# THE EFFECTS OF MOLYBDENUM AND SULFUR ON THE FLOW AND SOLUBILITY OF VARIOUS MINERALS ALONG THE DIGESTIVE TRACT OF STEERS

BY

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## ABSTRACT

The effect of high dietary Mo and S upon the flow and solubility of minerals along the bovine digestive tract was examined. Four Holstein steers ($\bar{x}$ = 235 kg), fitted with cannulae in the rumen, proximal duodenum (PD) and terminal ileum (TI) were continuously fed 4.5 kg day$^{-1}$ of a barleygrain-hay (67%-27%) ration containing: (1) no added Mo or S (LMLS); (2) added Mo at 10 mg kg$^{-1}$ (HMLS); (3) added S at 3.0 g kg$^{-1}$ (LMHS); and (4) added Mo at 10 mg kg$^{-1}$ and S at 3.0 g kg$^{-1}$ (HMHS), in a 4 x 4 Latin Square Design. Rations were topdressed with 100g barley pellets day$^{-1}$ containing 2 mg dysprosium g$^{-1}$ as a non-digestible marker.

A higher ($P < 0.01$) apparent digestibility of dry matter (DM) with HM diets was a reflection of a higher ($P = 0.051$) digestibility of DM before the PD. Added Mo stimulated DM digestion in the rumen.

Although the flow of total Cu was not different ($P > 0.05$), the solubility of Cu (supernatant fractions of centrifuged digesta) with HM and HS diets was lower ($P < 0.01$) at the PD and TI, with an effect ($P < 0.05$) of HM + HS measured at the TI. Fecal excretion of Cu tended to be higher ($P = 0.07$) with HM diets.

With HM diets, there was a lower ($P < 0.05$) net secretion of Mn before the PD with less ($P < 0.05$) Mn absorbed distal to the PD, but with no effect ($P > 0.05$) upon Mn excretion in feces.

The fecal excretion of Zn was higher ($P < 0.05$), a result of a lower ($P < 0.01$) absorption distal to the PD, and a lower ($P < 0.05$) solubility of Zn at the TI, which contributed to a lower absorption of

Zn from the large intestine with HM diets. The solubility of Zn at the PD was lower ($P < 0.01$) with HS diets, but absorption distal to the PD was not affected ($P < 0.05$).

A lower ($P < 0.01$) excretion of Fe (higher apparent digestibility, $P = 0.08$) was the result of a higher ( $P < 0.05$) absorption of Fe from the large intestine with HM diets.

Neither Mo nor S influenced any of the Ca parameters examined.

The Mg in the DM of supernatants was lower in rumen fluid ($P < 0.05$) with HM + HS, was lower ($P < 0.05$) at the PD with HM diets and higher ( $P < 0.05$) at the PD with HS diets, but with no significant effect ($P < 0.05$) upon the net secretion or net absorption of Mg.

A lower ($P < 0.05$) net secretion of P before the PD was followed by a lower ($P = 0.055$) net absorption of P distal to the PD with HM diets; with HS diets, the net absorption of P was higher ($P < 0.01$) distal to the PD.

Both the flows and solubilities of minerals in addition to Cu were influenced by high dietary levels of either Mo or S.

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# 1. INTRODUCTION

Many factors influence absorption of minerals from the digestive tract. These include: level of dietary intake relative to requirement, mineral status of the animal, age, anabolic demands such as growth, pregnancy and lactation, disease state, genetic factors, hormones, vitamins, chemical form of mineral and nature of diet (NAS-NRC, 1984).

Several nutrient relationships have a profound effect on the absorption of a mineral. Interactions between and among minerals are important determinants of mineral absorption. Inhibition of mineral absorption has been reported, and may be the result of several different mechanisms. One such group of interactions involves a competition between two cations for transport proteins in the brush border of the intestinal cell. Another involves a reaction between two or more minerals, resulting in the formation of an insoluble precipitate, thus reducing the solubility and potential availability of that mineral for absorption. (Georgievskii, 1982)

Extensive research has been directed towards an understanding of the complex metabolic interaction among Cu, Mo and S, both at the gut and systemic level in ruminants. Excessive dietary Mo decreases the efficiency of Cu utilization, leading to a Cu deficiency. An elevated intake of S, whether in feedstuffs or in water also contributes to a Cu deficiency, due to the formation of insoluble, non-absorbable, and hence, unavailable copper sulfide in the digestive tract (Mason, 1981). When high dietary concentrations of Mo and S are simultaneously present,

the combined effect of dietary Mo and S upon Cu status are synergistic. This has been attributed to the formation of thiomolybdates in the rumen (Dick et al., 1975).

Thiomolybdates are compounds which are formed by the progressive reduction of sulfide with molybdate. These compounds apparently interact with dietary Cu to produce Cu-thiomolybdates in the rumen. A Cu-thiomolybdate complex is insoluble in the digestive tract, thereby reducing the fraction of Cu in the digestive tract that is available for absorption (Mason, 1981).

Trace minerals, such as Mn and Zn, may be at deficient levels in feedstuffs in Western Canada (Boila et al., 1985; Frischke & Gieblehaus, 1987) relative to animal requirements. Molybdenum and S may be present at high levels in forages consumed by grazing livestock (Boila et al., 1984a) and the absorption and utilization of minerals in addition to Cu may be influenced.

The distribution of trace minerals which includes Cu, Zn, Mn and Fe, and macro-minerals which include Ca, P, and Mg, between the solid and liquid phases of digesta from locations within the digestive tract of ruminants fed various rations has been identified (Bremner, 1970; Ben-Ghedalia et al., 1975; Grace et al., 1977; Yano et al., 1979; Ivan et al., 1979; Ivan & Veira, 1981; Ivan & Veira, 1982; Ivan et al., 1983a, b; Rahnema and Fontenot, 1986).

The physiochemical properties of digesta varies as the chyme moves along the digestive tract. As a result, the proportions of soluble and insoluble mineral compounds in assimilatable and non-assimilatable forms

of trace and macroelements change as well (Georgievskii, 1982). Thus, a close association between pH of the digesta, the solubility of a mineral and potential absorption of a mineral has been demonstrated.

Diets with high Mo and S effectively reduce the solubility of Cu in the digestive tract of ruminants. Effects of Mo and S upon the net absorption and interaction of minerals in relation to the solubility of a mineral deserves evaluation.

This study was undertaken in order to examine the effects of Mo and S upon the flows and solubilities of minerals in the digestive tract, including Zn, Mn, Fe, Ca, P and Mg in addition to Cu and Mo. The soluble fraction of mineral in digesta, was that in the supernatant or liquid after removal of the particulate or solid phase using centrifugation.

# 2. LITERATURE REVIEW

## 2.1 Molybdenum

### 2.1.1 Requirements, Deficiency and Toxicity

The requirement for Mo by ruminants has not been clearly defined (NAS-NRC, 1984). An exact estimate of the requirement is impossible to make, because both S and Cu influence Mo metabolism (Mason, 1981). The research dealing with Mo metabolism has been directed primarily towards its interrelationship with Cu and S, rather than a specific requirement for Mo (NAS-NRC, 1984).

Signs of Mo deficiency, such as reduced food intake, poor weight gains, higher mortality at birth and a shorter life expectancy were observed when goats were fed a diet containing 0.06 mg Mo $kg^{-1}$ DM (Anke et al., 1977). A significant growth response and an improved digestibility of cellulose were reported in sheep when the basal diet which contained 0.36 Mo mg $kg^{-1}$ DM was supplemented with 2 mg Mo $kg^{-1}$ DM (Ellis et al., 1958). Thus, Mo is essential for normal health, growth and production. The requirement for Mo by ruminants is very low and a deficiency of Mo is rare.

Maximum tolerable levels of Mo for cattle appear to be 6.0 mg $kg^{-1}$ diet (NAS-NRC, 1984). The organic forms are most toxic (Miller et al., 1970a; NAS-NRC, 1984). Toxicity has been reported as a practical problem in the grazing ruminant (Underwood, 1977). Cattle are more susceptible than sheep to an excess of Mo (Marcilese et al., 1969).

2.1.2 Absorption and Secretion

Molybdenum is in group VI B in the Periodic Table. Although it can exist in several oxidation states, it is most stable in the hexavalent oxidation state where it is usually bound to four oxygens and exists as the oxy-anion, molybdate, $MoO_4^{2-}$ (Huisingh and Matrone, 1976).

Molybdenum, in the biologically active hexavalent form, is readily and rapidly absorbed from the digestive tract when available in the diet or present as an inorganic form of the element (Underwood, 1977; Mason et al., 1978; Georgievskii, 1982). Molybdenum in forage, most of which is water soluble, is well absorbed by cattle and interferes with Cu metabolism in cattle. The Mo in dried forage appears not to be as available as that from green forage. Thus, forages that interfere with Cu metabolism when grazed, do not cause difficulties when fed as dry, harvested forage (Underwood, 1977; NAS-NRC, 1984; Suttle, 1986).

The hexavalent water-soluble compounds, such as sodium and ammonium molybdate, and certain insoluble compounds such as $MgMoO_4$ and $CaMoO_4$ are readily absorbed. Absorption of Mo from the disulfide, $MoS_2$, is poor which is a partial explanation for the antagonistic effect of the interaction between Mo and sulfate (Cardin and Mason, 1976; Underwood, 1977; Georgievskii, 1982).

Molybdenum is readily absorbed by the ruminant. However, the low overall apparent absorption of Mo may be 20 to 30% of dietary intake (Hansard, 1983).

The extent of absorption is dependent upon the route of administration (Bell et al., 1964; Miller at al., 1972; Mason et al., 1978; Hansard, 1983). Only 29.8% of $^{99}MoO_4^{-2}$ was absorbed from rumen doses while 62.2% of dosed $^{99}MoO_4^{-2}$ was absorbed from the abomasum (Miller at al., 1972). Miller at al. (1972) found that $^{99}Mo$ was absorbed from each section of the digestive tract of cattle from the abomasum through the length of the small intestine. No appreciable absorption of $^{99}Mo$ was observed from the rumen or omasum; however, net $^{99}Mo$ absorption by calves was doubled when the rumen, reticulum and omasum were by-passed. The absorption of $^{99}Mo$ from the abomasum of calves and the stomach of pigs was 74% of the Mo that was dosed.

Most of the Mo orally administered to swine was absorbed and rapidly excreted in the urine (Bell et al., 1964). With cattle, a higher percentage of orally dosed $^{99}Mo$ was excreted in the feces and little was found in the urine (Bell et al., 1964). Duodenal administration of $^{99}MoO_4^{-2}$ in sheep was followed by rapid absorption and excretion in urine (Mason et al., 1978). This was the same pattern of excretion seen in orally-dosed non-ruminant animals or abomasally-dosed calves (Miller et al., 1972).

Ruminal administration of Mo gave an entirely different pattern of absorption and excretion when compared to duodenal administration (Mason et al., 1978). With ruminal administration, an initial rapid appearance of radioactivity in the blood was absent. Less than 2% of the dose was detected in the blood 20-24 hours post-administration. More radioactivity was excreted in the feces.

Molybdate is transformed into a poorly absorbed form in the rumen (Mason et al., 1978). The reduced absorption of $^{99}Mo$ after passage through the rumen was due to a physical or chemical change occurring in this section of the gastrointestinal tract (Miller at al., 1972). Mason et al. (1978) and Kelleher et al. (1983) provided evidence that both Cu and sulfate interact with Mo during passage through the rumen, with a consequent decrease in the efficiency of post-ruminal absorption.

Although the forestomach is not a major site of absorption for Mo, net secretion of Mo into the reticulo rumen and omasum via saliva or by secretion directly into these compartments have been reported (Miller et al., 1972; Grace and Suttle, 1979). Grace and Suttle (1979) observed a higher recycling of Mo via the saliva into the rumen when dietary Mo was high and dietary S was low. Radioactive Mo moves slowly through the digestive tract of cattle (Hansard, 1983). About 15% of the isotope administered was retained for a few days within the digestive tract. This was due to fixation by the microflora (Georgievskii, 1982 citing Anke et al., 1971).

Molybdenum is absorbed primarily between the duodenum and ileum (Miller et al., 1972; Mason et al., 1978). The highest level of absorption of $^{99}Mo$ as $Na_2MoO_4$ from everted intestinal sacs was in the ileal segments of rats and very low in the proximal portion of the small intestine (Cardin and Mason, 1976). Comparable results were obtained by incubating small pieces of the distal ileum of sheep. The distal ileum of the small intestine was also the site of maximal Mo absorption (Mason and Cardin, 1977).

The process of Mo absorption is both carrier mediated, involving active transport (Cardin and Mason, 1975, 1976; Mason and Cardin, 1977) and by diffusion (Kosarek, 1976; Kosarek and Winston, 1977), depending upon the level or concentration of Mo in the gut contents. Three pools of Mo are recognized (Cardin and Mason, 1976): (1) that portion in the luminal fluid or mucosal concentration, (M); (2) that portion in the gut cells or gut concentration, (G); and (3) that portion in the serosal fluid or serosal concentration, (S). It was assumed, that if there were active transport of Mo from the mucosal to the other pools, then the ratios between the final and initial concentrations would be greater than unity. Cardin and Mason (1976) found that this was indeed true; the ratios for both S/M and G/M were greater than unity, except for the highest concentration (1mM) of $MoO_4^{-2}$ used in the incubating fluid with everted sacs of rat ileum. They also suggested that the consistently higher ratios for S/M than for G/M were further support for their contention (Cardin & Mason, 1976) that active transport occurred in the ileum. They demonstrated that most of the Mo moved through the serosal fluid and did not accumulate in the cells.

Kosarek (1976) and Kosarek & Winston (1977) used high concentrations of $^{99}Mo$ in vivo and demonstrated that absorption rates were essentially the same over a 10-fold range of 10-100 mg $L^{-1}$. These data revealed that absorption was based on diffusion alone. The concentrations used by Kosarek (1976), and Kosarek and Winston (1977) fell within and around the range tested by Cardin and Mason (1976). Cardin and Mason (1976) had noted that there was no active transport at

similar high Mo concentrations. Thus, it is possible that molybdate is moved by diffusion and by active transport. At high concentrations, active transport contributes less to Mo absorption.

Molybdenum, as molybdate, partially inhibits sulfate transport in the rat intestine through competition for the same carrier (Cardin and Mason, 1975). When small pieces of ileum from sheep were incubated, Mason and Cardin (1977) found that added sulfate ($SO_4^{-2}$) and copper ($Cu^{+2}$) partially inhibited Mo transport.

Despite a low apparent absorption, much of the Mo that does become absorbed is excreted in the urine. Some Mo is also excreted into the bile and in milk (Hansard, 1983). The principal route of Mo excretion in steers is feces (Bell et al., 1964; Miller et al., 1972). In 7 days, the fecal excretion of an oral dose of $^{99}Mo$ administered to steers averaged 92.4%, whereas urinary excretion averaged 4.5% (Bell et al., 1964).

## 2.2 The Copper x Molybdenum x Sulfur Interrelationship

### 2.2.1 Introduction

The interrelationship between Cu, Mo and sulfate was first reported by Dick and Bull (1945), and Dick (1952, 1953a, b, c). The metabolic interactions among Cu, Mo and S have been researched extensively. Over the last 40 years, this interaction has perhaps attracted more interest and attention than each of the elements separately.

A physiological Cu deficiency can result from this complex interaction among Cu, Mo and S. The dietary Cu is converted into biologically unavailable and insoluble complexes both at the gut and at the systemic level (Mason, 1981).

While Mo and S both have direct effects on the availability of Cu, many aspects of the three-way interaction have been rationalized by the chemical reaction between molybdate and sulfate in the rumen. The products of such a reaction, namely thiomolybdates, are thought to reduce the availability of Cu in both the gut and interfere with Cu metabolism systemically (Mason, 1981).

### 2.2.2 Mo - Cu Antagonism

Molybdenosis, hypocuprosis and conditional Cu deficiency are all terms used to describe a metabolic disturbance caused by a high intake of Mo (Ward, 1978). The antagonistic effects of dietary Mo upon Cu metabolism in ruminants are well known. The clinical symptoms which follow are economically important and include: poor growth, reduced feed intake, infertility, diarrhea, anemia, and bone abnormalities (Pitt, 1976; Underwood, 1977; Ward, 1978). In most cases, these symptoms are accompanied by changes in tissue and blood Cu concentrations, and can be alleviated by appropriate administration of supplementary Cu (Suttle 1983).

There are inter-species differences in response to excessive dietary Mo. Non-ruminants are relatively insensitive to Mo, while ruminants are extremely sensitive. Cattle are the least tolerant,

followed by sheep. Rats, rabbits, pigs and poultry, as non-ruminants, are next in order of tolerance (Pitt, 1976).

The ratio of Cu to Mo in the diet is very important in avoiding a Mo toxicity, or a Cu deficiency (Miltimore and Mason, 1971; Alloway, 1973). There is disagreement as to what the exact or appropriate ratio should be. Miltimore and Mason (1971) indicated that the critical Cu:Mo ratio in the diet should be 2.0, and that a dietary Cu to Mo ratio of less than 2:1 results in a conditioned Cu deficiency. Alloway (1973) suggested that a ratio closer to 4:1 is necessary for avoiding hypocuprosis.

Dietary levels of Mo, lower than 8 mg $kg^{-1}$, interfere with Cu metabolism. Suttle (1974 ) reported that an increase of 2-4 mg Mo $kg^{-1}$ decreased the efficiency of Cu utilization. An increment of dietary Mo of 4 mg $kg^{-1}$ decreased Cu availability by 35%, when the basal diet contained 2 mg Mo $kg^{-1}$. Small increases in herbage Mo which accompany pasture improvement (Whitelaw et al., 1979) or which occur naturally on Mo-rich soils such as black shales (Thornton et al., 1972) also reduce the absorbability of Cu from fresh and conserved herbage and increase the incidence of hypocuprosis.

At a given S concentration, the antagonistic effect of Mo was proportionately less in hay than in fresh material (Suttle, 1986). Increasing the Mo content of both these forage types, however, still reduced the percent Cu available in each forage.

Studies of the antagonism between Mo and Cu in ruminants have revealed that when dietary Mo content increases, the way in which Mo

inhibits Cu metabolism also changes (Smith and Wright, 1975; Bremner and Young, 1978). At relatively low dietary contents of Mo, in the range of 1-10 mg $kg^{-1}$ DM, effects are attributable to marked decreases in the efficiency in which dietary Cu is absorbed. Dietary Mo in the range of 2-5 mg $kg^{-1}$ can decrease plasma Cu concentrations in ruminants, consistent with the development of Cu deficiency (Mills et al., 1977; Bremner, 1979). At greater Mo intakes, this is not always so. Higher concentrations of Cu in both plasma and kidneys have been observed in sheep receiving diets with approximately 25 mg Mo $kg^{-1}$, in spite of lower concentration of Cu in liver tissue (Smith and Wright, 1975; Bremner and Young, 1978).

In sheep, dietary Mo in excess of 50 mg $kg^{-1}$ DM produced clinical signs of Cu deficiency (Mason, 1981). Blood and hepatic Cu were at levels considered adequate to supply sufficient Cu for enzyme prosthetic groups and thus maintain normal metabolism. This indicated that there was a systemic effect of Mo which inhibited the distribution and motility of Cu from tissue reserves where Cu has a functional role. This situation can be compared to that arising from a lower dietary intake of Mo (less than 8 mg $kg^{-1}$), where the progressive depletion of Cu reserves and decreased blood Cu levels develop before the clinical signs of Cu deficiency appear (Dick, 1956).

Dowdy and Matrone (1968a,b) proposed that the antagonism between Mo and Cu is due to the formation of a Cu-Mo complex. They observed such a complex in vitro at a pH range near neutrality. Using X-ray diffraction analysis, Dowdy et al. (1969) revealed this complex to be similar to the

mineral lindgrenite, $2CuMoO_4 \cdot Cu(OH)_2$. This has been referred to as either "Cu(II) molybdate", or as "cupric molybdate" (Huisingh et al., 1973; Pitt, 1976). Dowdy et al. (1969) determined that the molar ratio of Cu to Mo in the complex was about 4:3, and contained no sulfur.

Dowdy and Matrone (1968a, b) observed that this Cu-Mo complex appeared to be absorbed but was metabolically unavailable in the body. In baby pigs, for instance, the cupric molybdate compound was absorbed. Serum Cu levels were similar to those in pigs fed supplemental copper sulfate. A measure of the activity of ceruloplasmin in plasma however, indicated that the Cu from cupric molybdate was biologically unavailable for synthesis of ceruloplasmin.

When the Cu-Mo complex was injected into sheep, both Cu and Mo were removed from the blood at the same rate. Dowdy and Matrone (1968a) concluded ath the Cu-Mo complex was transported as a unit, in vivo.

Some early studies (Dowdy and Matrone, 1968b) also demonstrated that the Cu-Mo complex bound-Mo was turned over slowly by the tissues. Tissue Mo levels were due to the presence of this complex were higher in pigs than Mo levels due to an equal amount of Mo, present as sodium molybdate.

The Cu-Mo complex has been examined more fully. Huisingh and Matrone (1976) demonstrated that the complex was not stable in serum. When cupric molybdate was added to serum and dialyzed against saline, the $Cu^{+2}$ remained bound within the serum, while the molybdate was freely dialyzable. This observation suggested that the Cu-Mo complex does not exist as such in the serum, but that the $Cu^{+2}$ becomes bound to serum

proteins. Molybdate remains, however, as a free ion. Huisingh and Matrone (1976) did note cupric molybdate could exist in rumen fluid.

### 2.2.3 Sulfur-Copper Antagonism

Sulfur, in either organic or inorganic forms, interacts with Cu in the ruminant animal and results in Cu deficiency (Suttle, 1974). This S-Cu antagonism is due to the formation of insoluble Cu sulfide (CuS) in the rumen, in the gastrointestinal tract beyond the rumen, and at the tissue or systemic level (Huisingh and Matrone, 1976). Copper sulfide represents an unavailable form of Cu at both the gut and systemic level.

#### 2.2.3.1 Production of Sulfide in the Rumen

Microorganisms in the rumen are capable of degrading both inorganic and organic forms of sulfur (Lewis, 1954; Bird & Hume, 1971; Gawthorne and Nader, 1976). Inorganic forms of S which are readily reduced to sulfide, include: sulfate, thiosulfate and sulfites. Organic forms of S include various S-amino acids, proteins and sulfated polysaccharides. Organic S, according to Hartmans and Bosman (1970), provides 60 to 70% of the S in herbage, as constituents of S-amino acids of leaf proteins, which contain the amino acids cysteine, cystine and methionine. Sulfide can also be released from these S-amino acids through microbial action (Gawthorne and Nader, 1976).

Ruminants, as opposed to monogastric species, are able to generate large quantities of hydrogen sulfide gas in the reticulo-rumen. This is achieved through the action of sulfide producing bacteria, such as

Desulfovibrio, which are present in rumen contents (Huisingh et al., 1974). Bray and Till (1975) reported that these bacteria have been found in high concentrations in the range of $10^2$ to $10^8$ $ml^{-1}$ of rumen fluid in sheep consuming sulfate-containing diets.

Sulfate reduction to sulfide occurs through a dissimilatory pathway. Dissimilatory reducing bacteria use sulfate as a terminal electron acceptor and produce large amounts of sulfide (Peck, 1962). Huisingh and Matrone (1976) indicated that the enzyme, adenosine triphosphate-sulfurylase is the first enzyme in the dissimilatory sulfate reducing pathway and catalyzes the activation of sulfate by adenosine triphosphate to form adenosine-5'-phosphophosphate (APS). This product will ultimately form hydrogen sulfide. Huisingh et al. (1974) stated that these dissimilatory sulfate-reducing bacteria make the major contribution to the total sulfate reduced in the rumen. Thus, both organic and inorganic forms of S are broken down readily to sulfide with the rate of sulfide production being very rapid.

The sulfide generated in the rumen may be absorbed, detoxified in the liver and incorporated into S-amino acid. This sulfide may be precipitated as the heavy metal, cupric sulfide (CuS) in the rumen and in post ruminal digestive tract, as well as in the tissues (Mason, 1981; Kandylis, 1984).

2.2.3.2 Effects of Sulfur on Copper Absorption and Systemic Effects

A reduced absorption of Cu and detrimental systemic effects of dietary S, in either inorganic forms such as $Na_2SO_4$, or organic forms, such as S amino acids have been observed in both conventional (Suttle, 1974) and semi-purified (Suttle, 1975a) diets. These diets contained up to 4 g S $kg^{-1}$ diet with a low dietary Mo level of approximately 0.5 mg $kg^{-1}$ diet.

Decreased plasma Cu was reported with both organic and inorganic S sources when Cu levels were considered adequate. Total plasma Cu was most marked with cysteine, followed by methionine (Suttle, 1975a). Suttle (1975a) also observed that other Cu parameters such as plasma direct reacting Cu, ceruloplasmin, and liver Cu were lowered with an increase of supplemental S from 1 to 4.0 g $kg^{-1}$ diet. Suttle (1974) estimated that S reduced the true availability ration of Cu from 0.062 to 0.041 when S levels were increased from 1 to 4.0 $kg^{-1}$ diet.

The addition of S as copper sulfide, which provided 5 mg Cu $kg^{-1}$ to the diet of hypocupraemic ewes had no influence upon plasma Cu or haemoglobin (Suttle, 1974). However, this same amount of Cu, provided as copper sulfate, resulted in a higher plasma Cu and elevated haemoglobin levels. Dietary S also had no effect on repletion rate when Cu was given by intravenous infusion. This evidence suggested that the site of the Cu-S interaction was within the alimentary tract (Suttle, 1974).

Increasing dietary S to 4 g $kg^{-1}$ diet has an effect on young growing ruminants (Goodrich and Tillman, 1966a; Ademosun and Munyabuntu, 1982). With Mo at 0.6 to 2 mg $kg^{-1}$ diet and Cu at low dietary concentrations, an increase of dietary S reduced weight gains and feed efficiency. Increasing S intakes from 1 to 4 g S $kg^{-1}$ ration also influenced both Cu status and performance of young growing lambs fed diets with 0.66 to 2.0 mg Mo $kg^{-1}$, and 4.1 to 10mg Cu $kg^{-1}$. Feed efficiency, ceruloplasmin activity and tissue Cu levels were lower with the higher S intakes.

Elevated dietary S also has been shown to have an inhibitory effect on Cu utilization by pregnant ewes and cows (Mills and Fell, 1960; Gooneratne, 1986). There were subsequent effects upon fetal growth, development, lowered fetal Cu stores and reduced live weights of both lambs and calves.

There is no simple correlation between sulfide concentrations and soluble Cu in the rumen and the availability of Cu (Mills et al., 1978; Simpson et al., 1982). Increasing the dietary S content from 1.8 to 3.2 g S $kg^{-1}$ in a semi-synthetic diet with 11.9 mg Cu and less than 0.05 mg Mo $kg^{-1}$ DM had no effect on the retention of Cu in the liver of young calves (Mills et al., 1977). Such increases in dietary S content resulted in high ruminal sulfide levels and decreased content of Cu in the soluble fraction of rumen liquor. A fall in liver Cu was expected but Mills et al. (1977) concluded that this was perhaps a species difference, calves vs lambs, or due to the very low Mo content of the basal diet. An increase in dietary S from 1.8 to 3.2 $kg^{-1}$ DM had no

influence upon Cu retention of young growing cattle, unless Mo was added to a basal diet low in Mo (Simpson et al., 1982).

#### 2.2.3.3 Copper Sulfide Formation at the Tissue Level

Sulfide is rapidly absorbed through the rumen wall, oxidized to sulfate in the liver and is excreted as sulfate in the urine (Huisingh and Matrone, 1976). The enzyme that is responsible for the oxidation of sulfide to sulfate is sulfide oxidase in liver tissue. This enzyme is known to be dependent upon the content of Cu available in the liver (Siegel and Monty, 1961). Copper increases sulfide oxidase activity while Mo lowers it. A reduced sulfide oidase activity leads to accumulation of sulfide in the liver and to a precipitation of an insoluble CuS in the liver (Halverson et al., 1960; Siegel and Monty, 1961; Spais et al., 1968). Copper sulfide is an unavailable chemical form of Cu.

#### 2.2.3.4 Influence of Molybdenum on Rumen Sulfide

Molybdenum inhibits the rate of reduction of sulfate to sulfide in-vitro (Huisingh and Matrone, 1972) and in vivo (Gawthorne and Nader, 1976). The enzyme in the first step in sulfate reduction in sulfate reducing bacteria is ATP-sulfurylase. This enzyme is inhibited by Mo. However, high dietary Mo at 50 mg $kg^{-1}$ enhanced the rate of sulfide production from methionine (Huisingh et al., 1975). The in vivo equilibrium sulfide concentration in the rumen fluid of both sheep (Mills, 1960) and cattle (Hartmans and Bosman, 1970) was higher with

50 mg Mo $kg^{-1}$ in the diet. Ruminal sulfide concentration was also high (Gawthorne and Nader, 1976) when molybdate was infused into the rumen, despite the known apparent inhibition by Mo on sulfide production reported by Huisingh et al. (1975). Thus, a second action of molybdate was an inhibition of sulfide absorption from the rumen (Gawthorne and Nader, 1976). The impaired absorption and turnover of sulfide resulted in a high rumen sulfide content in the experiment of Gawthorne and Nader (1976). The concentration of sulfide in the post-ruminal tract was reported to be 35% higher.

2.2.3.5 Effect of Forage Type and Dietary Protein

Much of the S in feedstuffs is present as S-amino acids in protein. Consequently, sulfide production is dependent upon the amount of protein degraded in the rumen. This in turn, depends upon the level of protein in the ration, the solubility of that protein and the nature of the forage consumed (Hartmans and Bosman, 1970; Ward, 1978; Ivan and Veira, 1981; Suttle, 1986).

There was a lower storage of Cu in the liver of cattle grazing fresh pasture than in cattle fed hay, harvested at the same growth stage. Both the fresh and conserved hay diets had similar mineral contents (Hartmans and Bosman, 1970). Suttle (1986) demonstrated, with Mo at 1 mg $kg^{-1}$ DM, that the effect of S upon Cu availability was more dramatic in fresh grass than silage. Suttle (1986) noted that with each increment of S the availability of Cu was always less for fresh grass than silage. Ivan and Veira (1981) observed that Cu solubility was

proportionately decreased in both rumen and abomasal digesta as dietary crude protein content increased from 7 to 19% with a low dietary Mo of 1.5 to 1.6 mg $kg^{-1}$ DM.

Ward (1978) had postulated that high levels of very soluble, degradable protein in fresh pasture resulted in formation of CuS, a result of sulfide generation from S-amino acids within the rumen. Beever et al. (1976) concluded that when forage is dried, rumen degradability of protein was reduced compared to fresh forage. This resulted in less ruminal sulfide production in sheep fed hay, than those given green forage. These facts offer an explanation for the symptoms resulting from normal Cu and low Mo intakes on pasture, in addition to the effects of forage preservation upon the induction of clinical signs of Cu deficiency in ruminants.

#### 2.2.3.6 Forage, Grain and Water Levels of S

The S in pasture can vary from 1.8 to 5.0 g $kg^{-1}$ DM, with the range of S levels in grasses and clovers being similar. In certain species, such as Kale or Brassica oleracea, extreme concentrations of up to 8 g S $kg^{-1}$ DM have been reported (Metsen and Saunders, 1978; Barry et al., 1981). Merry et al. (1983) suggested that diets in South Australia with appreciable quantities of cruciferous species which have low Mo, but high S concentrations, could induce a Cu deficiency in ruminants. Cereal grains, such as barley, corn and oats tend to be similar in S contents ranging from 1.4 to 2.3 g S $kg^{-1}$ DM (NAS-NRC, 1984).

Another source of S, in addition to organic S in feedstuffs and that which is supplemented in rations, is sulfate present in water. The levels of sulfate found in deep well water in many areas of Saskatchewan may be as high as 2000 mg $L^{-1}$. The S present in the water is 100% available in the rumen, as opposed to 80% available when present as a dietary supplement (Gooneratne, 1986). The total dietary S intake to a 500 kg cow, via both water and the feed could be as high as 0.31% (Gooneratne, 1986).

Toxicity of S has rarely been demonstrated at levels below 2.5 g S added $kg^{-1}$ to the diet as sulfate. The addition of 2 g S $kg^{-1}$ DM to diets containing 1 g S $kg^{-1}$ DM, however, can result in a 35% reduction in Cu absorption (Suttle, 1974, 1975a). Thus, some of the effects of high sulfate in water could be symptoms of a S toxicity superimposed upon a Cu deficiency.

### 2.2.4 Thiomolybdates and Copper Antagonism

#### 2.2.4.1 Thiomolybdate Formation

Thiomolybdates, with the general formula $[MoO_NS_{(4-N)}]^{-2}$, where N = 0 to 3, are formed in the rumen environment (Dick et al., 1975). Essential steps for their formation (Dick et al., 1975) include: (1) Reduction in the rumen of sulfate to sulfide; and (2) Progressive reaction of hydrogen sulfide with molybdate in the rumen, at relatively neutral pH to produce thiomolybdates. The thiomolybdates react with Cu to produce insoluble Cu-thiomolybdates. Copper, as Cu-thiomolybdate, is

not available for absorption and is excreted in the feces. Soluble thiomolybdates produced in excess and not combined with Cu within the digestive tract appear to be absorbed, and as a consequence, interfere with Cu metabolism systemically. Thus, thiomolybdates are responsible for the induction of Cu deficiency in ruminants fed diets high in both Mo and S contents.

Di-, tri- and tetra- thiomolybdates are apparently formed in the rumen, depending on specific conditions (Clarke and Laurie, 1980). Thiomolybdates exist in equilibrium with one another. The formation of a specific thiomolybdate is critically dependent upon the ratio of S to Mo in the rumen (Clarke and Laurie, 1980). Under conditions where the ratio is relatively low, the formation of dithiomolybdate $[MoO_2S_2]^{-2}$ and trithiomolybdate $[MoOS_3]^{-2}$ is favored. This would occur at normally low concentrations of 1 g S $kg^{-1}$ and 1 mg Mo $kg^{-1}$. At higher S:Mo ratios, and over long periods of time, extensive formation of tetrathiomolybdate $[MoS_4]^{-2}$ is favored. Of all the thiomolybdates, tetrathiomolybdate is considered the most potent, the most stable in the rumen (Gooneratne, 1986) and a very effective Cu antagonist.

2.2.4.2 Synthesis and Absorption of Thiomolybdates

Evidence for in vivo and in vitro synthesis of thiomolybdates (Mills et al., 1978; Hynes et al., 1985; Price et al., 1987) and their subsequent absorption from various sites of the gastrointestinal tract (Mason et al., 1980, 1982, a, b; Kelleher et al., 1983; Hynes et al., 1984, 1985) have been reported.

Mills et al. (1978) incubated suspensions of sieved rumen contents under anaerobic conditions with both ammonium molybdate to provide 5 or 10 mg Mo $L^{-1}$, original medium, and S sources to provide 20-50 mg total S $L^{-1}$. These conditions led to the appearance of the characteristic absorption spectrum of the $[MoS_4]^{-2}$ ion in the centrifuged supernatants of such cultures.

The characteristic absorption spectrum of tetrathiomolybdate was not detected in rumen fluid unless the Mo and S were well above that present at normal dietary ranges of Mo and S (Mills et al.; 1978). The absorption spectrum of tetrathiomolybdate was not detected in the supernatant of rumen contents obtained from cattle that were maintained on a diet that consisted of 5 mg Mo, 11 mg Cu and 3.8 g S $kg^{-1}$ DM (Mills et al., 1978). These authors (1978) suggested that the spectrophotometric techniques were insensitive to thiomolybdates in vivo at dietary concentrations less than 100 mg Mo $kg^{-1}$ diet. The failure to detect tetrathiomolybdate was due in part to both the dilution of ingested Mo in the liquid phase of rumen contents and the distribution of Mo between solid and liquid phases.

Thiomolybdate anions are water soluble (Price and Chesters, 1985) and are largely associated with the particulate matter in rumen digesta (Price et al, 1987). When tetrathiomolybdate was added in solution to rumen contents, it rapidly disappeared from the aqueous phase.

More recently, Price et al. (1987) provided direct evidence for thiomolybdate synthesis within the rumen of sheep, under dietary conditions similar to those encountered in field cases of Mo induced Cu

deficiency. At 16 hours after injection of $^{99}MoO_4$ into the rumen of sheep maintained on dried grass (6.2 mg Mo $kg^{-1}$ DM, 4.3 g S $kg^{-1}$ DM), thiomolybdates, mainly tri- and tetra-species were identified by Sephadex G25 chromatography in the rumen solids.

The absorption of thiomolybdates has been demonstrated in vivo (Mason et al., 1982a). A protein bound, trichloroacetic acid (TCA)-insoluble $^{99}Mo$ appeared in plasma a few hours after infusion of 30 mg $^{99}Mo$ into the rumen of sheep fed a concentrate diet supplemented with 3 g S $day^{-1}$. Most of the $^{99}Mo$ could be displaced from its protein carrier in vitro. The displaced labelled compounds were identified as di- and trithiomolybdates. However, tetrathiomolybdate was not detected in the plasma of sheep. $^{99}Mo$ labelled di- and trithiomolybdates, but not tetrathiomolybdate were also detected in the plasma of cattle, after the infusion of $^{99}Mo$ labelled molybdate into the rumen (Hynes et al., 1984, 1985).

