814	6.456
b: Insoluble degradable DM	23.243	34.126
(a+b):Total DM degradation	30.057	40.582
c: degradation rate constant	0.0069	0.0063

Key: **; Value in Co adequate were higher than in Co deficient at ($P<0.01$). a,b and c: see section 3.4.1.

Table 8. Disappearance of ADF (%) from hay incubated in the rumen.

Incubation time (h)	Percent ADF disappearance	
	Co adequate	Co deficient
6	6.6±1.22	4.2±1.22
12	7.1±1.20	4.5±1.23
24	7.3±1.21	5.5±1.21
48	10.0±1.20	8.9±1.22
72	13.3±1.23	13.9±1.24
a: Soluble ADF	5.328	1.756
b: Insoluble degradable ADF	70.828	78.705
(a+b): Total ADF degradation	76.156	80.461
c: degradation constant	0.0015	0.0022

Key: Effect of Co supplementation on ADF disappearance was not significant ($P>0.05$). a,b and c: see (section 3.4.1.)

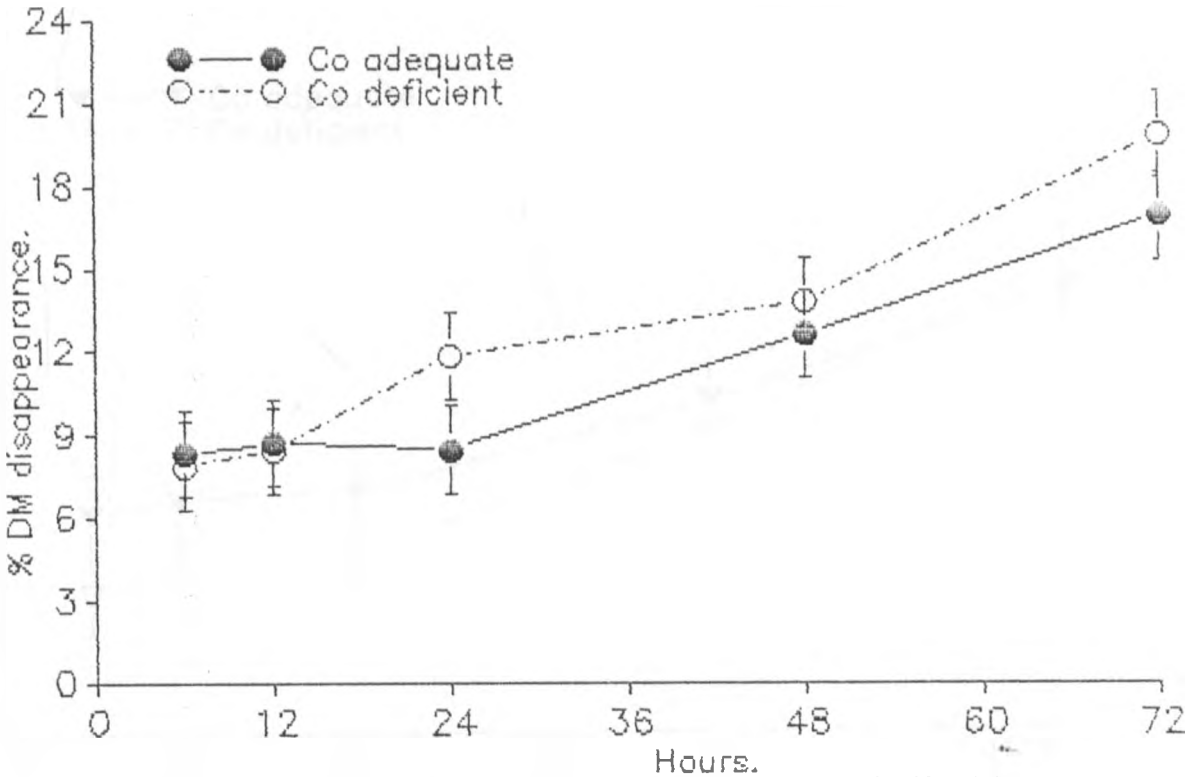

Fig. 7. Disappearance of hay from nylon bags. Vertical bars represent the standard error of the means.

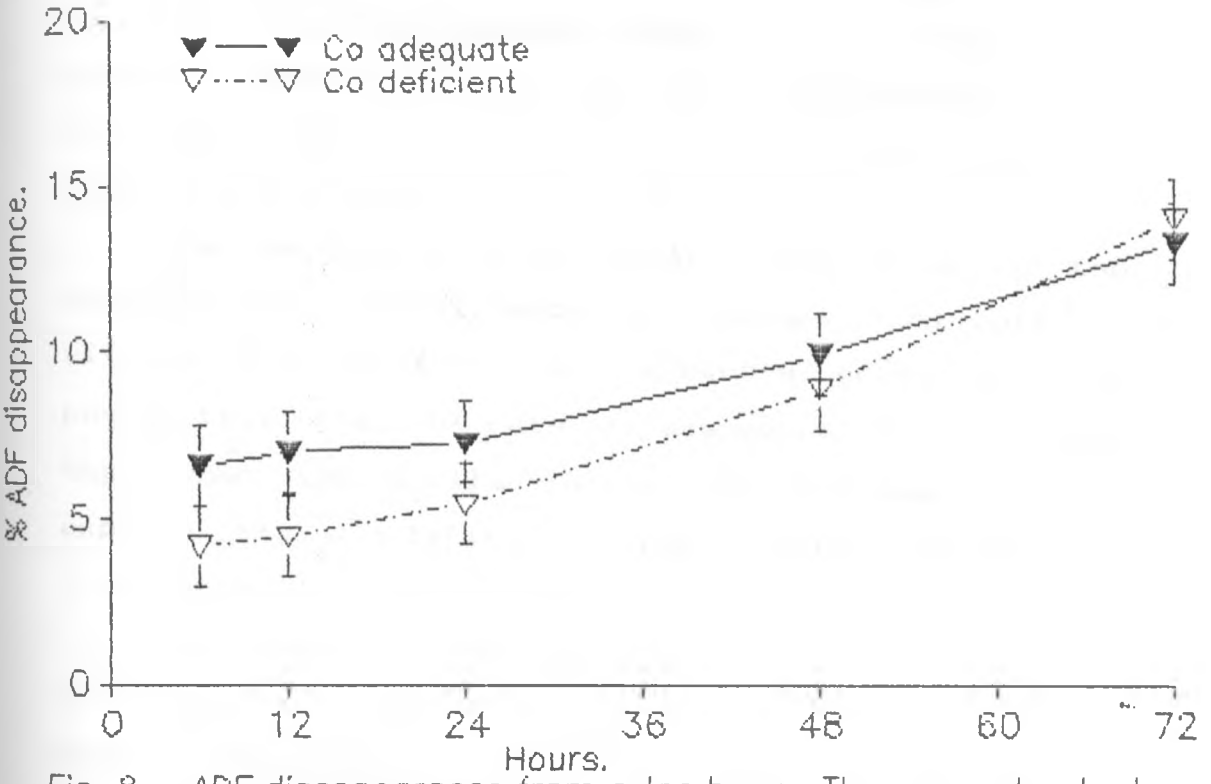

Fig. 8. ADF disappearance from nylon bags. The mean standard errors are represented by vertical bars.

diet while the cobalt deficient lambs showed a significantly higher coefficient of apparent digestibility for the fibrous fraction of the ration.

Supplementation of high levels of cobalt to calves (Saxena and Ranjhan, 1978) and to sheep (Pal *et al.*, 1980; Krasnodebska, 1984) has been shown to improve digestibility of cellulose, crude-fibre, ether-extracts and retention of nitrogen.

In support of previous findings by Becker and Smith (1949) the apparent coefficients of digestibility of dry and organic matter, ether and nitrogen free extracts were higher for the cobalt supplemented than deficient goats although the differences were not significant ($P>0.05$).

The absence of significant differences in the digestibility coefficients of dietary nutrients in vitamin B_{12} deficient and adequate goats and the possibility that the rate of passage of feed through the rumen was similar in the two treatments could explain the similarity in feed intake between the Treatments.

The course of digestion appears to be affected by the quality of the diet: hay fed to the goats had a high crude fibre and low crude protein contents as it was overmature at harvesting (Table 2) and therefore fermentation of the cellulosic material in the rumen was slow as depicted by the slow disappearance of dry matter and acid-detergent fibre from the nylon bags when incubated for 72 hours. However, the small differences in the digestibility coefficients between the two Treatments might reflect the effects of cobalt supplementation under the constraints imposed on the

rumen fermentation processes by the poor quality hay.

In their effects on fermentation of cellulose, it is possible that the quality of the feed takes precedence over the dietary level of cobalt and therefore, supplementation of cobalt does not confer significant benefits to the fermentation of cellulose in poor quality roughage.

The higher urinary nitrogen loss and reduced nitrogen retention in cobalt deficiency is explained by a reduction in the tissue levels of vitamin B_{12} containing enzyme ,5-methyltetrahydrofolate-homocystein methyltransferase, which participates in transmethylation of homocystein to methionine in the liver (Gawthorne and Smith, 1974). Methionine acts as the initiating amino acid in protein biosynthesis in the tissues and a deficiency of this amino acid results in reduced tissue protein retention.

4.3. Rumen liquor volatile fatty acids, pH and ammonia concentrations.

The concentrations of the main volatile fatty acids in the rumen liquor collected at various intervals for 15 hours following feeding hay to the goats are presented in Table 9 and Figures 9 to 11. The concentration of acetic acid was not significantly different ($P>0.05$) between cobalt adequate and deficient goats. Rumen liquor obtained from cobalt supplemented goats contained a significantly higher concentration of propionic, butyric, valeric ($P<0.01$) and isovaleric ($P<0.05$) acids than the cobalt deficient

Table 9. Effects of cobalt on volatile fatty acids concentration in rumen liquor.

Hour	Cobalt status	Sample No	Volatile fatty acids (mg/100 ml). Acetic	propionic	Butyric	Valeric	isovaleric
0	A	4	366.5±45.47	183.4±25.28**	35.8±4.98**	4.0±0.75**	3.2±1.19**
	D	4	301.5±45.56	69.0±25.00	21.9±5.97	1.5±0.75	1.8±1.17
3	A	4	311.2±45.46	138.9±24.88**	52.8±6.00**	5.7±0.75**	2.1±0.19**
	D	4	282.5±46.45	71.1±25.28	19.7±5.85	n.s	n.s
6	A	4	300.1±45.50	224.1±24.22**	53.9±6.86**	6.5±0.65**	1.3±1.16**
	D	4	294.6±44.90	85.3±20.55	20.8±5.91	n.s	n.s
9	A	4	311.6±43.95	192.1±26.93**	49.9±5.33**	3.0±0.76**	3.2±1.20**
	D	4	366.8±46.01	146.6±21.89	45.7±6.44	2.6±0.47	n.s
11	A	4	314.8±50.23	323.8±18.86**	48.7±6.33**	4.2±0.75**	1.2±0.20**
	D	4	390.4±45.47	184.2±26.75	35.8±6.43	3.9±0.72	n.s
15	A	4	324.7±45.85	224.3±26.13**	52.0±5.26**	6.1±0.71**	3.1±1.18**
	D	4	399.9±50.12	169.2±26.54	33.6±5.26	3.9±0.65	n.s
Mean	A	24	324.8±18.56	214.4±10.32**	48.6±4.44**	4.9±0.31**	2.6±0.49**
	D	24	338.6±16.83	120.9±10.32	29.4±2.49	2.6±0.32	0.8±0.50

Keys: A: Cobalt adequate; B: Cobalt deficient. **: VFA concentration in cobalt adequacy was higher than in deficiency during the same hour at (P<0.01). n.s: Insignificant VFA concentration.

ones.

A peak concentration of propionic acid was attained four hours after the goats were fed on the concentrate (Fig. 10). The rise in concentration of the other acids following the feeding of hay in the morning was gradual and inconsistent and the change following feeding of the concentrate was not as distinct as was for propionic acid. The acetate to propionate ratio was 1 :1.6 and 1 : 3.3 for coablt adequate and deficient goats respectively.

The pH of liquor obtained from the cobalt supplemented goats was consistently lower ($P<0.01$) than that from deficient goats over the 15 hours (Table 10 and Fig. 12). The average pH over the fifteen-hour period was 6.4 for cobalt adequate and 6.9 for the deficient goats respectively. In both Treatments, the lowest pH was attained two hours after feeding the goats on the concentrate and thereafter, a gradual rise to the pre-feeding value occured.

The mean free ammonia concentration in the liquor for the 15 hours was significantly ($P<0.01$) higher for cobalt adequate than deficient goats except two hours after offering the concentrate when, transiently, the concentration in both Treatments were equal (Fig. 13). The mean ammonia concentration was 101.9 $\pm$ 10.34 and 45.4 $\pm$ 9.42 mg/l (Table 10) of the liquor in the cobalt supplemented and deficient goats respectively.

The rumen liquor pH in cobalt adequate was lower than in deficient goats due to the consistently higher concentration of propionic acid in the liquor from the cobalt supplemented goats. In calves (Saxena *et al.*,1980) and in *in vitro* rumen cultures (Mcdonald and

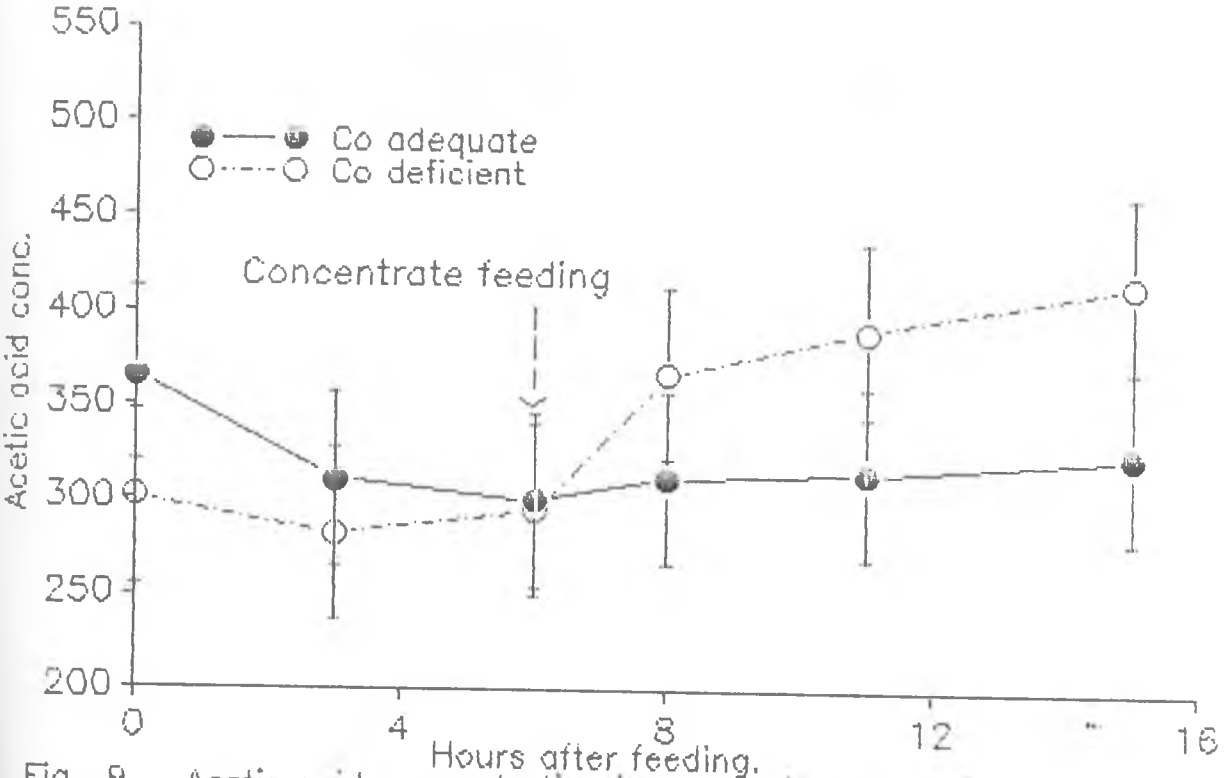

Fig. 9. Acetic acid concentration in rumen liquor (mg/100 ml). The mean standard errors are represented by vertical bars.

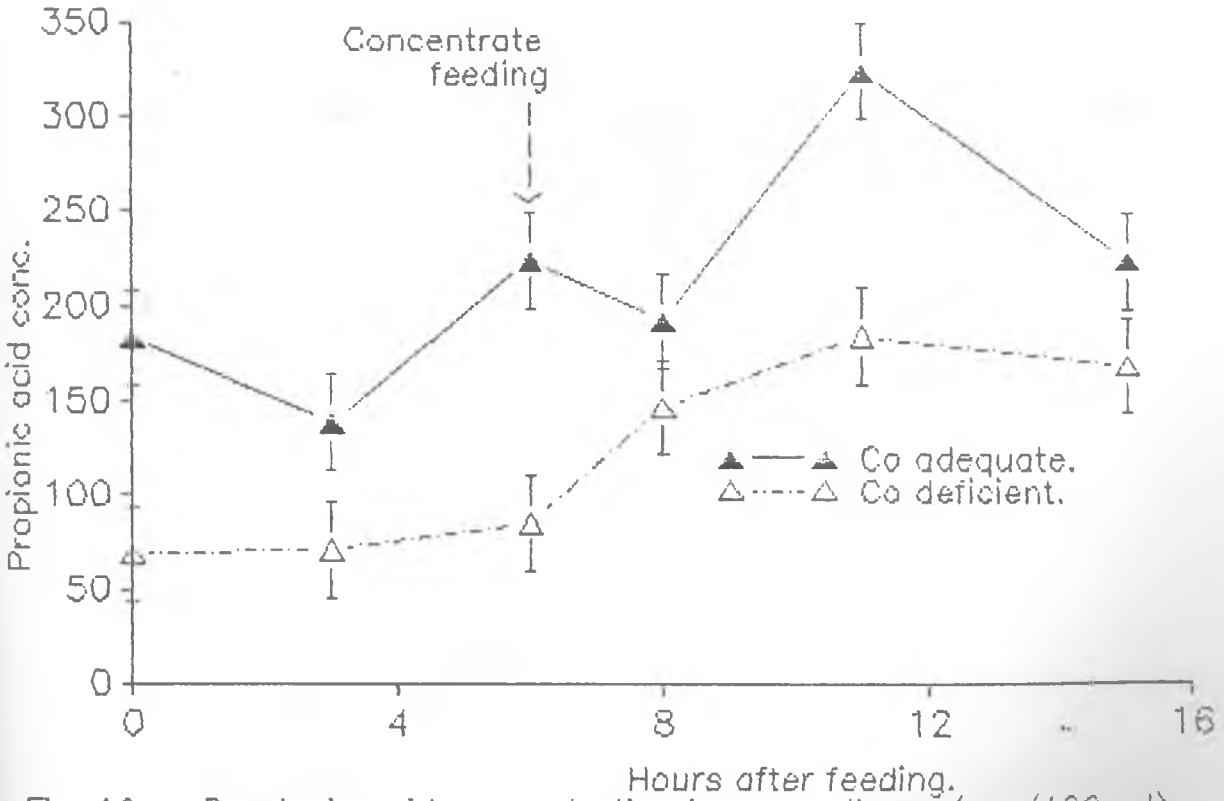

Fig. 10. Propionic acid concentration in rumen liquor (mg/100 ml). Vertical bars represent the mean standard errors.

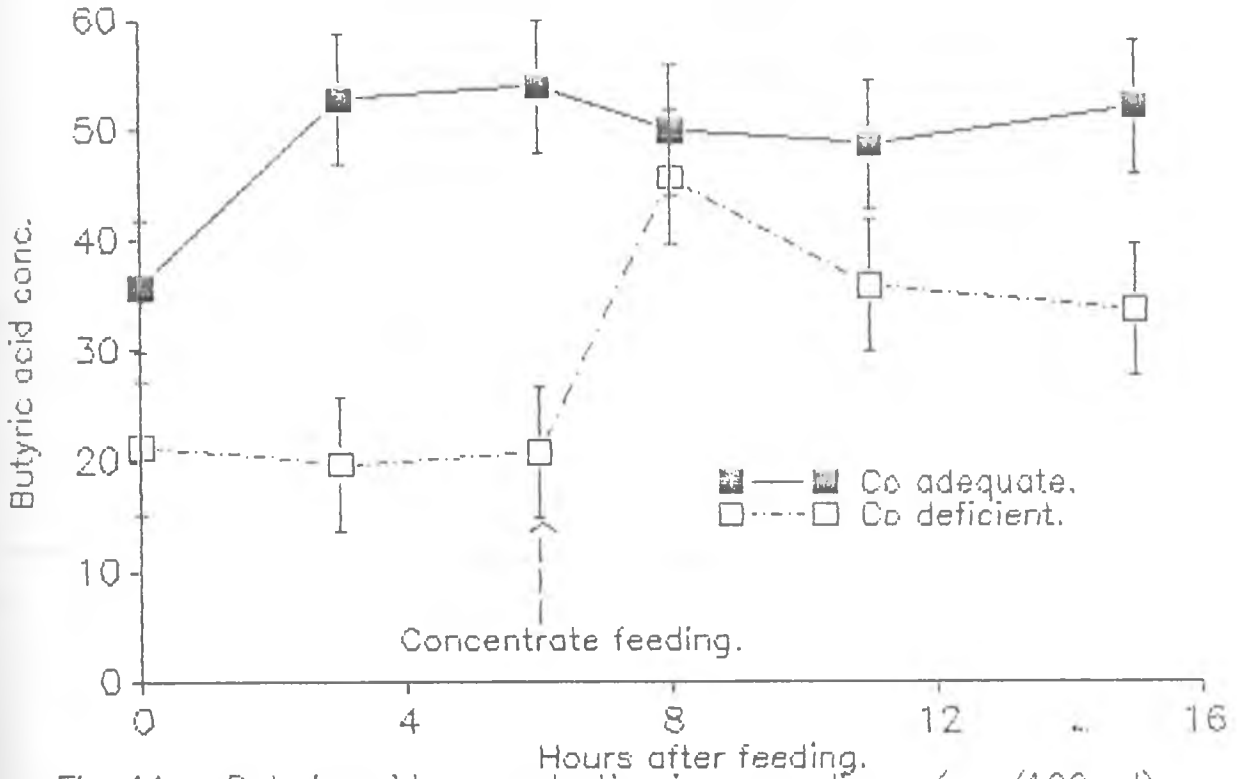

Fig. 11. Butyric acid concentration in rumen liquor (mg/100 ml). Vertical bars show the mean standard errors.