Price et al. (1987) reported that whether injected into the rumen of sheep as molybdate or as tetrathiomolybdate, bound $^{99}Mo$ that appeared in plasma was mainly present as di- and trithio-species. The tetrathio-species appeared in only trace amounts in plasma but only after injection of tetrathiomolybdate. This was noted in spite of the existence of tetrathiomolybdate as a major form of thiomolybdate in rumen digesta.

Infusion of $^{99}Mo$-labelled di-, tri- and tetrathiomolybdate into the duodenum (Mason et al., 1980; 1982 b) resulted in absorption of these compounds. Kelleher et al. (1983) found that both tri- and

tetrathiomolybdate $^{99}Mo$ were rapidly absorbed from the rumen and duodenum; they were found in the plasma in a protein-bound form. A significant proportion of the thiomolybdates survived the acid environment of the post abomasal digestive tract. This occurred in spite of their sensitivity to acid hydrolysis and the absorption process itself (Mason, 1981; Clarke and Laurie, 1980). Kelleher et al. (1983) observed that Mo from thiomolybdates that were absorbed from the duodenum, was detected in plasma in larger quantities in the TCA-soluble fraction, in addition to the normally high Mo component in the TCA-insoluble fraction.

#### 2.2.4.3 Effects of Thiomolybdates on Copper Availability: Gut and Systemic Sites of Action

Two separate sites of action of thiomolybdates in reducing Cu availability have been demonstrated. Firstly, thimolybdates react with Cu and lead to a reduction in Cu absorption with a resultant hypocupraemia. Secondly, absorption of excess thiomolybdates from the digestive tract will result in a lowered availability of Cu systemically, at various metabolic sites of action. These actions of thiomolybdates have been noted in sheep and cattle (Mason, 1981).

Some important aspects of the effects of thiomolybdates and, or high dietary levels of both S and Mo, on Cu absorption, Cu metabolism, and ultimately animal performance have been noted:

(1) Mature cattle and sheep, and growing ruminants respond

similarly when fed diets containing high levels of both Mo and S

(2) The effect of high Mo and high S on Cu absorption in ruminants has been mimicked by the addition of pre-formed thimolybdates to the diets of ruminants and non-ruminants such as rats (Mills et al., 1978; Bremner and Young, 1978)

(3) High Mo and S or tetrathiomolybdate, and their effects upon absorption of Cu, result in lower plasma and liver Cu levels and reduced enzyme activity of ceruloplasmin. These may occur when the dietary Cu levels are thought to be adequate (Goodrich and Tillman, 1966b; Mills et al., 1977, 1978; Ademosun and Munyabuntu, 1982; Robinson et al., 1987; Wittenberg and Boila, personal communication).

(4) Addition of high Mo and high S levels to the diet, in order to mimic the effects of thiomolybdates lead to decreased animal performance, reflected by poorer growth rates (Ademosun and Munyabuntu, 1982).

Mills et al. (1982) suggested that $[MoS_4]^{-2}$ is a more effective inhibitor of Cu absorption than any of the partially substituted molybdates. Also, $[MoS_4]^{-2}$ is a more effective inhibitor of Cu absorption, than sulfide (Mills et al., 1982).

The absorption of Cu from the digestive tract may be reduced by the formation of Cu-thiomolybdate-protein complexes that are poorly digested and absorbed (Gawthorne et al., 1982). Gawthorne et al. (1982) suggested that conditions for the formation of complexes and

interactions would be particularly favourable in ruminants because of the long retention time of digesta, sulfide concentration and neutral pH of the rumen environment.

Once thiomolybdates are absorbed, particularly as di- and tri-species (Price et al., 1987), they bind rapidly to plasma proteins that exhibit increased reactivity toward Cu ions. Thiomolybdates bind to plasma proteins such as albumin, in vivo. In a bound form, the thiomolybdate protein complex is relatively stable (Kelleher et al., 1983; Hynes et al., 1984).

Infusion of thiomolybdates led to the appearance of a TCA-insoluble fraction which had a great affinity for Cu. The increased proportion of Cu associated with the albumin results in an altered systemic metabolism (Hynes et al., 1984). Hynes et al. (1984) concluded that the accumulation of Cu on albumin does not occur because of a direct interaction with thiomolybdates that are carried on albumin. It is more likely the result of a modification by thiomolybdates of the way in which Cu is bound to the albumin molecule.

#### 2.2.4.4 Effects of Thiomolybdates on Copper Excretion

Gooneratne et al. (1981a, b) established that thiomolybdates, when injected intravenously, reduced liver Cu levels in sheep and increased Cu excretion via the urine and feces. They had stated that Cu excreted in the feces was derived from (a) biliary excretion, (b) endogenous secretions of saliva, gastric and intestinal juices, and (c) exogenous excretion of unabsorbed dietary Cu.

Bile has been considered to be a major pathway for excretion of Cu from the body (Underwood, 1977). The effect of thiomolybdates upon loss of Cu via bile was examined more fully. Gooneratne et al. (1985) reported that biliary excretion of Cu increased 1.5 to 3-fold in sheep injected intravenously with thiomolybdate. The effect was dramatic and the peak Cu concentration in bile was reached 3 hours post-injection, indicating the efficiency of thiomolybdate in increasing biliary Cu excretion. The excretion profile of Mo followed the same pattern. This indicated that both Cu and Mo were apparently excreted as a Cu-thiomolybdate complex. Gooneratne et al. (1985) suggested that a Cu-thiomolybdate complex, a TCA-insoluble component, similar to that in the plasma, is formed in the liver and then excreted in bile. This was supported by the coincidence of the Cu and Mo peaks in both plasma and bile, which occurred at approximately 12 hours post-injection. Thus, evidence for enhanced excretion of Cu by thiomolybdates via the biliary route has been reported.

In a subsequent study, Gooneratne et al. (1986) injected sheep intravenously with $^{67}Cu$, followed by an intravenous dose of $^{99}Mo$ labelled tetrathiomolybdate after 27 hours had passed. These sheep were fed diets with either 5 or 35 mg Cu kg $DM^{-1}$. Tetrathiomolybdate increased both $^{67}Cu$ and stable Cu excretion two to three fold in the bile. Furthermore, tetrathiomolybdate induced a major shift of Cu into high molecular weight proteins having a molecular weight greater than 80,000.

Tetrathiomolybdates induce a negative Cu balance in ruminants two ways: (1) by promoting biliary Cu excretion; and (2) by increasing the percent of Cu in the macromolecular fraction of bile, thus limiting enterohepatic circulation of Cu.

## 2.3 Physiology of Mineral Absorption: A General Overview

### 2.3.1 Intake of Minerals into the Digestive Tract

Minerals enter the digestive tract through exogenous and endogenous routes. Exogenous routes include intake of feedstuffs, water, soil and pre-formed mineral supplements. Endogenous routes include salivary, pancreatic, biliary and intestinal secretions, as well as desquamation of intestinal cells.

### 2.3.2 Mineral Absorption Events

Mineral absorption can be thought of as three events, which follow a specific sequence (Rosenberg and Solomons, 1984):

(1) Luminal Events which govern the preparation and delivery of a mineral for UPTAKE into the cells (enterocytes) of the digestive tract;

(2) Mucosal Events that determine the TRANSFER of a mineral through the cell to the basolateral surfaces; and

(3) Post-Absorptive Events which govern the TRANSPORT and disposition of the mineral into the mesenteric circulation toward the liver and peripheral tissues.

In ruminants, ruminal effects play a major role in the release of minerals, a result of the breakdown of feedstuffs through microbial activity.

2.3.2.1 Ruminal Events

Once ingested, feedstuffs enter the reticulo-rumen where they undergo pre-gastric modification. While resident in the rumen, feedstuffs are broken down through mastication or rumination and by fermentative action of microbial populations until feed particles are small enough to pass to the lower digestive tract (Oldham, 1985). Through these initial processes, minerals are released, become solubilized and become available to the microbial population in the rumen digesta (Playne et al., 1978).

Minerals within the microbial fraction are associated with microorganisms in several ways (Durand and Kawashima, 1980). Both macro and trace elements are present in microorganisms through an association of the element with the cell surface or cytoplasmic membranes, and to an uptake, an influx process. Various factors influence the association of a mineral element with a microorganism. These factors include ruminal pH, the binding ability of that mineral, the extent of release of a mineral from feedstuffs into a soluble or available form for the microbes, and the nature of the mineral in question.

Differences between the extent of mineral release (Playne et al., 1978; Rooke et al., 1983; Van Eys and Reid, 1987) and the in vivo solubility of minerals, as well as the concentrations of soluble

minerals in the rumen contents have been demonstrated for both macro and trace elements for various diets (Yano et al., 1979; Ivan and Veira, 1982; Ivan et al., 1983 a, b). A dynamic equilibrium exists between minerals in the free, soluble state and those that are in association with the solid ruminal digesta phase which includes rumen microorganisms and undegraded feed residue (Durand and Kawashima, 1980).

The digesta leaving the rumen contains food particles which are small enough to leave. Microbes can be attached to these particles or are in the fluid phase of digesta (Oldham, 1985). Minerals are found distributed in these fractions. Rumen digesta enters the abomasum (true stomach) where there is further digestion and breakdown of feedstuffs for subsequent absorption in the lumen of the segments of the intestinal tract (Cole & Garrett, 1980).

2.3.2.2 Luminal Events

After ingestion and subsequent ruminal events, dominant intraluminal events involve two major processes (Rosenberg and Solomons, 1984). The first process is a freeing of the mineral from its association with the matrix in the original feed or in microorganisms into a form sufficiently soluble to be absorbed. Secondly, there is propulsion of the mineral to the appropriate gastrointestinal segment in order for absorption to occur.

Digestion of proteins and dissolution of salt crystals are important processes. Proteolytic digestion of both metalloproteins and metalloenzymes is essential to the liberation of protein-bound minerals

(Rosenberg & Solomons, 1984). Gastric acid and the prevailing conditions of the abomasum are important for making such macrominerals as Ca, Mg and P available for absorption (Maynard et al., 1979; Field, 1981; Georgievskii, 1982; Rosenberg and Solomons, 1984).

The oxidation state of a mineral is a determinant of its solubility and of its subsequent uptake. The pH of luminal contents and the microenvironment of the unstirred layer adjacent to the brush border also influences the availability of minerals with variable valence states such as Cu and Fe (Rosenberg and Solomons, 1984).

Intraluminal mineral binding molecules or ligands of endogenous origin may assist the delivery of a mineral to the mucosal membrane (Evans, 1976; Bremner and Mills, 1981). These molecules act to reduce the susceptibility of the mineral to a formation of a complex and the precipitation of minerals with other dietary factors. In turn, ligands may provide for an assisted uptake of the mineral.

The lumen of the gastrointestinal tract contains free metal, low and high molecular weight chelates of the metal, metalloproteins, and other ligands. Depending upon the stability constant (which defines the relationship between a mineral and a ligand) of a ligand, the metal in question, the concentration of other metals, a consideration of insolubility and adsorption characteristics, a new dynamic equilibrium may be established in the lumen (Kratzer and Vohra, 1986). Intracellular proteins such as transferrin may act as lumen-to-cell shuttles for inorganic, nonheme forms of Fe and Cu (Ashmead and Christy, 1985).

2.3.2.3 Mucosal Events

The uptake of minerals across the mucosal membrane is a passive or an active process. These processes are also responsible for the transfer of minerals across the lateral or basal membranes to the intercellular space and capillaries (Rosenberg and Solomons, 1984).

Experimental phenomena including saturable transport or competitive inhibition and demonstration of genetic transport defects indicate that specific channels or carriers are involved in the uptake or transfer of some minerals. When the transport is concentrative, some form of energy-requiring "pump" that is either specific for the mineral or shared by other nutrient or non-nutrient minerals must be invoked (Rosenberg and Solomons, 1984).

Once a mineral enters an enterocyte, it may follow one of several routes of utilization. It may be appropriated by the cell itself for its own nutritional and metabolic needs, it may be designated for release into the body as a systemic nutrient or it may be trapped and withheld from both metabolic pathways of the cell and the transfer pathways through the cell into the body. Both translocation and partitioning of minerals appear to be mediated by specific carrier proteins within the cell. In addition, the irreversible capture of a mineral not required for use either by the enterocyte or for the nutrition of the host involves intracellular binding proteins (Rosenberg & Solomons, 1984). Metallothionein may play such a role for Zn

(Cousins, 1979), ferritin for Fe (Huebers et al., 1979) and Ca-binding protein for Ca (Pansu et al., 1981).

#### 2.3.2.4 Post-Absorptive Events

The transportation of a nutrient away from the intestinal epothelium to body tissues involves the participation of binding proteins. These are circulating proteins of systemic origin. Serum albumin and transferrin are non-specific and specific transport proteins, respectively, for various minerals (Bremner and Mills, 1981; Georgievskii, 1982; Hansard, 1983; Rosenberg and Solomons, 1984; Kratzer & Vohra, 1986).

### 2.3.3 Effects of Dietary Constituents on Absorption of Various Minerals

Both dietary constituents and the chemical form of a mineral in the diet influence mineral absorption.

#### 2.3.3.1 Copper

The absorption of Cu is influenced by the chemical form of Cu. Copper in porphyrin compounds and copper sulfide are poorly utilized (Georgievskii, 1982). Lassiter and Bell (1960) reported that with sheep, the Cu in Cu-wire was largely unavailable, while Cu in copper oxides was less available than that in water soluble Cu salts or the carbonate form of Cu. In turn, Chapman and Bell (1963) reported that

the relative appearance of $^{64}Cu$ in the blood of cattle was in the order of: $CuCO_3 > Cu(NO_3)_2 > CuSO_4 > CuCl_2 >$ CuO(powder)>CuO(needles)>Cu(wire).

Changes in the chemical forms of copper in plants affecting availability also occur because fresh green herbage is less effective in promoting body Cu stores than hay or dried herbage of equivalent total Cu content (Hartmans and Bosman, 1970). Apparently, changes in the chemical forms of the Cu occur during the curing or drying process which improve their absorption.

The availability of Cu not only differs between forage forms, hay vs fresh grass, but also differs depending upon forage species and composition of the ration (Grace, 1975; Stevenson and Unsworth, 1978). Grace (1975) reported that the apparent availability of Cu from fresh perennial ryegrass and white clover was 30 and 34%, respectively, while it was only 9% from red clover. Stevenson and Unsworth (1978) observed when the relative proportions of ground straw, ground barley and dried grass meal in the ration were varied, the availability of the dietary Cu was less variable and ranged from +6 to -15%.

The absorption of Cu is also related to the nutritional quality of the ration. The method of processing the cereal component of the diet can also influence the results obtained (Bertoni et al., 1976a; Lamand, 1978).

2.3.3.2 Manganese

Manganese exists as the ionic form in feedstuffs (Georgievskii, 1982). The exact chemical form(s) of Mn in forages and their effects in the ruminant are largely unknown.

Differences in Mn availability have been found when a range of rations have been fed (Grace, 1975; Bertoni et al., 1976a; Ivan and Grieve, 1976). These differences did not appear, however, to be related to a particular factor. Apparent Mn absorption was different among various silages fed to sheep (Ivan et al., 1983a).

2.3.3.3 Zinc

Inorganic salts of Zn have a high availability in the gut of ruminants (NAS-NRC, 1984). The chemical forms of Zn in grains, plant proteins and forages are considered only fair, in terms of availability and utilization (Jimenez, 1980).

Marked differences in the apparent Zn absorption by mature dairy cows fed rations containing grass and rolled barley, ground pelleted maize, or ground pelleted barley were noted by Bertoni et al. (1976a). Availability of Zn from the diet containing rolled barley was markedly greater than from either of the other two diets. Differences in apparent availability of Zn in pasture forages (Grace, 1975) and differences in the apparent absorption of Zn from various silage types (Ivan et al., 1983a) have been observed.

2.3.3.4 Iron

Ruminants can utilize the nonheme Fe present in plants and soil (Hansard, 1983). The Fe in feedstuffs is more readily assimilated than Fe of animal origin. The assimilation of heme Fe that has been found in feedstuffs is thought to be poor (Gieorgievskii, 1982).

Ferrous Fe is absorbed to a much greater degree than ferric Fe. Reduction of the ferric ion to ferrous ion occurs in the small intestine before absorption (Underwood, 1977).

Ammerman et al. (1967) have ranked Fe sources in decreasing order of availability, as ferrous sulfate, ferrous carbonate, ferric chloride and ferric oxide. Thompson and Raven (1959) reported that the Fe in grasses and legumes was less available than in ferric chloride.

2.3.3.5 Calcium

The true digestibility of Ca varies in feedstuffs (NAS-NRC, 1984). Calcium is normally bound to proteins and organic acid anions in plants. The Ca in carbonate or phosphate is readily available for absorption (Georgievskii, 1982).

2.3.3.6 Phosphorus

Phosphorus occurs in organic and inorganic forms in feedstuffs. Various feedstuffs have different availabilities of P (Field and Woolliams, 1984) and vary in their true digestibility of P (NAS-NRC, 1984).

The availability of P from inorganic P supplements has been ranked (Peeler, 1972) highest available to lowest available as: dicalcium phosphate, deflourinated phosphate, bone meal and soft phosphate. Sodium phosphates and ammonium polyphosphate are approximately equal to dicalcium phosphate in P availability. Orthophosphates are more available than metaphosphates and pyrophosphates. Although phytate P is unavailable to monogastrics (Maynard et al., 1979), ruminants are able to use this form of P satisfactorily.

#### 2.3.3.7 Magnesium

Magnesium in feeds is bound to proteins, anions of organic acid complexes and other organic compounds (Georgievskii, 1982). Feedstuffs vary widely in their availability of Mg. However, the availability of Mg increases with increasing plant maturity (Underwood, 1966).

Magnesium carbonate, oxide and sulfate are considered good, available forms of supplemental Mg. The Mg in magnesite and dolomitic limestone is not readily available to cattle (Gerken and Fontenot, 1967; Ammerman and Chicco, 1968).

### 2.3.4 Physiological Factors

The percentage of dietary mineral that is absorbed varies in relation to changes in dietary level and physiological needs. Physiological factors which influence mineral absorption include age of the animal, mineral status of the animal and physiological demands such as growth, pregnancy and lactation.

2.3.4.1 Age and Growth

Mineral absorption is both higher in absolute value and more efficient in young versus older animals for many minerals. The apparent availability of Cu was higher in young, milk fed ruminants, compared to more mature, adult ruminating animals (Suttle, 1975b). With lambs, the efficiency of Cu absorption was reported at 71% at 28 days before weaning, 47% at 14 days before weaning, but only 8-10% after weaning (Suttle, 1975b). With calves, the absorption from mouth to duodenum, mouth to ileum, and mouth to anus in milk fed animals was 10, 59 and 68% respectively, compared with 10, 19 and 27% post weaning (Bremner and Davies, 1980). The decline in absorption from the small intestine was mainly responsible for the decrease in net Cu absorption after weaning (Bremner and Davies, 1980).

Calves which suckle possess an efficient mechanism for Cu absorption (Bremner, 1980). The absorption coefficient of Cu for calves is 0.7 while for mature cattle, only 0.04 (ARC, 1980). Higher absorption of Cu by young calves, as opposed to mature cattle was due to a high retention of immobile Cu in the intestinal walls which later becomes mobile when the animal is weaned (Bremner, 1980).

In adult ruminants, the absorption of Zn is 20-40% of intake, while in young animals, the relative absorption is higher (Georgievskii, 1982). In dairy cattle, Miller and Cragle (1965) reported a difference in absorption due to age of animals. The apparent absorption of dietary Zn in mature dairy cattle was 12%, compared to 20% in 5 to 12 month old

calves of the same breed maintained on the same rations. Miller and Cragle (1965) concluded, that the effects of age per se, could not be separated from differences of growth rate. Both age and growth rate, thus have an effect on Zn absorption (Miller et al., 1968; Stake et al., 1973).

The absorption of Mn also decreases with age (Hansard, 1983). However, the absorption of Mn is low and the efficiency with which Mn is absorbed by cattle remains unchanged over a very wide range of Mn intakes (Sansom et al., 1978).

Increased absorption and efficiency of absorption for macro-minerals, such as Ca (Braithwaite and Riazuddin, 1971), P (Braithwaite, 1975) and Mg (Smith, 1962) have been reported for young, growing ruminants with high demands for these minerals.

2.3.4.2 Mineral Status

Miller et al. (1970b) found that the apparent absorption of a single dose of $^{65}Zn$ decreased as dietary Zn content increased from 33 to 633 mg $kg^{-1}$. This effect was due to dietary Zn content, and not Zn status, as all animals were fed the same ration before the experiment. Calves of low Zn status (Pate et al., 1970), which were maintained on a Zn-deficient diet, absorbed $^{65}Zn$ more rapidly and to a greater extent when dosed duodenally with $^{65}Zn$, than did Zn-repleted calves fed a similar diet.

The absorption of Fe is largely dictated by body needs. Absorption plays a major role in Fe homeostasis (Underwood, 1977). Once Fe is

absorbed, it is tenaciously held by the body and not excreted to an appreciable extent. Thus, given an adequate supply in the diet, an animal regulates Fe absorption in accordance with its physiological needs.

Adult animals generally absorb 5-10% of the Fe in natural feeds, but this proportion may reach 15-20% if the diet is deficient in Fe, during intense erythropoiesis and during depletion of Fe reserves (Georgievskii, 1982). Only 2 to 20% of an oral $^{59}Fe$ dose was absorbed by normal animals, while 20 to 60% was retained during dietary Fe deficiency (Hansard, 1983). The absorption of Fe is higher in anemia conditions due to deficiency and disease (Stake, 1977).

Cattle and sheep deficient and/or fed low levels of Ca, Mg or P demonstrated increased absorption of these minerals (Smith, 1962; Braithwaite, 1975; Schneider et al., 1985). Schneider et al. (1985) reported that the percentage of $^{32}P$ absorbed was higher in sheep on low P diets. There was an increased P absorption when diets of P deficient sheep were supplemented with P (Young et al., 1966). The absorption of P rose for the first 11 days and then fell to rates comparable to control sheep fed on P adequate diets.

#### 2.3.4.3 Pregnancy and Lactation

The demand for various elements is higher when animals are pregnant or lactating. Davies and Williams (1976) demonstrated that 54% of a single intragastric dose of $^{64}Cu$ was absorbed by pregnant rats, compared with only 26% by non-pregnant ones. The quantity of Zn absorbed from

isolated loops of duodenum from pregnant rats increased up to 80% in the late stages of gestation, but not at earlier stages (12 to 15 days), when fetal demand for Zn was lower (Davies and Williams, 1977). Lactation also increased both the amount and efficiency of Zn absorption (Neathery et al., 1973; Kratzer and Vohra, 1986).

The percentage of Ca absorbed from the small intestine of dairy cows was higher in response to the onset of lactation (Care et al., 1980). Care et al. (1980) observed a positive linear relationship between the percentage of absorption of Ca and the rate of milk secretion. It was calculated that 80% of the lactational loss of Ca was compensated by an increase in the efficiency of Ca absorption.

### 2.3.5 Influence of Genetics on Absorption of Specific Minerals

#### 2.3.5.1 Introduction

The efficiency of mineral absorption is subject both to systemic and to random variation. A variation due to genetic differences can be large for certain minerals, and is the main reason why certain animals do not absorb optimum quantities of specific minerals from their diets (Field, 1984).

A requirement for minerals may vary between breeds and among individuals of the same breed. This evidence has been based upon an estimate of an effect of genetic variation upon the incidence of disorders associated with the metabolism of minerals and in the concentration of minerals in both blood and various tissues (Wiener,

1979). The effect of a between animal variation upon the efficiency of absorption of minerals from the diet is not important for all elements. Most of the information relating to the effects of animal variation relates to P, Mg and Cu.

In the last few years, a new tool has become available for studying the genetic control of mineral metabolism. Sheep and cattle of very similar or the same genotype can be produced by blastomere separation by cleavage from 4 and 8- cell embryos (i.e. cloning), followed by transplantation of the micromanipulated embryos to ewes, one to two days after oestrus. The viability of the embryo is such that monozygotic twins are produced. A greater number of single blastomeres from two different embryos are fused prior to transplantation, and are termed chimaera-derived (Willadsen, 1981).

In mineral experiments, the use of chimaera animals avoids the largest component of individual variation and permits an efficient comparison of an effect of dietary modification between monozygotic twin sets (Field and Woolliams, 1984).

2.3.5.2 Phosphorus

Individual animals may differ 2-fold in the efficiency of absorption of P from the diet (Field, 1981). Animals which absorb dietary P with a high efficiency excreted P in the urine.

Phosphorus metabolism appears to be under genetic control (Field and Suttle, 1979). A variation in urinary excretion and hence

efficiency of absorption of P was much greater between than with three sets of monozygotic twin cows.

Additional evidence for the view that individual differences in P absorption had a genetic basis came from two studies involving chimaera-derived triplets (Field et al., 1983). The efficiencies of absorption of total P in the diet were very similar within, but were markedly different between four sets of triplets. Mean values for the efficiency of absorption, metabolic requirements relative to intake were 0.74, 0.65, 0.84 and 0.82. The fractional absorption of inorganic P supplement had the same ranking with the mean values of 0.67, 0.47, 0.92 and 0.85, respectively.

The availability of P was measured in a variety of feedstuffs, using the same chimaera-derived triplets (Field and Woolliams, 1984). Twelve feedstuffs were used; the efficiency of absorption was different between the sets of triplets, and between the diets. There was no evidence that the difference between the sets was influenced by the type of diet being fed. The ranking of the sets on each diet was identical for 10 out of the 12 diets.

#### 2.3.5.3 Magnesium

Hypomagnesaemic tetany is a disease that is common in beef cows grazing lush spring forages. This disease also affects large numbers of high producing dairy cows grazing on forages and is characterized by a metabolic deficiency of Mg (Greene et al., 1986). This deficiency of Mg may be due to a low dietary intake or a reduced availability of dietary

Mg. Hypomagnesaemia occurs most frequently during early lactation (Greene et al., 1986)

Field (1984, citing Butler et al. 1983) noted that a large variation between animals and breeds existed in relation to their susceptibility to hypomagnesaemia. Part of this difference was due to variation in the net requirements of Mg and part of the difference was due to the differences in the efficiency of absorption of dietary Mg among individuals. Kemp and Guerink (1978) reported variation in apparent absorption of 7-33% and Hutton et al. (1965) reported a range of 0-37%. The Agricultural Research Council (1980) has recognized this variability and recommended a dietary allowance for Mg.

It is not clear what part heredity plays in the absorption of Mg. The contribution of heredity has been reported by Field and Suttle (1979) with monozygotic twin cattle and Field and Woolliams (1984) with chimaera-derived sheep. Field and Suttle (1979) showed that there was a three-fold difference in the efficiency of absorption between sets of twins. Field and Woolliams (1984) observed that one set of triplets always excreted less Mg in their urine than the other three sets on each of 12 diets. They concluded that since urinary excretion represents Mg absorbed surplus to requirements, requirement for Mg was apparently controlled genetically.

These above studies measured apparent and not true absorption. Therefore, part of the differences in apparent absorption were likely attributable to differences in endogenous fecal excretion.

Significant differences in the true digestibility of Mg among cow breeds and their crosses were noted by Greene et al. (1986). Brahman cattle and their crosses were less susceptible to grass tetany. This was due to an increased ability to maintain a higher Mg digestibility, a contrast to other beef and dairy breeds. Greene et al. (1986) explained that Brahman cattle had a smaller digestive tract volume, which may be related to a shorter ruminal retention time. Thus, there was a more efficient absorption of Mg.

2.3.5.4 Copper

Different breeds of sheep have a different ability to maintain stores of Cu in their bodies. Some breeds of sheep have a higher efficiency of Cu absorption than others (Wiener et al., 1978).

While no genetic differences in Cu absorption have been reported in cattle, Rowlands et al. (1974) and Wiener et al. (1980) have reported a heritable component to the variation in concentrations of Cu in plasma of cattle. Field (1984, citing Gibson's unpublished results) stated that the Jersey breed of cattle have a higher requirement than the Friesian. Gooneratne (1986) remarked that a Cu deficiency was observed more frequently in Simmental cattle than in other breeds. This was due to an enhanced excretion of Cu in both bile and urine.

# 3. MATERIALS AND METHODS

## 3.1 Experimental Animals

Four Holstein steers, averaging 235 kg in weight at the beginning of the experiment, were fistulated in the rumen, proximal duodenum and terminal ileum (approximately 10-15 cm proximal from the ileal-cecal junction). Steers were approximately 11 months of age at the beginning of the experiment. The duodenal and ileal fistulae were each fitted with a soft, flexible, plastisol T-shaped cannula. Plastisol plugs were also prepared in order to prevent leakage and accumulation of material in the barrel of each cannula. The rumen fistulae were fitted with a Bar Diamond flexible cannula (Bar Diamond, Inc., Parma, Idaho). All steers were given approximately eight weeks to recover from surgery before the start of the experiment.

## 3.2 Experimental Diets

Four experimental pelleted rations were fed to steers, in a 4 x 4 Latin Square Design. All four rations (Table 1) consisted of barley and chopped hay with supplements. The four experimental rations with added minerals on an as-fed basis were: (1) a basal ration which contained no added Mo or S (low Mo - low S, LMLS); (2) the basal plus 0.3% added S (low Mo - high S, LMHS); (3) basal plus 10 mg $kg^{-1}$ added Mo (high Mo-low S, HMLS), and (4) the basal ration plus 0.3% added S and 10 mg $kg^{-1}$ added Mo (high Mo - high S, HMHS). The Mo and S were added as ammonium

Table 1. Ingredient composition of experimental rations fed to Holstein steers (percent composition on an as-fed basis)

| Item | Rations | | | |
|---|---|---|---|---|
| | LMLS | LMHS | HMLS | HMHS |
| Barley | 67.9 | 66.6 | 67.9 | 66.6 |
| Hay[1] | 27.1 | 27.1 | 27.1 | 27.1 |
| Molasses | 3.0 | 3.0 | 3.0 | 3.0 |
| Sodium sulfate[2] | - | 1.3 | - | 1.3 |
| Premix[3] | 1.0 | 1.0 | 1.0 | 1.0 |
| Limestone | 0.5 | 0.5 | 0.5 | 0.5 |
| Salt[4] | 0.5 | 0.5 | 0.5 | 0.5 |

[1]Hay was ground through a 13 mm screen for incorporation into pelleted diets.

[2]$Na_2SO_4 \cdot 5H_2O$ was added to supply 0.3% supplemental sulfur.

[3]Rations for treatments HMLS and HMHS each contained 4.50 g of ammonium molybdate $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$ in 2.5 kg wheat middlings. All rations contained both vitamins A and D in the wheat middlings filler to supply 13,200 IU $head^{-1}$ $day^{-1}$ and 1,650 IU $head^{-1}$ $day^{-1}$, respectively.

[4]Cobalt-iodized salt.

molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (Fisher Scientific, Fair Lawn, N.J.) and sodium sulfate, $Na_2SO_4 \cdot 5H_2O$.

The premixes for the treatments HMLS and HMHS were each prepared separately, according to the following procedure. 13.5 g of ammonium molybdate was first dissolved into 1 litre of warm, distilled-deionized water and then transferred to a 1 litre plastic spray bottle. This solution was sprayed onto 3 kg of wheat middlings in a step-wise fashion. Approximately one-third of the solution was sprayed onto 1 kg of wheat middlings and mixed for 15 minutes. Another 1 kg quantity of wheat middlings was added to this combination and a second third of molybdate solution was then applied. After mixing an additional 15 minutes, the last 1 kg quantity of wheat middlings was added with the remaining molybdate solution applied. The entire combination was mixed slowly for 45 minutes and dried in a forced-air oven at 60°C to constant weight. The dried wheat middlings were then mixed with an additional 2.25 kg of wheat middlings, 2.25 kg of chromic oxide and the required amounts of vitamins A and D to make three, 2.5 kg premixes per experimental ration. The mineral analysis of the four rations are given in Table 2.

## 3.3 Experimental Protocol

Each experimental period was 21 days in length. During this 21 day period, the steers were housed in raised metabolism crates and fed continuously using automatic belt conveyors. The belt conveyor was programmed to run for 30 seconds every 20 minutes. The pelleted rations

Table 2. Mineral analyses of experimental rations and dysprosium-barley pellets fed to Holstein steers

| | Ration | | | | |
|---|---|---|---|---|---|
| | LMLS mean (SE) | LMHS mean (SE) | HMLS mean (SE) | HMHS mean (SE) | Dysprosium - barley pellets mean (SE) |
| % DM | 95.7(0.13) | 95.6(0.04) | 96.2(0.27) | 95.7(0.14) | 95.2(0.13) |
| Mineral composition ($kg^{-1}$ DM) | | | | | |
| Cu, mg | 2.9(0.05) | 3.3(0.07) | 2.8(0.13) | 3.8(0.11) | 5.9(0.06) |
| Mo, mg | 1.7(0.13) | 1.9(0.29) | 13.2(0.25) | 13.3(0.72) | 1.5(0.25) |
| S, g | 1.2(0.08) | 3.9(0.09) | 1.3(0.08) | 3.9(0.05) | 1.2(0.14) |
| Zn, mg | 24.1(0.35) | 24.2(0.30) | 24.9(0.25) | 26.0(0.49) | 30.3(0.32) |
| Mn, mg | 18.9(0.27) | 19.0(0.43) | 19.7(0.47) | 19.3(0.20) | 13.6(0.73) |
| Fe, mg | 118(3.0) | 136(10.4) | 115(6.2) | 112(4.3) | 73.7(0.41) |
| Ca, g | 4.0(0.11) | 3.8(0.07) | 4.0(0.06) | 4.2(0.23) | 0.50(0.01) |
| Mg, g | 1.6(0.03) | 1.5(0.01) | 1.5(0.01) | 1.5(0.02) | 1.4(0.01) |
| P, g | 3.1(0.05) | 3.1(0.10) | 3.2(0.03) | 3.1(0.02) | 3.7(0.17) |

were spread evenly every morning on the conveyor belts of the automatic feeders, which moved towards the centre of the unit and dropped the ration into the respective wooden feed box of each steer. All steers received 4.5 kg $day^{-1}$ (as-fed) of their respective ration over each of the four experimental periods. Throughout the course of the experiment, all steers had free access to tap water. A constant amount (250 g) of each ration and tap water (30 mL) was sampled daily during the last eight days of each experimental period. Each ration was bulked to form one sample per experimental period, which was stored for subsequent analyses.

There were 10 days between each experimental period. During this time, all four steers were removed from their crates and placed outside where they were fed the basal ration twice daily at 9:00 a.m. and 4:30 p.m. in a common feeder.

### 3.4 Dysprosium as a Marker

In this experiment, dysprosium (Dy), as dysprosium chloride $DyCl_2 \cdot 6H_2O$ (Alfa Products, Danvers, Ma.) was used as a particulate marker in order to measure the digestibility and flow of minerals and dry matter through the duodenum, ileum and in the feces of the four steers. Rare earth elements, such as dysprosium (Dy), have become popular as non-digestible markers in nutrition studies where the flow of dry matter within the digestive tract, fecal dry matter excretion and digestibility of dietary constituents were estimated. Dysprosium is reliable and suitable as an inert, non-digestible marker in both cattle (Ellis, 1968;

Young et al., 1972, 1976) and swine (Kennelly et al., 1980a). Fecal grab-sampling in the above studies provided an alternative to the total collection technique. With Dy, there was a constant recovery, with minimal variation and a reliable procedure for analysis.

One of the distinguishing properties of Dy, in addition to being non-digestible and non-absorbable is that it possesses a strong affinity for particulate matter; Dy becomes adsorbed onto, and remains tenaciously bound to digesta particles. Thus, Dy flows through the digestive tract in close association with feed particles (Ellis, 1968). this strong affinity reduces variation in concentration of marker that has been attributed to the differential flow rates of feed particles and markers (Corbett et al., 1958, 1959).

While chromium sesquioxide is a commonly used marker in nutrition studies, the efficacy of this compound depends upon the purpose for which it is used (MacRae, 1974). Chromium sesquioxide is not closely associated with the solid phase of digesta. Consequently, in studies where this association is of importance, the validity of chromic sesquioxide as a marker may be questionable. In techniques where digesta samples are obtained from T-shaped cannulae, it has been assumed that chromic sesquioxide passes through the gastrointestinal tract at the same rate as the particulate phase of digesta, and is closely associated with that phase. Since MacRae (1974) and Faichney, (1975) have reported that chromium sesquioxide is not associated specifically with the particulate phase, and behaves independently of both the

particulate and liquid phases, chromium sesquioxide has a limited use in spot-sampling procedures.

The marker, $DyCl_3 \cdot 6H_2O$ was fed every day of each experimental period as an additive within a pellet of finely ground barley which was top-dressed along the conveyor belt on the daily ration allotment.