Table 10 Rumen liquor pH and ammonia concentration.

Hours after feeding	Liquor pH.			Ammonia concentration (mg/l)		
	No of samples	Co adequate	Co deficient	No of samples	Co adequate	Co deficient
0	4	6.6±0.12	7.2±0.12**	4	95.6±8.42**	39.2±8.42
3	4	6.5±0.11	7.1±0.12*	4	80.5±9.00**	32.9±7.56
6	4	6.6±0.13	6.9±0.13	4	102.2±6.24**	37.9±8.89
8	4	5.8±0.16	6.6±0.15*	4	109.7±8.44	105.0±9.32
11	4	6.3±0.14	6.5±0.16*	4	96.3±7.66**	68.7±9.60
15	4	6.3±0.12	6.8±0.13*	4	97.3±8.33**	28.9±8.23
Overall mean	24	6.3±0.13	6.9±0.16*	24	101.9±0.34**	45.42±0.34

Key : **, *; Liquor pH in Co deficiency was higher than in Co adequacy and the ammonia concentration in Co adequacy was higher than in deficiency at (P<0.01) and (P<0.05) respectively.

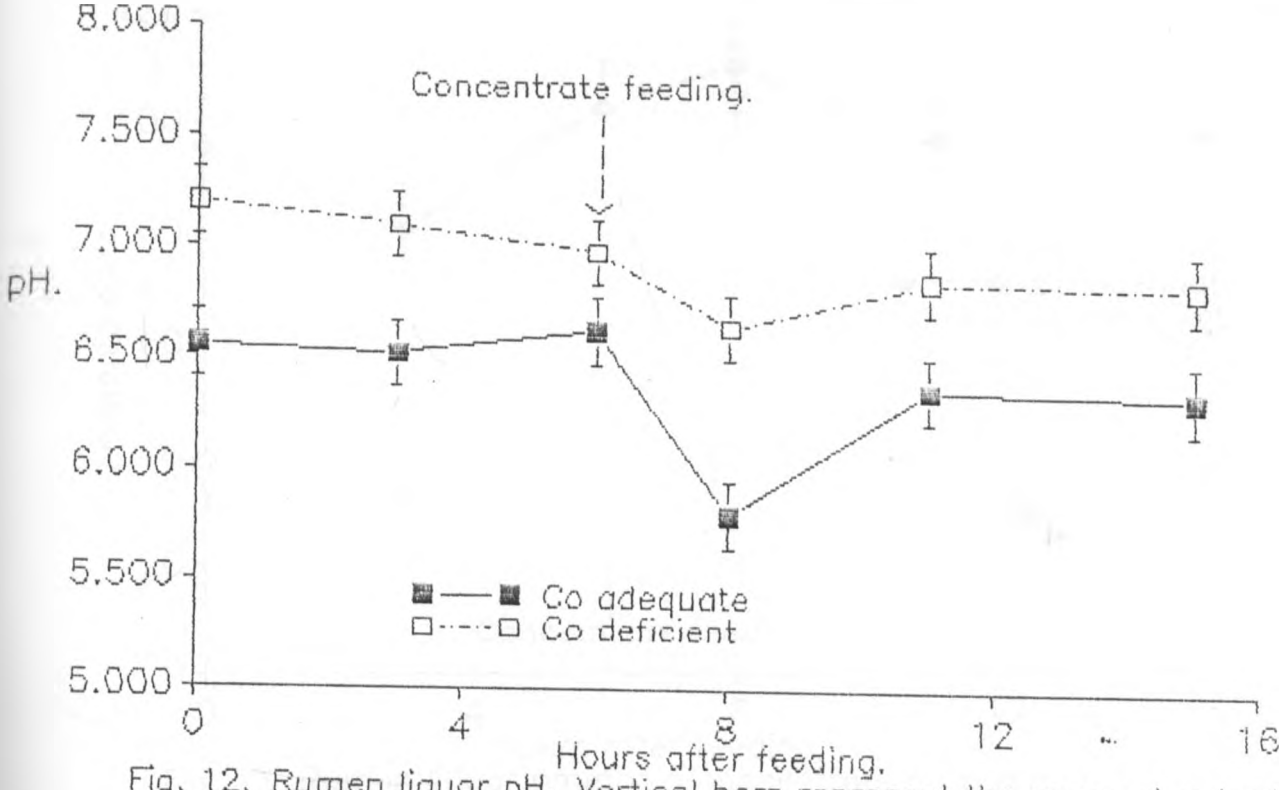

Fig. 12. Rumen liquor pH. Vertical bars represent the mean standard errors.

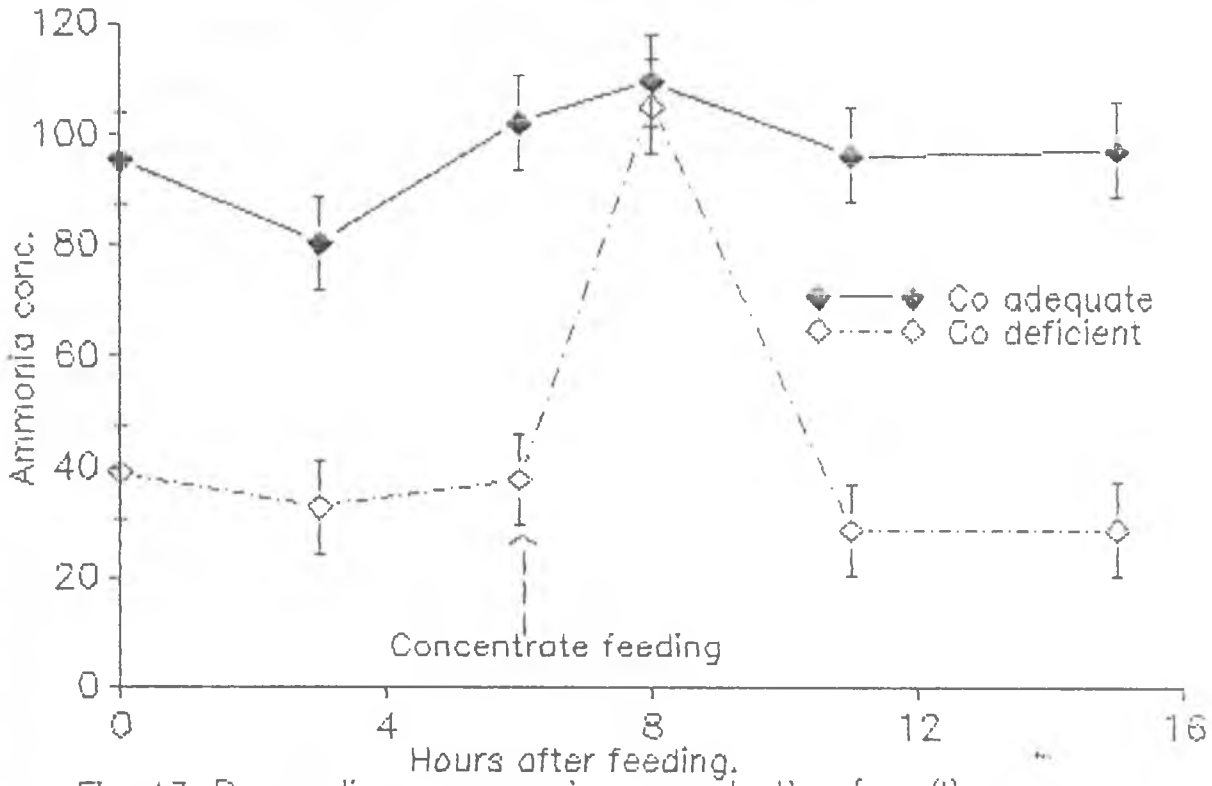

Fig. 13. Rumen liquor ammonia concentration (mg/l).
The mean standard errors are shown as vertical bars.

Suttle, 1986), supplementation of cobalt was shown to lower pH and increase the total volatile fatty acids in liquor. The mean liquor pH was within 5.9 - 7.0: the range within which cellulolysis occurs normally in the rumen (Mould and Orskov, 1984).

In addition to the limitations that the poor quality hay imposed on the rumen fermentation of cellulose, the similarity in liquor concentration of acetic acid and in the disappearance of ADF (see Tables 8 and 9) for the two treatments support the finding that in cobalt deficiency, the cellulose-splitting microorganisms in the rumen are not affected as has previously been reported (Becker and Smith, 1949). Degradation rate of cellulosic material, the main precursor of rumen acetate, was comparable between the two treatments.

Cobalt supplementation improved production of propionic, butyric, valeric and isovaleric acids in the rumen. The higher, albeit not significant, digestibility coefficients of some of the dietary components in the cobalt adequate compared to deficient goats (Table 5) were clearly reflected in the resultant end-products of rumen fermentation -the volatile fatty acids concentration. Rumen microbial population is high when dietary cobalt levels are adequate (Gall *et al.*, 1949) which consequently improved the volatile fatty acids production.

The low acetate to propionate ratio in cobalt adequacy was attributed to the higher concentration of propionate in the liquor of the cobalt supplemented goats as the acetate concentration was comparable between the two treatments. The fermentation of the

soluble fraction of the feed, as depicted by the low acetate:propionate ratio, benefited from cobalt supplementation. The improvement in the rumen breakdown of cellulose reported by Saxena *et al* (1980) is at variance with the present findings owing probably to the cobalt content (0.18 ppm) of the wheat bhusa fed to the calves which was higher than the 0.02 ppm in the hay fed to the goats in the present study.

In the rumen, most of the dietary protein is hydrolysed to ammonia and various carbon skeletons. Ammonia is the key intermediate in the synthesis of microbial protein. Estimates of the optimum concentration of ammonia in rumen liquor vary widely, from 85 to over 300 mg/l (McDonald *et al*, 1984). For optimal utilization of ammonia, the energy supplied by fermentation of feed in the rumen should meet the energy demand of rumen microbes for protein synthesis. Ammonia accumulates in the rumen and blood when energy supply is inadequate leading to a nitrogen loss in the form of urea (McDonald *et al*, 1984).

The concentration of ammonia in the rumen of cobalt supplemented goats was within while that in deficient goats was below the 85-300 mg/l range. Goats in both treatments were fed on the same diet therefore the lower ammonia values in cobalt deficiency reflect a slower rate of hydrolysis of dietary protein which was perhaps attributed to a reduction in rumen microbial population (Gall *et al.*, 1949). On feeding concentrate to the cobalt deficient goats and therefore raising the ammount of protein available to the microorganisms, a transient rise in ammonia concentration to 105 mg/l occured followed by a rapid decline to 68.7 mg/l three

hours thereafter. The pattern of variation in ammonia concentration was somewhat similar to the changes that occured in the liquor concentration of propionic and butyric acids (Figs. 10 and 11) in both treatments indicating that the fermentation of the whole diet was slower in deficient than in cobalt adequate status.

A reduction in rumen liquor concentration in cobalt deficient calves (Saxena and Ranjhan, 1980) and a reduction in microbial protein synthesis (Krasnodebska, 1984) have been reported.

4.4. Gas production from in vitro fermentation of rumen-contents.

Table 11 shows the gas-production in micromoles/g/h at STP from the *in vitro* fermentation of rumen-contents obtained for cobalt supplemented and deficient goats. Gas-production was significantly ($P<0.01$) higher for cobalt adequate than deficient goats. However, wide variation between the quantities of gas produced by fermentation of contents from different goats within the same Treatment and between different weeks for the same Treatment were wide.

The period of the day when the goats were killed (morning or afternoon) had no effect on the quantity of gas produced.

A mixture of carbon-dioxide, methane and a small amount of hydrogen are produced in the rumen as by-products of microbial metabolism. The quantity of gas produced is in a stoichiometric relationship with the amount of substrate fermented (Hungate, 1965). Hoppe *et al.*, (1977) found a direct relationship between gas and volatile fatty acids production using *in vitro*

Table 11. Gas-production from *in vitro* fermentation of rumen-contents.

week	Gas-production in micromoles/g/h at STP.			
	No of samples	Co adequate	No of samples	Co deficient
11	8	60.4 ± 3.21**	8	53.5 ± 4.15
24	8	64.4 ± 2.03**	8	57.7 ± 3.13
25	8	53.7 ± 4.15**	8	16.1 ± 5.20
Overall mean	24	59.5 ± 2.54**	24	42.2 ± 2.77

Key: ** Values for Co adequate higher than for Co deficient goats at ($P<0.01$).

fermentation of rumen-contents obtained from freely grazing wild ruminants.

Methane production in the rumen increases after feeding particularly when the diet is rich in concentrate (Mcdonald *et al.*, 1984). However, in freely grazing wild ruminants, Hoppe *et al.*, (1977) found no relationship between gas-production and the amount of time wild ruminants were allowed to graze before they were killed. In the present study, gas production was independent of the time of the day when the goats were killed.

Both gas-production from the rumen-contents and volatile fatty acids concentration in the rumen liquor were higher for cobalt supplemented than deficient goats. This observation is probably explained by the higher microbial counts reported in cobalt adequacy (Gall *et al.*, 1949).

Vogel *et al.*, (1980) and Stumm *et al.*,(1982) demonstrated the existence of a close physical relationship between methanogenic bacteria and ciliates in the rumen: the bacteria were found closely attatched to the pellicles of some species of rumen ciliates and the association was enhanced by development of anaerobic conditions in the rumen (Stumm *et al.*, 1982).

Some rumen ciliates produce hydrogen as a metabolic end-product which is taken up by the methanogenic bacteria and, together with carbon-dioxide, is utilised by the bacteria to generate energy and in the process, methane is produced as a metabolic end-product. The synthesis of methane from hydrogen involves biochemical transfer of a single carbon atom; a reaction in which vitamin B_{12} is envisaged to

participate (Czerkawski, 1986).

Cobalt deficiency reduces both the bacterial population (Gall *et al.*, 1949) and vitamin B_{12} production (Smith and Loosli, 1957; Hedrich *et al.*, 1973) in the rumen. When the rumen-contents were flushed with carbon-dioxide to establish anaerobiosis, it is expected that an association was established between the methanogens and the ciliates and, coupled with the higher bacterial count and vitamin B_{12} levels, led to methane production that contributed to the higher total gas-production from fermentation of contents from Cobalt adequate than deficient goats.

The association of methanogenic bacteria and rumen ciliates could explain the observation that the presence of ciliates appear to be necessary for bacteria to utilise organic cobalt for growth (Bonhomme *et al.*, 1982).

4.5 Changes in liveweight and body condition scores in cobalt deficiency.

At the start of the trial, the mean liveweights for cobalt-supplemented and unsupplemented goats were 20.8 $\pm$ 1.38 Kg and 23.1 $\pm$ 1.94 Kg respectively. At the twenty-third week, liveweight expressed as a percentage of that at the beginning of the trial was 95.2 $\pm$ 1.30 % for cobalt adequate and 97.7 $\pm$ 1.33 % for cobalt deficient goats respectively. A small and gradual loss of liveweight occured over the whole experimental period for goats in both Treatments. The difference in percentage weight loss was not significant ($P>0.05$) between the Treatments (Table 12 and Fig. 14).

Table 12. Body Condition Scores and percentage change in body weight.

Week	No. of goats	Body condition score		Body weight (%)	
		Co adequate	Co deficient	Co adequate	Co deficient
8	8	2.6 ± 0.14**	2.3 ± 0.14	95.2 ± 1.30	97.7 ± 1.33
10	10	2.6 ± 0.14**	2.3 ± 0.14	93.9 ± 1.30	95.8 ± 1.33
19	5	2.5 ± 0.18**	1.9 ± 0.20	87.0 ± 1.67	88.7 ± 1.88
23	4	2.7 ± 0.21**	1.7 ± 0.20	88.2 ± 1.33	82.1 ± 1.88
Mean	27	2.6 ± 0.11**	2.2 ± 0.99	90.7 ± 0.98	90.7 ± 0.92

Key: **; Scores for Co adequate were higher than for Co deficient goats at ($P<0.01$).

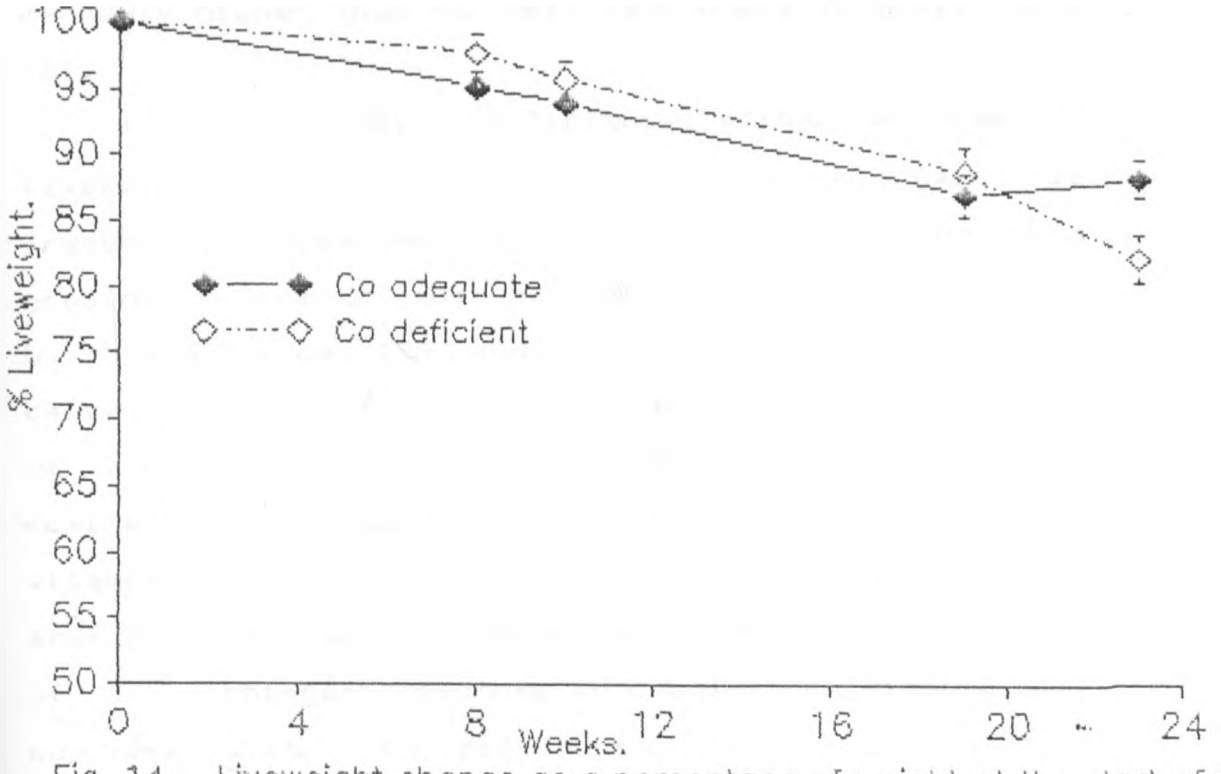

Fig. 14. Liveweight change as a percentage of weight at the start of the trial. Vertical bars represent the mean standard errors.

Between the 8th and 23rd weeks of the experimental period, when the body condition was assessed, cobalt deficient goats had lower scores ($P<0.05$) than their cobalt adequate counterparts (Table 12).

In both Treatment, scores were higher ($P<0.05$) for older than younger goats. When the age of the goats at the beginning of the trial was included as a covariate in the statistical model, the difference in body condition scores for the cobalt adequate were markedly higher than for deficient goats ($P<0.01$; Table 12).

In vitamin B_{12} dificiency, either a loss of liveweight or an absence of liveweight gain is frequently observed in the more susceptible young growing ruminants such as lambs (Rice *et al.*, 1988; Quirk and Norton, 1987 and Duncan *et al.*, 1987) and calves (Quirk and Norton, 1982). The occurence of a response in growth and production when deficient ruminants are supplemented with either cobalt or vitamin B_{12} is dependent on the age and vitamin B_{12} status of the animals (Underwood, 1981); an absence of a weight-gain response in cobalt or vitamin B_{12} supplementation to deficient adult sheep has been reported (Fisher and MacPherson, 1986; Mohamed *et al.*, 1983).

Small East African goats aged between 1-2 years and fed on hay containing less than 0.01 mg Co/Kg, gained in liveweight when supplemented with intrarumenal cobalt-oxide bullets during an experimental period lasting twenty-three weeks (Mgongo *et al.*,1981). On the contrary, Clark *et al.* (1986) failed to obtain a similar reponse in kids on a feed containing 0.035 mg

Co/Kg over a four months period during which, on three ocassions, the kids were supplemented with 1.5 mg injections of hydroxycobalamin.

Neither a liveweight gain nor a significant reduction in the gradual rate of weight-loss was observed in the cobalt supplemented goats in this study although the hay fed to the goats was obtained from the same cobalt-deficient area as that fed by Mgongo *et al.*(1981), the goats were comparable in age and were of the same breed. The hay fed to the goats contained higher cobalt than that fed by Mgongo (1979) probably because it was harvested in the dry season when the hay cobalt-content has been reported to be high (Naik, 1965).