Ten grams of $DyCl_2 \cdot 6H_2O$ was mixed with 1 litre of water, warmed gently and stirred continuously until all the crystals were dissolved. Then, the litre of solution was sprayed onto 2.5 kg of finely ground barley and mixed thoroughly in a large mixing bowl for 45 minutes. This combination was later dried on aluminum-foil lined pans in a forced-air oven at 60°C to constant weight. Twenty mixes of this dysprosium-barley preparation (2.5 kg per mix) were prepared and later pelleted into small pellets (1/8 inch diameter) using a small, Templewood Pelleter (Opperman Gears Ltd.; Newbury, England). The pellets were allowed to dry and bagged in 100g quantities. These pellets were sprinkled evenly over each daily allotment of ration to provide a theoretical intake of 240 mg of elemental Dy per 24 hour period to each steer. One 100 g bag of dysprosium-barley pellets was sampled daily during the last 10 days of each experimental period and bulked to form one sample for each experimental period to be used for later analyses. The mineral analysis of the dysprosium-barley pellets are presented in Table 2.

## 3.5 Collection of Digesta and Feces

During the last three days of each experimental period, ruminal contents, duodenal and ileal digesta and fecal samples were collected

from each steer, once daily over three different time periods. Sampling from each steer began first with the feces (a grab-sample from the rectum), and then from the cannulae at the ileum, duodenum and rumen.

Ruminal contents were immediately strained through four layers of cheesecloth. Each day, 100 - 150 mL of rumen fluid and approximately 250 mL of ileal and duodenal digesta were collected from each steer and placed into clean, acid-washed plastic bottles. Daily fecal grab-samples were placed into plastic bags and stored frozen.

Supernatant fractions of rumen fluid, duodenal and ileal digesta were obtained shortly after sampling each steer. Digesta and rumen fluid were centrifuged (72,000 x g, 18°C) using a Beckman, Model L3-50 Ultracentrifuge. The weights of the separated supernatant and precipitated fractions of both duodenal and ileal digesta were recorded after centrifugation. The supernatant fraction of rumen fluid, the supernatant and precipitated fractions of duodenal and ileal digesta and samples of the total duodenal and ileal digesta were all stored frozen at -15°C.

At the end of each period, total duodenal and total ileal digesta and their respective precipitated fractions were freeze-dried for 48 hours. After freeze-drying, all samples were then ground in a small Braun coffee mill. Daily total digesta and precipitated fractions from each steer were bulked to form one sample of each kind per steer per period. The ground samples were then stored in sealed Whirl-Pak bags.

Fecal grab-samples were oven-dried for 72 hours to a constant weight in a Coldstream forced-air oven, which was set at 60°C. Upon

removal, samples were allowed to equilibrate to room conditions before grinding. All samples of feces, dysprosium-barley pellets and rations were ground in a small Wiley mill (Standard Model No.3) through a 1 mm stainless steel screen. Fecal samples from each steer were bulked together to form one fecal sample per steer per period. The ground dysprosium-barley, the four rations and fecal samples were later re-ground finely in a small laboratory grinder (Tecator, Cyclotec 1093 Sample Mill) and stored for later analyses.

The supernatant fractions of rumen fluid, ileal and duodenal digesta from each steer were combined to form one sample of each kind per steer per period and stored frozen.

### 3.6 Chemical Analysis

The total S content of the rations and of the dysprosium-barley pellets was analyzed according to the procedure outlined by Boila et al. (1984a).

For mineral analyses, 1.5 g of ground rations, dysprosium-barley pellets, precipitated duodenal and ileal fractions and total duodenal and ileal digesta and feces were weighed into a 50 mL Kimax boro-silicate glass screw-top vial. These samples were ashed in these vials at a furnace temperature of 550°C for 12 hours. The vials containing the ash residue were removed from the furnace and allowed to cool to room temperature. Fifteen mL of 5N HCl containing 1% (vol/vol) concentrated nitric acid were added to the ash residue. Vials were capped with polypropylene screw caps and placed in a sonic water bath

set at 60°C for 1 hour. The capped vials were removed after this time, shaken, allowed to cool to room temperature and left to stand overnight.

Concentrations of Zn, Cu, Fe, Mn, Mg, Ca and Mo in this solution were determined by flame atomic absorption spectrophotometry using air-acetylene (Zn, Cu, Fe, Mn, Mg, Ca) and nitrous oxide-acetylene (Mo) flames (Instrumentation Laboratory AA/AE Spectrophotometer Model 551), according to Sotera and Stux (1979). Phosphorous content was determined colourimetrically (A.O.A.C., 1984) using a Baush and Lomb Spectronic 20.

Liquid samples (20 mL) of rumen fluid supernatants, duodenal and ileal digesta supernatants were weighted into crucibles made of silica glass, frozen and freeze-dried for 48 hours. The crucibles with the freeze-dried residues were then ashed at 550°C for 12 hours. The crucibles containing the ash residue were removed from the furnace and allowed to cool to room temperature. The following method is a modification of a procedure outlined by Boila et al., 1984b. Fifteen mL of 5N HCl containing 1% (vol/vol) concentrated nitric acid were added to the ash which was then heated on a hot plate such that there eventually was a gentle boil for approximately 15 minutes. The solution was cooled and filtered through ashless Whatman #42 filter paper. The filter paper was washed with 3 to 4 washings of distilled deionized water. Filtrates from each sample were brought up to a constant volume with distilled deionized water in a 25 -mL volumetric flask.

For some of the supernatant samples from digesta, the resulting solutions obtained with this above procedure contained concentrations of Mo approximately equal to that in the blanks. These samples were

prepared for Mo analysis using a modification of the procedure of Thompson and Blanchflower (1971). Ten mL of supernatant were weighted into a 25 mL glass vial. A 5 mL volume of a concentrated nitric-perchloric acid mixture (4:1 by volume) was added to each digestion vial and left to digest overnight at room temperature. The vials containing the acid mixture and sample were heated in an aluminum block until the yellow colour had disappeared. The clear, colourless digests were then boiled to dryness. Residues were dissolved in 3 mL of 5% (vol/vol) concentrated HCl. Solutions were analyzed for Mo as described earlier.

Standards used for mineral analyses were prepared with certified atomic absorption reference standards (Fisher Scientific). For Ca and Mg analysis, standards and sample solutions were diluted with lanthanum, with the resulting solutions containing 1% lanthanum to control interferences from silicon, aluminum, phosphate and sulfate. Similarly, sodium sulfate was added to samples and standards prior to Mo analysis at a rate of 10.4 mM to control interferences from other minerals during analysis. All glassware used for mineral analyses was acid washed with 10% nitric acid and then rinsed thoroughly first with distilled water followed by distilled-deionized water. Accuracy of technique was verified using citrus leaves (#1572) as a standard reference material (National Bureau of Standards, Washington, D.C.).

Ground dysprosium-barley (75 mg), total duodenal and ileal digesta (500 mg) and fecal samples (750 mg) were submitted to the Slowpoke Reactor Facility, University of Alberta, where concentrations of metastable dysprosium (Dym) were estimated using an automated

instrumental neutron activation analysis method (Kennelly et al., 1980a). Samples were irradiated at a neutron flux of $10^{11} Ncm^{-2}s^{-1}$ for 45 seconds, allowed to cool for 7 seconds and then counted for 45 seconds. The radiation released from 165 Dym emits at an energy level of 108 keV and has a half-life of 75.42 seconds with a standard error of 0.36 seconds.

## 3.7 Calculations

Dysprosium (Dy) was used for the calculation of DM flows at the proximal duodenum and terminal ileum and for fecal DM excretion, using the continuous infusion, time sequence sampling method described by Faichney (1975) which is shown in Equation I, below:

**Equation I**:

$$\text{kg DM flow day}^{-1} = \frac{(A)(B)}{C}$$

Where A = (kg DM intake $day^{-1}$)

= [(100 g Dy-barley pellets)(%DM in Dy-barley Pellets)]

+ [(4.5 kg Ration)(%DM in Ration)]

Where B = mg Dy $kg^{-1}$ dietary DM =

(100 g Dy-barley pellets)(%DM in Dy-barley pellets)

(mg Dy $g^{-1}$ Dy-barley pellets) $(A)^{-1}$

Where C = mg Dy $kg^{-1}$ digesta or fecal DM.

The flow of minerals at the cannulated duodenal and ileal sites and fecal mineral excretion were calculated by multiplying the flow of digesta DM or fecal DM excretion by the concentration of mineral in digesta or feces at that sampling site.

Equation II was used for the calculation of apparent digestibility of DM or of each mineral.

**Equation II**:

Apparent Digestibility

$$= 100 \left(\frac{\text{Total Intake - Total Fecal Excretion}}{\text{Total Intake}}\right)$$

Equation III was used to calculate the percent soluble mineral in the duodenal and ileal digesta.

**Equation III**:

$$\% \text{ Soluble Mineral} = \frac{(100)(D)}{(D + E)}$$

Where D = (total mineral in supernatant phase) = (concentration of mineral in supernatant phase)(total weight of supernatant phase).

Where E = (total mineral in precipitated phase) = (concentration of mineral in precipitated phase)(total weight of precipitated phase)

Note: (D + E) is a measure of the total mineral in the digesta,

which was centrifuged to obtain the respective supernatant and precipitated phases.

Equation IV was used to calculate the flow of soluble mineral at the duodenal and ileal cannulated sites.

**Equation IV**:

Soluble mineral flow =
(kg DM flow $day^{-1}$)(concentration mineral $kg^{-1}$ total digesta)(% soluble mineral in duodenal or ileal digesta)

Expressing mineral concentration in the supernatant phase on the basis of supernatant DM gives a measure of weight of soluble mineral per weight of soluble DM or [(g soluble mineral)(g soluble DM)$^{-1}$]

## 3.8 Statistical Analysis

All data were analyzed using the Latin square analysis of variance according to Snedecor and Cochran (1980).

Orthogonal contrasts were performed on the means from all the data presented. The three orthogonal contrasts selected are described below.

Contrast I:

A comparison of high Mo versus low Mo (HM vs LM) or, (HMLS + HMHS) vs (LMLS + LMHS).

Contrast II:

A comparison of high S versus low S (HS vs LS) or, (LMHS + HMHS) vs (LMLS + HMLS).

Contrast III:

This third contrast examined the effect of high Mo and high S together relative to high Mo and high S separately in the ration.

The four diets were:

LMLS = A

LMHS = B = A + (high S)

HMLS = C = A + (high Mo)

HMHS = D = A + (high Mo + high S)

Such that:

(high S) = (B - A)

(high Mo) = (C - A)

(high Mo + high S) = (D - A)

If there is no synergistic effect of Mo plus S, then:

(high Mo + high S) = (high S) + (high Mo)

(D - A) = (B - A) + (C - A)

D - A = B + C - 2A

D = B + C - A

The form of the contrast is

(A + D) vs (B + C)

or, (LMLS + HMHS) vs (LMHS + HMLS).

# 4. RESULTS

## 4.1 Analysis of Dysprosium in the Barley Pellets

Six samples of the prepared dysprosium-barley from each period were submitted for dysprosium analysis. The average concentration of dysprosium in the barley pellets fed to the cattle, among periods, ranged from 1.982 to 2.095 mg $g^{-1}$ DM (x=2.027) and the coefficient of variation among periods ranged between 2.7% and 3.2% (Appendix Table B).

## 4.2 Dry Matter

The average DM intake was 4.40 kg $day^{-1}$ (Table 3, Appendix Tables A1 and A2). Throughout the course of this experiment, there were no feed refusals.

The DM entering the proximal duodenum, expressed as a daily flow rate (P=0.052) or as a percent of intake (P=0.051), tended to be lower for the high molybdenum (HM) treatments; there was a tendency for a higher (P=0.055) DM loss with the HM treatments (Table 3).

The DM flow at the terminal ileum ranged between 1.21 and 1.47 kg $day^{-1}$, and accounted for 50.9% to 56.7% of the duodenal DM flow; ileal flow minus duodenal flow was not different (P>0.05) across all orthogonal contrasts.

The loss of DM in the large intestine, expressed as fecal DM excretion minus ileal DM flow, and fecal DM excretion when expressed as a percent of ileal DM flow, were not different (P>0.05).

Table 3. Dry matter intake, dry matter flow at the proximal duodenum, flow at the terminal ileum and fecal dry matter excretion for steers consuming diets high in molybdenum and sulfur contents

| Treatment | Intake (kg day$^{-1}$) | Entering proximal duodenum: Flow (kg day$^{-1}$) | Entering proximal duodenum: Percent of intake | Entering proximal duodenum: Duodenal flow minus intake (kg day$^{-1}$) | Leaving terminal ileum: Flow (kg day$^{-1}$) | Leaving terminal ileum: Percent of duodenal flow | Leaving terminal ileum: Ileal flow minus duodenal flow (kg day$^{-1}$) | Fecal excretion: Fecal excretion (kg day$^{-1}$) | Fecal excretion: Percent of ileal flow | Fecal excretion: Fecal excretion minus ileal flow (kg day$^{-1}$) | Fecal excretion: Intake minus fecal excretion (kg day$^{-1}$) | Fecal excretion: Apparent digestibility (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LMLS | 4.40 | 2.74 | 62.2 | -1.66 | 1.47 | 54.1 | -1.27 | 1.15 | 78.3 | -0.32 | 3.25 | 73.9 |
| LMHS | 4.40 | 2.81 | 63.8 | -1.59 | 1.42 | 50.9 | -1.39 | 1.19 | 84.5 | -0.23 | 3.21 | 73.0 |
| HMLS | 4.42 | 2.43 | 54.9 | -2.00 | 1.34 | 56.7 | -1.05 | 1.05 | 76.3 | -0.33 | 3.37 | 76.3 |
| HMHS | 4.40 | 2.34 | 53.1 | -2.06 | 1.21 | 51.8 | -1.13 | 0.97 | 80.2 | -0.24 | 3.44 | 78.0 |
| SE | - | 0.161 | 3.71 | 0.166 | 0.074 | 2.69 | 0.134 | 0.039 | 2.81 | 0.048 | 0.043 | 0.91 |
| Contrast† | | | | | | | | | | | | |
| (I) HM vs LM | - | NS(1) | NS(2) | NS(3) | NS | NS | NS | ** | NS | NS | ** | ** |
| (II) HS vs LS | - | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | - | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

†For explanation of orthogonal contrasts, refer to Materials and Methods section under the heading "Statistical Analysis".

NS = Not significant ($P>0.05$).

** = Orthogonal contrasts are different ($P<0.01$).

Various contrasts tended towards significance ($0.05<P\leq0.10$: (1) P = 0.052, (2) P = 0.051, (3) P = 0.055.

There was a lower ($P<0.01$) excretion of fecal DM, higher ($P<0.01$) total DM loss calculated as DM intake minus fecal DM excretion and a higher ($P<0.01$) apparent digestibility of DM with the HM treatments.

## 4.3 Copper

The intake of Cu (Table 4, Appendix Table A3) across all treatments ranged from 12.6 to 17.0 mg $day^{-1}$.

The total Cu entering the proximal duodenum (Table 4) was not different ($P>0.05$) and ranged between 23.1 and 24.5 mg $day^{-1}$, which represented a net addition, duodenal flow minus intake, of 6.1 to 10.7 mg $day^{-1}$ (Table 5, Appendix Table A4). Duodenal Cu flow, when expressed as a percent of intake (Table 5), was lower ($P<0.05$) for the high-sulfur (HS) treatments. The percent soluble Cu in the duodenal digesta (Table 4) was lower ($P<0.01$) with the HM and the HS treatments and as a consequence, the soluble Cu flow entering the duodenal digesta was lower ($P<0.01$) with the HM treatments and lower ($P<0.05$) with the HS treatments.

Total Cu flow at the terminal ileum expressed either as a flow rate (Table 4; 21.2 to 23.1 mg $day^{-1}$) or as a percent of duodenal flow (Table 5; 92.4% to 99.6%) was not different ($P>0.05$). The percent soluble Cu ($P<0.01$) and the flow of soluble Cu ($P<0.05$) in the ileal digesta (Table 4) were lower with the HM and the HS treatments. The addition of both Mo and S, compared to Mo and S alone in the diets (Contrast III) resulted in a lower ($P<0.05$) percent soluble Cu, but did not influence ($P=0.08$) the soluble Cu flow in ileal digesta.

Table 4. Copper intake, total and soluble flow of copper at the proximal duodenum and at the terminal ileum, and total fecal excretion of copper for steers consuming diets high in molybdenum and sulfur contents

| | | Entering proximal duodenum | | | Leaving terminal ileum | | | |
|---|---|---|---|---|---|---|---|---|
| | Intake (mg day$^{-1}$) | Total flow (mg day$^{-1}$) | Percent soluble | Soluble flow (mg day$^{-1}$) | Total flow (mg day$^{-1}$) | Percent soluble | Soluble flow (mg day$^{-1}$) | Excreted in feces (mg day$^{-1}$) |
| Treatment | | | | | | | | |
| LMLS | 13.0 | 23.2 | 22.2 | 5.15 | 21.6 | 63.8 | 13.8 | 10.1 |
| LMHS | 14.8 | 24.5 | 15.0 | 3.69 | 22.5 | 58.4 | 13.2 | 10.7 |
| HMLS | 12.6 | 23.3 | 13.8 | 3.24 | 23.1 | 57.7 | 13.4 | 12.4 |
| HMHS | 17.0 | 23.1 | 10.5 | 2.38 | 21.2 | 39.4 | 8.5 | 13.5 |
| SE | - | 1.07 | 1.36 | 0.418 | 1.15 | 2.33 | 1.03 | 1.21 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | - | NS | ** | ** | NS | ** | * | NS(1) |
| (II) HS vs LS | - | NS | ** | * | NS | ** | * | NS |
| (III) HMHS vs (HM) + (HS) | - | NS | NS | NS | NS | * | NS(2) | NS |

†Refer to Table 3.

NS = Not significant (P>0.05), * Orthogonal contrasts are different (P<0.05), ** (P<0.01).

Various contrasts tended towards significance (0.05<P≤0.10): (1) P = 0.07, (2) P = 0.08.

Table 5. Differences for copper along the digestive tract, between intake, flows at cannulated sites in the proximal duodenum and terminal ileum and in fecal excretion for steers consuming diets high in molybdenum and sulfur contents

| | Proximal duodenum | | Terminal ileum | | Fecal excretion | | | |
|---|---|---|---|---|---|---|---|---|
| | Duodenal flow minus intake (mg day$^{-1}$) | Duodenal flow as percent of intake | Ileal flow minus duodenal flow (mg day$^{-1}$) | Ileal flow as percent of duodenal flow | Fecal excretion minus ileal flow (mg day$^{-1}$) | Fecal excretion as percent of ileal flow | Intake minus fecal excretion (mg day$^{-1}$) | Apparent digestibility (%) |
| Treatment | | | | | | | | |
| LMLS | +10.3 | 179 | -1.6 | 93.1 | -11.5 | 46.9 | 2.8 | 21.3 |
| LMHS | +9.8 | 168 | -2.0 | 92.4 | -11.9 | 47.3 | 4.1 | 27.5 |
| HMLS | +10.7 | 185 | -0.2 | 99.6 | -10.4 | 55.7 | 0.2 | 1.5 |
| HMHS | +6.1 | 137 | -1.9 | 93.7 | -7.8 | 64.5 | 3.5 | 19.9 |
| SE | 1.23 | 9.5 | 1.50 | 6.32 | 1.72 | 6.64 | 1.15 | 7.20 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | NS | NS | NS | NS | NS | NS | NS | NS(1) |
| (II) HS vs LS | NS | * | NS | NS | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | NS | NS | NS | NS | NS | NS | NS | NS |

†Refer to Table 3.

NS = Not significant (P>0.05), * Orthogonal contrasts are different (P<0.05).

Various contrasts tended towards significance (0.05<P≤0.10): (1) P = 0.10.

Total fecal excretion of Cu (Table 4) and fecal excretion parameters listed in Table 5 were not significant ($P>0.05$). There was a tendency for a higher fecal excretion (Table 4) of Cu ($P=0.07$) and a lower ($P=0.10$) apparent digestibility of Cu (Table 5) with the HM diets.

The concentration of Cu in the soluble rumen fluid DM (Table 6, Appendix Table A5) was not different ($P>0.05$) and ranged between 4.28 to 12.2 $\mu g\ g^{-1}$ soluble DM. The concentration of Cu in the duodenal soluble DM (Table 6) was not different ($P>0.05$); however, it tended to be lower ($P=0.053$) for the HM treatments. The concentration of Cu in the ileal soluble DM was lower ($P<0.05$) with the HS treatments.

## 4.4 Molybdenum

The intake of Mo for the LM treatments were 7.65 and 8.65 mg $day^{-1}$, while that for the HM diets were 57.3 and 57.5 mg $day^{-1}$ (Table 7, Appendix Table A6).

The Mo flow at the proximal duodenum (Table 7) was higher ($P<0.01$) for the HM diets. Duodenal flow of Mo, when expressed as a percent of intake (Table 8, Appendix Table A7) was lower ($P<0.05$) for the HS diets. Disappearance of Mo between intake and proximal duodenum, expressed as duodenal flow minus intake, was higher ($P<0.05$) for the HM diets (Table 8). The percent soluble Mo and soluble Mo flow at the proximal duodenum were higher ($P<0.01$) with the HM treatments (Table 7).

The daily total flow of Mo at the terminal ileum was higher ($P<0.01$) with the HM treatments (Table 7). The flow of Mo at the terminal ileum was higher ($P<0.01$) for the HS treatments and when both

Table 6. Concentrations of soluble copper and molybdenum in the soluble dry matter in rumen fluid, duodenal and ileal digesta of steers consuming diets high in molybdenum and sulfur contents

| | Copper ($\mu g\ g^{-1}$ soluble DM) | | | Molybdenum ($\mu g\ g^{-1}$ soluble DM) | | |
|---|---|---|---|---|---|---|
| | Rumen fluid | Duodenal digesta | Ileal digesta | Rumen fluid | Duodenal digesta | Ileal digesta |
| Treatment | | | | | | |
| LMLS | 11.9 | 9.27 | 32.7 | 2.32 | 4.30 | 10.5 |
| LMHS | 6.24 | 7.35 | 23.3 | 1.79 | 3.07 | 10.1 |
| HMLS | 12.2 | 6.08 | 32.0 | 15.6 | 12.0 | 34.9 |
| HMHS | 4.28 | 4.68 | 18.6 | 15.4 | 10.7 | 36.9 |
| SE | 4.51 | 1.218 | 3.55 | 0.880 | 0.909 | 2.50 |
| Contrast† | | | | | | |
| (I) HM vs LM | NS | NS(1) | NS | ** | ** | ** |
| (II) HS vs LS | NS | NS | * | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | NS | NS | NS | NS | NS | NS |

†Refer to Table 3.

NS = Not significant ($P>0.05$), *Orthogonal contrasts are different ($P<0.05$), **($P<0.01$).

Various contrasts tended towards significance ($0.05<P\leq0.10$): (1) $P = 0.053$.

Table 7. Molybdenum intake, total and soluble flow of molybdenum at the proximal duodenum and at the terminal ileum, and total fecal excretion of molybdenum for steers consuming diets high in molybdenum and sulfur contents

| | Intake | Entering proximal duodenum | | | Leaving terminal ileum | | | Excreted in feces |
|---|---|---|---|---|---|---|---|---|
| | (mg day$^{-1}$) | Total flow (mg day$^{-1}$) | Percent soluble | Soluble flow (mg day$^{-1}$) | Total flow (mg day$^{-1}$) | Percent soluble | Soluble flow (mg day$^{-1}$) | (mg day$^{-1}$) |
| Treatment | | | | | | | | |
| LMLS | 7.65 | 7.95 | 17.1 | 1.39 | 5.08 | 43.1 | 2.15 | 4.38 |
| LMHS | 8.65 | 6.13 | 13.6 | 0.83 | 5.70 | 45.4 | 2.59 | 5.28 |
| HMLS | 57.3 | 51.5 | 23.8 | 12.3 | 27.7 | 32.2 | 8.72 | 30.3 |
| HMHS | 57.5 | 45.8 | 24.8 | 11.4 | 37.9 | 32.6 | 12.4 | 38.0 |
| SE | - | 2.029 | 1.13 | 0.661 | 1.278 | 3.47 | 0.895 | 1.584 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | - | ** | ** | ** | ** | * | ** | ** |
| (II) HS vs LS | - | NS | NS | NS | ** | NS | NS | * |
| (III) HMHS vs (HM) + (HS) | - | NS | NS(1) | NS | ** | NS | NS | NS(2) |

†Refer to Table 3.

NS = Not significant (P>0.05), * Orthogonal contrasts are different (P<0.05), ** (P<0.01).

Various contrasts tended towards significance (0.05<P≤0.10): (1) P = 0.09, (2) P = 0.07.

Table 8. Differences for molybdenum along the digestive tract, between intake, flows at cannulated sites in the proximal duodenum and terminal ileum and in fecal excretion for steers consuming diets high in molybdenum and sulfur contents

| | Proximal duodenum | | Terminal ileum | | Fecal excretion | | | |
|---|---|---|---|---|---|---|---|---|
| | Duodenal flow minus intake (mg day$^{-1}$) | Duodenal flow as percent of intake | Ileal flow minus duodenal flow (mg day$^{-1}$) | Ileal flow as percent of duodenal flow | Fecal excretion minus ileal flow (mg day$^{-1}$) | Fecal excretion as percent of ileal flow | Intake minus fecal excretion (mg day$^{-1}$) | Apparent digestibility (%) |
| Treatment | | | | | | | | |
| LMLS | +0.30 | 103.0 | -2.88 | 65.6 | -0.70 | 85.8 | 3.28 | 42.9 |
| LMHS | -2.38 | 76.3 | -0.43 | 93.3 | -0.43 | 92.9 | 3.23 | 34.4 |
| HMLS | -5.73 | 89.8 | -23.83 | 55.2 | -2.55 | 109.0 | 27.0 | 47.0 |
| HMHS | -11.6 | 80.4 | -7.90 | 82.6 | +0.08 | 100.0 | 19.5 | 33.4 |
| SE | 2.22 | 6.07 | 2.95 | 4.87 | 0.564 | 2.20 | 2.00 | 4.76 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | * | NS | ** | NS | * | ** | ** | NS |
| (II) HS vs LS | NS | * | * | ** | NS | NS | NS | NS (1) |
| (III) HMHS vs (HM) + (HS) | NS | NS | NS | NS | NS | * | NS | NS |

†Refer to Table 3.

NS = Not significant (P>0.05), * Orthogonal contrasts are different (P<0.05), ** (P<0.01).

Various contrasts tended towards significance (0.05<P≤0.10): (1) P = 0.06.

Mo and S together were added to the diets. The Mo flow at the terminal ileum, when expressed as a percent of duodenal flow (Table 8) ranged between 55.2% to 93.3% and was higher ($P<0.01$) for the HS treatments.

There was a net disappearance of Mo, ileal flow minus duodenal flow, between the proximal duodenum and terminal ileum (Table 8) for all treatments. The disappearance of Mo between these locations was higher ($P<0.01$) with the HM diets and lower ($P<0.05$) with the HS diets.

The percent soluble Mo at the terminal ileum was lower ($P<0.05$) with the HM diets while flow of soluble Mo was higher ($P<0.01$) for the HM diets (Table 7).

The Mo excreted in the feces (Table 7) was higher ($P<0.01$) for the HM diets. Fecal Mo excretion was also higher ($P<0.05$) with the HS diets and tended to be higher ($P=0.07$) for the HMHS diet. Fecal Mo excretion, expressed as a percent of ileal flow (Table 8) was higher ($P<0.01$) for the HM diets and was lower ($P<0.05$) when both Mo and S were added to the diet. There was a net appearance of Mo in the large intestine, expressed as fecal excretion minus ileal flow, with the HM diets compared to a net disappearance of Mo with the LM diets (Table 8).

Total Mo disappearance, calculated as intake minus fecal excretion (Table 8) was higher ($P<0.01$) for the HM treatments. Apparent Mo digestibility (Table 8) ranged from 33.4% to 47.0% and was not different ($P>0.05$). With the HS diets, apparent Mo digestibility tended to be lower ($P=0.06$).

The concentration of Mo in the rumen fluid, duodenal and ileal digesta supernatant DM were all higher ($P<0.01$) with the HM treatments

(Table 6). The HS treatments had no effect ($P>0.05$) upon the measures of soluble Mo.

4.5 Manganese

The intake of Mn (Table 9, Appendix Table A8) across all treatments ranged from 83.0 to 86.5 mg $day^{-1}$.

The HM, HS or HMHS treatments had no significant ($P>0.05$) effect upon the flow rates of Mn at the proximal duodenum, terminal ileum nor upon fecal Mn excretion (Table 9). However, the HM treatments had significant effects ($P<0.05$) when Mn flow at the proximal duodenum was compared relative to Mn intake (Table 10, Appendix Table A9). The difference, calculated as duodenal flow minus intake, was lower ($P<0.05$) with the HM treatments. When the Mn flow rate entering the proximal duodenum was expressed as a percent of intake Mn (Table 10), that percentage was also lower ($P<0.05$) with the HM treatments.

There was a net disappearance of Mn between the proximal duodenum and terminal ileum (Table 10) for all treatments, which ranged between 6.6 and 16.4 mg $day^{-1}$. The percent soluble Mn (Table 9) at the terminal ileum ranged between 8.8% to 13.7%; this was on average, a 7-fold decrease from that found at the proximal duodenum.

There was a further net disappearance of Mn between the terminal ileum and fecal excretion for all treatments and these ranged between 8.9 and 15.4 mg $day^{-1}$. Total Mn disappearance, calculated as intake minus fecal excretion for all treatments ranged between 9.9 and

Table 9. Manganese intake, total and soluble flow of manganese at the proximal duodenum and at the terminal ileum, and total fecal excretion of manganese for steers consuming diets high in molybdenum and sulfur contents

| | Intake | Entering proximal duodenum | | | Leaving terminal ileum | | | Excreted in feces |
|---|---|---|---|---|---|---|---|---|
| | (mg day$^{-1}$) | Total flow (mg day$^{-1}$) | Percent soluble | Soluble flow (mg day$^{-1}$) | Total flow (mg day$^{-1}$) | Percent soluble | Soluble flow (mg day$^{-1}$) | (mg day$^{-1}$) |
| Treatment | | | | | | | | |
| LMLS | 83.0 | 96.9 | 82.2 | 79.6 | 84.8 | 13.7 | 11.5 | 69.7 |
| LMHS | 83.4 | 102.0 | 80.5 | 82.3 | 85.7 | 13.6 | 11.7 | 70.3 |
| HMLS | 86.5 | 86.1 | 83.6 | 72.2 | 79.5 | 10.8 | 8.5 | 70.6 |
| HMHS | 84.9 | 95.8 | 81.4 | 78.1 | 84.8 | 8.8 | 7.6 | 75.0 |
| SE | - | 5.31 | 1.87 | 5.36 | 5.75 | 2.32 | 2.01 | 5.00 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | - | NS | NS | NS | NS | NS | NS | NS |
| (II) HS vs LS | - | NS | NS | NS | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | - | NS | NS | NS | NS | NS | NS | NS |

†Refer to Table 3.

NS = Not significant (P>0.05).

Table 10. Differences for manganese along the digestive tract, between intake, flows at cannulated sites in the proximal duodenum and terminal ileum and in fecal excretion for steers consuming diets high in molybdenum and sulfur contents

| | Proximal duodenum | | Terminal ileum | | Fecal excretion | | | |
|---|---|---|---|---|---|---|---|---|
| | Duodenal flow minus intake (mg day$^{-1}$) | Duodenal flow as percent of intake | Ileal flow minus duodenal flow (mg day$^{-1}$) | Ileal flow as percent of duodenal flow | Fecal excretion minus ileal flow (mg day$^{-1}$) | Fecal excretion as percent of ileal flow | Intake minus fecal excretion (mg day$^{-1}$) | Apparent digestibility (%) |
| Treatment | | | | | | | | |
| LMLS | +13.9 | 117 | -12.1 | 87.4 | -15.1 | 82.7 | 13.3 | 16.0 |
| LMHS | +18.6 | 122 | -16.4 | 84.4 | -15.4 | 83.3 | 13.1 | 15.4 |
| HMLS | -0.4 | 99.1 | -6.6 | 94.3 | -8.9 | 88.6 | 15.9 | 17.7 |
| HMHS | +11.0 | 113 | -11.1 | 88.6 | -9.8 | 88.4 | 9.9 | 11.4 |
| SE | 4.19 | 5.10 | 4.73 | 4.76 | 4.69 | 5.00 | 3.83 | 4.80 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | * | * | NS | NS | NS | NS | NS | NS |
| (II) HS vs LS | NS | NS | NS | NS | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | NS | NS | NS | NS | NS | NS | NS | NS |

†Refer to Table 3.

NS = Not significant (P>0.05), * Orthogonal contrasts are different (P<0.05).

15.9 mg $day^{-1}$ and apparent Mn digestibility was between 11.4% and 17.7% (Table 10).

The concentration of Mn in the soluble rumen fluid DM (Table 11, Appendix Table A10) was lower ($P<0.05$) for the HS treatments. The concentration of Mn in the duodenal and ileal soluble DM (Table 11) were not different ($P>0.05$).

## 4.6 Zinc

The intake of Zn (Table 12, Appendix Table A11) ranged between 107 and 115 mg $day^{-1}$.

Zinc entering the proximal duodenum, expressed as a daily flow (Table 12), or as a percent of intake (Table 13, Appendix Table A12) was not different ($P>0.05$). However, the concentration of Zn in the soluble DM (Table 11), the percent soluble Zn and the daily flow of soluble Zn (Table 12) at the proximal duodenum were lower ($P<0.01$) for the HS diets.

The flow of Zn at the terminal ileum, expressed as a total flow (Table 12), or as a percent of duodenal flow (Table 13) was not different ($P>0.05$). The concentration of Zn in the ileal soluble DM (Table 11) tended to be lower ($P=0.06$) for the HM diets. In addition, both the percent soluble Zn and the flow of soluble Zn at the terminal ileum (Table 12) were lower ($P<0.05$) for the HM diets.

Fecal excretion of Zn was higher ($P<0.05$) with the HM treatments (Table 12). That excretion of Zn in feces, when expressed as a percent of ileal flow (Table 13) was higher ($P<0.01$) for the HM diets.

Table 11. Concentrations of soluble manganese, zinc and iron in the soluble dry matter in rumen fluid, duodenal and ileal digesta of steers consuming diets high in molybdenum and sulfur contents

| | Manganese (μg $g^{-1}$ soluble DM) | | | Zinc (μg $g^{-1}$ soluble DM) | | | Iron (μg $g^{-1}$ soluble DM) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Rumen fluid | Duodenal digesta | Ileal digesta | Rumen fluid | Duodenal digesta | Ileal digesta | Rumen fluid | Duodenal digesta | Ileal digesta |
| Treatment | | | | | | | | | |
| LMLS | 62.2 | 119 | 28.9 | 31.3 | 265 | 99.4 | 97.1 | 420 | 431 |
| LMHS | 55.9 | 117 | 28.4 | 24.7 | 166 | 93.9 | 88.8 | 462 | 444 |
| HMLS | 71.4 | 115 | 28.3 | 51.5 | 231 | 87.1 | 88.7 | 371 | 359 |
| HMHS | 52.3 | 118 | 22.1 | 15.9 | 189 | 76.4 | 70.3 | 396 | 393 |
| SE | 5.07 | 9.2 | 5.42 | 11.43 | 18.5 | 6.49 | 24.73 | 33.4 | 45.2 |
| Contrast† | | | | | | | | | |
| (I) HM vs LM | NS | NS | NS | NS | NS | NS(1) | NS | NS | NS |
| (II) HS vs LS | * | NS | NS | NS | ** | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | NS | NS | NS | NS | NS | NS | NS | NS | NS |

†Refer to Table 3

NS = Not significant (P>0.05), * Orthogonal contrasts are different (P<0.05), ** (P<0.01).

Various contrasts tended towards significance (0.05<P≤0.10): (1) P = 0.06.

Table 12. Zinc intake, total and soluble flow of zinc at the proximal duodenum and at the terminal ileum, and total fecal excretion of zinc for steers consuming diets high in molybdenum and sulfur contents

| | Intake | Entering proximal duodenum | | | Leaving terminal ileum | | | |
|---|---|---|---|---|---|---|---|---|
| | (mg day$^{-1}$) | Total flow (mg day$^{-1}$) | Percent soluble | Soluble flow (mg day$^{-1}$) | Total flow (mg day$^{-1}$) | Percent soluble | Soluble flow (mg day$^{-1}$) | Excreted in feces (mg day$^{-1}$) |
| Treatment | | | | | | | | |
| LMLS | 107 | 201 | 80.4 | 162 | 162 | 41.4 | 66.4 | 41.3 |
| LMHS | 107 | 180 | 62.8 | 114 | 139 | 49.0 | 68.2 | 48.1 |
| HMLS | 110 | 189 | 79.8 | 150 | 162 | 30.7 | 49.8 | 86.5 |
| HMHS | 115 | 169 | 69.4 | 116 | 134 | 24.7 | 33.4 | 60.5 |
| SE | - | 13.5 | 2.42 | 10.0 | 15.6 | 5.04 | 7.37 | 11.63 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | - | NS | NS | NS | NS | * | * | * |
| (II) HS vs LS | - | NS | ** | ** | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | - | NS | NS | NS | NS | NS | NS | NS |

†Refer to Table 3.

NS = Not significant (P>0.05), * Orthogonal contrasts are different (P<0.05), ** (P<0.01).