An absence of weight gain or maintenance of the weight at the start of the trial for the cobalt supplemented goats suggests that other factors, besides the cobalt-content of the feed, might have been limiting: the hay fed to the goats was low in crude protein and high in crude fibre contents (table 2). It is possible that a nutritional inadequacy contributed to the gradual weight loss in both Treatments.

A 0.035 mg Co/Kg in pasture (Clark *et al.*, 1986) and the 0.02 mg co/kg of the hay fed in this trial were both more than twice the 0.01 mg/kg of the hay fed by Mgongo *et al.*, (1981). As suggested by Clark *et al.*(1986), the cobalt reference range for sheep might not define the requirements for goats; the latter species could be more resistant as an extension on cobalt deficient diet from 16 (Clark *et al.*, 1986) to 23 weeks in the present study did not affect either

liveweight or feed intake.

In goats a dietary cobalt level that would elicit a significant liveweight loss appear to be less than 0.02 mg/Kg DM; in this species, a weight loss response has been reported only when the diet contained less than 0.01 mg/Kg DM (Mgongo *et al.*,1981).

A loss of liveweight in a vitamin B_{12} trial in ruminants is an unreliable indicator of deficiency (Rice *et al.*, 1989) as weight loss or slow growth rate may be secondary to a loss of appetite (Robertson, 1971). Both feed intake and digestibility of dietary nutrients were not significantly different between the two treatments and could explain the absence of a significant weight loss in the cobalt deficient goats.

Despite the absence of significant differences in weight changes between the two Treatments, cobalt adequate goats attained higher body condition scores than deficient ones. Small East African goats deposit minimal subcutaneous fat as an adaptational measure to the heat stress in arid conditions where they thrive. Thus the body condition scoring method adopted in this study expresses the amount of palpable muscle at the lumber region in relation to the lumber vertebral bones (Honhold *et al.*, 1988).

At birth, the skeletal system is at a more advanced stage of development than the muscular system and muscle deposition attains its maximum rate at the point of inflection of the sigmoid growth curve (Carles, 1983). This accounts for the higher scores attained by older than younger goats in both treatments.

In an assessment of the nutritional status of

goats, Honhold *et al.*,(1988) found body condition scoring a more consistent criterion than a change in liveweight. Although a significant ($P<0.05$) weight gain response was observed when deficient lambs were supplemented with cobalt, comparatively higher body condition scores ($P<0.01$) were attained by supplemented compared with cobalt deficient lambs (Rice *et al*, 1989).

In cobalt deficiency, retention of protein in the tissues declines as a consequence of a deficiency of methylcobalamin, the vitamin B_{12} containing cofactor of the methionine synthetase enzyme, resulting in an impairment in recycling of methionine from homocysteine in the liver (Gawthorne and Smith, 1974). A methionine deficiency might become limiting in vitamin B_{12} deficiency unless the amino acid is adequately provided in the feed (Rice *et al.*, 1988). Supplementation of protein to cobalt deficient goats has been shown not to significantly reduce a loss of liveweight in deficient goats (Mgongo, 1979).

The lower scores for the cobalt deficient goats could have arisen from a reduction in rumenal microbial protein biosynthesis due to a decline in the bacterial count (Gall *et al.*,1949) and from an impairement of the tissue protein retention process owing to a failure to recycle methionine from homocystein in the liver: retention of nitrogen in the body tissues was significantly ($P<0.05$) lower and the urinary nitrogen loss was significantly higher($P<0.05$) in vitamin B_{12} deficient than adequate goats in the present study (see Table 6).

As suggested by Rice *et al.* (1989), vitamin B_{12}

deficiency could affect different body tissues variably: it is likely that the deficiency affects the animals more adversely at a stage of growth when the requirements for methionine in the biosynthesis of protein is high i.e., during the stage at which rapid muscle-deposition occurs; this probably explains the higher susceptibility of young stock to deficiency (Underwood, 1977).

Apart from the muscle, other body tissues appear to be affected to a lesser degree by cobalt deficiency making a change in liveweight a less sensitive indicator of deficiency.

4.6 Blood parameters in vitamin B_{12} deficient goats.

4.6.1 Serum Vitamin B_{12}.

The mean concentration of serum vitamin B_{12} over the entire experimental period for cobalt adequate, 289.6 $\pm$ 40.76 pg/ml, was significantly ($P<0.01$) higher than the 142.8 $\pm$ 28.27 pg/ml for cobalt deficient goats. After 10 weeks on experiment, the serum vitamin B_{12} concentration in the unsupplemented goats fell below 200-250 pg/ml which is considered to be the lower critical level for sheep (Tressol and Lamand, 1979; Rice *et al.*, 1987). The lowest level, 81.14 $\pm$ 28.27 pg/ml, was attained during the nineteenth week and a slight increase to 110.31 pg/ml occured at the twenty-fifth week (Table 13 and Fig. 15).

The variation in the serum vitamin B_{12} concentration between goats in the same treatment and between different weeks for the same goat was wide irrespective of the treatment as depicted by the large standard errors of serum concentration of the

Serum vitamin B_{12} (pg/ml).

Week	No. of goats	Co adequate	No. of goats.	Co deficient
3	6	366.4 ± 72.89**	5	235.4 ± 76.05
5	3	386.9 ± 97.97**	8	211.1 ± 60.81
10	4	249.7 ±104.28*	18	169.4 ± 56.61
19	7	350.7 ± 63.82**	5	81.1 ± 28.27
23	5	349.9 ± 78.53**	10	97.5 ± 53.50
25	4	231.3 ± 92.67	3	110.3 ± 97.48
Overall mean	29	289.6 ± 40.76**	52	142.8 ± 28.27

Key: **, *: Vitamin B_{12} higher in Co adequate than deficient goats at ($P<0.01$) and ($P<0.05$) respectively.

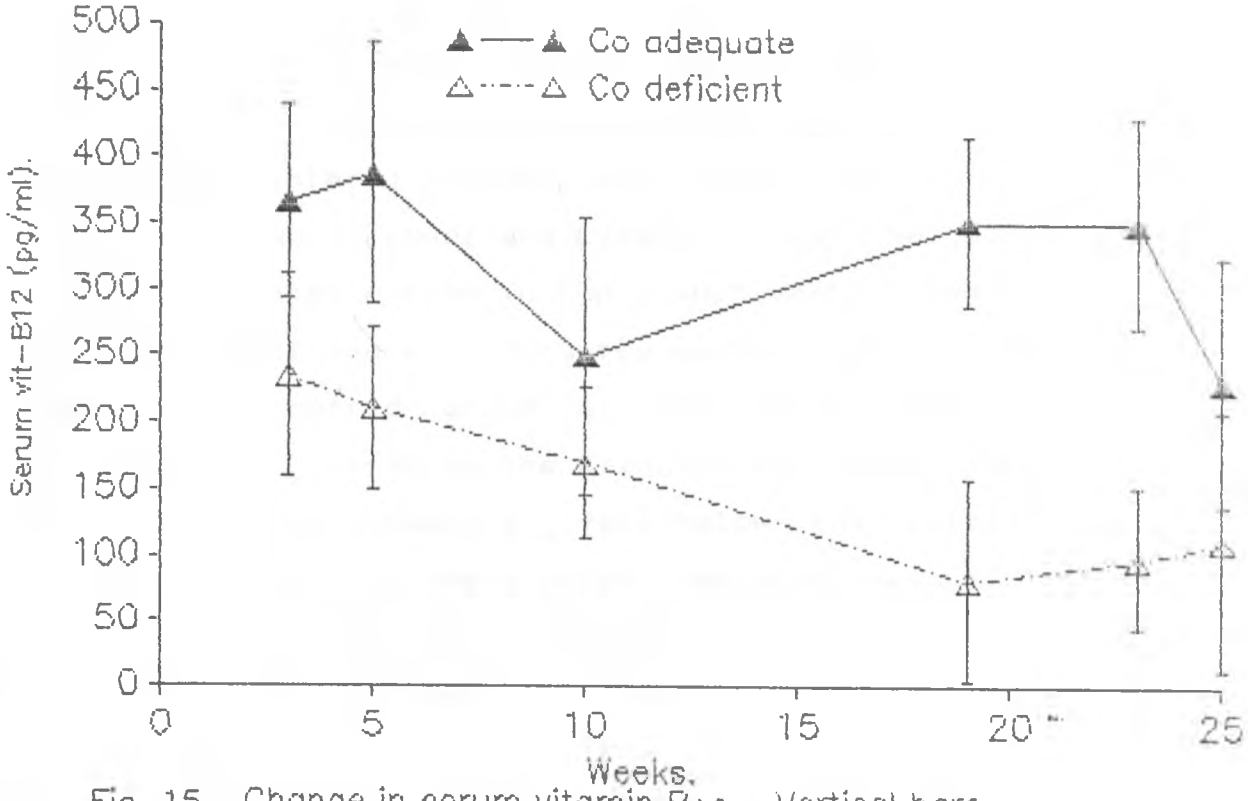

Fig. 15. Change in serum vitamin B_{12}. Vertical bars represent the mean standard errors.

vitamin. The variation was higher in the cobalt adequate (40.76 pg/ml) than deficient goats (28.27 pg/ml) over the entire experimental period.

When put on a diet low in cobalt, rumenal synthesis of vitamin B_{12} rapidly decreases as a consequence of a limitation in the proliferation of vitamin B_{12} synthesizing bacteria which leads to a a rapid decline in the levels of the serum vitamin B_{12}. The rate of decline is slowed by liberation of the liver vitamin reserves and, in sheep, a stabilization occurs at around 200 pg vitamin B_{12}/ml for several weeks before a further decline ensues as the liver reserves are depleted (Tressol and Lamand, 1979).

As in sheep (Tressol and Lamand, 1979), the serum vitamin B_{12} concentration in the unsupplemented pen-fed goats had declined significantly below that in the cobalt supplemented group at the third week of the experiment. Based on the standard for sheep (ARC, 1980), the serum vitamin B_{12} fell below the critical 200 pg/ml after 10 weeks which compares favourably with 8 weeks reported by Mgongo (1979); moreover, vitamin B_{12} was consistently below 200 pg/ml after the 10^{th} week and the variation in the vitamin levels were more marked for the cobalt adequate than deficient goats in both trials.

The serum vitamin B_{12} reference range differs between species (Suttle, 1986) and the reference for sheep used in this study might be inappropriate for goats as was suggested by Clark *et al.*, (1986). It appears, in goats, that a decline in serum vitamin B_{12} concentration to below 200 pg/ml is indicative of a deficient status.

Vitamin B_{12} assay using the Ammersham RIA kits has been shown to be reliable (Millar and Penrose, 1980) therefore, the variations observed in serum vitamin B_{12} were an indication of differences in the vitamin levels which probably reflected varying vitamin B_{12} status of goats in the same Treatment owing to their age differences. Studies in sheep have shown that serum vitamin B_{12} levels vary widely especially when sheep on cobalt deficient diets are orally supplemented with cobalt (Findlay, 1972). Owing to these variations, a single serum sample is not sufficient to establish the vitamin B_{12} status in goats any more than it is in sheep (Findlay, 1972).