Table 13. Differences for zinc along the digestive tract, between intake, flows at cannulated sites in the proximal duodenum and terminal ileum and in fecal excretion for steers consuming diets high in molybdenum and sulfur contents

| | Proximal duodenum | | Terminal ileum | | Fecal excretion | | | |
|---|---|---|---|---|---|---|---|---|
| | Duodenal flow minus intake (mg day$^{-1}$) | Duodenal flow as percent of intake | Ileal flow minus duodenal flow (mg day$^{-1}$) | Ileal flow as percent of duodenal flow | Fecal excretion minus ileal flow (mg day$^{-1}$) | Fecal excretion as percent of ileal flow | Intake minus fecal excretion (mg day$^{-1}$) | Apparent digestibility (%) |
| Treatment | | | | | | | | |
| LMLS | +94 | 189 | -38.5 | 80.3 | -121 | 27.2 | 65.7 | 61.4 |
| LMHS | +74 | 169 | -41.5 | 77.2 | -91 | 34.6 | 58.7 | 54.7 |
| HMLS | +79 | 174 | -27.3 | 86.1 | -75 | 50.7 | 23.9 | 18.4 |
| HMHS | +54 | 147 | -35.0 | 78.4 | -73 | 44.4 | 54.7 | 48.7 |
| SE | 14.7 | 15.8 | 8.49 | 5.14 | 12.2 | 3.97 | 13.61 | 12.27 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | NS | NS | NS | NS | * | ** | NS | NS(1) |
| (II) HS vs LS | NS | NS | NS | NS | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | NS | NS | NS | NS | NS | NS | NS | NS |

†Refer to Table 3.

NS = Not significant (P>0.05), * Orthogonal contrasts are different (P<0.05), ** (P<0.01).

Various contrasts tended towards significance (0.05<P≤0.10): (1) P = 0.09.

Total Zn disappearance along the digestive tract, calculated as intake minus fecal excretion (Table 12) was not different ($P>0.05$). However, the apparent digestibility of Zn (Table 13) tended to be lower ($P=0.09$) with the HM treatments.

## 4.7 Iron

The intake of Fe across all treatments (Table 14, Appendix Table A13) ranged between 489 and 593 mg $day^{-1}$.

There was no effect ($P>0.05$) of supplemental Mo or S upon the data obtained for Fe in the rumen fluid (Table 11), at the proximal duodenum and at the terminal ileum (Tables 11, 14 and 15, Appendix Table A14).

The soluble Fe flow in the ileal digesta tended to be lower ($P=0.09$) for the HM treatments (Table 14).

Iron disappearance, calculated as fecal excretion minus ileal flow (Table 15), tended to be higher ($P=0.06$) with the HM treatments, with less ($P<0.01$) Fe excreted in the feces of steers on these treatments (Table 14); fecal Fe excretion expressed as a percent of ileal flow was lower ($P<0.05$) for the HM treatments (Table 15).

Apparent Fe digestibility (Table 15) was negative across all treatments and tended to be higher ($P=0.08$) for the HM treatments.

## 4.8 Calcium

The intake of Ca (Table 16, Appendix Table A15) across all treatments ranged from 16.3 to 19.2 g $day^{-1}$.

Table 14. Iron intake, total and soluble flow of iron at the proximal duodenum and at the terminal ileum, and total fecal excretion of iron for steers consuming diets high in molybdenum and sulfur contents

| | Intake | Entering proximal duodenum | | | Leaving terminal ileum | | | |
|---|---|---|---|---|---|---|---|---|
| | (mg day$^{-1}$) | Total flow (mg day$^{-1}$) | Percent soluble | Soluble flow (mg day$^{-1}$) | Total flow (mg day$^{-1}$) | Percent soluble | Soluble flow (mg day$^{-1}$) | Excreted in feces (mg day$^{-1}$) |
| Treatment | | | | | | | | |
| LMLS | 518 | 886 | 48.9 | 430 | 640 | 20.1 | 127 | 640 |
| LMHS | 593 | 895 | 48.6 | 437 | 698 | 22.6 | 160 | 777 |
| HMLS | 506 | 779 | 45.2 | 352 | 590 | 15.8 | 94.0 | 535 |
| HMHS | 489 | 648 | 49.5 | 317 | 599 | 17.7 | 106 | 509 |
| SE | - | 104.8 | 3.44 | 18.0 | 35.8 | 2.53 | 21.8 | 41.1 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | - | NS | NS | NS | NS | NS | NS | ** |
| (II) HS vs LS | - | NS | NS | NS | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | - | NS | NS | NS | NS | NS | NS | NS |

†Refer to Table 3.

NS = Not significant (P>0.05), ** Orthogonal contrasts are different (P<0.01).

Table 15. Differences for iron along the digestive tract, between intake, flows at cannulated sites in the proximal duodenum and terminal ileum and in fecal excretion for steers consuming diets high in molybdenum and sulfur contents

| | Proximal duodenum | | Terminal ileum | | Fecal excretion | | | |
|---|---|---|---|---|---|---|---|---|
| | Duodenal flow minus intake (mg day$^{-1}$) | Duodenal flow as percent of intake | Ileal flow minus duodenal flow (mg day$^{-1}$) | Ileal flow as percent of duodenal flow | Fecal excretion minus ileal flow (mg day$^{-1}$) | Fecal excretion as percent of ileal flow | Intake minus fecal excretion (mg day$^{-1}$) | Apparent digestibility (%) |
| Treatment | | | | | | | | |
| LMLS | +368 | 170 | -246 | 76.8 | 0 | 100 | -122 | -23.6 |
| LMHS | +302 | 153 | -198 | 78.8 | +77 | 112 | -182 | -33.4 |
| HMLS | +274 | 156 | -189 | 79.1 | -55 | 90.3 | -29 | -7.1 |
| HMHS | +159 | 133 | -50 | 94.8 | -90 | 87.1 | -20 | -4.6 |
| SE | 107.6 | 21.0 | 119.5 | 11.99 | 47.1 | 6.69 | 52.4 | 10.48 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | NS | NS | NS | NS | NS(1) | * | NS | NS(2) |
| (II) HS vs LS | NS | NS | NS | NS | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | NS | NS | NS | NS | NS | NS | NS | NS |

†Refer to Table 3.

NS = Not significant (P>0.05), * Orthogonal contrasts are different (P<0.05).

Various contrasts tended towards significance (0.05<P≤0.10): (1) P = 0.06, (2) P = 0.08.

Table 16. Calcium intake, total and soluble flow of calcium at the proximal duodenum and at the terminal ileum, and total fecal excretion of calcium for steers consuming diets high in molybdenum and sulfur contents

| | Intake | Entering proximal duodenum | | | Leaving terminal ileum | | | |
|---|---|---|---|---|---|---|---|---|
| | ($g\ day^{-1}$) | Total flow ($g\ day^{-1}$) | Percent soluble | Soluble flow ($g\ day^{-1}$) | Total flow ($g\ day^{-1}$) | Percent soluble | Soluble flow ($g\ day^{-1}$) | Excreted in feces ($mg\ day^{-1}$) |
| Treatment | | | | | | | | |
| LMLS | 17.1 | 19.8 | 86.9 | 17.2 | 10.7 | 24.8 | 2.65 | 11.2 |
| LMHS | 16.3 | 19.6 | 83.7 | 16.4 | 9.35 | 29.3 | 2.77 | 10.0 |
| HMLS | 17.5 | 20.2 | 87.4 | 17.8 | 11.5 | 28.8 | 3.07 | 10.8 |
| HMHS | 19.2 | 23.3 | 87.1 | 20.3 | 12.2 | 21.3 | 2.59 | 11.4 |
| SE | - | 1.99 | 1.85 | 1.76 | 1.52 | 3.85 | 0.326 | 1.18 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | - | NS | NS | NS | NS | NS | NS | NS |
| (II) HS vs LS | - | NS | NS | NS | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | - | NS | NS | NS | NS | NS | NS | NS |

†Refer to Table 3

NS = Not significant ($P>0.05$).

Supplemental Mo and S had no effect ($P>0.05$) upon the various Ca parameters listed in Tables 16, 17 (Appendix Table A16) and 18 (Appendix Table A17).

Total Ca flow at the proximal duodenum was 115% to 121% of intake, which represented a net addition of 2.70 to 4.08 g $day^{-1}$ relative to intake (Table 17). The percent soluble Ca at the proximal duodenum ranged between 83.7% and 87.4% and the soluble Ca flow was between 16.4 and 20.3 g $day^{-1}$ (Table 16).

Calcium flow at the terminal ileum, when expressed as a percent of duodenal flow (Table 17) varied between 47.7% and 56.3%. The percent soluble and soluble Ca flow at this location ranged between 21.3% and 29.3%, and 2.59 and 3.07 g $day^{-1}$, respectively (Table 16).

Fecal Ca excretion ranged from 10.0 to 11.4 g $day^{-1}$ (Table 16) and represented 94.8% to 106% of ileal Ca flow. Apparent Ca digestibility was between 34.6% and 40.4% (Table 17).

The concentration of Ca in the soluble rumen fluid DM ranged from 6.43 to 6.90 mg $g^{-1}$ soluble DM and the concentrations of Ca in the duodenal and ileal soluble DM were from 23.2 to 29.1, and 8.32 to 9.07 mg $g^{-1}$ soluble DM, respectively (Table 18).

## 4.9 Magnesium

The intake of Mg (Table 19, Appendix Table A18) across all treatments ranged from 6.62 to 6.89 g $day^{-1}$.

There were no significant ($P>0.05$) effects of Mo and S upon the Mg parameters presented in Tables 19 and 20 (Appendix Table A19).

Table 17. Differences for calcium along the digestive tract, between intake, flows at cannulated sites in the proximal duodenum and terminal ileum and in fecal excretion for steers consuming diets high in molybdenum and sulfur contents

| | Proximal duodenum | | Terminal ileum | | Fecal excretion | | | |
|---|---|---|---|---|---|---|---|---|
| | Duodenal flow minus intake (g day$^{-1}$) | Duodenal flow as percent of intake | Ileal flow minus duodenal flow (g day$^{-1}$) | Ileal flow as percent of duodenal flow | Fecal excretion minus ileal flow (g day$^{-1}$) | Fecal excretion as percent of ileal flow | Intake minus fecal excretion (g day$^{-1}$) | Apparent digestibility (%) |
| Treatment | | | | | | | | |
| LMLS | +2.65 | 116 | -9.10 | 54.1 | +0.50 | 104 | 5.95 | 34.6 |
| LMHS | +3.38 | 121 | -10.28 | 47.7 | +0.60 | 106 | 6.30 | 38.7 |
| HMLS | +2.70 | 115 | -8.75 | 56.3 | -0.68 | 94.8 | 6.73 | 39.1 |
| HMHS | +4.08 | 121 | -11.10 | 52.5 | -0.75 | 96.6 | 7.78 | 40.4 |
| SE | 1.476 | 7.8 | 1.112 | 3.77 | 0.774 | 6.1 | 1.109 | 6.16 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | NS | NS | NS | NS | NS | NS | NS | NS |
| (II) HS vs LS | NS | NS | NS | NS | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | NS | NS | NS | NS | NS | NS | NS | NS |

†Refer to Table 3.

NS = Not significant (P>0.05).

Table 18. Concentrations of soluble calcium, magnesium and phosphorus in the soluble dry matter in rumen fluid, duodenal and ileal digesta of steers consuming diets high in molybdenum and sulfur contents

| | Calcium (mg $g^{-1}$ soluble DM) | | | Magnesium (mg $g^{-1}$ soluble DM) | | | Phosphorus (mg $g^{-1}$ soluble DM) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Rumen fluid | Duodenal digesta | Ileal digesta | Rumen fluid | Duodenal digesta | Ileal digesta | Rumen fluid | Duodenal digesta | Ileal digesta |
| Treatment | | | | | | | | | |
| LMLS | 6.43 | 24.7 | 8.78 | 4.58 | 5.51 | 5.51 | 36.5 | 26.8 | 8.73 |
| LMHS | 6.77 | 23.2 | 8.32 | 4.91 | 5.91 | 7.27 | 33.0 | 26.8 | 8.13 |
| HMLS | 6.90 | 25.4 | 9.07 | 4.82 | 4.86 | 8.27 | 39.5 | 25.3 | 7.35 |
| HMHS | 6.56 | 29.1 | 8.94 | 4.22 | 5.53 | 8.18 | 33.2 | 26.8 | 7.78 |
| SE | 1.167 | 1.51 | 0.966 | 0.183 | 0.209 | 0.470 | 2.63 | 2.25 | 0.776 |
| Contrast† | | | | | | | | | |
| (I) HM vs LM | NS | NS | NS | NS | * | NS(1) | NS | NS | NS |
| (II) HS vs LS | NS | NS | NS | NS | * | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | NS | NS | NS | * | NS | NS | NS | NS | NS |

†Refer to Table 3.

NS = Not significant ($P>0.05$), * Orthogonal contrasts are different ($P<0.05$).

Various contrasts tended towards significance ($0.05<P\leq0.10$): (1) P = 0.06.

Table 19. Magnesium intake, total and soluble flow of magnesium at the proximal duodenum and at the terminal ileum, and total fecal excretion of magnesium for steers consuming diets high in molybdenum and sulfur contents

| | Intake | Entering proximal duodenum | | | Leaving terminal ileum | | | |
|---|---|---|---|---|---|---|---|---|
| | ($g\ day^{-1}$) | Total flow ($g\ day^{-1}$) | Percent soluble | Soluble flow ($g\ day^{-1}$) | Total flow ($g\ day^{-1}$) | Percent soluble | Soluble flow ($g\ day^{-1}$) | Excreted in feces ($g\ day^{-1}$) |
| Treatment | | | | | | | | |
| LMLS | 6.89 | 4.04 | 90.4 | 3.65 | 3.67 | 60.6 | 2.22 | 3.72 |
| LMHS | 6.74 | 4.63 | 89.1 | 4.13 | 4.20 | 58.8 | 2.47 | 4.05 |
| HMLS | 6.62 | 3.60 | 89.0 | 3.22 | 3.59 | 60.6 | 2.18 | 3.53 |
| HMHS | 6.62 | 3.97 | 89.9 | 3.57 | 3.76 | 56.2 | 2.11 | 3.58 |
| SE | - | 0.255 | 1.35 | 0.242 | 0.180 | 2.07 | 0.112 | 0.253 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | - | NS(1) | NS | NS | NS | NS | NS | NS |
| (II) HS vs LS | - | NS | NS | NS | NS(2) | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | - | NS | NS | NS | NS | NS | NS | NS |

†Refer to Table 3.

NS = Not significant ($P>0.05$).

Various contrasts tended towards significance ($0.05<P\leq0.10$): (1) P = 0.08, (2) P = 0.10.

Table 20. Differences for magnesium along the digestive tract, between intake, flows at cannulated sites in the proximal duodenum and terminal ileum and in fecal excretion for steers consuming diets high in molybdenum and sulfur contents

| | Proximal duodenum | | Terminal ileum | | Fecal excretion | | | |
|---|---|---|---|---|---|---|---|---|
| | Duodenal flow minus intake (g day$^{-1}$) | Duodenal flow as percent of intake | Ileal flow minus duodenal flow (g day$^{-1}$) | Ileal flow as percent of duodenal flow | Fecal excretion minus ileal flow (g day$^{-1}$) | Fecal excretion as percent of ileal flow | Intake minus fecal excretion (g day$^{-1}$) | Apparent digestibility (%) |
| Treatment | | | | | | | | |
| LMLS | -2.85 | 58.6 | -0.37 | 91.1 | +0.06 | 101 | 3.17 | 45.9 |
| LMHS | -2.11 | 68.7 | -0.43 | 91.7 | -0.15 | 96.3 | 2.69 | 39.9 |
| HMLS | -3.01 | 54.4 | -0.01 | 99.7 | -0.06 | 98.0 | 3.08 | 46.7 |
| HMHS | -2.65 | 60.0 | -0.22 | 94.9 | -0.18 | 95.7 | 3.05 | 46.0 |
| SE | 0.251 | 3.72 | 0.225 | 5.06 | 0.137 | 3.60 | 0.248 | 3.71 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | NS | NS | NS | NS | NS | NS | NS | NS |
| (II) HS vs LS | NS(1) | NS(2) | NS | NS | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | NS | NS | NS | NS | NS | NS | NS | NS |

†Refer to Table 3.

NS = Not significant ($P>0.05$).

Various contrasts tended towards significance ($0.05<P\leq0.10$): (1) $P = 0.07$, (2) $P = 0.08$.

Significant effects ($P<0.05$) on soluble Mg levels in both the rumen fluid and in the duodenal digesta (Table 18) and various trends ($0.05<P\leq0.10$) are indicated below.

The flow of Mg at the proximal duodenum ranged between 54.4% to 68.7% of intake (Table 20). There was a tendency for a lower ($P=0.08$) Mg flow at the proximal duodenum with the HM treatments (Table 19). With the HS treatments, the net disappearance of Mg, calculated as duodenal flow minus intake, tended ($P=0.07$) to be lower (Table 20). There was a tendency for increased ($P=0.08$) flow of Mg at the proximal duodenum with the HS diets, when duodenal flow was expressed as a percent of Mg intake (Table 20). The concentration of Mg in the duodenal soluble DM (Table 18) was lower ($P<0.05$) with the HM diets and higher ($P<0.05$) with the HS diets.

The Mg flow at the terminal ileum, expressed as a percent of the duodenal flow (Table 20) ranged between 91.1% and 99.7%. The daily flow of Mg at the terminal ileum (Table 19) ranged from 3.59 to 4.20 g $day^{-1}$ and tended to be higher ($P=0.10$) for the HS diets. The concentration of Mg in the soluble DM in the ileal digesta (Table 18) tended to be higher ($P=0.06$) for the HM diets.

Fecal excretion of Mg, expressed as a percent of ileal flow ranged from 95.7% to 101% and apparent Mg digestibility ranged between 39.9% to 46.7% (Table 20).

## 4.10 Phosphorus

The intake of P (Table 21, Appendix Table A20) ranged from 13.6 to 14.1 g $day^{-1}$.

For P, parameters of duodenal flow, ileal flow and fecal excretion (Table 21) were not significant ($P>0.05$) among the treatments.

Phosphorus flow at the proximal duodenum (Table 21) ranged between 27.8 to 32.2 g $day^{-1}$, which represented 198% to 237% of intake (Table 22, Appendix Table A21). The flow of P at the proximal duodenum (Table 21) tended to be lower ($P=0.06$) with the HM treatments. The net appearance of P, calculated as duodenal flow minus intake (Table 22) was lower ($P<0.05$) with the HM treatments. Phosphorus flow at the proximal duodenum, with the HM treatments was also lower ($P<0.05$) when expressed as a percent of intake (Table 22). The percent soluble P at the proximal duodenum ranged between 59.2% to 63.1% and the soluble flow of P ranged between 17.1 and 19.3 g $day^{-1}$ (Table 21).

Phosphorus flow at the terminal ileum, expressed as a percent of duodenal flow (Table 22) ranged from 23.2% to 29.7%; this was not significant ($P>0.05$) in relation to the HM or HS diets, but it tended to be higher ($P=0.052$) when both Mo and S were added to the diets. The daily P flow at the terminal ileum (Table 21) ranged from 6.53 to 9.05 g $day^{-1}$ and tended to be lower ($P=0.09$) for the HM treatments. The net disappearance of P, calculated as ileal flow minus duodenal flow (Table 22) ranged between 21.3 to 24.4 g $day^{-1}$ and was higher ($P<0.05$) with the HS treatments and tended to be lower ($P=0.08$) with the HM treatments. The percent soluble P at the terminal ileum (Table 21)

Table 21. Phosphorus intake, total and soluble flow of phosphorus at the proximal duodenum and at the terminal ileum, and total fecal excretion of phosphorus for steers consuming diets high in molybdenum and sulfur contents

| | Intake | Entering proximal duodenum | | | Leaving terminal ileum | | | |
|---|---|---|---|---|---|---|---|---|
| | ($g\ day^{-1}$) | Total flow ($g\ day^{-1}$) | Percent soluble | Soluble flow ($g\ day^{-1}$) | Total flow ($g\ day^{-1}$) | Percent soluble | Soluble flow ($g\ day^{-1}$) | Excreted in feces ($g\ day^{-1}$) |
| Treatment | | | | | | | | |
| LMLS | 13.7 | 30.6 | 63.1 | 19.3 | 9.05 | 26.7 | 2.37 | 9.45 |
| LMHS | 13.6 | 32.2 | 59.2 | 19.2 | 7.88 | 33.8 | 2.65 | 7.63 |
| HMLS | 14.1 | 27.8 | 61.3 | 17.1 | 6.53 | 28.9 | 1.94 | 7.00 |
| HMHS | 13.9 | 29.5 | 59.4 | 17.7 | 7.45 | 23.0 | 1.72 | 7.13 |
| SE | - | 1.19 | 2.74 | 1.05 | 0.723 | 2.77 | 0.316 | 0.902 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | - | NS(1) | NS | NS | NS(2) | NS | NS(3) | NS |
| (II) HS vs LS | - | NS | NS | NS | NS | NS | NS | NS |
| (III) HMHS vs (HM) + (HS) | - | NS | NS | NS | NS | NS(4) | NS | NS |

†Refer to Table 3.

NS = Not significant ($P>0.05$).

Various contrasts tended towards significance ($0.05<P\leq0.10$): (1) $P = 0.06$, (2) $P = 0.09$, (3) $P = 0.08$, (4) $P = 0.06$.

Table 22. Differences for phosphorus along the digestive tract, between intake, flows at cannulated sites in the proximal duodenum and terminal ileum and in fecal excretion for steers consuming diets high in molybdenum and sulfur contents

| | Proximal duodenum | | Terminal ileum | | Fecal excretion | | | |
|---|---|---|---|---|---|---|---|---|
| | Duodenal flow minus intake (g day$^{-1}$) | Duodenal flow as percent of intake | Ileal flow minus duodenal flow (g day$^{-1}$) | Ileal flow as percent of duodenal flow | Fecal excretion minus ileal flow (g day$^{-1}$) | Fecal excretion as percent of ileal flow | Intake minus fecal excretion (g day$^{-1}$) | Apparent digestibility (%) |
| Treatment | | | | | | | | |
| LMLS | +16.9 | 224 | -21.5 | 29.7 | +0.40 | 105 | 4.23 | 30.8 |
| LMHS | +18.6 | 237 | -24.4 | 24.1 | -0.25 | 97.1 | 5.98 | 44.2 |
| HMLS | +13.8 | 198 | -21.3 | 23.2 | +0.48 | 107 | 7.08 | 50.3 |
| HMHS | +15.6 | 212 | -22.1 | 25.5 | -0.33 | 95.9 | 6.78 | 48.8 |
| SE | 1.17 | 8.6 | 0.60 | 1.61 | 0.271 | 3.71 | 0.925 | 6.59 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | * | * | NS (1) | NS | NS | NS | NS (2) | NS |
| (II) HS vs LS | NS | NS | * | NS | * | * | NS | NS |
| (III) HMHS vs (HM) + (HS) | NS | NS | NS | NS (3) | NS | NS | NS | NS |

†Refer to Table 3

NS = Not significant ($P>0.05$), * Orthogonal contrasts are different ($P<0.05$).

Various contrasts tended towards significance ($0.05<P\leq0.10$): (1) P = 0.08, (2) P = 0.10, (3) P = 0.052.

tended to be lower (P=0.06) when both Mo and S were added to the diets (Contrast III). The flow of soluble P at the terminal ileum (Table 21) tended to be lower (P=0.08) for the HM diets.

There was a net P disappearance, calculated as fecal excretion minus ileal flow (Table 22) with the HS treatments, while with the LS treatments there was a net appearance of P, a significant (P<0.05) effect of the HS treatments. Phosphorus fecal excretion, expressed as a percent of ileal flow was lower (P<0.05) with the HS diets. Total P disappearance, calculated as intake minus fecal excretion, ranged from 4.23 to 7.08 g $day^{-1}$ (Table 22) and was not different (P>0.05) among treatments. Total P disappearance tended to be higher (P=0.10) with the HM treatments (Table 22). Apparent digestibility of P ranged between 30.8% to 50.3% and was not different (P>0.05).

The concentration of P in the rumen fluid, duodenal and ileal digesta soluble DM was not different (P>0.05) within location (Table 18).

## 4.11 Fecal excretion minus duodenal flow

For Cu, Mo, Mn, Zn, Mg and P, but not Fe or Ca, significant effects (P<0.05) and trends (0.05<P≤0.10) were observed when mineral disappearance calculated as fecal excretion minus duodenal flow (Table 23, Appendix Tables A4, A7, A9, A12, A19, A21, A14, A16, respectively).

Disappearance of Cu tended to be lower (P=0.10) for the HM diets.

Table 23. Differences for minerals calculated as fecal excretion minus duodenal flow for steers consuming diets high in molybdenum and sulfur contents

| | Fecal excretion minus duodenal flow ($day^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Copper | Molybdenum | Manganese | Zinc | Iron | Calcium | Magnesium | Phosphorus |
| | mg | mg | mg | mg | mg | g | g | g |
| Treatment | | | | | | | | |
| LMLS | -13.1 | -3.58 | -27.2 | -159 | -246 | -8.6 | -0.32 | -21.1 |
| LMHS | -13.9 | -0.85 | -31.8 | -132 | -121 | -9.7 | -0.58 | -24.6 |
| HMLS | -10.7 | -21.3 | -15.5 | -103 | -245 | -9.4 | -0.07 | -20.8 |
| HMHS | -9.6 | -7.8 | -20.8 | -109 | -140 | -11.9 | -0.40 | -22.4 |
| SE | 1.71 | 3.34 | 2.55 | 9.3 | 109.8 | 1.46 | 0.141 | 0.53 |
| Contrast† | | | | | | | | |
| (I) HM vs LM | NS(1) | * | ** | ** | NS | NS | NS | NS(2) |
| (II) HS vs LS | NS | NS(3) | NS(4) | NS | NS | NS | NS(5) | ** |
| (III) HMHS vs (HM) + (HS) | NS | NS | NS | NS | NS | NS | NS | NS |

†Refer to Table 3.

NS = Not significant ($P>0.05$), * Orthogonal contrasts are different ($P<0.05$), ** ($P<0.01$).

Various contrasts tended towards significance ($0.05<P\leq0.10$): (1) P = 0.10, (2) P = 0.055, (3) P = 0.052, (4) P = 0.10, (5) P = 0.08.

Disappearance of Mo was higher ($P<0.05$) with the HM diets but tended to be lower ($P=0.052$) with the HS diets.

Disappearance of Mn and Zn were lower ($P<0.01$) with the HM diets, while the HS diets tended to result in higher ($P=0.10$) Mn disappearance.

Disappearance of Mg tended to be higher ($P=0.08$) with the HS diets. Disappearance of P was higher ($P<0.01$) with the HS diets but tended to be lower ($P=0.055$) with the HM diets.

# 5. DISCUSSION

Cannulae placed at the proximal duodenum and at the terminal ileum separated the digestive tract into three areas which entail: (1) the rumen, reticulum, omasum and abomasum, or stomach region; (2) the small intestine; and (3) the large intestine. Differences between daily intake, flows at the proximal duodenum, flows at the terminal ileum and fecal excretion were calculated. These differences identify dry matter digested,and the net absorption and net secretion of a mineral in each area.

## 5.1 Dry Matter

The apparent digestibility of DM for the HM treatments was higher than that with the LM treatments (Table 3). Apparent digestibility of DM with the HM and LM treatments averaged 77.2% and 73.5%, respectively. This difference between the HM and LM treatments was a reflection of the trend for a higher DM digestibility measured (Table 3) before the proximal duodenum. Dry matter flow at the proximal duodenum, averaged 2.39 and 2.78 kg $day^{-1}$ with the HM and LM treatments, respectively (Table 3, Fig. 1A). This flow represented an average DM disappearance from intake DM of -2.03 and -1.63 kg $day^{-1}$ for the HM and LM diets, respectively (Table 3, Fig. 1B). Of the total DM digested within the steers (Table 24), more DM disappeared in Region #1 before the proximal duodenum with the HM diets; this disappearance was an average of 10 percentage units higher for the HM diets at 59.6%, than with LM diets at

Table 24. Dry matter disappearance within the three regions, expressed as a percent of the total dry matter disappearance for steers consuming diets high in molybdenum and sulfur contents†

| | Region #1 | Region #2 | Region #3 |
|---|---|---|---|
| | Dry matter disappearance as a percent of total | Dry matter disappearance as a percent of total | Dry matter disappearance as a percent of total |
| Treatment | | | |
| LMLS | 50.8 | 39.3 | 9.9 |
| LMHS | 49.6 | 43.1 | 7.3 |
| HMLS | 59.1 | 31.2 | 9.7 |
| HMHS | 60.1 | 32.8 | 7.1 |
| SE | 4.76 | 4.12 | 1.53 |

†Region #1: Pre-intestinal tract, calculated as proximal duodenal flow minus intake.

Region #2: Small intestine, calculated as terminal ileal flow minus proximal duodenal flow.

Region #3: Large intestine, calculated as fecal excretion minus terminal ileal.

Region #1 + Region #2 + Region #3 = 100% of digested dry matter.

Figure 1A. Effects of high and low levels of dietary molybdenum upon dry matter flows at the proximal duodenum, terminal ileum and fecal excretion of dry matter.

Figure 1B. Effects of high and low levels of dietary molybdenum upon dry matter disappearance between various locations along the digestive tract.

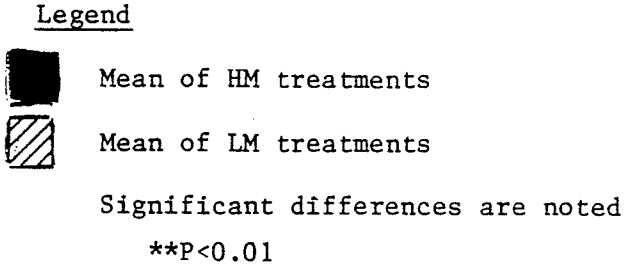


Figure 1A

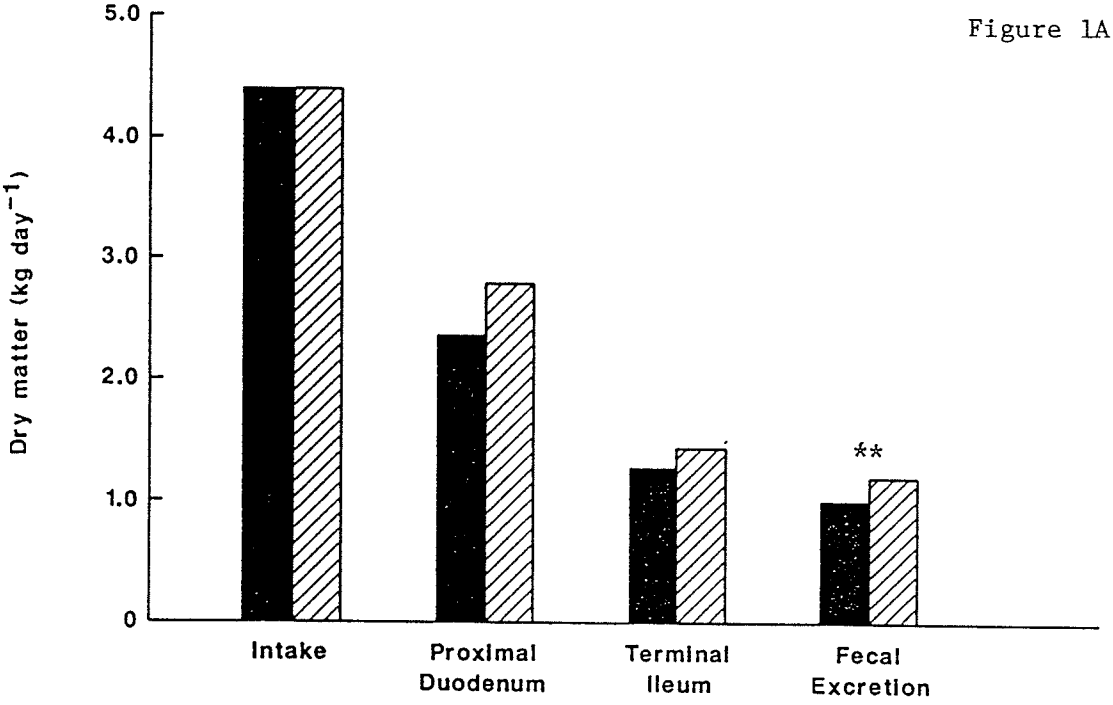
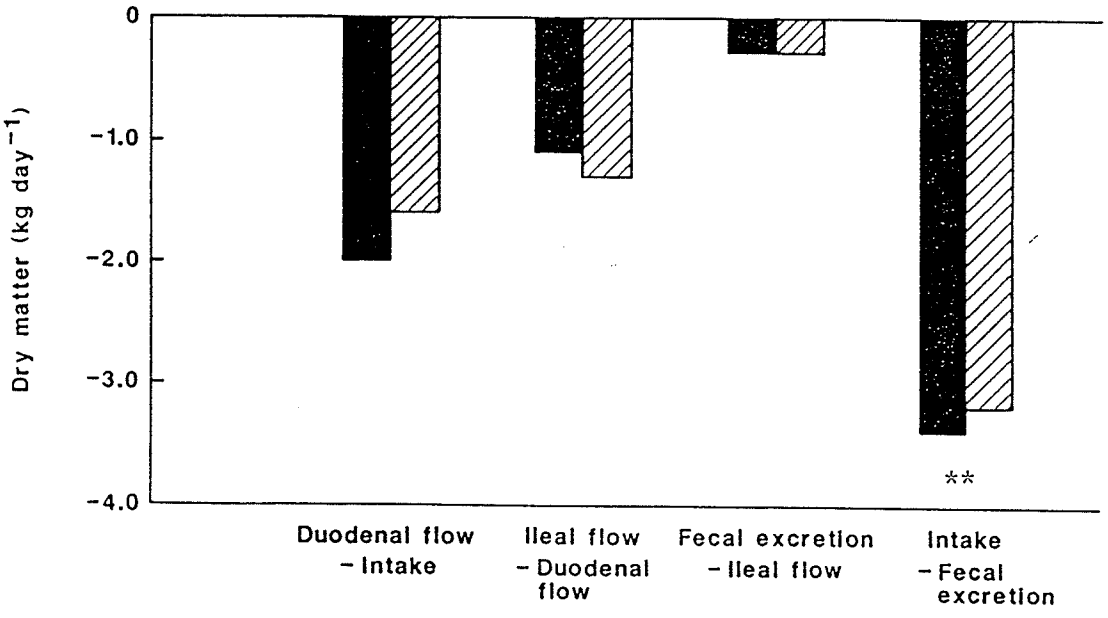



Figure 1B



50.2%. The higher dietary Mo content appeared to modify microbial metabolism thereby increasing DM digestibility by these organisms, and increasing DM digestibility overall (Table 3).

Cellulose digestion by microorganisms in the rumen was stimulated in vitro by the addition of 10 to 200 mg Mo $L^{-1}$ (Durand and Kawashima, 1980). Positive effects of Mo upon cellulose digestion have also been noted by Martinez and Church (1970). McNaught et al. (1950) and Sala (1957) found a negative effect of Mo upon cellulose digestion. The presence or absence of rumen fluid in the in-vitro incubation and interaction with other elements may explain the different responses (Ellis et al., 1958). When both Cu and Mo were included in incubation media (Ellis et al., 1958), cellulose digestion was stimulated.

A high digestibility of cellulose, and possibly total cell wall with added Mo may offer one explanation to the higher DM digestibility by rumen microbes. Other factors to consider are the physical form of the diet and the proportion of hay to grain in the diet. The hay was ground to 1.5 cm before being pelleted along with barley and other constituents (Table 1). The digestibility of DM is consistently lower with fine ground and pelleted roughages relative to chopped roughages (Blaxter and Graham, 1956; Johnson et al., 1964). The grinding and pelleting of roughages will depress the DM digestibility of these feedstuffs up to 5 percentage units (NAS-NRC, 1984).

The positive effect of Mo upon fibre digestion as the sole contribution to DM digestibility is questionable. Without Mo present, the small particle size, digestion of those particles and rate of

passage considerations (Warner, 1981) of the hay in this experiment would contribute to DM digestion in a direction opposite to that measured (Table 3).

Barley was a major component in the diet (Table 1). If the major effect of Mo was not upon cellulose digestion, then perhaps the digestion of dietary components, such as starch from the barley was modified.

## 5.2 Copper

The concentration of Cu in the diets was low at 2.8 to 3.8 mg $kg^{-1}$ DM (Table 2). A suggested requirement for Cu is 8 mg $kg^{-1}$ DM, with a range of 4 to 10 mg $kg^{-1}$ DM designated as acceptable, depending upon the Mo and S status of the diet (NAS-NRC, 1984). Copper status of steers before and during the experiment was not assessed. It was assumed that Cu status for all steers before the experiment was similar.

The mean concentration of Cu in the rumen fluid supernatant was 8.65 $\mu g\ g^{-1}$ soluble DM (Table 6). Low concentrations of trace elements such as Cu, are found in the rumen fluid supernatant due to the formation of insoluble complexes and accumulation of Cu in microorganisms. Considerable accumulation of Cu in the cell walls of microorganisms has been noted (Durand and Kawashima, 1980). The concentration of soluble Cu is much lower in the rumen than in the feed (Bremner, 1970; Ivan and Veira, 1981). Almost 88% of the Cu present in the rumen digesta of sheep fed dried grass was associated with the solid phase, with over 50% of the total Cu present in the large particulate

material. The remaining fraction of Cu was equally distributed among the various bacterial and protozoal fractions, and the liquid or soluble phase (Price and Chesters, 1985).

The concentration of soluble Cu in the rumen fluid supernatant did not differ in response to Mo or S; however, numerical differences were noted (Table 6). The mean concentration of soluble Cu was 8.24 and 9.07$\mu g\ g^{-1}$ DM for the HM and LM diets, respectively, and 5.26 and 12.1$\mu g\ g^{-1}$ DM for the HS and LS diets, respectively, the concentration of Cu for the HMHS treatment was numerically the lowest at 4.28 $\mu g\ g^{-1}$ DM. A high variation among individual measures may have precluded showing a significant difference (Table 6).

Effects of dietary Mo or S, or Mo plus S upon the solubility of Cu in the rumen fluid vary. Mills (1960) and Mills et al., (1977) reported a lower concentration of Cu in the rumen fluid of sheep and cattle, consuming diets with added Mo and S. In contrast, Chesters et al. (1982) suggested that a lack of an effect on soluble Cu in the rumen was due to changes within the microbial cells and not in the soluble phase as the result of an interaction with thiomolybdates. Ivan and Veira (1985) found that soluble Cu was not different with low or high dietary Mo, but decreased proportionately with the provision of supplemental Cu to sheep. Price and Chesters (1985) observed that, although added Mo increased the concentration of Mo in digesta fractions in the rumen, Mo did not alter the concentration of Cu in rumen fluid supernatant. A low dietary content of Cu (Table 2) may preclude a measured effect of Mo and S upon soluble Cu in the rumen.

The Cu entering the proximal duodenum ranged from 135 to 185% of that ingested (Table 5). This represented a net secretion of 6 to 10 mg Cu $day^{-1}$ before the proximal duodenum (Table 5). Water consumption by steers would have contributed about 2.3 mg Cu $day^{-1}$ (Appendix Table C). A considerable secretion of Cu into the digestive tract, before the proximal duodenum in the order of approximately 4 to 8 mg $day^{-1}$ is noted. The higher amounts of Cu entering the proximal duodenum relative to that consumed originates via the saliva and also by direct secretion through the rumen epithelium (Stevenson and Unsworth, 1978; Grace et al., 1982; Georgievskii, 1982; Grace and Gooden, 1980). These endogenous secretions would likely account for the increased Cu flow at the proximal duodenum. Bremner and Davies (1980) suggested that the abomasum could also be a major site of Cu secretion before the proximal duodenum. The net secretion of Cu before the proximal duodenum varies considerably in sheep (Grace, 1975; Stevenson and Unsworth, 1978; Ivan et al; 1979; Ivan and Veira, 1982) and cattle (Bertoni et al., 1976a; Ivan and Grieve, 1976).

The flow of Cu at the proximal duodenum as a percent of intake was lower with the HS diets (Table 5) and averaged 153% and 182% for the HS and LS diets, respectively. It is possible that a higher dietary S intake modified the recycling of Cu via the saliva or reduced other endogenous Cu secretions before the proximal duodenum.

The percent soluble Cu and the flow of soluble Cu at the proximal duodenum were all lower with the HM and HS diets (Table 4, Figures 2A and 3A). The mean proportion of soluble Cu at the proximal duodenum

Figure 2A. Effects of high and low levels of dietary molybdenum upon the total and soluble flows of Cu at the proximal duodenum and terminal ileum, and total fecal excretion of copper.

Figure 2B. Effects of high and low levels of dietary molybdenum upon the net movement of copper between various locations along the digestive tract.


Significant differences for soluble Cu flow are noted.

*P<0.05

**P<0.01

Figure 2A

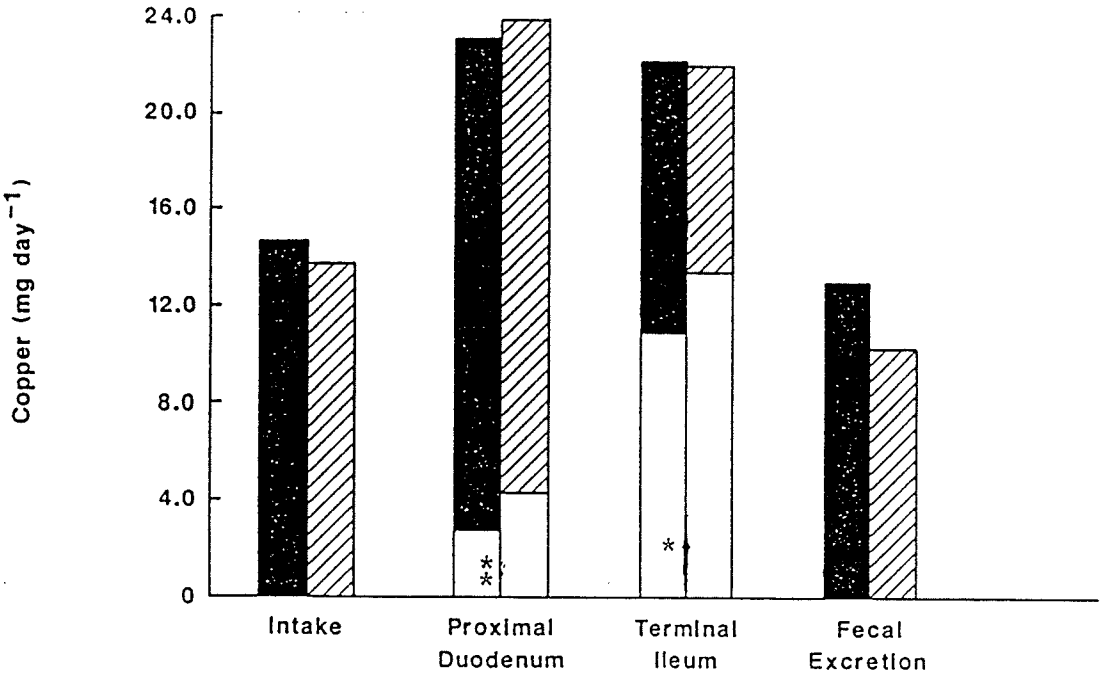



Figure 2B

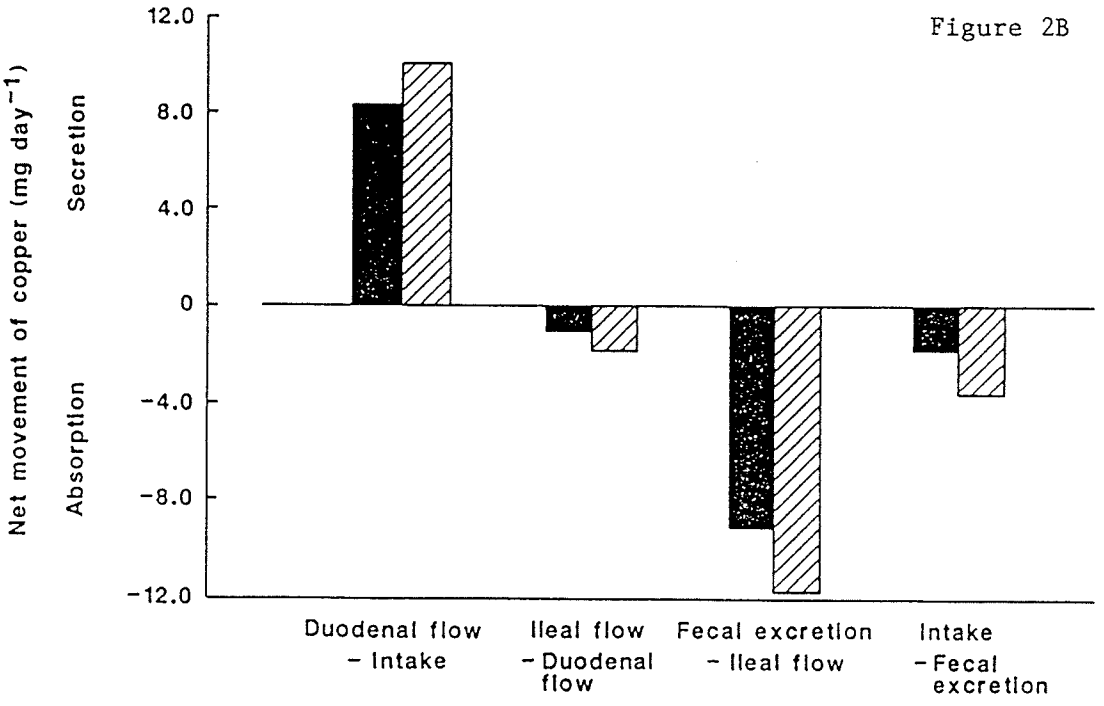


Figure 3A. Effects of high and low levels of dietary sulfur upon the total and soluble flows of copper at the proximal duodenum and terminal ileum, and total fecal excretion of copper.

Figure 3B. Effects of high and low levels of dietary sulfur upon the net movement of copper between various locations along the digestive tract.

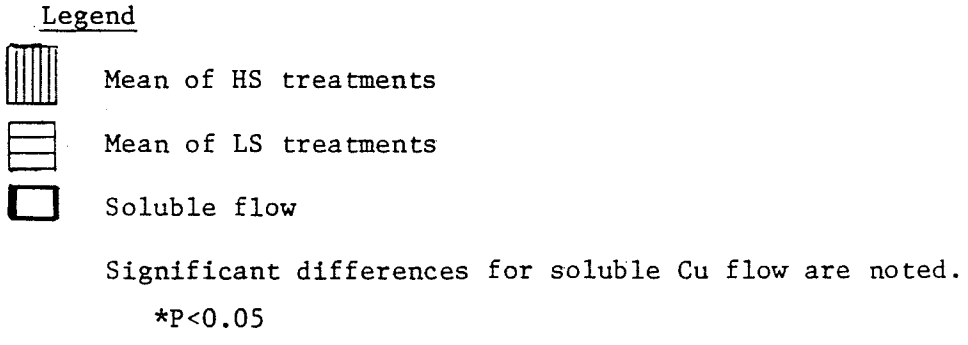


Figure 3A

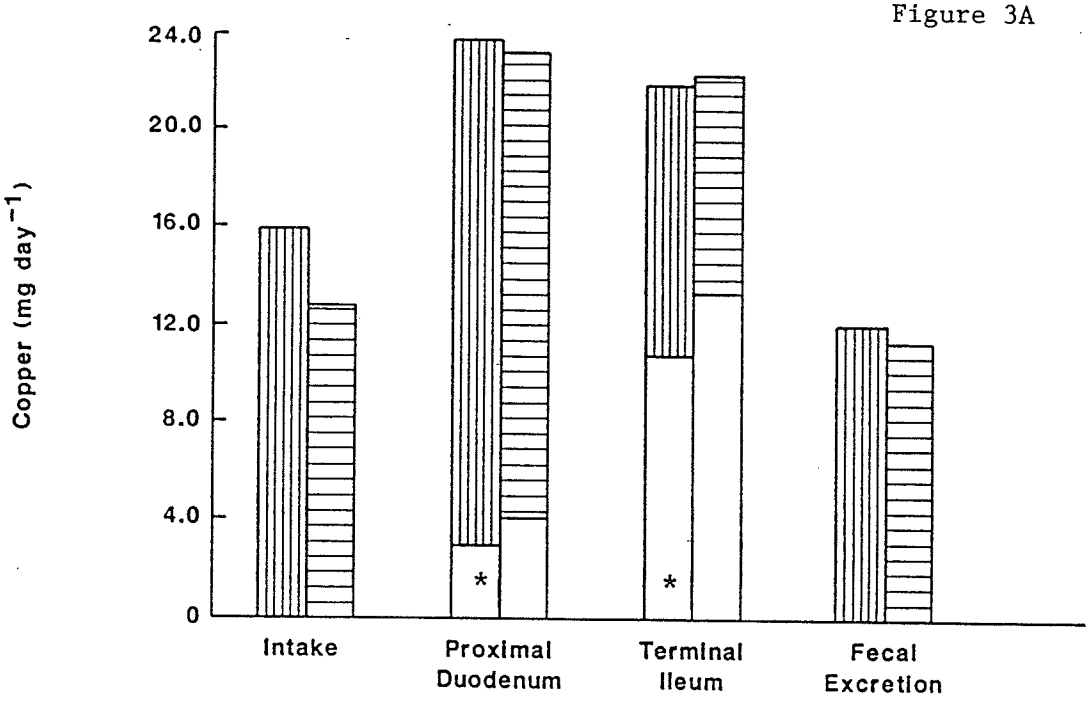
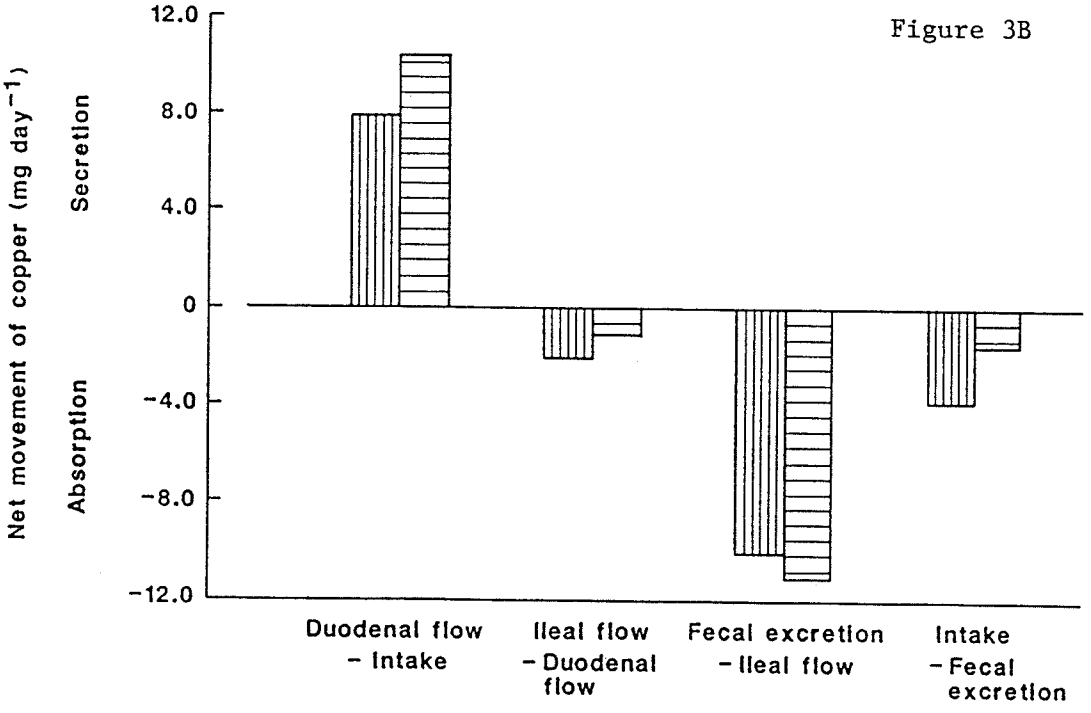



Figure 3B



with the HM and LM diets was 12.2% and 18.6% (Table 4) respectively, with corresponding soluble Cu flows at 2.8 and 4.4 mg $day^{-1}$ (Table 4, Fig. 2A). The mean proportion of soluble Cu at the proximal duodenum with the HS and LS diets was 12.8% and 18.0%, respectively (Table 4) with corresponding soluble Cu flows at 3.04 and 4.20 mg $day^{-1}$, respectively (Table 4, Fig. 3A). Both Mo and S were exerting an independent effect upon Cu solubility at the proximal duodenum.

Chesters et al. (1982) observed that during passage of digesta from rumen to duodenum, the proportion of soluble Cu remained constant with low Mo diets, but decreased by about 50% with high Mo diets relative to the low Mo diets. Gawthorne et al. (1985) reported that in vitro and in vivo addition of ammonium molybdate at 25 mg $kg^{-1}$ Mo halved the proportions of acid-soluble (TCA) Cu in various fractions in rumen digesta. The reduction of Cu solubility by S is thought to be a result of the formation of an insoluble cupric sulfide complex (CuS) which would be formed under favorable rumen conditions (Mason, 1981).

While the HMHS contrast was not significant for the percent soluble Cu at the proximal duodenum (Table 4, Contrast III), the HMHS value at 10.5% was numerically the lowest, however, compared to the other three treatments. Both Mo and S were synergistic in decreasing the solubility of Cu compared to Mo or S alone. In addition, Mo absorption was reduced by the HS diets in the small intestine (Table 8, Fig. 4B). Thus the effect of Mo and S upon Cu solubility (Table 4) and S upon Mo absorption(Table 7) would indicate the presence of a Cu-Mo-S complex in

Figure 4A. Effects of high and low dietary levels of molybdenum upon the apparent digestibility of molybdenum and various % flow relationships along the digestive tract.

Figure 4B. Effects of high and low dietary levels of sulfur upon the apparent digestibility of molybdenum and various % flow relationships along the digestive tract.

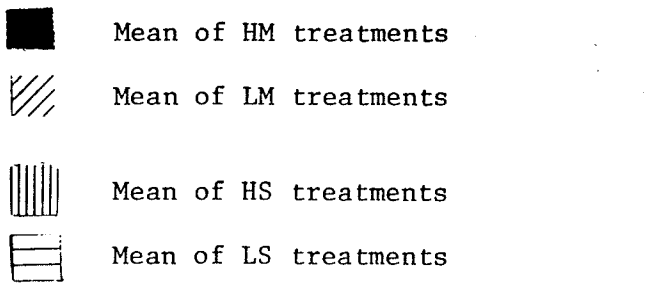


Figure 4A

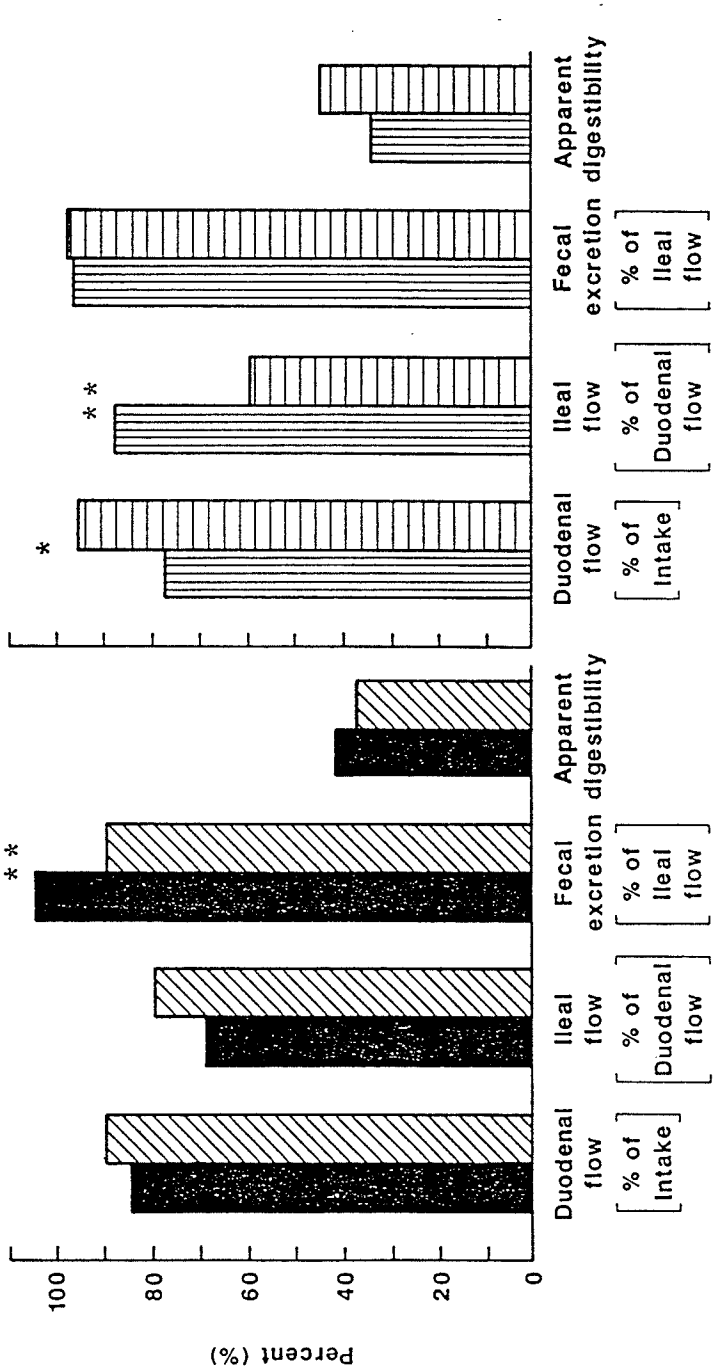



Figure 4B

the small intestine. Therefore, it is likely thiomolybdates were formed, thereby reducing the solubility of Cu.

At the concentrations of Mo and S used in this experiment (Table 2), the synthesis of thiomolybdates is likely. Rumen synthesis of di-, tri-, and tetrathiomolybdates have been demonstrated in both sheep (Price et al., 1987) and cattle (Hynes et al., 1985) consuming diets high in Mo and S.

Price et al. (1987) reported that almost 70% of the radioactive $^{99}Mo$ was associated with the solids of the rumen digesta, mainly as tri- and tetrathiomolybdates. Gawthorne et al. (1985) demonstrated that tetramolybdate at 5 mg Mo $kg^{-1}$ consistently decreased the TCA-solubility of Cu in rumen digesta. This TCA-insoluble Cu was bound to particulate matter and proteins, together with Mo. Gawthorne et al. (1985) concluded that thiomolybdates rapidly react with particulate matter to form complexes that bind Cu strongly and reduce the solubility of Cu in TCA.

Kelleher et al. (1983) reported that a significant proportion of the thiomolybdates formed in the rumen escape destruction in the acid environment of the abomasum. Price et al. (1987) observed differences in the proportions of the Mo containing constituents between ruminal and duodenal digesta solids. The persistence of bound tetra-thiomolybdate was noted. The relative proportion of tri-thiomolybdate was also lower in the duodenal digesta than in ruminal digesta.

The percent soluble Cu at the terminal ileum was higher than that of the proximal duodenum (Table 4). This difference in the percent

soluble Cu fraction at the ileum has been demonstrated by Bremner (1970) and Ivan et al. (1983a). Gollan et al. (1971) have found substances that formed soluble complexes with Cu under alkaline conditions in human alimentary secretions, despite the high pH at the lower small intestine.

At the terminal ileum, the mean percent soluble Cu for the HM and LM diets (Table 4) respectively was 48.6% and 61.1% while the corresponding soluble Cu flow was 11.0 and 13.5 mg $day^{-1}$ (Table 4, Fig. 2A). The mean percent soluble Cu for the HS and LS diets (Table 4) respectively, was 48.1% and 60.8%, and the corresponding soluble flow was 10.9 and 13.6 mg $day^{-1}$ (Table 4, Fig. 2B).

When the effect of Mo and S was investigated using contrast 3 (Table 4), the solubility of Cu at the terminal ileum was also lower with a tendency for lower flow of soluble Cu into the large intestine (Table 4). A formation of thiomolybdates and a binding of Cu to those thiomolybdates in the particulate fraction would explain the effect of Mo and S upon the solubility of Cu (Price et al., 1987).

Price et al. (1987) reported that free thiomolybdates were not detected in the liquid phase of digesta at the terminal ileum.

Neither HM nor HS influenced the absorption of Cu in either the small or large intestine (Table 5, Figures 2B and 3B). However, overall Cu absorption distal to the proximal duodenum (Table 23) tended to be lower with the HM diets, with a tendency for an increased fecal excretion of Cu (Table 4, Fig. 2B) and a tendency for decreased apparent digestibility for Cu with the HM diets (Table 5).

It is not clear why HM or HS diets did not influence Cu absorption within the small intestine. Gooneratne et al. (1986) have demonstrated a high endogenous loss of Cu in the bile when thiomolybdates were intravenously administered, and proposed that endogenous losses of Cu could also occur from other intestinal secretions. There is a possibility that endogenous secretion was equal to the amount of Cu that was absorbed.

A high urinary output of Cu also occurs when diets were high in Mo and S (Gooneratne et al., 1981a, b; Ammerman and Goodrich, 1983). Gooneratne et al. (1985, citing Fisher et al., 1976) observed increases in fecal Cu excretion in cattle given high Mo diets.

Considerable absorption of Cu occurred distal to the proximal duodenum. (Table 23). This has been observed by Grace et al. (1974), Bertoni et al. (1976a), Stevenson and Unsworth (1978) and Ivan et al. (1979). In the present experiment, more Cu was absorbed in the large intestine (Table 5) than in the small intestine. Bertoni et al. (1976a) reported a considerable absorption of Cu in the large intestine, but a variable absorption of Cu in the small intestine of cattle. Grace (1975) and Ivan et al. (1983a) also observed considerable Cu absorption in the large intestine of sheep.

The apparent digestibility of Cu across treatments (Table 5) ranged from 1.3 to 27.5%. It can range from -15 to +35% (Grace, 1975; Bertoni et al., 1976a; Stevenson and Unsworth, 1978; Ivan et al., 1979; Grace and Gooden, 1980; Ivan et al., 1983a). True absorption of Cu is low, at about 5%. A high proportion of dietary Cu appears in the feces. Also

there is active excretion of Cu via bile, a major route of Cu excretion (Underwood, 1977; Grace, 1983).

## 5.3 Molybdenum

### 5.3.1 Influence of dietary Mo

The intake of Mo with the HM diets were approximately 7-fold higher than that with the LM diets (Table 7). Despite the higher flow of Mo at both the proximal duodenum and terminal ileum with the HM diets (Table 7), the same proportion of Mo was absorbed before the proximal duodenum and in the small intestine (Table 8, Fig. 4A). Duodenal flow was 85.1% and 89.7% of intake for the HM and LM diets, respectively (Table 8, Fig. 4A). Ileal flow was at 58.9% and 79.5% of duodenal flow for the HM and LM diets, respectively (Table 8, Fig. 4A). Since the amount of Mo absorbed before, and within the small intestine were the same regardless of the dietary Mo content, level of dietary Mo had no effect upon the fraction of Mo absorbed.

Kosarek (1976) and Kosarek and Winston (1977) found that at higher Mo intakes and at resultant higher concentrations of Mo in the lumen, Mo was absorbed by passive diffusion. At low intakes, absorption of Mo occurs by active transport (Cardin and Mason, 1975, 1976; Mason and Cardin, 1977). Absorption by passive diffusion demonstrates that there is little control over the amount of Mo absorbed (Winston, 1981). Plasma and tissue Mo levels are markedly higher with increased dietary Mo intakes, a reflection of an increased absorption of Mo (Huber et al.,

1971; Grace and Suttle, 1979; Van Ryssen and Stielau, 1981; Ademosun and Munyabuntu, 1982; Ivan and Veira, 1985).

Similar proportions of Mo were absorbed (Table 8), whether Mo was a part of the feed matrix in the LM diets, or as a Mo salt in the HM diets. Molybdenum is known to be readily absorbed from most diets and in inorganic forms (Underwood, 1977; Georgievskii, 1982).

On average, 8.67 and 1.04 mg $day^{-1}$ of Mo were absorbed before the proximal duodenum with the HM and LM diets, respectively (Table 8). There was also net absorption of Mo from the small intestine, which was 15.9 and 1.7 mg $day^{-1}$ for the HM and LM diets, respectively (Table 8). Miller et al. (1972) demonstrated that no appreciable absorption of $^{99}Mo$ occurred in the rumen and omasum, while Mason et al. (1978) reported that no radioactivity from $^{99}Mo$ was absorbed from the rumen. Miller et al. (1972) did report an absorption of 10% of the daily intake of Mo from the abomasum. The principal site of Mo absorption in both sheep and cattle is the small intestine (Miller et al., 1972; Mason et al., 1978). More absorption of $^{99}Mo$ in cattle had occurred in the caudal third of the small intestine (Miller et al., 1972).

A small net absorption of Mo was noted in the large intestine with the LM diets, while a small net secretion occurred with the HM diets (Table 8, Fig. 4A). The fecal excretion of Mo was 105% and 89% of ileal flow with HM diets and LM diets, respectively (Table 8, Fig. 4A).

The apparent digestibility of Mo for the HM and LM diets was 40.2% and 38.7% respectively (Table 8, Fig. 4A). While Mo in feedstuffs and from inorganic sources are readily absorbed, apparent absorption is low

and has ranged from 30 to 36% (Comar et al., 1949; Miller et al., 1972; Mason et al., 1978) in cattle and sheep. Grace and Suttle (1979) observed that fecal endogenous losses of Mo can be as great as 38 to 52% of the total Mo fecal excretion with endogenous biliary excretion contributing to fecal excretion (Scaife, 1956). True absorption of dietary Mo can be as high as 84% (Grace and Suttle, 1979).

### 5.3.2 Influence of dietary S

High dietary levels of S resulted in a marked reduction in Mo absorption within the small intestine (Table 8, Fig. 4B). Ileal flow of Mo was 88% and 60.4% of duodenal flow for the HS and LS diets respectively (Table 8, Fig. 4B). Actual Mo absorption within the small intestine was 4.17 and 13.4 mg $day^{-1}$ for the HS and LS diets, respectively (Table 8). The lower Mo absorption with the HS diets within the small intestine led to increased fecal Mo excretion with the HS diets (Table 8).

Both the absorption of Mo in the small intestine and the fecal excretion of Mo in feces is dependent upon the S status of the diet. A lower absorption of Mo due to elevated dietary S levels is manifested by a lower blood and tissue level of Mo in both cattle (Cunningham et al., 1959; Huber et al., 1971; Wittenberg and Boila, personal communication) and in sheep (Mason et al., 1978; Grace and Suttle, 1979) along with increased fecal excretion of Mo (Mason et al., 1978; Grace and Suttle, 1979).

The influence of sulfur upon Mo absorption was partially explained by Dick (1956). According to Dick (1956), inorganic sulfate interferes with Mo absorption when the concentration of S is high enough by preventing the transport of Mo across the intestinal membranes. Cardin and Mason (1975, 1976), and Mason and Cardin (1977) recognized that both sulfate ($SO_4^{-2}$) and molybdate ($MoO_4^{-2}$) because of a similar size, charge and configuration, compete for sites on a common transport system in the bovine ileum in a manner similar to that in the rat intestine. In the ovine intestine, the sulfate ion competes effectively with molybdate, while molybdate is less competitive relative to sulfate.

The formation of S-Mo complexes which are largely unavailable for absorption (Dick et al., 1975; Mason, 1981) reduced the absorption of Mo in the digestive tract. Thiomolybdates which form under conditions of high dietary S levels remain primarily associated with the solid fraction in digesta. This association allows for a stability of the Mo-S compounds (Price et al., 1987).

The HS diets contributed to a lower Mo flow at the proximal duodenum. The flow of Mo at the proximal duodenum was 78.4 and 96.4% of intake for the HS and LS diets, respectively (Table 8, Fig. 4B). A recycling of Mo has been described by Suttle and Grace (1978) and by Grace and Suttle (1979). Sulfur compounds in the diet increase urinary Mo excretion (Mason et al., 1978), decrease Mo retention in the body and decrease the recycling of Mo to the rumen (Grace and Suttle, 1979). A high dietary S content reduced the Mo recycled via saliva by reducing

the amount of Mo in the body that was available for secretion (Grace and Suttle, 1979).

The apparent digestibility of Mo tended to be lower with the HS diets (Table 8, Fig. 4B). Apparent digestibility of Mo for the HS and LS diets was 33.9% and 45%, respectively (Table 8, Fig. 4B). Fecal Mo excretion increased 1.25X as S levels were elevated to 3.9 g $kg^{-1}$ DM (Table 7). Fecal Mo excretion in sheep was increased an average of 1.7X when S intake was increased from 0.93 to 3.23 g $day^{-1}$ (Grace and Suttle, 1979). That elevated intake of S also reduced the true absorption of dietary Mo from 80% to 37%.

## 5.4 Manganese

There was a lower concentration of soluble Mn in the DM of the rumen fluid supernatant with the HS diets (Table 11). The concentration of Mn within the rumen fluid supernatant was 54.1 and 66.8 $\mu g\ g^{-1}$ Mn DM for the HS and LS diets, respectively.

Low concentrations of soluble trace elements such as Mn, are normally found in the supernatant fraction of rumen fluid. This is due to an accumulation of Mn in bacteria and protozoa and due to the formation of insoluble Mn complexes (Durand and Kawashima, 1980). Ivan and Veira (1981) and Bremner (1970) have reported that the percent soluble Mn is four to five fold lower in rumen fluid than in feed.

Much of dietary Mn is converted into insoluble complexes in the rumen (Bremner, 1970). Trace elements form complexes with anionic ligands. These ligands may be inorganic compounds such as sulfide, or

organic compounds such as phytic acid, amino acids and fatty acids which are end products of microbial fermentation (Wetzel and Menke, 1978). Bremner (1970) detected several soluble cationic and anionic Mn containing complexes and a major neutral Mn complex in the rumen fluid of sheep.

High levels of supplemental S result in higher sulfide-S concentrations in the rumen fluid (Kandylis and Bray, 1987). A soluble Mn complex may have reacted with the sulfide to form an insoluble Mn-S complex in the rumen.

The solubility of Mn is higher at a low rumen pH (Nikolic et al., 1978). Although the rumen pH was not examined, it is possible that the pH of the rumen was low enough to enhance the solubility of Mn; consequently, a Mn-S interaction is possible.

Suttle (1984) suggested that Mn, like Fe, is capable of trapping sulfide gas to produce acid-labile sulfides. If dietary Mn reacted with sulfide solely within the rumen, then the lack of an effect of sulfur upon Mn in the rest of the digestive tract (Tables 9, 10 and 11) is explainable.

The concentration of Mn in the duodenal supernatant DM was about twice that in rumen fluid (Table 11). The solubility of Mn in the duodenal digesta was 81.9% (Table 9). The increase in the concentration of soluble Mn and the high solubility at the proximal duodenum upon passage through the rumen was in agreement with the results of Bremner (1970), Ivan and Veira (1981) and Ivan et al. (1983a). According to Bremner (1970), a large increase in the solubility of Mn occurs during

the passage of digesta from the rumen to the abomasum. This apparently arises from the dissociation at low pH of insoluble Mn complexes that are not sufficiently stable to exist intact in the abomasum. Ivan and Veira (1981) and Bremner (1970) observed that the solubility of Mn in the abomasal digesta was four to five times higher than that in the rumen.

The solubility of Mn at the terminal ileum was 11.7% and was seven-fold lower than that at the proximal duodenum (Table 9). Similar changes in Mn solubility have been reported (Bremner, 1970; Ivan et al., 1983a).

The apparent digestibility of Mn ranged from 11.4% to 17.7% (Table 10) and lies within the range of 1 to 25% reported for cattle (Bertoni et al., 1976a) and for sheep (Grace, 1975; Ivan et al., 1979; Ivan et al., 1983a). Absorption of Mn from feeds is considered to be low with adult ruminants and can range between 10 to 18% of intake (Georgievskii, 1982). Gibbons et al. (1976) and Sansom et al. (1978) suggested that a constant fraction of dietary Mn of approximately 1% is absorbed from the intestines, over a wide range of concentration of Mn in the diet.

Manganese is known to be excreted almost entirely in the feces, with a small fraction excreted in the urine (Grace, 1975; Ivan et al., 1979; Georgievskii, 1982; Ivan et al., 1983a). About 84.5% of the Mn consumed by cattle was excreted in the feces (Table 9). Homeostatic control of Mn retention is almost entirely achieved by the regulation of fecal endogenous secretion, with bile serving as the principal route of

Mn excretion into the digestive tract (Bertinchamps et al., 1966). The excretion of Mn in bile is related directly to the absorption of Mn from the gastro-intestinal tract (Symonds and Mallinson, 1982). Excretion of Mn also occurs in pancreatic juice and other intestinal secretions to a lesser extent (Underwood, 1977).

The increased flow of Mn at the proximal duodenum relative to intake was similar to that measured for net absorption along the small intestine (Table 10). This would suggest that the level of endogenous Mn secretion before the proximal duodenum were similar to that absorbed within the small intestine. Grace (1975), Bertoni et al. (1976a) and Ivan et al. (1983a) have observed that the small intestine was a region of net secretion, with the level of Mn leaving the small intestine greater than that which entered. In this experiment, flow of Mn at the terminal ileum was similar to that ingested by steers (Table 9). Manganese flow at the terminal ileum ranged from 84.4 to 94.3% of duodenal flow (Table 10).

Manganese is equally well absorbed throughout the length of the small intestine (Underwood, 1977). It is apparently absorbed in the ionic ($Mn^{+2}$) form (Bremner, 1970). The principal site of Mn absorption within the small intestine is the duodenum (Georgievskii, 1982).

Absorption of Mn also occurs in the large intestine (Grace, 1975; Bertoni et al., 1976a; Ivan et al., 1983a). Fecal Mn excretion was 83.3 to 88.6% of ileal flow (Table 10). Similar values have been reported by Bertoni et al. (1976a) and Grace (1975).