The cobalt supplemented goats had a consistently higher serum vitamin B_{12} concentration than the unsupplemented suggesting that serum vitamin B_{12} in goats, as in sheep (Findlay, 1972), reflects the level of dietary cobalt intake.

Weight loss, anorexia, anaemia and low serum vitamin B_{12} concentration in sheep on cobalt deficient diets have been observed after either 16 (Jones and Anthony, 1970) or 20 weeks (Mohammed and Lamand, 1986). The serum vitamin B_{12} fell below 200 pg/ml after 12 weeks while signs of deficiency appeared 8 weeks later (Mohammed and Lamand, 1986). In goats, vitamin B_{12} declined to less than 200 pg/ml after 10 weeks on the cobalt deficient hay without manifestations of clear signs of deficiency. The results of this study suggests that goats are more resistant than sheep to cobalt deficiency and that the lower reference point for serum vitamin B_{12} should be regarded as being below 200 pg/ml.

4.6.2 Hematological values.

Table 14 shows the mean hematological values for the twenty-three weeks on experiment for the two groups of goats.

The cobalt supplemented goats had significantly ($P<0.01$) higher erythroyte count, packed cell volume, hemoglobin concentration, mean cell volume and total leucocyte count than those in the unsupplemented group although the values for both groups were within the normal range for goats (Schalm, 1965; Benjamin, 1974). The trends in above blood values for the two treatments over the experimental period are shown in Figures 16 to 18 and appendices 1 to 5. Cobalt supplementation had no effect on the mean plasma protein ($P>0.05$) between cobalt adequate, (7.20 $\pm$ 0.15 g/100 ml) and deficient (6.97 $\pm$ 0.08 g/100 ml) goats over the entire experimental period.

Anaemia, defined as a reduction below the normal range of erythrocyte number and/or hemoglobin concentration (Schalm, 1965), did not develop within the experimental period in the unsupplemented goats (Table 14).

A decrease in hemoglobin concetration, packed cell volume and erythrocyte count in three months and in one to two years old cobalt deficient goats has been reported by Gombe and Verjee (1976) and Mgongo *et al.* (1981) respectively: the latter, at the end of a twenty-three weeks experimental period, classified the anaemia as macrocytic and normochromic.

In cobalt deficiency, anaemia accompanies other signs of deficiency (Jones and Anthony, 1970; Mohammed and Lamand, 1986) and is among the last symptoms to

Table 14. Effects of cobalt deficiency on mean blood values.

Parameter	Co adequate	Co deficient	Normal Value
Erythrocyte x $10^{6}/mm^{3}$	14.9 ± 1.13*	13.7 ± 0.61	12-20
Packed cell volume %	27.2 ± 0.92**	23.2 ± 0.49	24-48
Hemoglobin g/100 ml	9.7 ± 0.39**	8.4 ± 0.21	8-14
Mean cell volume (c.u)	19.9 ± 0.27*	19.2 ± 0.14	15.5-30
Leucocyte count x $10^{3}/mm^{3}$	14.2 ± 0.83*	12.0 ± 0.44	6-16

KEY: *, **; Blood value for Co adequate were higher than for Co deficient goats at ($P<0.05$) and ($P<0.01$) respectively.

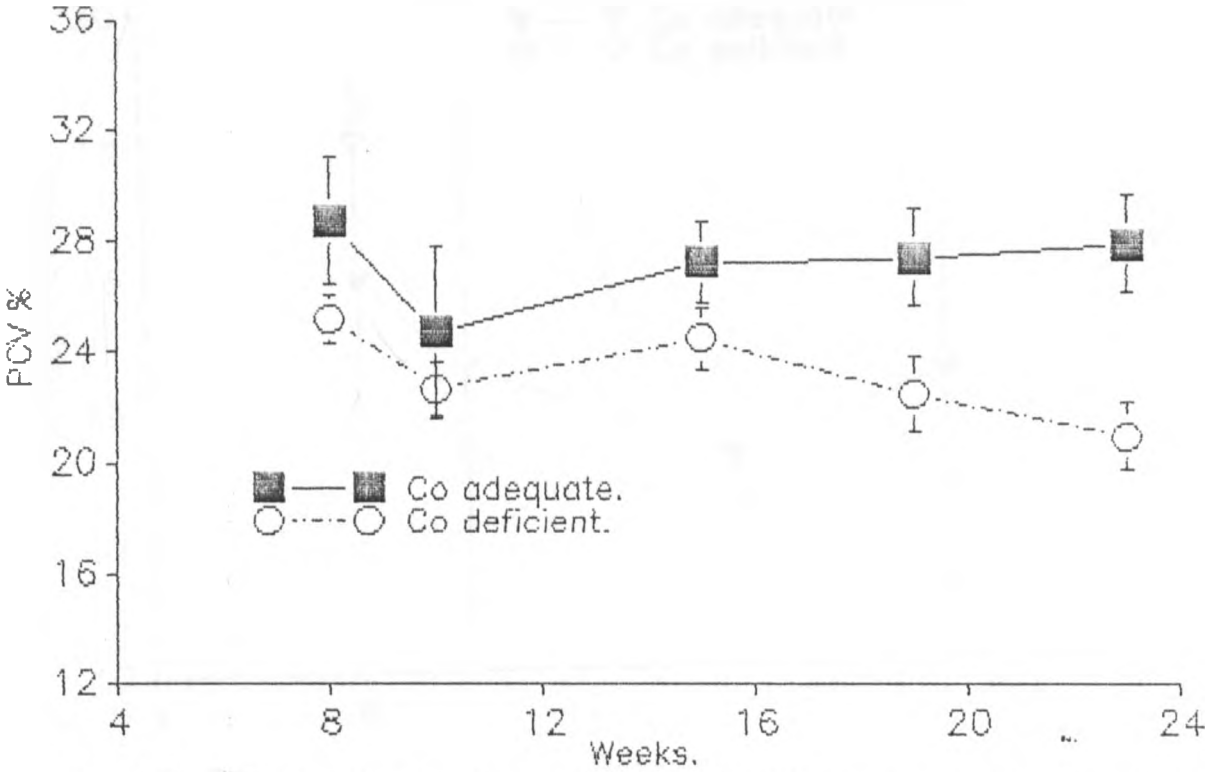

Fig. 16. Packed cell volume (%). Vertical bars represent the mean standard errors.

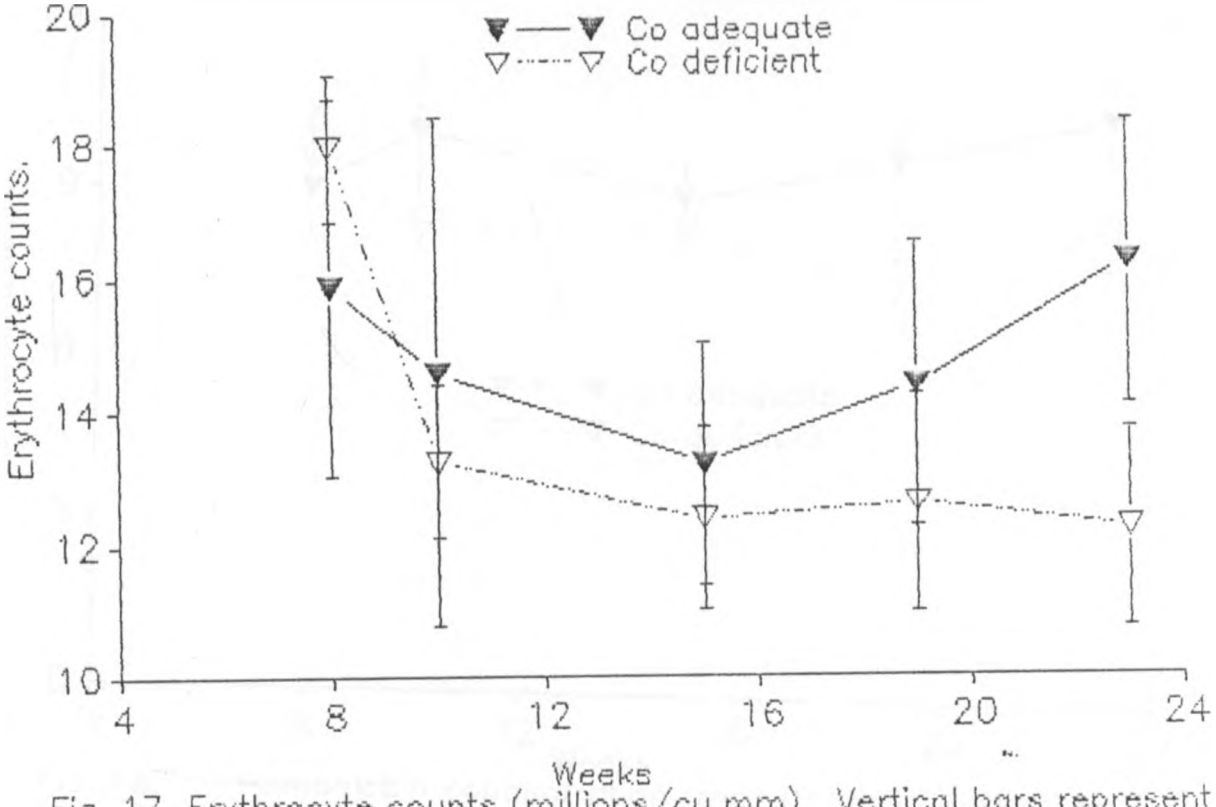

Fig. 17. Erythrocyte counts (millions/cu.mm). Vertical bars represent the mean standard errors.

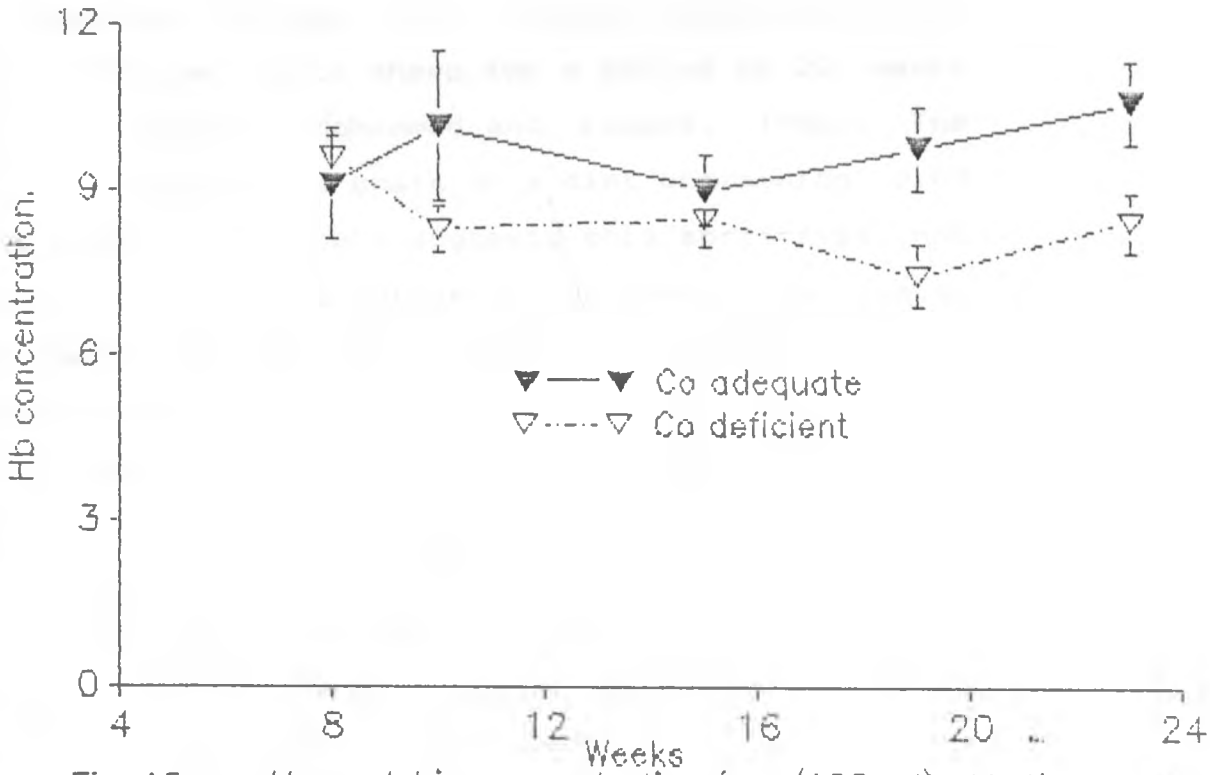

Fig. 18. Hemoglobin concentration (mg/100 ml). Vertical bars represent the mean standard errors.

return to normal following treatment with either vitamin B_{12} or cobalt (Smith and Loosli, 1957; Somers and Gawthorne, 1969). Development of anaemia, like other signs of cobalt deficiency, is dependent on the dietary level of cobalt, the age and the liver vitamin B_{12} status of the animal. In subclinical deficiency anaemia may altogether be absent (Underwood, 1981).

Vitamin B_{12} is required in the process of erythrocyte maturation in the bone marrow (Schalm, 1965; Gombe and Verjee, 1976). A diet containing 0.06 mg Co/Kg DM when fed to sheep for a period of 20 weeks leads to anaemia (Mohammed and Lamand, 1986). The absence of anaemia in goats on a diet containing 0.02 mg Co/Kg DM for 23 weeks suggests this species is not as prone to low cobalt intake as the sheep. In goats, a course of development of anaemia comparable to that in sheep has been described when the feed cobalt content was less than 0.01 mg/Kg DM (Mgongo *et al.*, 1981).

The significantly lower erythrocyte count, packed cell volume and hemoglobin concentration in the deficient compared with cobalt supplemented goats indicates a low degree of impairment of erythropoiesis which could probably have led to anaemia had the period of this study been prolonged.

4.7 Serum thyroid hormones and metabolic rate in vitamin B_{12} deficiency.

4.7.1 Thyroid hormones.

The average serum concentrations of total thyroxine, free triiodothyronine and free

tetraiodothyronine for serum samples taken over the experimental period are presented in table 15. Serum TT_4 concentration was significantly ($P<0.01$) higher in cobalt deficient than adequate goats (Table 15). Serum TT_4 were, on average, 59.0 $\pm$ 1.70 and 51.6 $\pm$ 2.45 nMol/l in vitamin B_{12} deficient and adequate goats respectively. A difference ranging from 10 to 15 nMol/l between the highest and lowest TT_4 concentrations were observed in both treatments (Table 15). However, a clear trend in serum TT_4 concentration over the experimental period was not established in both treatments (Fig. 19).

Serum concentrations of FT_3 and FT_4 were neither affected significantly ($P>0.05$) by the cobalt status, the period on experiment nor the sex of the goats. During the entire experimental period, the serum concentration of the thyronines were fairly constant at around 10 pMol/l (Table 15).

In vitamin B_{12} deprivation, enlargement of the thyroid glands occurs in chicks and piglets while in man, the prevalence of goitre has been shown not only to be dependent on iodine but also on the ratio of iodine to cobalt in areas where the two elements are deficient (Underwood, 1975). In vitamin B_{12} deficient goats, Mgongo *et al.*, (1981) observed hypertrophy and hyperplasia of the thyroid gland as was evidenced by an increase in the size of the thyroid follicles and height of glandular epithelial cells.

A direct involvement of vitamin B_{12} in the etiology of thyroid gland disorders has not been proved unequivocally. Mgongo (1979) suggested the thyroid abnomalities observed in vitamin B_{12} deficient goats

Table 15. Levels of serum thyroid hormones.

Week	Cobalt status	No of samples	Total serum thyroxine (nMol/l)	Serum FT_4 (pMol/l)	Serum FT_3 (pMol/l)
3	A	5	65.8 ± 4.40	9.4 ± 0.54	9.5 ± 0.52
	D	5	66.4 ± 4.58	9.7 ± 0.56	9.7 ± 0.56
5	A	4	60.1 ± 2.90	10.1 ± 0.72	10.1 ± 0.72
	D	8	67.4 ± 1.66**	9.9 ± 0.45	10.0 ± 0.45
10	A	4	59.6 ± 6.28	10.9 ± 1.27	10.9 ± 1.27
	D	19	62.0 ± 3.54	10.6 ± 0.39	10.2 ± 0.41
19	A	7	55.3 ± 3.84	10.6 ± 0.47	11.0 ± 0.76
	D	5	51.7 ± 4.66	9.9 ± 0.57	10.6 ± 0.39
23	A	5	59.7 ± 4.73	9.3 ± 0.57	9.3 ± 0.58
	D	10	57.6 ± 3.22	10.2 ± 0.39	10.2 ± 0.39
25	A	4	45.7 ± 5.58	10.4 ± 0.68	10.4 ± 0.68
	D	3	63.5 ± 7.38**	10.3 ± 0.90	9.4 ± 0.72
mean	A	29	51.6 ± 2.45	10.2 ± 0.30	10.2 ± 0.30
	D	52	59.0 ± 1.70**	10.0 ± 0.21	10.0 ± 0.21

Key: A: Cobalt adequate; D: Cobalt deficient. **: value in Co deficient were higher than in Co adequate goats at ($P<0.01$).

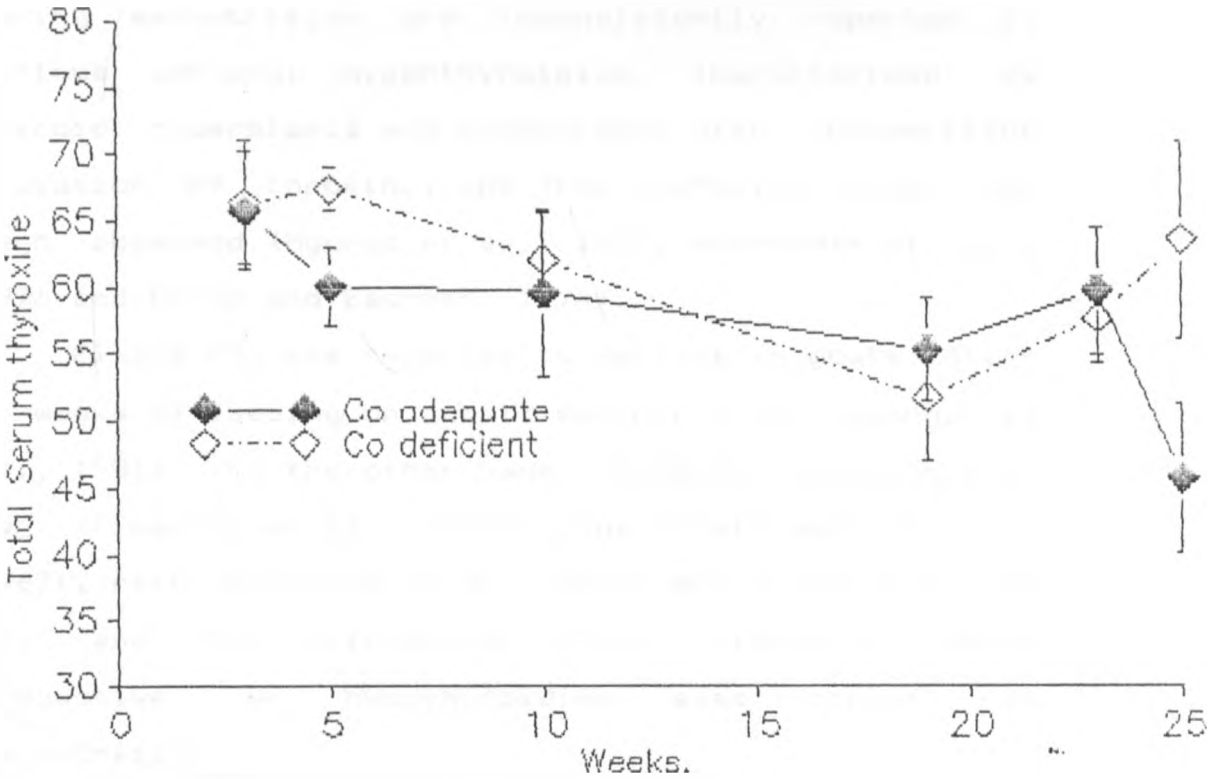

Fig. 19 . Total serum thyroxine (nMOl/l).
Mean standard errors are shown as vertical bars.

were due to a protein-calorie malnutrition resulting from disorders of propionate and methionine metabolism. The findings of the present study support this suggestion as both the ammonia and volatile fatty acids concentration in the rumen liquor of cobalt deficient goats were low which consequently reduce ruminal protein synthesis and energy supply to the goats.

Thyroid gland histopathological findings and changes in the serum thyroid hormones concentration during malnutrition are inconsistently reported by various workers: hyperthyroidism, characterised by thyroid hyperplasia and hypertrophy with concomittant elevation of throxine, and free thyroxine index has been observed (Mgongo *et al.*, 1981; Florsheim *et al.*, 1976 and Burke and Eastman, 1974).

Plasma FT_3 was reported to decline in goats after 6 weeks of feeding on cobalt deficient hay (Mgongo *et al.*, 1981). On the other hand, glandular hypoplasia in man (Pimstone *et al.*, 1973), pigs (Platt and Stewart, 1967), rats (Florheim *et al.*, 1970) and a reduction in FT_4 and FT_3 (Florsheim, 1970; Pimstone, 1976) suggestive of hypothyroidism also occurs in malnutrition.

Whether malnutrition results in hypo or hyperthyroidism, it is clear that a breakdown in the hypothalamo-pituitary homeostatic axis occurs but the precise location affected is uncertain. The suggested locations include a primary thyroid gland disturbance (Pimstone *et al.*, 1973), an impairment of iodine excretory capacity of the kidneys leading to accumulation of iodine in the extracellular pool which inhibits thyroid hormones' production (Florsheim *et*

al., 1970) or a direct impairment of both the hypothalamus and pituitary (Mgongo *et al.*, 1981).