The mean concentration of Mn in the diet fed to steers was 19.2 mg $kg^{-1}$ DM (Table 2). The suggested requirement is 40 mg $kg^{-1}$ DM for growing steers, with a range of 20 to 50 mg $kg^{-1}$ DM (NAS-NRC, 1984). Underwood (1977) suggested that 20 mg $kg^{-1}$ DM is adequate for growing-finishing cattle. The Mn level in the diets (Table 2) was low in terms of an absolute requirement. This would account for the high net absorption of Mn from the small intestine (Table 10).

The feeding of high dietary Mo reduced the flow of Mn at the proximal duodenum (Table 10) and reduced the net absorption of Mn distal to the proximal duodenum (Table 23). The mechanism of a Mo x Mn interaction is not identifiable. The total net absorption of Mn along the digestive tract (fecal excretion relative to that consumed), was not affected by Mo (Table 10). Dietary Mo had no effect upon the total solubility and total absorbability of Mn in the intestinal tract (Table 9).

## 5.5 Zinc

The higher flow of Zn at the proximal duodenum relative to that consumed, at 147-189% of intake (Table 13) has been reported for sheep (Grace, 1975; Stevenson and Unsworth, 1978; Ivan et al., 1979) and cattle (Miller and Cragle, 1965; Hiers et al., 1968; Bertoni et al., 1976a). Duodenal flow of Zn as a percent of intake has ranged from 105% to as high as 280% in the above listed references.

Considerable quantities of Zn were secreted before the proximal duodenum in the range of 54 to 94 mg $day^{-1}$ (Table 15). An estimated

input of Zn in tap water was 17.4 mg $day^{-1}$ (Appendix Table C). A net secretion of Zn into the rumen and reticulum has been demonstrated (Hiers et al., 1968). Large quantities of Zn are secreted via the saliva (Grace et al., 1982) and are transported through the rumen epithelium (Watson and Kastelic, 1967). Gastric secretions may also contribute to the higher flow of Zn at the proximal duodenum; however, the abomasum is considered a region of net absorption of Zn (Hiers et al., 1968; Miller and Cragle, 1965). Miller and Cragle (1965) reported an apparent absorption of 35% of dietary $^{65}Zn$ between the mouth and abomasum of young calves and mature cows.

The concentration of Zn in the duodenal supernatant (Table 11) like that of Mn, was higher than that in the rumen fluid supernatant. The lower concentration of Zn in the rumen fluid is due to the formation of insoluble complexes and accumulation of Zn in bacteria (Durand and Kawashima, 1980). Zinc is present in bacterial cell walls and may accumulate in certain organisms in extremely high concentrations. Much of the cell associated Zn is bound non-specifically to cell surfaces (Durand and Kawashima, 1980).

Both the concentration of soluble Zn and the percent solubility of Zn change with the passage of digesta through the abomasum. There is dissociation of insoluble Zn complexes at low pH (Bremner, 1970). Ivan and Veira (1981) reported that percent solubility of Zn in the rumen was 12%, but was 91% in the abomasum. Bremner (1970) observed a similar change of solubility of Zn from 6% in the rumen to 50.4% in the abomasum and 59.9% in the duodenum. Ivan et al. (1983a) also found higher

concentrations of Zn in the supernatant fraction of duodenal digesta in contrast to that measured in rumen fluid.

A reduction in the percent solubility and flow of soluble Zn (Table 12) in the duodenal digesta were noted for the HS diets. The HS diets also reduced the concentration of Zn in the duodenal digesta supernatant (Table 11). The mean solubility of Zn at the proximal duodenum with the HS and LS diets was 66.1% and 80.1% respectively. The mean soluble flow of Zn for the HS and LS diets were 115 and 156 mg $day^{-1}$; the mean concentrations of Zn in the duodenal supernatant were 178 and 227 $\mu g\ g^{-1}$ soluble DM, respectively. A reduction in these parameters with the HS diets did not influence the overall absorption of Zn distal to the proximal duodenum (Table 23), within the small or large intestine (Table 13) or the overall apparent digestibility of Zn (Table 13).

Soluble Zn in the abomasum, duodenum and upper jejunum is in the free, ionic ($Zn^{+2}$) form and is not complexed (Bremner, 1970). In an ionic state, $Zn^{+2}$ is free to form coordination complexes with both exogenous and endogenous ligands. These Zn-complexes vary greatly in stability. Some complexes render the metal unavailable for absorption (Solomons and Cousins, 1984).

Zinc sulfide is soluble in acid and therefore, it is unlikely that sulfide was responsible for the decreased solubility of Zn at the proximal duodenum (Table 12). Most of the sulfur that enters the small intestine is protein—S, rather than sulfide—S. Protein—S is either of dietary or microbial origin (Bray and Till, 1975; Grace, 1983). If

sulfide did not result in a lower Zn solubility at this location, than an end-product of S metabolism in the rumen or a product of protein-S breakdown in the abomasum contributed to the lowered solubility of Zn. These may have been peptides or poly-peptides capable of rendering Zn acid-insoluble at the low duodenal pH, without a subsequent effect on Zn absorption within the small or large intestine.

Within the intestinal tract, Zn is associated with a variety of ligands which may influence the absorption process. Some ligands are in the diet while others are produced by digestion, or are present in endogenous secretions (Kratzer and Vohra, 1986). There is an enormous competition for binding of Zn among the many ligands in the intestinal tract. These ligands in turn, may have a large influence upon the availability of Zn for transport to, and possibly across the brush border of the intestinal epithelium (Kratzer and Vohra, 1986). Small molecular weight binding agents, such as amino acids, and polypeptides enhance the mucosal uptake and ultimately the absorption of Zn (Mertz, 1986). Sulfur-amino acids, such as cysteine, a product of digestion, has been shown to facilitate the absorption of Zn (Sandstead, 1981).

The net absorption of Zn in the small intestine was 35.6 mg $day^{-1}$ (Table 13). Zinc is absorbed in the abomasum and throughout the small intestine (Miller and Cragle, 1965; Hiers et al., 1968; Georgievskii, 1982; Grace, 1983). Endogenous Zn is excreted in pancreatic, bile and intestinal juices, as well as in the large intestine (Underwood, 1977; Georgievskii, 1982; Grace, et al., 1982; Grace, 1983). Zinc

homeostasis is maintained by both absorption and endogenous secretion of Zn (Georgievskii, 1982).

A net Zn absorption of 73 to 121 mg Zn $day^{-1}$ occurred in the large intestine (Table 13). On average, this net absorption was 2.5X higher compared to the net absorption of Zn within the small intestine. Considerable net absorption has been reported for the large intestine of sheep (Grace, 1975) and cattle (Bertoni et al., 1976a). In some instances, Zn absorption from the large intestine was higher than absorption from the small intestine.

The absorption of Zn distal to the proximal duodenum (Table 23) was lower with the HM diets and averaged 106 mg $day^{-1}$. A reduced Zn absorption in the large intestine (Table 13, Fig. 5B) contributed to the lowered overall Zn absorption beyond the proximal duodenum, with HM diets. Absorption of Zn in the large intestine averaged 75 and 106 mg $day^{-1}$ with the HM and LM diets, respectively (Table 13, Fig. 5B). Fecal excretion as a percent of ileal flow averaged 47.6% and 30.9% with the HM and LM diets, respectively; fecal excretion with the HM and LM diets averaged 73.5 and 44.7 mg $day^{-1}$, respectively (Table 13, Fig. 5A). The reduced Zn absorption in the large intestine was due to a lower solubility of Zn and flow of soluble Zn at the terminal ileum (Table 12, Fig. 5A). Soluble Zn at the terminal ileum for the HM and LM treatments was 27.7% and 45.2% of total Zn, respectively (Table 12), while flow of soluble Zn at the terminal ileum for these corresponding treatments averaged 41.6 and 67.3 mg $day^{-1}$ (Table 12, Fig. 5A).

Figure 5A. Effects of high and low levels of dietary molybdenum upon the total and soluble flows of zinc at the proximal duodenum and terminal ileum, and total fecal excretion of zinc.

Figure 5B. Effects of high and low levels of dietary molybdenum upon the net movement of zinc between various locations along the digestive tract.

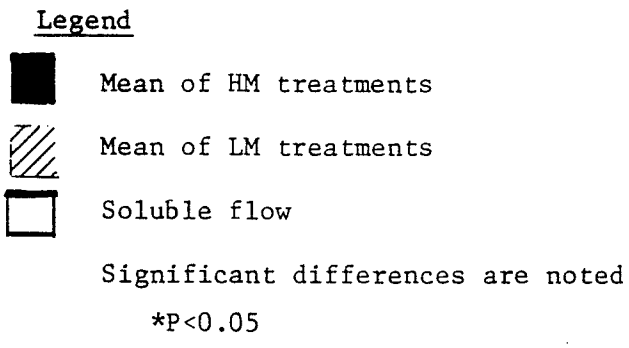


Figure 5A

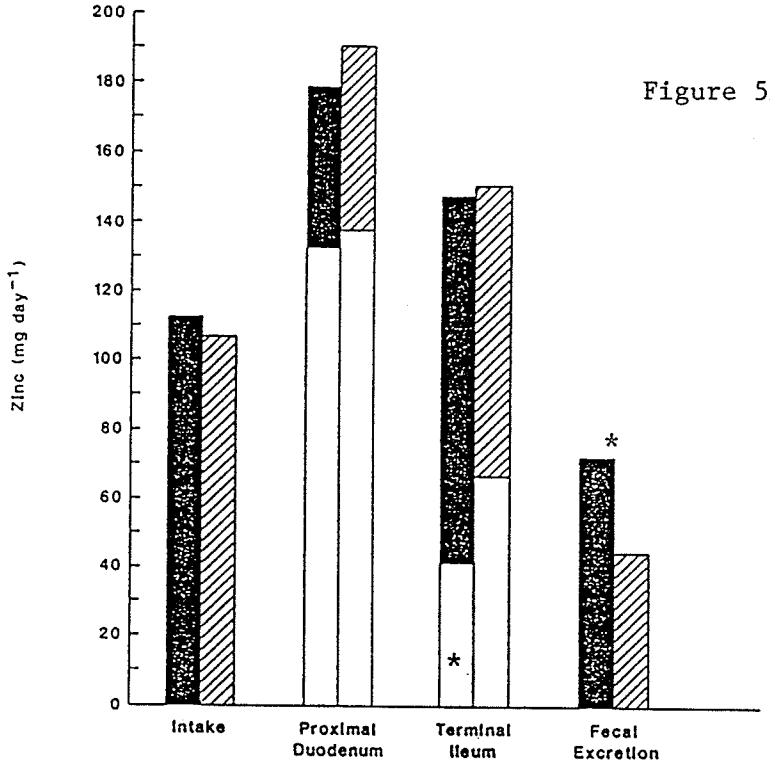
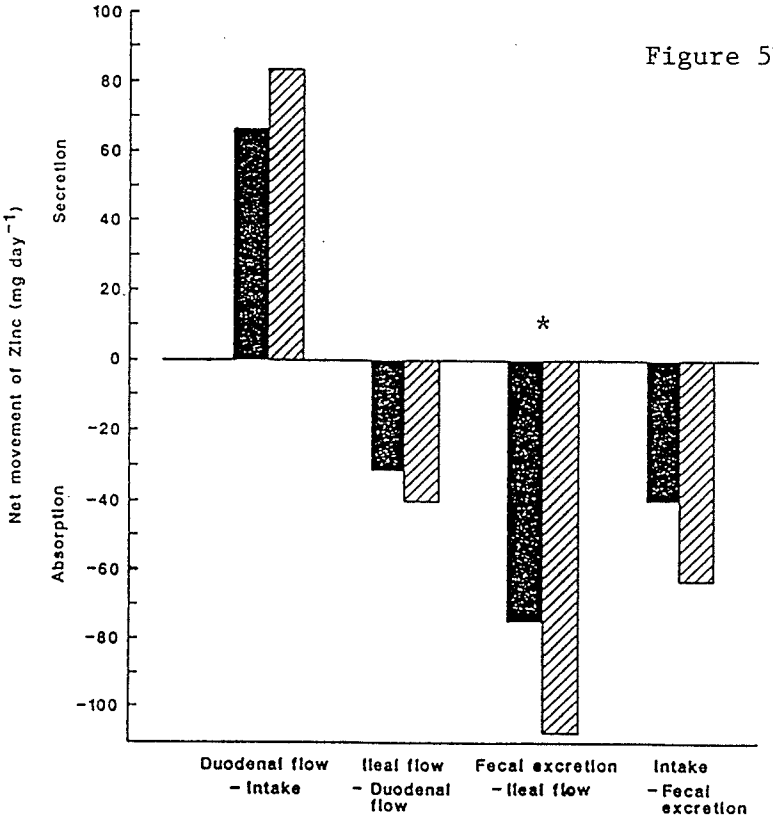



Figure 5B



Little is known about Zn absorption in the large intestine. Soluble Zn does exist in an anionic complex of high molecular weight and a high percentage of Zn is bound to macro-molecular material in the lower jejunum and terminal ileum (Bremner, 1970). Dietary Mo may bind ligand(s) and reduce the solubility of those ligands in the lower small intestine rendering those ligands unable to form a soluble Zn-ligand complex at the near neutral pH in the large intestine.

The concentration of Zn in the diet was was 24.8 mg $kg^{-1}$ DM (Table 2). While requirements for Zn are not precisely defined, a suggested value of 30 mg $kg^{-1}$ DM, with a range of 20 to 40 mg $kg^{-1}$ DM is given (NAS-NRC, 1984). The apparent digestibility of Zn tended to be lower with HM diets (Table 13). A requirement for Zn may be altered by other dietary minerals through an effect upon absorption and utilization (NAS-NRC, 1984).

## 5.6 Iron

There was a large net secretion of Fe before the proximal duodenum. Duodenal flow of Fe was 153% of that ingested (Table 15). A net increase in duodenal Fe flow relative to that ingested has been reported with sheep (Ivan et al., 1979) and cattle (Bertoni et al. 1976a). Water consumption by cattle was estimated to contribute approximately 8 to 9 mg Fe $day^{-1}$ (Appendix Table C). Salivary secretions may also contribute to the high flows of Fe at the proximal duodenum (Grace et al., 1982). Secretion of Fe across the rumen epithelium and gastric

secretions of Fe may also account for an increased flow at the proximal duodenum.

The concentration of Fe in the duodenal digesta supernatant DM was approximately four to six times higher than that in the rumen fluid supernatant DM (Table 11). Low concentrations of Fe are found in the supernatant fraction of rumen fluid both due to the formation of insoluble complexes or precipitates and to the accumulation of Fe in microbial fractions (Bremner, 1970; Durand and Kawashima, 1980). Ivan et al. (1983a) reported a high concentration of soluble Fe in the duodenal digesta compared to rumen fluid supernatant of sheep fed corn or alfalfa silage diets. Ivan et al. (1979, 1983a) reported that the proportion of soluble Fe at the proximal duodenum averaged about 25%. The average solubility of Fe at the proximal duodenum in this present experiment was 48.1% (Table 14).

The concentration (Table 11) and proportion of soluble Fe (Table 14) was higher in duodenal digesta than the rumen fluid after passage through the abomasum. This is due to the action of gastric juice which splits the many Fe complexes formed in the rumen and allows formation of Fe salts, such as $FeCl_2$. These salts are readily ionized and absorbed in the small intestine (Underwood, 1977; Jacobs and Worwood, 1981; Georgievskii, 1982). Ivan and Veira (1981) reported a low solubility for Fe in the rumen digesta of 4.3%, but a substantially higher solubility of Fe in the abomasal digesta of 20.1%. Non-heme Fe, which is mainly found in cereals and plants is well utilized by ruminants (Georgievskii, 1982; Hansard, 1983) and is released as soluble hydrated

$Fe^{+2}$ or $Fe^{+3}$ by the gastric juice (Jacobs and Worwood, 1981) for absorption in the intestinal tract.

Absorption of Fe distal to the proximal duodenum (Table 23) varied considerably from 121 to 246 mg $day^{-1}$. Much of the net absorption of Fe from the digestive tract occurred within the small intestine (Table 15), except for the HMHS treatment. A large variation in Fe absorption occurred in the small intestine with absorption across all treatments in the range of 50 to 246 mg $day^{-1}$. Ileal flow of Fe was 77 to 95% of duodenal flow (Table 15), indicating that about 5 to 13% of the Fe that entered the small intestine was absorbed in the small intestine. Iron is absorbed from all sections of the small intestine, with a principal site of absorption being the duodenum (Van Campen and Mitchell, 1965; Georgievskii, 1982; NAS-NRC, 1984).

There was a variable net absorption or secretion of Fe in the large intestine (Table 15). Absorption of Fe does occur from the large intestine of cattle (Bertoni et al., 1976a) and sheep (Ivan et al., 1983a).

More Fe was excreted in the feces than was consumed, resulting in the overall negative apparent digestibility (Table 15). Fecal Fe excretion ranged from 509 to 777 mg $day^{-1}$ (Table 14). Much of dietary Fe is excreted in the feces with only small losses occurring via the urine (Ammerman et al., 1967; Grace, 1983; Hansard, 1983). Fecal Fe includes that Fe unabsorbed from feeds, endogenous losses of Fe due to desquamated intestinal mucosal cells, and excretion of Fe in bile (Georgievskii, 1982; Hansard, 1983).

Intestinal absorption of Fe ranges between 2 and 20%; however, 4% is usually the average (Georgievskii, 1982; Hansard, 1983; Ashmead and Christy, 1985). The amount of Fe absorbed is controlled and related to the animal's absolute requirement and Fe status of the body. Animals of low Fe status or receiving diets deficient in Fe will absorb and retain more Fe (Forth, 1974; Grace, 1983; Hansard, 1983; NAS-NRC, 1984).

A requirement for Fe by cattle is not well established. Calves have a higher Fe requirement than older cattle. According to ARC (1980) and NAS-NRC (1984), the requirement for cattle weighing less than 150 kg is at 100 mg $kg^{-1}$ DM, while that for older cattle weighing greater than 150 kg is 30 to 50 mg $kg^{-1}$ DM. The steers in this experiment were not Fe deficient based on an Fe content of 120 mg $kg^{-1}$ DM in the diet (Table 2).

With the HM diets, there was a higher apparent absorption of Fe in the large intestine (Table 15), which resulted in a lower fecal Fe excretion (Table 14) that influenced the overall apparent digestibility of Fe (Table 15). Since there was a tendency for a lower flow of Fe at the terminal ileum with HM diets, there was a higher efficiency of Fe absorption in the large intestine. The enhanced Fe absorption in the large intestine may be due to a modification by Mo of the Fe transport process in the large intestine.

Since the level of storage Fe is related to Fe absorption (Jacobs and Worwood, 1981; Lynch, 1984), Mo may have modified Fe storage in the spleen or liver, thereby stimulating Fe uptake from the lumen of the large intestine. Williams et al. (1983, citing Lee et al., 1963) have

shown that Fe accumulates in the intestinal mucosa and submucosa of Cu deficient swine. Since Mo was present in high levels along with low Cu levels with the HM diets (Table 2), it is possible that a potential Cu deficiency led to increased Fe uptake from the large intestine. However, based on our present knowledge of interactions among minerals, it is not clear what is the actual mechanism involved that allows enhanced Fe absorption in the large intestine by Mo.

## 5.7 Calcium

Neither Mo nor S had an effect ($P > 0.05$) upon Ca within the digestive tract of steers. The following discussion compares the results obtained in the present experiment (Tables 16, 17 and 18) to that reported in the literature.

The flow of Ca at the proximal duodenum was 3.2 g $day^{-1}$ higher (Table 17) than the daily intake of Ca at 17.5 g $day^{-1}$ (Table 16). The flow of Ca at the proximal duodenum was 118% of intake (Table 17). Duodenal flow of Ca relative to intake of Ca by sheep (Pfeffer et al., 1970; Ben-Ghedalia et al., 1975; Grace et al., 1977; Stevenson and Unsworth, 1978; Wylie et al., 1985; Rahnema and Fontenot, 1986) and cattle (Perry et al., 1967; Kemp et al., 1973; Bertoni et al. , 1976b; Goetsch and Owens, 1985) has ranged from 110 to 140%.

Goetsch and Owens (1985) suggested that more Ca was recycled to the rumeno-reticulum, omasum, or abomasum with a high grain diet in order to aid the activation of amylase for starch breakdown. Recycling of Ca in

saliva could contribute 1 to 2 g Ca daily. Water consumed by the steers contributed an additional 0.6 g $day^{-1}$ (Appendix Table C).

Grace et al. (1974), Liebholz (1974), Greene et al. (1983), Ivan et al. (1983b) and Grings and Males (1987) have found a net absorption of Ca between mouth and duodenum. However, endogenous secretions of Ca may exceed the net absorption before the proximal duodenum (Ben-Ghedalia et al., 1975). Furthermore, diet composition, physical form of the ration, source of Ca and availability of that source may influence the result (Bertoni et al., 1976b).

A large proportion of Ca entering the proximal duodenum was absorbed in the small intestine (Table 17). The mean ileal flow of Ca was approximately 53% of duodenal flow of Ca. The small intestine is the principal site of Ca absorption in both cattle and sheep (Perry et al., 1967; Kemp et al., 1973; Ben-Bhedalia et al., 1975; Bertoni et al., 1976b; Grace et al., 1977; Greene et al., 1983; Skylan and Hurwitz, 1985; Wylie et al., 1985; Goetsch and Owens, 1985).

Absorption of Ca within the small intestine occurs primarily in the duodenum and jejunum. Absorption from the duodenum is via active transport and facilitated by Vitamin D; absorption of Ca from the jejunum is by passive diffusion (Ammerman and Goodrich, 1983). The solubility of Ca compounds and hence the absorption of Ca is favored by acid conditions and hindered by alkaline conditions in the lower small intestine (Georgievskii, 1982).

A mean of 86.3% soluble Ca at the proximal duodenum (Table 16) lies in the range of 50 to 92.2% reported by Ben-Ghedalia et al. (1975),

Grace et al. (1977), Yano et al. (1979) and Ivan et al. (1983b). The mean concentration of Ca in the duodenal supernatant at 25.6 mg $g^{-1}$ soluble DM was about four-fold higher than that found in the rumen fluid supernatant (Table 18). Grace et al. (1977), Yano et al. (1979), Ivan et al. (1983b) and Goetsch and Owens (1985) have also found a corresponding lower concentration of Ca in rumen fluid relative to the soluble fraction in the duodenum.

The solubility of Ca in the rumen is pH dependent with a significant negative correlation between pH and soluble Ca content. The pH affects both the formation of insoluble salts as well as the binding of Ca to microorganisms or feedstuffs (Grace et al., 1977; Durand and Kawashima, 1980). Grace et al. (1977) observed that 40% of the Ca was associated with the alkali insoluble fraction in the rumen contents. The insoluble fraction was largely fiber constituents.

The observed difference for solubility of Ca at the duodenum, relative to the rumen, is due to the effect of the low pH in the abomasum upon Ca. The abomasum secretes the strong acid HCl which is responsible for the conversion of calcium compounds to $CaCl_2$. This $CaCl_2$ completely dissociates into ions which results in the release of Ca in an ultra-filterable form (Storry, 1961; Yano et al., 1979; Georgievskii, 1982).

The solubility of Ca in the digesta of the small intestine declines as the digesta proceeds distally from the proximal duodenum (Ben-Ghedalia et al., 1975; Yano et al., 1979). The percent soluble Ca at the terminal ileum of steers (Table 16) was about 26%, and represented a

3.3-fold decrease relative to the solubility of Ca in the proximal duodenum. The solubility of Ca at the terminal ileum (Table 16) was in the range of 3.2 to 49.9% reported by Storry (1961), Ben-Ghedalia et al. (1975), Grace et al. (1977), Yano et al. (1979), Ivan et al (1983b), and Rahnema and Fontenot (1986) for sheep and cattle. The higher pH of the lower small intestine, like that of the rumen, favors the formation of insoluble phosphates, oxalates, sulfates and carbonates of Ca (Georgievskii, 1982).

Either a small net absorption or a small net secretion of Ca occurred within the large intestine (Table 17), which balances to no change across four experimental diets. Under various experimental conditions, both net absorption (Pfeffer et al., 1970; Grace et al., 1974; Ben-Ghedalia et al., 1975; Bertoni et al., 1976b; Grace et al., 1977; Skylan and Hurwitz, 1985) and net secretion (Ivan et al., 1983b; Wylie et al., 1985) have been demonstrated for the large intestine. A net absorption occurs despite a lower solubility for Ca in this section of the digestive tract (Yano et al., 1979). Grace et al. (1977) indicated that Ca can also be absorbed from regions of the digestive tract where the concentration of water soluble or ionic Ca in the digesta is low.

The apparent digestibility of Ca was 38.2% (Table 17), with less Ca excreted in the feces than was consumed. Apparent digestibilities of Ca that have been reported show tremendous variation. Negative apparent Ca digestibilities have ranged from -10 to -32% (Pfeffer et al., 1970; Ivan et al., 1983b: Rahnema and Fontenot, 1986) while positive apparent Ca

digestibilities have ranged from as low as +5 and +8% (Pfeffer et al., 1970; Ivan et al., 1983b) to +45% (Grace et al., 1974; Ben-Ghedalia et al., 1975; Bertoni et al., 1976b; Grace et al., 1977; Greene et al., 1983; Wylie et al., 1985). Values as high as +78 to +87% have also been reported (Goetsch and Owens, 1985).

Calcium is mainly excreted in feces, with urine being a minor excretory route (NAS-NRC, 1984). Fecal endogenous loss of Ca is independent of the amount of Ca in the diet, or amount that is absorbed, and will be directly proportional to the live weight of an animal. The absorption of Ca is regulated at the gut level and is dependent upon the needs of the body (ARC, 1980).

## 5.8 Magnesium

A substantial amount of the Mg ingested was absorbed before the proximal duodenum (Table 20). The flow of Mg at the proximal duodenum was 54 to 69% of intake. This large net absorption of 30 to 45% of dietary Mg before the proximal duodenum has been reported for sheep (Pfeffer et al., 1970; Grace et al., 1974; Ben-Ghedalia et al., 1975; Axford et al., 1975; Tomas and Potter, 1976; Field and Munro, 1977; Stevenson and Unsworth, 1978; Ivan et al., 1983b; Wylie et al., 1985; Rahnema and Fontenot, 1986) and cattle (Bertoni et al., 1976b; Greene et al., 1983; Banks and Smith, 1984). These reports indicate that 10 to 50% of the Mg ingested is absorbed before the small intestine with a 30% overall average. Passage of Mg to the duodenum is strongly correlated with intake (Axford et al., 1975; Field and Munro, 1977).

The reticulo-rumen of sheep is the main site of absorption for Mg (Tomas and Potter, 1976; Field and Munro, 1977) with absorption being an active, sodium-linked transport (Marten, 1983, citing Martens, 1978). In steers, however, Horn and Smith (1978) found only negligible amounts of Mg were absorbed from the reticulo-rumen and concluded that the main site of pre-intestinal Mg absorption was the omasum. This was confirmed by Edrise and Smith (1979). However, Banks and Smith (1984) noted that at low Mg intakes, there was a net addition of Mg in the reticulo-rumen followed by a net absorption in the abomasum. Only at higher Mg intakes, was there an appreciable absorption of Mg from the reticulo-rumen, in agreement with Martens (1983).

Little absorption of Mg occurred in the small intestine (Table 20). This occurred despite a high solubility of 90% for Mg at the proximal duodenum (Table 19). Magnesium in part, is converted into its ionized form ($Mg^{+2}$) by the gastric juice of the abomasum (Georgievskii, 1982). In an ionized form, Mg is absorbed in the upper small intestine and in the initial segment of the large intestine (Ben-Ghedalia et al., 1975; Georgievskii, 1982).

Pfeffer et al. (1970) indicated that absorption of Mg occurs throughout the small intestine. In turn, endogenous secretions of Mg also occur throughout the intestinal tract. Biliary and pancreatic secretions contain a significant amount of Mg (Pfeffer et al., 1970). Thus, endogenous secretion of Mg may exceed absorption in the small intestine.

The solubility of Mg at the terminal ileum was 59%, and about two-thirds that in the proximal duodenum (Table 19). The net absorption of Mg in the large intestine was marginal (Table 20). Bertoni et al. (1976b), Greene et al. (1983) and Grace et al. (1974) observed a net secretion of Mg within the small intestine and a net absorption in the large intestine. By contrast, Wylie et al. (1985) and Pfeffer et al. (1970) observed a net absorption in the small intestine with a net secretion into the large intestine. Thus, there seems to be an effective recycling of Mg within the intestinal tract with variable results occurring due to perhaps, differences between ration type, age and, or physiological demand of animals for Mg or availability of Mg in feedstuffs.

The apparent digestibility of Mg (Table 20) was similar to that reported by Grace and Macrae (1972), Bertoni et al. (1976b), Stevenson and Unsworth (1978) and Ivan et al. (1983b).

When both Mo and S were at high levels in the diet (HMHS vs HM + HS), there was a lower concentration of Mg in the rumen fluid supernatant (Table 18). Either there was an enhanced absorption of Mg from the soluble fraction or there was a greater association of Mg with the insoluble digesta fraction.

It is possible that under conditions when both Mo and S were in excess, there was a modified binding of Mg to the particulate fraction which encompassed microorganisms and feed particles. This lower concentration of Mg in the soluble DM (Table 18) may contribute to the

tendency of a lower absorption of Mg before the proximal duodenum (Table 20).

With HS diets, there was a higher concentration of Mg in the supernatant DM of duodenal digesta (Table 18) which contributed to a higher net absorption of Mg distal to the proximal duodenum (Table 23). By contrast, with HM diets, there was a lower concentration of Mg in the supernatant. DM of duodenal digesta (Table 18), together with a tendency toward a lower flow of Mg at the proximal duodenum (Table 19). However, there was no effect upon absorption distal to the proximal duodenum (Table 23).

## 5.9 Phosphorus

The high flow of P at the proximal duodenum was about twice that in the diet fed to steers (Table 21). A flow of P entering the small intestine in the order of 1.1 to 5.0 times that consumed has been reported in the literature for sheep and cattle (Pfeffer et al., 1970; Grace et al., 1974; Leibholz, 1974; Bertoni et al., 1976b; Grace et al., 1977; Stevenson and Unsworth, 1978; Ivan et al., 1983b: Greene et al., 1983; Banks and Smith, 1984; Wylie et al., 1985; Grings and Males, 1987).

A high P flow at the proximal duodenum is a reflection of both the amount of salivary flow and the quantity of P present in the saliva (McDougall, 1948; Kay, 1960; Yarns et al., 1965). The contribution of P in water consumed by cattle was negligible (Appendix Table C).

The daily production of saliva by cattle may range from 40 to 190 L $day^{-1}$ (Hastings, 1944; Bailey and Balch, 1961; Yarns et al.,1965) with some estimates as low as 22 L $day^{-1}$ (Edrise et al., 1986). It may be calculated by scaling from the results of Yarns et al. (1965) and Edrise et al. (1986) on saliva production rates in steers, that for the present steers and diets, average saliva flow was 35 to 45 L $day^{-1}$. At this flow of saliva, and with an estimate of P flow by Banks and Smith (1984), the contribution of P via the saliva at the proximal duodenum would have been 25 to 30 g $day^{-1}$ in addition to a dietary input of 13.8 g $day^{-1}$ (Table 21).

Salivary flow is subject to wide variation and can be reduced with pelleted diets (such as that used in this experiment) and with concentrate diets (Yarns et al., 1965; Durand and Kawashima, 1980). There is an inverse relationship between the concentration of P in saliva and the volume of salivary flow. Thus, the quantity of P entering the rumen does not change markedly when salivary flow is reduced (Durand and Kawashima, 1980).

There is little net exchange of P in the abomasum (Banks and Smith, 1984). However, there is an appreciable net absorption of P in the omasum (Edrise and Smith, 1979; Banks and Smith, 1984). Banks and Smith (1984) suggested that as much as 30% of the P which flows into the rumeno-reticulum is absorbed in the omasum.

Flow of P at the proximal duodenum was 205% of intake with the HM diets compared to 231% with the LM diets (Table 22). This was a reflection of a trend for a reduced flow of 14.7 g P $day^{-1}$ at the

proximal duodenum with the HM diets compared to 17.8 g P $day^{-1}$ with the LM diets (Table 21). There was either an enhanced absorption, or a reduced saliva secretion or endogenous input of P before the proximal duodenum with the HM diets.

About 40 to 61% of the water which leaves the reticulum may be reabsorbed in the omasum (Edrise et al., 1986). Large quantities of P that enter the rumeno-reticulum may be reabsorbed in the omasum (Banks and Smith 1984). Added Mo (as sodium molybdate) may influence the electrolyte concentration within the omasal fluid and perhaps modified absorption of both water and phosphate at that site.

The rumeno-reticulum is not a major region for P absorption (Georgievskii, 1982). Since the concentration of soluble P in the rumen fluid supernatant (Table 18) was not influenced by Mo addition, it is doubtful that a Mo x P interaction occurred within the rumeno-reticulum. Therefore, Mo would not have influenced the solubility or availability of phosphate and absorption of P in the rumeno-reticulum.

The absorption of P distal to the proximal duodenum (Table 23) tended to be lower with the HM diets. The net absorption of P distal to the proximal duodenum (Table 23) was 21.6 g $day^{-1}$ and 22.9 g $day^{-1}$ with the HM and LM diets, respectively, a difference of 1.3 g $day^{-1}$. Phosphorus absorption from the small intestine (Table 22) was 21.7 g $day^{-1}$ and 23.0 g $day^{-1}$ for the HM and LM diets respectively, a difference of 1.3 g $day^{-1}$.

The HM diets influenced neither the soluble P at the proximal duodenum (Table 21) nor the concentration of P in the duodenal digesta

supernatant (Table 18). The flow of soluble P at the proximal duodenum for the HM and LM diets was 19.3 and 17.4 g $day^{-1}$ (Table 21). Although not significant, the difference between these values, 1.9 g $day^{-1}$, is similar to 1.3 g $day^{-1}$, that was calculated for the difference between HM and LM diets for absorbed P in the small intestine. This suggests that the level of soluble P or flow of soluble P at the proximal duodenum may limit P absorption in this region.

Interactions between Mo and P (Shirley et al., 1950) and between Mo and S, relative to P (Goodrich and Tillman, 1966b) have been described. Goodrich and Tillman (1966b) reported that when the dietary level of Mo was increased from 2 to 8 mg $kg^{-1}$ with 1 g S $kg^{-1}$, plasma P levels were lower in sheep, but had no effect when the diet contained 4 g S $kg^{-1}$. They also observed that with an increase of S level from 1 to 4 g $kg^{-1}$ in diets having 2 mg $kg^{-1}$ Mo plasma P levels were lower, with no effect when the diet contained 8 mg Mo $kg^{-1}$.

There is a high correlation between the concentration of P in saliva and P in rumen contents with that in serum (Tomas et al., 1967). Young et al. (1966) concluded that the rate of secretion of phosphate via saliva might be regulated by the concentration of phosphate in serum. High plasma levels are accompanied by a high concentration of phosphate in saliva (Care et al., 1980). An intravenous infusion of P in sheep increased the phosphate content of saliva (Scott & Beastall, 1978).

It was concluded that Mo reduced absorption of P in the small intestine (Table 22). This then resulted in a level of P in plasma

which in turn modified the secretion of P in saliva which was in evidence as a tendency for reduced flow of P at the proximal duodenum (Table 21).

There was an enhanced net absorption of P distal to the proximal duodenum with the HS diets (Table 23). This higher absorption of P was supported by a higher net absorption in both the small and large intestine (Table 22).

The effect of S upon an enhanced P absorption from the small intestine (Table 22) cannot be resolved in a direct manner. Goodrich and Tillman (1966b) observed an effect of the Cu x Mo x S interaction upon plasma P. Sheep fed 4 g S $kg^{-1}$ diet had lower plasma P, except when the diet had both 40 mg Cu $kg^{-1}$ diet and 8 mg Mo $kg^{-1}$ diet. Dietary supplementation of both Cu and Mo were required to return plasma P to normal.

High dietary S lowers the retention of Cu (Pitt, 1976). Adequate dietary Cu is required for proper P metabolism (Goodrich & Tillman, 1966b). Goodrich & Tillman (1966b) found that Mo acts to alleviate the adverse effect of a high level of sulfate; additional Cu is required because the level of Mo did not completely counteract the effect of sulfate. Since dietary Cu in this experiment was low, (Table 2), the tendency for a lower absorption of P from the small intestine, ileal flow relative to duodenal flow, was noted when both Mo and S were in the diet (Table 22). This effect of added S, when Mo is also present, may be explained by the formation of thiomolybdates, thereby reducing the

amount of available Mo that may interact with P to reduce the absorption of P in the small intestine.

Shirley et al. (1950) reported losses of P in feces in steers were two to three times normal when the dietary content of Mo was high, while losses of P in urine were reduced. The excretion of P in the feces (Table 21) was not influenced by added Mo, and averaged 7.80 g $day^{-1}$. It is not possible to separate endogenous or exogenous P in the feces. Either component could have been increased in the feces. Furthermore, there may have been increased urinary P excretion.

The small intestine is the principal site for a net absorption of P in the digestive tract. This has been reported for cattle (Bertoni et al., 1976b; Greene et al., 1983) and sheep (Leibholz, 1974; Grace et al., 1974, 1977; Ivan et al., 1983b; Wylie et al., 1985). Approximately 70-77% of the P entering the proximal duodenum was absorbed in the small intestine (Table 22). About 130 to 150% as much P was absorbed in the small intestine as was secreted before the proximal duodenum. The net secretion of P before the pylorus is closely matched by net absorption in the small intestine (Bertoni et al., 1976b; Pfeffer et al., 1970).

The absorption of P, like that of Ca, is dependent upon the solubility of P at the site of absorption and is influenced by the low pH of the upper small intestine (Maynard et al., 1979; Field, 1981). Gastric juice within the abomasum solubilizes the insoluble P leaving the rumeno-reticulum followed by absorption of that P in the small intestine (Georgievskii, 1982).

The solubility of P at the proximal duodenum and at the terminal ileum was 60.8% and 21.1%, respectively (Table 21). The solubilities of P reported by Ben-Ghedalia et al. (1975), Grace et al. (1977), Yano et al. (1979) and Ivan et al. (1983b) were 62 to 83% at the proximal duodenum, and 16 to 52% at the terminal ileum. The rise in pH as the digesta moves along the intestinal tract is accompanied by a concomitant decrease in P solubility (Ben-Ghedalia et al., 1975). Bile and pancreatic juices, as well as other intestinal secretions act as major factors in reducing the solubility of P in the digesta flowing through the lower small intestine (Ben-Ghedalia et al., 1975).

Either a small net absorption or a small net secretion of P occurred in the large intestine (Table 22). A small net P absorption has been reported (Pfeffer et al., 1970; Bertoni et al., 1976b; Greene et al., 1983; Ivan et al., 1983b; Wylie et al., 1985). Absorption of P, rather than secretion of P is thought to be more intensive in the large intestine (Georgievskii, 1982).

# 6. SUMMARY

Four steers, cannulated in the rumen, proximal duodenum, and terminal ileum, were fed barley grain-hay (67%-27%) pelleted diets in a 4 x 4 Latin Square Design. The diets contained: (1) no added Mo or S, (2) added Mo at 10 mg $kg^{-1}$, (3) added S at 3.0 g $kg^{-1}$, and (4) both Mo and S at the above-stated levels. The effect of Mo and S upon the flows and solubilities of various minerals along the digestive tract of steers were examined.

Dry Matter

The apparent digestibility of DM was higher ($P < 0.01$) with HM diets. This was a reflection of a higher ($P = 0.051$) DM digestibility before the proximal duodenum.

Copper

Although flows of total Cu at the proximal duodenum and terminal ileum were not different ($P > 0.05$), the solubility and soluble flows of Cu at the proximal duodenum and terminal ileum were lower ($P < 0.05$) with HM and HS diets. There was a synergistic effect ($P < 0.05$) of HM plus HS on the solubility of Cu at the terminal ileum. Fecal excretion tended to be higher ($P = 0.07$) with HM diets.

Molybdenum

Although absolute flows of Mo differed ($P < 0.05$) between HM and LM diets, duodenal flow as a percent of intake and ileal flow as a percent of duodenal flow did not differ ($P > 0.05$). The apparent digestibility of Mo was not different ($P > 0.05$) in response to supplemental Mo.

The absorption of Mo was lower ($P < 0.05$) while fecal excretion of Mo was higher ($P < 0.05$) with HS diets.

Manganese

The secretion of Mn before the proximal duodenum was lower ($P < 0.05$) and the absorption of Mn distal to the proximal duodenum was lower ($P < 0.01$) with the HM diets. The solubility of Mn at the proximal duodenum and terminal ileum and the net absorption of Mn from the digestive tract did not differ ($P > 0.05$) in response to supplemental Mo.

With HS diets, the concentration of Mn in rumen fluid supernatant DM was lower ($P < 0.05$) while the solubility of Mn at the proximal duodenum and terminal ileum, and net absorption along the digestive tract did not differ ($P > 0.05$).

Zinc

A higher ($p < 0.05$) fecal excretion of Zn with the HM diet was due to a lower ($P < 0.01$) absorption of Zn distal to the proximal duodenum, a lower ($P < 0.05$) solubility and flow of soluble Zn at the terminal

ileum which contributed to a lower ($P < 0.05$) absorption of Zn in the large intestine.

The solubility of Zn and flow of that soluble Zn at the proximal duodenum were lower ($P < 0.01$) with the HS diets; however, the HS diets did not influence ($P > 0.05$) absorption of Zn distal to the proximal duodenum.

Iron

A lower ($P < 0.01$) excretion of Fe in feces was due to a higher ($P < 0.05$) absorption of Fe in the large intestine with the HM diets.

Calcium

The flow and solubility of Ca in the digestive tract was not influenced ($P > 0.05$) by HM or HS diets.

Magnesium

The concentration of Mg in the rumen fluid supernatant DM was lower ($P < 0.05$) when a high concentration of Mo and S were in the diet. The concentration of Mg in supernatant DM at the proximal duodenum was lower ($P < 0.05$) with HM diets and higher ($P < 0.05$) with HS diets. Other parameters relating to flow, and the absorption or secretion of Mg were not affected by HM or HS diets.

Phosphorus

A lower ($P < 0.05$) net secretion of P before the proximal duodenum was followed by a lower ($P = 0.055$) net absorption of P distal to the proximal duodenum with HM diets.

With HS diets, the absorption of P distal to the proximal duodenum was higher ($P < 0.05$) in both the small and large intestine.

The soluble P at the proximal duodenum and at the terminal ileum as well as the net absorption of P from the total length of the digestive tract was not different with the HM or HS diets.

# 7.0 CONCLUSIONS

The effects of Mo + S upon digestibility of DM and flows and solubility of minerals was evaluated. Only with Ca was there no effect of Mo or S.

With Mo, there was a higher digestibility of DM, a beneficial effect of Mo upon digestion by microorganisms in the rumen.

High dietary Mo and S had separate and synergistic effects upon the solubility of Cu within the digestive tract, and the HM diets resulted in an increased fecal excretion of Cu. High dietary Mo had no effect upon the fraction of Mo absorbed, while high dietary S reduced the net absorption of Mo from the small intestine, with a concomitant high excretion of Mo in feces. Evidence that the Cu-Mo-S interrelationship was functioning within the digestive tract has been presented. A formation of thiomolybdates explains the observed results.

Effects of Mo

(1) There was a lower net secretion of Mn before the proximal duodenum followed by a lower net absorption after the proximal duodenum but with no effect upon Mn balance overall.

(2) With Zn, there was a lower absorption from the large intestine, followed by a high excretion in feces, while with Fe, there was a higher absorption from the large intestine followed by a lower excretion of Fe in feces.

(3) There was a lower concentration of Mg in the supernatant dry matter at the proximal duodenum, but without other effects upon Mg.
(4) A lower net secretion of P before the proximal duodenum followed by a lower net absorption of P after the proximal duodenum, but no change in overall P balance within the animal.

Effects of S

With high dietary S, there was a lower concentration of Mn in the supernatant dry matter of rumen contents, a lower solubility of Zn at the proximal duodenum, and a higher concentration of Mg in the supernatant dry matter at the proximal duodenum.

There was a higher absorption of P distal to the proximal duodenum, with the effect of S occurring in both the small and large intestine. Although these effects of S were noted, there were no effects of S upon the balance, for Mn, Zn, Mg and P, as indicated by no effects upon fecal excretion or apparent digestibility.

Effects of Mo + S

Only with Mg and Cu, as noted above, was there an effect of Mo + S in the diet. For Mg, there was a lower concentration of Mg in the supernatant dry matter of rumen contents. There were indications of a Mo x S effect upon Mg and P along the digestive tract of steers. However, these effects were not identified as significant effects in this experiment.

The implications of this experiment are that excessive dietary levels of Mo and S modify the balance of minerals in addition to Cu within the digestive tract. A detrimental effect of Mo and S upon Cu was verified. When fecal excretion and apparent digestibility of a mineral was evaluated, high Mo had an adverse effect upon Zn and a beneficial effect upon Fe. There were times when an effect of Mo or S was noted at sites along the digestive tract. However, the overall balance of minerals such as Mg, P and Mn, fecal excretion relative to that consumed was not different.

This experiment was of a short-term nature. Over a long period of time, effects upon the overall balance of Mg, P and Mn may occur. Only with Ca was an effect of Mo, or S not obtained.

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# 9. APPENDICES

# 9. APPENDICES

## Explanation of abbreviations for Appendix Tables A1-A21:

| | |
|---|---|
| Du: | Duodenal |
| Il: | Ileal |
| Fec excr: | Fecal excretion |
| App dig: | Apparent digestibility |
| Tot Du Flow: | Total duodenal flow |
| % Sol Du: | Percent soluble in duodenal digesta |
| Sol Du Flow: | Soluble mineral flow in duodenal digesta |
| Tot Il Flow: | Total ileal flow |
| % Sol Il: | Percent soluble in ileal digesta |
| Sol Il Flow: | Soluble mineral flow in ileal digesta |
| Tot Fec Excr: | Total fecal excretion |
| RFSDM: | Rumen fluid soluble dry matter |
| DUSDM: | Duodenal soluble dry matter |
| ILSDM: | Ileal soluble dry matter |

Appendix Table A1: Analysis of variance for various DM flow parameters of the proximal duodenum and terminal ileum

| Source | Df | Mean squares | | | | | |
|---|---|---|---|---|---|---|---|
| | | Du Flow | Du Flow % Intake | Du Flow - Intake | Il Flow | Il Flow % Du Flow | Il Flow - Du Flow |
| Animal | 3 | 130762.3 | 63.9 | 118497.9 | 59395.2 | 27.1 | 43870.1 |
| Period | 3 | 59238.5 | 31.1 | 61401.6 | 5946.0 | 30.5 | 57883.8 |
| Treatment | 3 | 210449.4 | 112.1 | 223389.5 | 50096.0 | 26.7 | 89008.1 |
| Orthogonal treatment contrasts | | | | | | | |
| HM vs LM | 1 | 607347.5 | 325.4 | 651733.3 | 90932.4 | 12.6 | 228269.0 |
| HS vs LS | 1 | 446.27 | 0.033 | 7.02 | 45945.9 | 64.8 | 37336.0 |
| HMHS vs HM + HS | 1 | 23554.6 | 10.8 | 18428.1 | 13409.6 | 2.61 | 1419.4 |
| Error | 6 | 103731.0 | 55.2 | 109545.6 | 22022.3 | 28.8 | 72147.8 |

Appendix Table A2: Analysis of variance for various flow parameters relating to fecal DM excretion

| Source | Df | Mean squares | | | | |
|---|---|---|---|---|---|---|
| | | Fec Excr | Fec Excr % Il Flow | Fec Excr - Il Flow | Intake - Fec Excr | App Dig |
| Animal | 3 | 24624.5 | 13.3 | 8405.8 | 22703.0 | 12.43 |
| Period | 3 | 10558.1 | 135.0 | 28737.9 | 11802.4 | 5.59 |
| Treatment | 3 | 39372.6* | 49.1 | 10244.9 | 43771.9* | 20.83* |
| Orthogonal treatment contrasts | | | | | | |
| HM vs LM | 1 | 101314.0** | 40.1 | 280.6 | 119906.4** | 54.5** |
| HS vs LS | 1 | 1600.0 | 101.9 | 30397.9 | 263.3 | 0.62 |
| HMHS vs HM + HS | 1 | 15202.9 | 5.37 | 56.3 | 11146.1 | 7.39 |
| Error | 6 | 6132.8 | 31.4 | 9172.8 | 7336.3 | 3.30 |

*P<0.05

**P<0.01

Appendix Table A3: Analysis of variance for various Cu parameters: total, percent soluble and soluble flows of Cu at the proximal duodenum and terminal ileum and total fecal excretion

| | | Mean squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source | Df | Tot Du Flow | % Sol Du | Sol Du Flow | Tot Il Flow | % Sol Il | Sol Il Flow | Tot Fec Excr |
| Animal | 3 | 4.33 | 7.01 | 0.19 | 2.34 | 62.2 | 5.80 | 11.7 |
| Period | 3 | 7.81 | 4.61 | 0.20 | 7.82 | 330.6** | 21.0* | 22.0 |
| Treatment | 3 | 1.83 | 96.4** | 5.37* | 2.86 | 450.6** | 24.9* | 10.0 |
| Orthogonal treatment contrasts | | | | | | | | |
| HM vs LM | 1 | 1.89 | 164.0** | 10.4** | 0.031 | 627.8** | 25.7* | 28.1 |
| HS vs LS | 1 | 1.27 | 110.4** | 5.38* | 0.85 | 557.9** | 30.2* | 1.82 |
| HMHS vs HM + HS | 1 | 2.33 | 14.8 | 0.35 | 7.70 | 166.2* | 18.7 | 0.090 |
| Error | 6 | 4.56 | 7.40 | 0.70 | 5.29 | 21.8 | 4.27 | 5.89 |

*P<0.05

**P<0.01

Appendix Table A3: Analysis of variance for various Cu parameters: total, percent soluble and soluble flows of Cu at the proximal duodenum and terminal ileum and total fecal excretion

| | | Mean squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source | Df | Tot Du Flow | % Sol Du | Sol Du Flow | Tot Il Flow | % Sol Il | Sol Il Flow | Tot Fec Excr |
| Animal | 3 | 4.33 | 7.01 | 0.19 | 2.34 | 62.2 | 5.80 | 11.7 |
| Period | 3 | 7.81 | 4.61 | 0.20 | 7.82 | 330.6** | 21.0* | 22.0 |
| Treatment | 3 | 1.83 | 96.4** | 5.37* | 2.86 | 450.6** | 24.9* | 10.0 |
| Orthogonal treatment contrasts | | | | | | | | |
| HM vs LM | 1 | 1.89 | 164.0** | 10.4** | 0.031 | 627.8** | 25.7* | 28.1 |
| HS vs LS | 1 | 1.27 | 110.4** | 5.38* | 0.85 | 557.9** | 30.2* | 1.82 |
| HMHS vs HM + HS | 1 | 2.33 | 14.8 | 0.35 | 7.70 | 166.2* | 18.7 | 0.090 |
| Error | 6 | 4.56 | 7.40 | 0.70 | 5.29 | 21.8 | 4.27 | 5.89 |

*P<0.05

**P<0.01

Appendix Table A4: Analysis of variance for various Cu parameters: Differences in flows and % of flows among various locations

| Source | Df | Mean squares | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Du Flow-Intake | Du Flow % Intake | Il Flow-Du Flow | Il Flow % Du Flow | Fec Excr-Il Flow | Fec Excr % Il Flow | Fec Excr-Du Flow | Intake-Fec Excr | App Dig |
| Animal | 3 | 1.42 | 24.2 | 6.07 | 88.7 | 7.99 | 188.5 | 4.68 | 6.8 | 451.71 |
| Period | 3 | 7.18 | 262.0 | 25.7 | 437.0 | 37.8 | 623.4 | 36.9 | 30.2* | 1291.78* |
| Treatment | 3 | 17.6 | 1903.4* | 2.72 | 43.6 | 13.6 | 278.0 | 16.0 | 13.5 | 510.5 |
| Orthogonal treatment contrasts | | | | | | | | | | |
| HM vs LM | 1 | 10.4 | 679.7 | 2.40 | 60.0 | 26.3 | 679.2 | 44.6 | 11.9 | 759.7 |
| HS vs LS | 1 | 25.8 | 3648.1* | 4.20 | 44.4 | 5.18 | 85.0 | 0.05 | 23.5 | 614.8 |
| HMHS vs HM + HS | 1 | 16.6 | 1382.6 | 1.56 | 26.7 | 9.46 | 70.0 | 3.33 | 5.06 | 156.9 |
| Error | 6 | 6.02 | 363.3 | 9.03 | 159.6 | 12.0 | 176.5 | 11.7 | 4.42 | 207.26 |

*P<0.05

Appendix Table A5: Analysis of variance for various Cu and Mo soluble DM parameters

| | | Mean squares | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cu | | | Mo | | |
| Source | Df | RFSDM | DUSDM | ILSDM | RFSDM | DUSDM | ILSDM |
| Animal | 3 | 89.9 | 7.30 | 43.5 | 9.00 | 2.96 | 39.3 |
| Period | 3 | 20.9 | 7.19 | 245.2* | 1.83 | 2.92 | 7.73 |
| Treatment | 3 | 63.6 | 15.2 | 188.6 | 240.4** | 80.7** | 876.6** |
| Orthogonal treatment contrasts | | | | | | | |
| HM vs LM | 1 | 2.58 | 34.4 | 29.4 | 720.6** | 235.5** | 2621.7** |
| HS vs LS | 1 | 182.9 | 11.0 | 519.6* | 0.49 | 6.73 | 2.43 |
| HMHS vs HM + HS | 1 | 5.35 | 0.27 | 16.7 | 0.13 | 0.018 | 5.75 |
| Error | 6 | 81.4 | 5.94 | 50.4 | 3.10 | 3.30 | 25.0 |

*P<0.05

**P<0.01

Appendix Table A6: Analysis of variance for various Mo parameters: total, percent soluble and soluble flows of Mo at the proximal duodenum and terminal, ileum and total fecal excretion

| | | Mean squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source | Df | Tot Du Flow | % Sol Du | Sol Du Flow | Tot Il Flow | % Sol Il | Sol Il Flow | Tot Fec Excr |
| Animal | 3 | 23.6 | 4.09 | 2.75 | 6.14 | 168.4 | 7.16 | 15.1 |
| Period | 3 | 22.8 | 4.38 | 2.25 | 21.9 | 3.82 | 4.50 | 33.9 |
| Treatment | 3 | 2335.5** | 115.6** | 154.8** | 1072.8** | 190.8 | 99.2** | 1185.2** |
| Orthogonal treatment contrasts | | | | | | | | |
| HM vs LM | 1 | 6934.7** | 319.8** | 461.9** | 3008.5** | 561.5* | 269.6** | 3434.0** |
| HS vs LS | 1 | 56.6 | 6.41 | 2.33 | 117.7** | 7.16 | 17.4 | 74.8* |
| HMHS vs HM + HS | 1 | 15.0 | 20.6 | 0.16 | 92.2** | 3.92 | 10.8 | 46.9 |
| Error | 6 | 16.5 | 5.08 | 1.75 | 6.53 | 48.2 | 3.21 | 10.0 |

*P<0.05

**P<0.01

Appendix Table A7: Analysis of variance for various Mo parameters: Differences in flows and % of flows among various locations

| Source | Df | Mean squares | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Du Flow-Intake | Du Flow % Intake | Il Flow-Du Flow | Il Flow % Du Flow | Fec Excr-Il Flow | Fec Excr % Il Flow | Fec Excr-Du Flow | Intake-Fec Excr | App Dig |
| Animal | 3 | 19.0 | 156.8 | 13.6 | 26.0 | 2.26 | 73.9 | 16.5 | 16.9 | 52.8 |
| Period | 3 | 35.0 | 339.1 | 50.2 | 269.5 | 3.65 | 38.6 | 59.3 | 67.9 | 280.5 |
| Treatment | 3 | 105.8* | 581.2 | 442.4** | 1160.3** | 8.82* | 382.9** | 328.5* | 570.0** | 176.1 |
| Orthogonal treatment contrasts | | | | | | | | | | |
| HM vs LM | 1 | 233.3* | 89.5 | 808.0** | 444.9 | 14.1* | 902.7** | 608.9* | 1596.0** | 9.13 |
| HS vs LS | 1 | 73.5 | 1339.8** | 337.6* | 3035.9** | 4.84 | 2.25 | 261.6 | 57.8 | 492.3 |
| HMHS vs HM + HS | 1 | 10.4 | 314.2 | 181.6 | 0.12 | 7.56 | 243.6* | 115.0 | 56.3 | 26.8 |
| Error | 6 | 19.7 | 147.5 | 34.8 | 95.0 | 1.27 | 19.4 | 44.7 | 16.0 | 90.7 |

*P<0.05

**P<0.01

Appendix Table A8: Analysis of variance for various Mn parameters: total, percent soluble and soluble flows of Mn at the proximal duodenum and terminal ileum and total fecal excretion

| | | Mean squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source | Df | Tot Du Flow | % Sol Du | Sol Du Flow | Tot Il Flow | % Sol Il | Sol Il Flow | Tot Fec Excr |
| Animal | 3 | 29.6 | 15.9 | 35.0 | 67.6 | 23.8 | 12.7 | 80.3 |
| Period | 3 | 351.5 | 61.4 | 368.6 | 58.6 | 10.5 | 13.7 | 94.2 |
| Treatment | 3 | 177.4 | 6.85 | 73.5 | 32.2 | 22.9 | 17.5 | 23.7 |
| Orthogonal treatment contrasts | | | | | | | | |
| HM vs LM | 1 | 289.0 | 5.20 | 135.7 | 39.1 | 59.9 | 50.7 | 31.6 |
| HS vs LS | 1 | 222.0 | 15.0 | 74.2 | 37.8 | 4.59 | 0.59 | 24.8 |
| HMHS vs HM + HS | 1 | 21.2 | 0.35 | 10.4 | 19.8 | 4.11 | 1.22 | 14.6 |
| Error | 6 | 112.7 | 13.9 | 115.0 | 132.2 | 21.6 | 16.2 | 100.1 |

Appendix Table A9: Analysis of variance for various Mn parameters: Differences in flows and % of flows among various locations

| Source | Df | Mean squares | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Du Flow-Intake | Du Flow % Intake | Il Flow-Du Flow | Il Flow % Du Flow | Fec Excr-Il Flow | Fec Excr % Il Flow | Fec Excr-Du Flow | Intake-Fec Excr | App Dig |
| Animal | 3 | 32.1 | 44.5 | 118.1 | 113.6 | 86.0 | 87.0 | 140.8* | 92.2 | 119.7 |
| Period | 3 | 86.9 | 107.2 | 156.8 | 114.7 | 159.5 | 183.8 | 628.9** | 275.0 | 325.4 |
| Treatment | 3 | 261.3 | 385.8 | 64.0 | 68.9 | 47.6 | 40.0 | 203.7* | 24.2 | 28.4 |
| Orthogonal treatment contrasts | | | | | | | | | | |
| HM vs LM | 1 | 480.7* | 716.9* | 115.6 | 123.5 | 141.0 | 119.2 | 511.9** | 0.49 | 4.69 |
| HS vs LS | 1 | 260.0 | 369.8 | 76.6 | 76.0 | 76.0 | 0.22 | 98.5 | 38.4 | 47.7 |
| HMHS vs HM + HS | 1 | 43.2 | 70.6 | 0.023 | 7.26 | 0.39 | 0.59 | 0.60 | 33.6 | 32.8 |
| Error | 6 | 70.3 | 104.0 | 89.7 | 90.8 | 87.8 | 100.0 | 26.0 | 58.6 | 92.1 |

*P<0.05

**P<0.01

Appendix Table A10: Analysis of variance for various Mn, Zn and Fe soluble DM parameters

| Source | Df | Mean squares | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mn | | | Zn | | | Fe | | |
| | | RFSDM | DUSDM | ILSDM | RFSDM | DUSDM | ILSDM | RFSDM | DUSDM | ILSDM |
| Animal | 3 | 948.7* | 254.6 | 36.5 | 634.0 | 2727.3 | 132.6 | 122.3 | 912.8 | 6652.3 |
| Period | 3 | 30.1 | 354.3 | 24.1 | 255.6 | 4120.8 | 695.0 | 732.6 | 661.0 | 339.7 |
| Treatment | 3 | 279.2 | 9.19 | 41.3 | 916.8 | 7772.5* | 391.3 | 510.2 | 6027.9 | 6019.7 |
| Orthogonal treatment contrasts | | | | | | | | | | |
| HM vs LM | 1 | 31.8 | 4.94 | 46.8 | 135.7 | 137.8 | 884.3 | 718.4 | 13290.1 | 15415.7 |
| HS vs LS | 1 | 642.0* | 0.81 | 45.2 | 1769.5 | 20032.9** | 262.8 | 711.7 | 4511.5 | 2214.2 |
| HMHS vs HM + HS | 1 | 163.9 | 21.8 | 31.9 | 845.1 | 3146.9 | 26.8 | 100.7 | 282.2 | 429.3 |
| Error | 6 | 103.0 | 336.6 | 117.7 | 522.6 | 1370.7 | 168.5 | 2446.5 | 4454.7 | 8176.7 |

*P<0.05

**P<0.01

Appendix Table A11: Analysis of variance for various Zn parameters: total, percent soluble and soluble flows of Zn at the proximal duodenum and terminal ileum and total fecal excretion

| | | Mean squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source | Df | Tot Du Flow | % Sol Du | Sol Du Flow | Tot Il Flow | % Sol Il | Sol Il Flow | Tot Fec Excr |
| Animal | 3 | 1922.0 | 87.3 | 2002.1* | 1371.8 | 51.6 | 617.3 | 240.7 |
| Period | 3 | 2184.8 | 41.7 | 774.3 | 2365.5 | 69.2 | 362.2 | 2125.0 |
| Treatment | 3 | 731.3 | 292.5** | 2344.1* | 897.4 | 476.1 | 1059.3* | 1593.1 |
| Orthogonal treatment contrasts | | | | | | | | |
| HM vs LM | 1 | 539.4 | 35.8 | 109.3 | 30.0 | 1249.1* | 2633.7* | 3332.2* |
| HS vs LS | 1 | 1654.5 | 789.9** | 6713.4** | 2639.4 | 3.60 | 213.8 | 369.6 |
| HMHS vs HM + HS | 1 | 0.016 | 51.9 | 209.5 | 22.8 | 175.5 | 330.5 | 1077.5 |
| Error | 6 | 730.3 | 23.4 | 401.3 | 977.3 | 101.7 | 217.2 | 540.5 |

*P<0.05

**P<0.01

Appendix Table A12: Analysis of variance for various Zn parameters: Differences in flows and % of flows among various locations

| | | Mean squares | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | Df | Du Flow-Intake | Du Flow % Intake | Il Flow-Du Flow | Il Flow % Du Flow | Fec Excr-Il Flow | Fec Excr % Il Flow | Fec Excr-Du Flow | Intake-Fec Excr | App Dig |
| Animal | 3 | 2783.2 | 3073.9 | 234.0 | 45.0 | 643.8 | 34.4 | 1024.6 | 493.4 | 334.4 |
| Period | 3 | 1364.9 | 1034.1 | 478.3 | 142.3 | 1111.4 | 377.6 | 2658.3* | 2022.8 | 1747.2 |
| Treatment | 3 | 1103.1 | 1204.5 | 150.4 | 62.0 | 1938.2 | 432.9 | 2694.1* | 1364.8 | 1440.3 |
| Orthogonal treatment contrasts | | | | | | | | | | |
| HM vs LM | 1 | 1233.8 | 1381.4 | 315.1 | 48.3 | 3994.2* | 1109.1** | 6552.9** | 2100.0 | 2399.8 |
| HS vs LS | 1 | 2049.8 | 2161.6 | 114.5 | 116.2 | 1033.6 | 1.35 | 460.1 | 567.6 | 555.5 |
| HMHS vs HM + HS | 1 | 25.8 | 70.6 | 21.6 | 21.5 | 786.8 | 188.2 | 1069.3 | 1427.0 | 1365.6 |
| Error | 6 | 868.3 | 1000.1 | 288.2 | 105.6 | 595.9 | 63.1 | 343.6 | 741.2 | 602.1 |

*P<0.05

**P<0.01

Appendix Table A13: Analysis of variance for various Fe parameters: total, percent soluble and soluble flows of Fe at the proximal duodenum and terminal ileum and total fecal excretion

| | | Mean squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source | Df | Tot Du Flow | % Sol Du | Sol Du Flow | Tot Il Flow | % Sol Il | Sol Il Flow | Tot Fec Excr |
| Animal | 3 | 28739.5 | 16.9 | 9907.5 | 2323.9 | 16.1 | 797.9 | 5582.2 |
| Period | 3 | 31202.0 | 46.5 | 1387.9 | 13959.4 | 18.5 | 823.6 | 9587.2 |
| Treatment | 3 | 53195.5 | 14.7 | 13923.3 | 9614.1 | 34.4 | 3288.7 | 58478.8* |
| Orthogonal treatment contrasts | | | | | | | | |
| HM vs LM | 1 | 124962.3 | 7.82 | 39222.1 | 21978.1 | 83.1 | 7516.1 | 138012.3** |
| HS vs LS | 1 | 14884.0 | 16.0 | 748.3 | 4389.1 | 19.6 | 1956.4 | 11664.0 |
| HMHS vs HM + HS | 1 | 19740.3 | 20.2 | 1799.5 | 2475.1 | 0.48 | 393.7 | 25760.3 |
| Error | 6 | 43886.2 | 47.3 | 12891.7 | 5120.3 | 25.7 | 1892.3 | 6770.9 |

*P<0.05

**P<0.01

Appendix Table A14: Analysis of variance for various Fe parameters: Differences in flows and % of flows among various locations

| Source | Df | Mean squares | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Du Flow-Intake | Du Flow % Intake | Il Flow-Du Flow | Il Flow % Du Flow | Fec Excr-Il Flow | Fec Excr % Il Flow | Fec Excr-Du Flow | Intake-Fec Excr | App Dig |
| Animal | 3 | 27952.7 | 929.8 | 25866.2 | 163.6 | 6621.1 | 123.5 | 9257.2 | 6272.1 | 237.5 |
| Period | 3 | 23927.4 | 870.3 | 38541.1 | 476.0 | 11053.9 | 237.0 | 27905.2 | 18690.6 | 772.0 |
| Treatment | 3 | 30322.1 | 913.6 | 28600.4 | 279.4 | 21283.1 | 495.28 | 17874.7 | 24056.1 | 751.4 |
| Orthogonal treatment contrasts | | | | | | | | | | |
| HM vs LM | 1 | 56050.6 | 1101.6 | 42127.6 | 334.6 | 49840.6 | 1176.9* | 324.0 | 64897.6 | 2050.9 |
| HS vs LS | 1 | 32490.1 | 1598.7 | 35438.1 | 315.6 | 1743.1 | 77.8 | 52900.0 | 2475.1 | 52.5 |
| HMHS vs HM + HS | 1 | 2425.6 | 40.6 | 8235.6 | 188.1 | 12265.6 | 231.2 | 400.0 | 4795.6 | 150.8 |
| Error | 6 | 46292.9 | 1760.4 | 57121.8 | 574.6 | 8882.8 | 179.0 | 48182.5 | 10992.5 | 470.4 |

*P<0.05

Appendix Table A15: Analysis of variance for various Ca parameters: total, percent soluble and soluble flows of Ca in the duodenum and ileum and total fecal excretion

| | | Mean squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source | Df | Tot Du Flow | % Sol Du | Sol Du Flow | Tot Il Flow | % Sol Il | Sol Il Flow | Tot Fec Excr |
| Animal | 3 | 8.37 | 1.46 | 5.94 | 3.80 | 23.2 | 0.32 | 7.28 |
| Period | 3 | 13.8 | 34.8 | 18.5 | 5.30 | 34.1 | 0.77 | 2.97 |
| Treatment | 3 | 11.9 | 11.8 | 11.1 | 5.86 | 55.3 | 0.18 | 1.65 |
| Orthogonal treatment contrasts | | | | | | | | |
| HM vs LM | 1 | 16.8 | 14.9 | 19.8 | 13.1 | 15.6 | 0.064 | 1.21 |
| HS vs LS | 1 | 8.70 | 11.8 | 3.12 | 0.33 | 9.32 | 0.13 | 0.30 |
| HMHS vs HM + HS | 1 | 10.2 | 8.53 | 10.5 | 4.10 | 140.8 | 0.36 | 3.42 |
| Error | 6 | 15.8 | 13.7 | 12.4 | 9.18 | 59.3 | 0.42 | 5.57 |

Appendix Table A16: Analysis of variance for various Ca parameters: Differences in flows and % of flows among various locations

| | | Mean squares | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | Df | Du Flow- Intake | Du Flow % Intake | Il Flow- Du Flow | Il Flow % Du Flow | Fec Excr- Il Flow | Fec Excr % Il Flow | Fec Excr- Du Flow | Intake- Fec Excr | App Dig |
| Animal | 3 | 4.60 | 117.8 | 1.65 | 17.7 | 1.32 | 134.5 | 0.05 | 3.80 | 161.6 |
| Period | 3 | 8.94 | 268.8 | 11.2 | 116.0 | 0.84 | 60.7 | 8.29 | 2.48 | 75.4 |
| Treatment | 3 | 1.80 | 43.4 | 4.68 | 53.2 | 2.14 | 120.9 | 7.69 | 2.50 | 25.7 |
| Orthogonal treatment contrasts | | | | | | | | | | |
| HM vs LM | 1 | 0.56 | 1.28 | 0.23 | 49.5 | 6.38 | 346.1 | 9.00 | 5.06 | 39.3 |
| HS vs LS | 1 | 4.41 | 128.3 | 12.4 | 103.3 | 0.00 | 16.4 | 12.3 | 1.96 | 29.6 |
| HMHS vs HM + HS | 1 | 0.42 | 0.64 | 1.38 | 6.65 | 0.031 | 0.16 | 1.82 | 0.49 | 8.21 |
| Error | 6 | 8.71 | 241.0 | 4.94 | 56.9 | 2.40 | 149.8 | 8.51 | 4.92 | 151.9 |

Appendix Table A17: Analysis of variance for various Ca, Mg and P soluble DM parameters

| Source | Df | Mean squares | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Ca | | | Mg | | | P | | |
| | | RFSDM | DUSDM | ILSDM | RFSDM | DUSDM | ILSDM | RFSDM | DUSDM | ILSDM |
| Animal | 3 | 14.9 | 21.8 | 11.0 | 0.62 | 0.30 | 1.50 | 113.4 | 26.5 | 1.39 |
| Period | 3 | 11.8 | 17.2 | 0.55 | 0.36 | 0.061 | 0.99 | 54.2 | 10.6 | 6.58 |
| Treatment | 3 | 0.18 | 24.5 | 0.42 | 0.38 | 0.91 | 1.54 | 37.8 | 2.27 | 1.35 |
| Orthogonal treatment contrasts | | | | | | | | | | |
| HM vs LM | 1 | 0.067 | 42.1 | 0.81 | 0.20 | 1.06* | 4.51 | 11.8 | 2.47 | 2.97 |
| HS vs LS | 1 | 0.00 | 4.54 | 0.34 | 0.071 | 1.14* | 0.019 | 94.0 | 1.97 | 0.030 |
| HMHS vs HM + HS | 1 | 0.47 | 26.9 | 0.10 | 0.87* | 0.077 | 0.098 | 7.55 | 2.36 | 1.05 |
| Error | 6 | 5:44 | 18.2 | 3.73 | 0.13 | 0.17 | 0.88 | 27.8 | 20.2 | 2.41 |

*P<0.05

Appendix Table A18: Analysis of variance for various Mg parameters: total, percent soluble and soluble flows of Mg at the proximal duodenum and terminal ileum and total fecal excretion

| | | Mean squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source | Df | Tot Du Flow | % Sol Du | Sol Du Flow | Tot Il Flow | % Sol Il | Sol Il Flow | Tot Fec Excr |
| Animal | 3 | 0.17 | 5.53 | 0.15 | 0.020 | 18.7 | 0.057 | 0.028 |
| Period | 3 | 0.26 | 20.4 | 0.35 | 0.12 | 15.5 | 0.093 | 0.057 |
| Treatment | 3 | 0.72 | 1.82 | 0.57 | 0.30 | 17.5 | 0.096 | 0.22 |
| Orthogonal treatment contrasts | | | | | | | | |
| HM vs LM | 1 | 1.19 | 0.43 | 0.99 | 0.27 | 6.73 | 0.16 | 0.43 |
| HS vs LS | 1 | 0.92 | 0.10 | 0.71 | 0.49 | 38.6 | 0.033 | 0.14 |
| HMHS vs HM + HS | 1 | 0.048 | 4.94 | 0.017 | 0.13 | 7.21 | 0.093 | 0.080 |
| Error | 6 | 0.26 | 7.25 | 0.23 | 0.13 | 34.3 | 0.050 | 0.26 |

Appendix Table A19: Analysis of variance for various Mg parameters: Differences in flows and % of flows among various locations

| | | Mean squares | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | Df | Du Flow-Intake | Du Flow % Intake | Il Flow-Du Flow | Il Flow % Du Flow | Fec Excr-Il Flow | Fec Excr % Il Flow | Fec Excr-Du Flow | Intake-Fec Excr | App Dig |
| Animal | 3 | 0.21 | 41.5 | 0.22 | 125.7 | 0.045 | 33.7 | 0.17 | 0.12 | 15.6 |
| Period | 3 | 0.19 | 45.8 | 0.33 | 170.2 | 0.017 | 9.13 | 0.32 | 0.020 | 7.43 |
| Treatment | 3 | 0.62 | 143.7 | 0.14 | 82.0 | 0.046 | 26.5 | 0.18 | 0.18 | 39.6 |
| Orthogonal treatment contrasts | | | | | | | | | | |
| HM vs LM | 1 | 0.49 | 166.0 | 0.33 | 172.4 | 0.020 | 17.1 | 0.19 | 0.073 | 46.2 |
| HS vs LS | 1 | 1.23 | 245.9 | 0.068 | 29.0 | 0.11 | 54.5 | 0.35 | 0.27 | 44.9 |
| HMHS vs HM + HS | 1 | 0.14 | 19.3 | 0.021 | 44.6 | 0.0068 | 7.85 | 0.0039 | 0.19 | 27.8 |
| Error | 6 | 0.25 | 55.3 | 0.20 | 102.2 | 0.075 | 51.8 | 0.080 | 0.25 | 55.1 |