This study was aimed at relating the thyroid changes directly to the physiological functions of the gland in vitamin B_{12} deficient goats. The decline in vitamin B_{12} in the cobalt deficient goats was similar to that reported by Mgongo (1979) but the differences in thyroid hormone levels -particularly the free thyronines- were marked: an elevation of TT_4 in the present study was unaccompanied by changes in either serum FT_3 or FT_4 concentrations.

Serum TT_4 concentration is usually many folds that of FT_4 (Schwartz *et al.*, 1971) while the physiological potency of FT_3 is at least three to four times that of FT_4 (Blackburn *et al.*, 1954). There is evidence that thyroid functions at the tissue level are exerted by FT_3 and that thyroxine is converted, by monodeiodination, to FT_3 (Oppenheimer *et al.*,1972; Schwartz *et al.*, 1971). The wide variations in serum total thyroxine in vitamin B_{12} adequate and deficient goats and the absence of appreciable changes in resting metabolism in both treatments supports the finding that the physiological potency of TT_4 is low.

Radioimmunoassay of FT_3 reliably discriminates between hypothyroid, euthyroid and hyperthyroid conditions (Eastman *et al.*, 1973). Vitamin B_{12} deficiency did not affect serum FT_3 as the latter was comparable between the cobalt deficient and sufficient goats. However, the elevation of TT_4 in cobalt deficiency concurs with the findings of Mgongo *et al.* (1981) and is indicative of some degree of thyroid disturbance.

The absence of clear manifestations of clinical signs of cobalt deficiency and thyroid dysfunction were in agreement in this study. If the dietary cobalt intake proportionately reflects the nutritional stress imposed on the goats, then the lower dietary cobalt fed to goats by Mgongo (1979) subjected the goats to a higher level of stress than in the present study and may be the underlying factor responsible for the differences in the findings of the two studies. On supplementation of energy and protein to the vitamin B_{12} deficient goats (and thus reducing the nutritional stress) Mgongo observed a partial alleviation of the structural glandular disorder.

A subclinical vitamin B_{12} is likely to have developed in treatment 2 causing a mild change in thyroid functions as was reflected by an elevation in the total thyroxine with no effects either on FT_3 or FT_4 concentration in the serum. It is possible that the total serum thyroxine is elevated before commencement of a decline in serum triiodothyronine concentration in the course of development of avitaminosis B_{12}.

4.7.2 Resting metabolism.

The resting metabolism, depicted by the rate of oxygen consumption, was not significantly ($P>0.05$) different between the vitamin B_{12} sufficient and deficient goats. The rate of oxygen consumption at the 6^{th} and between the 24^{th} to 27^{th} weeks of the experimental period are presented in Table 16.

Oxygen consumption varied significantly ($P<0.01$) between measurements taken in different weeks of the

Table 16. Rate of resting oxygen consumption.

Week	NO of observations	Co adequate	NO of observations	Co deficient
		Oxygen-consumption (ml/g/h at STP)		
6	27	0.21 ± 0.007	15	0.20 ± 0.010
24	20	0.27 ± 0.010	60	0.22 ± 0.005
25	40	0.24 ± 0.006	20	0.33 ± 0.010
26	40	0.19 ± 0.007	40	0.16 ± 0.007
27	20	0.29 ± 0.010	27	0.29 ± 0.016
Mean	147	0.24 ± 0.003	155	0.24 ± 0.004

Key: Oxygen consumption was not significantly ($P>0.05$) different between the two treatments.

experimental period but there were no significant ($P>0.05$) differences in oxygen consumption between the deficient and vitamin B_{12} adequate goats.

Serum TT_4 was elevated in the cobalt deficient goats but without effects on the resting metabolism as the latter is dependent on the plasma FT_3 concentration regardless of the total thyroxine levels (Burke and Eastman, 1974).

The absence of significant differences in the rate of resting metabolism between the vitamin B_{12} deficient and adequate goats is in agreement with the comparable serum free thyroid hormone -especially the free triiodothyronine- concentration in both groups of goats. These results suggests the free thyroid hormones largely determine the rate of resting metabolism in goats. Cobalt deficiency had neither effect on free thyronines nor on resting metabolism in the present study.

CONCLUSION.

Goats fed on hay containing 0.02 mg/Kg DM for a twenty-three weeks experimental period developed cobalt deficiency after the tenth week when the serum concentration of vitamin B_{12} declined to below 200 pg/ml. However, vitamin B_{12} deficiency signs such as inappetence, weight loss and anaemia did not develop in the goats.

Between the eighth and the twenty-third weeks of the experiment the body condition of the goats was assessed by manual palpation of the lumber region; Vitamin B_{12} deficient goats attained significantly ($P<0.01$) lower scores than the cobalt supplemented controls. A loss of body condition occured without an appreciable loss of weight in the cobalt deficient goats. It is suggested that both the biosynthesis and retention of protein in the muscles is impaired before commencement of weight loss in vitamin B_{12} deficiency.

The coefficients of apparent digestibility of dry matter, organic matter, ether extracts, crude protein and nitrogen free extracts were higher -but not significantly ($P>0.05$) -in the cobalt supplemented than deficient goats. The rumen liquor concentration of propionic, butyric, valeric and isovaleric acids were significantly ($P<0.05$) lower in the cobalt deficient goats. The rumen liquor concentration of acetic acid and the degradability of dry matter and cellulose in the cobalt deficient hay were not significantly ($P>0.05$) different between the deficient and cobalt adequate goats. Cobalt supplementation improved rumen

fermentation of the soluble fraction of the diet but not that of cellulose contained in the low quality hay fed to the goats.

Rumen liquor concentration of free ammonia and the gas production from *in vitro* fermentation of rumen contents were significantly ($P<0.01$) lower while the liquor pH was significantly higher ($P<0.01$) in cobalt deficient than supplemented goats. It is concluded that in cobalt deficiency, the activity of the rumen microorganisms diminishes resulting in a reduction in the provision of volatile fatty acids and microbial proteins to the host ruminant which gives rise to signs of deficiency undifferentiable from undernutrition or chronic helminthiasis.

The concentration of total serum thyroxine was significantly ($P<0.01$) higher in the cobalt deficient than supplemented goats but no significant ($P>0.05$) differences were detected in the serum concentration of either free tetraiodothyronine or triiodothyronine. Consequently, the rate of resting metabolism was not significantly ($P>0.05$) different between the two groups of goats. Neither hypothyroidism nor hyperthyroidism previously reported in work on cobalt deficiency in goats or in energy-protein malnutrition in various animal species occured in this study. Feeding goats on hay containing 0.02 mg Co/Kg DM for twenty-three weeks resulted in an elevation of serum thyroxine which, Perhaps, preceeds other thyroid disorders.

A subclinical vitamin B_{12} deficiency characterized by inapparent manifestation of clinical signs developed in goats in this study. Comparatively, goats are likely to be more resistant than sheep to

cobalt deficiency as vitamin B_{12} deficiency signs have been described in sheep on diets comparable in cobalt content and for a duration as long as that of the present study. Further work is required to establish the critical serum vitamin B_{12} below which signs of deficiency become apparent and the duration on various low dietary cobalt levels after which vitamin B_{12} deficiency develops in goats.

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APPENDICES.

1. Erythrocyte counts.

	Erythrocyte count $x10^6$.			
week	no of samples	Co adequate	No of samples	Co deficient
8	6	15.8±2.83	5	17.9±1.11
10	8	14.5±3.82	7	13.3±1.16
15	7	13.2±1.18	8	12.4±1.37
19	6	14.4±2.13	5	12.6±1.63
23	4	16.2±2.13	4	12.2±1.49
Overall Mean	31	14.8±1.13**	29	13.7±0.61

Key: **: Erythrocyte counts for Co adequate were higher than for Co deficient at ($P<0.01$).

2. Packed cell volume.

	Packed cell volume (PCV) %.			
week	No of samples	Co adequate	No of samples	Co deficient
8	6	28.7±2.31	5	25.2±0.90
10	8	24.7±3.12	7	22.7±0.94
15	7	27.2±1.48	8	22.5±1.12
19	6	27.4±1.74	5	22.5±1.33
23	4	27.9±1.74	4	21.0±1.22
Overall Mean	23	27.2±0.92**	29	23.2±0.49

Key: **: PCV for Co adequate was higher than for Co deficient goats at ($P<0.01$).

Table 3. Hemoglobin concentration.

	Hemoglobin concentration (g/100 ml).			
week	No of samples	Co adequate	No of samples	Co deficient
8	6	9.1±0.99	5	9.6±0.39
10	8	10.2±1.35	7	8.3±0.41
15	7	9.0±0.64	8	8.5±0.49
19	6	9.8±0.75	5	7.5±0.57
23	4	10.6±0.75	4	8.4±0.53
Overall Mean	23	9.7±0.39**	29	8.4±0.21

Key: **: Hemoglobin concentration for Co adequate was higher than for Co deficient goats at ($P<0.01$).

4. Mean cell volume (MCV)

	Mean cell volume (cubic microns).			
week	No of samples	Co adequate	No of samples	Co deficient
8	6	23.6±0.66	5	21.1±0.25
10	8	19.2±0.89	7	18.4±0.27
15	7	18.4±0.42	8	18.2±0.32
19	6	19.3±0.50	5	19.1±0.38
23	4	19.2±0.50	4	19.1±0.35
Overall Mean	23	19.9±0.27	29	19.2±0.14

Key: MCV for the two treatments was not significantly different at ($P<0.05$).

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week	No of samples	Co adequate	No of samples	Co deficient
8	6	9.1±0.99	5	9.6±0.39
10	8	10.2±1.35	7	8.3±0.41
15	7	9.0±0.64	8	8.5±0.49
19	6	9.8±0.75	5	7.5±0.57
23	4	10.6±0.75	4	8.4±0.53
Overall Mean	23	9.7±0.39**	29	8.4±0.21

Key: **: Hemoglobin concentration for Co adequate was higher than for Co deficient goats at ($P<0.01$).

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week	No of samples	Co adequate	No of samples	Co deficient
8	6	23.6±0.66	5	21.1±0.25
10	8	19.2±0.89	7	18.4±0.27
15	7	18.4±0.42	8	18.2±0.32
19	6	19.3±0.50	5	19.1±0.38
23	4	19.2±0.50	4	19.1±0.35
Overall Mean	23	19.9±0.27	29	19.2±0.14

Key: MCV for the two treatments was not significantly different at ($P<0.05$).

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5. Leucocyte counts.

Total leucocyte count (x $10^3/mm^3$).				
week	No of samples	Co adequate	No of samples	Co deficient
8	6	12.1±2.07	5	12.6±0.81
10	8	16.7±2.79	7	11.9±0.84
15	7	13.1±1.33	8	10.7±1.05
19	6	13.9±1.56	5	12.2±1.19
23	4	15.2±1.56	4	12.5±1.09
Overall Mean	23	14.2±0.85**	29	12.0±0.44

Key: **: Leucocyte counts for Co adequate were higher than for Co deficient goats at ($P<0.01$).