Appendix Table A20: Analysis of variance for various P parameters: total, percent soluble and soluble flows of P at the proximal duodenum and terminal ileum and total fecal excretion

| | | Mean squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source | Df | Tot Du Flow | % Sol Du | Sol Du Flow | Tot Il Flow | % Sol Il | Sol Il Flow | Tot Fec Excr |
| Animal | 3 | 16.4 | 95.1 | 23.0 | 3.21 | 60.6 | 0.051 | 3.61 |
| Period | 3 | 19.7 | 140.6 | 28.0* | 4.45 | 28.8 | 0.89 | 2.46 |
| Treatment | 3 | 13.6 | 13.3 | 4.70* | 4.39 | 82.1 | 0.70 | 5.13 |
| Orthogonal treatment contrasts | | | | | | | | |
| HM vs LM | 1 | 29.7 | 2.40 | 13.1 | 8.70 | 73.4 | 1.84 | 8.70 |
| HS vs LS | 1 | 11.2 | 33.6 | 0.32 | 0.063 | 1.42 | 0.0033 | 2.89 |
| HMHS vs HM + HS | 1 | 0.0025 | 4.00 | 0.65 | 4.41 | 171.5 | 0.25 | 3.80 |
| Error | 6 | 5.64 | 30.0 | 4.40 | 2.09 | 30.8 | 0.40 | 3.25 |

*P<0.05

Appendix Table A21: Analysis of variance for various P parameters: Differences in flows and % of flows among various locations

| Source | Df | Mean squares | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Du Flow-Intake | Du Flow % Intake | Il Flow-Du Flow | Il Flow % Du Flow | Fec Excr-Il Flow | Fec Excr % Il Flow | Fec Excr-Du Flow | Intake-Fec Excr | App Dig |
| Animal | 3 | 14.4 | 619.1 | 12.9* | 32.3 | 1.30 | 191.7 | 9.66* | 3.26 | 183.5 |
| Period | 3 | 16.8 | 750.9 | 13.1* | 36.0 | 0.90 | 140.7 | 10.3* | 1.16 | 85.1 |
| Treatment | 3 | 16.9 | 1092.4 | 7.80* | 32.4 | 0.71 | 122.2 | 11.8** | 6.54 | 314.8 |
| Orthogonal treatment contrasts | | | | | | | | | | |
| HM vs LM | 1 | 37.8* | 2519.3* | 6.25 | 26.0 | 0.00 | 0.28 | 6.25 | 13.3 | 578.3 |
| HS vs LS | 1 | 13.0 | 755.5 | 13.0* | 10.5 | 2.10* | 357.3* | 25.5** | 2.10 | 140.9 |
| HMHS vs HM + HS | 1 | 0.023 | 2.43 | 4.20 | 60.9 | 0.023 | 9.11 | 3.61 | 4.20 | 225.3 |
| Error | 6 | 5.43 | 293.6 | 1.44 | 10.4 | 0.294 | 55.1 | 1.10 | 3.43 | 173.9 |

*P<0.05

**P<0.01

Appendix Table B: Concentration and coefficient of variation for analysis of Dy in Dy-barley pellets across four experimental periods

| | Period | | | |
|---|---|---|---|---|
| Replicate | 1 | 2 | 3 | 4 |
| Dy content (mg $g^{-1}$ DM) | | | | |
| 1 | 2.061 | 2.015 | 1.999 | 2.215 |
| 2 | 2.000 | 2.073 | 2.020 | 2.057 |
| 3 | 2.063 | 1.942 | 1.941 | 2.097 |
| 4 | 2.063 | 1.945 | 2.000 | 2.085 |
| 5 | 2.080 | 1.938 | 1.995 | 2.013 |
| 6 | 1.979 | 1.982 | 2.112 | 2.099 |
| Mean | 2.018 | 1.982 | 2.013 | 2.095 |
| SE | 0.024 | 0.021 | 0.022 | 0.026 |
| Coefficient of variation (%) | 3.03 | 2.71 | 2.80 | 3.22 |

Appendix Table B: Concentration and coefficient of variation for analysis of Dy in Dy-barley pellets across four experimental periods

| | Period | | | |
|---|---|---|---|---|
| Replicate | 1 | 2 | 3 | 4 |
| Dy content (mg $g^{-1}$ DM) | | | | |
| 1 | 2.061 | 2.015 | 1.999 | 2.215 |
| 2 | 2.000 | 2.073 | 2.020 | 2.057 |
| 3 | 2.063 | 1.942 | 1.941 | 2.097 |
| 4 | 2.063 | 1.945 | 2.000 | 2.085 |
| 5 | 2.080 | 1.938 | 1.995 | 2.013 |
| 6 | 1.979 | 1.982 | 2.112 | 2.099 |
| Mean | 2.018 | 1.982 | 2.013 | 2.095 |
| SE | 0.024 | 0.021 | 0.022 | 0.026 |
| Coefficient of variation (%) | 3.03 | 2.71 | 2.80 | 3.22 |

Appendix Table C: The contribution of water to the dietary mineral intake of steers

| Mineral | Concentration ($\mu g\ ml^{-1}$) | Estimated mineral intake† ($mg\ day^{-1}$) |
|---|---|---|
| | Mean (SD) | |
| Ca | 24.53(0.165) | 613.3 |
| Mg | 5.77(0.193) | 144.3 |
| P | ND | - |
| S | 4.16(0.248) | 104.0 |
| Cu | 0.092(0.0145) | 2.3 |
| Mo | ND | - |
| Fe | 0.341(0.2251) | 8.53 |
| Mn | 0.018(0.0055) | 0.45 |
| Zn | 0.697(0.1875) | 17.4 |

ND - Not detectable using the procedure for feed analysis.

†The water consumption by cattle weighing between 235 and 273 kg was estimated to be 25 L $day^{-1}$ at an environmental temperature range of 18 to 26°C (NAS-NRC, 1984).

Explanation for Appendix Tables D1-D10, containing raw data that was used for the preparation of Data Tables 1-24.

Abbreviations:

TRT - Refers to the following treatments:

A = LMLS

B = LMHS

C = HMLS

D = HMHS

PD - Periods 1 to 4 of the Latin-Square

ANIM - Animal I.D. Number

PCTDM - Percent dry matter

Note: All mineral analyses for rations (Table D1), Dy-barley (Table D2), total duodenal (Table D4) and total ileal (Table 7) digesta and feces (Table D10) are expressed on a dry matter basis. Analyses for duodenal (Table D5) and ileal (Table D8) digesta particulate fractions are expressed on a freeze-dried to constant weight of 97% DM basis. Analyses for duodenal (Table D6) and ileal (Table D9) digesta supernatant fractions are expressed on a per gram of liquid supernatant. Analyses for rumen fluid supernatants (Table D3) are expressed on a dry matter basis.

Dy - Dysprosium:

$mg\ g^{-1}$ (Table D2)

$\mu g\ g^{-1}$ (Tables D4, D7 and D10)

CA - Calcium, mg $g^{-1}$

MG - Magnesium, mg $g^{-1}$

P - Phosphorus, mg $g^{-1}$

S - Sulfur, mg $g^{-1}$

CU - Copper, µg $g^{-1}$

MO - Molybdenum, µg $g^{-1}$

FE - Iron, µg $g^{-1}$

MN - Manganese, µg $g^{-1}$

ZN - Zinc, µg $g^{-1}$

TOTWT - Total weight, g

Note: Total weight (g) of duodenal and ileal digesta supernatant and total freeze-dried weight of duodenal and ileal digesta particulate fractions obtained upon centrifugation. These weights were used for the calculation of % soluble mineral as described in Equation III (see Materials and Methods section, under "Calculations").

APPENDIX TABLE D1: CONCENTRATION OF MINERALS IN FOUR EXPERIMENTAL RATIONS ACROSS FOUR PERIODS

| OBS | TRT | PD | ANIM | PCTDM | CA | MG | P | S | CU | MO | FE | MN | ZN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | 1 | 24 | 95.8 | 3.92 | 1.57 | 3.10 | 1.10 | 2.80 | 1.97 | 116.8 | 17.84 | 23.54 |
| 2 | B | 1 | 33 | 95.5 | 3.55 | 1.44 | 3.20 | 4.10 | 3.61 | 1.80 | 130.1 | 18.33 | 25.92 |
| 3 | C | 1 | 29 | 96.3 | 3.73 | 1.45 | 3.00 | 1.10 | 2.47 | 13.10 | 129.9 | 16.79 | 21.79 |
| 4 | D | 1 | 27 | 95.9 | 3.91 | 1.41 | 3.00 | 3.98 | 3.90 | 12.52 | 118.6 | 18.49 | 29.39 |
| 5 | A | 2 | 29 | 95.8 | 3.42 | 1.49 | 2.91 | 1.03 | 2.72 | 1.58 | 110.5 | 17.94 | 22.14 |
| 6 | B | 2 | 24 | 95.6 | 3.42 | 1.47 | 3.07 | 3.99 | 3.32 | 2.65 | 138.6 | 18.95 | 23.65 |
| 7 | C | 2 | 27 | 96.4 | 3.81 | 1.44 | 3.12 | 1.44 | 2.62 | 12.99 | 106.3 | 18.68 | 24.31 |
| 8 | D | 2 | 33 | 95.8 | 4.22 | 1.41 | 3.07 | 3.72 | 3.75 | 14.10 | 102.0 | 19.13 | 23.51 |
| 9 | A | 3 | 33 | 95.3 | 3.71 | 1.44 | 2.87 | 1.36 | 2.59 | 1.73 | 107.0 | 17.88 | 23.32 |
| 10 | B | 3 | 27 | 95.6 | 3.69 | 1.48 | 2.73 | 3.71 | 2.65 | 1.45 | 102.6 | 17.07 | 20.15 |
| 11 | C | 3 | 24 | 96.6 | 3.58 | 1.41 | 3.00 | 1.39 | 2.54 | 12.78 | 105.1 | 16.76 | 21.15 |
| 12 | D | 3 | 29 | 95.3 | 4.79 | 1.47 | 2.98 | 3.83 | 3.55 | 13.40 | 107.4 | 18.26 | 22.33 |
| 13 | A | 4 | 27 | 95.8 | 3.71 | 1.51 | 3.04 | 1.26 | 2.84 | 1.37 | 120.0 | 18.96 | 23.49 |
| 14 | B | 4 | 29 | 95.7 | 3.69 | 1.48 | 2.96 | 3.83 | 3.03 | 1.53 | 149.4 | 18.61 | 23.34 |
| 15 | C | 4 | 33 | 95.4 | 3.58 | 1.46 | 3.04 | 1.26 | 3.00 | 11.91 | 103.0 | 23.45 | 28.34 |
| 16 | D | 4 | 24 | 95.9 | 4.79 | 1.50 | 3.03 | 3.84 | 3.32 | 10.92 | 100.5 | 18.38 | 24.60 |

APPENDIX TABLE D2: CONCENTRATION OF MINERALS IN DY-BARLEY ACROSS FOUR PERIODS

| OBS | PD | PCTDM | DY | CA | MG | P | S | CU | MO | FE | MN | ZN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 95.1 | 2.018 | 0.51 | 1.35 | 3.60 | 0.78 | 5.90 | 1.97 | 70.00 | 11.77 | 29.32 |
| 2 | 2 | 95.6 | 1.982 | 0.49 | 1.34 | 3.75 | 1.28 | 8.18 | 1.47 | 71.22 | 15.00 | 28.71 |
| 3 | 3 | 95.2 | 2.013 | 0.47 | 1.29 | 3.52 | 1.21 | 5.51 | 0.82 | 69.08 | 12.28 | 26.33 |
| 4 | 4 | 95.0 | 2.095 | 0.47 | 1.31 | 3.65 | 1.45 | 6.18 | 1.27 | 70.32 | 12.86 | 30.97 |

APPENDIX TABLE D3: CONCENTRATION OF MINERALS IN RUMEN FLUID SUPERNATANT OF STEERS FED DIETS HIGH IN DIETARY MO AND S

| OBS | TRT | PD | ANIM | PCTDM | CA | MG | P | CU | MO | FE | MN | ZN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | 1 | 24 | 2.55 | 9.73 | 4.38 | 29.61 | 5.05 | 1.26 | 141.13 | 57.41 | 44.72 |
| 2 | B | 1 | 33 | 2.14 | 8.42 | 5.71 | 36.03 | 5.06 | 1.88 | 92.05 | 70.28 | 10.90 |
| 3 | C | 1 | 29 | 1.70 | 4.68 | 5.24 | 38.16 | 11.10 | 18.15 | 100.83 | 56.48 | 64.94 |
| 4 | D | 1 | 27 | 1.73 | 12.61 | 4.92 | 23.72 | 3.43 | 13.05 | 38.36 | 47.67 | 11.36 |
| 5 | A | 2 | 29 | 1.67 | 4.87 | 4.49 | 32.13 | 28.09 | 3.95 | 48.74 | 43.06 | 18.81 |
| 6 | B | 2 | 24 | 1.73 | 5.58 | 4.15 | 31.09 | 4.54 | 2.05 | 30.29 | 49.33 | 8.11 |
| 7 | C | 2 | 27 | 1.72 | 8.30 | 4.83 | 38.70 | 4.00 | 13.57 | 126.46 | 85.91 | 93.69 |
| 8 | D | 2 | 33 | 1.76 | 9.00 | 4.53 | 42.12 | 2.77 | 12.09 | 58.25 | 77.45 | 13.95 |
| 9 | A | 3 | 33 | 2.07 | 6.15 | 5.20 | 48.60 | 7.54 | 1.65 | 121.47 | 89.77 | 16.73 |
| 10 | B | 3 | 27 | 1.77 | 8.38 | 4.74 | 26.63 | 3.16 | 1.51 | 90.24 | 52.82 | 23.94 |
| 11 | C | 3 | 24 | 1.81 | 7.62 | 4.16 | 34.91 | 29.08 | 16.58 | 77.14 | 52.91 | 16.20 |
| 12 | D | 3 | 29 | 1.69 | 2.22 | 3.45 | 25.52 | 4.90 | 18.14 | 79.37 | 49.68 | 19.77 |
| 13 | A | 4 | 27 | 2.07 | 4.95 | 4.25 | 35.46 | 6.70 | 2.44 | 76.94 | 58.42 | 44.25 |
| 14 | B | 4 | 29 | 1.81 | 4.68 | 5.05 | 38.15 | 12.20 | 1.74 | 142.41 | 51.16 | 55.57 |
| 15 | C | 4 | 33 | 1.83 | 6.99 | 5.05 | 46.40 | 4.71 | 13.96 | 50.31 | 90.24 | 31.12 |
| 16 | D | 4 | 24 | 1.85 | 2.40 | 3.99 | 41.91 | 6.02 | 18.30 | 105.34 | 34.46 | 18.60 |

APPENDIX TABLE D4: CONCENTRATION OF MINERALS IN TOTAL DUODENAL DIGESTA OF STEERS FED DIETS HIGH IN DIETARY MO AND S

| OBS | TRT | PD | ANIM | PCTDM | DY | CA | MG | P | CU | MO | FE | MN | ZN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | 1 | 24 | 97.0 | 70.38 | 6.91 | 1.46 | 12.09 | 8.56 | 4.01 | 487.46 | 33.35 | 76.10 |
| 2 | B | 1 | 33 | 97.5 | 84.79 | 7.59 | 1.79 | 13.67 | 9.95 | 2.65 | 362.42 | 38.75 | 77.40 |
| 3 | C | 1 | 29 | 96.9 | 80.02 | 6.95 | 1.38 | 11.71 | 9.60 | 26.65 | 316.13 | 34.05 | 109.45 |
| 4 | D | 1 | 27 | 97.4 | 78.81 | 9.28 | 1.61 | 10.82 | 8.36 | 18.38 | 236.25 | 38.86 | 79.36 |
| 5 | A | 2 | 29 | 97.7 | 77.88 | 7.05 | 1.49 | 10.81 | 9.19 | 2.83 | 267.44 | 40.28 | 108.13 |
| 6 | B | 2 | 24 | 97.6 | 57.31 | 6.14 | 1.67 | 10.69 | 8.29 | 1.84 | 317.45 | 34.52 | 56.15 |
| 7 | C | 2 | 27 | 97.8 | 76.85 | 7.23 | 1.35 | 10.45 | 9.21 | 20.34 | 426.54 | 33.19 | 60.89 |
| 8 | D | 2 | 33 | 97.1 | 86.62 | 10.92 | 1.89 | 13.79 | 12.85 | 20.60 | 339.55 | 43.05 | 80.94 |
| 9 | A | 3 | 33 | 98.0 | 77.79 | 8.84 | 1.62 | 13.09 | 9.43 | 2.90 | 304.11 | 40.61 | 63.43 |
| 10 | B | 3 | 27 | 97.7 | 65.39 | 7.61 | 1.56 | 9.44 | 7.80 | 2.05 | 269.83 | 31.48 | 55.35 |
| 11 | C | 3 | 24 | 97.5 | 85.06 | 8.56 | 1.45 | 11.09 | 9.56 | 20.37 | 275.30 | 30.48 | 68.45 |
| 12 | D | 3 | 29 | 97.8 | 78.90 | 12.00 | 1.54 | 11.09 | 9.09 | 20.01 | 245.51 | 36.28 | 59.84 |
| 13 | A | 4 | 27 | 97.8 | 59.68 | 6.37 | 1.36 | 9.24 | 7.16 | 2.07 | 244.23 | 29.54 | 52.64 |
| 14 | B | 4 | 29 | 97.4 | 73.10 | 6.87 | 1.60 | 12.83 | 9.30 | 2.34 | 337.59 | 41.83 | 72.83 |
| 15 | C | 4 | 33 | 97.8 | 76.85 | 10.44 | 1.75 | 12.50 | 9.99 | 17.82 | 265.46 | 43.23 | 73.10 |
| 16 | D | 4 | 24 | 97.4 | 86.31 | 7.60 | 1.77 | 14.99 | 9.37 | 19.49 | 294.00 | 46.11 | 68.97 |

APPENDIX TABLE D5: CONCENTRATION OF MINERALS IN DUODENAL PARTICULATE DIGESTA OF STEERS FED DIETS HIGH IN DIETARY MO AND S

| OBS | TRT | PD | ANIM | TOTWT | CA | MG | P | CU | MO | FE | MN | ZN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | 1 | 24 | 9.894 | 1.41 | 0.228 | 5.42 | 8.09 | 6.81 | 176.19 | 7.12 | 25.53 |
| 2 | B | 1 | 33 | 10.239 | 1.59 | 0.328 | 7.38 | 12.32 | 4.43 | 249.52 | 11.00 | 42.25 |
| 3 | C | 1 | 29 | 7.209 | 2.31 | 0.413 | 8.76 | 21.79 | 15.67 | 208.57 | 14.47 | 40.60 |
| 4 | D | 1 | 27 | 8.044 | 2.32 | 0.329 | 8.63 | 12.21 | 11.55 | 156.16 | 13.77 | 46.45 |
| 5 | A | 2 | 29 | 8.447 | 1.03 | 0.169 | 5.18 | 10.98 | 3.57 | 162.32 | 11.77 | 24.63 |
| 6 | B | 2 | 24 | 8.019 | 2.08 | 0.216 | 6.52 | 11.59 | 9.33 | 145.69 | 9.11 | 26.25 |
| 7 | C | 2 | 27 | 10.662 | 1.22 | 0.203 | 6.47 | 8.85 | 12.45 | 163.18 | 7.97 | 19.63 |
| 8 | D | 2 | 33 | 7.946 | 1.86 | 0.273 | 8.73 | 12.61 | 8.24 | 192.91 | 11.59 | 42.27 |
| 9 | A | 3 | 33 | 8.957 | 1.89 | 0.252 | 6.93 | 14.18 | 15.14 | 154.71 | 10.91 | 24.29 |
| 10 | B | 3 | 27 | 8.393 | 1.50 | 0.245 | 5.68 | 17.75 | 4.88 | 125.06 | 8.22 | 34.89 |
| 11 | C | 3 | 24 | 6.449 | 1.22 | 0.210 | 5.30 | 19.82 | 21.64 | 250.12 | 6.37 | 19.02 |
| 12 | D | 3 | 29 | 5.053 | 1.71 | 0.198 | 6.17 | 18.30 | 16.57 | 143.82 | 8.25 | 24.00 |
| 13 | A | 4 | 27 | 9.448 | 1.33 | 0.231 | 5.78 | 15.60 | 6.31 | 163.17 | 8.97 | 18.69 |
| 14 | B | 4 | 29 | 6.736 | 1.23 | 0.233 | 6.73 | 15.86 | 9.21 | 200.27 | 12.31 | 37.53 |
| 15 | C | 4 | 33 | 6.097 | 1.51 | 0.216 | 7.33 | 16.46 | 18.49 | 177.12 | 9.68 | 23.25 |
| 16 | D | 4 | 24 | 7.559 | 1.07 | 0.198 | 5.86 | 25.91 | 19.09 | 176.92 | 9.27 | 25.50 |

APPENDIX TABLE D6: CONCENTRATION OF MINERALS IN DUODENAL DIGESTA SUPERNATANT OF STEERS FED DIETS HIGH IN DIETARY MO AND S

| OBS | TRT | PD | ANIM | TOTWT | PCTDM | CA | MG | P | CU | MO | FE | MN | ZN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | 1 | 24 | 191.305 | 1.78 | 0.402 | 0.095 | 0.429 | 0.122 | 0.089 | 8.16 | 1.73 | 6.10 |
| 2 | B | 1 | 33 | 181.693 | 1.84 | 0.433 | 0.133 | 0.535 | 0.087 | 0.028 | 9.49 | 2.18 | 3.28 |
| 3 | C | 1 | 29 | 120.239 | 2.48 | 0.563 | 0.120 | 0.621 | 0.232 | 0.324 | 9.04 | 2.61 | 7.07 |
| 4 | D | 1 | 27 | 173.687 | 1.90 | 0.620 | 0.113 | 0.417 | 0.068 | 0.175 | 7.70 | 2.35 | 4.26 |
| 5 | A | 2 | 29 | 175.796 | 1.76 | 0.403 | 0.093 | 0.457 | 0.133 | 0.032 | 7.43 | 2.21 | 5.78 |
| 6 | B | 2 | 24 | 183.808 | 1.54 | 0.342 | 0.098 | 0.388 | 0.092 | 0.075 | 6.38 | 1.62 | 2.20 |
| 7 | C | 2 | 27 | 175.872 | 1.93 | 0.501 | 0.098 | 0.462 | 0.088 | 0.229 | 8.16 | 2.17 | 3.83 |
| 8 | D | 2 | 33 | 142.659 | 1.66 | 0.463 | 0.091 | 0.420 | 0.056 | 0.145 | 6.05 | 1.70 | 3.09 |
| 9 | A | 3 | 33 | 207.450 | 1.90 | 0.569 | 0.155 | 0.662 | 0.182 | 0.137 | 8.70 | 2.72 | 4.09 |
| 10 | B | 3 | 27 | 171.593 | 1.51 | 0.403 | 0.090 | 0.356 | 0.183 | 0.050 | 6.25 | 1.72 | 2.28 |
| 11 | C | 3 | 24 | 218.206 | 1.57 | 0.373 | 0.067 | 0.401 | 0.073 | 0.197 | 6.10 | 1.50 | 3.75 |
| 12 | D | 3 | 29 | 124.090 | 1.96 | 0.663 | 0.100 | 0.470 | 0.138 | 0.235 | 6.88 | 2.14 | 3.34 |
| 13 | A | 4 | 27 | 180.349 | 1.87 | 0.441 | 0.101 | 0.421 | 0.245 | 0.060 | 6.44 | 2.05 | 3.28 |
| 14 | B | 4 | 29 | 166.428 | 1.87 | 0.378 | 0.096 | 0.542 | 0.122 | 0.047 | 9.38 | 2.42 | 3.58 |
| 15 | C | 4 | 33 | 186.406 | 1.68 | 0.490 | 0.088 | 0.446 | 0.096 | 0.178 | 5.48 | 2.48 | 3.40 |
| 16 | D | 4 | 24 | 194.501 | 1.91 | 0.417 | 0.107 | 0.683 | 0.091 | 0.243 | 8.89 | 2.63 | 3.31 |

APPENDIX TABLE D7· CONCENTRATION OF MINRALS IN TOTAL ILEAL DIGESTA OF STEERS FED DIETS HIGH IN DIETARY MO AND S

| OBS | TRT | PD | ANIM | PCTDM | DY | CA | MG | P | CU | MO | FE | MN | ZN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | 1 | 24 | 97.5 | 129.79 | 7.03 | 2.41 | 7.72 | 13.83 | 3.77 | 456.76 | 48.57 | 113.41 |
| 2 | B | 1 | 33 | 97.0 | 156.07 | 7.29 | 3.38 | 5.81 | 18.85 | 4.83 | 571.37 | 64.58 | 122.51 |
| 3 | C | 1 | 29 | 97.2 | 146.92 | 8.57 | 2.91 | 6.88 | 21.68 | 19.75 | 506.01 | 65.66 | 195.35 |
| 4 | D | 1 | 27 | 97.3 | 135.24 | 10.48 | 2.96 | 5.34 | 17.32 | 25.62 | 561.97 | 67.77 | 134.84 |
| 5 | A | 2 | 29 | 97.4 | 127.16 | 6.55 | 2.27 | 5.68 | 14.97 | 3.46 | 388.70 | 60.87 | 149.06 |
| 6 | B | 2 | 24 | 97.1 | 124.75 | 6.06 | 2.89 | 6.37 | 14.81 | 3.79 | 447.18 | 57.22 | 80.12 |
| 7 | C | 2 | 27 | 97.4 | 130.24 | 5.95 | 2.45 | 3.76 | 13.92 | 16.91 | 376.58 | 55.87 | 84.79 |
| 8 | D | 2 | 33 | 97.1 | 153.12 | 7.84 | 2.50 | 5.30 | 16.90 | 28.56 | 373.14 | 56.49 | 96.73 |
| 9 | A | 3 | 33 | 97.7 | 148.35 | 9.29 | 3.00 | 6.48 | 18.06 | 4.17 | 519.84 | 72.74 | 94.30 |
| 10 | B | 3 | 27 | 97.6 | 112.40 | 6.37 | 2.35 | 3.16 | 12.97 | 3.50 | 385.54 | 45.55 | 76.53 |
| 11 | C | 3 | 24 | 97.4 | 152.68 | 7.23 | 2.52 | 3.16 | 17.59 | 26.96 | 469.01 | 54.13 | 123.98 |
| 12 | D | 3 | 29 | 97.3 | 177.43 | 13.89 | 3.47 | 6.83 | 17.66 | 41.12 | 493.94 | 73.77 | 94.58 |
| 13 | A | 4 | 27 | 98.0 | 122.88 | 6.45 | 2.37 | 4.85 | 12.54 | 2.54 | 390.99 | 51.14 | 84.41 |
| 14 | B | 4 | 29 | 97.7 | 161.86 | 6.77 | 3.46 | 7.55 | 18.03 | 4.12 | 610.48 | 80.29 | 124.05 |
| 15 | C | 4 | 33 | 97.5 | 133.32 | 11.23 | 2.58 | 5.07 | 14.38 | 17.84 | 377.75 | 55.49 | 95.84 |
| 16 | D | 4 | 24 | 97.1 | 181.98 | 8.53 | 3.60 | 7.39 | 18.18 | 31.87 | 542.51 | 84.01 | 109.63 |

APPENDIX TABLE D8: CONCENTRATION OF MINERALS IN ILEAL PARTICULATE DIGESTA OF STEERS FED DIETS HIGH IN DIETARY MO AND S

| OBS | TRT | PD | ANIM | TOTWT | CA | MG | P | CU | MO | FE | MN | ZN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | 1 | 24 | 12.453 | 3.98 | 0.72 | 7.31 | 4.49 | 4.98 | 432.87 | 25.52 | 38.08 |
| 2 | B | 1 | 33 | 12.424 | 5.74 | 1.57 | 4.53 | 5.72 | 4.61 | 616.16 | 64.17 | 54.06 |
| 3 | C | 1 | 29 | 9.679 | 6.96 | 1.29 | 5.42 | 5.77 | 17.77 | 513.20 | 57.10 | 62.29 |
| 4 | D | 1 | 27 | 14.043 | 11.23 | 1.58 | 7.51 | 7.95 | 13.24 | 604.05 | 86.71 | 93.35 |
| 5 | A | 2 | 29 | 16.904 | 6.14 | 1.13 | 5.71 | 4.19 | 3.02 | 315.26 | 42.49 | 32.28 |
| 6 | B | 2 | 24 | 16.599 | 6.65 | 1.81 | 6.88 | 4.09 | 3.92 | 531.57 | 58.17 | 20.35 |
| 7 | C | 2 | 27 | 14.824 | 4.99 | 1.17 | 3.09 | 5.15 | 10.13 | 407.81 | 58.28 | 38.95 |
| 8 | D | 2 | 33 | 19.255 | 7.81 | 1.40 | 5.56 | 4.65 | 27.78 | 378.87 | 53.21 | 44.36 |
| 9 | A | 3 | 33 | 18.565 | 7.79 | 1.56 | 5.85 | 4.63 | 3.08 | 448.48 | 62.80 | 30.23 |
| 10 | B | 3 | 27 | 19.797 | 4.58 | 1.14 | 2.70 | 4.38 | 3.04 | 397.87 | 46.68 | 34.91 |
| 11 | C | 3 | 24 | 16.240 | 5.12 | 1.19 | 3.85 | 5.33 | 28.05 | 470.40 | 45.62 | 37.99 |
| 12 | D | 3 | 29 | 11.740 | 6.41 | 1.45 | 6.77 | 7.64 | 12.03 | 496.64 | 79.25 | 55.93 |
| 13 | A | 4 | 27 | 18.754 | 6.75 | 0.97 | 3.97 | 2.90 | 2.42 | 410.90 | 43.95 | 30.28 |
| 14 | B | 4 | 29 | 9.030 | 10.26 | 2.12 | 7.33 | 6.37 | 3.95 | 454.00 | 64.67 | 23.38 |
| 15 | C | 4 | 33 | 17.657 | 10.89 | 1.40 | 5.31 | 4.62 | 19.18 | 422.75 | 67.12 | 54.00 |
| 16 | D | 4 | 24 | 18.507 | 8.95 | 2.30 | 7.56 | 10.84 | 36.06 | 464.97 | 85.32 | 55.26 |

APPENDIX TABLE D9: CONCENTRATION OF MINERALS IN ILEAL DIGESTA SUPERNATANT OF STEERS FED DIETS HIGH IN DIETARY MO AND S

| OBS | TRT | PD | ANIM | TOTWT | PCTDM | CA | MG | P | CU | MO | FE | MN | ZN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | 1 | 24 | 81.686 | 2.94 | 0.170 | 0.177 | 0.330 | 0.995 | 0.319 | 11.10 | 0.531 | 3.23 |
| 2 | B | 1 | 33 | 111.875 | 3.66 | 0.377 | 0.318 | 0.301 | 1.212 | 0.305 | 16.49 | 1.186 | 4.07 |
| 3 | C | 1 | 29 | 93.596 | 3.11 | 0.293 | 0.206 | 0.299 | 1.159 | 1.117 | 11.84 | 0.922 | 3.42 |
| 4 | D | 1 | 27 | 135.143 | 3.38 | 0.262 | 0.255 | 0.302 | 0.706 | 1.087 | 12.80 | 0.584 | 3.44 |
| 5 | A | 2 | 29 | 153.356 | 2.44 | 0.176 | 0.166 | 0.164 | 1.266 | 0.254 | 11.12 | 0.551 | 2.44 |
| 6 | B | 2 | 24 | 154.195 | 3.64 | 0.280 | 0.263 | 0.387 | 0.948 | 0.392 | 16.09 | 1.377 | 3.35 |
| 7 | C | 2 | 27 | 121.469 | 2.89 | 0.287 | 0.215 | 0.222 | 1.194 | 0.788 | 9.76 | 0.675 | 2.40 |
| 8 | D | 2 | 33 | 140.286 | 3.00 | 0.356 | 0.250 | 0.205 | 0.620 | 1.188 | 11.11 | 0.550 | 1.91 |
| 9 | A | 3 | 33 | 143.203 | 3.24 | 0.306 | 0.256 | 0.267 | 0.803 | 0.368 | 18.29 | 0.977 | 3.19 |
| 10 | B | 3 | 27 | 181.681 | 2.67 | 0.238 | 0.183 | 0.148 | 0.435 | 0.353 | 9.05 | 0.568 | 1.64 |
| 11 | C | 3 | 24 | 130.189 | 2.49 | 0.376 | 0.240 | 0.125 | 0.394 | 1.067 | 10.32 | 0.957 | 2.05 |
| 12 | D | 3 | 29 | 84.793 | 2.96 | 0.279 | 0.268 | 0.198 | 0.455 | 0.897 | 10.34 | 0.664 | 2.03 |
| 13 | A | 4 | 27 | 159.848 | 3.16 | 0.400 | 0.237 | 0.275 | 0.639 | 0.300 | 10.33 | 1.416 | 2.82 |
| 14 | B | 4 | 29 | 112.699 | 3.11 | 0.198 | 0.197 | 0.253 | 0.554 | 0.254 | 16.97 | 0.686 | 3.45 |
| 15 | C | 4 | 33 | 135.345 | 2.93 | 0.294 | 0.274 | 0.210 | 0.982 | 0.986 | 8.81 | 0.637 | 2.15 |
| 16 | D | 4 | 24 | 164.712 | 3.35 | 0.224 | 0.260 | 0.290 | 0.580 | 1.525 | 15.78 | 1.856 | 2.40 |

APPENDIX TABLE D10: CONCENTRATION OF MINERALS IN FECES OF STEERS FED DIETS HIGH IN DIETARY MO AND S

| OBS | TRT | PD | ANIM | PCTDM | DY | CA | MG | P | CU | MO | FE | MN | ZN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | A | 1 | 24 | 96.0 | 163.69 | 8.78 | 3.17 | 9.28 | 6.26 | 4.42 | 632.31 | 57.06 | 38.75 |
| 2 | B | 1 | 33 | 96.2 | 177.35 | 8.84 | 3.67 | 6.08 | 11.59 | 4.86 | 677.79 | 66.89 | 65.64 |
| 3 | C | 1 | 29 | 96.8 | 181.43 | 10.79 | 3.59 | 8.23 | 11.16 | 27.02 | 610.28 | 72.42 | 145.93 |
| 4 | D | 1 | 27 | 96.4 | 171.85 | 11.19 | 3.46 | 5.77 | 11.24 | 33.70 | 517.40 | 82.09 | 89.13 |
| 5 | A | 2 | 29 | 96.6 | 183.19 | 8.99 | 3.19 | 8.47 | 8.06 | 4.46 | 541.69 | 64.21 | 39.22 |
| 6 | B | 2 | 24 | 96.0 | 172.09 | 8.91 | 4.10 | 8.36 | 7.92 | 4.78 | 829.93 | 61.66 | 29.38 |
| 7 | C | 2 | 27 | 96.5 | 180.75 | 8.67 | 3.22 | 5.84 | 8.23 | 22.95 | 460.88 | 64.27 | 40.32 |
| 8 | D | 2 | 33 | 96.1 | 212.83 | 12.13 | 3.58 | 7.87 | 11.35 | 36.87 | 537.98 | 66.61 | 48.90 |
| 9 | A | 3 | 33 | 96.8 | 171.64 | 13.10 | 3.57 | 9.40 | 11.67 | 3.92 | 611.74 | 69.44 | 45.92 |
| 10 | B | 3 | 27 | 97.0 | 137.69 | 8.61 | 2.81 | 3.93 | 7.02 | 3.82 | 581.29 | 56.14 | 41.27 |
| 11 | C | 3 | 24 | 96.6 | 202.36 | 8.03 | 3.00 | 4.12 | 10.43 | 40.91 | 539.93 | 60.05 | 87.94 |
| 12 | D | 3 | 29 | 97.4 | 216.13 | 14.93 | 4.29 | 7.92 | 18.74 | 51.68 | 534.67 | 88.68 | 57.72 |
| 13 | A | 4 | 27 | 97.6 | 157.48 | 8.25 | 3.06 | 6.05 | 9.45 | 2.63 | 456.21 | 53.75 | 27.11 |
| 14 | B | 4 | 29 | 97.6 | 173.00 | 7.14 | 3.22 | 7.79 | 9.73 | 4.38 | 544.42 | 53.16 | 26.65 |
| 15 | C | 4 | 33 | 97.8 | 173.95 | 13.09 | 3.59 | 8.16 | 17.68 | 25.84 | 436.24 | 71.20 | 57.77 |
| 16 | D | 4 | 24 | 97.7 | 203.78 | 9.38 | 3.52 | 8.28 | 14.79 | 36.62 | 515.45 | 71.98 | 48.97 |